**Abstract.**—The reproductive biology of Atlantic croaker, *Micropogonias undulatus*, collected during 1990–91 from commercial catches in Chesapeake Bay and in Virginia and North Carolina coastal waters (*n*=3,091), was studied by using macroscopic and microscopic gonad staging, the gonadosomatic index, oocyte diameter distributions, and histological analysis. Atlantic croaker are multiple spawners with asynchronous oocyte development and indeterminate fecundity. Mean length at first maturity for males and females was 182 and 173 mm TL, respectively. More than 85% of both sexes were mature by the end of their first year and all were mature by age 2. Spawning extends over a protracted period (July–December), but individual fish apparently spawn over a shorter interval. Eleven gravid and running-ripe females were collected within the Chesapeake Bay suggesting some spawning occurs in estuarine waters. Monthly sex ratios indicated a strong predominance of females during the main period of spawning. A high incidence of atretic, advanced yolked oocytes in spawning females collected throughout the spawning season suggests that a surplus production of yolked oocytes may be part of the reproductive strategy of Atlantic croaker.

# Maturity, spawning, and ovarian cycle of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters*

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The Atlantic croaker, *Micropogonias undulatus* (Linnaeus), ranges from Cape Cod, Massachusetts, to the Bay of Campeche, Mexico (Welsh and Breder, 1923; Johnson, 1978). Although not common north of New Jersey (Hildebrand and Schroeder, 1928; McHugh, 1981), it is one of the most abundant inshore, demersal species of the Atlantic and Gulf of Mexico coasts of the United States (Joseph, 1972).

Despite the large number of studies describing spawning periodicity of Atlantic croaker in the mid-Atlantic and Chesapeake regions (e.g. Hildebrand and Schroeder, 1928; Wallace, 1940; Johnson, 1978; Colton et al., 1979; Morse, 1980; Norcross and Austin, 1988), studies on reproductive biology are rare and mostly incomplete. Information on sexual maturity, fecundity, and sex ratios has been reported (Hildebrand and Schroeder, 1928; Wallace, 1940; Morse, 1980). However, speculation on whether or not Atlantic croaker spawn within Chesapeake Bay (Welsh and Breder, 1923; Pearson, 1941; Haven, 1957) has not been investigated; estimates of size at maturity (Wallace, 1940; Morse,

1980) do not agree; estimates of age at maturity (Welsh and Breder, 1923; Wallace, 1940) were based on length frequency and scale ageing, which have been shown to be less accurate than otolith ageing for Atlantic croaker (Joseph, 1972; Barbieri et al., 1994); and available fecundity estimates (Morse, 1980) cannot be used without an evaluation of Atlantic croaker's fecundity pattern, i.e. whether they have determinate or indeterminate annual fecundity.

Traditionally, estimates of fish fecundity have been based on the assumption that the total number of eggs spawned by a female each year (annual fecundity) is fixed prior to the onset of spawning, a condition known as determinate fecundity (Hunter et al., 1992). However, recent evidence (Hunter and Goldberg, 1980; Hunter and Macewicz, 1985a; Hunter et al., 1985; Horwood and Greer Walker, 1990) indicates that in many temperate and tropical fish annual fecundity cannot be estimated from the standing stock of advanced oocytes because unyolked oocytes continue to be matured and spawned through-

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out the spawning season. This condition is called indeterminate fecundity (Hunter et al., 1992). The only way to estimate annual fecundity, therefore, is by estimating batch fecundity—the number of eggs released during each spawning—and multiplying it by spawning frequency—the number of times an average female spawns during the spawning season (Hunter and Macewicz, 1985a; Hunter et al., 1985, 1992). Although the extended spawning season of Atlantic croaker (Wallace, 1940; Colton et al., 1979; Warlen, 1982) suggests it is a multiple spawner with indeterminate fecundity, no attempt has been made to evaluate its fecundity pattern.

In this study we test the assumption of determinate annual fecundity and describe spawning periodicity, size and age at maturity, sex ratios, ovarian cycle, and oocyte atresia for Atlantic croaker in the Chesapeake Bay and adjacent coastal waters.

## Methods

Four approaches were used to sample Atlantic croaker for this study. In 1990 and 1991 fish were collected from commercial poundnet, haul-seine, and gillnet fisheries that operate from late spring to early fall in the lower Chesapeake Bay (Fig. 1). Local fish processing houses and seafood dealers were contacted weekly during April–October 1990 and 1991, and one 22.7 kg (50 lb) box of fish of each available market grade (small, medium, or large) was purchased for processing. Since Atlantic croaker migrate out of Chesapeake Bay in midfall to overwinter offshore (Haven, 1959), monthly samples from November to March 1990 and from November through December 1991 were obtained from commercial trawlers operating in Virginia and North Carolina shelf waters. In addition to these collections, daily samples from a gill net in the lower York River were obtained during the periods August–October 1990 and July–October 1991, except on weekends. In 1991 the net was emptied twice a day: in the early morning (6:00–8:00 am) and in the evening (5:00–7:00 pm). Time of death was recorded for fish alive at the time the net was emptied.

Daily gillnet samples were used to monitor small-scale (less than weekly) changes in Atlantic croaker reproductive condition and to collect hydrated or recently spawned females. Finally, collections from the commercial fisheries were supplemented by fish obtained from the Virginia Institute of Marine Science (VIMS) juvenile bottom trawl survey. The VIMS trawl survey used a monthly stratified random sampling program in the lower Chesapeake Bay and monthly
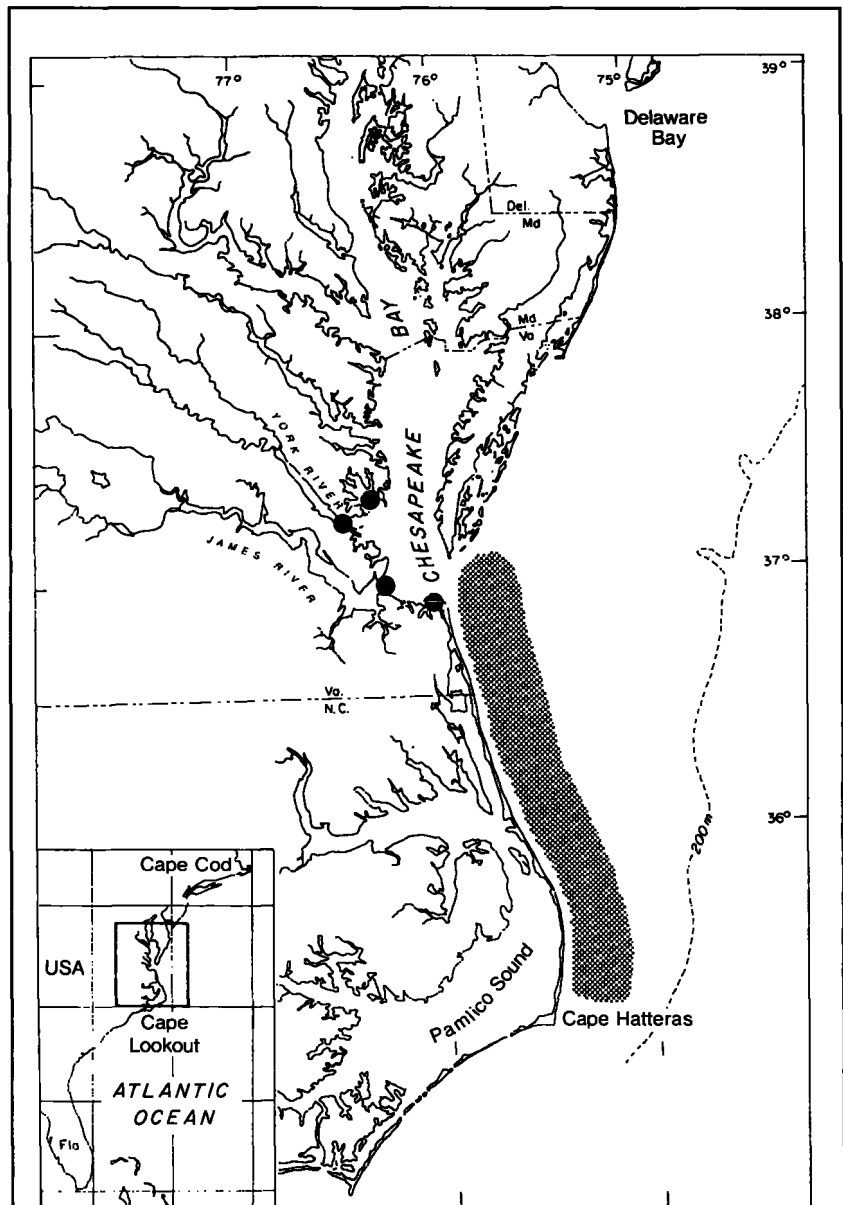


**Figure 1**

Map of the Chesapeake Bay and mid-Atlantic region. Black dots in Chesapeake Bay indicate poundnet, haul-seine, or gillnet collection sites. Hatched area off Virginia and North Carolina indicates where otter trawl collections of Atlantic croaker, *Micropogonias undulatus*, were obtained.

fixed midchannel stations in the York, James, and Rappahannock rivers.

Fish were measured for total length (TL, ±1.0 mm), total weight (TW, ±1.0 g), and gonad weight (GW, ±1.0 mm), sexed, and both sagittal otoliths were removed and stored dry. The left otolith was sectioned through the core with the diamond blade of a Buehler low-speed Isomet saw. Sections 350–500 μm thick were then mounted on glass slides with Flo-texx clear mounting medium and aged under a dissecting microscope (6–12×) following criteria described in Barbieri et al. (1994). The gonadosomatic index (GSI) was calculated for individual fish as (GW/(TW–GW)·100). Females were assigned a macroscopic gonad maturity stage (Table 1). Males were classified only as sexually mature or immature. Female macroscopic stages were verified microscopically by inspecting fresh oocyte samples and histology slides of a randomly selected subsample of ovaries in each maturity stage. Fresh oocytes were removed from one ovary, spread on a microscope slide, and examined under a dissecting microscope (12–50×). Color photographs were used to permanently record the appearance of fresh oocyte samples. This technique allowed fresh oocytes to be compared with histology slides in assessing gonad maturity stage and the

## Table 1

Description of gonad maturity stages for female Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters. Macroscopic appearance refers to fresh ovaries. Gonad stages 3, 4, and 5 are in spawning phase. FOM = final oocyte maturation; POF's = postovulatory follicles.

| Stage | Macroscopic appearance | Microscopic appearance |
|---|---|---|
| (1) Immature | Ovaries very small, light pink in color; translucent. | Only primary growth oocytes present; no atresia; ovarian membrane thin. |
| (2) Developing | Ovaries ranging from small to medium (≤ 25% of body cavity); yellow to light orange in color; no opaque (advanced yolked) oocytes present. | Only primary growth, cortical alveoli and a few partially yolked oocytes present; no major atresia. |
| (3) Fully developed | Ovaries ranging from large (25–50% of body cavity) to very large (~100% of space available in body cavity); creamy yellow to orange in color; opaque oocytes prevalent and easily detected; if partially spent, may have some left-over clear (hydrated) oocytes present at the posterior end of the ovarian lumen. | Primary growth to advanced yolked oocytes present; may have some left-over hydrated oocytes from previous spawning; often major atresia of advanced yolked oocytes, but no major atresia of other oocytes. |
| (4) Gravid | Ovaries ranging from large (25–50% of body cavity) to very large (~100% of space available in body cavity); creamy yellow to light orange in color; unovulated clear (hydrated) oocytes visible amongst opaque oocytes, giving a speckled appearance; clear oocytes not collected in the ovarian lumen. | Primary growth to FOM/hydrated oocytes present; often major atresia of advanced yolked oocytes, but no major atresia of other oocytes; hydrated oocytes are still in follicles (unovulated). |
| (5) Running-ripe | Ovaries ranging from large (25–50% of body cavity) to very large (~100% of space available in body cavity); creamy yellow to light orange in color; most clear (hydrated) oocytes are collected in the ovarian lumen (ovulated). | Primary growth to FOM/hydrated oocytes present; often major atresia of advanced yolked oocytes, but no major atresia of other oocytes; hydrated oocytes not in follicles (ovulated); may have POF's. |
| (6) Regressing | Ovaries ranging from small to medium (≤25% of body cavity); mustard yellow to light orange in color; more flaccid than previous stages; often contain clear fluid; can detect a few opaque oocytes. | Primary growth to advanced yolked oocytes present, but the number of yolked oocytes relative to unyolked oocytes is now much smaller; major atresia of cortical alveoli, partially yolked and advanced yolked oocytes. |
| (7) Resting | Ovaries very small; dark orange to reddish in color; no opaque oocytes present; ovarian membrane thickened and more opaque than in immature fish. | The majority (>90%) of oocytes are primary growth; may have other oocytes in late stages of atresia; more follicular tissue than immature fish. |

occurrence and intensity of oocyte atresia. For histological preparation, tissue samples were fixed in 10% neutrally buffered formalin for 24 hours, soaked in water another 24 hours, and stored in 70% ethanol. Samples were embedded in paraffin, sectioned to 5–6 μm thickness and stained with Harris' Hematoxylin and Eosin Y. Histological classification of ovaries (Table 1) was based on the occurrence and relative abundance of five stages of oocyte development (primary growth, cortical alveoli, partially yolked, advanced yolked, and hydrated) and on the occurrence and intensity of alpha (α) atresia. Terminology for stages of oocyte development and ovarian atresia follows Wallace and Selman (1981), Hunter and Macewicz (1985b) and Hunter et al. (1992).

Fecundity pattern was evaluated through monthly oocyte diameter distributions of fully developed (gonad stage 3) females collected during the spawning season. Before measurements were taken, oocytes were hydraulically separated from each other and from the ovarian membrane and preserved in 2%



**Figure 2**

Percentage of mature male and female Atlantic croaker, *Micropogonias undulatus*, by 10-mm total length intervals, with a logistic function (continuous line) fitted to the data. Arrows indicate mean length at first maturity ($L_{50}$). *n*=sample size.

formalin following the method of Lowerre-Barbieri and Barbieri (1993). Oocyte measurements were taken after a preservation period of at least 24 hours. Samples were stirred before oocytes were removed to reduce bias from settling differences caused by oocyte size or density. Oocytes ≥0.1 mm were measured (±0.02 mm) with an ocular micrometer in a dissecting microscope. Measurements were taken along the median axis of the oocyte parallel to the horizontal micrometer gradations (Macer, 1974; DeMartini and Fountain, 1981).

To estimate mean length at first maturity ($L_{50}$) for males and females, the fraction of mature fish per 10 mm length intervals was fit to the logistic function by nonlinear regression (Marquardt method), by using the statistical software program FISHPARM (Saila et al., 1988). $L_{50}$ was defined as the smallest length interval in which 50% of the individuals were sexually mature. Females were considered sexually mature if they were in gonad stage 2 (developing) or higher (Table 1). However, to avoid classifying resting (reproductively inactive) or early developing fish as immature, and thus obtaining biased estimates of $L_{50}$, only fish collected in September, when no resting or developing stages were found, were used for this analysis.

Monthly sex ratios were tested statistically for significant deviations from the expected 1:1 ratio with a chi-square test (α=0.05).

## Results

### Size and age at maturity

Atlantic croaker mature at a small size and early age. Males and females started to mature at 170 and 150 mm, respectively; at lengths greater than these the percentages of mature fish increased rapidly (Fig. 2). Estimated mean length at first maturity ($L_{50}$) was 182 mm for males (SE=1.46 mm) and 173 mm for females (SE=1.33 mm). For both sexes, all individuals ≥250–260 mm were mature.

The percentage of mature fish by age showed a similar pattern of early maturation. More than 85% of both males and females were sexually mature by the end of their first year and all were mature by the end of their second.

### Spawning

Spawning of Atlantic croaker in the Chesapeake Bay and adjacent coastal waters extends over a protracted period. Females in spawning phase (gonad stages: fully developed (3), gravid (4), or running-ripe (5); Table 1) were collected from July through December
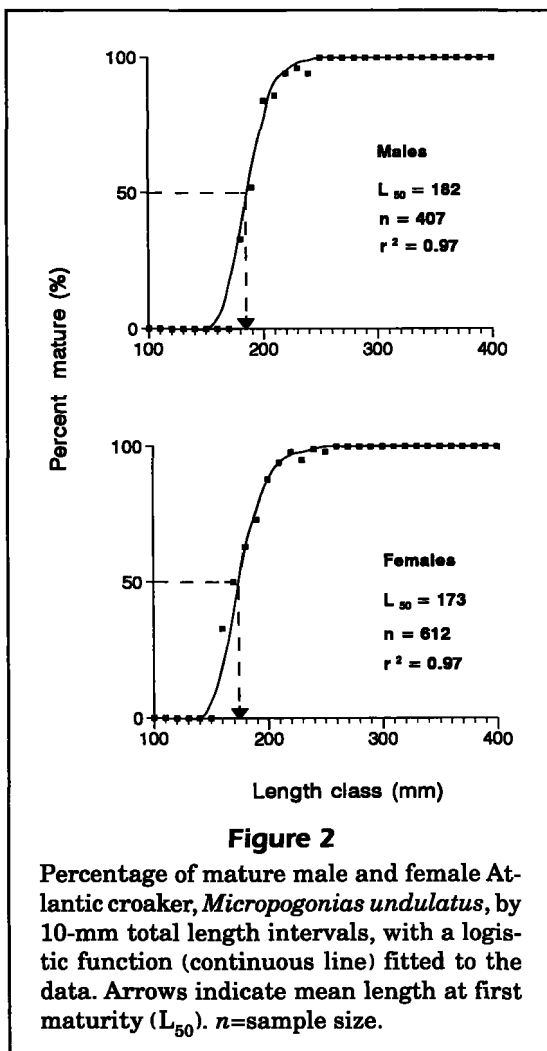
(Fig. 3). However, the capture of developing females (stage 2) from May through August and regressing females (stage 6) from September through December indicates that spawning initiation and cessation were not synchronous among individuals. Although the population spawned over a six-month interval (July–December), individuals apparently spawned over a shorter period. Most females appeared to spawn for 3–4 months as indicated by the large percentages of fully developed (stage 3) ovaries from August through November.

The pattern of gonad development in males provided further evidence of an extended spawning season for Atlantic croaker. Mean and maximum GSI values increased sharply during July and August, and remained relatively high until November or December, depending on the year (Fig. 4). In addition, males with very large testes and free-running milt were common during August–September in collections from all locations and sampling gears, indicating intense male spawning during this period.

Spawning of Atlantic croaker occurred in the estuary as well as in coastal oceanic waters. Females with hydrated oocytes (gonad stages 4 and 5), indicative of imminent spawning, were collected in the lower York and James rivers (n=8 in 1990; n=3 in 1991; Fig. 3) and from coastal waters off Virginia and North Carolina (n=1 in 1990; n=3 in 1991; Fig. 3). Collections of spawning fish (gonad stages 3–5) in Chesapeake Bay during the period July–October (n=649 in 1990; n=277 in 1991; Fig. 3) and from offshore waters during November–December (n=39 in 1990; n=11 in 1991; Fig. 3) suggest spawning continues offshore and south as Atlantic croaker migrate from the estuary. However, the presence during September–October of regressing and resting females in Chesapeake Bay (n=39 in 1990; n=24 in 1991; Fig. 3) indicates that some individuals may complete their spawning in estuarine waters.

Although gravid and running-ripe females were collected during most of the spawning season (August–November, Fig. 3), they were present in very low numbers. During both years of sampling only seven gravid and eight running-ripe females were collected. In Chesapeake Bay, despite the large number of poundnet and haul-seine collections (1,422 mature females processed), gravid or running-ripe females were obtained only from gill nets and mainly from collections from the lower James River (six gravid and four running-ripe females). Daily gillnet collections in the lower York River during August–October 1990 and July–October 1991 (456 mature females processed) showed only one running-ripe and one partially spent female, i.e. a fully developed female that had fresh left-over hydrated oocytes in the
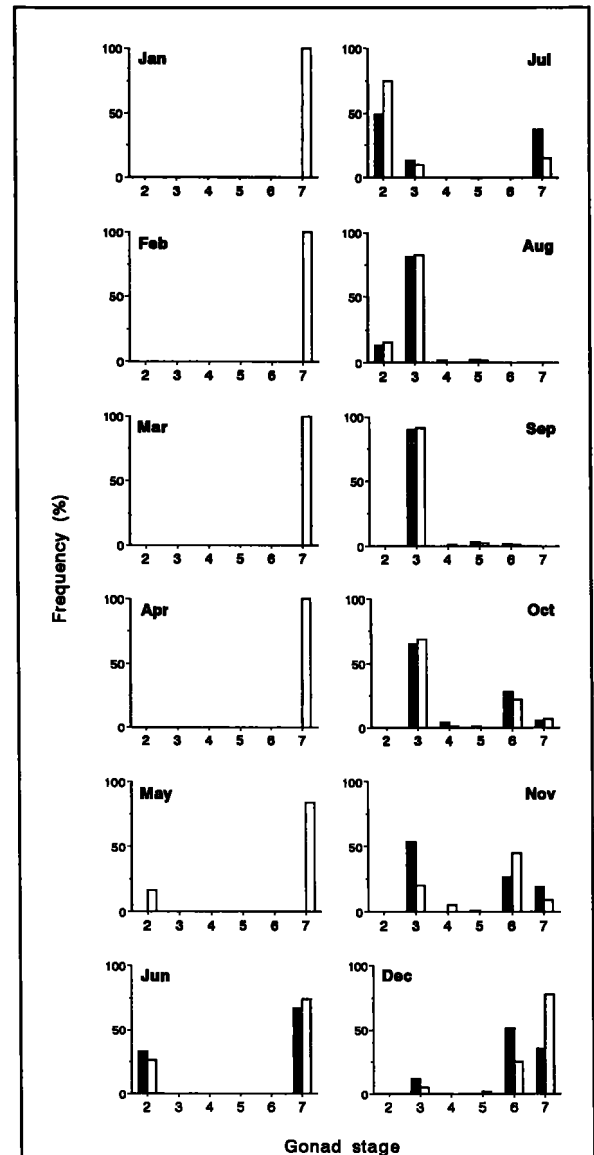


**Figure 3**

Percentage of gonad maturity stages by month for mature female Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters. Black bars=1990 data; open bars=1991 data. Gonad stages are (2) developing; (3) fully developed; (4) gravid; (5) running-ripe; (6) regressing; and (7) resting. Monthly sample sizes are presented in Table 2. Samples from April to October are from Chesapeake Bay; samples from November to March are from coastal waters off Virginia and North Carolina.

ovarian lumen indicating recent spawning but that still had a large number of advanced yolked oocytes and could potentially spawn again. Offshore collections during November–December of 1990 and 1991
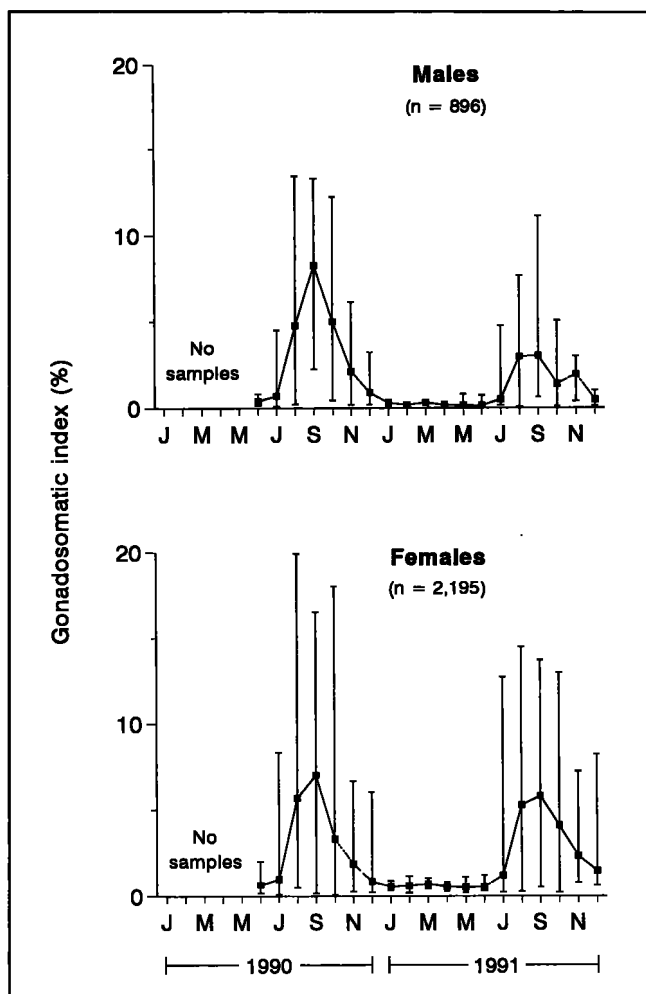
**Figure 4**

Monthly mean gonadosomatic index for male and female Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters, 1990–91. Vertical bars are ranges. *n*=sample size. Monthly sample sizes are presented in Table 2.
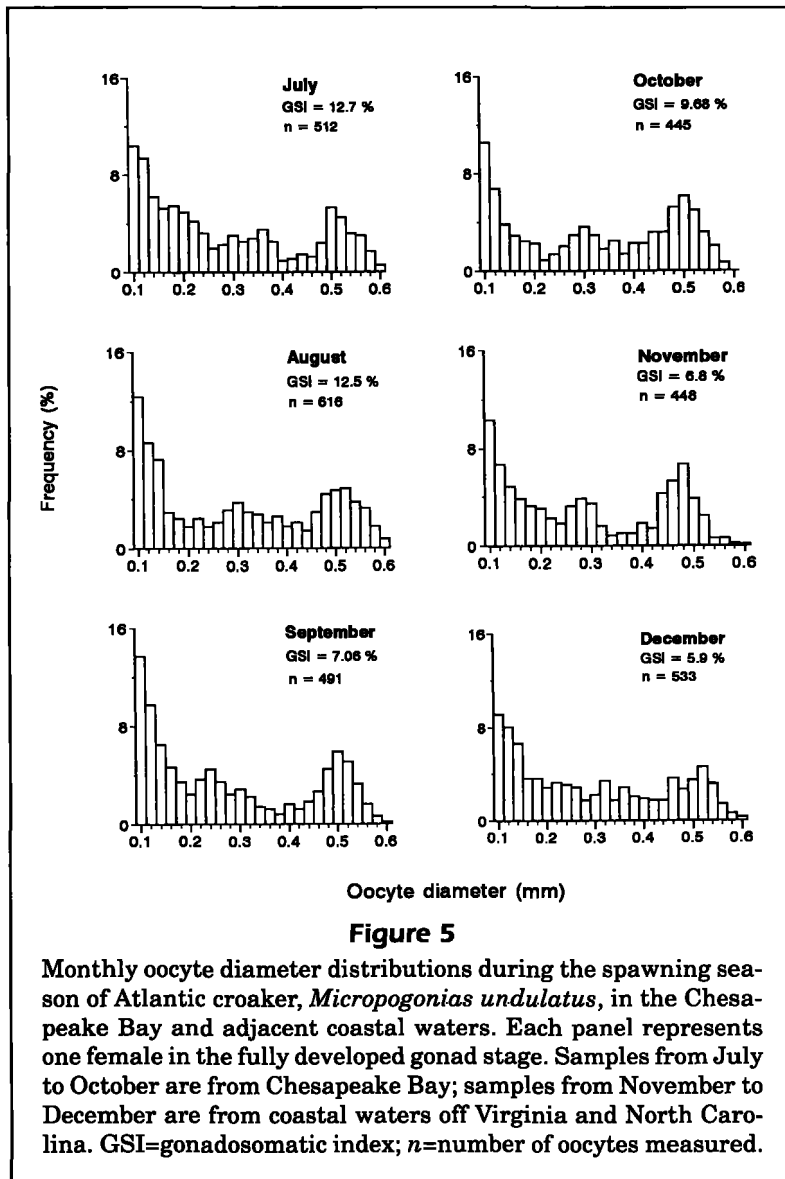
**Table 2**

Number of males and females by month and chi-square tests for the monthly sex ratios of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters, 1990–91. $**=P<0.01$.

| Year | Month | Number of | | Chi-square |
| | | males | females | |
|---|---|---|---|---|
| 1990 | Jun | 107 | 71 | 3.64 |
| | Jul | 185 | 358 | 27.80** |
| | Aug | 132 | 357 | 51.74** |
| | Sep | 40 | 249 | 74.91** |
| | Oct | 33 | 99 | 16.50** |
| | Nov | 56 | 64 | 0.22 |
| | Dec | 41 | 33 | 0.37 |
| 1991 | Jan | 22 | 26 | 0.04 |
| | Feb | 27 | 27 | — |
| | Mar | 25 | 23 | 0.04 |
| | Apr | 36 | 51 | 1.29 |
| | May | 98 | 121 | 1.10 |
| | Jun | 52 | 129 | 15.96** |
| | Jul | 44 | 103 | 11.84** |
| | Aug | 21 | 122 | 34.96** |
| | Sep | 16 | 119 | 38.99** |
| | Oct | 9 | 75 | 25.61** |
| | Nov | 15 | 33 | 3.37 |
| | Dec | 32 | 40 | 0.44 |

also showed a small percentage of gravid and running-ripe females (Fig. 3).

## Sex ratios

Atlantic croaker showed wide temporal fluctuations in sex ratio. During both years, the frequency of males in samples decreased in June and July at the beginning of the spawning season, reached a minimum in the period of September–October, and increased again during November–December. Chi-square tests (Table 2) showed highly significant differences ($P<0.01$) in sex ratios between months over the periods July–October 1990 and June–October 1991.

## Oocyte development and spawning pattern

Monthly oocyte diameter distributions of fully developed females collected throughout the spawning season showed three main groups of oocytes (Fig. 5). However, oocyte development appears to be asynchronous; there is a large degree of overlap and no clearly defined limits between modal groups. Histological analysis showed that the first group, ranging approximately from 0.06 to 0.24 mm diameter, is composed mainly of primary growth and cortical alveolus oocytes but may include a few partially yolked oocytes in the beginning stages of yolk deposition (0.22–0.24 mm diameter). The second group, ranging approximately from 0.26 to 0.38 mm diameter, is composed of partially yolked oocytes in several stages of yolk deposition. The third group, ranging approximately from 0.40 to 0.60 mm diameter, is formed by advanced yolked oocytes and probably represents the group from which individual spawning batches will be formed.

Although frequency distributions of gonad stages and oocyte diameters (Figs. 3 and 5) indicated Atlantic croaker are multiple spawners with indeterminate fecundity, postovulatory follicles (POF's) were identified only in recently ovulated, running-ripe fe-

Oocyte diameter (mm)

**Figure 5**

Monthly oocyte diameter distributions during the spawning season of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters. Each panel represents one female in the fully developed gonad stage. Samples from July to October are from Chesapeake Bay; samples from November to December are from coastal waters off Virginia and North Carolina. GSI=gonadosomatic index; n=number of oocytes measured.

showed healthy advanced yolked oocytes, atretic advanced yolked oocytes in different stages of degeneration, and atretic follicles (β-, γ-, and δ-stage atresia) in the same ovary.

Compared with healthy oocytes (Fig. 6A), early phases of α atresia of advanced yolked oocytes in Atlantic croaker are characterized by the disintegration of the nucleus, which loses its integrity, becoming amorphous and slightly basophilic, and by the disintegration of yolk globules, which begin to dissolve, forming a continuous, amorphous mass, especially around the nucleus (Fig. 6B). At this stage, the majority of yolk granules at the periphery of the cytoplasm still maintain their structural integrity, spherical shape, and strong acidophilic staining. At intermediate stages, disintegration of yolk globules progresses towards the peripheral cytoplasm, which by now may have a band of dark, basophilic material (Fig. 6C), and the zona radiata begins to deteriorate. At late stages of α atresia (Fig. 6D), the nucleus has completely disappeared, the zona radiata has lost its structural integrity, and the cytoplasm has been invaded by phagocytizing granulosa cells. Only portions of dissolved yolk and a few yolk globules remain at this stage. However, atresia will continue until the oocyte is completely resorbed, leaving only the remaining follicle. After this phase, α-stage atresia has been completed and follicular atresia begins with the resorption of the remaining granulosa and thecal cells.

Comparisons of fresh oocyte samples with histology slides confirmed the high incidence of α atresia of advanced yolked oocytes in Atlantic croaker. Although the histological method appeared more sensitive in detecting earlier stages of atresia (Fig. 7A), the use of fresh oocytes was indispensable. Fresh oocytes provided an easy, fast way to assess gonad condition and to identify oocyte atresia. A large proportion of atretic advanced yolked oocytes could be easily identified by clumping and darkening of the yolk granules, formation of a clear zone in the peripheral cytoplasm (Fig. 7B), and at later stages, formation of several light yellow vacuoles (Fig. 7C).

males. No POF's were found in fully developed females, even those with left-over hydrated oocytes in the posterior end of the ovarian lumen. As a result, it was usually impossible to distinguish fully developed females spawning for the first time from those that had spawned at least once before (partially spent females).

## Atresia of advanced yolked oocytes

Spawning-phase Atlantic croaker females (gonad stages 3–5; Table 1) showed a high incidence of α atresia of advanced yolked oocytes throughout the spawning season (July–December). However, the exact proportion of atretic oocytes could not be determined because of the difficulty in identifying oocytes in very early stages of atresia. Some females

## Description of the ovarian cycle

A diagrammatic representation of the Atlantic croaker ovarian cycle, based on the temporal distri-
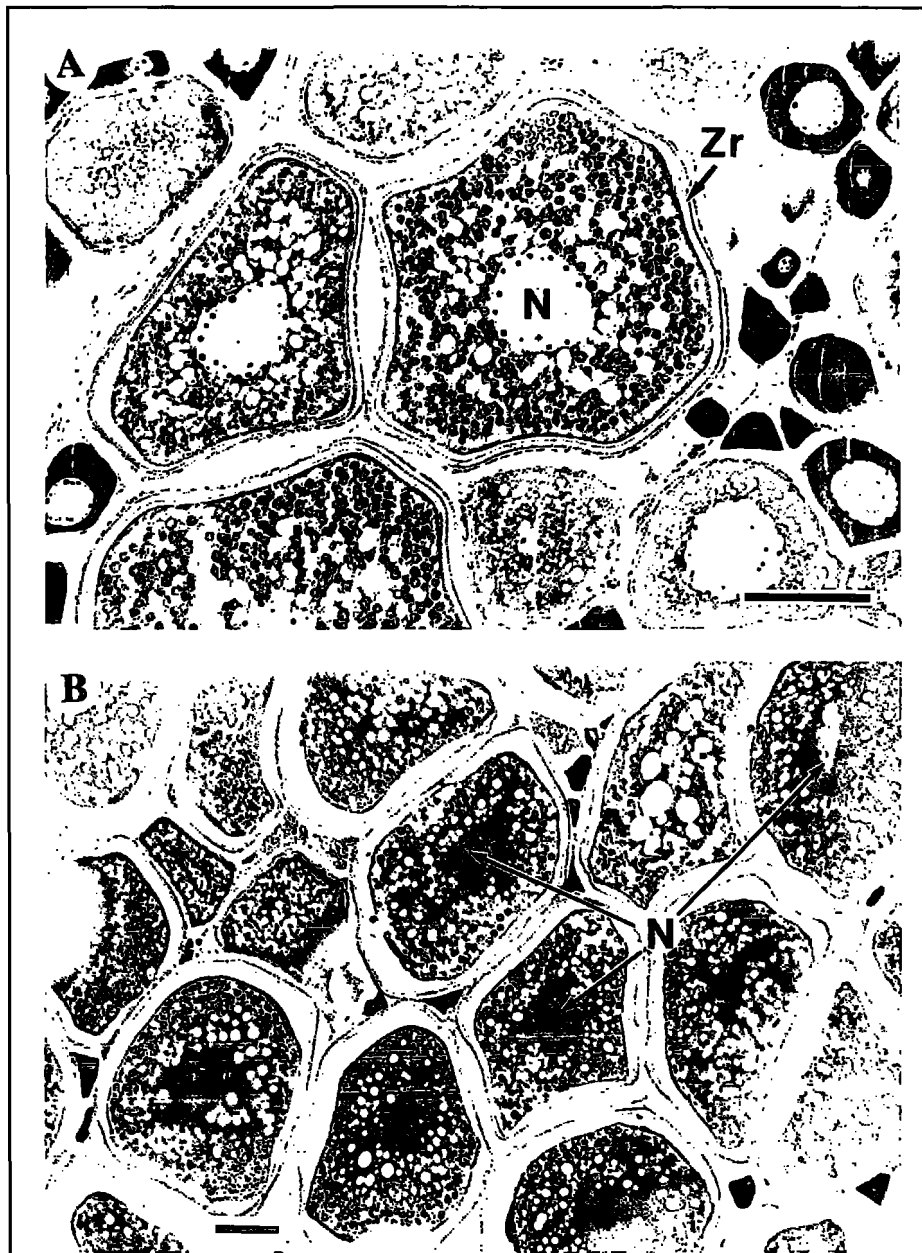
**Figure 6**

Appearance of advanced yolked oocytes of Atlantic croaker, *Micropogonias undulatus*. (A) healthy (nonatretic) oocyte; (B) oocytes in early stage of α atresia; (C) oocyte in intermediate stage of α atresia; (D) oocytes in late stage of α atresia. N=nucleus; Zr=zona radiata; Pc=peripheral cytoplasm; La=late stage of α atresia. Bars=0.1 mm.

bution of maturity stages and the pattern of oocyte development is presented in Figure 8. The cycle can start either with immature females, which enter the cycle for the first time by reaching sexual maturity, or with adult resting females, which restart the cycle by entering the developing stage at the beginning of

each spawning season. After the first batch of advanced yolked oocytes is completed, females, now in the fully developed stage, go through a smaller cycle (spawning phase) that characterizes the pattern of multiple spawning and indeterminate fecundity of Atlantic croaker. During this phase, fully developed
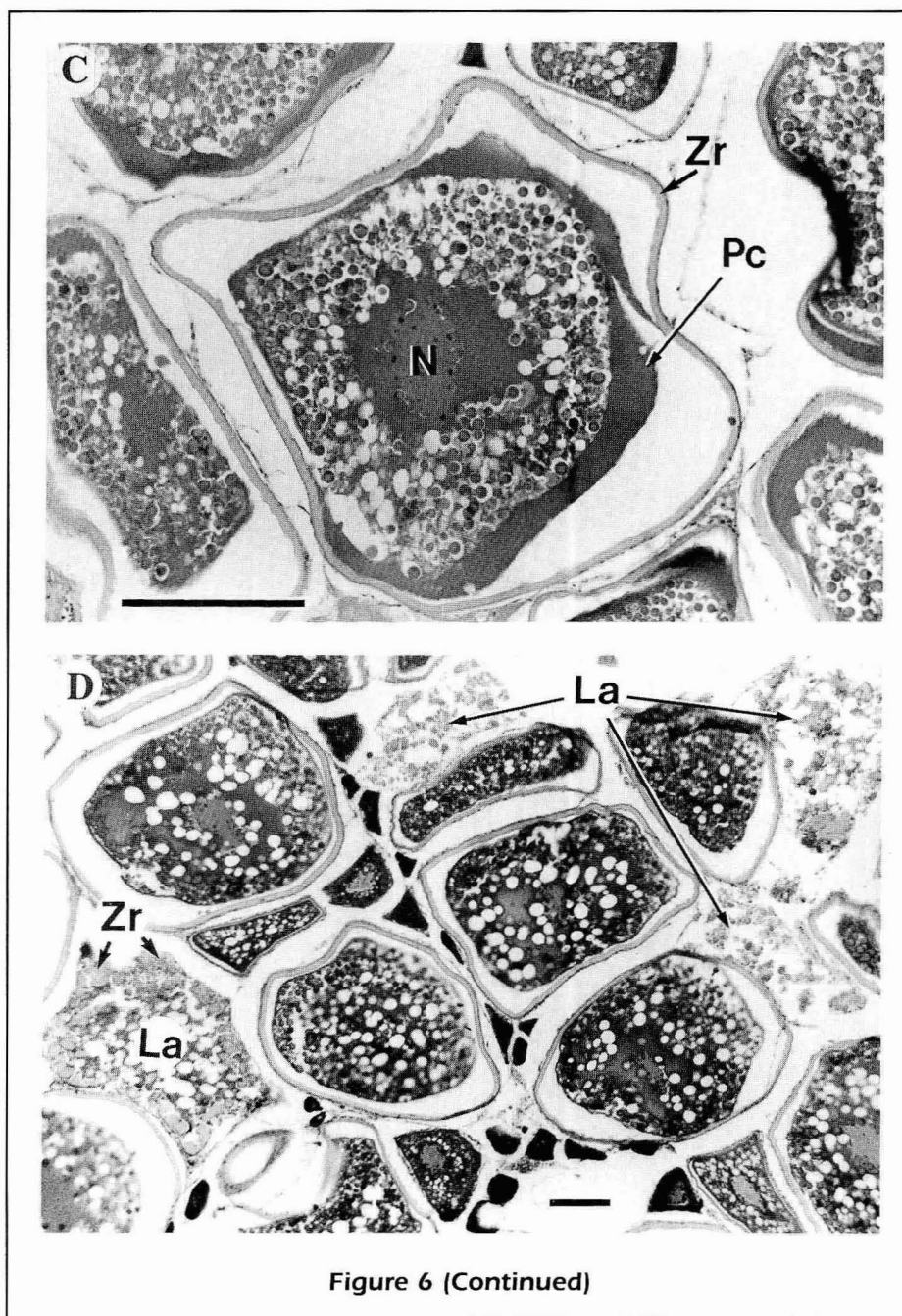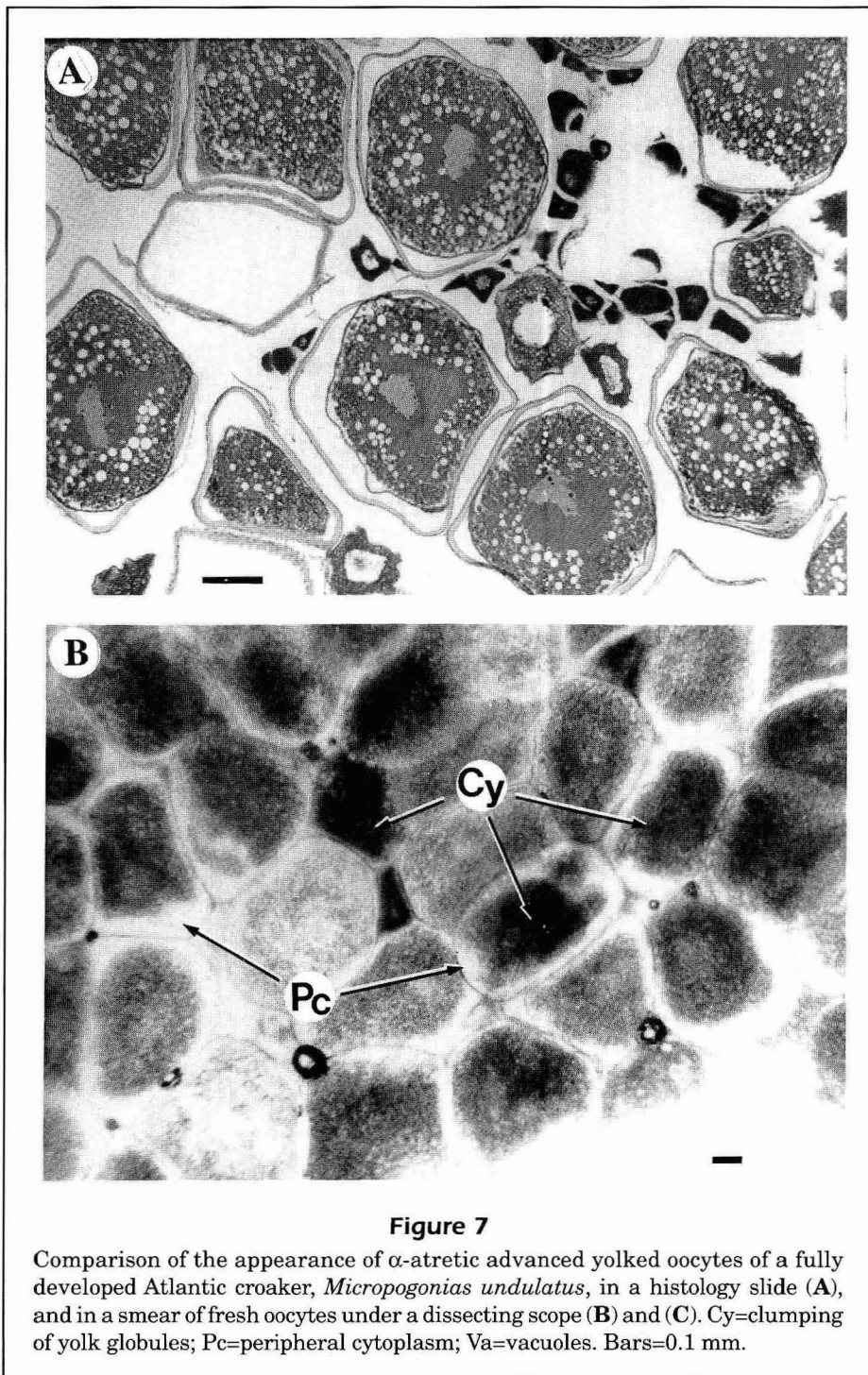
**Figure 6 (Continued)**

females cycle through the gravid and running-ripe stages by undergoing the processes of hydration, ovulation, and spawning. If spawning has not been completed, left-over advanced yolked oocytes are resorbed, a new batch of advanced yolked oocytes is recruited from the group of partially yolked oocytes (redeveloping process), and females are ready to go through the cycle again. If spawning is completed, females will then move to the regressing stage, where, through the process of oocyte atresia, left-over oocytes (cortical alveoli to advanced yolked stage) will be resorbed, after which ovaries return to the resting stage.

## Discussion

### Spawning periodicity

Our results on spawning periodicity of Atlantic croaker agree with previous reports for the Chesapeake Bay and mid-Atlantic regions. Prior studies

**Figure 7**
Comparison of the appearance of α-atretic advanced yolked oocytes of a fully developed Atlantic croaker, *Micropogonias undulatus*, in a histology slide (**A**), and in a smear of fresh oocytes under a dissecting scope (**B**) and (**C**). Cy=clumping of yolk globules; Pc=peripheral cytoplasm; Va=vacuoles. Bars=0.1 mm.

(Welsh and Breder, 1923; Wallace, 1940; Johnson, 1978; Colton et al., 1979; Morse, 1980) describe a protracted spawning season, extending from July/August through November/December, with peak spawning during September/October. However, reports of spawning from September/October through March/April along the South Atlantic Bight (Hildebrand and Cable, 1930; Bearden, 1964; Warlen, 1982; Lewis and Judy, 1983) indicate that south of Cape Hatteras, North Carolina, spawning seems to start a little later and to continue through early spring, perhaps as a result of the southward late summer/early fall migration of Atlantic croaker (Hildebrand and Schroeder, 1928; Wallace, 1940; Haven, 1959).
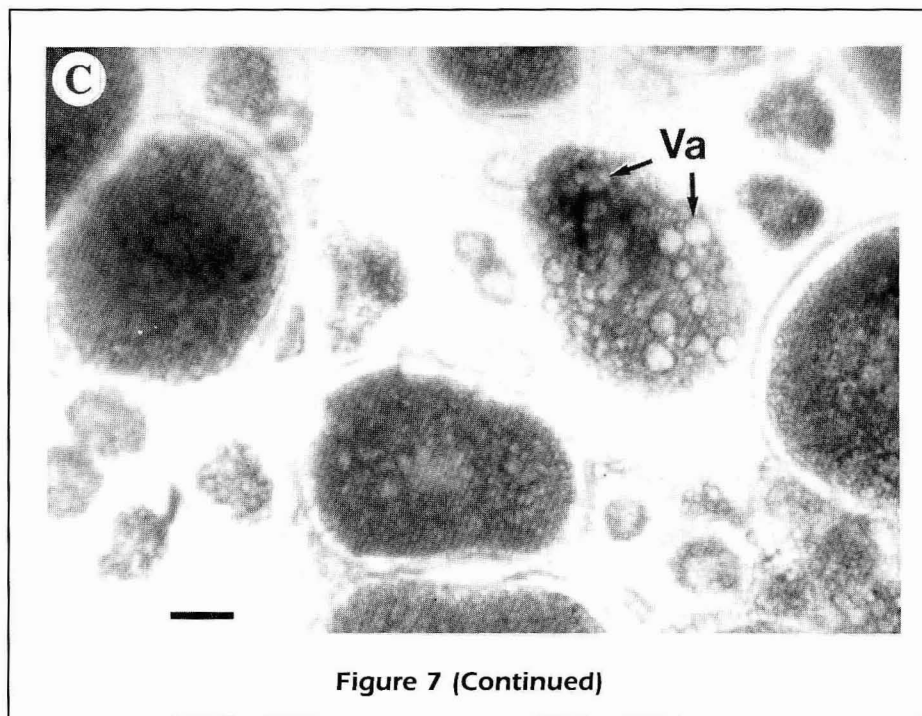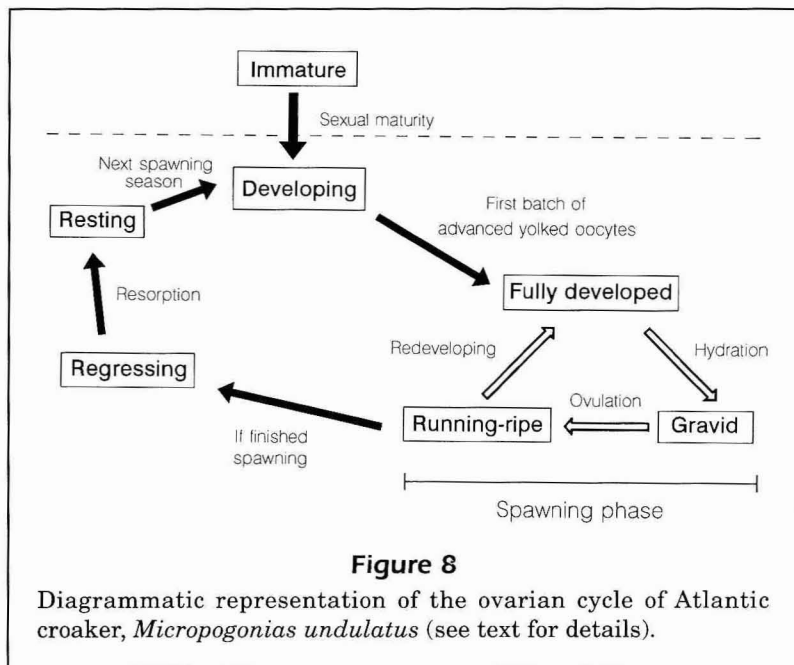
**Figure 7 (Continued)**



**Figure 8**

Diagrammatic representation of the ovarian cycle of Atlantic croaker, *Micropogonias undulatus* (see text for details).

The presence of small juveniles (<20 mm TL) in the York River from August/September through May/June has prompted suggestions that north of Cape Hatteras spawning of Atlantic croaker may also continue through spring (Haven, 1957; Chao and Musick, 1977). However, our results confirm previous reports (Wallace, 1940; Colton et al., 1979; Morse, 1980) that in the Chesapeake Bay and mid-Atlantic regions spawning is essentially completed by the end of December.

Although Welsh and Breder (1923) suggested that spawning might take place in large estuaries such as the Delaware and Chesapeake bays, this study represents the first documented report of estuarine spawning for Atlantic croaker. Previous studies have consistently described Atlantic croaker as strict marine spawners whose larval and juvenile stages migrate into estuarine nursery areas (Pearson, 1929; Hildebrand and Cable, 1930; Wallace, 1940; Haven,

1957; Warlen, 1982; Lewis and Judy, 1983; Setzler-Hamilton, 1987). However, the fact that during both years we found spawning-phase females (stages 3–5) in Chesapeake Bay from July through October and that regressing and resting females—which probably had completed spawning for the season—were collected in the estuary indicates that the role of estuaries as additional spawning areas for Atlantic croaker may be more important than previously thought. Other sciaenids that were believed to be strict marine spawners have also been reported to spawn occasionally in estuaries (Castello, 1985; Johnson and Funicelli, 1991). However, whether significant spawning of Atlantic croaker occurs in Chesapeake Bay or other estuaries requires further investigation.

The fact that spawning-phase Atlantic croaker have not previously been found in Chesapeake Bay can be attributed, at least in part, to their pattern of multiple spawning and indeterminate fecundity. Because in multiple spawning fishes the processes of hydration, ovulation, and spawning usually occur within a matter of hours (Hunter and Macewicz, 1985a; Brown-Peterson et al., 1988), the probability of collecting gravid or running-ripe females is much lower compared with other maturity stages. Additionally, contrary to what happens with total spawners, partially spent ovaries contain oocytes ranging from primary growth to advanced yolked stage, making the macroscopic identification of post-spawning fish very difficult (Hunter and Macewicz, 1985a). In most cases, we were not able to distinguish macroscopically between fully developed and partially spent ovaries, and this also may have been a problem with previous studies (e.g. Wallace, 1940).

Diel periodicity of spawning could also influence the occurrence of hydrated females in samples from different gears. The thousands of adult Atlantic croaker examined by Haven (1957) and Wallace (1940) were collected primarily from Chesapeake Bay commercial pound nets and haul seines, which are usually fished in the predawn or early morning hours (Reid, 1955; Chittenden, 1991). During the rest of the day and through most of the night, fish remain alive in the pound-head or in the seine-bag until the nets can be fished (emptied), usually during slack water, and between 4:00 and 9:00 am. We hypothesize that during this period Atlantic croaker spawn within the nets at their usual spawning time of dusk (Holt et al., 1985). Females collected from these nets the following morning would probably show little or no signs of spawning and be identified as "developing" (Wallace, 1940) or fully developed (this study). However, contrary to what happens with pound nets and haul seines, gill nets usually kill the fish within a short time after capture. Females undergoing hy-

dration or ovulation, especially those caught a few hours before dusk, would die before they finished spawning, and the presence of hydrated oocytes in the ovaries could be recorded. This may explain why we observed hydrated or recently spent females only in gillnet collections. A similar pattern has also been observed for weakfish, *Cynoscion regalis*, which, like Atlantic croaker, spawn primarily between 6:00 and 9:00 pm.[1]

## Size and age at maturity

Our estimates of size and age at maturity are generally below values previously reported for Atlantic croaker in the Chesapeake Bay and mid-Atlantic regions. Disagreement with previous reports can be attributed to three main factors: 1) failure of at least some studies (Wallace, 1940; Morse, 1980) to sample small, young fish from fishery-independent sampling programs; 2) the inclusion of samples collected from a period when resting (reproductively inactive) fish were present in the estimation of the proportion of mature fish by size or age; and 3) disagreement with previous estimates of age at maturity probably reflects problems with age-determination methods previously used for Atlantic croaker. Because of the difficulty in distinguishing resting and immature gonads, estimates based on samples pooled over the entire spawning season or during a period when resting fish were present (e.g. Wallace, 1940; Morse, 1980) are probably biased towards larger sizes or older ages. Hunter et al. (1992) found that estimates of $L_{50}$ for Dover sole were higher when females were taken during the spawning season than when they were sampled before spawning began. They suggested that estimates of length or age at first maturity should always be based on samples collected prior to the onset of spawning, when postspawning females with highly regressed ovaries are rare. However, for species like Atlantic croaker, which show individually asynchronous gonadal maturation, sampling before the onset of spawning will not prevent the occurrence of prespawning, resting fish. To avoid this problem we used only fish collected in September, when no resting or developing stages occurred, to estimate size and age at first maturity. Finally, disagreement with previous estimates of age at maturity probably reflects problems with age-determination methods previously used for Atlantic croaker. The use of length frequencies (Welsh and Breder,

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[1] Lowerre-Barbieri, S. K., M. E. Chittenden Jr., and L. R. Barbieri. 1993. The multiple spawning pattern of weakfish, *Cynoscion regalis*, in the Chesapeake Bay and mid-Atlantic Bight, with a discussion of annual fluctuations in reproductive output. Virginia Institute of Marine Science, Gloucester Point, VA 23062. Unpubl. manuscr., 61 p.

1923) requires considerable subjective interpretation given the extended spawning season of Atlantic croaker, the generally asymptotic growth after age 2, and the great overlap in observed sizes at age (Barbieri et al., 1994). Although Welsh and Breder (1923) and Wallace (1940) have also used scales, problems in applying this method to Atlantic croaker have been reported (Joseph, 1972).

## Sex ratios

Our results on temporal fluctuations in Atlantic croaker sex ratios agree well with previous reports for the Chesapeake Bay and mid-Atlantic regions (Welsh and Breder, 1923; Wallace, 1940). The predominance of females during the first 3–4 months of spawning may indicate that either males start leaving the estuary earlier than females as fish migrate out of Chesapeake Bay or that spawning-phase females are more susceptible to the fishing gears used in Chesapeake Bay (pound nets, haul seines, and gill nets). During both years, the frequency of males decreased during the first two months of spawning and began increasing again in October/November when the first offshore trawl collections were obtained. Mark-recapture studies are necessary to better evaluate the migratory patterns of Atlantic croaker in Chesapeake Bay and the mid-Atlantic region.

## Atresia of advanced yolked oocytes

High levels of atresia typically have been used to identify regressing ovaries, and for many teleosts, have been described as representing a key histological marker for the cessation of spawning (Hunter and Macewicz, 1985, a and b; Hunter et al., 1986; Dickerson et al., 1992). However, our results with Atlantic croaker indicate that high levels of atresia do not necessarily imply the end of spawning. Although we found significant atresia of cortical alveoli and partially yolked oocytes only in regressing ovaries, indicating it could in fact be used to mark the end of spawning, major atresia of advanced yolked oocytes was observed in actively spawning females throughout the spawning season suggesting it may represent a normal part of the reproductive biology of Atlantic croaker. The fact that hydrated females— either actively spawning or just about to spawn— showed advanced yolked oocytes undergoing atresia suggests that a portion of these oocytes are never matured and spawned. In other words, it appears that a surplus production of advanced yolked oocytes is part of the reproductive strategy of Atlantic croaker. Fully developed females may hydrate and spawn more or less of these oocytes depending, for example, on environmental conditions.

Evidence from laboratory studies seems to support this hypothesis. Middaugh and Yoakum (1974) used chorionic gonadotropin to induce laboratory spawning of Atlantic croaker. They found that although the abdomen of females became extremely distended as a result of oocyte hydration, only a limited number of eggs could be stripped from each fish. More recently, Trant and Thomas (1988) and Patiño and Thomas (1990) evaluated in vitro germinal vesicle breakdown (GVBD, an index of final oocyte maturation) in laboratory-spawned Atlantic croaker. They reported that in this species there is always a residual number of "advanced oocytes" which fail to complete GVBD or even enter the morphological maturation process, suggesting that not all oocytes in a spawning batch would be matured and spawned.

## Conclusion

Because of the small number of gravid females collected and the fact that POF's could be identified only in running-ripe females, we were not able to estimate batch fecundity and spawning frequency for Atlantic croaker. However, our results have shown that 1) Atlantic croaker mature at a smaller size and earlier age than previously thought; 2) Atlantic croaker are capable of spawning in the estuary, although the magnitude of estuarine spawning is still unclear; 3) they are multiple spawners with indeterminate fecundity, indicating that the only available estimates of fecundity (Morse, 1980)—those based on the assumption of determinate fecundity— should not be used for management; and 4) the oocyte size-frequency method (MacGregor, 1957) should not be used to estimate batch fecundity for this species, because of the high levels of atresia of advanced yolked oocytes observed in spawning females. Future studies on the reproductive biology of Atlantic croaker in Chesapeake Bay and the mid-Atlantic region should concentrate on offshore, preferably fishery-independent, trawl collections to obtain gravid females for batch fecundity estimates following the hydrated oocyte method (Hunter et al., 1985). Rates of deterioration and resorption of POF's must also be evaluated in laboratory-spawned fish to determine if the postovulatory follicle method (Hunter and Macewicz, 1985a) can be used to estimate spawning frequency for this species.

## Acknowledgments

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