Reproductive Biology of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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ABSTRACT

This study addressed the reproductive biology of Atlantic croaker in order to determine the occurrence and periodicity of spawning in different regions within the Chesapeake Bay, and to investigate previous findings of elevated levels of atresia in females within the Bay. Collections of Atlantic croaker were made from the Eastern and Western Shores of the Chesapeake Bay and offshore between Virginia Beach and Cape Hatteras in 1998-2000 and ovaries were examined for maturity stage and atresia.

Ovarian maturity stages as well as gonadosomatic indices indicated that Atlantic croaker spawn in the Chesapeake Bay region from July through October and that peak spawning occurs there around September or early October. However, the absence of many Gravid or Running Ripe Stage females suggested that very few females spawn within the Bay itself. No significant difference was found in the frequencies of gonad maturity stages and gonadosomatic indices from the Eastern Shore, Western Shore and Offshore.

High incidence of Alpha atresia of Atlantic croaker oocytes was found only at the end of the spawning period. Therefore, the presence of high levels of alpha atresia may be a rough indicator that the spawning period is near its close. Determining levels of atresia can be an important tool in studying reproductive biology of multiple spawners such as Atlantic croaker.

Reproductive Biology of Atlantic croaker,

Micropogonias undulatus, in the Chesapeake Bay

INTRODUCTION

The Atlantic croaker, *Micropogonias undulatus*, (Figure 1) is a seasonally abundant demersal inshore species that ranges from the Gulf of Maine to South America (Welsh and Breder 1923; Joseph 1972; Chittenden and McEachran 1976; Chao and Musick 1977). It is one of the most abundant and commercially valuable species in the Chesapeake Bay region, where adults are typically present from spring to fall. Most fish enter the Chesapeake Bay in early spring, move up-river or up-bay in spring and early summer, move more or less randomly in the tributaries in summer and then move down-river or down-bay in fall as they migrate offshore, where they remain during the winter.(Pearson 1932; Wallace 1940; and Haven 1959). Adults are generally found on a variety of substrates including mixtures of mud, sand and shell, as well as sponge and coral (Bearden 1964). Larvae and juveniles use estuarine waters as a nursery (Weinstein 1979; Lewis and Judy 1983) and usually occur in deep channels of tidal streams where the bottom is composed of soft mud and decomposing plant material (Wallace 1940; Haven 1957; Bearden 1964). Atlantic croaker spend their first winter in the deeper parts of bays and rivers (Welsh and Breder 1923), and leave the Bay the following year (Haven 1957; Chao and Musick 1977; and Norcross 1983).

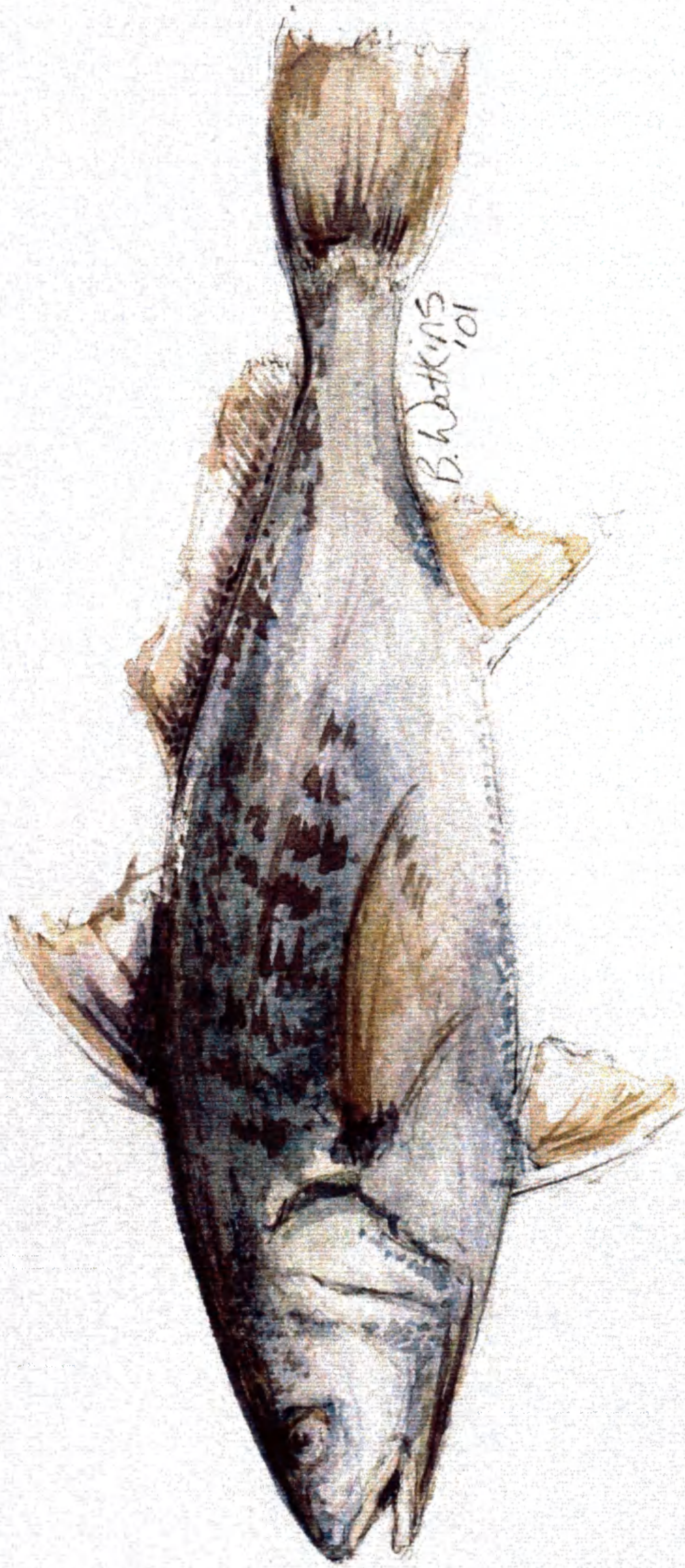
Much work has been done on Atlantic croaker in the Chesapeake Bay, including general reproductive biology (Hildebrand and Schroeder 1928; Wallace 1940; Johnson 1978; Colton et al 1979; Morse 1980; Norcross and Austin 1988; and Barbieri et al 1994), ovarian development patterns and age/length at maturity (Barbieri et al. 1994), spawning period (Barbieri et al. 1994; Holt et al. 1985; Hildebrand and Cable 1930; Pearson 1929; Wallace 1940; Haven 1957; Colton et al. 1979; Warlen 1982; Welsh and Breder 1923; and Hildebrand and Schroeder 1928), larval life history and transport (Middaugh and Yoakum 1974; Wallace 1940; Norcross 1991), and fecundity (Morse 1980, Barbieri et al. 1994; Campbell 1985; Prager et al. 1987).

Despite all these studies, information on Atlantic croaker reproduction in the Chesapeake Bay region is incomplete and many questions are unanswered. For example, there has been much disagreement on the occurrence and amount of spawning in the Chesapeake Bay. Many studies have suggested the possibility of Bay spawning (Haven 1957; Welsh and Breder 1923; and Pearson 1941), but this possibility has not been proved or disproved. Also, large numbers of Atlantic croaker appear on the Eastern Shore of the Bay during the spawning period. Previous studies however, have not examined fish from this area; and reasons for their sudden seasonal appearance are not known. And although many studies have described the spawning grounds of Atlantic croaker outside the Bay (Colton et al. 1979; Fahay 1975; Hildebrand and Cable 1930; Morse 1980; Norcross and Austin 1988; Norcross 1991; Pearson 1929), none has described spawning grounds within the Bay itself. In addition, while the spawning period of Atlantic croaker has been studied extensively along the coast (Hildebrand and Cable 1930; Pearson 1929; Wallace 1940; Haven 1957; Colton et al. 1979; Warlen 1982; Welsh and Breder 1923; and Hildebrand and Schroeder 1928), no study has described spawning periodicity within the Chesapeake Bay.

Many studies have been done on atresia (the resorption of oocytes and follicles by the ovary) in multiple spawning species (Hunter and Macewicz 1985b; Bretschneider and Duyvene de Wit 1947; Lambert 1970; Vizziano et al. 1991; Guraya 1994, 1993, 1986; and Agarwal and Singh 1990). Only one study, conducted by Barbieri et al. (1994), has investigated atresia in Atlantic croaker. In their analysis, they found a very high level of atresia throughout the entire spawning period in Atlantic croaker in the Chesapeake Bay. These findings were not examined in detail and the reasons for the observed atresia remain unclear. High levels of atresia could be a normal part of Atlantic croaker reproductive life history; however, it also may be a signal of some environmental stress such as increased toxicity in spawning areas.

This study addressed the reproductive biology of Atlantic croaker in order to determine the occurrence and periodicity of spawning along the Eastern Shore and Western Shore of the Chesapeake Bay, and to investigate previous findings of elevated levels of atresia in females along both the Eastern and Western Shores. In doing so, I describe microscopic staging criteria, compare microscopic and macroscopic staging in order to assess the relative accuracy of different staging methods techniques, present evidence of spawning activity by time and location, and measure the incidence of atresia by time and location.

Fig. 1 Illustration of the Atlantic croaker, *Micropogonias undulatus*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MATERIALS AND METHODS

Data Collection

I obtained collections of Atlantic croaker in 1998 and 1999 from haul seine, pound and gill net, and otter trawl fisheries along the Eastern and Western Shores of the Chesapeake Bay, as well as offshore along the Atlantic coast between Virginia Beach, VA and Cape Hatteras, NC (Figure 2). Collections were purchased from commercial fish houses several times a month. Fish were obtained in several size grades; small, medium, large and, occasionally, jumbo. Approximately 5000 fish were collected, about 3500 of which were females; 416 were collected on the Eastern Shore, 410 were collected offshore, and 2660 on the Western Shore of the Chesapeake Bay.

I obtained my data from both fresh collections and from preserved ovaries. I processed each fresh collection immediately upon return to the laboratory. For each individual in a fresh collection, the sex, total length (mm), standard length (mm), total weight (g), and gonad weight (g), and gonad stage was recorded. Gonad stage was also recorded microscopically from preserved ovaries.

Fig. 2. Map of the Chesapeake Bay Region. Black marks indicate collection sites. Diamonds – Western Shore sites. Circles – Eastern shore sites. Squares – Offshore/Coastal sites.

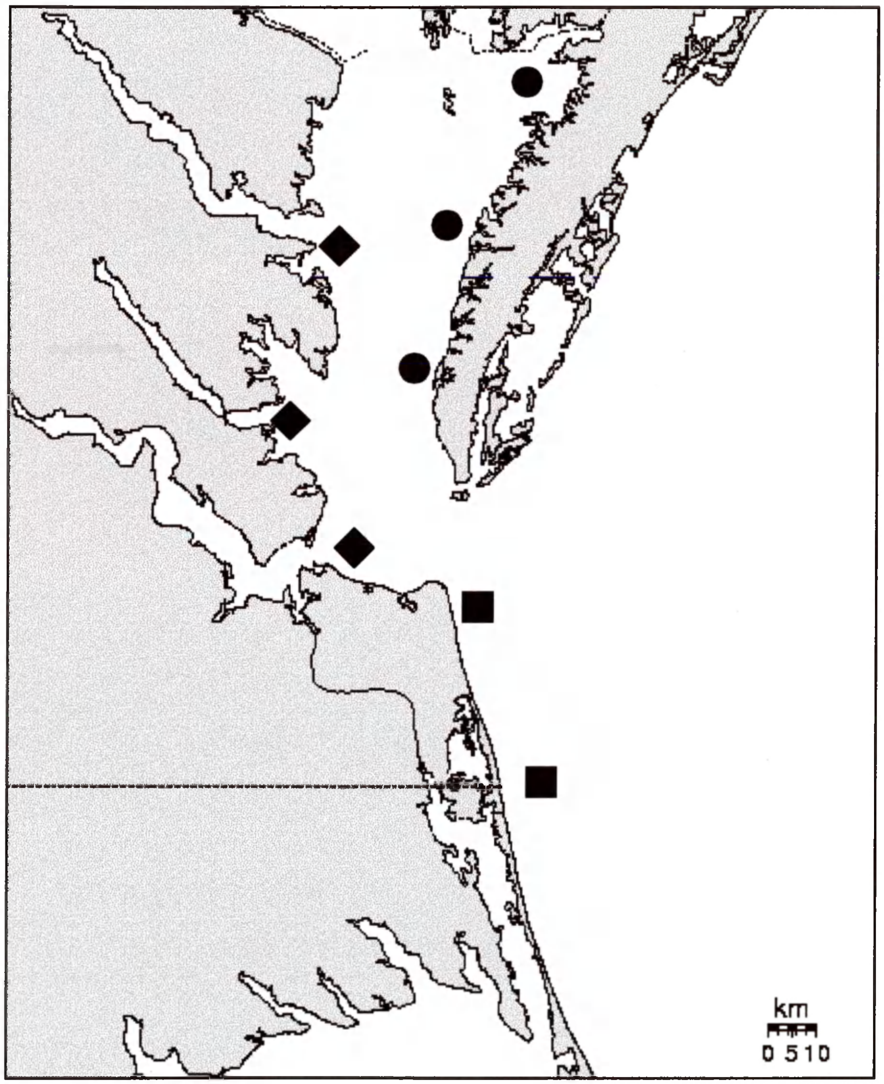

km
0 5 10

Table 1. Description of Ovarian Maturity Stages for Atlantic croaker, following Barbieri et al. (1994).

Stage	Macroscopic Appearance	Microscopic Appearance
(1) Immature	Ovaries very small, light pink; translucent.	Only Primary Growth oocytes present; no atresia; thin ovarian membrane.
(2) Developing	Ovaries small to medium (≤ 25% of body cavity); yellow to light orange; some opaque oocytes present but most not opaque.	Only Primary Growth; cortical alveoli and a few partially yolked oocytes present; no major atresia.
(3) Fully Developed (3b) Partially Spent	Ovaries large to very large (25% to almost 100 % of body cavity); yellow to light orange; opaque oocytes present.	Primary growth to advanced yolked oocytes; may be major atresia of advanced yolked oocytes in Partially Spent (3b) but no major atresia of other oocytes.
(4) Gravid	Ovaries large to very large; yellow to light orange; hydrated oocytes intermixed with opaque oocytes and not collected in the ovarian lumen (unovulated).	Primary Growth to hydrated oocytes present; may be major atresia of advanced yolked oocytes but no major atresia of other oocytes; hydrated oocytes associated with follicles.
(5) Running Ripe	Ovaries large to very large; yellow to light orange; most hydrated oocytes are collected in the lumen (ovulated); some may still be intermixed with opaque oocytes.	Primary Growth to hydrated oocytes present; may be major atresia of advanced yolked oocytes but no major atresia of other oocytes; hydrated oocytes ovulated into lumen.
(6) Spent/ Regressing	Ovaries small to medium; light orange to reddish orange; flaccid and watery; opaque oocytes present.	Primary Growth to advanced yolked oocytes present; number of yolked oocytes relative to unyolked is much smaller; major atresia of cortical alveoli, particularly yolked and advanced yolked oocytes
(7) Resting	Ovaries very small; dark orange to reddish; no opaque oocytes.	>90% of oocytes are Primary Growth; may have other oocytes in late stages of atresia; thick ovarian membrane.

Ovarian Stages

The indirect detection of spawning necessitates a methodology for determining spawning status based on ovarian condition. The macroscopic appearance of the ovary is dependent on and should reflect the microscopic development of oocytes within the ovary. Before discussing the methodology used to determine ovarian stage, a summary of oocyte and ovarian development is provided.

There are four phases of oocyte maturation in the Atlantic croaker: 1) the Primary Growth Phase, 2) Yolk Vesicle Formation, 3) Vitellogenesis, and 4) the Hydration and Ovulation Phase. These four phases correspond to 7 stages of ovarian development. The relationship between macroscopically determined ovarian stage and microscopically determined oocyte maturation phase is outlined in Table 1.

The Primary Growth Phase of oocyte maturation includes the establishment of the primary oocyte and is itself divided into two subphases, the Chromatin Nucleolar Phase and the Perinucleolar Phase. Ovaries in the Immature and Resting stages are dominated by oocytes in the Primary Growth Phase. The second phase is called Yolk Vesicle (or Cortical Alveoli) Formation. As oocytes mature, yolk vesicles appear in the cytoplasm and migrate towards the periphery of the cell to give rise to cortical alveoli. Oocytes in this phase are common in Developing Stage ovaries. The third phase, or Vitellogenesis, includes the accumulation of yolk within the cytoplasm of the oocyte. This occurs as large numbers of yolk granules appear in the cytoplasm. In addition, the micropyle forms late in Vitellogenesis and early in the succeeding

Hydration and Ovulation Phase. Fully Developed and Partially Spent Stage ovaries contain oocytes in the Vitellogenesis phase. The final oocyte maturation phase includes both Hydration and Ovulation. Hydration occurs when the oocytes rapidly absorb fluid from the surrounding water and become very large and amoeboid in shape. Gravid and Running Ripe ovaries contain hydrated oocytes. The hydrated oocytes are still associated with the follicle in Gravid stage females, but in Running Ripe stage ovaries the hydrated oocytes undergo Ovulation. During Ovulation, the follicle bursts open, releasing the hydrated oocyte into the lumen of the ovary, and contracts.

Following Ovulation, the follicle (Post Ovulatory Follicle, or POF) remains visible for a brief period, on the order of days, within the ovary before it is resorbed. POFs characterize ovaries in the Spent or Regressing stages. Although the ages and appearance of POFs are not known in Atlantic croaker, fish that spawn in warmer waters, 17-19°C, have short-lived POFs that are readily distinguishable for less than 24 hours, and are completely degraded within 2-3 days (Yamamoto and Yoshioka 1964; Hunter and Goldberg 1980). Bay temperatures when Atlantic croaker spawn generally exceed 20° C; therefore I assume that POF's persist in Atlantic croaker in the Chesapeake Bay for a maximum of 24 hours.

Staging Methodology

Macroscopic Staging

I determined the macroscopic ovarian maturity stages of individual females based on stage criteria and terminology in Wallace and Selman (1981), Hunter and Macewicz (1985a), Hunter et al. (1992), Barbieri (1993), and Barbieri et al. (1994) as summarized in Table 1 and West (1990). The macroscopic staging was based on gross morphological criteria such as ovary size relative to the individual's body cavity, ovary color and the presence or absence of large visible yolked oocytes or hydrated oocytes.

Microscopic Staging

Using histological techniques, I used microscopic staging to verify macroscopic ovarian maturity stages. Microscopic staging was based on the maturation phase of oocytes within the ovary and their appearance under stain, rather than the appearance of the fresh whole ovary itself. Microscopic staging terminology used herein follows Wallace and Selman (1981), Hunter and Macewicz (1985a), Hunter et al. (1992), Barbieri (1993); and Barbieri et al. (1994) as summarized in Table 1 and West (1990). Ovaries were preserved in 10% neutral buffered formalin for at least one week. Once the ovaries were fully preserved I cut cross-sections about 5mm in width from the middle. The cross-sections were then rinsed in tap water to remove traces of formalin and placed in 70% ethanol for at least 24 h. The sections were run through a series of dehydrating solutions and embedded in paraffin, sectioned to 5-6 μm thickness. The sections were stained with Harris Hematoxylin and Eosin. Finally, the stained tissue was examined microscopically to determine phase of oocyte development and incidence of atresia. A total of 1647 ovaries were processed histologically (821 from the Western Shore, 416 from the Eastern Shore, and 410 from Offshore collections).

Spawning Location

In order to detect the occurrence of spawning along the Eastern Shore and Western Shore of the Bay, I used both macroscopic and microscopic staging methodologies to observe the presence or absence of Gravid, Running Ripe and Spent ovarian stages. As described above, these ovarian stages are coincident with the microscopic observation of hydrated and ovulated oocytes and post ovulatory follicles. In a multiple spawning species, like Atlantic croaker (Barbieri et al 1994), hydration and ovulation of the oocytes occur quickly, typically over only a few hours (Hunter and Macewicz 1985a; Brown-Peterson et al. 1988). Therefore, the presence of hydrated oocytes indicates imminent spawning. Because Barbieri et al (1994) successfully observed Gravid and Running Ripe fish only in gillnet collections, I made special gillnet collections weekly during the spawning period to increase my chances of collecting fish with hydrated oocytes. To do so, nets were set in late afternoon or early evening and fished near dusk whenever possible.

The presence of Spent or Partially Spent stage ovaries or the presence of POFs would suggest an individual has recently spawned. Therefore the presence of POFs was an important criterion in determining if spawning occurs along the Eastern and Western shores of the Bay. Due to the transient nature of POFs only collections obtained from gillnets fished at dusk, and collections that were caught and processed within 24 hours were analyzed for POF occurrence.

Spawning Period

Using gonad maturity stages and the gonadosomatic index (GSI), I estimated duration and peak in spawning on both the Eastern and Western Shore of the Chesapeake Bay as well as the area near the mouth of the Bay and Virginia Beach, VA. Spawning period was also estimated by calculating the proportion of females in the Spent Stage over time following Harry (1959). The gonadosomatic index is useful as a general indicator of ovarian cycles or spawning periodicity (DeVlaming et al. 1982), and is essentially the percentage of the total body weight devoted to gonad material. It is given by:

$$GSI = GW/TW \times 100$$

where GW = Gonad weight (g);

TW = Total weight of the individual (g);

Incidence of Atresia

Microscopic methods were used to determine the incidence of atresia. Ovaries were preserved and processed for sectioning as described above. Terminology and criteria used for identifying atretic oocytes follows Bretschneider and Duyvene de Wit (1947), Lambert (1970), and Hunter and Macewicz (1985b) who described four stages of atresia: Alpha (α) atresia, which is the resorption of the entire oocyte by the follicular granulosa cells; Beta (β) atresia, which involves the major resorption of the follicle itself; and Delta (δ) and Gamma (γ) atresia, which involve the continuing resorption of the follicle. In order to describe patterns in the incidence of atresia, I determined the percentage of yolked oocytes undergoing alpha atresia in all ovaries. In addition, ovaries were classified according to the percentage of yolked oocytes undergoing alpha (α) atresia (Table 2).

I performed univariate analysis of variance (ANOVA) tests with multiple factors on levels of atresia to determine if the index differed between collection areas or between years. I also determined if atretic classification states varied over time and between collection areas using the Kruskal Wallis test for nonparametric variables.

Table 2. Classification of levels of atresia.

Atretic State	Description
0	Ovary contains no atresia
1	Ovary contains less than 25% Alpha (α) atresia of yolked oocytes
2	Ovary contains between 25 and 50% α atresia of yolked oocytes
3	Ovary contains between 50 and 75% α atresia of yolked oocytes
4	Ovary contains over 75% α atresia of yolked oocytes

RESULTS

Description of Microscopic Gonad Stages

Atlantic croaker are multiple spawners, with each individual spawning several times over a protracted period. Throughout the spawning period, Atlantic croaker females progress through seven stages of ovarian maturity, described below, spawning and redeveloping until their final spawn. After their final spawn, females advance into the Spent and Resting Stages (Figure 3). Ovarian maturity stage can be determined accurately based on the microscopic appearance of the ovary under stain.

The appearance of Resting Stage females was highly variable. Most ovaries contained large numbers of both atretic oocytes and follicles, which ranged in age from recent to very old. In addition, these ovaries contained thick ovarian membranes and loosely arranged primary growth oocytes (Figure 4). In contrast, some ovaries contained densely packed clusters of primary growth oocytes with no atretic oocytes and a very thin ovarian membrane.

Immature Stage ovaries were similar in appearance to Resting Stage ovaries. These ovaries had a very dense arrangement of primary growth oocytes and a thin ovarian wall (Figure 5). In general, Immature Stage females could be distinguished

Fig. 3. Diagrammatic description of the Atlantic croaker ovarian maturity cycle.

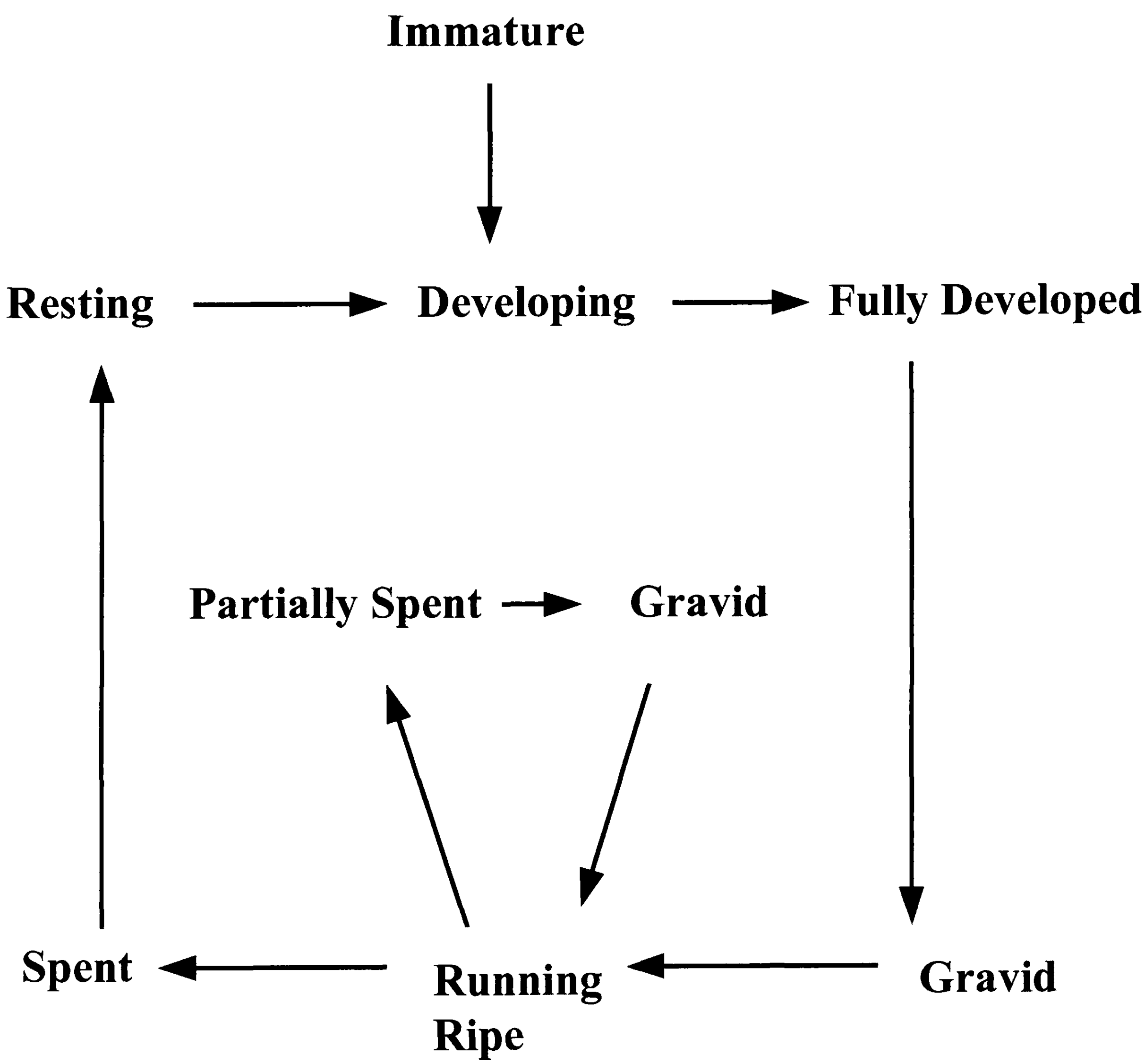

Immature
Resting
Developing
Fully Developed
Partially Spent
Gravid
Spent
Running Ripe
Gravid

Fig. 4. Histological appearance of Ovaries in Resting Maturity Stage.
PG – primary growth oocyte. OW –ovarian wall.

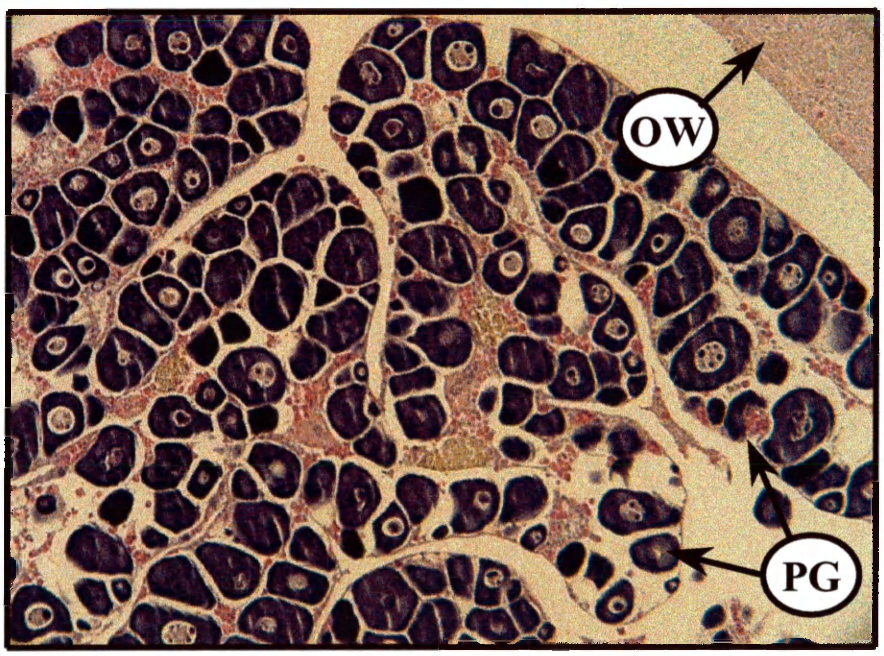
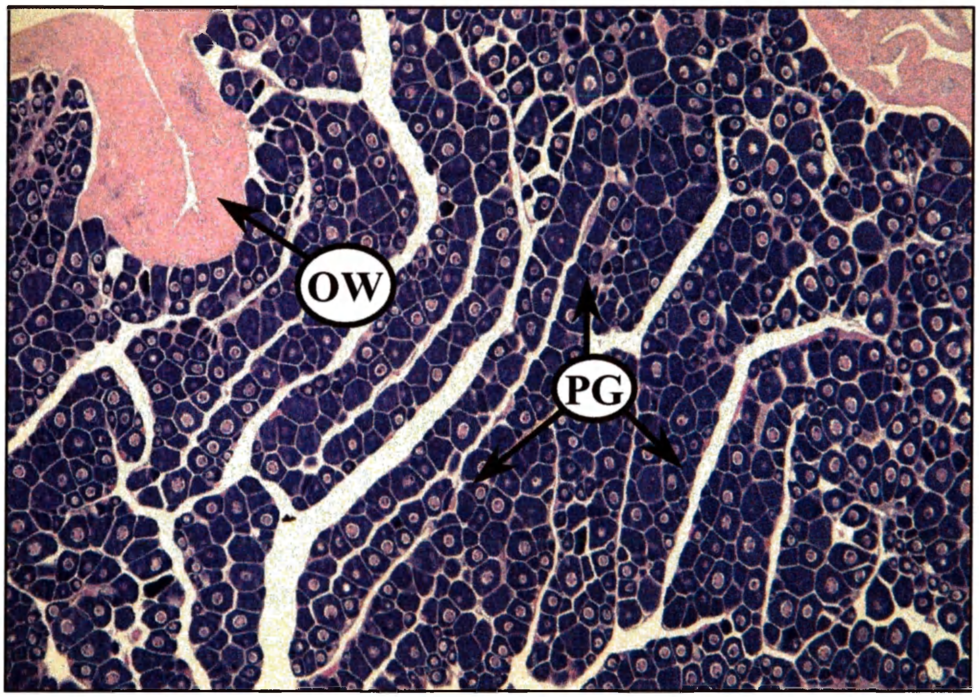

OW
PG

Fig. 5. Histological appearance of ovaries in Immature Maturity Stage. PG – primary growth oocyte. OW – ovarian wall.



OW
PG

Fig. 6. Histological appearance of ovaries in (a) Developing, (b) Fully Developed, and (c) Fully Developed/Partially Spent Stages. AO – atretic oocyte. CA – cortical alveoli. PG – primary growth oocyte. YO – yolked oocyte. YG – yolk granules.

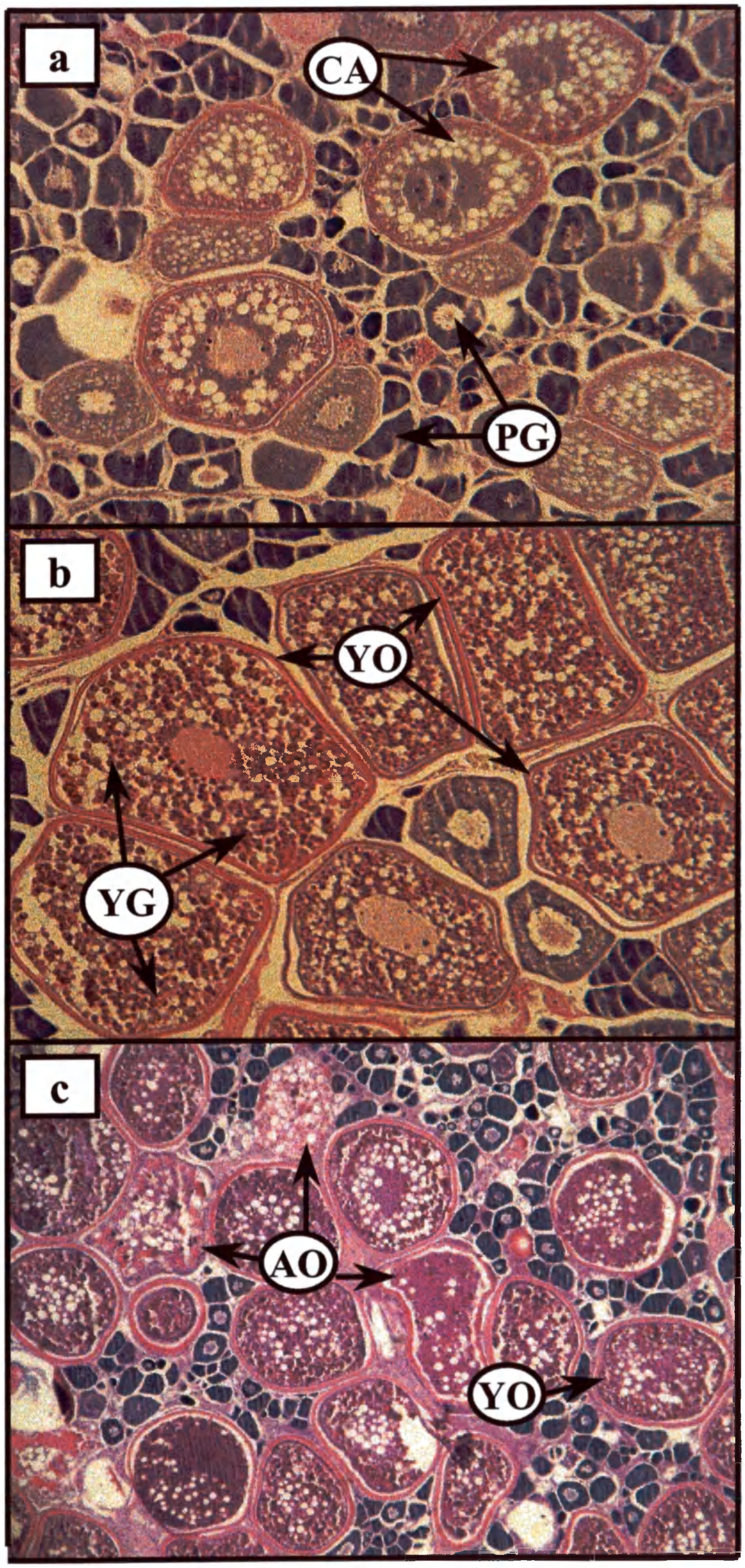

a
CA
PG
b
YO
YG
c
AO
YO

Fig. 7. Histological appearance of ovaries in Spent Maturity Stage. Histological appearance of Alpha (α) atresia. αA – Alpha atretic oocyte. Lα - late Alpha atretic oocyte. HYO – healthy yolked oocyte. ZR – Zona Radiata.

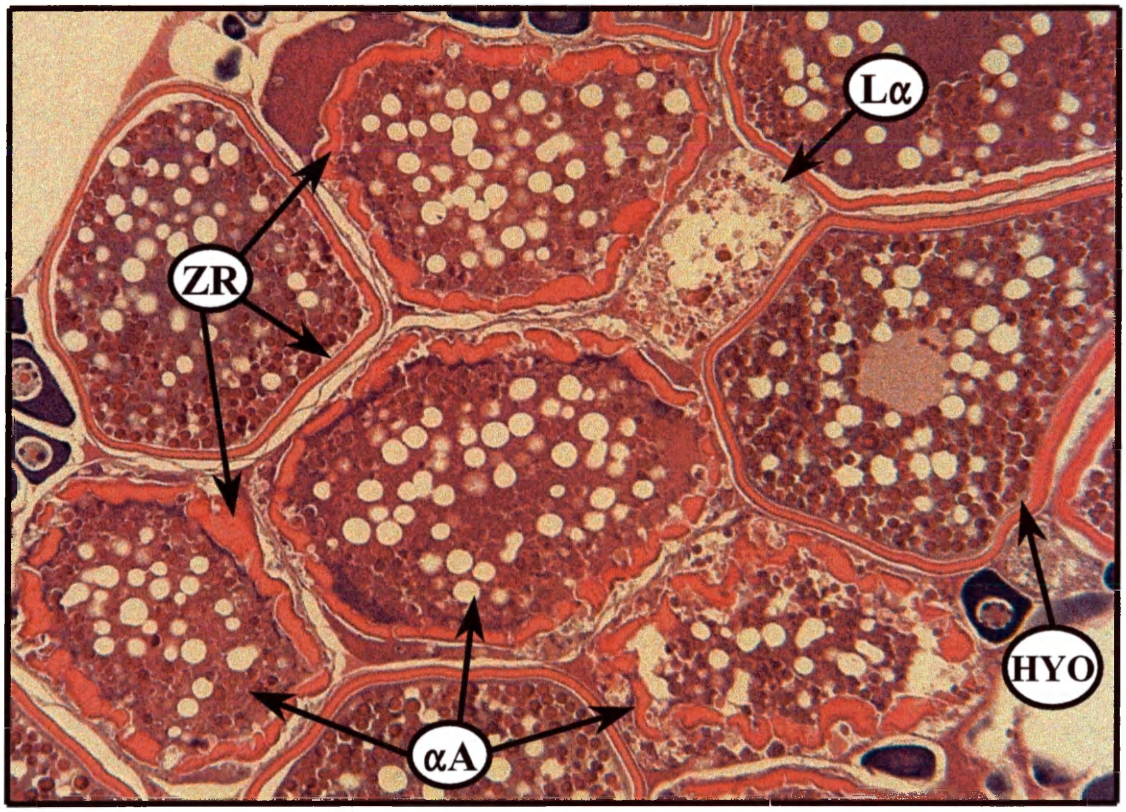

Lα
ZR
αA
HYO

Fig. 8. Histological appearance of an old Post Ovulatory Follicle in a Spent Stage female. POF – Post Ovulatory Follicle; YO – yolked oocyte; AO – atretic oocyte.

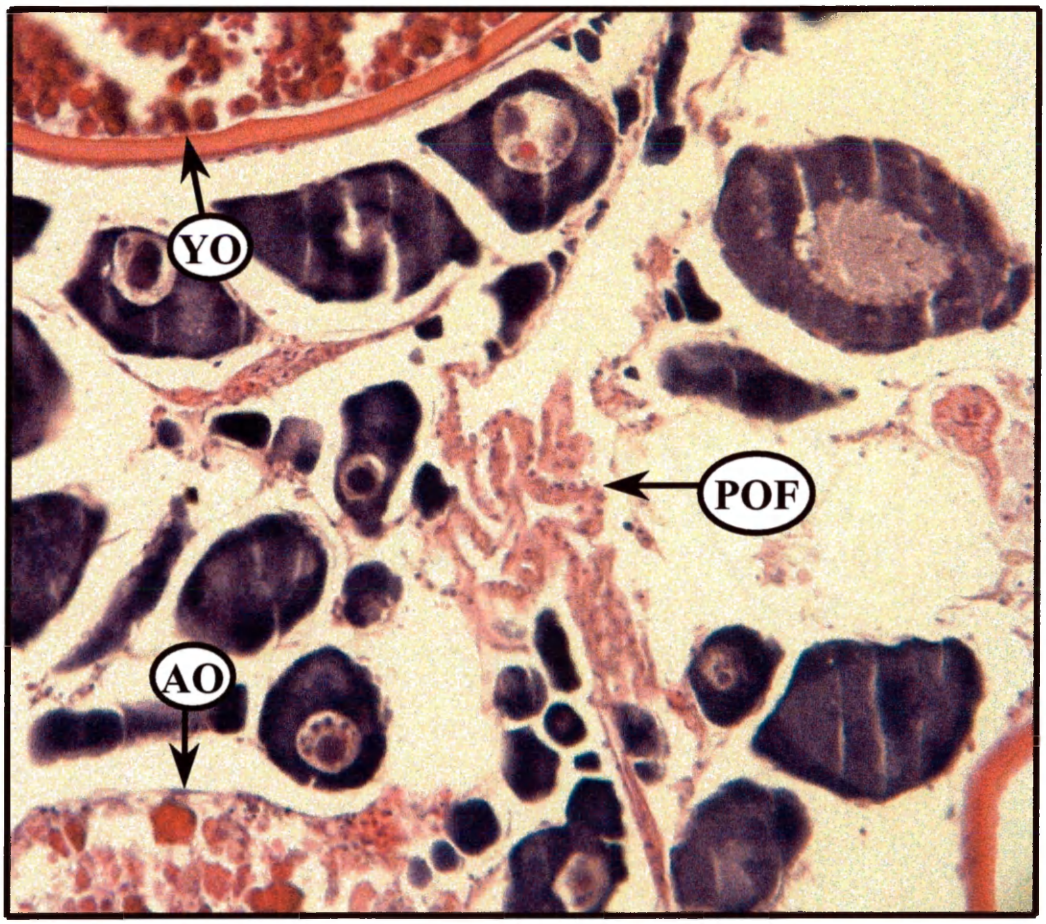

YO
POF
AO

Fig. 9. Percent agreement between macroscopic and microscopic staging methods in female Atlantic croaker by stage.

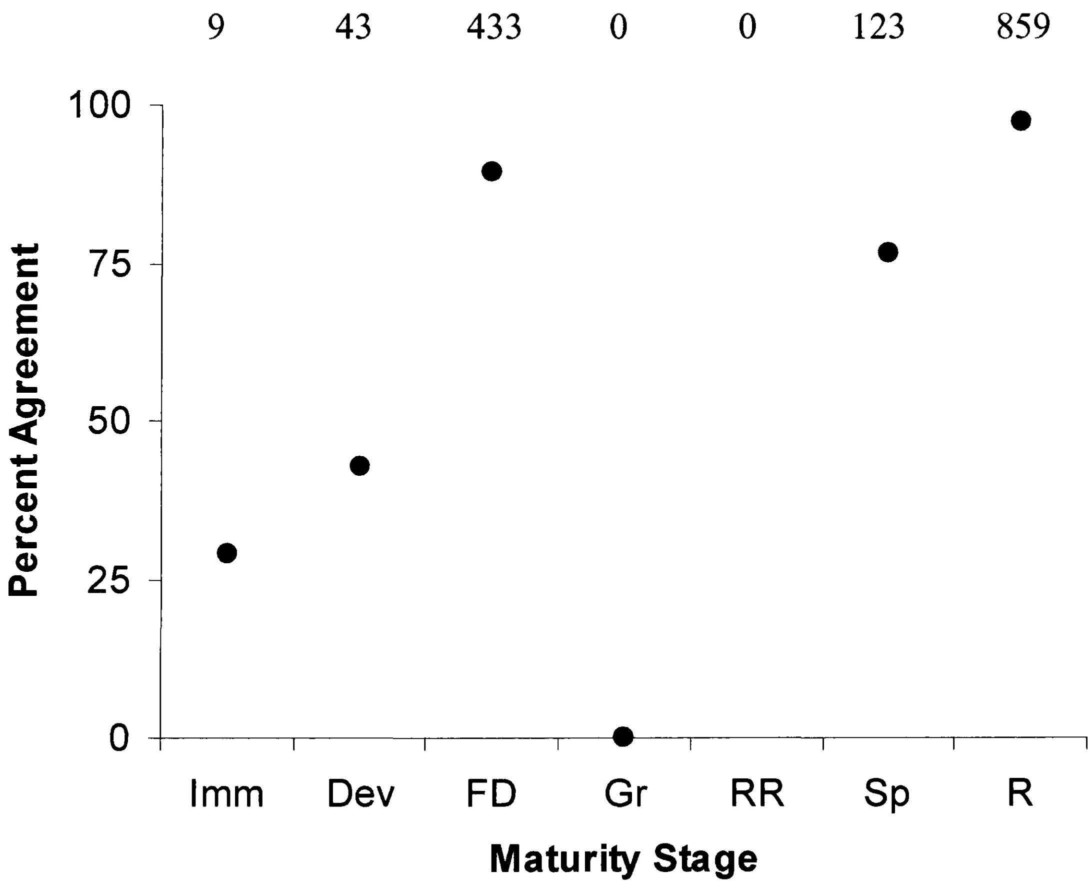

9
43
433
0
0
123
859
100
75
50
25
0
Percent Agreement
Imm
Dev
FD
Gr
RR
Sp
R
Maturity Stage

Table 3. Agreement between macroscopic and microscopic Staging methods. N=881. Total Agreement = 87.29 %.

	Macroscopic Stage						
Microscopic Stage	Imm	Dev	FD	Gr	RR	S	R
Imm	9	1	--	--	--	--	--
Dev	--	33	33	--	--	5	12
FD	--	12	433	9	--	9	4
Gr	--	--	--	--	--	--	--
RR	--	--	--	--	--	--	--
S	--	--	12	--	--	123	10
R	22	31	6	--	--	24	859
Percent agreement	29.03%	42.86%	89.46%	0%	N/A	76.04%	97.06%

Table 4: Combined Ovarian Maturity Stages: Revised description of Ovarian Maturity Stages for Atlantic croaker, *Micropogonias undulatus*. Adapted from Table 1 and Cooksey et al (1996).

Stage	Macroscopic Appearance
(1) Non Spawning	Ovaries very small, light pink to reddish; no opaque oocytes. Includes Immature and Resting Stages from Table 1.
(2) Maturing	Ovaries small to large; yellow to light orange; opaque oocytes present. May contain unovulated hydrated oocytes. Includes Developing, Fully Developed and Gravid Stages from Table 1.
(3) Running Ripe	Ovaries large to very large; yellow to light orange; most hydrated oocytes are collected in the lumen (ovulated); some may still be intermixed with opaque oocytes. Included Running Ripe Stage from Table 1.
(4) Spent/ Regressing	Ovaries small to medium; light orange to reddish orange; flaccid and watery; opaque oocytes present. Includes Spent Stage from Table 1.

from Resting Stage ovaries by the presence of late atretic oocytes. I found few Immature Stage females since my collections mostly included larger, adult fish.

Developing Stage ovaries varied in their appearance. These ovaries contained mostly primary growth oocytes and some cortical alveoli. More developed females also contained small numbers of early yolked oocytes (Figure 6a).

When advanced yolked oocytes were present, the ovary was classified as Fully Developed. Many Fully Developed Ovaries contained large numbers of advanced yolked oocytes as well as partially yolked oocytes, cortical alveoli, and primary growth oocytes, and few to no atretic oocytes (Figure 6b). Others contained large numbers of atretic yolked oocytes in addition to healthy advanced yolked oocytes (Figure 6c). The appearance of both atretic yolked oocytes and healthy advanced yolked oocytes indicates these females had spawned once already in the year but were maturing another batch of oocytes to be spawned. Therefore, ovaries found in this condition were noted as being Fully Developed Stage/Partially Spent

I did not find any Gravid or Running Ripe Stage females in my collections. I macroscopically staged 9 females as Gravid, but microscopic examination proved that those females were actually Fully Developed.

Spent Stage ovaries were often difficult to distinguish from Fully Developed or Partially Spent ovaries. Generally, Spent stage females contained much higher levels of atresia of yolked and unyolked oocytes (Figure 7). Some Spent Stage females also contained very old POFs (Figure 8).

Agreement Between Macroscopic and Microscopic Gonad Staging Methods

Agreement between the two staging methods was 88.5 % (Figure 9, Table 3). Agreement was highest for Resting Stage females, at 97.1 % but was much lower, only 76.4 %, for Spent Stage females. Agreement was very low for Developing Stage females, 42.9%, and for Immature Stage females, only 29 %, but high for Fully Developed Stage females, 89.5 %. A more detailed description regarding the nature of agreement between staging methodologies for each of the seven stages follows.

The very high agreement for Resting Stage ovaries results from their distinct macroscopic appearance. Resting Stage ovaries differ in size, color and texture from other stages, making them easier to stage macroscopically.

Agreement was lower for Spent females, because they are macroscopically similar to Fully Developed/Partially Spent and Resting stages. Both Fully Developed and Spent stage ovaries contain opaque oocytes and can be creamy yellow in appearance. In addition, Spent stage ovaries can also be smaller, flaccid and a darker orange, like Resting Stage ovaries. In contrast to these macroscopic similarities, the microscopic observation of both reduced numbers of yolked oocytes and high levels of atresia readily distinguish Spent Stage ovaries from other stages. Agreement for Immature females was very low due to similarities in macroscopic appearance between Immature and Resting Stage Ovaries.

Due to the similar macroscopic appearance of Developing Stage and Fully Developed Stage ovaries, most of the females incorrectly assessed as Fully

Developed macroscopically were classified as Developing Stage microscopically. The agreement for Fully Developed Stage females was much higher due to the more common occurrence of this stage, as well as the brief nature of the Developing Stage and the fact that upon microscopic examination, more females in question were classified as Fully Developed.

As seen above, stages can be difficult to distinguish without examining the oocytes themselves, because ovarian development occurs continuously, not in discrete jumps. Because of low agreement between macroscopic and microscopic staging in Atlantic croaker, I suggest using modified criteria that describe general spawning state of the female, similar to Cooksey's (1996) recommended alternate staging method for Spanish Mackerel. By combining stages that are developmentally similar and similar in appearance, error in staging may be reduced. This results in four stages for describing ovarian development: Non-Spawning, Maturing, Running Ripe and Spent.

The Non-Spawning Stage includes both immature and mature females that are not developing, actively spawning, or recovering from a previous spawn (Table 4). The Maturing Stage includes those females developing oocytes to be spawned in the future; Developing, Fully Developed, and Gravid (Table 4). Running Ripe Stage and Spent or Regressing Stage females are developmentally distinct and remain separate stages (Table 4).

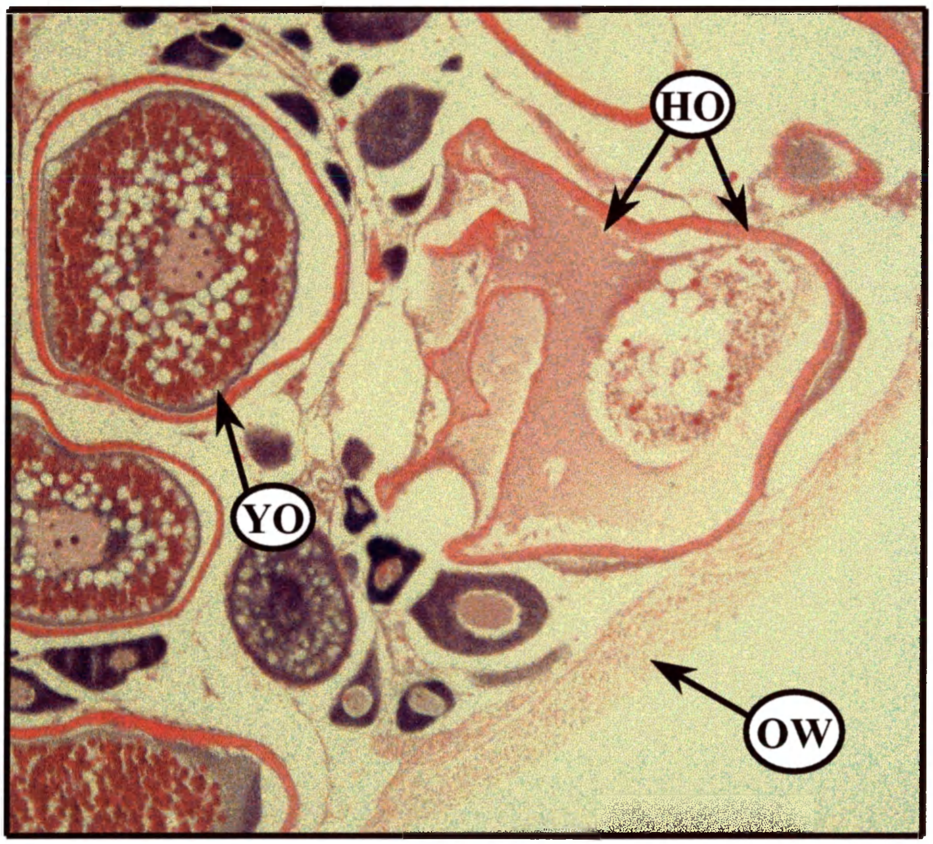
Spawning Results

Spawning Location

The occurence of Gravid or Running Ripe Stage females, the presence of Post-Ovulatory Follicles (POFs) and the observation of Spent Stage females were all used as indicators of recent spawning at the locations used in this study. The absence of Gravid or Running Ripe Stage females indicates few females spawn within the Chesapeake Bay itself.. Although five ovaries were identified as Gravid during macroscopic staging, none contained significant numbers of hydrated oocytes and were staged as Fully Developed microscopically. The few oocytes that appeared to be hydrated upon macroscopic inspection were found to be either very advanced yolked or early hydrated upon microscopic inspection (Figure 10). I did not find any females containing ovulated hydrated oocytes.

The presence of POFs in my collections suggests that some spawning does occur in the Chesapeake Bay. POFs were often difficult to distinguish due to extensive degradation. This implies that those POFs may be as old as 12 to 24 hours and cannot accurately indicate specific or small scale spawning sites, only general spawning area. I found 42 females with POFs in the Bay (Table 5) on both the Eastern and Western Shores, and 50 females with POFs in Offshore collections, indicating that spawning had occurred within 12 to 24 hours before my collections. These structures first appeared in collections in June of 1998 and July of 1999 and

Fig. 10.Histological appearance of early hydrated oocyte. HO – hydrated oocyte. OW – ovarian wall. YO – yolked oocyte.



HO
YO
OW

Table 5. Occurrence of Post Ovulatory Follicles in females collected in the Chesapeake Bay. Number of females containing POFs by area; Western Shore, Eastern Shore, and Offshore (with percentage of total collection in parenthesis).

Month	Total Number of Females with POFs (%)	Females with POFs Western Shore	Females with POFs Eastern Shore	Females with POFs Offshore
1998 April	0	0		
May	0	0		
June	1 (1.28%)	1 (1.28%)		
July	10 (6.49%)	2 (2.99%)	8 (9.20%)	
August	2 (4.76%)	2 (4.76%)		
Sept	4 (4.87%)	0	4 (7.41%)	
October	6 (11.11%)	6 (11.11%)		
November	11 (10.89%)	3 (6.67%)		8 (14.29%)
December		0		
1999 February		0		
March		0		0
April		0		
May		0	0	
June		0	0	
July	8 (4.52%)	0	8 (7.48%)	0
August	2 (1.89%)	0	1 (2.13%)	1 (2.63%)
Sept	3 (3%)	0	3 (6.98%)	
October	22 (30.56%)	4 (10.26%)		18 (54.55%)
November	23 (20.72%)	0		23 (21.50%)
December				0

Table 6. Occurrence of Spent Stage females in the Chesapeake Bay. Number of Spent Stage females collected by area; Western Shore, Eastern Shore, and Offshore (with percentage of total collection in parenthesis).

Month	Number of Spent Stage Females (%)	Spent Females Western Shore	Spent Females Eastern Shore	Spent Females Offshore
1998 April	0	0		
May	0	0		
June	0	0		
July	5 (3.25%)	0	5 (5.75%)	
August	1 (2.38%)	1 (2.38%)		
Sept	0	0	0	
October	21 (38.89%)	21 (38.89%)		
Nov	39 (38.61%)	21 (46.67%)		18 (32.14%)
Dec	5 (23.81%)	5 (23.81%)		
1999 Feb		0		
March		0		0
April		0		
May		0	0	
June		0	0	
July	6 (3.39%)	0	5 (4.67%)	1 (5.88%)
August	1 (0.94%)	0	0	1 (2.63%)
Sept		0	0	
October	32 (44.44%)	15 (38.46%)		17 (51.52%)
Nov	34 (30.63%)	0		34 (31.78%)
Dec				0

Fig.11. Frequencies of Ovarian Maturity Stages in all collections over time (White bars = 1998, Black bars = 1999).

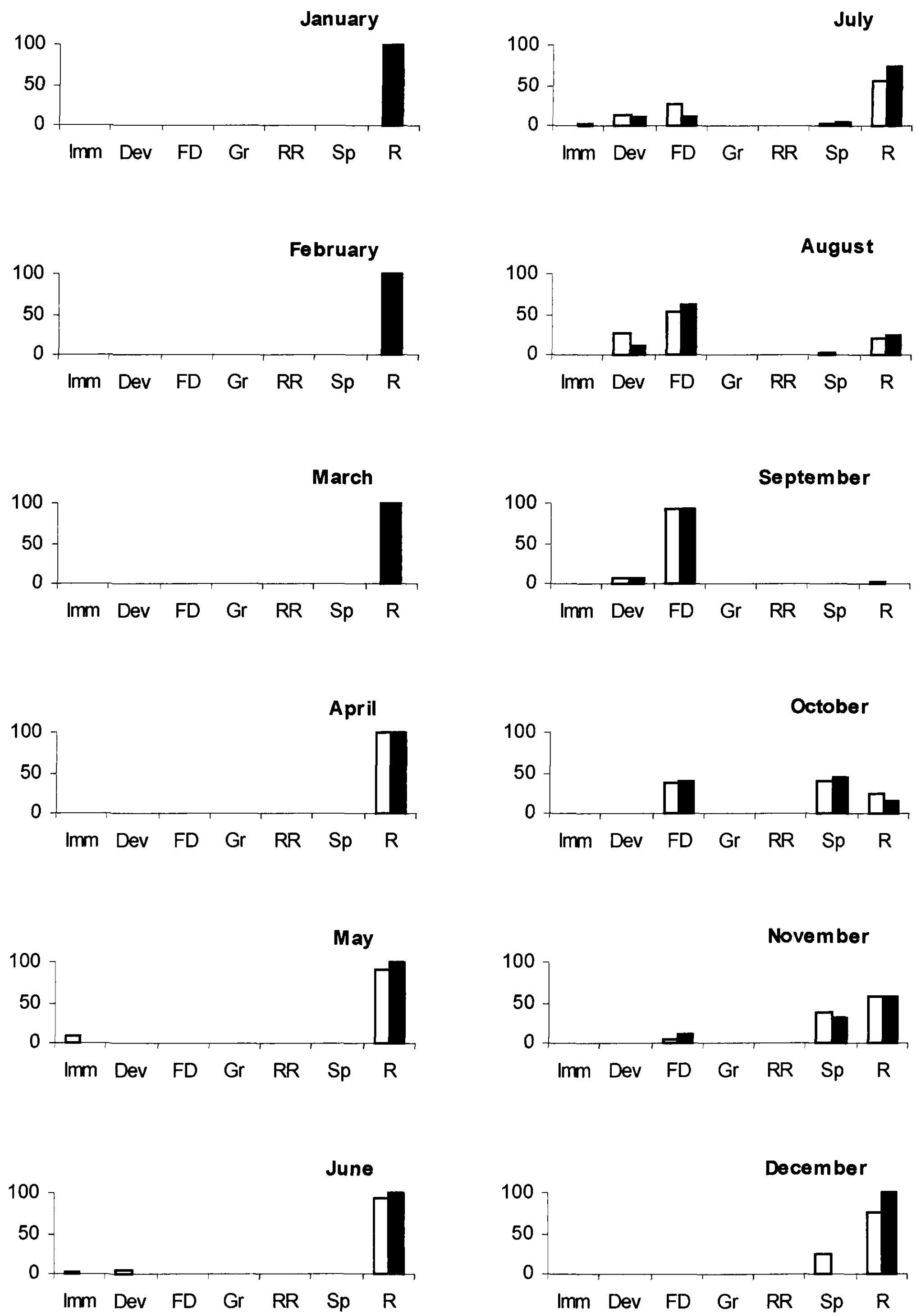
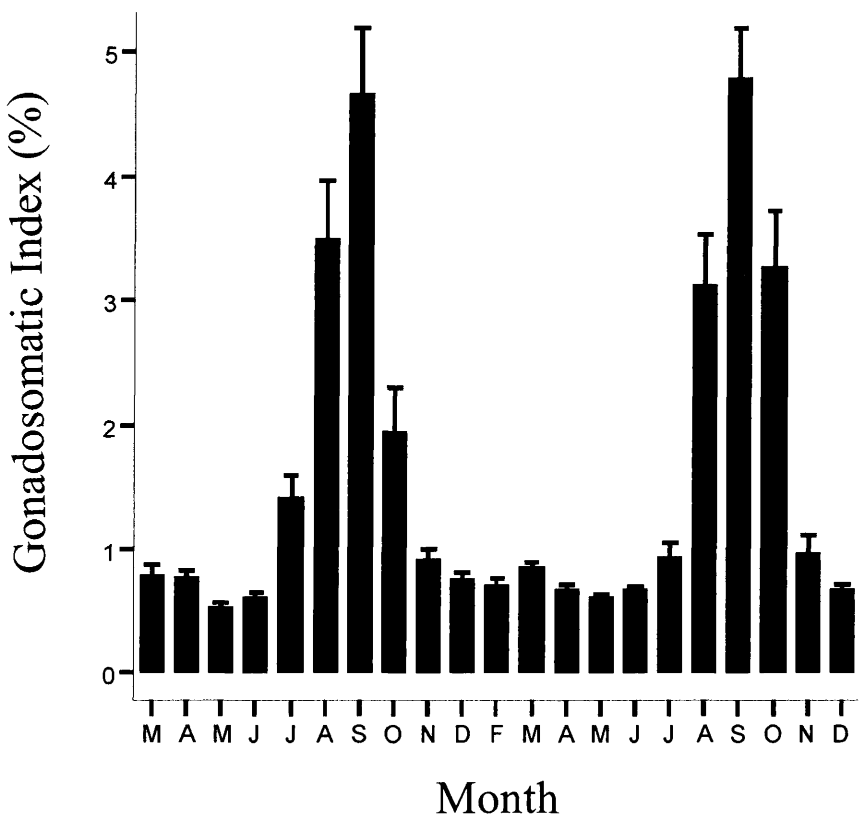

January
February
March
April
May
June
July
August
September
October
November
December
100
50
0
Imm Dev FD Gr RR Sp R

Fig.12. Mean monthly gonadosomatic indices for Atlantic croaker, 1998-2000 (Bar = mean. Error line = upper 95% Confidence Interval).



Gonadosomatic Index (%)
0
1
2
3
4
5
M A M J J A S O N D F M A M J J A S O N D
Month

Fig.13. Frequencies of Ovarian Maturity Stages in collections from the Western Shore of the Chesapeake Bay (White bars = 1998, Black bars = 1999).

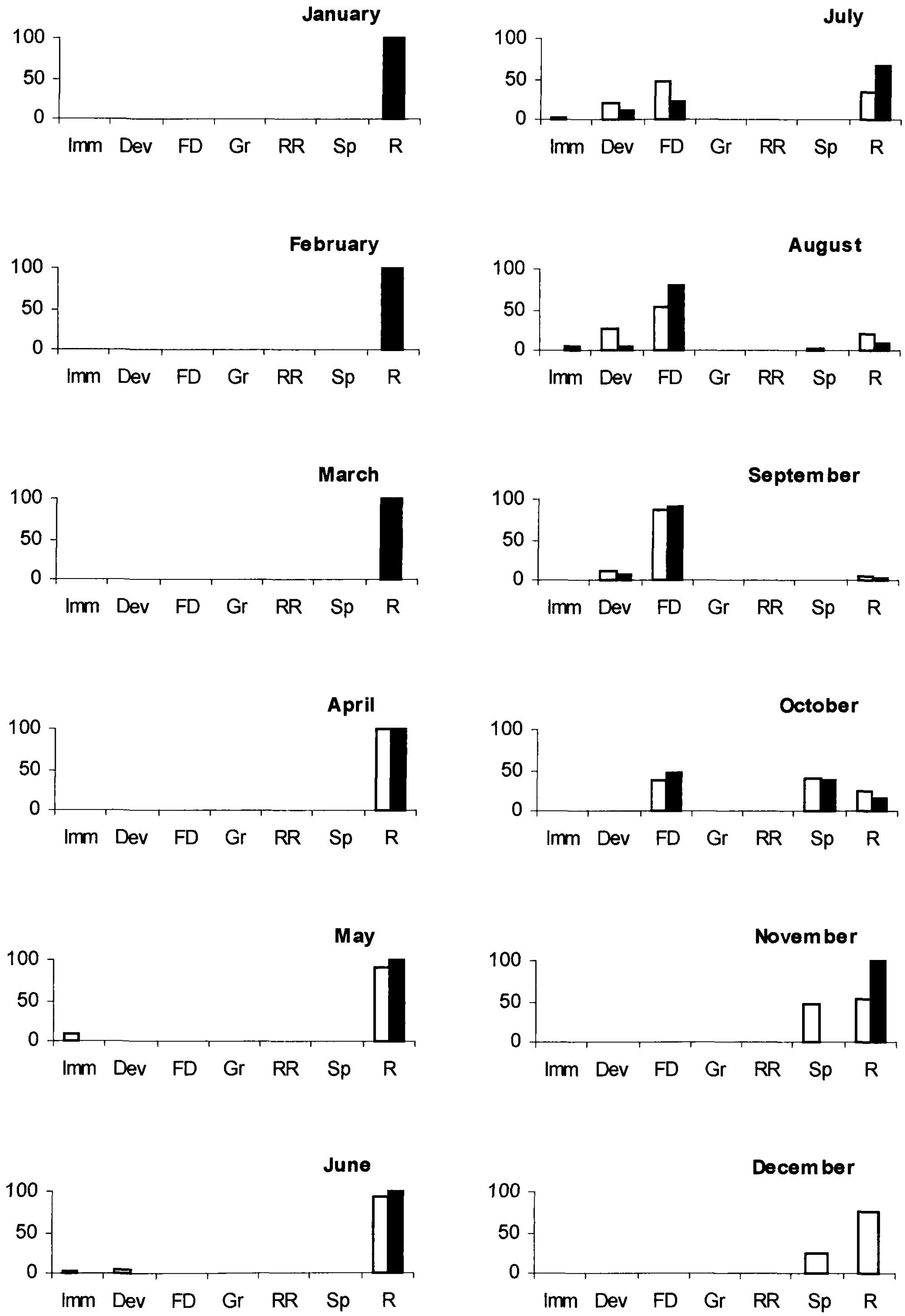

January
July
February
August
March
September
April
October
May
November
June
December
100
50
0
Imm
Dev
FD
Gr
RR
Sp
R

Fig.14. Frequencies of Ovarian Maturity Stages in collections from the Eastern Shore of the Chesapeake Bay (White bars = 1998, Black bars = 1999).

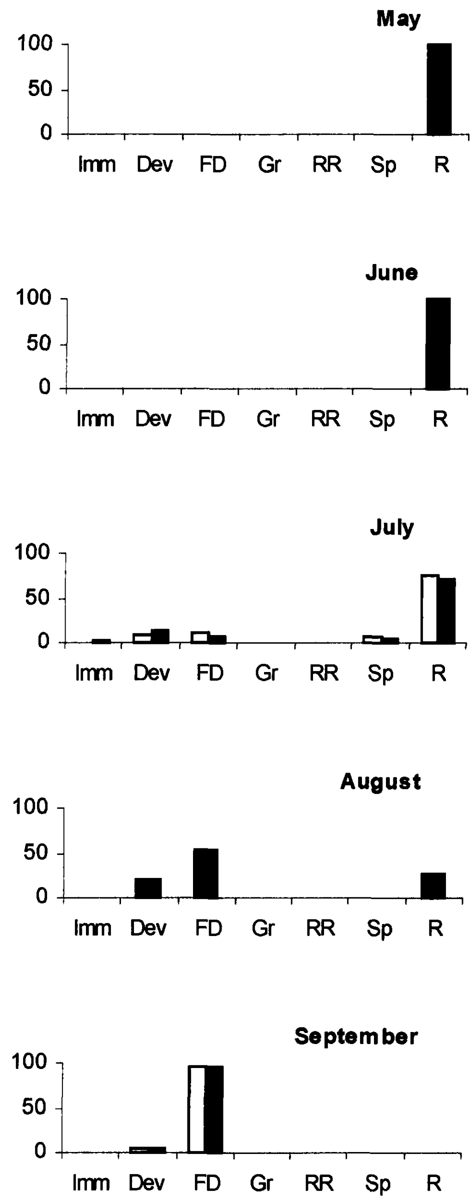

May
100
50
0
Imm
Dev
FD
Gr
RR
Sp
R
June
100
50
0
Imm
Dev
FD
Gr
RR
Sp
R
July
100
50
0
Imm
Dev
FD
Gr
RR
Sp
R
August
100
50
0
Imm
Dev
FD
Gr
RR
Sp
R
September
100
50
0
Imm
Dev
FD
Gr
RR
Sp
R

Fig.15. Frequencies of Ovarian Maturity Stages in Offshore collections
(White bars = 1998, Black bars = 1999).

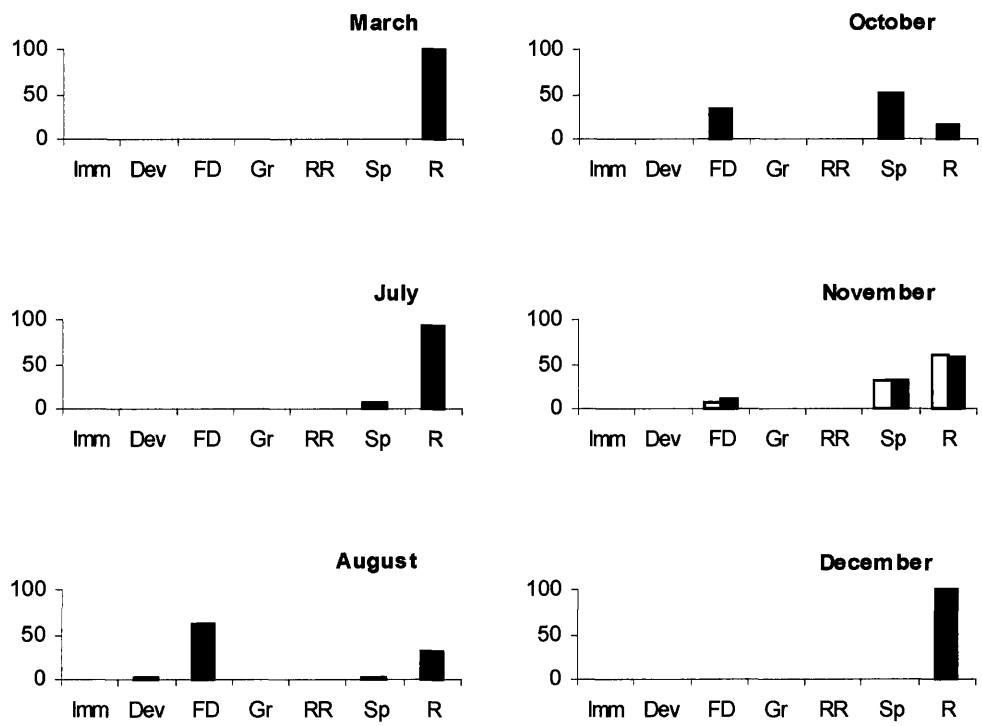

March
October
July
November
August
December
100
50
0
Imm
Dev
FD
Gr
RR
Sp
R

Fig.16. Mean monthly gonadosomatic indices for Female Atlantic croaker from the Western Shore and Eastern Shore of the Chesapeake Bay 1998-2000 (Bar = mean. Error line = upper 95% Confidence Interval).

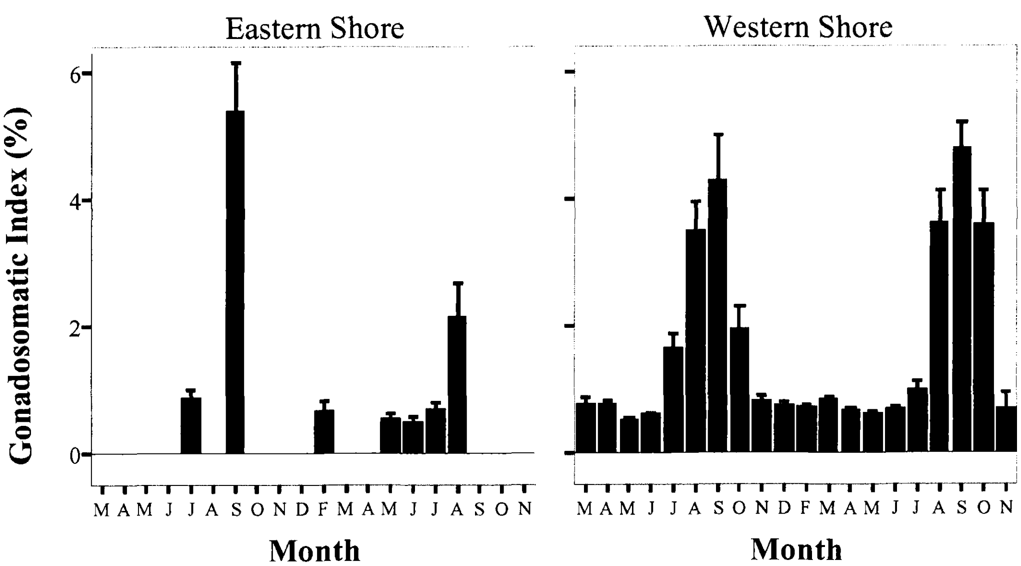
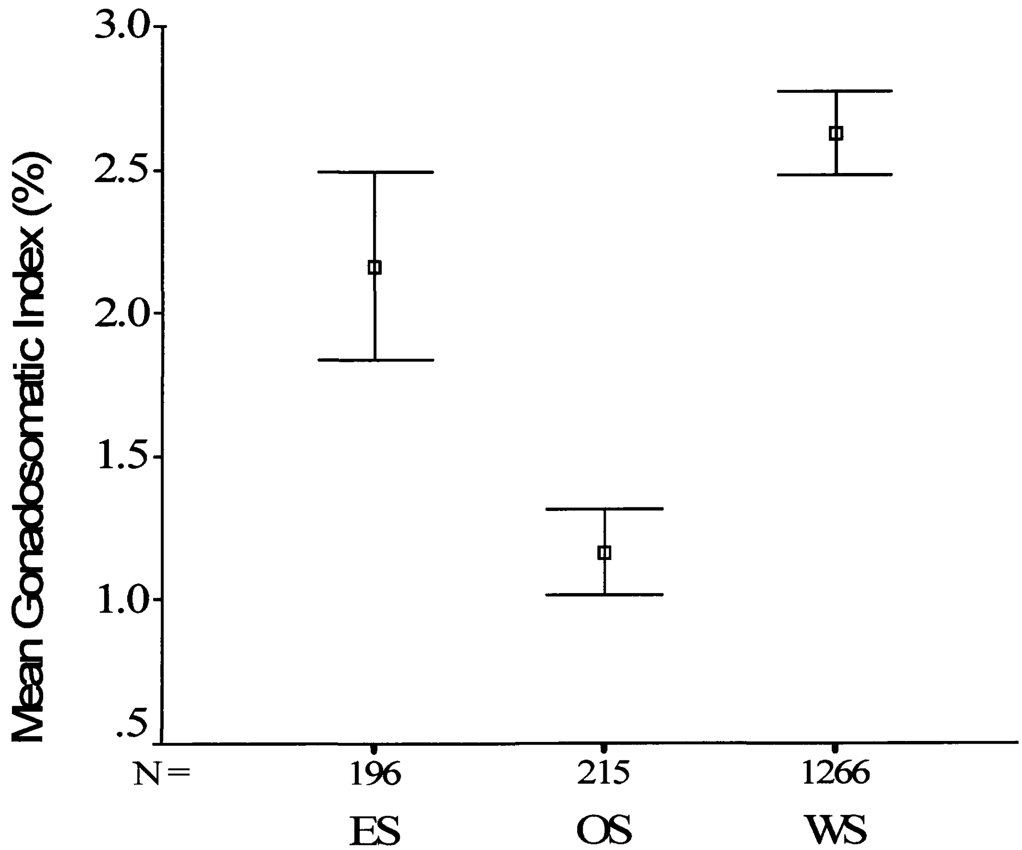

Eastern Shore
Western Shore
Gonadosomatic Index (%)
6
4
2
0
M A M J J A S O N D F M A M J J A S O N
M A M J J A S O N D F M A M J J A S O N
Month
Month

Fig.17. Comparison of GSI mean over spawning period between Eastern Shore, Western Shore and Offshore (Box = mean. Error line = 95% Confidence Interval).


Mean Gonadosomatic Index (%)
3.0
2.5
2.0
1.5
1.0
.5
N =
196
215
1266
ES
OS
WS

increased throughout the spawning period. Between 10 to 30% of females collected contained POFs in October and November. These structures were absent in all areas from December through May (Table 5). They were present on the Western Shore from June through November of 1998, as well as in October of 1999. On the Eastern Shore, I observed POFs from July to September in both years. POFs were present in Offshore collections in November of 1998 and 1999, as well as August and October of 1999.

The presence of Spent Stage females suggests some spawning occurs within the Chesapeake Bay. I found 73 Spent Stage females in the Chesapeake Bay and 71 Offshore from July through December of both 1998 and 1999 (Table 6). Spent Stage females were present on the Western Shore in August, and October through December of 1998, as well as in October of 1999 (Table 6). I also found Spent Stage females on the Eastern Shore in July of both 1998 and 1999. Spent females were present from July through November in 1999 and as well as in November of 1998. This indicates that those females may have completed their final spawn of the year near those areas shortly before capture.

Spawning Period

Both Ovarian maturity stages and Gonadosomatic indices suggest that Atlantic croaker spawn in the Chesapeake Bay region from July through October. Developing and Fully Developed females began to appear in June or July as the proportions of Resting females decreased to 60 - 75 %. More than half of the females were Fully Developed in August, and more than 90 % of the females were Fully Developed in September, indicating peak spawning at that time (Figure 11). After October, the number of Fully Developed females began to decrease. Although Spent Stage females were observed from July through December, Spent Stage females were most abundant in October and November, making up about 40 % of the females at that time. Nearly all females were in the Resting Stage from December to June (Figure 11), so little or no spawning could occur then.

Gonadosomatic indices of females provide a pattern similar to the one derived from gonad maturity stages. Mean gonadosomatic index was low, 0.5% - 1% from November through July and began to increase in late July (Figure 12). Mean gonadosomatic index rapidly increased to peak values 3.5 - 4.5% in August and September, coinciding with the estimated peak in spawning derived from gonad maturity stages. Mean gonadosomatic index remained high in October of 1999 at 3% but decreased rapidly in October of 1998 to less than 2%, indicating a decrease in spawning after September. In 1999, the GSI had decreased greatly by November.

Spawning Period by Location

Frequencies of gonad maturity stages were similar for the both the Eastern and Western shores as well as Offshore (Figures 13, 14, and 15, respectively). As described above, spawning occurred in all locations from July through October, with peak spawning occurring from September to early October. Fully Developed ovaries first appeared in July on the Eastern and Western shores and in August Offshore, and were present until October on the Western Shore and November Offshore. On both the Eastern and Western shores, the frequency of Fully Developed Stage females was highest in September at over 90%, indicating that peak spawning occurred shortly thereafter. The occurrence of Fully Developed Stage females probably peaked in September Offshore, since the frequency of Spent Stage females peaked in October; but fish were not available there at that time. I found Spent Stage females on the Western Shore from October through December and Offshore in October and November. Spent Stage females made up 25 to 50 % of the catch in October and November on the Western Shore and Offshore, indicating that many females had completed their final spawn in those areas shortly before capture.

If the gonadosomatic index is an accurate indicator of spawning effort, the spawning effort of Atlantic croaker is probably similar between the Eastern and Western shores (Figure 16). The mean gonadosomatic index for the spawning period was significantly higher on the Western and Eastern shores than Offshore (Figure 17). However, the low GSI values for Offshore collections (Figure 17) may be misleading since fish were unavailable during the peak spawning month of September.

Description of Microscopic Atretic States

Atlantic croaker oocytes pass through four states of atresia that are readily distinguished based on their microscopic appearance under stain: 1) Alpha (α) Atresia, 2) Beta (β) Atresia, 3) Gamma (γ) Atresia and 4) Delta (δ) Atresia. Oocytes showing α atresia can be distinguished from other healthy and atretic oocytes by the

Fig.18. Histological appearance of Beta (β) atresia of Atlantic croaker oocytes. βA – Beta atretic oocyte.

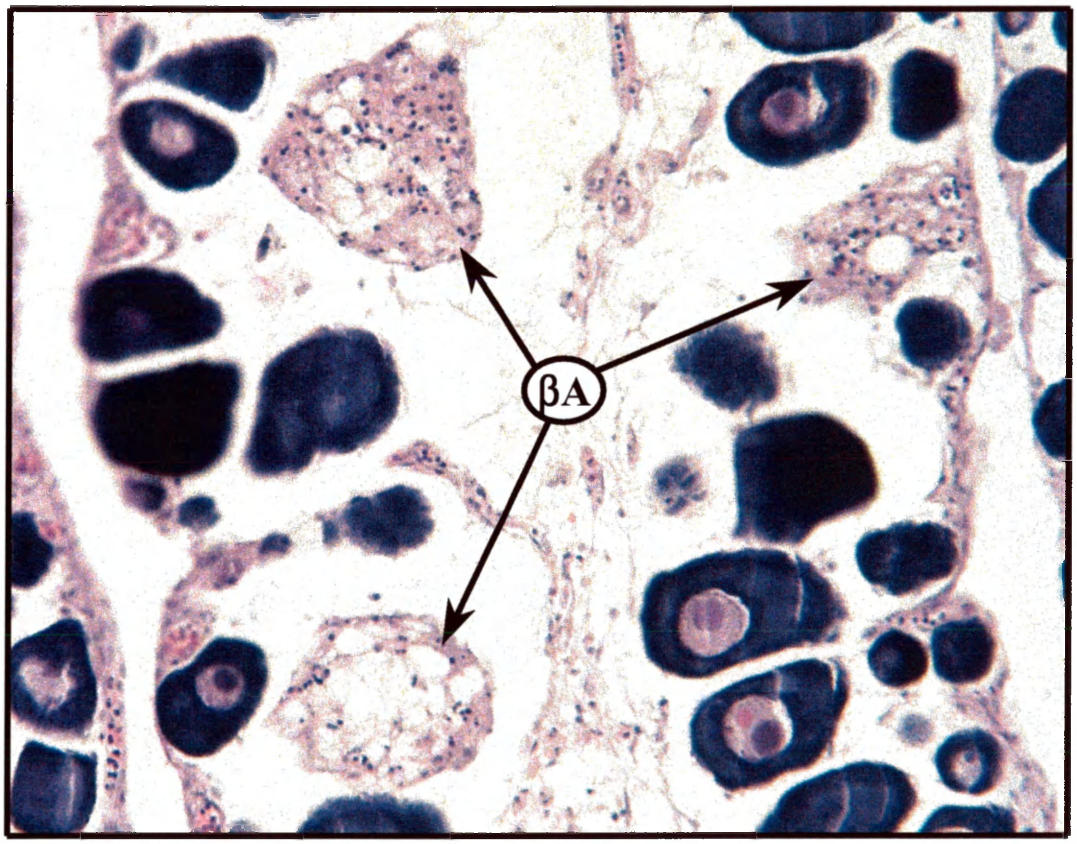

βA

Fig.19. Histological appearance of Gamma and Delta atresia of Atlantic croaker oocytes. δA – Delta atretic oocyte. PG – primary growth oocyte.

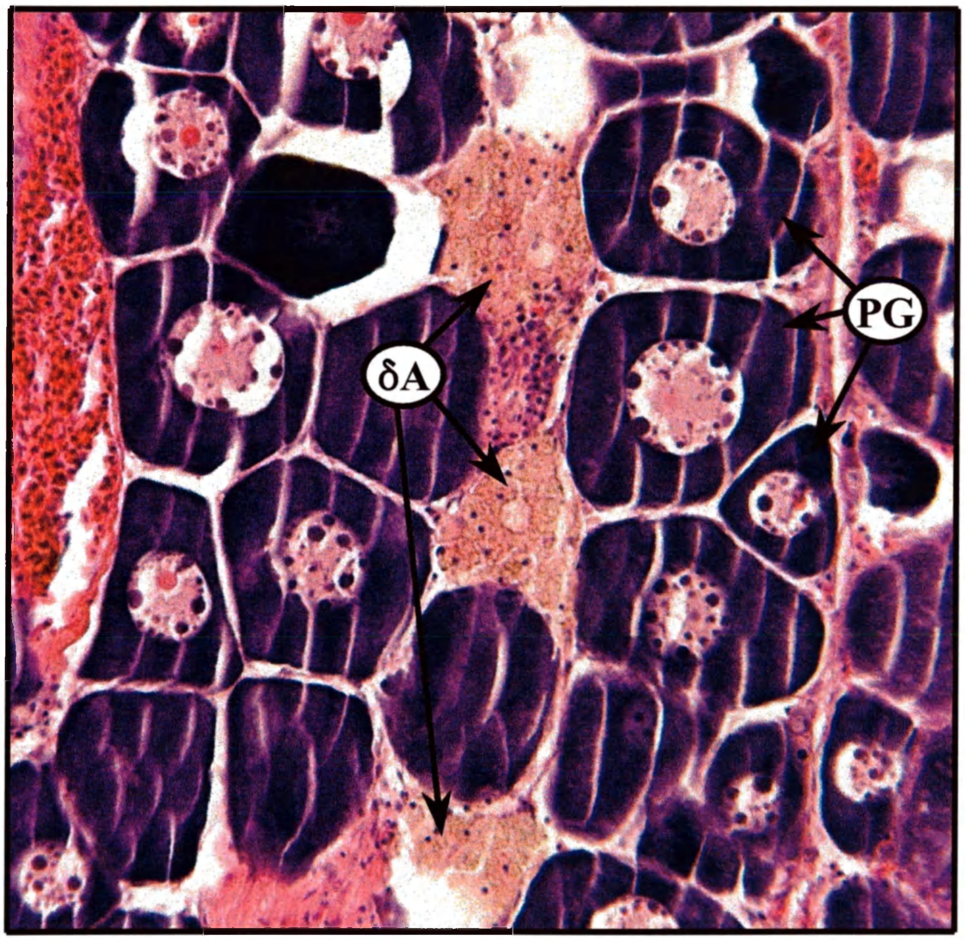

PG
δA

Fig.20. Frequency (percentage of total females collected) of occurrence of atresia in the Chesapeake Bay. (a) No atresia present (α, β, δ or γ) in collections; (b) Alpha (α) atresia present in collections; (c) Beta (β) atresia present in collections; (d) Delta (γ) or Gamma (δ) atresia present in collections.

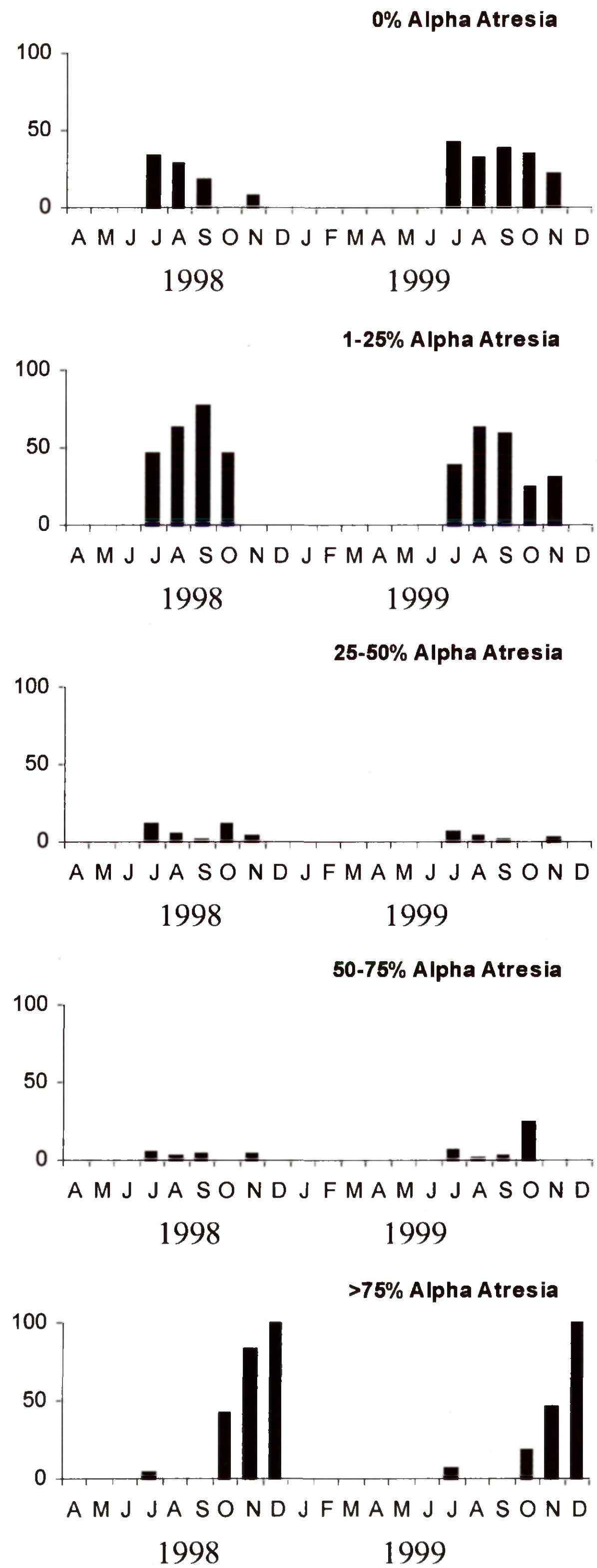

0% Alpha Atresia
1-25% Alpha Atresia
25-50% Alpha Atresia
50-75% Alpha Atresia
>75% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999

Fig.21. Frequency of occurrence of females in five alpha (α) atretic states by month in the Bay (White bars = 1998, Black bars = 1999).

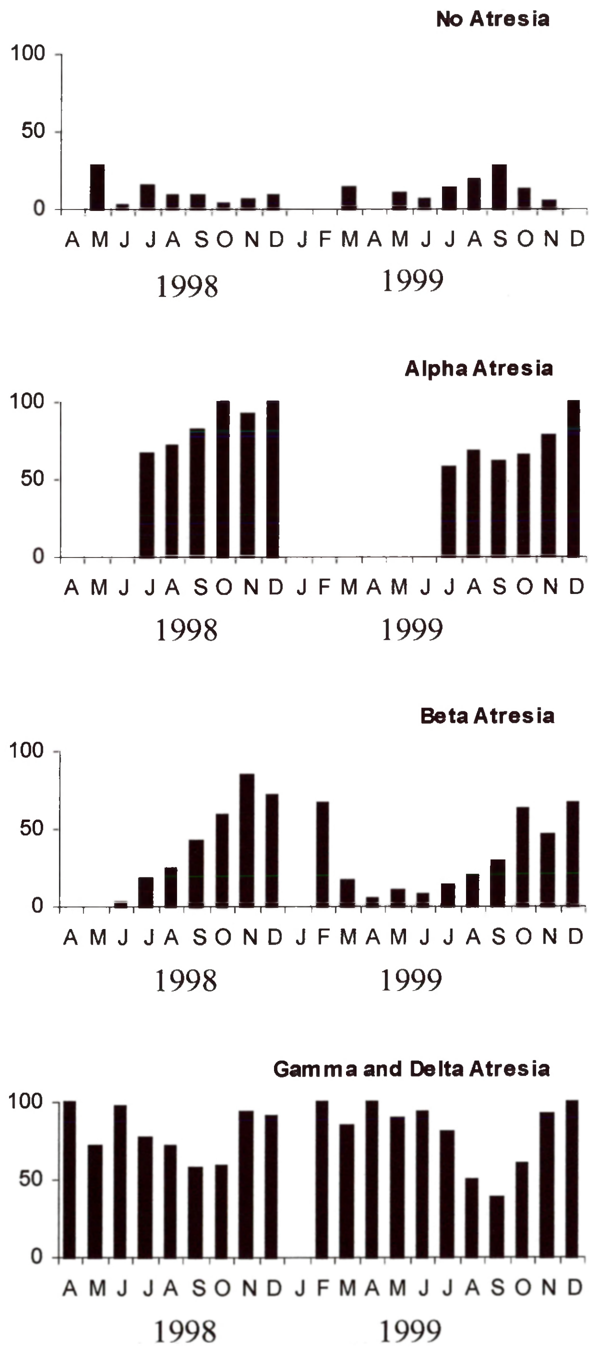
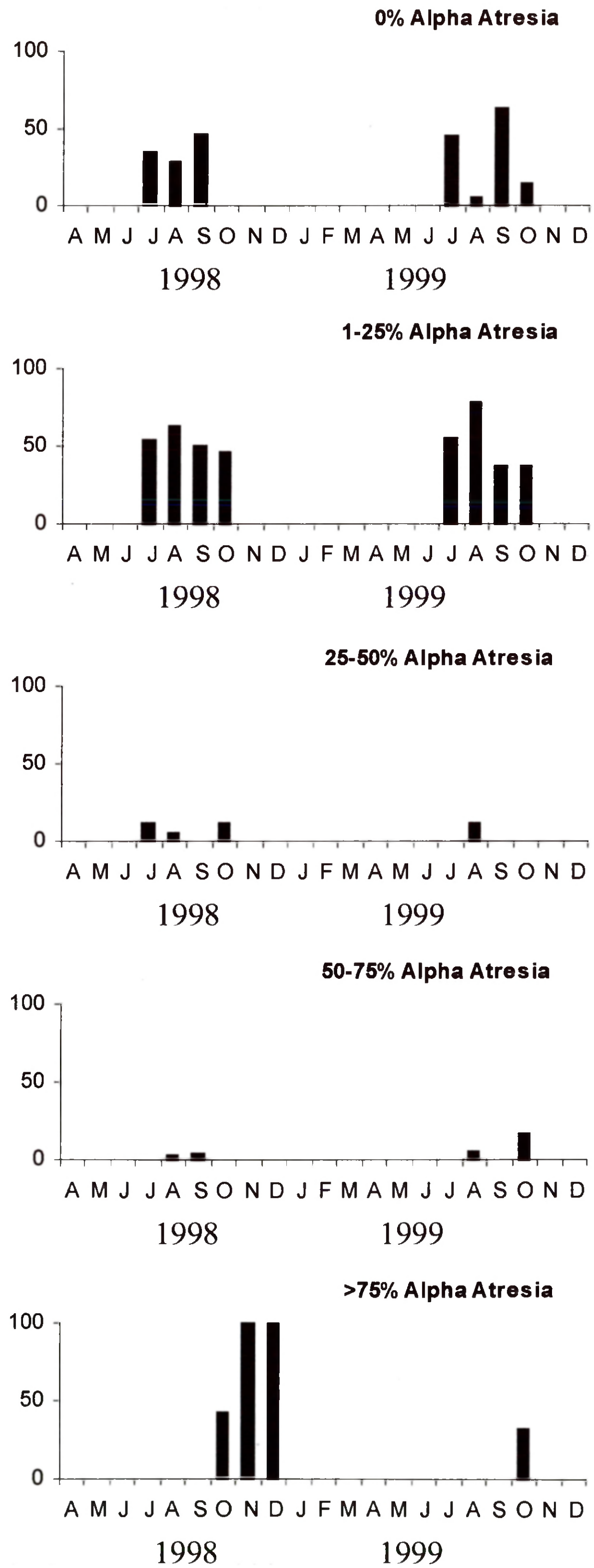

No Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
Beta Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
Gamma and Delta Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999

Fig.22. Frequency of occurrence of females in five alpha (α) atretic states by month in collections from the Western Shore of the Chesapeake Bay (White bars = 1998, Black bars = 1999).



0% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
1-25% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
25-50% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
50-75% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
>75% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999

Fig.23. Frequency of occurrence of females in five alpha (α) atretic states by month in collections from the Eastern Shore of the Chesapeake Bay (White bars = 1998, Black bars = 1999).

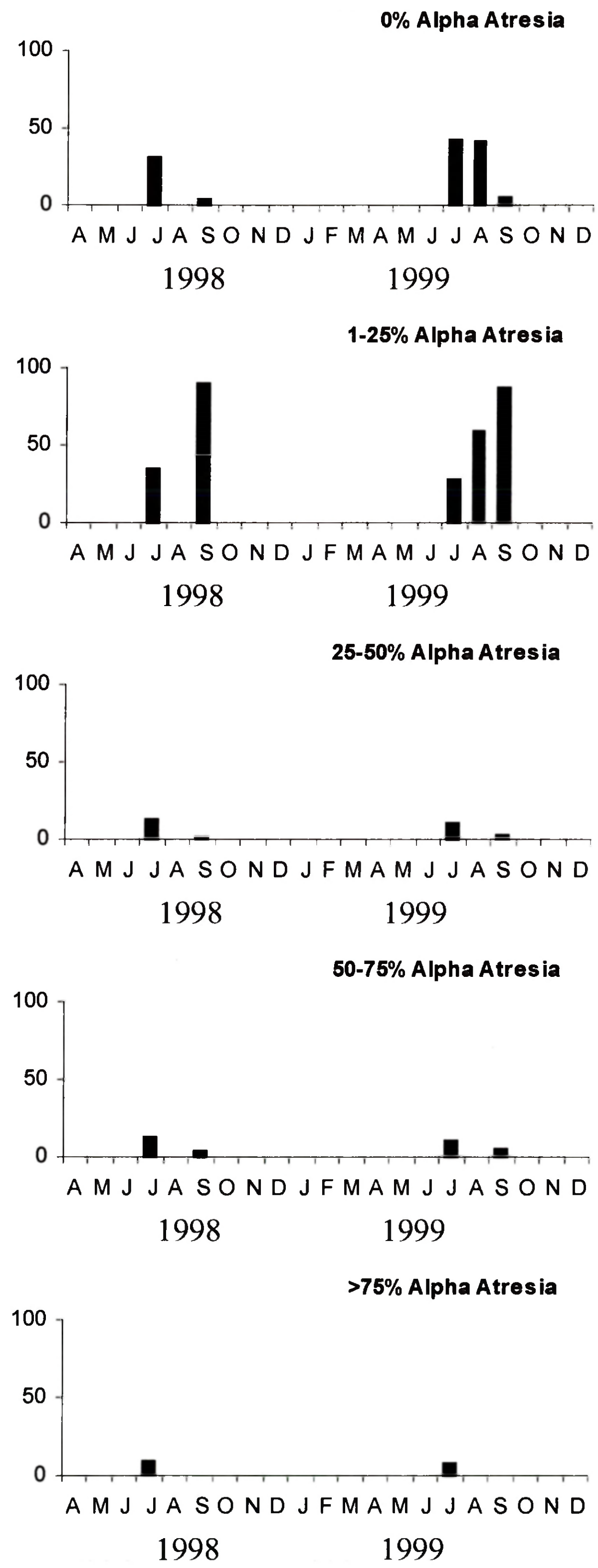

0% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
1-25% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
25-50% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
50-75% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
>75% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999

Fig.24. Frequency of occurrence of females in five alpha (α) atretic states by month in Offshore collections (White bars = 1998, Black bars = 1999).

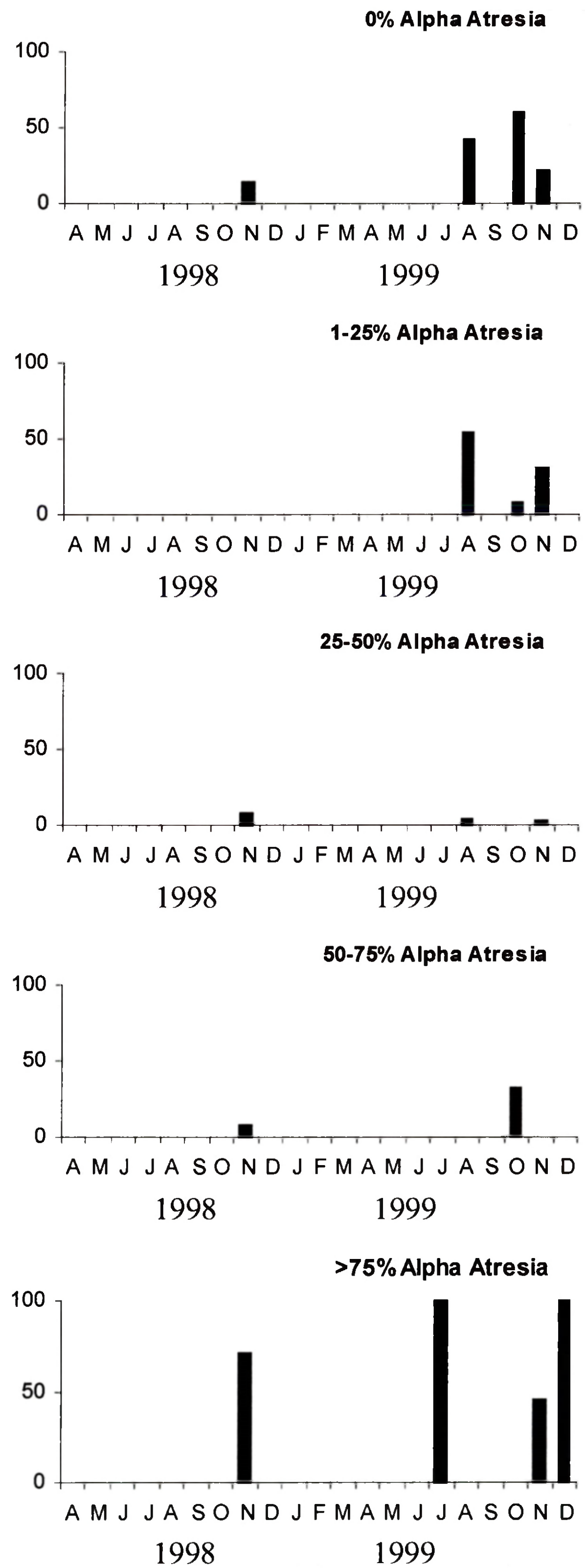
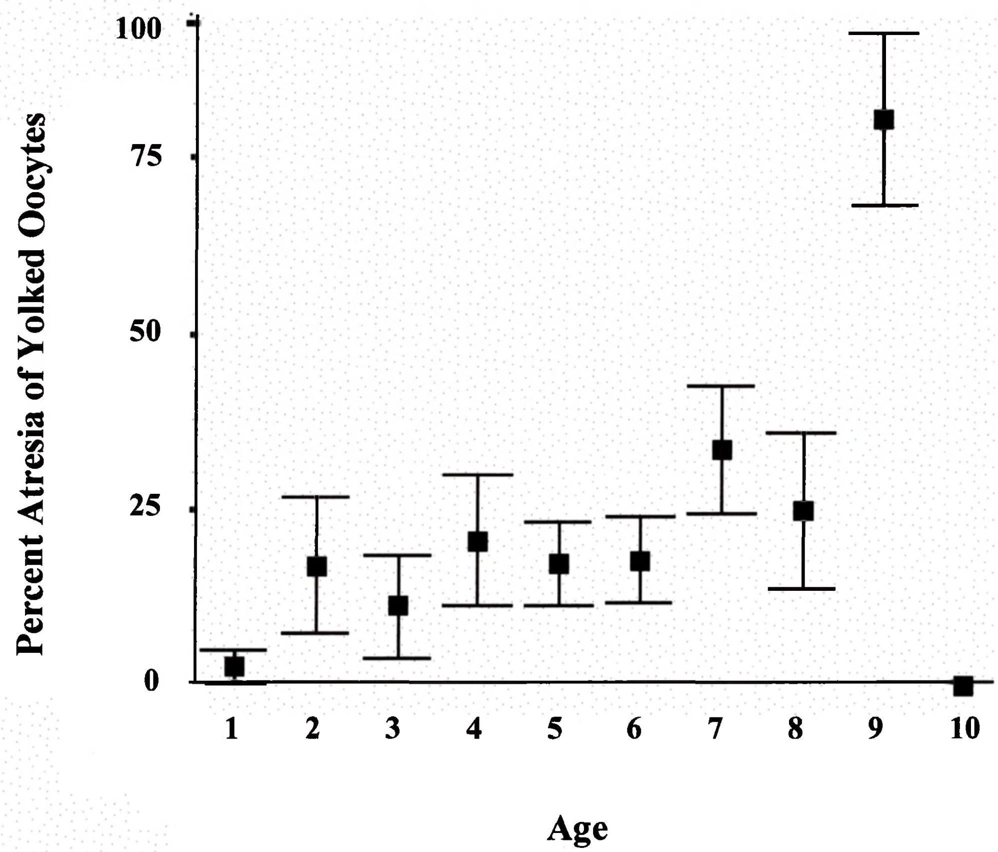

0% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
1-25% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
25-50% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
50-75% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999
>75% Alpha Atresia
100
50
0
A M J J A S O N D J F M A M J J A S O N D
1998
1999

Fig.25. Comparison of mean incidence of alpha atresia over spawning period between ages (Box = mean. Error line = 95% Confidence Interval).


Percent Atresia of Yolked Oocytes
100
75
50
25
0
1
2
3
4
5
6
7
8
9
10
Age

presence of the follicle, disintegration of yolk globules if present, rupture and degradation of the zona radiata, and nuclear disintegration (Figure 7). Oocytes showing β atresia are characterized by degradation of the granulosa and thecal layer of the follicle and often the presence of large vacuolar structures throughout the cytoplasm (Figure 18). Oocytes displaying γ and δ atresia appear as dense yellow-brown pigmented material (Figure 19), and they are difficult to distinguish from one another. Gamma atresia is generally rare (Hunter and Macewicz 1985b), so for this study the Gamma and Delta Stages were combined. The percent occurrence of α atresia can be used to described five atretic states (0-4), as described in Table 2.

Incidence of Atresia

Incidence of Atresia by Type

Atresia occurred in Atlantic croaker ovaries in the Chesapeake Bay throughout the year. Over 60-70% of all females showed some atresia during all months, and during April of 1998 and January, February and December of 1999 all females showed some a of atresia (Figure 20a). Beta atresia was present throughout the year, except during April and May of 1998. Beta atresia occurred in about 10% of the females in March through June, increased from July through September, and occurred in over 50 % of females in October through December (Figure 20c). Delta and Gamma atresia were also present throughout the year. The proportion of females showing this stage of atresia was high, 60 – 100%, from November to July, but decreased in August through October to around 50-60 % in 1998 and 40-55% in 1999 (Figure 20d). Alpha atretic oocytes were present in most females from July through December. Females generally showed no α atresia outside the spawning period. This is not surprising since I looked only at α atresia of yolked oocytes, which are only present in females approaching or in spawning stages, or in Spent Stage. Alpha atresia generally appeared in over 50 % of females in July, and increased steadily to approximately 100% of females in December (Figure 20b).

Incidence of α Atresia by State

Females showing no signs of α atresia were present throughout the spawning period. The percentage of females containing yolked oocytes showing no α atresia was approximately 30 - 50% in July (Figure 21a). By the end of the spawning period, atresia-free females accounted for only about 10 – 20% of the population in November and 0% in December. This indicates that more females had developed and completed spawning at this time, resulting in more females with α atretic oocytes.

Females showing up to 25 % α atresia (Atretic State 1) were also very common throughout the spawning period. Females in State 1 first appeared in July and increased to a peak in frequency in August and September, approximately 60 – 75% (Figure 21b). The frequency of these State 1 females decreased in October and November and was 0 by December of both years. Fewer than approximately 10% of females collected contained 25 – 50% α atresia (Atretic State 2) over the spawning period (Figure 21c). This indicates that large-scale atresia of yolked oocytes does not occur until females are no longer spawning or capable of spawning, and that they then rapidly become atretic simultaneously, bypassing mid-levels of atresia. Few females showed 50-75% α atresia (State 3) over the spawning period, with only 5 – 10% observed in July through November (Figure 21d). Therefore, large numbers of oocytes remain viable after successive spawns until the individual completes their final spawn. The frequency of females in this state increased to almost 25% in October of 1999, suggesting that those females may have completed their final spawn just prior to that time. Very high levels, >75 % (State 4) of α atresia are common only near the end of the spawning period and may be a useful predictor of spawning cessation. Females in State 4 began to appear in October of both 1998 and 1999, reaching peak frequencies in November - December of both years. Although Atretic State 4 occurs only in females unable to spawn again, it is not known how long α atretic oocytes remain within the Atlantic croaker ovary. Therefore, I cannot be certain of the exact time when these females completed their final spawn.

Incidence of Atresia by Location

Females from the both the Eastern and Western Shores of the Chesapeake Bay showed a similar pattern for Atretic States 0-3. Less than 50% of females collected showed no α atresia over most of the spawning period (Figures 22 & 23). Approximately 40-60% of females collected on the Western Shore were in Atretic

State 1, but less than 10% were in Atretic State 2 from July through October. Higher levels of Atretic State 1 (~75%) were found on the Eastern Shore during the same time. About 40% of females on the Western Shore showed greater than 75% α atresia in October of both years, increasing to 100% of females collected in November and December of 1998. Low numbers of Atretic State 4 females were observed in Eastern Shore collections, because collections were not available on the Eastern Shore in October or November, the months during which I would expect many females to conclude spawning.

Females from Offshore collections showing no α atresia were present throughout the spawning period (Figure 24). Females in Atretic State 1 were present in high numbers, over 50% of the collection, in August. Lower frequencies of Atretic State 1 were also observed in October (~10%) and November (~25%). Very few females were found in atretic states 2 or 3 throughout the spawning period. Nearly 100% of all females collected in November and December were in Atretic State 4, meaning that spawning ends for many females in the Offshore area at that time. Only one individual with yolked oocytes was observed in July. Therefore, the 100% occurrence of Atretic State 4 during the month of July is likely an artifact of small sample size.

In summary, the incidence of α atresia in Atlantic croaker does not vary significantly between the areas or between the spawning periods. I found no significance between years for the spawning period including the months of July through November ($p= 0.1146$) or between sites ($p = 0.5867$). I also found no significant difference between sites within years (1998; $p = 0.4653$ and 1999; $p = 0.3742$).

Incidence of Atresia by Age

Incidence of atresia varied with age. Younger females had significantly lower atresia during the spawning period and within peak spawning months than did older females ($F=2.58$, $df=9$, $p<0.01$). Mean atresia was very low, <1% of yolked oocytes, for age one females, which are fish spawning for the first time (Figure 25). Mean atresia was approximately 10% over the period for females age two through six, and over 25% for females age seven and over. Individual fecundities may vary with age, since a larger proportion of developing or yolked eggs in younger females appears to remain viable compared to those in older females, which may have spawned many times before. Low sample sizes probably affected the mean atresia for the oldest ages. Only one age ten female was collected during the spawning season in August, a time when spawning is beginning and atretic oocytes are uncommon. In contrast, 33 age nine females were caught during the spawning season. All but four were caught in October and November, when spawning is at an end and many females contain high levels of alpha atresia.

Kruskal-Wallis analysis of variance for atresia classification states showed no significant differences between locations ($p>0.10$), but did show significant variation over time ($p< 0.0001$), as expected. There was no significant difference in atretic classification states between ages early in the spawning period when most atretic females contained low levels of alpha atresia. However, there was a significant difference in atretic states between ages near the end of the spawning period ($p<0.0001$) when high levels of atresia first begin to appear, which further indicates that atresia may impact fecundity in young fish to a lesser degree than in older fish.

Occurrence of Remnant Chorion

Atlantic croaker ovaries often contained structures that probably arise from oocytes that become nonviable during their development and degrade within the ovary until only the large chorion and remnants of the zona radiata remain (Figure 26) (personal communication with Beverly Macewicz, National Marine Fisheries Service, Southwest fisheries Science Center, La Jolla, CA). Some of these remnant chorion remained in the follicle and I could not determine whether they arose from yolked or hydrated oocytes (Figure 26a). Others were much larger and occurred in the lumen, suggesting that they were remains of previously ovulated hydrated oocytes (Figure 26b). Ovulated remnant chorion probably arise when oocytes are ovulated into a part of the lumen next to the ovarian wall and become trapped between the wall and part of the connective tissue/ ovigerous folds. The presence of remnant chorion from hydrated oocytes could be interpreted as evidence of prior spawning, or prior attempt at spawning. They appear to persist for very long periods of time, however, and probably do not reliably indicate recent spawning. Remnant chorion often were present as large masses within the lumen of the ovary (Figure 26c). If these structures are left-over hydrated oocytes, they may impact the fecundity of spawning Atlantic croaker.

Remnant Chorion by Location

Remnant chorion were present nearly all months in the Chesapeake Bay, with the exception of August of 1998 and October and November of 1999. They were most frequent from December to March, occurring in approximately 30 to 40 percent of females collected at that time (Table 7). Remnant chorion were absent from the Western Shore in August of 1998 and 1999 and September through November of 1999 (Table 7). Thirty to 40% of females from the Western Shore contained Remnant chorion in December and March. The frequency of these structures on the Western Shore remained high, around 15% of females, in April and May. Females containing Remnant chorion were seen on the Eastern Shore for all available months. Remnant chorion were most common, in 10-20% of females collected, in September of 1998 and May and June of 1999. Females from Offshore collections showed a similar pattern. Between 10 to 20% of females collected contained Remnant chorion in November of both 1998 and 1999, and March, July and December of 1999. Remnant chorion may be similar to late stages of atresia, since they appear to last for very long periods of time. Because of this, they are probably not useful in describing spawning activity. The reason for these structures and their importance remain unknown.

Fig.26. Histological appearance of Remnant Chorion from hydrated oocytes (RC). (a) Remnant Chorion in follicle; (b) ovulated Remant Chorion; (c) ovulated Remnant Chorion mass (RCM). OL – Ovary Lumen.

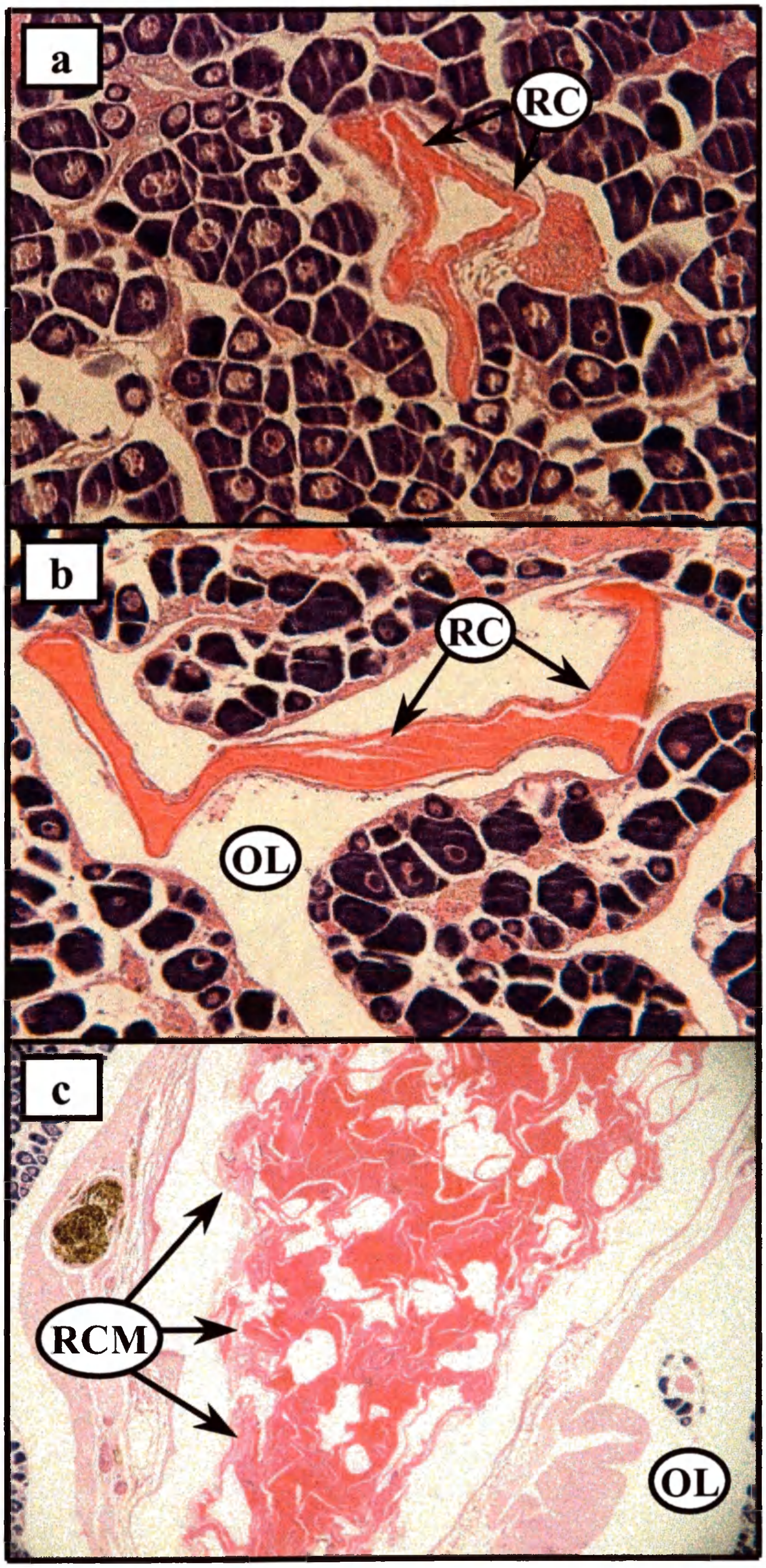

a
RC
b
RC
OL
c
RCM
OL

Table 7. Occurrence of Remnant Chorion in females collected in the Chesapeake Bay. Number of females containing Remnant Chorion by area; Western Shore, Eastern Shore, and Offshore (with percentage of total collection in parenthesis).

Month	Females with Remnant Chorion (%)	R. Chorion Western Shore (%)	R. Chorion Eastern Shore (%)	R. Chorion Offshore (%)
1998 April	2 (16.67%)	2 (16.67%)		
May	3 (9.38%)	3 (9.38%)		
June	5 (6.41%)	5 (6.41%)		
July	6 (3.90%)	5 (7.46%)	1 (1.15%)	
August	0	0		
Sept	7 (8.54%)	1 (3.57%)	6 (11.11%)	
October	8 (14.81%)	8 (14.81%)		
November	9 (8.91%)	3 (6.67%)		6 (10.71%)
December	9 (42.86%)	9 (42.86%)		
1999 February	1 (33.33%)	1 (33.33%)		
March	35 (27.78%)	23 (32.39%)		12 (21.82%)
April	12 (14.29%)	12 (14.29%)		
May	7 (17.5%)	4 (16.67%)	3 (18.75%)	
June	17 (24.64%)	15 (30%)	2 (10.53%)	
July	7 (3.95%)	2 (3.77%)	1 (0.93%)	3 (17.65%)
August	3 (2.83%)	0	2 (4.26%)	1 (2.63%)
Sept	4 (4%)	0	4 (9.30%)	
October	0	0		0
November	16 (14.41%)	0		16 (14.95%)
	0			11 (16.67%)

DISCUSSION

Gonad Staging

Because Atlantic croaker are multiple spawners whose oocytes develop continuously, macroscopic staging can be difficult. The changes in the ovary at the cellular level are not apparent at the whole ovary level, and this can lead to error when using macroscopic staging methods alone (Cooksey 1996). I found a low overall agreement between macroscopic and microscopic staging methods for Atlantic croaker. Therefore I suggested using modified general staging criteria similar to that recommended for Spanish mackerel, also a multiple spawning species, by Cooksey (1996). Identifying ovaries by general spawning state rather than specific ovarian maturation stage allows researchers to achieve a quick and accurate assessment of ovarian condition without microscopic examination.

Spawning Location

Some Atlantic croaker spawning does occur within the Chesapeake Bay itself. However, most spawning seems to occur elsewhere, probably along the western Atlantic coast, south of Virginia Beach. These findings agree with Barbieri et al. (1994) who found few females with hydrated oocytes as well as Spent Stage females in the lower Chesapeake Bay, lower York and James Rivers and coastal waters off Virginia and North Carolina. The lack of Gravid or Running Ripe females may reflect the transient nature of these ovarian stages. The presence of POFs indicate that some Atlantic croaker spawning probably occurs within the Bay. These findings disagree with previous studies, which conclude that Atlantic croaker are probably strict marine spawners and use the estuary only as a nursery (Pearson 1929; Hildebrand and Cable 1930; Wallace 1940; Haven 1957; Warlen 1982; and Lewis and Judy 1983.) Other sciaenids once believed to be strict marine spawners also have been reported to occasionally spawn in estuaries. For example, whitemouth, *Micropogonias furnieri,* normally spawn offshore of southern Brazil but also may spawn in Lagoa dos Patos when there is strong salt-water intrusion (Castello 1985). It is likely that many factors, such as food and habitat availability, overall health of the stock, and freshwater flow or bay stratification, affect the degree to which individuals spawn in different areas.

It has been thought that there is not significant spawning of Atlantic croaker in the Chesapeake Bay, since no larvae under 10 mm had been found there before 1957 (Haven 1957). Olney (1983), for example, did not find any larvae in the Bay in surface and oblique plankton tows in 1983, but 1.5 to 15 mm larvae and postlarvae have been caught in subsurface and bottom tows at the mouth of the Bay (Pearson 1941; Norcross 1991). In addition, young have been found in the James, Rappahanock, Pamunkey and Mattaponi rivers (Massman 1954; Raney and Massman 1953). Reiss and McConaugha (1999) found that Atlantic croaker larvae increased in length with distance offshore, suggesting that spawning may occur closer to the Bay than previously thought, and they related Atlantic croaker transport strategy to that of the blue crab, *Callinectes sapidus*. These findings imply that Atlantic croaker spawned within the Chesapeake Bay may be able to utilize seaward surface currents as buoyant eggs and deeper up-Bay currents as demersal larvae to move into nursery areas in Bay tributaries.

Spawning Period

Atlantic croaker spawn in the Chesapeake Bay over a protracted period, from July through November or December, with little difference in timing between the Western and Eastern shores of the Bay or the coastal area just south of the mouth of the Bay. The spawning period for Atlantic croaker along the coast and south of the Bay is similar (Wallace 1940; Colton et al. 1979; Warlen 1982; Welsh and Breder 1923; and Hildebrand and Schroeder 1928). Although some spawning occurs throughout the entire period, most spawning occurs in a shorter period, from August through October. Again, this agrees with previous work on spawning outside of the Bay (Haven 1957).

Incidence of Atresia

Although α atresia was present throughout the spawning period, I did not find that alpha atresia of Atlantic croaker oocytes persisted in unusually high quantities until the end of the spawning period. The peak in levels of α atresia occurs in October, which is the start of the decline in spawning activity. This disagrees with the Barbieri et al. (1994) study, which described females as showing an unusually high incidence of atresia throughout the spawning period.

The presence of alpha atresia is apparently an accurate marker of the beginning of the spawning period and a rough indicator that the spawning period is near its close, which agrees with other studies (Hunter and Macewicz 1985a, 1985b; Hunter et al. 1985; Agarwal and Singh 1990; Vizziano et al. 1991; and Guraya 1986, 1993, 1994). However, the presence of alpha atresia alone does not accurately mark the close of the spawning period, only the end of spawning for the individual in which α atresia is observed. I did not find highly atretic ovaries (greater than 50% atresia) during the entire spawning period; they appeared one month after the peak in spawning occurred in September.

Atresia also can be useful as an indicator of environmental stress since it has been found to arise due to a number of stresses such as starvation (Perez and Figueiredo 1992; Ma et al. 1998; and Kurita et al. 2000), extremes in temperature (Jafri 1989, and Saxena and Sandhu 1994), and toxins (Das 1988, and Sukumar and Karpagaganapathy 1992). Incidence of α atresia of yolked oocytes was similar in all locations sampled, implying that local environmental stresses probably do not control atresia in the Chesapeake Bay. Large-scale seasonal cues, such as declining temperature and photoperiod, and change in summer winds, cause the cessation of spawning in Atlantic croaker (Norcross et al. 1988; Norcross 1991), and these factors did not vary enough between the three sampling regions to affect atresia levels significantly.

Finally, the observation of lower levels of atresia in younger fish than older fish differs from previous work on the Northern Anchovy, *Engraulis mordax* (Hunter and Macewicz 1985b.) That study found higher levels of atresia in smaller or younger fish regardless of month, and attributed the difference to a shorter or more infrequent spawning period for younger fish. In contrast to *Engraulis*, the spawning period appears to be similar between ages in Atlantic croaker, although the percentage of developing oocytes that remain viable may vary with age or size.

CONCLUSIONS

I examined the occurrence of spawning within the Chesapeake Bay in order to determine whether Atlantic croaker use the Bay, in particular the Eastern Shore, as a significant spawning ground. Despite the finding that some Atlantic croaker spawning likely occurs within the Chesapeake Bay, it appears that most spawning occurs elsewhere and that Atlantic croaker probably do not use the Eastern Shore as a significant spawning ground. In addition, it is probable that the frequency of spawning in Bay areas varies greatly interannually due to variations in environmental conditions such as temperature and salinity stratification. Further work is necessary to determine whether the Bay is a significant spawning ground for Atlantic croaker and whether recruitment of the population is affected by this local spawning.

I also determined the spawning period along both the Eastern and Western Shores of the Chesapeake Bay as well as Offshore, near the Virginia Beach, VA area. I found that the spawning period is similar for each of these regions beginning around July and ending around November, with peak spawning occurring between September and October. The timing of spawning is also affected by variability in the environment, especially summer temperatures and wind changes during the spawning season.

I investigated the previously observed high levels of α atresia in Atlantic croaker in order to answer the question of whether there exists some environmental stress on the population. I found that high proportions of females showing over 50% alpha atresia only appear near the end of the spawning period and may be useful as predictors of this period. To predict the end of the spawning period more accurately, the average age or duration of atretic oocytes must be estimated by inducing spawning of Atlantic croaker in the laboratory. Determining levels of atresia can be an important tool in studying reproductive biology of multiple spawners such as Atlantic croaker.

Finally, Atlantic croaker fecundity remains unknown. To estimate fecundity, large numbers of Gravid Stage females must be observed, and these stages are difficult to obtain. The accurate determination of fecundity may require an even more intensive sampling of females targeted during peak spawning times as well as historically documented offshore spawning grounds. Since the sample size for this study exceeded previous studies, a laboratory spawning study may be necessary to determine batch fecundity. Spawning frequency estimates require extensive sampling within a spawning period as well as improved knowledge of POF aging. In addition, fecundity differences between different age or size fish may be compounded by different levels of atresia inherent to age and size-classes (Walsh et al. 1990; Vizziano et al. 1991; Ntiba and Jaccarini 1992; Walker et al. 1994; Barker et al 1998; Marshall et al. 1999; Kurita et al. 2000; and Macchi et al. 2000). These cohort-specific factors should be taken into consideration in future estimates of Atlantic croaker fecundity.

LITERATURE CITED

Agarwal, N. K., and H. R. Singh. 1990. Pre-ovulatory follicular atresia (corpora atretica) in snowtrout *Schizothorax plagiostomus* (Heckel). J. Anim. Morphol. Physiol. 37:29-33.

Barbieri, L. R. 1993. Life history, population dynamics and yield-per-recruit modeling of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay area. Ph.D. Diss., College of William and Mary, School of Marine Science, Gloucester Point, VA. 140 p.

Barbieri, L.R., M.E. Chittenden, Jr., and S. K. Lowerre-Barbieri. 1994. Maturity, spawning, and ovarian cycle of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters. Fish. Bull. 92:671-685.

Barker, L. A., B. J. Morrison, B. J. Wicks, and F. W. H. Beamish. 1998. Potential fecundity of landlocked sea lamprey larvae, *Petromyzon marinus*, with typical and atypical gonads. Copeia 4:1070-1075.

Bearden, C. M. 1964. Distribution and abundance of Atlantic croaker, *Micropogon undulatus*, in South Carolina. Contrib. Bears Bluff Lab., Rept. No. 40:23 p.

Bretschneider, L. H., and J. J. Duyvene de Wit. 1947. Sexual endocrinology of non-mammalian vertebrates. Monogr. Prog. Res., Vol. II, Elsevier, N. Y.

Brown-Peterson, N., P. Thomas, and C. R. Arnold. 1988. Reproductive biology of the spotted seatrout, *Cynoscion regalis*, in South Texas. Fish. Bull. 86:373-388.

Campbell, A. 1985. Application of a yield and eggs-per-recruit model to the lobster fishery in the Bay of Fundy. North Amer. J. Fish. Mgmt. 5:91-104.

Castello, J. P. 1985. The ecology of consumers from dos Patos Lagoon estuary, Brazil. In A. Yáñez-Arancibia (ed.), Fish community ecology in estuaries and coastal lagoons: towards an ecosystem integration, p. 383-406. DR (R) UNAM Press, Mexico.

Chao, L. N., and J. A. Musick. 1977. Life history, feeding habits, and functional morphology of juvenile scaienid fishes in the York River estuary, Virginia. Fish. Bull., U. S. 75:657-702.

Chittenden, M. E., Jr., and J. D. McEachran. 1976. Composition, ecology, and dynamics of demersal fish communities on the northwestern Gulf of Mexico continental shelf, with a similar synopsis for the entire Gulf. Texas A&M Univ., TAMU-SG-76-208, 104 p.

Colton, Jr., J. B., W. G. Smith, A. W. Kendall, Jr., P. L. Berrien and M. P. Fahay. 1979. Principal spawning areas and times of marine fishes, Cape Sable to Cape Hatteras. Fish. Bull., 76:911-915.

Cooksey, C. L. 1996. Reproductive biology of Spanish mackerel, Scomberomorus maculatus, in the lower Chesapeake Bay. M.S. Thesis. College of William and Mary, School of Marine Science, Gloucester Point, VA. 68 p.

Das, R. C. 1988. Cadmium toxicity to gonads in a freshwater fish, *Labeo bata* (Hamilton). Arch. Hydrobiol. 112: 467-474.

DeVlaming, V. G. Grossman, and F. Chapman. 1982. On the use of the Gonadosomatic index. Comp. Biochem. Physiol. 73A:31-39.

Fahay, M. P. 1975. An annotated list of larval and juvenile fishes captured with surface-towed meter net in the South Atlantic Bight during four RV DOLPHIN cruises between May 1967 and February 1968. NOAA Tech. Rept. NMFS SSRF-685, 39 p.

Guraya, S. S. 1986. The cell and molecular biology of fish oogenesis. Monogr. Dev. Biol. 18: 223 p.

Guraya, S. S. 1993. Follicular (or oocyte) atresia and its causes and functional significance in the fish ovary. *In*. B. r. Singh (ed.) Advances in Fish Research, Vol. 1. Narenda Publishing House. India. pg. 313-332.

Guraya, S. S. 1994. Gonadal development and production of gametes in fish. Proc. Indian Natl. Sci. Acad. 60: 15-32.

Harry, G. Y. Jr. 1959. Time of spawning, length of maturity, and fecundity of the English, petrale, and Dover soles (*Parophrys vetulus*, *Eopsetta jordani*, and *Microstomus pacificus*, respectively). Oreg. Fish Comm. Res. Briefs 7:5-13.

Haven, D. S. 1957. Distribution, growth and availability of juvenile croaker, *Micropogon undulatus*. Ecology 38: 88-97.

Haven, D. S. 1959. Migration of croaker, *Micropogon undulatus*. Copeia 1:25-30.

Hildebrand, S. F. and L. E. Cable. 1930. Development and life history of fourteen teleostean fishes at Beaufort, North Carolina. Bull. U. S. Bur. Fish. 46:383-488.

Hildebrand, S. P. and W. C. Schroeder. 1928. Fishes of the Chesapeake Bay. Fish. Bull. 43:1-366.

Hunter, J. R. and S. R. Goldberg. 1980. Spawning incidence and batch fecundity in the northern anchovy, *Engraulis mordax*. Fish. Bull. 77:641-652.

Hunter, J. R. and B. J. Macewicz. 1985a. Measurement of spawning frequency in multiple spawning fishes. *In*: R. Lasker (ed.), an egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*. pg. 79-94. U. S. Dep. Comm., NOAA Tech. Rep. NMFS 36.

Hunter, J. R. and B. J. Macewicz. 1985b. Rates of atresia in the ovary of captive and wild Northern anchovy, *Engraulis mordax*. Fish. Bull. 83:119-136.

Hunter, J.R., N. C. H. Lo and R. J. H. Leong. 1985. Batch fecundity in multiple spawning fishes. *In*: R. Lasker (ed.), an egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*. pg. 67-77. U. S. Dep. Comm., NOAA Tech. Rep. NMFS 36.

Hunter, J. R., B. J. Macewicz, N. C. H. Lo and C. A. Kimbrell. 1992. Fecundity, spawning and maturity of female Dover sole, *Microstomus pacificus*, with an evaluation of assumptions and precision. Fish. Bull. 90:101-128.

Jafri, S. I. H. 1989. The effects of photoperiod and temperature manipulation on reproduction in the roach, *Rutilus rutilus* (L.) (Teleostei). Pak. J. Zool. 21: 289-299.

Johnson, G. D. 1978. Micropogonias undulatus (Linnaeus), Atlantic croaker, pp. 2270233. In: Development of fishes of the Mid-Atlantic Bight: An atlas of egg, larval and juvenile stages. Vol IV. Carangidae through Ephippidae. FWS/OBS-78/12 Fish & Wildl. Serv.

Joseph, E. B. 1972. The status of the sciaenid stocks of the middle Atlantic coast. Chesapeake Sci. 13:87-100.

Kurita, Y., A. Thorsen, M. Fonn, A. Svardal, and O. S. Kjesbu. 2000. Oocyte growth and fecundity regulation of Atlantic herring (*Clupea harengus*) in relation to declining body reserves during overwintering. 6. Int. Symp. on the Reproductive Physiology of Fish. 4-9 Jul 1999: Bergen.

Lambert, J.G. D. 1970. The ovary of the guppy, Peocilia reticulata. The atretic follicle, a Corpus atreticum, or a Corpus luteum praeovulationis. Z. Zellforsch 107:54-67.

Lewis, R. M. and M. H. Judy. 1983. The occurrence of spot, *Leiostomus xanthurus*, and Atlantic croaker, *Micropogonias undulatus*, larvae in Onslow Bay and Newport River estuary, North Carolina. Fish. Bull. 81: 405-412.

Ma, Y., O. S. Kjesbu, and T. Jergensen. 1998. Effects of ration on maturation and fecundity in captive Atlantic herring (*Clupea harengus*). Can J. Fish Aquat. Sci. 55:900-908.

Macchi, G. J., E. M. Acha, C. A. Lasta. 2000. Spawning frequency and batch fecundity of the whitemouth croaker (*Micropogonias furnieri*) of the Rio de la Plata Estuary, Argentina-Uruguay. 6. Int. Symp. on the Reproductive Physiology of Fish. 4-9 Jul 1999: Bergen.

Marshall, C. T., O. S. Kjesbu, A. Thorsen, and P. Solemdal. 1999. Spawner quality effects on two measures of reproductive potential: Have we been counting our eggs before they've hatched? Counc. Meet. Int. Counc. Expl. Sea. Stockholm. 1999.

Massman, W. H. 1954. Marine fishes in fresh and brackish waters of Virginia rivers. Ecology 35: 75-78.

Middaugh, D. P. and R. L. Yoakum. 1974. The use of chorionic gonadotropin to induce laboratory spawning of the Atlantic croaker, *Micropogonias undulatus*, with notes on subsequent embryonic development. Ches. Sci. 15: 110-114.

Morse, W. W. 1980. Maturity, spawning and fecundity of Atlantic croaker, *Micropogonias undulatus*, occurring north of Cape Hatteras, North Carolina. Fish. Bull. 78:190-195.

Norcross, B. L. 1983. Climate scale environmental factors affecting year-class fluctuations of Atlantic croaker (*Micropogonias undulatus*) in the Chesapeake

Bay, Virginia. Ph.D. Thesis, School of Marine Science, College of William and Mary, School of Marine Science, Gloucester Point, VA 388 p.

Norcross, B. L. 1991. Estuarine recruitment mechanisms of larval Atlantic croakers. Trans. Am. Fish. Soc. 120: 673-683.

Norcross, B. L., and H.M. Austin. 1988. Middle Atlantic Bight meridional wind component effect on bottom water temperatures and spawning distribution of Atlantic croaker. Cont. Shelf Res. 8:69-88.

Ntiba, M. J., and V. Jaccarini. 1992. The effect of oocytic atresia on fecundity estimates of the rabbit fish *Siganus sutor* (Pisces: Siganidae) of the Kenyan marine inshore waters. Hydrobiologia, 247:1-3.

Olney, J. E. 1983. Eggs and larvae, *Anchoa mitchilli*, and the weakfish, *Cynoscion regalis*, in lower Chesapeake Bay with notes on associated ichthyoplankton. Estuaries 6:20-35.

Pearson, J. C. 1929. Natural history and conservation of the redfish and other commercial sciaenids on the Texas coast. Bull. Bur. Fish. 44(1046):129-214.

Pearson, J.C. 1932. Winter trawl fishery off the Virginia and North Carolina coasts. U.S. Bur. Fish., Invest. Rep. No. 10, Washington, 31 p.

Pearson, J. C. 1941. The young of some marine fishes taken in lower Chesapeake Bay, with special reference to the grey seatrout *Cynoscion regalis* (Bloch). Bull. U. S. Fish. Wildl. Serv. 50:79-102.

Perez, N., and I. Firgueiredo. 1992. First approach to the study of atresia in the ovary of sardine, *Sardina pilchardus* (Walb.). Biol. Inst. Esp. Oceanogr. 8:191-199.

Prager, M. H., J. F. O'Brien, and S. B. Saila. 1987. Using lifetime fecundity to compare management strategies: a case history for striped bass. North Amer. J. Fish. Mgmt. 7:403-409.

Raney, E. C. and W. H. Massman. 1953. The fishes of the tidewater section of the Pamunkey River, Virginia. J. Wash. Acad. Sci. 43:424-432.

Reiss, C. S. and J. R. McConaugha. 1999. Cross-frontal transport and distribution of ichthyoplankton associated with Chesapeake Bay plume dynamics. Cont Shelf Res. 19:151-170.

Saxena, P. K., and R. Sandhu. 1994. Influence of temperature on ovarian recrudescence in the Indian catfish, *Heteropneustes fossilis*. J. Fish Biol. 44:168-171.

Sucumar, A., and P. R. Karpagaganapathy. 1992. Pesticide-induced atresia in ovary of a freshwater fish, *Colisa lalia* (Hamilton-Buchanan). Bull. Environ. Contam. Toxicol. 48(3):457-462.

Vizziano, D., M. A. Pravia, E. Lopez de Leon, and C. Laninno. 1991. Estimation of the percentage of atresia in the different ovarian stages of the king weakfish (*Macrodon ancylodon*). Frente Marit. 8:69-71.

Walker, M. G., P. R. Witthames, and I. Bautista de los Santos. 1994. Is the fecundity of the Atlantic mackerel (*Scomber scombrus*: Scombridae) determinate? Sarsia. 79: 13-26.

Wallace, D. H. 1940. Sexual development of the croaker, *Micropogon undulatus*, and distribution of the early stages in Chesapeake Bay. Trans. Am. Fish. Soc. 70:475-482.

Wallace, R. A. and K. Selman. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. Amer. Zool. 21:323-343.

Walsh, M., P. Hopkins, P.Whittames, M. Greer-Walker, and J. Watson. 1990. Estimation of total potential fecundity and atresia in the western mackerel stock. Counc. Meet. Int. Counc. Expl. Sea. Copenhagen. 1990.

Warlen, S. M. 1982. Age and growth of larvae and spawning time of Atlantic croaker in North Carolina. Proc. Annu. Conf., S. E. Assoc. Fish Wildl. Agencies 34:204-214.

Weinstein, M. P. 1979. Shallow marsh habitats as primary nurseries for fishes and shellfish. Cape Fear River, North Carolina. Fish. Bull. 77: 339-357.

Welsh, W. W., and C. M. Breder. 1923. Contributions to life histories of Sciaenidae of the eastern United States coast. Bull. U. S. Bur. Fish. 39:141-201.

West, G. 1990. Methods of assessing ovarian development in fishes: A review. Aust. J. Mar. Freshwater Res. 41:199-222.

Yamamoto, K., and H. Yoshioka. 1964. Rhythm of development in the oocyte of the medaka, Oryzias latipes. Bull. Fac. Fish., Hokkaido Univ. 15:5-19.

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