Ovarian morphology, oogenesis, and changes through the annual reproductive cycle of the female blue crab, Callinectis sapidus Rathbun, in Tampa Bay

Catalina E. Brown

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Ovarian Morphology, Oogenesis, and Changes through the Annual Reproductive Cycle of the Female Blue Crab, *Callinectes sapidus* Rathbun, in Tampa Bay

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
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Keywords: Germinal zone, Primary Growth, Secondary Growth, Atresia, Follicle Cells, Ovarian Lobe

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DEDICATION

To Alex

For your strength, dedication, support and love. For all the times you knew what to say, and everything you say is just right. Mostly for all the smiles and laughter.

To Ariana

For your compassion, understanding, sweetness, and caring, loving words. You are a light of joy and a source of love and happiness always.

To Erika

For your sense of humor and continual running commentary and curiosity on everything. I hope you learned something.

I love you all, and I am so glad that we made it through this. We move on forward to the next adventure.
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I thank the FWC–FWRI Crustacean Fisheries group, especially Charles Crawford and Angie Machniak, for their assistance in collecting samples and Joe O’Hop for helpful statistical advice. I extend a special thanks to Noretta Perry and Yvonne Waters for all their histology knowledge.
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ABSTRACT

The blue crab, Callinectes sapidus Rathbun, 1896, was studied because of its high dollar value to Florida’s commercial and recreational fisheries. The purpose of this study was to describe the structure of the ovary and oogenesis in the blue crab and the morphological changes in the female reproductive developmental stages over time. Histological techniques for high-resolution light microscopy were used to determine sexual maturity of female blue crabs. The ovarian morphology, oogenesis, and changes through the annual reproductive cycle of blue crabs in Tampa Bay were investigated for a period of two years, from January 2005 to January 2007. Ovarian structure was assessed by analyzing histological sections embedded in plastic epoxy resin, which provided a higher resolution than any other embedding material previously used in research on blue crab reproduction. Qualitative analyses of female gonads were made by describing the structure of the oocytes and determining the developmental stage of the oocytes from oogonia to full-grown oocytes. This study developed and introduced a new reproductive staging criteria for the species. Morphological characteristics of ovarian tissues and oocytes were determined to develop a classification for oocyte maturation stages. Morphological changes in the oocytes are well defined, and these were used to develop the staging schema.

In this study, it was found that carapace width is not a good indicator of maturity or developmental stage. Examination of the annual reproductive cycle indicates that late secondary growth occurs from July to March, and gravid crabs were found during November and December. Histological examination of ovarian tissue is essential for determining maturity in female blue crabs. By observing ovarian characteristics and by establishing the length of secondary growth during oogenesis in blue crabs of Tampa Bay, a more thorough understanding of the cyclic reproductive aspects of this species was obtained and specifically that animals at a carapace width between 100 mm and 125 mm may have mature oocytes, yet external features may not indicate that they are mature.
INTRODUCTION

The study of the morphology and physiology of the reproductive systems is essential to define the reproductive cycles of an animal species. It is established that reproduction is adapted to environmental conditions, in particular, temperature, photoperiod, food supply, and predators. Consequently, reproduction is cyclical and based on the season of the year in which conditions are adequate for the survival of the offspring. The foundation of reproduction for any species originates from the development of germinal cells during the process of gametogenesis, which is oogenesis in the females and spermatogenesis in the males. Therefore, the development of oocytes in the females and of spermatozoa in the males is a fundamental function of reproduction. Oogenesis is a complex process of cellular and molecular changes that occur during the formation, growth, and maturation of the female germinal cells. The development of the oocyte is remarkable, and the study of this process is essential to the understanding of reproduction.

The determination of the annual activity of the ovaries of blue crabs, *Callinectes sapidus* Rathbun (Portunidae), and the characterization of the developmental stages of the oocyte, are fundamental for an efficient management of the blue crab fishery. Blue crab fisheries are significant to the state of Florida and are an integral part of the state’s economic and cultural livelihood. In 2007, commercial landings of blue crabs in Florida were estimated to be over 10,300,000 pounds (Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute [FWC–FWRI] Fisheries-Dependent Monitoring [FDM] data, 2006–2008). Even though recreational fisheries of blue crabs are not monitored, blue crabs also support a large recreational fishery in the state. Statewide, Florida commercial blue crab landings in 2006 were 11,919,742 pounds, having a total value of $10,936,905. In 2007, commercial landings were 10,371,523 pounds, having an estimated value of $10,302,312. Unofficial landings data for 2008 show the total pounds of blue crabs in the Tampa Bay area to be about six million pounds (Table 1; FWC–FWRI, FDM data, 2006–2008).

Blue crabs occupy a large geographical distribution and a diversity of habitats, ranging along the eastern seaboard of North America, throughout the Gulf of Mexico, into the Caribbean, and down to northern Argentina (Steele and Bert, 1994)

The blue crab life history entails a comprehensive cycle of planktonic, nektonic, and benthic stages that occur throughout the estuarine and nearshore marine environments, depending upon the specific physiological requirements of each life-history stage. *Callinectes sapidus* are classified as true crabs (Table 2).
The blue crab produces large numbers of young, grows rapidly, and attains early sexual maturity. Offsetting their fecundity are high mortality rates and a short life span (Steele, 1982).

Three types of migration have been described: alimental, climatic, and gametic. Gametic migration involves reproduction and spawning (Steele, 1982). Blue crabs have a divided life cycle defined by migration, in which the female blue crab are catadromous and migrate from hyposaline waters (<35) to higher salinity waters to spawn and hatch their eggs. The high salinity of oceanic waters not only serves as habitat for the spawning female but also aids in the development of the larvae, improves dispersal capabilities, reduces osmoregulatory stress, and decreases predation (Steele, 1982).

The distribution of the species varies depending mostly on season, sex, and age. Adult blue crabs inhabit a range of bottom types including fresh, estuarine, and shallow oceanic waters. Large blue crabs are prevalent in larger bays and bayous (Oesterling, 1976). Differences in salinity, temperature, and habitat are among the factors that affect blue crab distribution and abundance (Steele and Bert, 1994). Although adult blue crabs are ubiquitous throughout an estuarine system, they are distributed seasonally with

---

Table 1. Blue crab landings in Florida for the years 2006 to 2008, with only preliminary data for 2008. (FWC–FWRI, 2008)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total of blue crabs (hard, soft)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pounds</td>
</tr>
<tr>
<td>2006</td>
<td>11,919,742</td>
</tr>
<tr>
<td>2007</td>
<td>10,371,523</td>
</tr>
<tr>
<td>2008</td>
<td>5,923,996</td>
</tr>
</tbody>
</table>

Table 2. Blue crab taxonomy. (Zinski, 2006)

<table>
<thead>
<tr>
<th>SUPERKINGDOM: EUKARYOTA</th>
<th>CLASS: MALACOSTRACA</th>
<th>SUPERFAMILY: PORTUNOIDEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom: Animalia</td>
<td>Subclass: Eumalacostraca</td>
<td>Family: Portunidae</td>
</tr>
<tr>
<td>Subkingdom: Metazoa</td>
<td>Superorder: Eucarida</td>
<td>Subfamily: Portunoidea</td>
</tr>
<tr>
<td>Phylum: Arthropoda</td>
<td>Order: Decapoda</td>
<td>Genus: Callinectes</td>
</tr>
<tr>
<td>Subphylum: Crustacea</td>
<td>Infraorder: Brachyura</td>
<td>Species: sapidus</td>
</tr>
</tbody>
</table>
respect to salinity and sex. In general, males are more prevalent in low-salinity areas, whereas females predominate in high-salinity areas (Oesterling, 1976).

Some studies have shown that blue crab populations are cyclic, and their abundance can fluctuate dramatically from year to year. These inter-annual fluctuations in abundance are thought to be due to physical, chemical, and biological factors that strongly influence the characteristics of the populations (Steele and Bert, 1994).

Blue crabs are heterosexual and exhibit distinct sexual dimorphism. Female blue crabs look distinctly different from male blue crabs. Macroscopic examination of the animals shows that females have red pinchers, and males have blue pinchers. Male and females are also easily distinguishable by the shape of their aprons. The male blue crab has the same shape apron throughout its life. The female’s apron changes from a narrow shape to a broader, wider apron as she become sexually mature (Fig. 1). Hermaphroditic blue crabs have been observed in Chesapeake Bay, Virginia (Johnson, 1980).

Figure 1. Mature and immature female aprons. Mature females have a broader apron than immature females.

Females attain sexual maturity in 12 to 14 months, reaching adult size at approximately 130–139 mm or 5.25–5.50 in. after 18–20 molts. When they molt at maturity, growth ends; consequently, it is known as the “terminal molt” (FWC–FWRI, 2006). Females mate only once, but males may mate numerous times. Mating takes place in brackish water after the female’s terminal molt. Unlike most marine organisms, blue crab mating and spawning (shedding of eggs) occur at different times (Steele, 1982).

The sperm is stored in the female’s body for up to a year and so will be accessible for successive spawnings, even though mating for the female is a one-time occurrence. She may spawn one to nine months after mating, depending on water temperatures. After mating, females migrate to high-salinity waters in lower estuaries, sounds, and nearshore spawning areas (FWC–FWRI, 2006).

In brachyurans, the spermatophores are deposited directly into the seminal receptacle of the female. After entering her pubertal molt, the female blue crab is carried by the male. Directly following the molting of the cuticula, known as ecdysis, copulation occurs. Unlike blue crabs, females of other brachyurans undergo more than one molt and thus successive mating (Johnson, 1980).
Following the pubertal-molt mating, the female enters a prolonged period without ecdysis known as anecdysis and normally will not molt or mate again. Sperm are stored in the seminal receptacles and used during both ovulations that normally occur in this species (Steele and Bert, 1994). Eggs are laid and held together by adhesion to the setae of the endopodites of the abdominal segments and the maturing egg mass (Johnson, 1980).

A precise analysis of the microscopic structure of the ovaries provides a better comprehension of the development of the female germ cell, the oocyte during the process of oogenesis, and its cyclical changes that define the reproduction of this species. Oogenesis is the sequence of stages that oocytes undergo, from the oogonium to oocyte maturation. To properly describe the process of oogenesis, several essential aspects need to be studied: the examination of the germinal zone of the ovary, in order to distinguish where the oogonia are located; and the identification of the process through which oogonia give rise to the oocytes, including the cellular morphological characteristics during the different stages of development, from early primary growth to late secondary growth. The development of the oocyte involves active and complex increases of ooplasm, and the deposit of abundant nutrients enclosed in structures such as yolk platelets and lipid droplets in the ooplasm during a precise sequence of changes. Morphological changes in the ooplasm that occur during this process aid in identifying the stages of the oocytes throughout oogenesis and thus the phase of the reproductive cycle of the specimen. This maturation sequence is the basis of the analyses of reproduction in the species and can be identified and quantified through histological examination.
OBJECTIVES

• Characterize the structure of the ovary and its morphological changes in blue crab populations of Tampa Bay.
• Identify and describe the morphological characteristics of the germ cells during the process of oogenesis and particularly the germinal zone of the ovary, providing an accurate description of the oocytes by using histological techniques.
• Define the annual development and seasonal changes of the oocytes in blue crab populations of the Tampa Bay area.
• Introduce a histology-based staging schema for oocyte developmental stages in blue crabs.
• Correlate oocyte developmental stages to carapace width.
LITERATURE REVIEW

Several authors have studied the reproductive characteristics of females of several species of decapoda and have described with macroscopical and microscopical observations the condition of the ovaries at sexual maturity, the annual reproductive cycles, and the morphology of the ovary and the process of oogenesis.

General Biology of the Female Blue Crab

Female blue crabs have four pairs of pleopods on abdominal segments 2 through 5. The first coxa of a pleopod is connected to the body by a soft and flexible articulating membrane. The coxa is undersized and barely calcified; but the next article, the basis, is large and harder. Two rami, the exopod and endopod, arise from the basis. (Fig. 2a). The oocytes pass through the seminal receptacles, where they are fertilized as they move out from the ovaries. The fertilized oocytes are then expelled from the gonopores and attach to the setae of the pleopods to form a large, cohesive mass or “sponge” that remains attached to the female until they hatch. This constitutes an ovigerous female (Fig. 2b).

Exterior Structure of the Ovary

The ovaries lie dorsal to the hepatopancreas and extend on both sides of the anterior margin of the body cavity to the cephalothorax. The hepatopancreas and the ovaries are intermingled along the anterior marginal dorsal portion of the carapace (Fig. 3a). The ovaries continue down towards the cardiac stomach in a posteromedial course. Then they

Figure 2a. Ventral view of mature female blue crab.

Figure 2b. Fertilized oocytes attach to the setae of the pleopods to form a large mass referred to as a “sponge” in a female blue crab.
are attached by a medial connection that joins the left and right ovaries at the level of the medial stomach. The ovary continues downward toward the posterior stomach and cover it. The ovaries show several gross stages as they go from immature to mature stages. The immature ovary resembles a small pink filament. At both distal regions of the oviduct are the seminal receptacles. These are contained in a hard, chitinous wall. The seminal receptacles are positioned laterally to the midline between the stomach and heart along the posterolateral border of the stomach. The size and color of the seminal receptacles vary depending on the maturity level of the ovary. Seminal receptacles change from small, thin, white, and membranous disks (Fig. 3b) to large, hard, and pink rounded spheres (Fig. 3c). As the ovary matures, the seminal receptacles shrink and the ovaries become thicker. The ovaries change from the light, soft pink of the immature ovary to the bright orange of the mature ovary (Fig. 3d).

Sexual Maturity

In Chesapeake Bay, female blue crabs reach sexual maturity after 18–20 post-larval molts, at the age of 1 to 1.5 years (Williams, 1965). Mature condition of the female blue crabs that have completed the pubertal molt is determined by examining the semicircular abdomen (Mense and Wenner, 1989; Steele and Bert, 1994). In stone crabs, *Menippe mercenaria*, mature condition of the ovaries was determined by gross analysis of the

Figure 3a. Dorsal ovary covering cardiac stomach.

Figure 3b. Immature ovaries and immature seminal receptacles.

Figure 3c. Mature seminal receptacles.

Figure 3d. Mature ovary and medial connection.
organ and by establishing a color-coded staging system based upon the color of the
ovaries (Gerhart and Bert, 2008). This color-coded system includes physiologically
mature females as having orange gonads, which determined that the female was gravid.
In blue crabs, it has been observed that size at maturity is inconsistent. Tagatz (1968)
noticed that maturity of blue crabs could be attained at various widths. Tagatz (1968)
recorded a mature female with a carapace width of 99 mm and an immature female with
a carapace width of 177 mm. It was noted that overlap in size ranges of immature and
mature female blue crabs was considerable.

Fischler (1959) recorded the smallest adult female blue crab in an ovigerous
condition with a carapace width of 55 mm, off the North Carolina coast. It was not
established whether these dwarf forms of C. sapidus normally occurred in the areas in
which they were collected, or whether they were the result of genetic or environmental
factors influencing growth and thereby causing dwarfism (Fischler, 1959). In contrast,
Steele and Bert (1994) found that the size at sexual maturity shows no obvious latitudinal
variation; most female blue crabs attain sexual maturity within the carapace width range
of 130–139 mm. In addition, it has been suggested that variations in temperature may
affect the development of mature oocytes. Steele and Bert (1994) found that there is a
virtual cessation of spawning by females in July. This observation suggests that
midsummer water temperatures produce physiological stress that inhibits reproduction.

Annual Reproductive Cycle

Hard (1942) described the macroscopic aspects of the ovaries in the blue crab during the
annual reproductive cycle, dividing the process into five stages (Table 3).

Table 3. Macroscopic changes and staging of the ovaries of blue crab, Callinectes
sapidus, during the reproductive cycle. (Hard, 1942)

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Stage I</td>
<td>Immediately after last molt, small white ovaries</td>
</tr>
<tr>
<td>Stage II</td>
<td>From August to May, ovaries are orange and increase in length and diameter.</td>
</tr>
<tr>
<td>Stage III</td>
<td>Mature ovaries preceding the first ovulation, ovaries bright orange and large, no sponge.</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Period between first and second ovulations, ovaries remain orange, sponge present.</td>
</tr>
<tr>
<td>Stage V</td>
<td>Ovaries collapsed, grey or brown, sponge still present on the swimmerets.</td>
</tr>
</tbody>
</table>
Perry (1975) described characteristics of the reproductive cycle in female blue crabs with developing ovaries and recently mated females during the spring, summer, and fall. Females with mature ovaries occurred throughout the year, and “berried” females were present in March and April. It was noted that females in the area spawned when water temperatures began to rise in the spring.

Females of *Portunus trituberculatus* (Hamasaki et al., 2006) were shown to have vitellogenic oocytes from October to December, and their developmental stage did not change over the winter from December to March.

Blue crab females from Chesapeake Bay showed arrested vitellogenesis during the winter months. Crabs collected in the same locality for histological examination during the winter months showed that no progression of oocyte development occurred during those months (Johnson, 1980).

In the paddle crab, *Ovalipes catharus* (Armstrong, 1988), it was observed that the percentage of females with moderately developed to well-developed ovaries increased steadily from March to October. Females with well-developed ovaries appeared in September. After the females with well-developed ovaries were collected in October, there was an increase of females in early stages of oocyte development. This would indicate that females of the paddle crab undergo a period of oogenesis over the winter months followed by spawning in the spring (Armstrong, 1988).

Minagawa et al. (1993) found that in the Izu islands of Japan, spawning females of the red frog crab, *Ranina ranina*, occurred only during the months of May to September. No oocytes were observed to develop beyond the primary yolk platelet stage during December and January. From August to November, most oocytes remained in stages younger than the secondary growth stage. Minagawa et al. (1993) divided oocyte development into stages based on the morphological characteristics of each stage.

Sastry (1983) indicated that the duration of reproductive cycles may differ in populations of the same species of crabs according to environmental conditions. Swiney and Shirley (2001) found that females of the dungeness crab, *Cancer magister*, spawned every two years in high-altitude populations in Alaska. Swiney and Shirley (2001) observed that the crab females do not extrude eggs annually, but could extrude eggs biannually.

Other authors propose that adding specific hormones brings about changes in the process of oocyte development. In the freshwater rice field crab, *Oziotelphusa senex senex*, Nagaraju et al. (2006) induced ovarian growth and vitellogenesis with the introduction of hormones, suggesting that vitellogenesis is a process in which female crabs store nutrients for subsequent use by developing oocytes.

**Ovary and Oogenesis**

Minagawa et al. (1993) divided the process of oogenesis in *Ranina ranina* into five main stages and ten substages based on the morphology of the oocytes. They described the process of proliferation as occurring in the oogonia and all of the following stages occurring in the oocytes (Table 4).
Lee et al. (1996) compared the levels of vitellin-immunoreactive proteins with the developmental changes in relation to vitellogenesis of the blue crab and established five staging levels. These are as follows:

Stage 1: Immature females. In this stage, the ovary was composed of previtellogenic oocytes with scant basophilic ooplasm, with a mean oocyte diameter of 16–24 µm. A layer of prefollicle cells enclosed the oocytes.

Stage 2: Females recently completing pubertal molt. This stage is characterized by the presence of swollen seminal receptacles in the oviduct of the females. The previtellogenic oocyte diameter ranged 30–60 µm. The ooplasm was intensely basophilic and contained a distinctive perinuclear yolk complex. Prefollicle cells migrating to the lobule periphery appeared to be in the process of surrounding the oocytes.

Stage 3: Early vitellogenesis. During this stage, the mean oocyte diameter ranged 66–100 µm. The perinuclear yolk complex had disappeared from the ooplasm, which now contained eosinophilic yolk bodies. The previtellogenic oocytes in stage 2 were found in the central regions of the ovarian lobes.

Stage 4: Mid-vitellogenesis. During this stage, the mean diameter of the oocytes was 103–160 µm. The ooplasm was full of eosinophilic yolk bodies.

Stage 5: Late vitellogenesis. During this stage, the mean oocyte diameter was 168–288 µm. Similar to stage 4; a layer of flattened follicle cells enclosed the oocytes. The ooplasm was full of eosinophilic yolk bodies.
Perinuclear Yolk Complex

During primary growth, a body known as the perinuclear yolk complex becomes apparent proximal to the nucleus. Several authors have described the perinuclear yolk complex in different species, including fish (Gülsoy, 2007) and hermit crabs (Komm and Hinsch, 2005). The perinuclear yolk complex of blue crabs has not been described in detail. Johnson’s (1980) description of this structure in blue crabs is limited; she only refers to it as a single large spherical inclusion that is PAS positive. In the trout *Oncorhynchus mykiss*, Gülsoy (2007) describes an inclusion that appears during primary growth in the basophilic cytoplasm of the trout. This inclusion is further portrayed as an area with the appearance of loosely aggregated particles that begins to emerge around the nucleus during primary growth. Gülsoy (2007) explains that this structure may be called perinuclear yolk body in different articles, but that the structure is not yolk; yet this term has widely been used for a long time in fish literature. Similarly, Nayyar (1964) agrees that authors throughout the literature have diverse views regarding the origin and the morphology of the perinuclear yolk complex, but that the majority of authors believe it to be related in some way with yolk formation; hence the term “yolk nucleus” has been universally accepted. Azevedo (1984) explains that this dense material consists mainly of fine granulo-fibrillar components and seems to be a common structure in germinal cells within different animal groups. Nayyar (1964) similarly illustrates the perinuclear yolk complex. In his study, the author describes the process of the yolk nucleus appearing as a mass of lipid granules and mitochondria located at first beside the nucleus. As the perinuclear yolk complex becomes spherical, it migrates to the peripheral region of the cytoplasm to lie just below the cell membrane (Nayyar, 1964). Nayyar (1964) compared this structure in eight species of fish, and no differences were found in the morphology of the yolk nucleus among these species.

Reproductive Characteristics of the Blue Crab

Florida blue crabs mate from March to December when water temperatures exceed 22°C. Female blue crabs mate once in their lifetime following the last, or terminal, molt (Steele, 1982). In Chesapeake Bay, blue crabs mate from May to October (Millikin and Williams, 1984), occurring regularly in areas with low salinity such as upper estuaries or lower portions of rivers (Tagatz, 1968).

According to Hard (1942), female blue crabs in Chesapeake Bay ovulate twice after maturation and attain sexual maturity in 12–14 months at a size of 5–7 inches. During years with longer and colder winters, the spawning season was significantly shorter (Daugherty, 1952).

In the Mississippi region, a project carried out by the Gulf Coast Research Laboratory studied the distribution and abundance of blue crabs in 1975. During this project, they collected and examined mature blue crab females. They found that spawning of blue crabs in northern gulf waters is protracted and that egg-bearing females occur in coastal and estuarine waters in the spring, summer, and fall (Perry, 1975).
MATERIALS AND METHODS

A total of 74 females were collected monthly from January 2005 to December 2006 from the Tampa Bay area (Fig. 4).

Blue crabs were harvested following collection protocols described in Steele and Bert (1994). For the duration of the study, temperatures were recorded at collection times. Mean temperatures (four measurements per month) in the Tampa bay area during these months ranged 20°–28°C. Temperature ranges showed little variation. Blue crab carapace texture was rated according to the subjective scale described in the protocol that the FWC–FWRI Crustacean Fisheries group uses for their data (Table 5).

Table 5. Crab carapace texture scale according to the Crustacean Fisheries group, FWC–FWRI. (C. Crawford, personal communication, 2007)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SOFT</td>
</tr>
<tr>
<td>2</td>
<td>PAPER-SOFT SHELL</td>
</tr>
<tr>
<td>3</td>
<td>WHITE LINE (PRE MOLT BY ~1 WEEK)</td>
</tr>
<tr>
<td>4</td>
<td>PINK LINE (PRE MOLT BY 2-3 DAYS)</td>
</tr>
<tr>
<td>5</td>
<td>RED LINE (PRE MOLT BY HOURS)</td>
</tr>
<tr>
<td>6</td>
<td>HARD</td>
</tr>
</tbody>
</table>
Live crabs were transported to the FWC–FWRI histology lab where they were placed on ice for 20 minutes for anesthetizing prior to dissection. All crabs were weighed on a Denver Instruments® scientific balance, model 4100, to the nearest 0.1 g. Carapace width (CW) was defined as the distance between the anterior lateral spine and the most-posterior lateral spine (Fig. 5). Carapace length (CL) was defined as the distance between the centers of the frontal interorbital carapace margin and the posterior margin. Carapace width and length were measured to the nearest millimeter (mm). Crabs with missing limbs, broken carapaces, or any signs of disease were not used.

Histology

Dissection followed methods described in Johnson (1980). The first carapace cut was made from the dorsal articulation above the right posterior leg, anterior to the frontal margin at the head. A second cut was made parallel to the first cut, on the left side. The lateral cuts were then joined by a transverse cut at the posterior margin of the carapace (Fig. 6a). The blue crab ovaries are located covering the cardiac stomach and under the spines, intermingled with the hepatopancreas. Gills are located to the side of the ovaries.

Figure 5. Carapace width (CW) and carapace length (CL).

Figure 6a. Necropsy of the blue crab carapace to dissect ovaries.
Ovaries were removed from the abdominal cavity and sectioned for histology as anterior, mid, and posterior regions. Each section was processed in order to identify the reproductive stage of the ovary (Fig. 6c).

Ovarian samples were placed in a fixative solution of 5% paraformaldehyde (PFMA) 0.1 molar phosphate buffer (Humason, 1972) for 24 hours (Appendix, Table A-1). Following fixation, the ovaries were thoroughly rinsed in tap water for an hour and placed in 70% ethanol inside scintillation vials. The dehydration process continued with 95% ethanol and an infiltration progression to 100% fresh glycol methacrylate resin, JB-4® resin (Appendix, Table A-2).

Ovaries were then embedded in JB-4®, an epoxy resin distributed by Electron Microscopy Sciences. Embedded tissue was sectioned at 4-µm thickness on a Leica® RM 2165 microtome using a 9-mm glass knife. A minimum separation of 60 µm (the approximate maximum diameter of an oocyte) was made between sections. The sections were mounted on Fisher® slides that are pretreated with an acid-cleaned 0.1% HCl solution. (Appendix, Table A-3). Three slides per tissue sample were made. One was stained with hematoxylin and eosin (H&E) (Appendix, Table A-4). A second slide was stained with periodic acid Schiff’s/metanil yellow (PAS/MY) (Quintero-Hunter et al., 1991; Appendix, Table A-5). A third slide was kept unstained. Stained sections were examined at a total magnification of 100–1000× on an Olympus® compound microscope. Each ovary was assigned to a reproductive stage following a classification scheme based on oocyte diameter ranges and oocyte morphological characteristics.

Power of the test for a two-sample t-test comparison was used to determine how many oocytes needed to be measured per ovarian lobe to get an accurate mean and variance. Only oocytes sectioned through the nucleus were measured (Armstrong, 1988) (Fig. 7). Some samples had fewer cells inside the ovarian lobe; in that case, all the cells that had a fully viewable nucleus were counted.
Using an Olympus® BH2 model teaching microscope, oocyte diameters were measured to the nearest µm with an ocular micrometer. Then with the calibration of 2-mm divisions into units of 100, the conversion per magnification was obtained (Table 6). Photomicrographs of sections illustrating the classification criteria were made using the Olympus® Vanox-T AH-2 camera.

Table 6. Conversion table showing objective size and conversion factor to change diameter into microns conversion factor.

<table>
<thead>
<tr>
<th>Objective Size</th>
<th>Conversion Factor to get Microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>4X</td>
<td>29.0</td>
</tr>
<tr>
<td>10X</td>
<td>12.2</td>
</tr>
<tr>
<td>20X</td>
<td>5.68</td>
</tr>
<tr>
<td>40X</td>
<td>2.85</td>
</tr>
<tr>
<td>100X</td>
<td>1.14</td>
</tr>
</tbody>
</table>

General Morphometric Analyses

Blue crabs were weighed and measured in the field, and the results were analyzed by linear regression methods after log transformation of body weight and carapace width. Linear regressions were used to derive the parameters of the power curve. Because there was a significant relationship between body weight and carapace width, analysis of covariance (ANCOVA; Zar, 1996) was used to examine the relationship of body weight and reproductive stage (determined from histological samples from female blue crab gonads) using carapace width (log-transformed) as the covariate. The covariate, carapace width, adjusts the observations of body weight (log-transformed) for the average body size of crabs in the samples and allows the comparison of body weight versus the oocyte developmental stages defined in this study.

Figure 7. Oocytes were measured only when the whole nucleus was visible.
RESULTS

Oocyte Descriptive Statistics

Descriptive statistics for the average diameters of oogonia and oocytes by developmental stage are shown in Table 7. Fifty-five cells were measured to obtain a meaningful average of oocyte size range. Confidence intervals are tight, and a reasonable difference in means was detected.

Table 7. Descriptive statistics for oocyte diameter ranges.

<table>
<thead>
<tr>
<th>DEVELOPMENTAL STAGE</th>
<th>MEAN OOCYTE DIAMETER (MM)</th>
<th>SD</th>
<th>N</th>
<th>SE (MM)</th>
<th>UPPER 95% CI (MM)</th>
<th>LOWER 95% CI (MM)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Primary Growth</td>
<td>32.56</td>
<td>2.95</td>
<td>40</td>
<td>0.47</td>
<td>33.5</td>
<td>31.6</td>
<td>9.1%</td>
</tr>
<tr>
<td>Primary Growth</td>
<td>38.7</td>
<td>4.3</td>
<td>55</td>
<td>0.6</td>
<td>39.8</td>
<td>37.5</td>
<td>12.77%</td>
</tr>
<tr>
<td>Early to Mid-Vitelligenic</td>
<td>68.2</td>
<td>10.4</td>
<td>55</td>
<td>1.41</td>
<td>71.0</td>
<td>65.3</td>
<td>10.61%</td>
</tr>
<tr>
<td>Late Vitelligenic</td>
<td>147.8</td>
<td>19.8</td>
<td>55</td>
<td>2.67</td>
<td>153.1</td>
<td>142.4</td>
<td>14.95%</td>
</tr>
<tr>
<td>Full-Grown</td>
<td>172.3</td>
<td>11.24</td>
<td>31</td>
<td>2.02</td>
<td>176.5</td>
<td>168.2</td>
<td>6.5%</td>
</tr>
</tbody>
</table>

Histological Structure of the Ovary

The ovary is contained inside units called ovarian lobes or ovarian pouches (Fig. 8a,b). Ando and Makioka (1999) used the later nomenclature. The ovarian lobes encompass the oocytes. Most oocytes inside the ovarian lobes are at about the same developmental stage.

Oogenesis is a continuous process in which oocytes undergo development from primary growth to secondary growth in a somewhat rapid fashion. Therefore, the percentage of oocytes inside an ovarian lobe at a specific developmental stage can correspond with the occurrence of another developmental stage and overlap at roughly 75% to 25% of each stage. Some lobes are configured with two main stages, but they may also have a small percentage of a third stage in them. Therefore, an ovarian lobe that is mainly in mid-secondary growth (75%) can have some oocytes in primary growth (23%) and a few oocytes representing the early primary growth stage (2%). Development
is a fluid process and oogonia are always present, but they may not always be evident. Early primary growth oocytes are also always present because along with oogonia, they compose the germinal zone, and all ovarian lobes have a germinal zone. Therefore, there can be more than one developmental stage inside one ovarian lobe, but mostly the ovarian lobes are in the same developmental stage per crab (Fig. 9).

The structure of the blue crab ovary consists of oocytes that develop from the center of the ovarian lobe to the periphery as oogenesis advances. This distribution of germinal cells in the ovarian lobes is characterized by the developmental progress of cells

![Figure 8a. Ovarian lobe containing secondary-growth oocytes. GZ = germinal zone.](image1)

![Figure 8b. Several ovarian lobes (OL) containing secondary-growth oocytes and germinal zones (GZ).](image2)

![Figure 9. Percentage of oocytes at a specific developmental stage inside ovarian lobes of four randomly chosen female blue crabs. This figure illustrates that oocytes at different stages of development can be found inside a specimen’s ovarian lobe. OOG = oogonia, ISG = late secondary growth, emSG = early mid-secondary growth, IPG = late primary growth, ePG = early primary growth.](image3)
from oogonia, which are found in a central germinal zone also known as a germaria (Fig. 10a,b), to late secondary growth stage, which are found in the periphery of the ovarian lobes (Fig. 10c,d).

The germinal zone is a row of germinal cells at the center of the ovary. During primary growth, there are no well-defined germinal zones and oogonia are difficult to recognize (Fig. 11a). In secondary growth, the germinal zones are well-defined and consist mostly of primary growth oocytes. Germinal zones provide new cells for continual development of oocytes as cells mature when they reach the periphery (Fig. 11b).

Figure 10a. Germinal zone (GZ) or germaria (PAS/MY).
Figure 10b. Germinal zone (GZ) or germaria in a mid-secondary growth oocyte (H&E).
Figure 10c. Mid-secondary-growth oocytes in periphery (H&E).
Figure 10d. Late secondary-growth oocytes on periphery of ovarian lobe (PAS/MY).
Figure 11a. Germinal zone is not evident in ovarian lobe in primary growth (PAS/MY).
Figure 11b. Defined germinal zone in late secondary growth ovarian lobe (PAS/MY).
Oocyte Staging Schema

A new staging schema is introduced in this study. This schema is based on morphological changes that occur through development. The oocyte staging schema is detailed in Table 8. Oocyte developmental stages and carapace-width diameters are compared and summarized in Table 9.

Table 8. Oocyte staging schema with developmental stages, cellular steps, and morphological characteristics. (n = 59)

<table>
<thead>
<tr>
<th>TYPE OF GERMINAL CELL</th>
<th>STAGES</th>
<th>MORPHOLOGICAL CHARACTERISTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oogonia</td>
<td>Mitotic Proliferation</td>
<td>Diameter range: 9–12 μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scant, clear cells</td>
</tr>
<tr>
<td>Oocyte</td>
<td>Early Primary Growth (ePG)</td>
<td>Oocyte diameter range: 12–50 μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scant, clear cells similar to oogonia, with augmentation of Ooplasm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>due to formation of cellular organelles</td>
</tr>
<tr>
<td></td>
<td>Late Primary Growth (IPG)</td>
<td>Oocyte diameter range: 51–114 μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basophilic cytoplasm-&gt; Active cell (organelles and ribosomes are</td>
</tr>
<tr>
<td></td>
<td></td>
<td>accumulating)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perinuclear yolk nucleus complex</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lambrush chromosomes are present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oil droplets and cortical alveoli begin to form</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreasing basophilia</td>
</tr>
<tr>
<td></td>
<td>Secondary Growth (SG)</td>
<td>Characterized by the onset formation of yolk globules and their</td>
</tr>
<tr>
<td></td>
<td></td>
<td>continual increase in volume through secondary growth. Secondary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>growth consists of early, mid-, and late vitellogenesis. Oocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>diameter range from 150-357 μm</td>
</tr>
<tr>
<td></td>
<td>Early to Mid-Secondary</td>
<td>Oocyte diameter range: 115–150 μm</td>
</tr>
<tr>
<td></td>
<td>Growth (emsSG)</td>
<td>Yolk platelet size range: 33–65 μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yolk platelets formation continues, increasing in size. Ooplasm is</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acidophilic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Well-defined germinal zone</td>
</tr>
<tr>
<td></td>
<td>Late Secondary Growth (lSC)</td>
<td>Oocyte diameter range: 151–357 μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yolk platelet size range: 91–95 μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yolk platelets continue to increase in size and amount</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oocytes become fully yoked, germinal zone is less evident</td>
</tr>
</tbody>
</table>

19
Table 9. Oocyte developmental stages, showing oocyte diameter ranges in microns and carapace-width ranges in mm. (n = 36)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Stage ID</th>
<th>Oocyte Diameter (µm)</th>
<th>Carapace Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oogonia</td>
<td>OOG</td>
<td>9 µm – 12 µm</td>
<td>112–117</td>
</tr>
<tr>
<td>Early Primary Growth</td>
<td>ePG</td>
<td>12 µm – 50 µm</td>
<td>96–171</td>
</tr>
<tr>
<td>Late Primary Growth</td>
<td>IPG</td>
<td>51 µm – 114 µm</td>
<td>135–178</td>
</tr>
<tr>
<td>Early to Mid-Secondary Growth</td>
<td>emSC</td>
<td>115 µm – 150 µm</td>
<td>147–178</td>
</tr>
<tr>
<td>Late Secondary Growth</td>
<td>ISC</td>
<td>151 µm – 357 µm</td>
<td>132–167</td>
</tr>
</tbody>
</table>

Oogonia

Oogonia are the female germinal cells, small oval cells found in the germinal zone. These cells cannot be classified as oocytes because oogonia are capable of dividing via mitosis to form other oogonia or via meiosis to form an oocyte. They are diploid cells in which meiosis has not started.

Oogonia have a characteristic scant, very clear ooplasm. The nucleus is pale, and a single nucleolus is prominent (Fig. 12). Oogonia appear to have interphase chromosomes prior to commencement of meiosis. Interphase chromosomes are found on the periphery of the cell. Basal lamina is evident in the germinal zone where oogonia are found. Oogonia range in diameter 9–12 µm.

Figure 12. Oogonia (OOG) with characteristic clear ooplasm, pale nucleus, and interphase chromosomes (IC) on periphery of ooplasm. GZ = germinal zone, BL = basal lamina.
Early Primary Growth

Early primary growth oocytes have begun meiosis, and therefore the cell has become a primary oocyte. The appearance of the early primary growth oocytes is similar to oogonia; however, the ooplasm is characterized by ooplasmic basophilia (Fig. 13a). When the ooplasm changes from the clear, scant appearance that it had during oogonia to a blue hue, that is the indication that the oogonia is now in early primary growth (Fig. 13b). These cells are larger than oogonia, ranging 15–50 µm, and can no longer divide via mitosis again. Therefore, the early primary growth oocyte is an oocyte and remains as such throughout the rest of its development, until full-grown. Oocytes, unlike oogonia, are diploid cells in meiosis that have duplicated chromosomes.

Late Primary Growth

The beginning of late primary growth is characterized by gradual basophilia of the ooplasm and by the absence of yolk. The basophilic ooplasm indicates that the cell is active with production of organelles, which increase the volume of the ooplasm, another marker for this stage. Oocyte diameter range for this stage is 51–114 µm.

Throughout late primary growth, the formation of organelles such as mitochondria, Golgi complexes, and abundant quantities of endoplasmic reticulum, ribosome, and fragmented glycogen may contribute to the blue staining of the ooplasm. For example, ribosomes are basophilic organelles that have a chemical attraction for basic stains, like hematoxylin stains, which give the cell its unique blue hue, characteristic of late primary growth. During this stage, the germinal zone is not as evident as it will be in more developed oocytes, yet the germinal zone with the oogonia
and the early primary growth oocytes is always present. Hemolymph is evident, and follicular cells and somatic cells are also present but not as obvious (Fig. 14).

In the course of the beginning stages of late primary growth stage, a distinct PAS positive body is present in the ooplasm, usually near the nucleus (Fig. 15a,b). The PAS positive body, also known as the perinuclear yolk complex, is only evident during the early stages of late primary growth, and it disperses before the cortical alveoli can be observed.

Although the biochemical properties of this body were not analyzed in this study, its description is similar to the description given for the inclusion called the perinuclear yolk complex by other authors (Nayyar, 1964; Komm and Hinsch, 2005; Gülsoy, 2007). The function of the perinuclear yolk complex may be to assist in assembling the proteins that later form the yolk globules. The PAS positive body is a cellular structure that will organize the yolk. The perinuclear yolk complex disappears as the oocyte develops and growth continues into the next stage. The PAS positive body disperses its contents into the ooplasm as late primary growth continues.

Once the perinuclear yolk complex has dispersed, filaments similar in appearance to lambrush chromosomes are observed in the nucleus of blue crab oocytes. These structures are an indication that the oocyte is in arrested meiosis (Fig. 16a,b).
In late primary growth, somatic cells and follicular cells are evident. These cells do not disappear during secondary growth and have always been present, but it is at the end of late primary growth and at the beginning of early secondary growth that follicular cells, basal lamina, and somatic cells become more evident. Follicle cells are distinguishable because of their elongated nucleus, and they can be found surrounding the oocytes. Follicle cells surround the oocyte, the follicle, and are found in the germinal compartment. The elongated nucleus of the follicle cells is more apparent in the later stages of development (Fig. 17).

Somatic cells are found (Fig. 18) outside the basal lamina and within the germinal compartment. Somatic cells can become follicle cells, but until they are not found in the
Figure 19a. Cortical alveoli (CA) in the periphery of the oocytes.

Figure 19b. Cortical alveoli (CA), basal lamina (BL), and follicle cell nucleus (FCN).

germinal compartment and surround the follicle, they are not described as follicle cells but only as somatic cells. Basal lamina is the structure located at the base of all epithelium and acts as a barrier between connective tissue and epithelium. Basal lamina had not been described in past studies of blue crabs.

Cortical alveoli are another morphological characteristic that emerge during late primary growth, and they occur immediately prior to the onset of secondary growth. At times, formation of cortical alveoli and the formation of yolk may proceed simultaneously in the latest stages of late primary growth. The cortical alveoli have never been described in studies of blue crabs. Although this study did not include a biochemical analysis, the staining procedures indicate that these may be cortical alveoli because they are PAS positive, and cortical alveoli contain polysaccharide components and protein components that would give them a strong purple hue (H. Grier, personal communication). The cortical alveoli are synthesized by the oocyte and become visible in the perimeter of the ooplasm. When cortical alveoli appear, they remain for the duration of development. This is the only morphological feature that was found in late primary growth and throughout secondary growth (Fig. 19a,b).

During the final stages of late primary growth, there are increasing numbers of lipid droplets in the ooplasm. The oil droplets are identified in tissue that has been infiltrated as vacuoles in the tissue. The lipids that compose these oil droplets dissipate during the infiltration process with ethanol. Oil droplets are also evident during early secondary growth but not mid- or late secondary growth (Fig. 20).

Secondary Growth

Secondary growth, or vitellogenesis, begins with the inclusion of yolk globules in the ooplasm. This initialization of yolk proteins in the ooplasm indicates that the oocyte has moved into secondary growth. Secondary growth was divided into 3 steps: early, mid-,
and late secondary growth. Each stage was based upon the size of oocyte diameter, yolk globules, and varialbness of the ooplasm, such as the polymorphism of yolk globules.

When secondary growth begins, the ooplasm loses the basophilic characteristics, becoming clearer in appearance and staining less blue/purple and more a lighter pink (Fig. 21). In early to mid-secondary growth, oocyte diameter range was 115–150 µm and the yolk globules size range was 33–65 µm. During early secondary growth, the PAS positive body or perinuclear yolk complex that was apparent in primary growth disappears; however, the follicle cells still surround the oocyte.

During mid-secondary growth, the yolk globules are more evident in the ooplasm as they grow larger. There is also a well-differentiated germinal zone that contains only primary growth oocytes (Fig. 22a,b).

During late secondary growth, the ooplasm stains a bright pink, and the germinal zone is evident. When the yolk globules reach a maximum diameter, the oocyte is in late secondary growth. Vitelline becomes more fluid and lighter in color. Large yolk globules coalesce, and the ooplasm has a more homogenous consistency (Fig. 23).

Near the germinal zone, several other cellular structures can be observed. Somatic cells are found outside the basal lamina and the germinal compartment, and follicle cells are found within the germinal compartment. Follicle cells surround the oocytes, and their nuclei are clearly seen around oocytes. Somatic cells can become follicle cells; but while they are found outside the germinal compartment, they are described as somatic cells. A basal lamina is also present. This structure is located at the base of the epithelium, separating the connective tissue from the epithelium and acting as a barrier (Fig. 24).
Figure 23. Late secondary growth oocytes are bright pink, and yolk globules have reached maximum diameter.

Figure 24. Late secondary growth: large yolk globules (YG), somatic cells (SC), and basal lamina (BL).

Full-Grown Oocytes

Full-grown oocytes have reached their maximum size. Most of the ooplasm retains a bright-pink stain. With the absence of large accumulation of cell organelles, the tissue now has a greater affinity for acid stains, and as a result, eosin stains them pink. Yolk becomes fused in full-grown oocytes (Fig. 25a). These oocytes are found mostly on the perimeter of the ovarian lobe at first; then when development continues, the whole ovarian lobe will appear with fused yolk oocytes. When full-grown, oocytes retain follicle cells, and yolk platelets are at their maximum size of 95 µm (Fig. 25b).
Figure 25a. Full-grown oocytes showing fused yolk globules (arrows) in oocytes at the periphery of the ovarian lobe.

Figure 25b. Follicle cells (arrows) in full-grown oocytes.

Figure 26. Mature fertilized egg in ovigerous female, showing yolk globules similar to those in full-grown oocytes.

Figure 27. Ovaries of ovigerous females in early and late primary growth. GZ = germinal zone.

Ovigerous Females

After the oocytes are full-grown, they mature and are fertilized. This signifies the end of oogenesis. The oocyte reactivates meiosis. The appearance of yolk in the mature fertilized egg is very similar to the appearance of the full-grown oocyte (Fig. 26).

Fertilized eggs make up the sponge. The ovaries of ovigerous females were found to always be in primary growth (Fig. 27).

The fertilized egg mass itself or the sponge area can be described as having fertilized eggs in various stages of development that may range from an early division stage to almost fully developed (Fig. 28).

Atresia

Some of the oocytes that were studied presented a condition similar to what has been described as atypical oocytes or atresia (Fig. 29a). The atresia stage is characterized by
evidence of oocyte disintegration. Atresia was observed in small females with a carapace width usually less than 100 mm. During atresia, oocytes showed a lack of structural integrity, with a tendency for oocyte membranes to fall apart and appear to fuse between oocytes. Oocytes undergoing atresia exhibit shrinking of the ooplasm, folding or collapsing of the oocyte membranes (Fig. 29b), and fusion of nucleus (Fig. 29c). The oocyte structure degenerates to form one continuous mass. This step was found only in samples from crabs measuring less than 100 mm CW and only during early primary growth.

Figure 29a. Collapsing membranes among oocytes (arrows), a condition referred to as atresia or atypical oocytes.

Figure 29b. Atresia in oocytes, showing fusion and collapsing of ooplasm among oocytes (arrows).

Figure 29c. Atresia in oocytes.
Annual Reproductive Cycle

Tampa Bay blue crabs with oocytes in the late secondary growth stage occurred more frequently from December to March than at any other time of the year. Of nine samples collected in December, seven had late vitellogenic oocytes, one was in mid-vitellogenesis, and one was a sponged female. Oocytes in the late vitellogenesis step were also observed in specimens collected during July, August, and September. From April to June, oocytes in most females were undergoing primary growth or early vitellogenesis, and no oocytes in the late vitellogenesis stage were observed in any of the specimens. The only sponged females collected were found in November and December. Blue crabs with oocytes in early primary growth and juvenile crabs were found mostly during the months of March, May, and June (Fig. 30); however, sample size restricts this conclusion. While sponged crabs were found only in November and December, it is possible that sponged crabs may be found at other times of the year. A larger sample size would be needed to determine whether ovigerous females are present in other months of the year. During this study, no evidence was found of blue crabs in a state of arrested development (Johnson, 1980) during the winter months. Temperature ranges in the sampling locations were not extreme, and subtropical regions such as Tampa Bay may provide favorable environmental conditions that support year-round production of eggs. Therefore, the annual reproductive cycle of the blue crab in the Tampa Bay area may differ from the annual cycle of the blue crabs in locations where water temperature ranges are cooler during winter months. Tampa Bay area blue crabs appeared to have a reproductive cycle that is continuous throughout the year.

Figure 30. Annual reproductive cycle of blue crabs in Tampa Bay. SPG = sponged or ovigerous female, ISG = late secondary growth, emSG = early mid-secondary growth, IPG = late primary growth, ePG = early primary growth, OOG = oogonia, JUV = Juvenile.
Correlation of Carapace Width and Oocyte Diameter with Developmental Stages

The average oocyte diameter per month for female blue crabs in the Tampa Bay area had a large variance, as would be expected (Table 10). During this study, crabs in all developmental stages were found during most of the year. Therefore, there is a large standard deviation each month, which signifies that the center of mass is spread wider about the mean.

The relationships between carapace width (CW) and oocyte diameters with gonad developmental stages were examined. Carapace width varied for blue crabs in different gonadal stages and was not clearly correlated with the progression in gonadal development. Although size (e.g., CW) is likely to be related to reproductive output (e.g., number of eggs produced), size alone may not be a good indicator of sexual maturity or developmental stage in blue crabs. The average diameters of oocytes appeared to be more strongly correlated to gonad developmental stage from oocyte primary growth through final oocyte maturation (OM). A slight trend of increasing carapace width from primary growth to early and mid-secondary growth was observed, but the sample sizes were too small to make any definite statements on this relationship if it exists. Blue crabs in later stages of gonad development were sometimes smaller than those in earlier stages of development, and it is probable that carapace width alone is not the best indicator of maturity or developmental stage in blue crabs.

Table 10. Descriptive statistics for the average diameters of oogonia and oocytes by developmental stage.

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>mean diameter (μm)</th>
<th>sd</th>
<th>n</th>
<th>se (μm)</th>
<th>upper 95%CI (μm)</th>
<th>lower 95%CI (μm)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Primary Growth</td>
<td>32.56</td>
<td>2.95</td>
<td>40</td>
<td>0.47</td>
<td>33.5</td>
<td>31.6</td>
<td>9.1%</td>
</tr>
<tr>
<td>Primary Growth</td>
<td>38.7</td>
<td>4.3</td>
<td>55</td>
<td>0.6</td>
<td>39.8</td>
<td>37.5</td>
<td>12.77%</td>
</tr>
<tr>
<td>Early/Mid Vitellogenic</td>
<td>68.2</td>
<td>10.4</td>
<td>55</td>
<td>1.41</td>
<td>71.0</td>
<td>65.3</td>
<td>10.61%</td>
</tr>
<tr>
<td>Late Vitellogenic</td>
<td>147.8</td>
<td>19.8</td>
<td>55</td>
<td>2.67</td>
<td>153.1</td>
<td>142.4</td>
<td>14.95%</td>
</tr>
<tr>
<td>Full-grown</td>
<td>172.3</td>
<td>11.24</td>
<td>31</td>
<td>2.02</td>
<td>176.5</td>
<td>168.2</td>
<td>6.5%</td>
</tr>
</tbody>
</table>
Field data shows that 88% of females at 130 mm CW are externally mature, and most females do not show external sexual maturity characteristics until they reach 125 mm CW (Fig. 31). There is a range from 80 mm CW to 120 mm CW in which females are scored as being immature but may be internally mature. Twelve percent of females under 130 mm CW were scored as mature, and one percent of females over 130 mm CW were scored as immature based on external characteristics, but they are most likely mature internally.

Figure 31. Field data showing how Tampa Bay blue crab females are scored for maturity and immaturity.
Percentage of Females in Different Developmental Stages

During this study, specimens at any size were found in different developmental stages (Fig. 32). Females of 90–124 mm CW included immature and mature individuals. Immature individuals were characterized by having no obvious oogonia, oogonia only, or oocytes in the preprimary growth stage. Individuals that were scored as mature (even if they had not yet undergone the molt to the adult instar) showed oocytes undergoing primary growth or secondary growth (early to late). Animals with the larger carapace widths had ovaries in any of the developmental stages from primary growth to late secondary growth.

![Histogram of the percentage of females in different developmental stages vs. carapace width. ISG = late secondary growth, emSG = early mid-secondary growth, IPG = late primary growth, ePG = early primary growth, OOG = oogonia.](image)

Figure 32. Histogram of the percentage of females in different developmental stages vs. carapace width. ISG = late secondary growth, emSG = early mid-secondary growth, IPG = late primary growth, ePG = early primary growth, OOG = oogonia.
DISCUSSION

One of the most significant processes in the reproductive biology of any animal species is the development of oogonia into mature oocytes. Current investigations into oocyte maturation have classified the process into discrete stages based upon microscopic characteristics, from oogonia to mature oocytes. The characteristics of the ovary of blue crabs have not been extensively analyzed using modern histological methods (Johnson, 1980). No previous assessment of blue crab oocyte development using glycol methacrylate embedding resin JB-4®, the embedding medium used during this study, has been published. This embedding technique allows the tissue to be sectioned at 4 µm, providing much better resolution of the cells with minimal artifact or distortion. Furthermore, the infiltration process itself is less damaging to the tissue than the traditional paraffin infiltration process. Cells do not collapse, shrink, or swell during processing as they do when other embedding techniques are used.

Overall, using the glycol methacrylate resin JB-4® embedding technique increases clarity and resolution, reduces distortions, and provides a higher-quality histological section that presents much more cellular detail than tissue processed by traditional paraffin methods. Using this embedding technique, the reproductive cycle and the ovarian morphology of the blue crab in the Tampa bay area can be better depicted. For this research, the process of oocyte maturation in blue crabs is described and a classification system for stages is developed to best categorize the morphological changes occurring throughout the maturation process of the oocytes. The morphological characteristics of blue crab oogenesis have never been depicted as well and with such high definition as they have for this study. Because of these advanced techniques, an updated oocyte development schema for blue crabs is introduced.

In blue crabs, oocytes are contained within an ovarian lobe. The connective tissue that surrounds the ovarian lobe and retains all the oocytes inside is visible. As oocytes mature, they move to the periphery of the ovarian lobe, and only oogonia and early primary growth oocytes are found closer to the center of the ovarian lobe. This area, where the younger stages originate, is known as the germinal zone or germaria, which is represented as specialized germ cell areas. In a study by Hinsch (1972), it was observed that in the spider crab, Libinia emarginata, the oogonia and primary growth oocytes were found in central regions of the ovary.

In the freshwater crab, Potamon dehaani, when the oogonia reach about 20 µm in diameter, they move out of the germinal zone. Ando and Makioka (1999) noted that in P. dehaani, the oogonia are not enclosed in the same regions where larger oocytes are found. Ando and Makioka (1999) observed that oocytes larger than 100 µm in diameter are enclosed within their own regions. The authors call these regions the oogenetic lobes.
Hence, in *P. dehaani*, there are large oogenetic lobes that contain only mature eggs (Ando and Makioka, 1999). In contrast, during this study it was noted that blue crab oocyte stages are only partially synchronous because cells are in different developmental stages, and all can be found in the same histological section. Different developmental stages were found within one ovarian lobe. Contained by the ovarian lobes, a large percent of the oocytes are all in a comparable developmental stage (Fig. 8a). Ovarian lobes were noted to have two main oocyte developmental stages strongly represented; for example, mid-vitellogenic and primary growth, or late vitellogenic and mid-vitellogenic. With the more mature stage germinal cells encompassing the majority of the oocytes present, the younger developmental stage oocytes are represented in smaller percentages of the total oocyte count. Oogenesis is a fluid process, and the different developmental stages of oocytes inside the ovarian lobes correspond to the different times in the annual reproductive cycle in which the crab will spawn. The younger oocytes, occupying less of the total percentage of the ovarian lobe, will eventually develop to occupy most of the ovarian lobe as they become mature. Similarly, Lee et al. (1996) found that germ cells of various developmental stages were present simultaneously within the ovaries of blue crabs. However, for this reason, when Lee et al. (1996) measured the oocytes, they measured only the most developmentally advanced oocytes within that ovary. For the study of blue crabs in Tampa Bay, in order to decide the number of oocytes to be measured, power of the test calculations for two-sample t-test was used. It was determined that 55 oocytes within the ovarian lobe needed to be measured to obtain a meaningful average of oocyte size range.

In blue crabs, oogonia and early primary growth oocytes are found in a germinal zone located in the center of the ovary. Ovarian lobes that contained mostly late vitellogenic oocytes had a distinct germinal zone assembled by primary growth oocytes. Therefore, ovarian lobes with secondary growth oocytes were noted to have an evident germinal zone. This germinal zone in the center of the ovarian lobe contains the late vitellogenic oocytes, and it consists of oocytes in an earlier stage of maturation. The germinal zone characteristic of ovarian lobes in late secondary growth, made up mainly of primary growth oocytes, ranged 40–50 µm in oocyte diameter.

The germinal zone is made up of oogonia and oocytes in early primary growth (Fig. 10a). In the freshwater crab, *P. dehaani*, Ando and Makioka (1999) recognized a similar germinal zone, which they called “germaria.” In *P. dehaani*, Ando and Makioka (1999) noticed that germaria are located in an ovarian epithelium. In contrast, blue crabs did not appear to have a separate epithelium, but rather the germinal zone is found in the center of the ovarian lobe, and the germinal zone contained the oogonia and early preprimary growth.

In the freshwater crab, *P. dehaani*, oogonia were defined as being basophilic and smaller than 20 µm in diameter. In blue crabs, oogonia have a scant ooplasm that is clear and range 9–12 µm in diameter. The oogonia of blue crabs have interphase chromosomes in the periphery.

Basal lamina can also be observed near the oogonia (Fig. 12). Ando and Makioka (1999) observed that in *P. dehaani*, the oogonia are found only in the germaria, which in
turn is found inside an ovarian epithelium. This similarity is shared by the freshwater crab, *P. dehaani*, and blue crabs. In both species, the oogonia are noted to be found only inside the germinal zone. However, in blue crabs, early primary growth oocytes were also found in the germinal zone, not just oogonia. This is why the germinal zone is less evident during the early primary growth stage (Fig. 11a).

Unlike the freshwater crab, *P. dehaani*, no ovarian epithelium was evident in the blue crab. Basal lamina was evident in blue crabs (Fig. 24). The advanced histological techniques used during this project provided outstanding resolution. The tissues obtained corroborated the results described by Johnson (1980), that oogonia in blue crabs are found in the germinal zone. Furthermore, this study advances to show that not only oogonia but also primary growth oocytes are in the germinal zone. The improved resolution that comes from using glycol methacrylate resin to embed the tissue provides evidence that oogonia are even more scant and smaller than described by Johnson (1980). The interphase chromosomes of oogonia were also observed.

Early and late primary growth oocytes in blue crabs are characterized by cytological changes in the ooplasm. Primary growth oocytes have a basophilic ooplasm. As oogonia develop into primary growth oocytes, there is an increase in basophilia (see staging schema, Table 8). Throughout development, one nucleus and one nucleolus are present (Fig. 16b). These are particularly evident during primary growth. A perinuclear yolk complex, also known as the PAS positive body, appears during primary growth (Fig. 15a,b).

This structure is observed as one of the early stages of late primary growth. This perinuclear yolk complex, or PAS positive body, aids in the future production of yolk. When it is present in the primary growth oocyte, it is assembled of organelles, not vitelline. The PAS positive bodies are noted first near the nucleus and later disappear by dispersing in the ooplasm as the oocyte develops further (Fig. 15a). With high-resolution light microscopy, the elements in this structure can be described as containing polysaccharides because it is PAS positive.

In the course of primary growth, the nucleus appears to have a filamentous characteristic. These filaments suggest that there may be lampbrush chromosomes in the nucleus (Fig. 16a), which would indicate that an oocyte with this structure might be in arrested meiosis I. This aspect of development needs to be studied further by using EM and comparing fixatives.

Follicle cells and somatic cells are always present in the oocyte; however, they are more apparent during the later stages of development. Nuclei of follicle cells can be observed surrounding the oocytes and are found inside the germinal compartment (Fig. 17). This relationship between the follicle cells and the oocyte is evident throughout the developmental process. Follicle cells do not disappear during secondary growth. After ovulation, the follicle is broken and the oocyte comes out of the follicle cells that surround it. The follicle cells remain in the ovarian lobe, and the oocyte comes out of the ovarian lobe, where it is fertilized and embryonic development begins. Therefore, the embryo is not surrounded by the follicle cells that are seen enclosing the oocytes prior to fertilization. Ando and Makioka (1999) did not observe true follicle cells surrounding the
oocytes and eggs throughout oogenesis in the freshwater crab, *P. dehaani*. In the spider crab, *L. emarginata*, Hinsch (1972) identifies follicular cells surrounding the oocytes as they differentiated. Throughout development, the encompassing follicular cells become located on the external boundaries in the spider crab (Hinsch, 1972).

Lee et al. (1996), using paraffin preparations, also observed that the blue crab ovarian lobes contained oocytes and that a layer of follicle cells enclosed these lobes, but that follicle cells were rarely found surrounding individual oocytes. It is possible that because this study used JB-4® epoxy resin and therefore obtained better resolution, the follicle cells surrounding the oocytes became apparent; and that Lee et al. (1996) were not able to distinguish the follicle cells because of the lower resolution achieved with methods using paraffin. Somatic cells are located outside the germinal compartment and do not surround the follicle. Basal lamina acts as a barrier from the connective tissue. (Fig. 18).

Cortical alveoli appear in the periphery of the oocyte in late primary growth and remain through development (Fig. 19a,b). Cortical alveoli have not been described in studies of blue crabs. The empty vesicles that had contained lipids become evident during the last phases of late primary growth. Lipids are lost during the infiltration processes used for histological tissue preparation because the 70% and 95% ethanol dissolve the lipids. Therefore, the vesicles that had contained the lipids appear microscopically as empty vesicles (Fig. 20). In blue crabs, the lipid droplets can still be observed during the period between early secondary growth and late primary growth; they are a fluid feature that progresses from one stage to the next and then disperses. These empty vesicles are confirmation that lipids were formed. This observation is concurrent with Ando and Makioka’s (1999) description of small oil droplets occurring in the ooplasms around the germinal vesicles of the largest previtellogenic oocytes seen during their study.

Secondary growth is a clear and evident process involving morphological changes in the oocyte. During secondary growth in blue crabs, the ooplasms shows a significant decrease in basophilia. Oocytes increase in size because of a considerable increase in the volume of yolk globules. Yolk globules begin to appear in one hemisphere of the oocyte (see Fig. 21), and as the yolk continues to grow, the oocyte becomes more acidophilic. As development continues, the yolk globules increase in volume (see Fig. 22a). Yolk globule size is the main distinguishing difference between early to mid-vitellogenic and late vitellogenic oocytes. This process coincides with the observations made by Ando and Makioka (1999) of the freshwater crab, *P. dehaani*, where it was noted that fine yolk granules appear first at the periphery of the ooplasm of the earliest vitellogenic oocytes. Then yolk granules increase in size and coalesce to become larger and fill the ooplasm. Hinsch (1972) noted that as vitellogenesis progresses in the spider crab, *L. emarginata*, there is a large amount of endoplasmic reticulum in the vitellogenic oocyte (Hinsch, 1972). In blue crabs during late secondary growth, the ooplasm is acidophilic.

During secondary growth, the germinal zone becomes more evident. Ovarian lobes with oocytes in the late secondary growth stage have a significant germinal zone that comprises primary growth oocytes and oogonia (Fig. 8b).
As the oocyte develops into full growth, the ooplasm is less basophilic and becomes more acidophilic and more fluid. The appearance of a full-growth oocyte is then similar to the appearance of the ovulated eggs (Fig. 26).

In this study, it was observed that some oocytes had ooplasm and cellular membranes that fused. This fusion of ooplasm and cellular membranes most likely represents atresia, a state in which the cellular material of the oocyte is being resorbed by the ovary (Fig. 29c). Atresia or atypical oocytes were observed in small females of less than 100 mm CW, and some atresia was observed in the ovarian lobes of sponged crabs (post-spawning). During atresia, oocytes are characterized by a lack of structural integrity, with a tendency for oocyte membranes to fall apart and appear to fuse between oocytes. Fusion of ooplasm and folding of membranes was typical of this condition (Fig. 29b). Lee et al. (1996) also found atypical oocytes in the blue crabs. In their study, Lee et al. (1996) referred to oocytes experiencing atresia as atypical oocytes and described them as lacking distinct structures such as nuclei and yolk bodies. The atypical oocytes or atretic oocytes seen during this study were mostly in primary growth; therefore, no disintegration of yolk globules was observed.

Lee et al. (1996) assigned each oocyte to a level from one to six based on its maturation. This classification system was valuable to this analysis to establish a baseline. However, there are considerable size differences that arise from using different fixatives and from using paraffin instead of JB-4®. The diameter ranges found in this study corroborate that because of the better resolution, more detail can be distinguished and smaller cells can be measured and described. Maturation stages for this study were based on the diameter ranges of the 55 oocytes per ovarian lobe. This sample size was based on the power of the test calculations, which concluded that measuring 55 oocytes would provide an accurate mean and an accurate variance.

Lee et al. (1996) established that in the blue crabs they analyzed, ovaries in immature females contained oocytes 16–24 µm wide. In this study, the comparable stage is oogonia, which ranged 9–12 µm in diameter. For stage 2, Lee et al. (1996) had blue crab oocyte diameter ranges of 30–60 µm. In this study, the early primary growth oocytes had a diameter range of 15–50 µm. In primary growth, the diameter ranges in this study were 51–144 µm, which is comparable to Lee et al. (1996) stage-three diameters ranging 66–100 µm. Early to mid-secondary growth oocytes in this study ranged 115–150 µm wide. There was no stage in Lee et al.’s (1996) study that was comparable to the early to mid-secondary growth stage found in the Tampa Bay blue crabs. It is possible that because of the higher resolution and the different embedding procedures used in this study, the early to mid-vitellogenic stage was more apparent than it would have been using the paraffin histology used by Lee et al. (1996). This study has secondary growth oocytes measuring 151–357 µm, and Lee et al. (1996) observed oocyte ranges in this stage of 168–288 µm. Lee et al. (1996) noted that the appearance of the most advanced oocytes of the post-spawning females was similar to the oocytes in early developmental stages (Table 11). During this study, it was also noted that females that carried fertilized eggs in a sponge had primary growth oocytes in the ovarian lobes. This is an important
feature because when determining sexual maturity based on carapace width, females that have had a sponge will have ovarian lobes in the primary growth developmental stage and yet they are sexually mature.

In this study, a new staging schema is introduced (Table 8). This is a very important contribution because the staging schemas used for blue crabs either are not based on histology or lack morphological detail. In recent publications, less than one paragraph is dedicated to the internal structure of the female blue crab, and these are based on descriptions from staging schemas done by Hard (1942). It is important to have a consistent staging schema for the species. Data needs to be compared from one publication to the next based on a common staging schema. Staging schemas based on gross morphology are not detailed enough and do not provide the information necessary to detect whether the oocytes have truly entered into secondary growth or whether they are still immature. Histology provides the only means to determine oocyte developmental stages.

External features of blue crabs in the Tampa Bay area are not clear indicators of the degree of gonadal maturity; for example, a large carapace width does not absolutely correlate to mature gonads (Table 9). Gonads in blue crab females may mature prior to the external features that are used as an indicator of sexual maturity. Using size alone to determine maturity would only provide a window of when the gonads are expected to be mature. Carapace width can overlap at each stage, providing an unreliable indicator of gonad stage (Fig. 31). Female crabs over 100 mm CW can be developing at different rates and therefore have ovarian lobes in different developmental stages. Sexual maturity should be determined by histological staging of the gonads. Additionally, the percentage of mature females in the different carapace-width ranges further suggests that carapace width is not a good indicator of gonadal development. Some females with smaller carapace widths in the 94–124-mm CW range were found to be vitellogenic (Fig. 32).

<table>
<thead>
<tr>
<th>Lee et al. (1996)</th>
<th>Diameter ranges (µm)</th>
<th>Tampa Bay blue crabs</th>
<th>Diameter ranges (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature females</td>
<td>16–24</td>
<td>oogonia</td>
<td>12–14</td>
</tr>
<tr>
<td>Stage two</td>
<td>30–60</td>
<td>early primary growth</td>
<td>15–50</td>
</tr>
<tr>
<td>Stage three</td>
<td>66–100</td>
<td>Late primary growth</td>
<td>51–144</td>
</tr>
<tr>
<td>No comparable stage</td>
<td>115–150</td>
<td>early to mid-secondary growth</td>
<td>115–150</td>
</tr>
<tr>
<td>Stage four</td>
<td>168–288</td>
<td>late secondary growth</td>
<td>151–357</td>
</tr>
</tbody>
</table>

Table 11. Comparison of oocyte diameter ranges between this study and the study by Lee et al. (1996).
Crabs with the larger carapace widths had ovaries in any of the developmental stages from primary growth to late secondary growth (Table 9). There are three main implications of these findings. First, carapace width alone is not an adequate indicator of maturity. Second, some females in the 90–124-mm CW range that had not yet molted into the adult instar showed obvious oocyte maturation and thus should be considered as maturing in analyses of size-at-maturity. Third, maturation must be determined histologically if female blue crabs have not yet molted into the adult instar.

Some females with larger carapace widths were found to have ovarian lobes in earlier developmental stages. This result concurs with the finding that the ovaries in ovigerous females were in primary growth. Female blue crabs may begin a new developmental cycle after a batch of eggs has been fertilized, and that is why oocytes in larger females are found to be in early developmental stages. Ovigerous females with oocytes in primary growth were found not only in this study but also in studies by Lee et al. (1996) and Hinsch (1972).

Tampa Bay blue crabs with oocytes in the late secondary growth stage occurred more frequently from December to March than at any other time of the year (see Fig. 30). Oocytes in the late secondary growth stage were also observed in specimens collected during July, August, and September. October was the only month during this study in which no vitellogenic crabs were observed. During April to June, oocytes in most females were undergoing primary growth or early secondary growth, and no oocytes in the late secondary growth stage were observed in any of the specimens. The only sponged females collected were found in November and December. Blue crabs with oocytes in early primary growth and juvenile crabs were found mostly during March, May, and June. Although sponged crabs were found only in November and December, sponged crabs may be found at other times of the year. A larger sample size would be useful in determining whether ovigerous females are present in other months of the year. There was no evidence that blue crabs in the Tampa Bay area may undergo a state of arrested development during the winter months (Johnson, 1980). Temperature ranges in the sampling locations did not fluctuate to extremes, and subtropical regions such as Tampa Bay provide favorable environmental conditions that allow year-round production of oocytes. The annual reproductive cycle of blue crabs in the Tampa Bay area may differ from the annual cycle of blue crabs in locations where water temperature ranges are cooler. As other studies corroborate (Steele and Bert, 1994), Tampa Bay area blue crabs have a reproductive cycle that is continuous throughout the year.
CONCLUSION

This study gives a significantly detailed description of the ovarian structure of the species. In blue crab, the ovaries are enclosed within distinct connective tissue that makes up the ovarian lobes. Inside these units, all oocytes are in a similar developmental stage. Ovarian pouches have a germinal zone, also referred to as the germaria, which is located at or around the center of the ovarian pouches and which contains the oogonia and the early primary growth oocytes. Oocytes in more advanced developmental states move to the periphery of the ovarian pouch. This distribution of cells from the center of the ovarian pouch to the periphery illustrates the advance of oogenesis.

A new staging schema for the species is introduced with this study and is based on the histological morphology of the ovaries. The new staging schema, based on high-resolution light microscopy, is important for the analysis of data sets that involve oogenesis. Developmental stages of blue crabs in this study progress from oogonia to primary growth, which is divided into early primary growth and late primary growth, and then to secondary growth. Secondary growth consists of early secondary growth to mid-secondary growth and late secondary growth. Oogonia were studied and found to be clear with scant ooplasm and with interphase chromosomes in the periphery. Primary growth involves the formation of a perinuclear yolk complex (i.e., PAS positive body), which disperses its content into the ooplasm. After the perinuclear yolk complex disperses, observable morphological characteristics such as lambrush chromosomes, cortical alveoli, and lipids deposit are noted. Therefore, during primary growth, the oocyte is basophilic, and vesicles containing oil droplets were seen as clear (i.e., empty, because of the slide preparation process) vesicles. In the nucleus of primary growth oocytes, filaments were observed. These filaments are indicative of lambrush chromosomes and could signify that preprimary growth oocytes have entered into arrested meiosis I.

The developmental stages continue into secondary growth (vitellogenesis), the onset of which is signified by the formation of yolk globules. During secondary growth, oocytes become more acidophilic and yolk globules increase in volume and numbers. Full-grown oocytes have a fluid ooplasm where the yolk globules have coalesced. After fertilization, the female crab carries fertilized eggs in a sponge outside her abdomen. While a female blue crab carries the sponge eggs, ovaries have only primary growth oocytes. Throughout development, follicular cells within the ovaries can be observed. They have an elongated nucleus and surround the oocytes. These cells do not multiply; they remain as a single layer of squamous cells throughout oogenesis. Basal lamina and somatic cells are also described. Neither of these cells had previously been described in studies of the species. It was noted that follicle cells are always inside the germinal compartment and that somatic cells are outside the germinal compartment. This study
corroborates studies of different species of the family Portunidae, that the germinal zone is found in the center of the ovarian lobe and that the oocytes, as they develop, move outwards to the periphery of the ovarian lobe. A concise description of the process of oogenesis by describing development from preprimary growth to full-grown is given.

The reproductive cycle of the blue crab in the Tampa Bay area showed that female blue crabs undergoing late secondary growth were found mostly from December to March. Blue crabs in the Tampa Bay area have secondary growth oocytes during the winter months, and spawning occurs from winter to summer. Some specimens in the late secondary growth stage were also observed during July, August, and September. During the April to June, no females undergoing late secondary growth were observed. Most of the females during those months were undergoing primary growth or early secondary growth. The only sponged females collected were found in November and December. Blue crabs with oocytes in early primary growth and juvenile crabs were noted mostly during the March, May, and June. Blue crabs in the Tampa bay area do not have an arrested secondary growth (vitellogenesis) during the winter months. Not all females in the population are at the same developmental stage, and therefore there is a wide spread between oocyte diameters per month, as expected. The carapace width of female blue crabs in the different developmental stages overlapped and was not a good indicator of maturity or developmental stage. Females that exhibit immature external features could show internal features of maturity. It is known in published studies of the species that blue crabs reach adult size at 130–139 mm CW. However, this study finds that blue crabs with a smaller carapace width can be undergoing secondary growth. Furthermore, animals with large carapace widths were found that they could be in all developmental stages. Some females in the 90–124-mm CW range that had not yet molted in the adult instar showed obvious oocyte maturation and thus should be considered as maturing in analyses of size at maturity.

Size at maturity of female blue crabs should be determined by histological means if the female has not yet molted into the adult instar.
REFERENCES


**APPENDIX**

Table A-1. 20% Paraformaldehyde stock solution (Hayat, 1981)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraformaldehyde</td>
<td>500 g</td>
</tr>
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<td>Distilled water</td>
<td>2090 mL</td>
</tr>
</tbody>
</table>

PMFA is dissolved in distilled water and heated to 60°C under the hood. 1N NaOH is added to depolymerize the solution, approximately 20 mL. The solution needs to cool down to room temperature, and then the final volume is adjusted to 2500 mL. The solution is filtered and refrigerated.

Table A-2. 5% Paraformaldehyde (PFMA) 0.1 molar phosphate buffer (Humason, 1972)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% PFMA</td>
<td>1000 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 mL</td>
</tr>
<tr>
<td>0.2 M phosphate buffer</td>
<td>2000 mL</td>
</tr>
</tbody>
</table>

Dilute 20% PFMA stock solution 1:1 with distilled water. Dilute that 1:1 with 0.2 M phosphate buffer, pH 7.4.

Table A-3. Infiltration Routine

<table>
<thead>
<tr>
<th>Component</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% EtOH</td>
<td>Sample can remain indefinitely</td>
</tr>
<tr>
<td>95% EtOH</td>
<td>Two to three hours depending on tissue size and thickness</td>
</tr>
<tr>
<td>50% JB-4</td>
<td>Four days</td>
</tr>
<tr>
<td>100% JB-4</td>
<td>Four days</td>
</tr>
<tr>
<td>Activated JB-4</td>
<td>Four days</td>
</tr>
</tbody>
</table>

Sample is embedded.
APPENDIX (Continued)

Table A-4. Hematoxylin and Eosin (H&E)

<table>
<thead>
<tr>
<th>Weigert’s Hematoxylin: 5% Hematoxylin stock solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoxylin</td>
</tr>
<tr>
<td>95% Ethanol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weigert’s Solution A: 1% Hematoxylin in 95% Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% Hematoxylin stock solution</td>
</tr>
<tr>
<td>95% Ethanol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weigert’s Solution B</th>
</tr>
</thead>
<tbody>
<tr>
<td>29% Aqueous ferric chloride</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
<tr>
<td>HCl</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weigert’s Working Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A</td>
</tr>
<tr>
<td>Solution B</td>
</tr>
</tbody>
</table>
Table A-5. Periodic Acid Schiff’s/Metanil Yellow (PAS/MY)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1% Periodic Acid</strong></td>
<td></td>
</tr>
<tr>
<td>Periodic acid</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>250 mL</td>
</tr>
<tr>
<td><strong>Schiff’s Reagent</strong></td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>900 mL</td>
</tr>
<tr>
<td>Basic fuchsin</td>
<td>5 g</td>
</tr>
<tr>
<td>1N hydrochloric acid</td>
<td>100 mL</td>
</tr>
<tr>
<td>Potassium metabisulfite</td>
<td>10 g</td>
</tr>
<tr>
<td><strong>Weigert’s Working Solution</strong></td>
<td></td>
</tr>
<tr>
<td>Solution A</td>
<td>150 mL</td>
</tr>
<tr>
<td>Solution B</td>
<td>150 mL</td>
</tr>
<tr>
<td><strong>Metanil Yellow Counterstain</strong></td>
<td></td>
</tr>
<tr>
<td>Stock Solution A: 0.1% aqueous metanil yellow</td>
<td></td>
</tr>
<tr>
<td>Metanil yellow</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>500 mL</td>
</tr>
<tr>
<td>Stock Solution B: 0.5% glacial acetic acid</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>995 mL</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>5 mL</td>
</tr>
<tr>
<td><strong>0.02% Metanil Yellow in 0.25% in Acetic Acid, Working Solution</strong></td>
<td></td>
</tr>
<tr>
<td>Stock Solution A</td>
<td>50 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>75 mL</td>
</tr>
<tr>
<td>Stock Solution B</td>
<td>125 mL</td>
</tr>
</tbody>
</table>