# A COMPARATIVE ANATOMICAL AND HISTOLOGICAL STUDY OF THE FEMALE REPRODUCTIVE TRACTS OF THE BROWN SPOTTED SHRIMP, *PENAEUS AZTECUS* (IVES); THE PINK SHRIMP, *PENAEUS DUORARUM* (BURKENROAD); AND THE WHITE SHRIMP, *PENAEUS SETIFERUS* (LINNAEUS)

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LOVINGLY DEDICATED TO

MY HUSBAND, HUBERT

AND DAUGHTER, KIM

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ABSTRACT

Ann Cottle Burden. A COMPARATIVE ANATOMICAL AND HISTOLOGICAL STUDY OF THE FEMALE REPRODUCTIVE TRACTS OF THE BROWN SPOTTED SHRIMP, PENAEUS AZTECUS (IVES); THE PINK SHRIMP, PENAEUS DUORARUM (BURKENROAD); AND THE WHITE SHRIMP, PENAEUS SETIFERUS (LINNAEUS). (Under the direction of Edward P. Ryan) Department of Biology, August, 1967.

A description and comparison are given of the anatomy and histology of the female reproductive systems of the brown spotted shrimp, *Penaeus aztecus*; the pink shrimp, *P. duorarum*; and the white shrimp, *P. setiferus* during ovarian development.

Four stages of ovarian development (undeveloped, developing, nearly ripe, and ripe) and the spent stage were determined for the three species on the basis of color of the ovary, width of the ovary, and percentage of ova in various size groups in the ovary.

The length of the ovary is related to the total length of the shrimp rather than to the stage of ovarian development. However, the width of the ovary is a probable indication of its developmental stage.

The female reproductive tracts of these species of penaeid shrimp were found to be basically alike in their anatomy, histology, and stages during ovarian maturation.

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# INTRODUCTION

The purpose of this investigation is to make a comparative anatomical and histological study of the female reproductive systems of the brown spotted shrimp, *Penaeus aztecus* (Ives); the pink shrimp, *P. duorarum* (Burkenroad); and the white shrimp, *P. setiferus* (Linnaeus) during various stages of ovarian maturation. These three species are the basis of North Carolina's shrimp fishery. A comparative histological study of their reproductive anatomy and stages of ovarian development is not available and is essential for an understanding of penaeid shrimp reproduction.

## Background Literature

### *Penaeus aztecus*

Burkenroad (1939) noted that the proportion of brown spotted males to females near shore was one to one, whereas offshore, females outnumbered males two to one. Shrimp with mature ovaries were obtained from deep water at various times of the year. He proposed that females spawned a number of times during a poorly defined spawning season. Spawning grounds of the North Carolina population were not located (Burkenroad, 1949), however, Williams (1965) theorized that the spawning site of brown spotted shrimp is deeper and farther offshore than that of pink or white shrimp. The spawning season of brown spotted shrimp in North Carolina is during the winter months (Williams, 1965).

### *Penaeus duorarum*

Williams (1953, 1955, 1958, 1959, 1960) studied growth, postlarval characters, and ecology of the pink shrimp as well as brown spotted and white shrimp. Geographical distribution and habitat were studied by Gunther (1961, 1962a). However, only limited information is available pertaining to reproductive anatomy of the species.

Dobkin (1961) described eggs that had been recently spawned as yellow-brown and opaque with a diameter of 0.31-0.33 mm. In the same year, Cummings observed various stages of ovarian maturation and noted a probable spawning season in Florida from April to July. The spawning season is comparable in North Carolina (Williams, 1965). Cummings (1961) noted that multiple spawning probably occurs in pink shrimp as in *P. setiferus* and *P. japonicus*.

*Penaeus setiferus*

Spaulding (1908) briefly described the external and internal gross anatomy of the reproductive system of white shrimp. Young (1959) in his extensive study of *P. setiferus* described the genital apparatus and presented illustrations.

In 1931 the United States Bureau of Fisheries established Gulf Investigations with the purpose of gathering information about the biology of white shrimp. Lindner and Anderson (1956) reported that, in 1931, *P. setiferus* composed over 98 per cent of the commercial catch in the Gulf of Mexico. As a result, they studied this species extensively.

The spawning season of white shrimp varies in different localities, but it generally begins in late March and is nearly completed by late September (Anderson, 1955). White shrimp spawn at sea rather than in estuaries (Lindner and Anderson, 1954).

Spaulding (1908) noted that at each ovulation, female shrimp released eggs into the water rather than attaching them to swimmerets. Penaeus setiferus has an extremely high reproductive potential and releases 500,000 to 1,000,000 eggs at a single spawning (Anderson, King, and Lindner; 1949a).

Recently spawned eggs of white shrimp are demersal, nonadhesive and spherical (Pearson, 1935). The mean diameter for live eggs is 0.28 mm (Pearson, 1939). Hudinaga (1942) reported a similar size for P. japonicus. These eggs of P. japonicus possessed a thin, delicate chorion which appeared bluish-purple under the microscope.

Much work has been done with postlarval identification of white shrimp (Williams, 1953), although little information has been recorded about gonad maturation. King (1948) described developmental stages of male and female gonads. He attempted to correlate ovarian microscopic anatomy with age and spawning frequency, but was unable to establish an age index. He did, however, indicate that a female may spawn more than once per breeding season.

## Commercial Importance

In North Carolina the commercial shrimp fishery ranks first in dollar value and is exceeded in volume only by the menhaden fishery (North Carolina Commercial Fisheries Newsletter, 1967). The annual catch in North Carolina increased ten-fold from 1918 to 1948 following the development of the Otter trawl (Broad, 1948).

Accompanying the expansion of the shrimp industry is a growing concern about possible depletion of this important resource (Anderson, Lindner, and King; 1949b). The existence and development of the industry depend upon survival of animals which may require management by various conservation agencies. In order to insure a maximum sustained yield, these agencies will need information relating to most aspects of shrimp biology, especially reproductive biology.

## Taxonomy

Seventeen species of the family Penaeidae inhabit coastal waters of North and South Carolina (Williams, 1965). In contrast to almost all other decapod crustaceans, penaeid shrimp do not carry their eggs until they hatch, and hatch from eggs at a stage earlier than a zoea (Schmitt, 1965).

There has been much controversy concerning usage of the specific name *setiferus* for white shrimp. Gunther (1962, p.108) states, "Rigid interpretation of the Rules and literature shows that the name of the South American white shrimp is *Penaeus setiferus* (Linnaeus). The name of the North American white shrimp is *Penaeus fluvialitis* (Say)." Nevertheless, the name of the South American species is so well established in the literature for white shrimp caught in the North Atlantic, that it is retained here.

## Life History

Larval development requires from two to three weeks and includes at least ten distinct stages excluding the egg. These consist of five naupliar, three protozoeal, and two mysis stages (Anderson, 1955).

Nauplii emerge about 20 to 24 hours after eggs are spawned (Pearson, 1939). The nauplis undergoes five molts before becoming a protozoea. After several additional molts the terminal mysis stage molts into the first of two postlarval stages. These are planktonic as were preceeding stages (Anderson, 1948).

During early developmental stages, the shrimp are transported by currents from offshore spawning sites into brackish inland waters. These estuaries are designated as "nursery grounds" and here young shrimp adopt a benthic existence (Anderson, 1948).

## Geographical Range

The Atlantic range of brown spotted shrimp is from New Jersey through the West Indies to Uruguay (Williams, 1965). Brown spotted shrimp are most often caught over a muddy bottom. The major fishery for this species is located along the Texas coast (Gunther, 1956).

Pink shrimp range from Chesapeake Bay to the Gulf of Mexico, the West Indies, Brazil, Bermuda, and West Africa from Mauritania to Angola (Williams, 1956). *Penaeus duorarum* inhabits estuaries and inner littoral regions and is found on coral-sand substrates usually containing mollusk shells. The greatest concentration of pink shrimp is located along Campeche Banks, Mexico (Gunther, 1956).

White shrimp (see note on taxonomy, p. 4) are found on the Atlantic Coast from Fire Island, New York, to Cape Kennedy, Florida; Gulf of Mexico from Pensacola to Campeche, Mexico; Cuba; and Jamaica. The greatest production of white shrimp has been from the Louisiana coast (Gunther, 1961). Most active of the three species, white shrimp inhabit estuaries and inner littoral regions with muddy substrates (Williams, 1965).

## Relative Importance in the Catch

According to Anderson (1948) *P. aztecus* is the dominant species in the Atlantic catch (48 per cent). Second is *P. duorarum* composing about 26 per cent yield and last is *P. setiferus* with 22 per cent yield. In North Carolina, from 1948 to 1949, 68 per cent of the commercial catch was *P. aztecus*; 17 per cent, *P. setiferus*; and 14 per cent *P. duorarum* (Broad, 1951).

## MATERIALS AND METHODS

The tissues used in this work included preserved shrimp ovaries provided by Dr. Austin B. Williams of the Institute of Marine Sciences, University of North Carolina, and live shrimp collected at various times from April through August, 1967. Fresh material was secured in an attempt to supply any developmental stages of the ovary that were lacking in Dr. Williams's collection.

Dr. Williams collected his specimens from 1952 through 1957. He fixed the ovaries in Bouin's fluid and stored them in a solution of 70 per cent alcohol and glycerine. Samples were dated, sites of collection were noted, and ovaries staged macroscopically according to color, relative size, and turgidity.

Live shrimp were caught in a trawl along the North Carolina coast, either in the Atlantic Ocean off Beaufort Inlet, in Core Creek, or in Bogue Sound. They were retained in a chilled state until dissected.

Immediately prior to dissection, external measurements were made with a vernier micrometer. Total length was considered as the distance from tip of rostrum to tip of telson.

Following dissection, ovarian lengths were measured *in situ* for each available stage of development. Widths of preserved ovaries (anterior region of the abdominal lobe) were measured later with an ocular micrometer in a compound microscope.

Oviducts were located by teasing the lateral muscles just anterior to the abdomen. Frequently the surface was flooded with Bouin's fluid. This facilitated removal of the duct by reducing its flaccidity. Oviduct lengths were measured *in situ* with a vernier micrometer and after preservation, their widths were measured with an ocular micrometer.

Excess Bouin's fluid was removed by soaking tissues in several changes of Lenoir's fluid (Gray, 1958). Brittleness of ripe ovaries after this treatment was prevented by dealcoholizing with cedarwood oil and by changing paraffin several times to insure maximum impregnation.

Sections were cut at six to eight microns with a rotary microtome. Before cutting each section, the face of the tissue block was brushed with a 0.5 per cent solution of collodion in acetone or absolute alcohol to prevent shattering.

Sections were stained with Harris's or Delafield's hematoxylin and counterstained with eosin y for general study. For study of nuclear structure, random sections were stained with Heidenhain's iron hematoxylin. Mallory's triple connective tissue stain was employed for detection of muscle.

The anterior, median, and posterior regions of the ovary of P. aztecus were compared to determine if they were homogeneous in structure and developmental stage. Generally they were found to be so. Thereafter, only the anterior region of the abdominal lobe was used for comparative purposes. The method for determining ova widths was adapted from Clarke (1934).

Random measurements of ova from ovaries that were in various stages of development, were made with an ocular micrometer. Mean widths and ranges were determined and ova were categorized according to size. After size groups were established, ova in each category were counted for each stage of ovarian development and their relative percentages were plotted on a bar graph.

Photomicrographs were taken with a Praktina 35 mm camera on Kodak Plus X (ASA 125) film at one-fifieth of a second.

RESULTS

## Gross Anatomy

### General Location

The female reproductive tract of *Penaeus aztecus* (Figure 1) consists of paired ovaries and oviducts. The ovaries extend from the anterior region of the cephalothorax to the base of the telson. Each ovary has one slender anterior lobe which is dorsolateral to the esophagus and stomach, seven or eight lateral or median lobes which are ventral to the heart and dorsal to the hepatopancreas, and one posterior or abdominal lobe which extends the entire length of the abdomen. The posterior lobe is ventral to the dorsal abdominal artery and dorsal to the intestine. The oviducts are attached to the tips of the sixth or seventh lateral lobes and open externally at the bases of the third pereiopods.

The gross anatomy of the ovaries and oviducts of *P. duorarum* were observed to be similar to those of *P. aztecus*. Freshly dissected tracts of *P. setiferus* were not collected. According to King (1948), the general anatomy of the ovary in the white shrimp was similar to that seen in *P. aztecus* and *P. duorarum*.

## Characteristics of the Ovary During Development

There are four stages of ovarian development in the species observed. These include: undeveloped, developing, nearly ripe, and ripe (hereafter abbreviated as U, D, NR, and R respectively). These stages can be determined macroscopically by color, presence or absence of chromatophores, and in most cases, turgidity (Table 1).

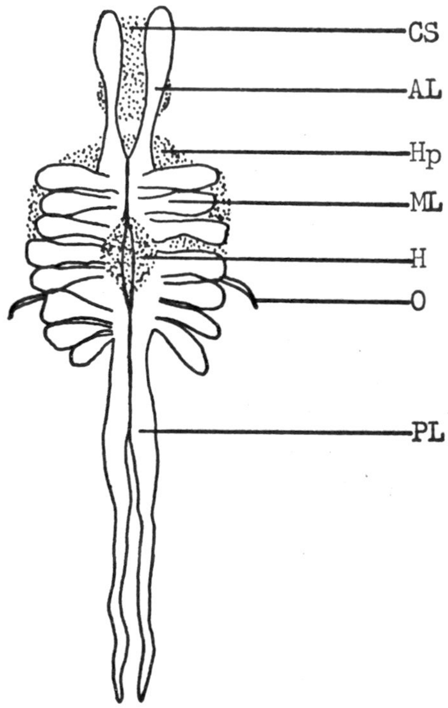



Figure 1. Diagram of female reproductive tract of Penaeus aztecus (Ives) with reference organs. CS-cardiac stomach. AL-anterior lobe. Hp-hepatopancreas. ML-median lobe. H-heart. O-oviduct. PL-posterior lobe. Ovary X 2.

Table 1. Macroscopic characteristics of ovary during developmental stages

| Species | Characteristic | Condition of ovary during developmental stages | | | |
|---|---|---|---|---|---|
| | | U | D | NR | R |
| | Color | clear | white-lt. blue | blue-green | drab olive-green |
| *P. aztecus* | Chromatophore | none | few | many | not apparent |
| | Turgidity | very flaccid | slightly flaccid | slightly turgid | very turgid |
| | Color | clear | white-lt. blue | blue-green | drab olive-green |
| *P. duorarum* | Chromatophore | none | few | many | not apparent |
| | Turgidity | very flaccid | slightly flaccid | slightly turgid | very turgid |
| | Color | clear | opaque, white | yellow-orange | drab olive-brown |
| *P. setiferus** | Chromatophore | none | few | many | not apparent |
| | Turgidity | very flaccid | slightly flaccid | slightly turgid | very turgid |

*Living specimens not observed here; from King (1948)

Spent ovaries are particularly difficult to recognize by macroscopic examination alone. Spent ovaries of brown spotted shrimp were not obtained during this study. Spent ovaries of pink shrimp are drab gray-green, lighter than when ripe, and collapsed. Spent ovaries of white shrimp are light drab olive-brown and reduced in size from the ripe state.

Correlations of Ovarian Measurements

Mean lengths of ovaries and mean total lengths of *P. aztecus* and *P. duorarum* during maturation were determined (Table 2).

Table 2. Mean total lengths and mean lengths of ovaries in *P. aztecus* and *P. duorarum*

| Species | Stage | Total length (mm) | Ovarian length (mm) | Ovarian length expressed as mm/100 mm total length |
|---|---|---|---|---|
| *P. aztecus* | U | 139.6 | 92.1 | 66.6 ±1.63* |
| | D | 142.2 | 91.6 | 64.1 ±2.18 |
| | NR | 123.5 | 81.0 | 65.7 ±2.64 |
| | R | not observed | | |
| *P. duorarum* | U | 133.8 | 88.5 | 66.0 ±1.15 |
| | D | 136.2 | 90.6 | 66.4 ±1.05 |
| | NR | 139.0 | 93.7 | 67.3 ±1.29 |
| | R | 132.3 | 86.5 | 65.4 ±0.14 |

*Mean ±standard error (five observations for each stage)

There is a high coefficient of correlation between ovary length and total body length of *P. aztecus* and *P. duorarum* (r = 0.95 and 0.97 respectively). However, there is little variation in mean ovary length in either species during maturation. Based on these observations, it is likely that the ovary length of *P. setiferus* would not be a direct indication of degree of maturation.

Mean widths of preserved ovaries of brown spotted, pink, and white shrimp during developmental stages were determined (Table 3).

Table 3. Mean widths of preserved ovaries during developmental stages

| Species | Stage | Mean width of ovaries (μ)* |
|---|---|---|
| | U | 1461.6 |
| | D | 1601.6 |
| | NR | 1758.4 |
| | R | 2049.6 |
| | Sp** | 1036.0 |
| | U | 1388.8 |
| | D | 1652.0 |
| | NR | 2232.0 |
| | R | 2514.4 |
| | Sp** | 1164.8 |
| | U | 1663.2 |
| | D | 1864.8 |
| | NR | 2302.8 |
| | R | 2699.2 |
| | Sp** | 800.8 |

*Five observations for each stage of development in each species
**Spent

There is a highly significant difference between widths of ovaries of each species during development as well as after spawning ($p < 0.001$). Thus ovary width is a probable indication of its stage of development. However, ovarian length does not vary significantly with developmental stages but rather is related to total body length.

## Microscopic Anatomy

A study was made of ova widths in the anterior, median, and posterior regions of an early developing ovary of P. aztecus. Table 4 contains mean measurements of those ova.

Table 4. Mean widths of ova from anterior, median and posterior regions of P. aztecus (very early developing ovary)

| Ovarian region | Mean widths of ova (μ)* |
|---|---|
| Anterior | $32.3 \pm 1.97$ |
| Median | $31.6 \pm 1.95$ |
| Posterior | $34.8 \pm 2.23$ |

*Mean $\pm$standard error (50 ova measured in each ovarian region)

No significant difference between the mean ova widths of the three ovary regions was shown by use of a "t" test. In each case $p > 0.05$, therefore homogeneous ovary structure is inferred.

Table 5 is a summary of mean widths of ova from ovaries in various stages of development. Mean ovary width increases with maturation.

Figure 2 presents the percentage of egg sizes in each stage of ovarian development. Each stage of maturation is characterized by a majority of one ova size category. These stages coincide with those that had earlier been staged macroscopically.

Spent ovaries are identified microscopically by the presence of many follicle cells left by a previous spawning and peripherally located resorbed ova. If there are any intact ova present, they are of the immature size group.

Table 5. Ranges and widths for size groups of ova

| Ova size group* | Range (μ) | Mean width (μ)** |
|---|---|---|
| Immature | less than 50 | 23.9 |
| Maturing | 50-120 | 90.5 |
| ***Nearly mature | 121-148 | 140.6 |
| ***Mature | over 150 | 157.2 |

*These size groups are defined thusly to avoid confusion between reference to degree of ova development and stage of ovary maturation.
**50 ova measured for each stage of development
***Data are mean figures and actual measurements overlap in some instances.

## General Histology of the Ovary

The general histology of the ovary is similar in brown spotted, pink, and white shrimp. In a cross sectional view, the ovary is divided into six or seven lobules by longitudinal septa of connective tissue.

In each species the ovary wall consists of three layers, an outer layer of pavement epithelium (mean thickness 13.6 μ), a middle layer of connective tissue (mean thickness 25.5 μ), and an inner layer of germinal epithelium (Figure 3). The outer two layers are continuous around the ovary but the inner layer is confined to an area called the "zone of proliferation" (Gutsell, 1936) or germinative zone (Figure 4) which gives rise to oogonia. Mitotic figures were observed in this region. The mean width of the germinative zone is 637.5 μ.

Figure 2. Percentage of ova size groups for stages of ovarian development

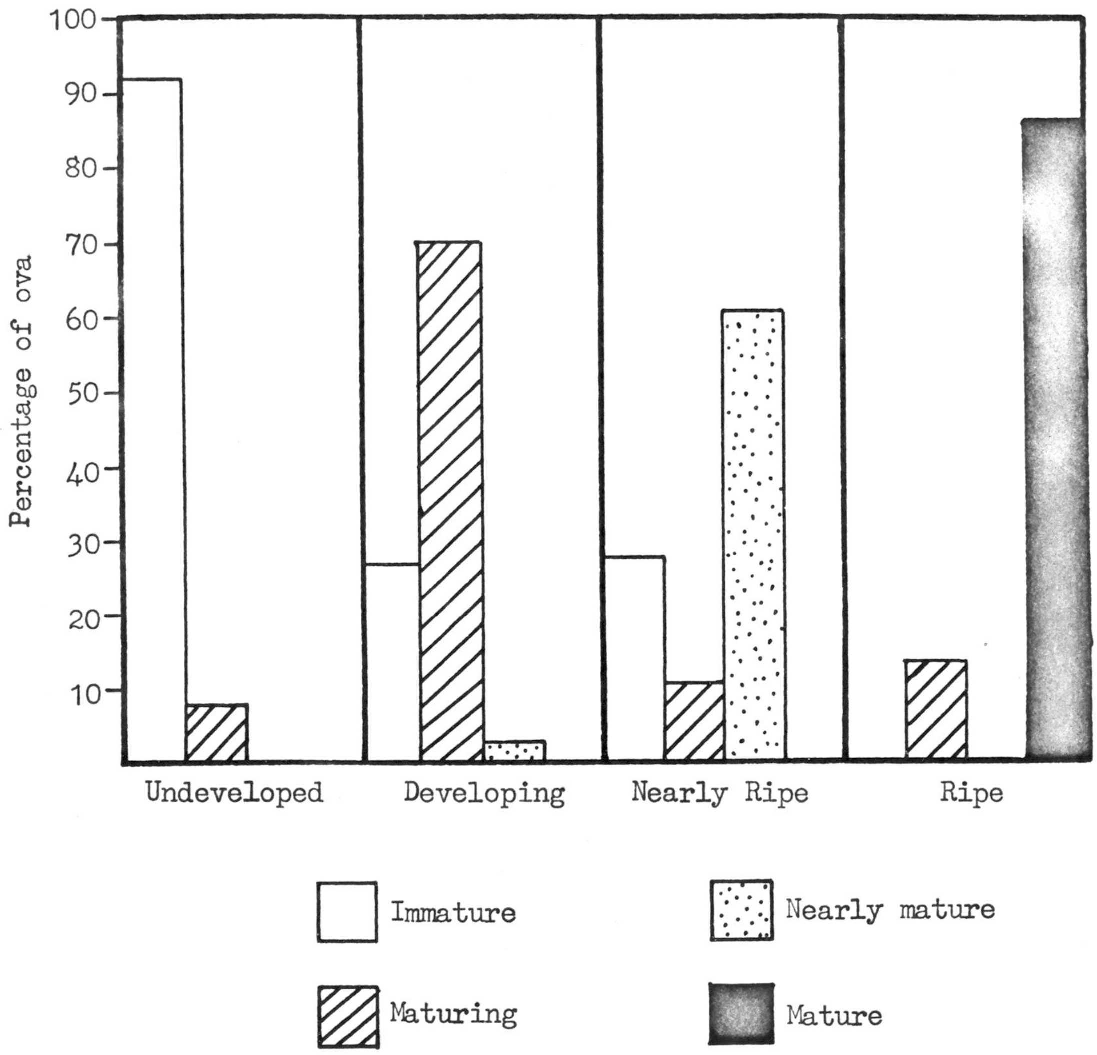
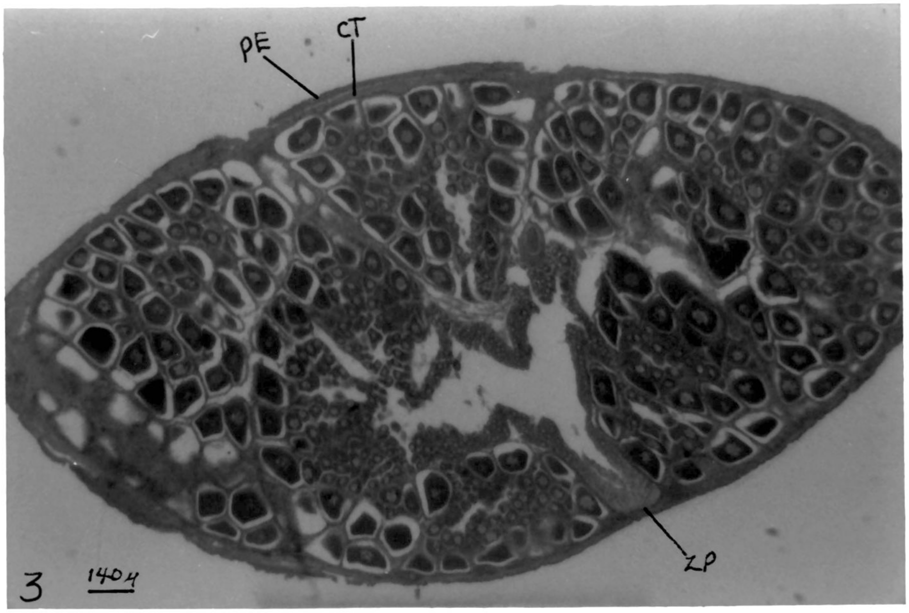
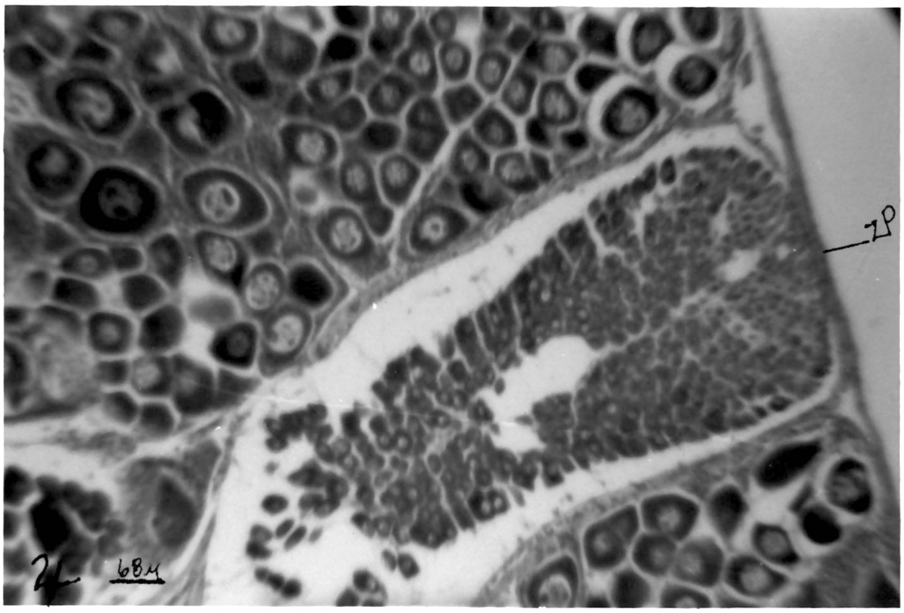


Figure 3. Section of developing ovary of *Penaeus aztecus*. PE-pavement epithelium. CT-connective tissue. ZP-zone of proliferation. X 35.

Figure 4. Section of undeveloped ovary of *Penaeus duorarum*. Note zone of proliferation-ZP. X 100.


PE
CT
ZP
3
140μ


ZP
4
68μ

In the anterior and median lobes, the germinative zone is located in a ventral position, whereas in the posterior lobe, it is more ventromedial.

Blood vessels or sinuses (Figure 5) can be seen in most cross sections. No muscle was detected in the ovary wall in any of the three species by Mallory's triple connective tissue stain.

## General Histology of the Oviduct

Oviduct structure is similar in all three species. The oviduct wall consists of three layers, an outer layer of epithelium (thickness of 12.5-17.5 μ), a middle layer of connective tissue (thickness of 25.0-30.0 μ), and an inner layer of columnar epithelium (thickness of 45.0-50 μ). No muscle was detected by Mallory's stain.
Figures 6 and 7 are photographs of oviducts of brown spotted and pink shrimp with developing ovaries. Figure 8 is a photograph of an oviduct from a white shrimp with ripe ovaries.

The only oviduct observed for *Penaeus setiferus* was one from a shrimp with ripe ovaries. Ripe oocytes were seen within the duct (width 700.0 μ) The mean widths of oviducts of *P. aztecus* and *P. duorarum* were 350 μ and 430 μ respectively.

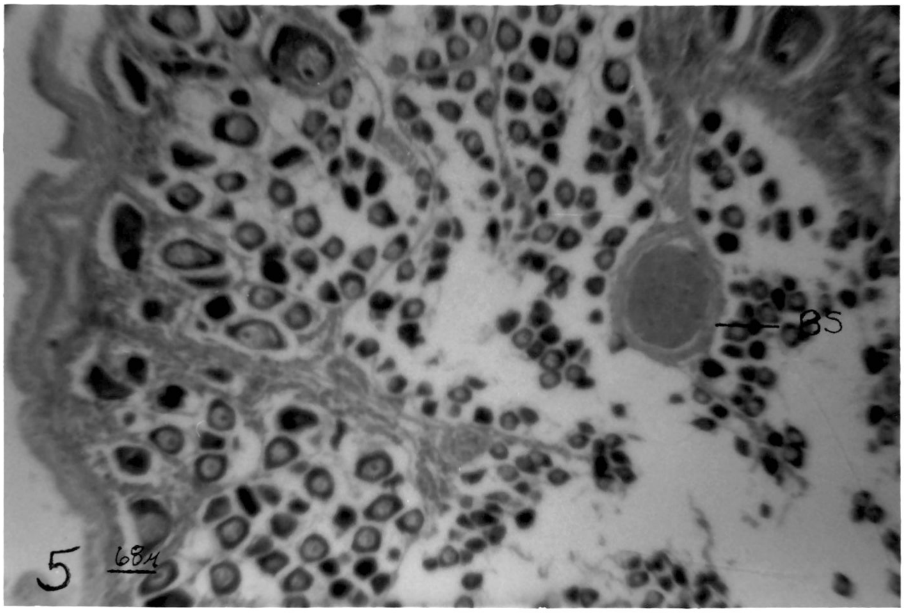
## Ovarian Maturation of *Penaeus aztecus*

### Undeveloped

Undeveloped ovaries (Figure 9) are characterized by approximately 92 per cent immature ova (mean width 23.9 μ) and eight per cent maturing ova (mean width 90.5 μ). The nongranular cytoplasm of immature ova is basophilic. The nucleus (mean width 17.6 μ) is

Figure 5. Section of ovary of *Penaeus aztecus*. Note blood sinus. X 100.

Figure 6. Section of oviduct of *Penaeus aztecus* with nearly ripe ovaries. E-epithelium. CT-connective tissue. CE-columnar epithelium. X 100.

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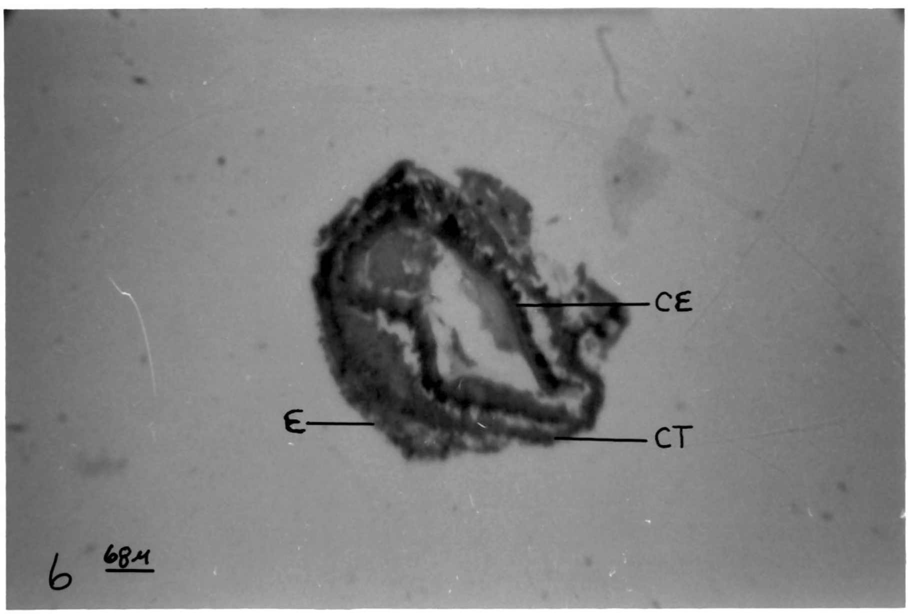
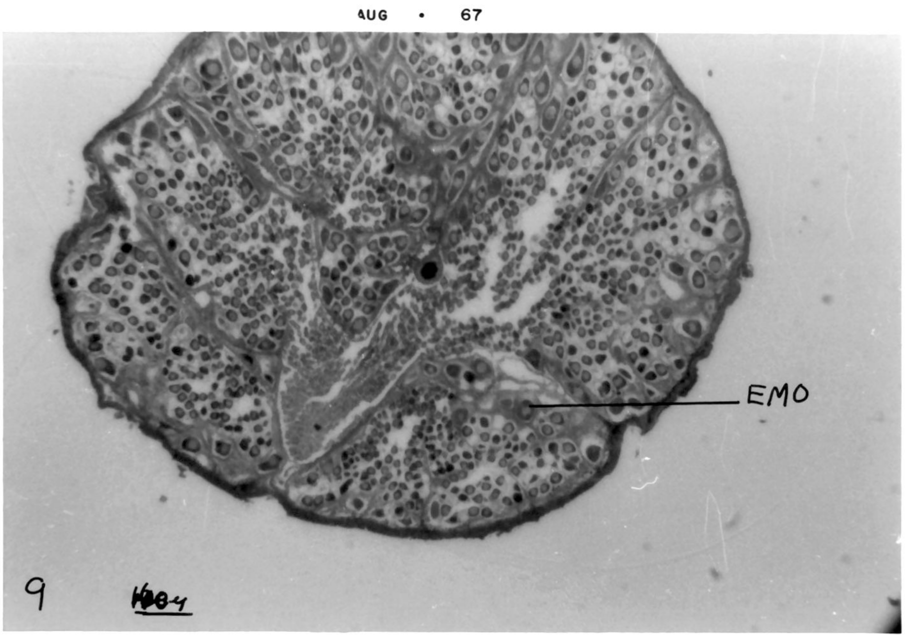


Figure 7. Section of oviduct of <u>Penaeus duorarum</u> with nearly ripe ovaries. E-epithelium. CT-connective tissue. CE-columnar epithelium. X 100.

Figure 8. Section of oviduct of <u>P. setiferus</u> with ripe ovaries. Ripe ovum in lumen. (Clear streaks in section caused by shattered tissue) X 100.

Figure 9. Section of undeveloped ovary of P. *aztecus*.
EMO-early maturing ovum. X 35.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EMO
9

highly vesicular and no distinct nucleoli were detected with Harris's or Delafield's hematoxylin. With Heidenhain's iron hematoxylin, many (approximately 20) chromatin particles can be seen within the nuclear membrane. A few early maturing ova can be seen near the periphery of the ovary, indicating movement toward the outer limits of the ovary during maturation.

Developing

As the ova begin to mature, one or two nucleoli are present and a ring of fine chromatin particles can be seen just inside the nuclear membrane. The finely granular cytoplasm is moderately basophilic.

Figure 10 shows an early developing ovary and its location relative to the dorsal abdominal artery.

The developing ovary (Figure 11) contains about 70 per cent maturing ova, 27 per cent immature ova, and three per cent nearly mature ova.

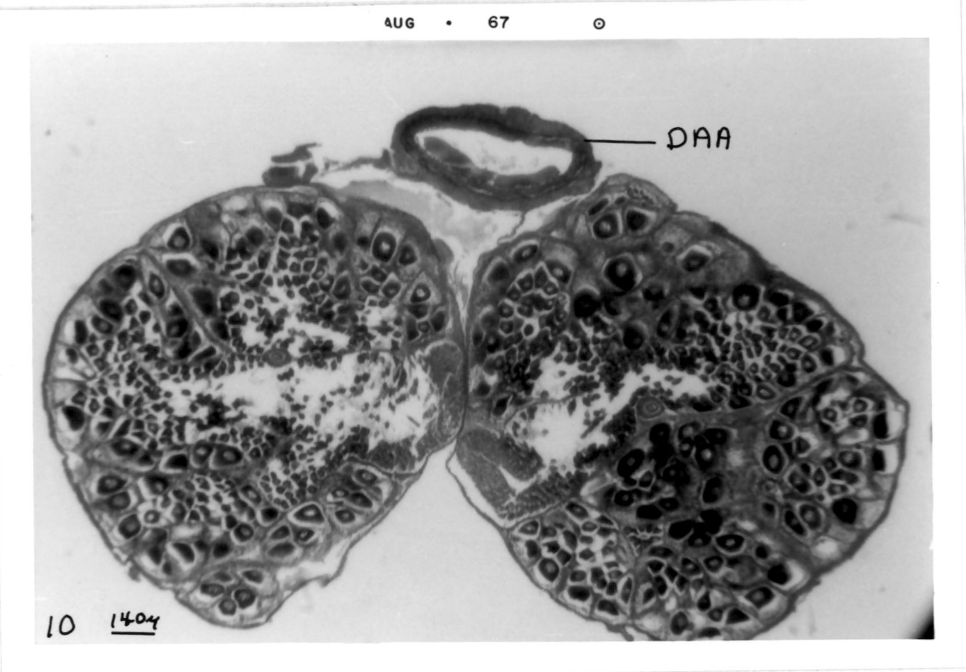
There are five to nine (usually seven) nucleoli in a nucleus (mean width 32.9 $\mu$) of a late developing ovum. These often are in a ring just inside the nuclear membrane with diffuse nuclear material concentrated inside the ring (Figure 12). Note absence of follicle cells around immature ova.

Nearly Ripe

The granular cytoplasm of a nearly mature ovum is acidophilic. There are no large nucleoli but rather 35-50 scattered chromatin particles which appear as a diffuse mass (Figure 13). As development advances, the nucleus "fades" and seems to be clear (Figure 14) and approximately 61 per cent immature nearly mature ova and 11 per cent maturing ova can be seen (Figure 15).

Figure 10. Section of early maturing ovaries of P. aztecus. DAA-dorsal abdominal artery. X 35.

Figure 11. Section of a developing ovary of P. aztecus. Note relative sizes and numbers of ova. X 35.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DAA
10
140μ

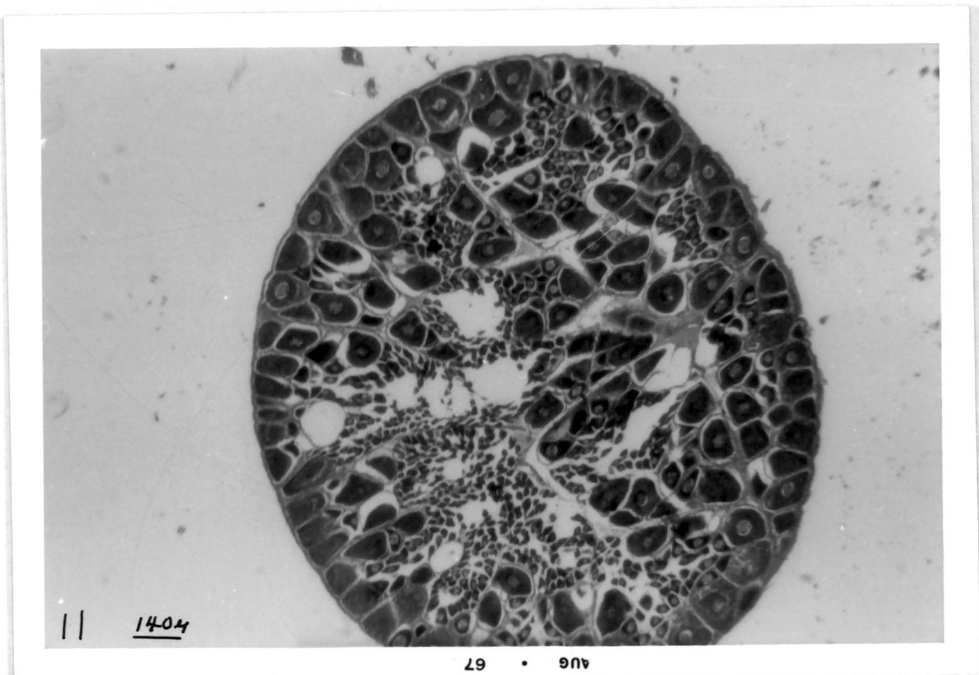
11
140μ
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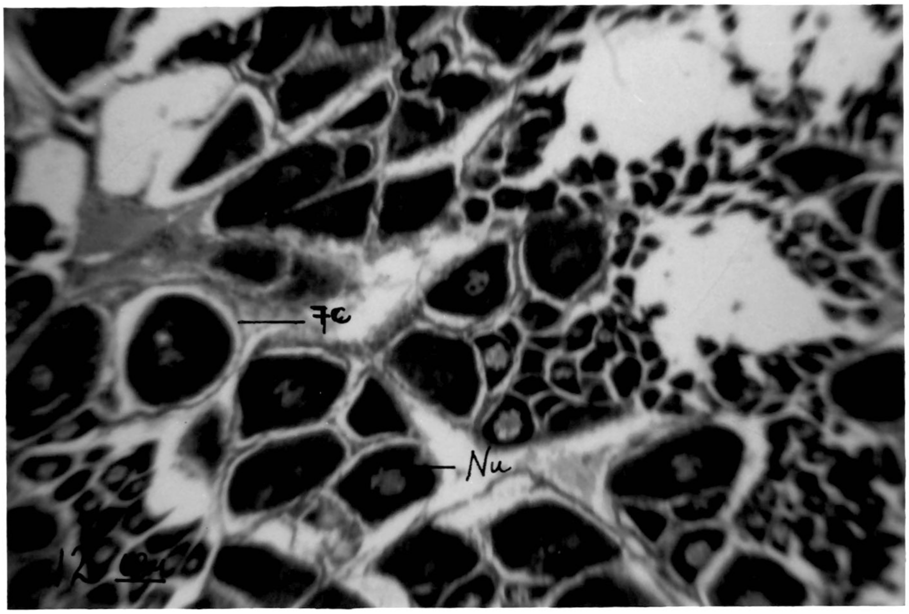
Figure 12. Section of developing ovary of P. *aztecus*. Note ring of nucleoli-Nu and follicle cell layer-FC around maturing ova only. X 100.

Figure 13. Section of a nearly ripe ovary of P. *aztecus*. Diffuse nucleus-N of nearly mature oocyte is shown. X 100.


FC
Nu
12

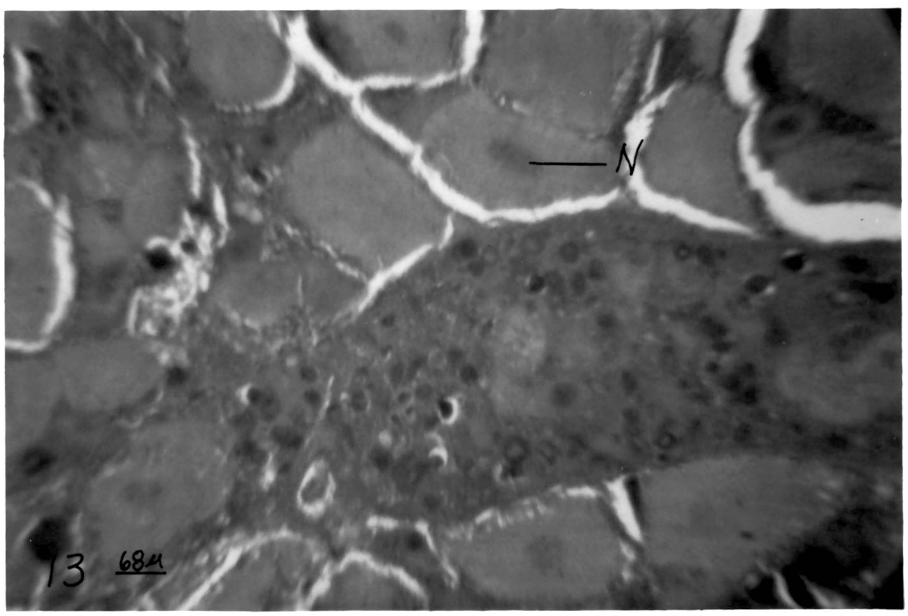
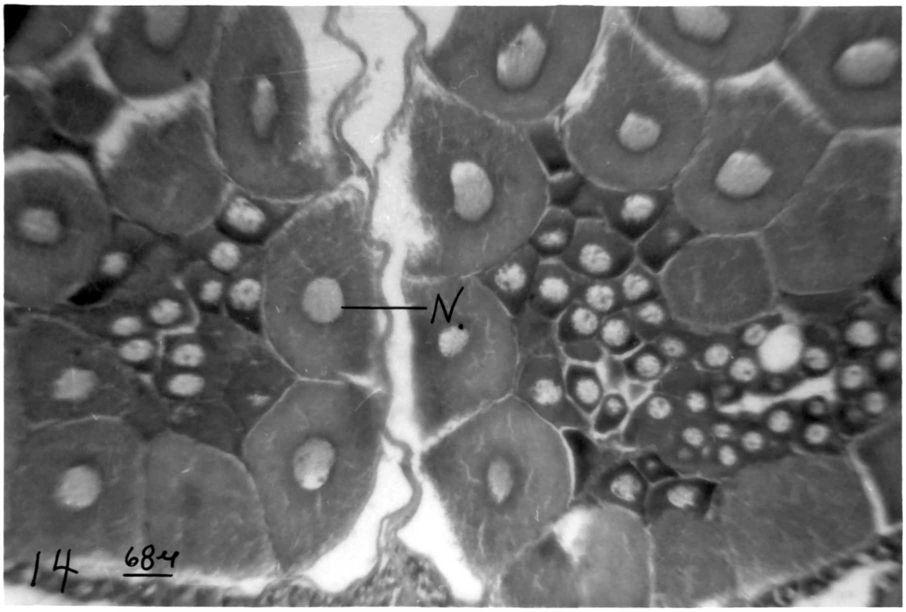
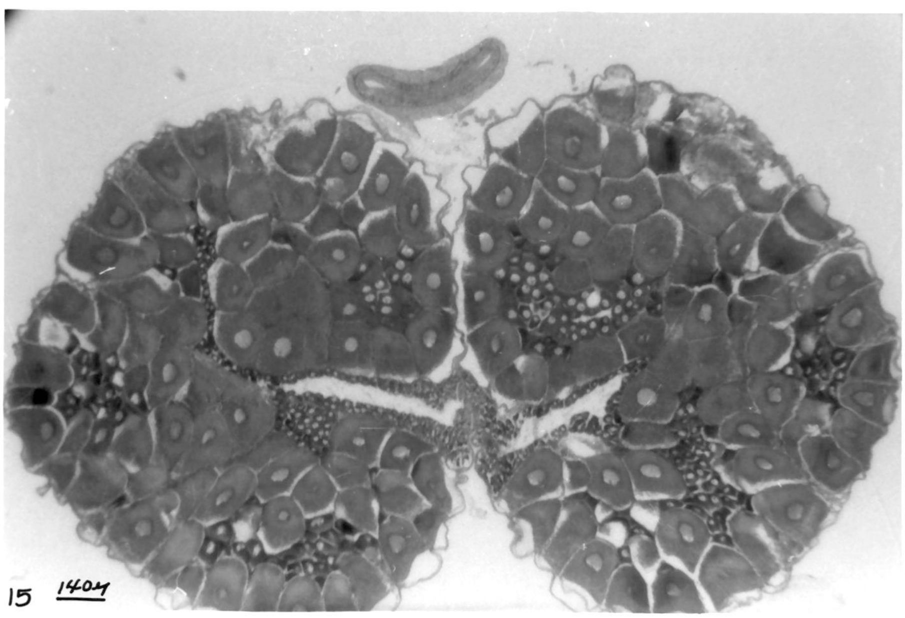

N
13
68μ

Figure 14. Section of a nearly ripe ovary of P. *aztecus*. Note the "clear" or faded nucleus-N. X 100.

Figure 15. Section of nearly ripe ovaries of P. *aztecus*. Note sizes and relative numbers of oocytes. X 35.


N.
14 68µ


15 140µ

Ripe

Approximately 80 per cent mature ova and 14 per cent maturing ova can be seen in Figure 16. In the ripe ovary, there are few immature ova and the zone of proliferation is often not seen.

The most distinctive feature of a mature ovum is the presence of peripheral rod-like bodies which are embedded in the cytoplasm. These are radially arranged at the periphery of the egg. They are eosinophilic as is the cytoplasm. The mean length of these rods is 63 $\mu$ and mean width 25 $\mu$ (Figure 16).

The nucleus is generally not visible but when seen appears as a purple, amorphous mass in the center of the egg (Figure 17).

Follicle cells appear as a fine line located adjacent to the mature ovum.

Spent

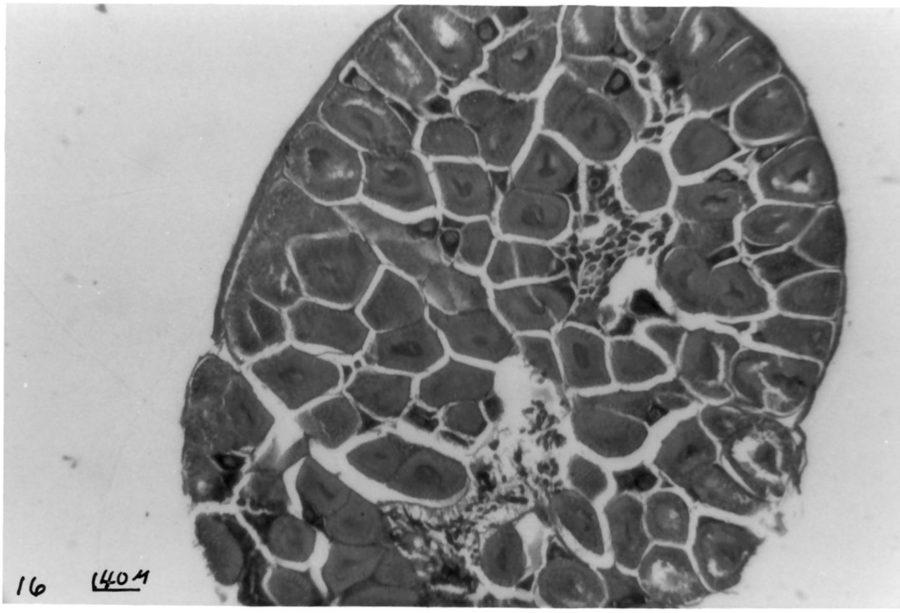
An ovary of P. aztecus was not observed immediately after spawning, however a partially recovered spent ovary was studied (Figure 18). In this condition large numbers of follicle cells and portions of partially resorbed eggs can be seen. Vacant areas surrounded by remaining follicle cells are present throughout the ovary.

Maturation of Ovary in P. duorarum and P. setiferus

In general, ovary development in pink and white shrimp is similar to that of brown spotted shrimp. Percentages of ova size groups for each stage of ovary development are essentially the same. To avoid repetition, only major similarities and differences will be noted.

Figure 16. Section of a ripe ovary of P. aztecus. X 35.

Figure 17. Section of a ripe ovary of P. aztecus. PR-peripheral rods. N-nucleus. FC-follicle cells. X 100.


16
140μ

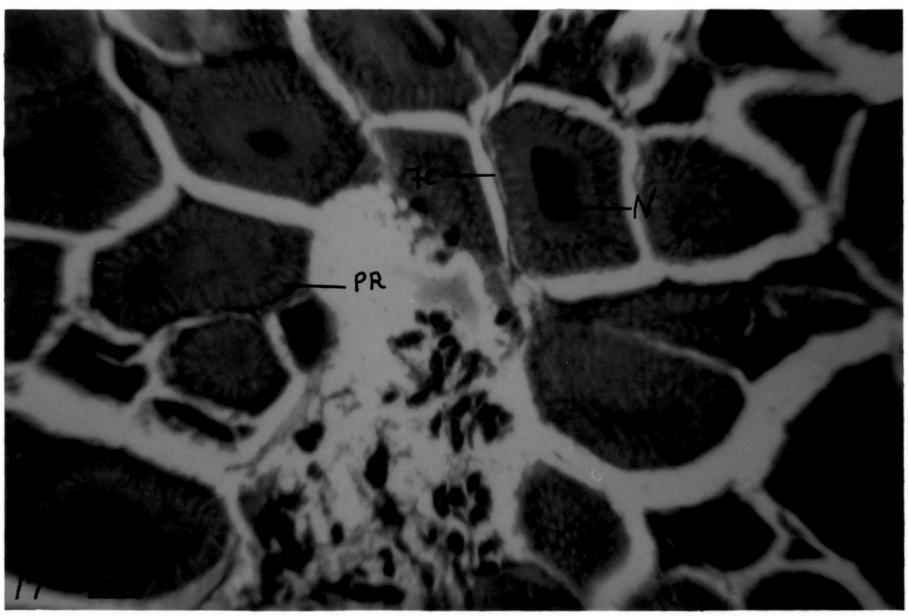
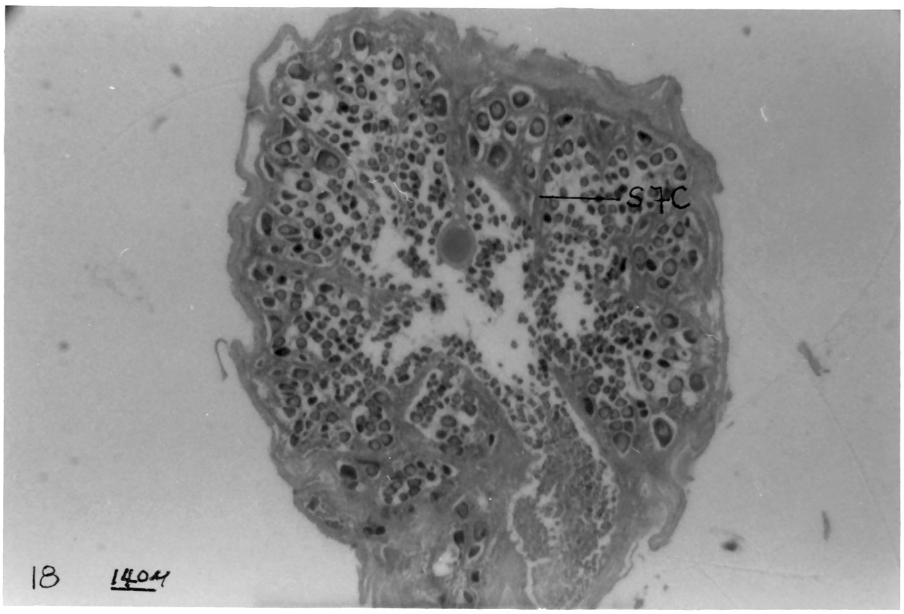

FC
N
PR
17

Figure 18. Section of a spent ovary of P. *aztecus*.
SFC-strands of follicle cells. X 35.


STC
18
140µ

Undeveloped

Cytoplasm of immature ova in pink (Figure 19) and white shrimp (Figure 20) is nongranular and basophilic. Nuclei of these cells are vesicular and contain diffuse chromatin material. The apparent difference between these ovaries is that the latter is partially recovered from a previous spawning. There are no follicle cells surrounding immature ova of either species (Figure 21). A closer view of remaining follicle cells can be seen in this photomicrograph.

Developing

Slightly granular cytoplasm of maturing ova of *P. duorarum* and *P. setiferus* stains moderately basophilic. There are usually five to six nucleoli within the nucleus of maturing ova (Figures 22 and 23). This is a slightly lower figure than for *P. aztecus* which had seven. Follicle cells surrounding the maturing ova are in a continuous layer about 10 µ thick. At no other time during development is this layer so prominent. As the ova grow in diameter, the follicle cell layer decreases in thickness (Figure 24). Mature ova of freshly preserved ovaries are uniformly oval and less reduced in size than those of prolonged preservation (Figures 25 and 26).

Nearly Ripe

Cytoplasm of the nearly ripe ovum is eosinophilic and contains many small granules. The nuclei appear as polymorphic masses in the center of the cells (Figures 27 and 28). When visible, there are usually about five nucleoli within the nucleus.

Figure 19. Section of undeveloped ovaries of P. *duorarum*. Note the uniformly oval ova. X 35.

Figure 20. Section of undeveloped ovary of P. *setiferus*. FC-follicle cells. X 35.

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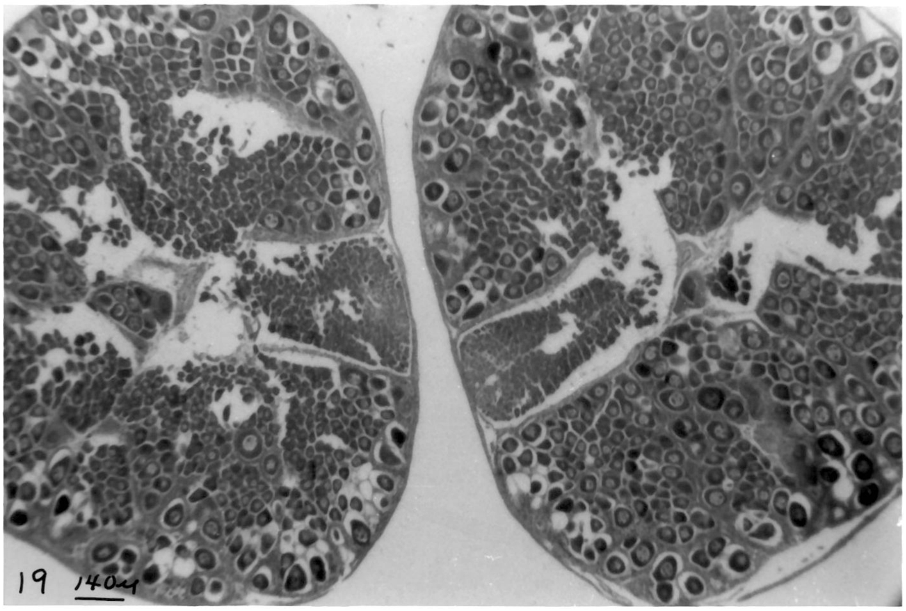


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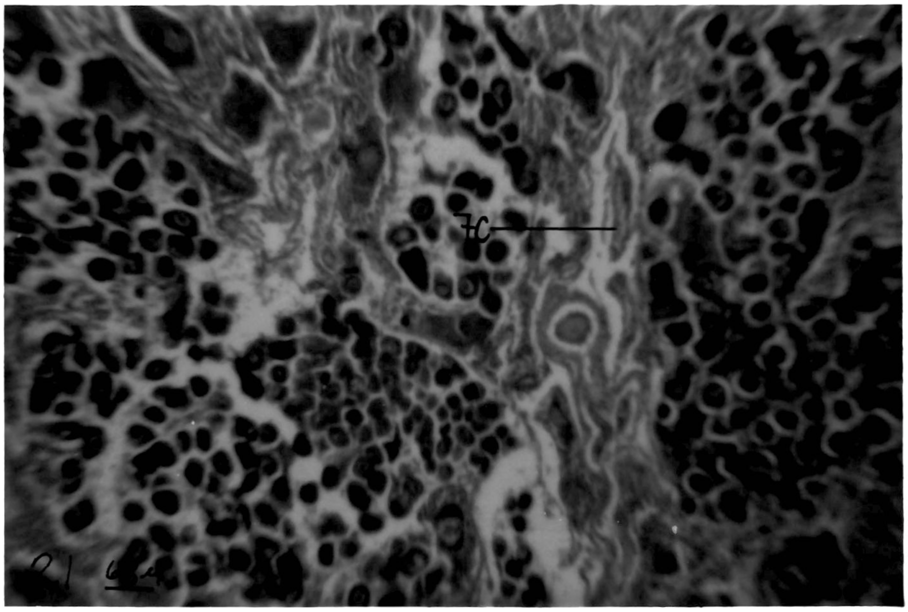
Figure 21. Section of undeveloped ovary of P. setiferus. Note absence of follicle cells around immature ova. Strands of remaining follicle cells are shown. FC-follicle cells. X 100.

Figure 22. Section of developing ovary of P. duorarum. A ring of six or seven nucleoli-Nu is indicated. X 100.


FC
21



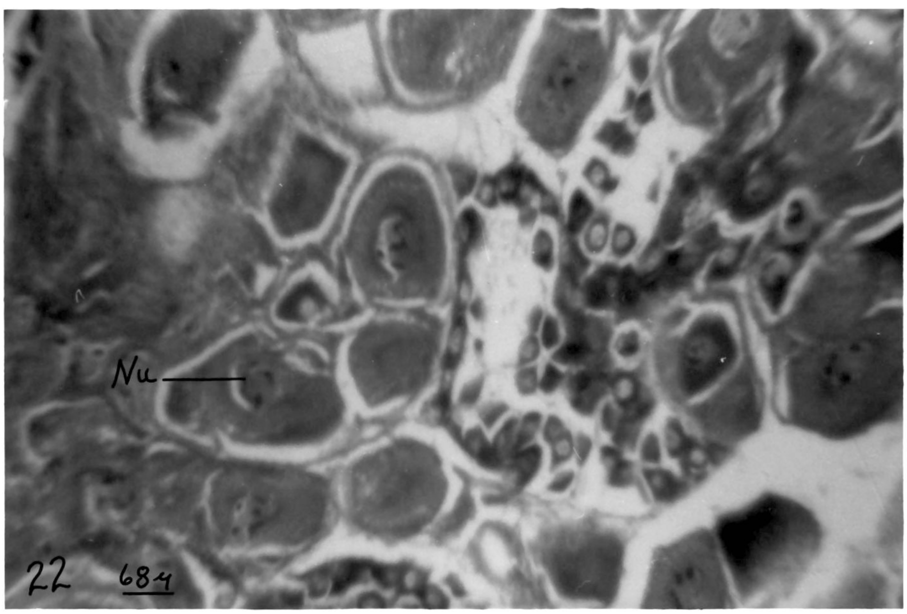

Nu
22 684

Figure 23. Section of developing ovary of P. setiferus. Note ring of nucleoli-Nu. X 100.

Figure 24. Section of developing ovary of P. duorarum. Note thickness of follicle cell layer-FCL. X 35.

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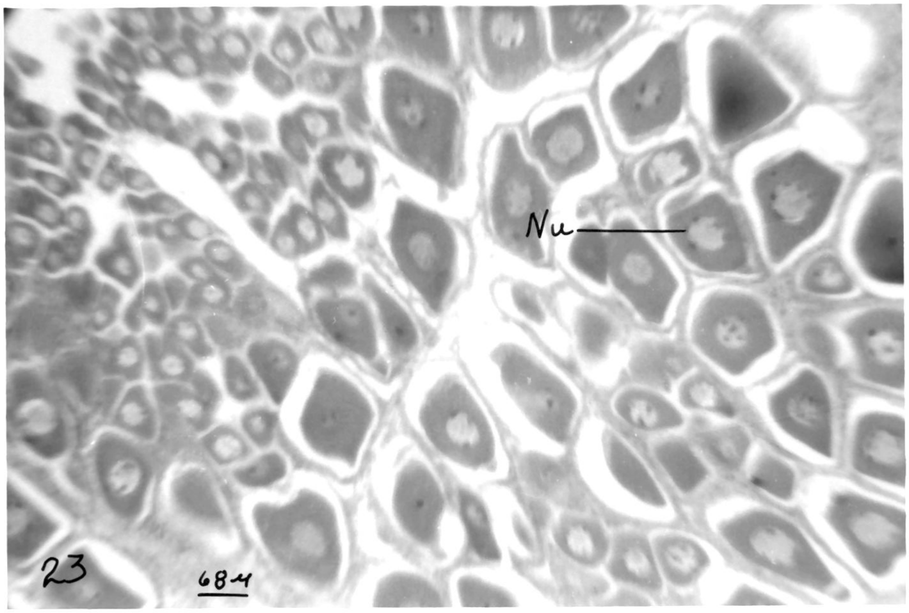


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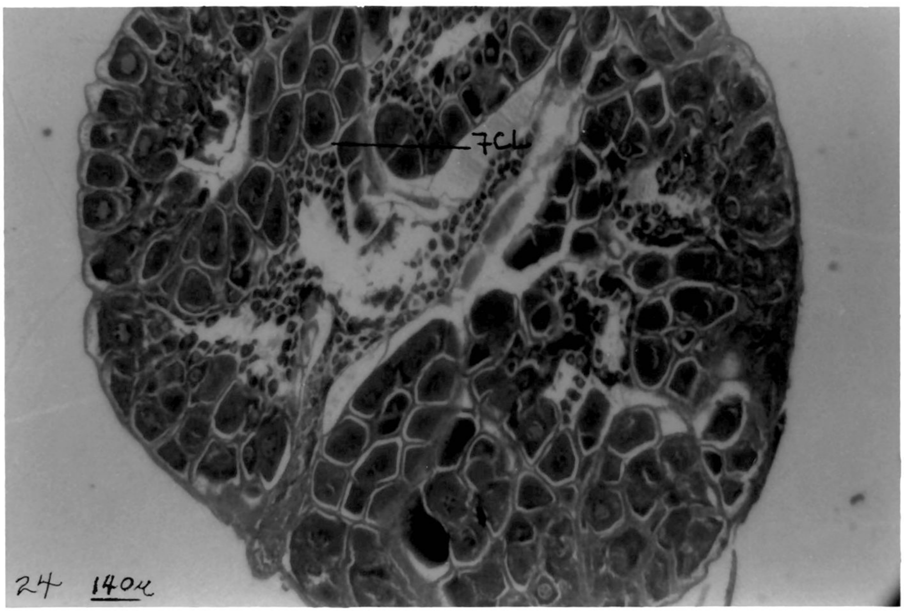

Figure 25. Section of early developing ovary of P. duorarum. Note uniformly oval cells. X 100.

Figure 26. Section of early developing ovary of P. duorarum. Note irregular, shrunken ova. X 100.

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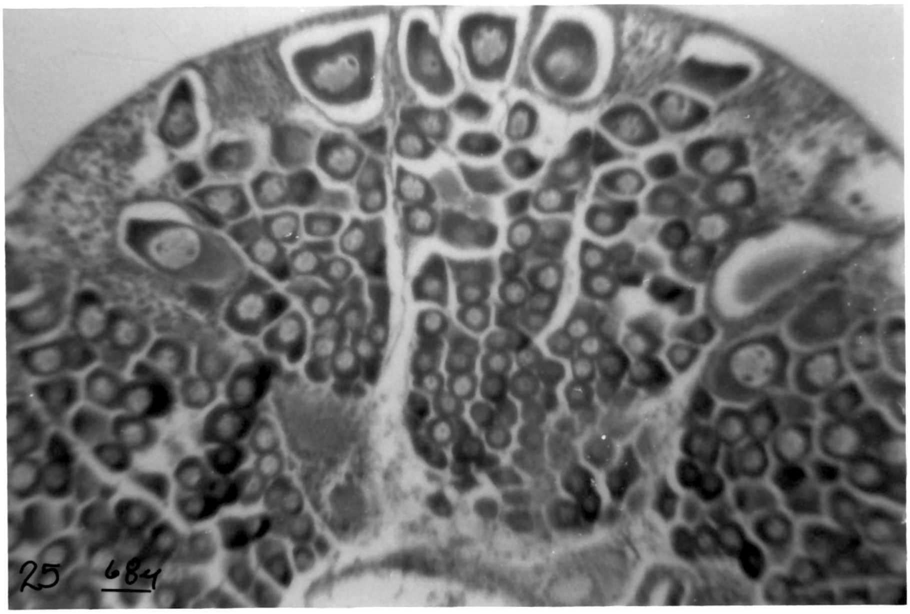


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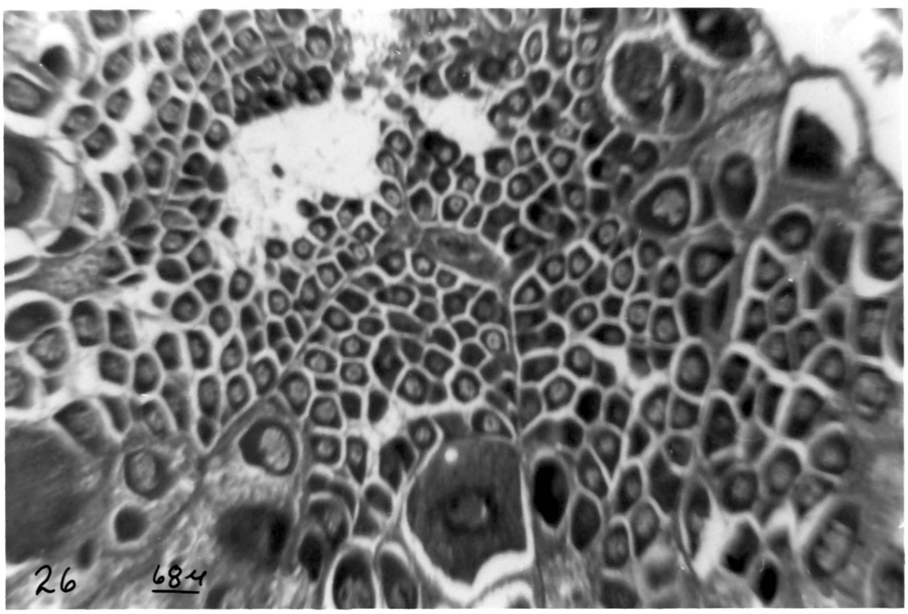

Figure 27. Section of nearly ripe ovaries of P. duorarum. Note amorphous nucleus-N. X 35.

Figure 28. Section of nearly ripe ovary of P. setiferus. Note nucleus-N. X 35.

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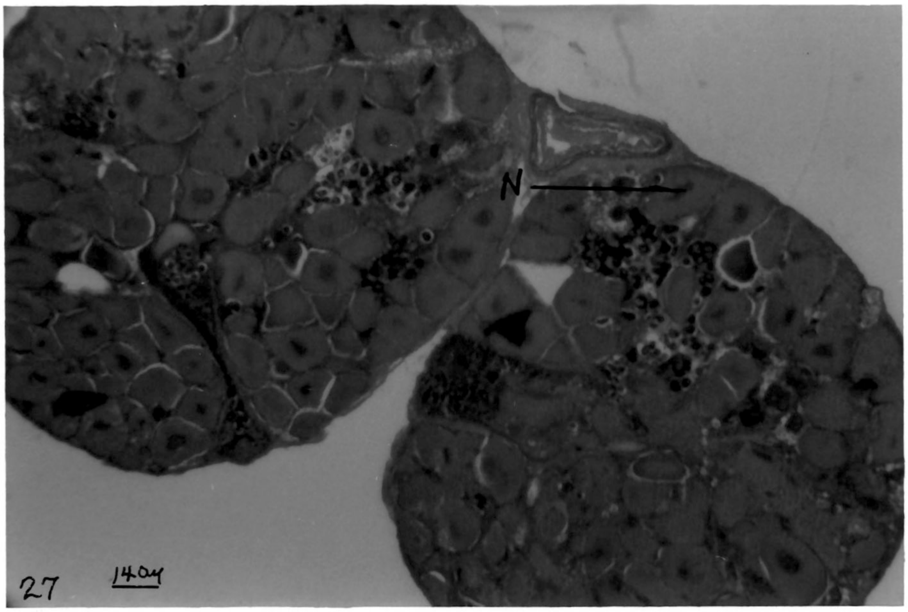


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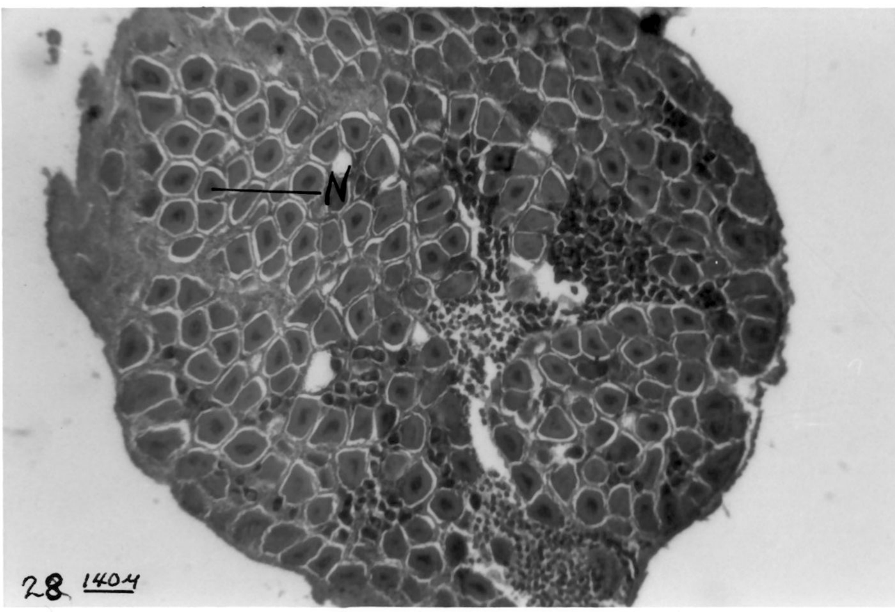


The layer of follicle cells is approximately five microns thick and lies against the ovum (Figure 29).

In the late nearly ripe stage of P. duorarum, the peripheral cytoplasm becomes extremely granular and amorphous globules become apparent. These bodies are eosinophilic and probably are the precursors of the peripheral rods. These globules are arranged in a somewhat radial manner about the nucleus (Figure 30).

Ripe

Mature ova of pink and white shrimp have eosinophilic cytoplasm containing many granules. The nuclei of these ova are basophilic and are centrally located amorphous structures (Figures 31 and 32). As the ovum reaches full maturity, the nucleus disappears from view (Figures 33 and 34).

These ova have distinct peripheral rod-like structures embedded in the cytoplasm. The nongranular, acidophilic rods are radially arranged about the center of the egg.

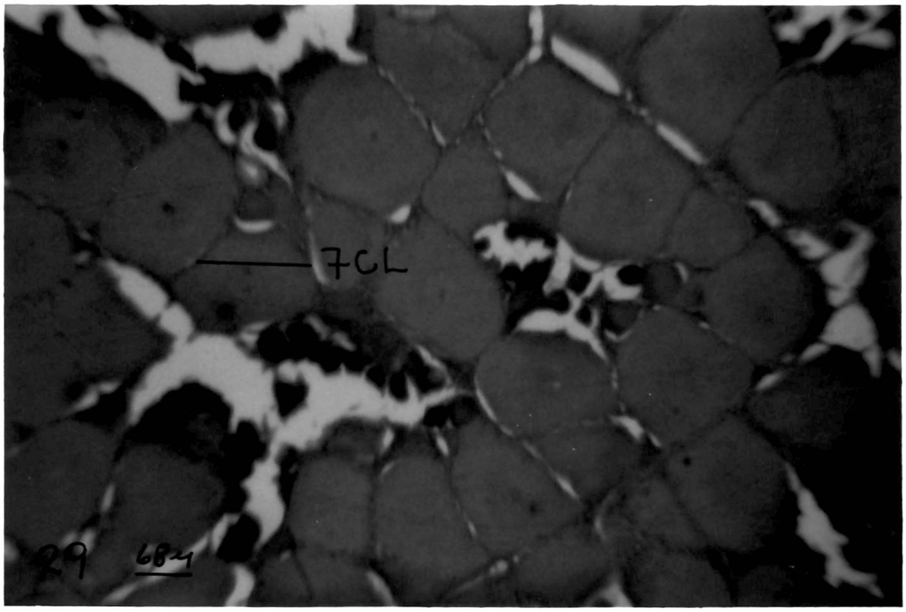
The follicle cell layer is reduced in thickness and appears as a fine line surrounding the mature ovum. The thickness of the follicle cell layer is less than two microns.

Spent

There are no major differences between the spent ovaries of P. duorarum and P. setiferus (Figures 35 and 36). There are many strands of remaining follicle cells in both. A few partially resorbed ova can be seen in Figure 35. Many vacant areas are distributed throughout the ovary.

Figure 29. Section of a nearly ripe ovary of P. duorarum. Note thin layer of follicle cells-FCL. X 100.

Figure 30. Section of late nearly ripe ovary of P. duorarum. PG-peripheral globules. X 100.

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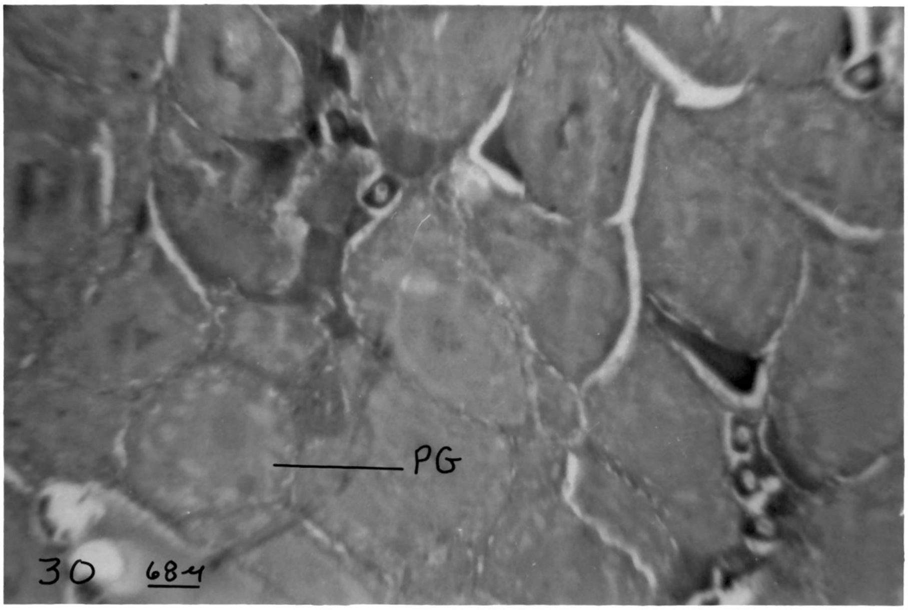
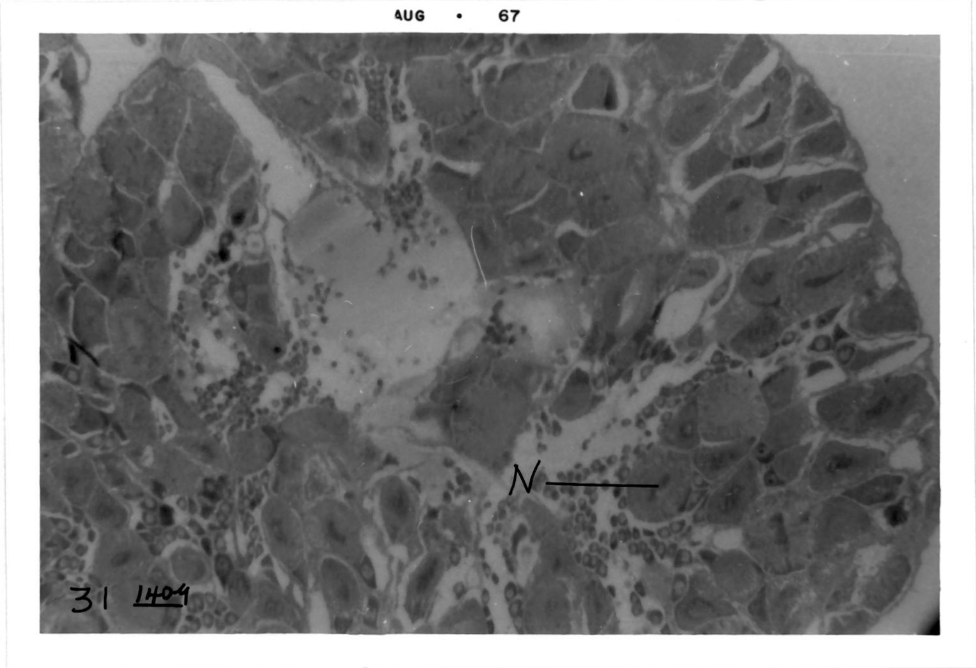


Figure 31. Section of a ripe ovary of P. duorarum. Note condition of nucleus-N. X 35.

Figure 32. Section of a ripe ovary of P. setiferus. Note condition of nucleus-N. X 35.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N
31 1404

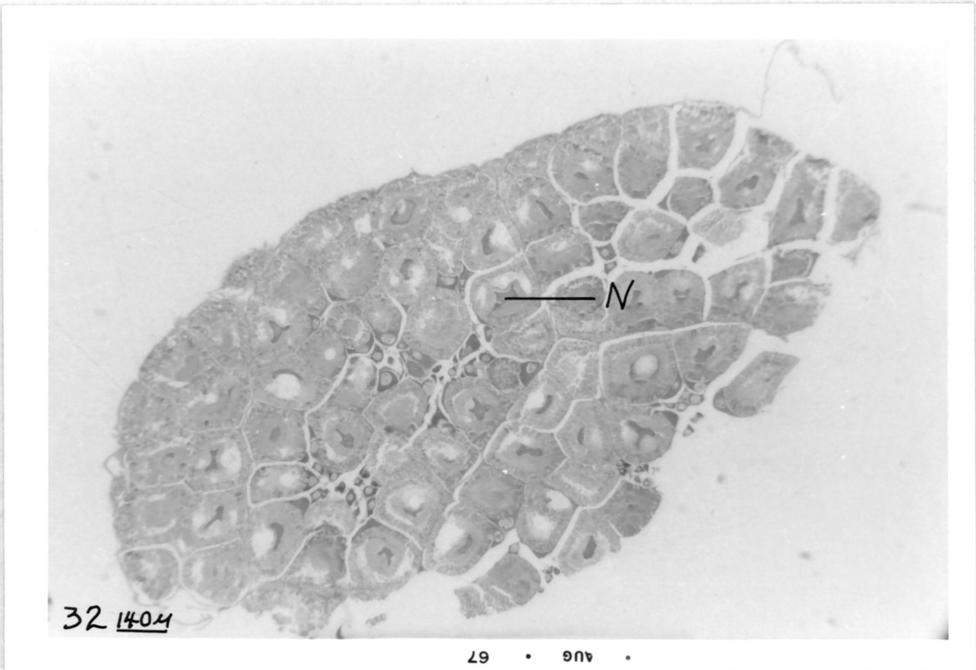
N
32 1404

Figure 33. Section of a ripe ovary of P. duorarum. PR-peripheral rods. N-nucleus. X 100.

Figure 34. Section of a ripe ovary of P. setiferus. PR-peripheral rods. N-nucleus. X 100.

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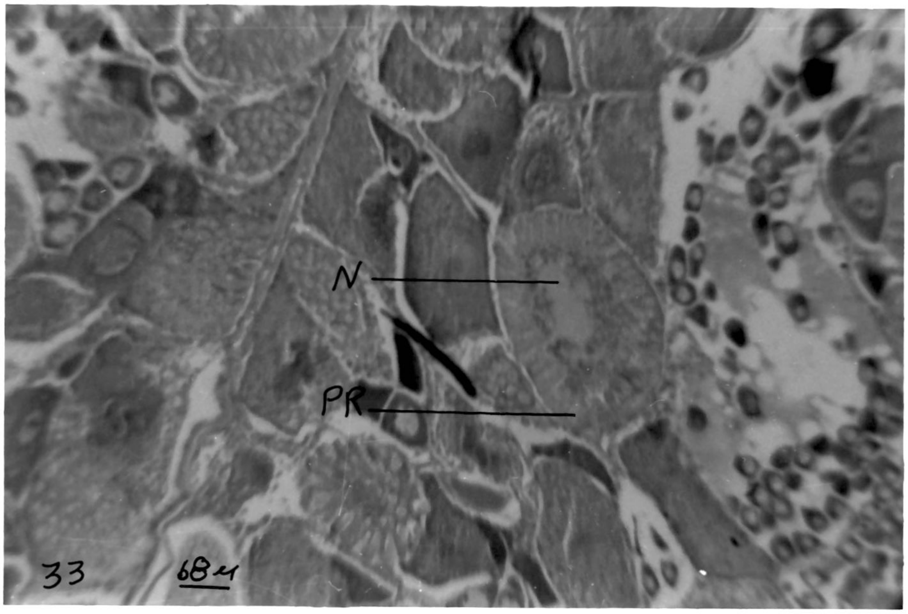


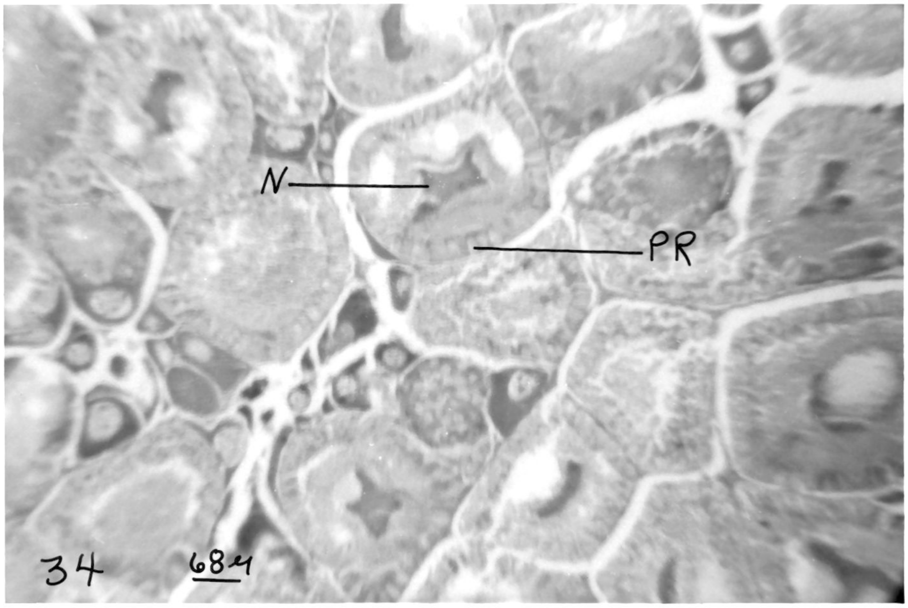


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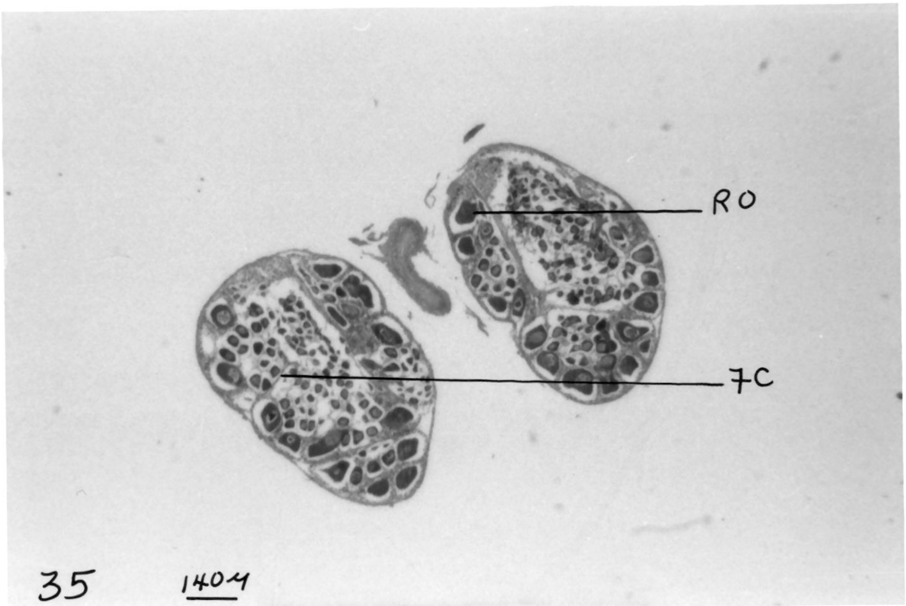
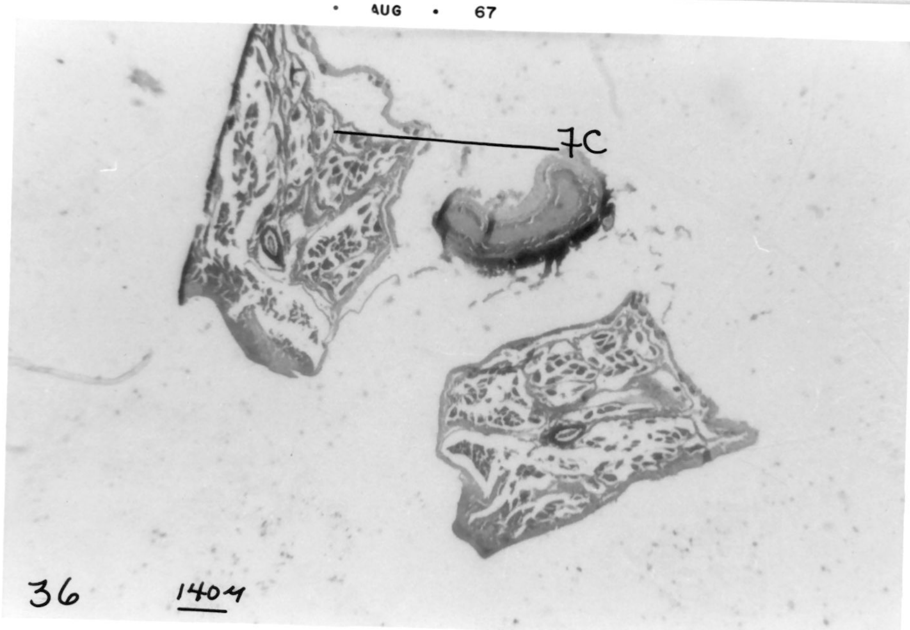
Figure 35. Section of spent ovaries of P. duorarum. Note strands of remaining follicle cells and many vacant areas in the ovary. Also apparent are the intact immature ova. RO-resorbed ovum. FC-follicle cells. X 35.

Figure 36. Section of spent ovaries of P. setiferus. Note reduced size of ovaries. FC-follicle cells. X 35.


RO
7C
35
140µ






7C
36
140µ

Intact ova of the spent ovary in *P. setiferus* are all immature, however, a few in that of *P. duorarum* are early maturing indicating that the latter is in a more recovered condition.

## DISCUSSION

### Gross Anatomy

Although the gross anatomy of the female reproductive systems of *Penaeus aztecus*, *P. duorarum*, and *P. setiferus* is similar, color of the ovaries during development differs.

The undeveloped ovaries of the three species were clear. Developing ovaries of *P. aztecus* and *P. duorarum* were white to light blue. However, King (1948) did not observe a blue color for comparable ovaries of *P. setiferus*. Cummings (1961) noted that the color of developing ovaries of *P. duorarum* was white (opaque)-pale olive buff. Nearly ripe ovaries of *P. aztecus* and *P. duorarum* were blue-green. Cummings reported that ovaries in this stage were a glaucous color in *P. duorarum* and King observed yellow or yellow-orange nearly ripe ovaries in *P. setiferus*. Yellow or yellow-orange nearly ripe ovaries were not observed for either *P. aztecus* or *P. duorarum*. However, Mr. L. Hardee encountered orange-colored, yellow-roed specimens of *P. aztecus* off the coast of Louisiana (Burkenroad, 1949).

No significant difference was found in the lengths of the ovaries of *P. aztecus* and *P. duorarum* during maturation. There is, however, a high correlation between the ovary length and total body length of these species during all developmental stages. These observations are the basis for the conclusion that ovary length is related to total length and not ovarian stage of development. Since this was found to be so for two species, it is possible that this would also be true for *P. setiferus*.

The ovarian width is probably an indication of the stage of maturation in the three species. Based on these ovary measurements, it is theorized that the ovaries of the brown spotted, pink, or white shrimp do not increase significantly in length during development but rather in width. Oocytes increase in width as a result of vitellogenesis and therefore, width of the ovary increases. The decrease in width of a spent ovary is apparent.

## Microscopic Anatomy

There is no significant difference among the mean widths of oocytes in the anterior, median, and posterior regions of the ovary in *P. aztecus*. Therefore, it is postulated that the ovary is homogeneous in structure and stage of development.

Ova in each of the four size categories were counted for each stage of ovarian development. Undeveloped ovaries are characterized by a majority (92 per cent) of immature ova, developing ovaries by (70 per cent) maturing ova, nearly ripe ovaries by (61 per cent) nearly mature ova, and ripe ovaries by (86 per cent) mature ova (Figure 2). Intact ova if present in a spent ovary are immature.

Cummings (1961) reported that all ova of undeveloped ovaries were small and there was no separate group of maturing ova. However, it was found in this study that eight per cent of the ova were early developing and in each case the ovary had been macroscopically staged as undeveloped.

### General Histology of the Ovary

The general histology of the ovary is very similar in brown spotted, pink and white shrimp. There was no evidence of muscle in the ovary wall. It was hypothesized (King, 1948) that muscular contractions of the cephalothorax and abdomen would induce spawning.

In the zone of proliferation oogonia arise by mitosis. Immature ova are centrally located in the ovary whereas more mature ova are positioned at the periphery. This apparently indicates a peripheral movement as maturation proceeds. The maturing ova are forced to this area by the rapid proliferation of oogonia (King, 1948).

The oviducts of P. aztecus, P. duorarum, and P. setiferus contain no muscle. They consist of three layers: outer epithelium, middle connective tissue, and inner columnar epithelium. The lumen of the duct in some cross sections appeared to be filled with some type of fluid residue. King (1948) postulated that this fluid is secreted by the columnar epithelium and probably facilitated the passage of eggs down the tube. Oviducts of shrimp with ripe ovaries were filled with mature eggs.

Maturation of the Ovary

Ovarian maturation is similar in the species observed. There are four stages of oocyte maturation based primarily on widths established as arbitrary indices for degree of maturation. Cytoplasmic and nuclear changes also characterize these stages.

Cytoplasm of immature and maturing ova is finely granular and basophilic. As maturation proceeds, the cytoplasm of nearly mature and mature ova becomes eosinophilic and contains more and larger granules. The change in cytoplasmic staining is probably a result of vitellogenesis. In contrast, Cummings (1961) postulated that this change resulted from the accumulation of proteins. Eosinophilic rod-like bodies are embedded in peripheral cytoplasm of mature ova. King (1948) found that these bodies in P. setiferus did not stain with a variety of fat specific stains or react with ninhydrin, a protein specific microchemical reagent. Bhatia and Nath (1931) believed the bodies to be albuminous yolk derived from mitochondria. Hudinaga (1942) considered similar globules in P. japonicus to be the precursor of jelly-like substance which is extruded around the egg immediately after it is spawned. Tuma (1966) also observed such bodies in P. merguiensis (DeMan) but he did not speculate on their function.

Nuclei of immature ova are vesicular with diffuse chromatin. As the ova mature, five to nine distinct nucleoli appear. With further development, nuclei of nearly mature ova begin to "fade from view" and in the mature oocyte, the nucleus is no longer visible.

Mean widths for ova of various degrees of maturity do not differ significantly for the three species.

A follicle cell layer is evident surrounding a maturing ovum. This layer is relatively thick but as the ovum reaches maturity, it is reduced in thickness. The follicle layer surrounding a mature ovum is so thin it appears as a fine line against the egg's surface. Hudinaga (1942) theorized that egg growth is facilitated by the absorption of these follicle cells.

Spent ovaries of P. aztecus, P. duorarum, and P. setiferus are characterized by strands of residual follicle cells. Partially resorbed ripe ova were seen in some spent ovaries. Only a few such ova were observed in the three species. According to Burkenroad (1949) the mean number of eggs that remained after spawning of *P. setiferus* was less than two per 0.1 ml of teased ovary and with a maximum number of ten.

Results obtained in this investigation indicate that the female reproductive systems of *P. aztecus*, *P. duorarum*, and *P. setiferus* are similar in their anatomy and histology. For the most part, results of this study concur with previous investigations.

## SUMMARY

1. The general anatomy and histology of the female reproductive tracts of the brown spotted shrimp, Penaeus aztecus (Ives); the pink shrimp, P. duorarum (Burkenroad); and the white shrimp, P. setiferus (Linnaeus) have been described and compared. These shrimp are of foremost commercial importance in North Carolina.
2. The ovaries of brown spotted, pink, and white shrimp differ in color during comparable stages of maturation.
3. The length of the ovary is related to total length of the shrimp and not to the ovarian stage of maturation.
4. The width of the ovary is a probable indication of its stage of development.
5. Cytoplasm of immature and maturing oocytes is basophilic and becomes acidophilic as the ova mature. The nucleus is a vesicular structure in immature oocytes. It becomes less apparent with further development, and in a ripe ovum, is not visible.
6. A relatively thick layer of follicle cells surrounds the maturing oocytes. The apparent reduction in the size of follicle cells as the ova size increases, suggests that the follicle cells might be absorbed by the ova.
7. The female reproductive systems of P. aztecus, P. duorarum, and P. setiferus are similar in their anatomy and histology. Maturation of the ovaries in the three species is essentially the same.

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