THE REPRODUCTIVE CYCLE AND
REPRODUCTIVE ANATOMY OF THE
HORSENECK CLAM, TRESUS MUTTALLII
IN TOMALES BAY, CALIFORNIA

by
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in partial fulfillment of the requirements
for the degree of

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in

Biology

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DATED May 5, 1981 (Signature)
Purpose of the Study:
The horseneck clam, *Tresus nuttallii*, is a common mudflat-dwelling bivalve belonging to the Family Mactridae. The chief predator of adult clams is man. The California Department of Fish and Game is responsible for the management of this game species. Life history information is essential for proper game management, and while many mactrids have been extensively studied, very little is known about *T. nuttallii*.

The purpose of this study is to examine the reproductive aspect of the life history of *Tresus nuttallii*. Two topics have been given special attention: the reproductive cycle and the anatomy of the reproductive system.

Procedure:
To determine the reproductive cycle of *T. nuttallii*, this study used the standard histological techniques for light microscopy on samples of gonadal tissue taken from animals periodically collected from the study site. Examination of the slides of gonadal tissue allowed for each individual to be placed into one of five reproductive categories. The proportion of animals in each category changes as the reproductive cycle progresses. The relative amount of follicular tissue in the gonad was used to confirm the results of the reproductive cycle study.

To examine the anatomy of the reproductive system of *T. nuttallii*, gross dissections and serial light microscope sections were made.

Findings:
Four periods of spawning were observed during this 24 month study: spring 1979 and 1980, and in the fall of 1978 and winter of 1979. The amount of follicular tissue in the gonad fluctuated cyclically; maxima coincided with early gametogenesis and minima with spawning. No evidence for the interconversion of follicular and gametogenic tissue was found. The anatomy of the reproductive system follows the pattern
commonly found in bivalves.

Conclusions:

Spawning occurs reliably in the spring, sporadically in the fall and winter. The follicular tissue is apparently the site for the storage of food reserves used for gametogenesis. Primordial germ cells are not derived from follicular tissue.

Chairperson: ____________________________  April 22, 1981

M. A. Program: Biology
Sonoma State University  Date
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Table of Contents

I. Introduction ........................................ 1

II. Materials and Methods
A. The Study Site ...................................... 4
B. Collecting *Træsus nuttallii* .................... 6
C. The Collections .................................... 8
D. Microtechnique .................................... 8
E. The Relationship Between the Alveolar and Follicular Tissues ... 9

III. Results
A. Observations ....................................... 10
B. Phases of the Reproductive Cycle ............... 18
C. The Reproductive Cycle ............................ 22
D. The Relationship Between the Reproductive Cycle and the Amount of Follicular Tissue .......................... 24

IV. Discussion
A. Methods ............................................ 25
B. The Reticulum in Male Alveoli .................. 26
C. The Reproductive Cycle ............................ 27
D. Phases of the Reproductive Cycle ............... 28
E. Confirming the Functions of the Reproductive Phases .................. 33
F. The Relationship Between the Alveolar and Follicular Tissues .... 36

V. Summary ............................................. 39

VI. Literature Cited .................................... 41

VII. Figures ............................................ 43
List of Figures

Figure 1. *Tresus nuttallii* .................. 43
Figure 2. *Tresus capax* ...................... 43
Figure 3. Visceral Skirt of *T. capax* .......... 44
Figure 4. Map of Study Site .................... 44
Figure 5. Visceral Mass of *T. nuttallii* ........ 45
Figure 6. Phases of Male Alveoli ............... 46
Figure 7. Phases of Female Alveoli .............. 47
Figure 8. Diagram of Right Collecting Duct ...... 48
Figure 9. Accessory Ducting ..................... 48
Figure 10. Graph of Reproductive Cycle ......... 49
Figure 11. Graph of Amount of Follicular Tissue .. 50
Figure 12. Reticulum in Male Alveolus .......... 51
Figure 13. Phases of Male Gonad ............... 52
Figure 14. Phases of Female Gonad .............. 53
I. Introduction:

The horseneck clam, *Tresus nuttallii*, is one of the larger mudflat dwelling bivalves on the Californian coast (Fig. 1). Variously known as the gaper, the summer clam, the rubberneck clam, and the great Washington clam (Ricketts and Calvin, 1974), it is closely related to *Tresus capax* (Fig. 2), with which it shares many of these common names. These two species have different geographical distributions, but in Northern California, their ranges overlap. *T. capax* is found from Northern California to Alaska (Machell and DeMartini, 1971), while *T. nuttallii* ranges from Baja California to British Columbia (Addicott, 1963). The more northerly distribution of *T. capax* appears to be due to its ability to withstand colder temperatures better than *T. nuttallii* (Pearce, 1965). Both species occur in the intertidal mudflats of Tomales Bay, California, but *T. capax* is only occasionally collected.

The siphons of both species appear identical at the surface of the mudflat. The differences between these two species are subtle, but in most cases, they are distinguishable by the external morphology of their shells. The best way to distinguish them, however, is by a flap of tissue, an elongation of the inner palp lamella termed the visceral skirt, which encircles much of the posterior region of the visceral mass in *T. capax* (Fig. 3) and is absent in *T. nuttallii* (Pearce, 1965).

The genus *Tresus* belongs to the family Mactridae, and
until recently, many aspects of mactrid life histories were either unknown or assumed to follow the pattern exhibited by other bivalves. With increased interest in ocean farming and aquaculture, however, more basic research on the reproduction of invertebrates that may be of commercial value is needed.

Knowledge of a species' reproductive cycle is essential when considering its niche, and it may give clues to the environmental stimuli that determine periods of reproduction. Reproductive cycles of the following mactrids have been studied: *Spisula solidissima* in New Jersey (Ropes, 1968), *Mulinia lateralis* in Connecticut (Calabrese, 1970), *Tresus capax* in South Humboldt Bay, California (Machell and DeMartini, 1971), and in British Columbia (Bourne and Smith, 1972a), and *Tresus nuttallii* in Elkhorn Slough, California (Clark *et al.*, 1975). Both species of *Tresus* are important for sport or food in California. Many aspects of the life history of *T. capax* have been studied, including recruitment, settling, mortality, and growth (Dinnel, 1971), breeding and growth (Bourne and Smith, 1972a), effects of temperature on larvae (Bourne and Smith, 1972b), diet and stored glycogen (Reid, 1969), but very little is known about the life history of *T. nuttallii*. The reproductive cycle of *T. nuttallii* in Elkhorn Slough, California has been studied (Clark *et al.*, 1975), but the sample sizes were too small to make definite conclusions about the time of spawning.

Recognizing the horseneck clam's popularity and its
potential commercial importance, this study was initiated to understand its reproductive biology. Two aspects of this problem have been given special attention: the reproductive cycle and the anatomy of the reproductive system.
II. Materials and Methods

A. The Study Site

Tomales Bay, Marin County, California is 64 km north of San Francisco at 39 degrees 15 minutes N latitude and 123 degrees 00 minutes W longitude. The bay is approximately 20 km long and varies from 0.7 to 2.7 km in width. Its depth averages 3.7 m and its greatest depth is 18.5 m below mean lower low tide. The lower, northern end of the bay is strongly influenced by oceanic conditions. In this area, salinities range from 30 to 35 0/00 and water temperatures vary from 12 to 15 degrees Celsius (Johnson, 1971). The animals studied were collected from Brazil's Beach on the east shore near the north end of Tomales Bay (Fig. 4).

The mudflat at Brazil's Beach consists of a layer of dark sand approximately 30 cm thick overlain with a layer of diatoms. At depths greater than 30 cm, the substrate varies from dark sand to rocks, dense clay, shells, and coarse gravel. The composition of the lower layer can change drastically within a few meters. The clams extend to an average depth of 40 cm below the surface, and the composition of the lower layer affects the ease of collection. On the surface of the mudflat numerous shallow depressions retain seawater when the tide recedes. During summer months, eelgrass covers the middle and lower reaches of the mudflat and hampers collecting.

Lower low tides of the mixed semidiurnal tidal cycle at
the study site ranged from a plus 1.9 feet to a minus 1.5 feet with respect to mean lower low water. At tidal heights less than 0.3 feet above mean lower low water, the mudflat was sufficiently exposed to permit collecting. The mudflat slopes very gradually and is maximally exposed when the tide level is about 1.0 feet below mean lower low water. Tides below that level increase the area if the mudflat very little, but they increase the amount of time available for collecting.

Weather also affected collecting conditions. On dark days, visibility in the hole being dug was severely limited. Occasionally, stiff northwest winds prevented the mudflat from draining and greatly limited the areas available for collecting. In spite of these difficulties, samples of near uniform size were collected at regular intervals.
B. Collecting *Tresus nuttallii*

As the tide recedes and exposes the mudflats, the clams withdraw their siphons 2 to 3 cm below the surface of the mud. This results in shallow, oblong depressions (about 3 cm wide, 4 cm long, and 1 cm deep) that experienced clammers can distinguish from depressions made from other animals. A shallow (about 3 cm) divot is gently taken with the tip of the shovel over the suspected clam burrow. If a clam is present, it will eject a stream of water. Gentle contact with the shovel blade does not injure the clam siphon because its tip is protected by two horny plates (Fig. 1).

Speed and technique are far more important than brute strength in digging horseneck clams. Speed is necessary because the sand is unstable and may collapse into the hole more rapidly than it is removed. Technique is important because the shell is relatively thin and fragile. The clam digger cannot use the shovel to pry against the clam to loosen it. A hole must be dug beside each clam to expose at least one-half of the shell. Only then can the clam be pried on to loosen it from the sand. Minimum force must be used.

Inexperienced clammers mistakenly dig a large (0.6 to 0.8 m in diameter) hole directly over the siphonal hole. This method involves far too much work. A rapidly dug small hole will suffice. The clam is easily lost in the loose mud when the hole is made directly over the clam's siphon. The only indicator of the clam's position is the siphonal hole,
and its position is difficult to monitor in mud that is being dug. Furthermore, the clam is rarely directly under the depression at the surface because siphons frequently are not perpendicular to the surface. Clams are often crushed or their siphons amputated when clammers lose the position of the clam.

The author prefers a small, straight shovel with a long handle. The smaller blade allows smaller (about 0.5 m in diameter) holes to be dug. The longer handle allows less stooping. Smaller holes are more stable than large ones. This increases the amount of time before the sides collapse and increases the percentage of clams taken.
C. The Collections

The specimens were collected at the study site from June 1978 to May 1980. No fewer than 10 clams ranging in size from 10 cm to 18 cm maximum shell length were taken in each collection. Collections were made every two weeks, weather permitting. The mean number of animals collected per month was 20. The clams were transported in seawater to Sonoma State University where samples of gonads were removed for histological examination.

D. Microtechnique

Gonadal tissue was taken from the right side of each specimen between the labial palp and the ctenidium (Fig. 5). Samples of gonadal tissue were fixed in Bouin's Fluid for 48 to 72 hours, dehydrated in alcohol, cleared in xylene, and embedded in 56 degree paraffin. Tissue sections 10 micrometers thick were mounted on glass slides and stained with Delafield's hematoxylin and eosin. One slide per clam was made. Serial 10 micrometer sections were cut and identically stained for investigation into the conversion of follicular to gametogenic tissue.
E. The Relationship Between the Aveolar and Follicular Tissues

The relative amounts of follicular and alveolar tissue were compared in different samples by the following method. One section of the gonadal sample was randomly selected and the area of the section covered by the follicular tissue was estimated and encoded as follows.

<table>
<thead>
<tr>
<th>Area of Section Covered by Follicular Tissue</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-25%</td>
<td>1</td>
</tr>
<tr>
<td>25-50%</td>
<td>2</td>
</tr>
<tr>
<td>50-75%</td>
<td>3</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>4</td>
</tr>
</tbody>
</table>

The classification of only the follicular tissue was sufficient because the gonad is visibly composed of only these two types of tissue.

For each month, all individuals in the collection were encoded. The code numbers were summed, and the mean taken. The mean percentage of the follicular tissue for each collection, given by the average of the codes, was graphed and compared to the reproductive state of the sample.
III. Results

A. Observations

**General Description of the Gonad**

In *Tresus nuttallii*, the sexes are separate. Each contains a pair of gonads that form a superficial layer one to five millimeters thick over the entire visceral mass, except the foot. There are no cross-connections between the right and left gonads. Gonadal tissue consists of a highly branched system of dorso-ventrally oriented tubules, termed alveoli, surrounded by a matrix of follicular tissue. Hematoxylin and eosin stain follicular tissue pink, gametes varying shades of purple. All individuals examined were sexually mature.
Description of the Alveoli

The alveoli give rise to the gametes in both sexes. Alveoli are round, tubular structures whose inner surface is the germinal epithelium. Each alveolus will pass through the following four phases during one reproductive cycle:

i) active
ii) ripe
iii) partially spawned
iv) spent

i) Gametogenesis occurs in the active alveolus. An active male alveolus has a thick germinal epithelium and a reticulate pattern of sperm in the lumen (Fig. 6a). In females, an active alveolus has developing oocytes in the lumen attached to the alveolar wall by stalks (Fig. 7a).

ii) The ripe male alveolus has a thin, basophilic germinal epithelium. The reticulate pattern of the active phase is gone; the lumen is now filled with sperm (Fig. 6b). In females, the ripe alveolus has more ripe eggs floating free in the lumen than are developing (Fig. 7b).

iii) The partially spawned male alveolus is characterized by a reduction in the amount of sperm in the lumen compared to the ripe phase (Fig. 6c). The germinal epithelium remains thin. An ovarian alveolus that is partially spawned has a reduced number of ripe eggs in its lumen, compared to the ripe phase (Fig. 7c).
iv) The beginning of the spent phase is marked by a large quantity of debris in the lumen. This debris consists of the sluffed germinal epithelium and residual sperm in males (Fig. 6d), and of residual oocytes in various stages of development in females (Fig. 7d). As the spent phase progresses, the debris is removed by amoebocytes. The spent phase continues until gametogenesis begins again.

The alveoli used in the previous spawning cycle do not degenerate; they retain their gametogenic capacity and are reused.

Alveolar walls vary in staining properties with the reproductive phase. The walls are pink at the start of the active phase. As the cycle progresses, the staining properties of the walls gradually change from eosinophily to basophilic, and when the ripe phase is reached, the walls are thin and basophilic. The alveolar walls remain basophilic through the partially spawned phase. During the spent phase, the area of the walls contiguous with the follicular tissue become eosinophilic while the area adjacent to the lumen remains basophilic.
**Gametogenesis**

Oocytes begin development by budding off the alveolar wall. They first appear as small, round basophilic cells. As the oocytes develop, they increase in size and push into the lumen of the alveolus, remaining attached to the wall by a thin stalk (Fig. 7a). The oocytes maintain contact with the alveolar wall throughout development, and when ripe, the stalks detach and the eggs lie free in the lumen. Ripe oocytes are approximately 44 micrometers in diameter.

The sperm develop in radial columns in the alveolus, with the tails of the sperm oriented toward the lumen (Fig. 6a). Spermatogonial proliferation occurs at the basal lamina. This increase in the number of cells results in a thickening of the germinal epithelium. Rapid cell division at or near the basal lamina pushes the dividing spermatocytes toward the lumen. The meiotic divisions occur en route from the basal lamina to the lumen, and upon reaching the lumen, the developing sperm are at least to the spermatid stage, and are frequently mature spermatozoa. Nurse cells were not observed.
Accessory Reproductive Structures

Ripe gametes move from the alveoli to a dorsally located collecting duct (Fig. 8). In both sexes, the collecting duct in each gonad extends posteriorly to a spawn vesicle or seminal receptacle that holds the ripe gametes awaiting release. A short duct connects the vesicle to the gonopores. The gonopores open on tiny papillae about 3mm long, 1mm high, by 1mm wide, on the dorsal surface of the visceral mass, immediately anterior to the posterior foot retractor muscle.

Each ctenidium is joined along its dorsal length to the visceral mass. The junction of these two structures produces a duct that guides the gametes to the excurrent siphon after they are released into the suprabranchial chamber (Fig. 9). From the excurrent siphon, the gametes are shed into the sea, where fertilization occurs.
Description of the Follicular Tissue

The follicular tissue is organized in discrete, randomly arranged bundles or cords that branch in a manner similar to the alveoli. The role of the follicular tissue is more than mechanical support for the alveoli; preliminary observations suggest the following functions:

1) the food reserves for gametogenesis are stored in the follicular tissue

2) the alveoli are derived from the follicular tissue

The amount of follicular tissue follows a cyclical pattern increasing and decreasing according to the reproductive state of the gonad. Follicular tissue is maximal immediately prior to gametogenesis. As the gametes ripen and accumulate, there is a concurrent decrease in the follicular tissue, reaching a minimal amount at the time of spawning. The amount of follicular tissue increases during the post-spawning or spent phase.

The evidence for the conversion of follicular tissue into alveolar tissue is their differential staining properties. Both vary in their affinities for stain. The alveolar walls range from eosinophilic to basophilic, while the follicular tissue varies only in its affinity for eosin. At times the two tissues are equally eosinophilic. This overlap in staining properties was examined and there was good evidence albeit circumstantial, that the follicular tissue was the structural and functional precursor of the gametogenic tissue.
The initial changes in the conversion are similar in males and females. The first recognizable change is a subtle increase in affinity for eosin exhibited by highly localized areas of follicular tissue. This change results in a cluster of darker cells. The cluster of cells can range from 40 to 140 micrometers in diameter, but whether further differentiation is size dependent is unknown. These clusters of cells are present in most individuals which have open areas of follicular tissue, regardless of reproductive phase. These localized differences were thought to be artifacts, but they appeared in many slides and persisted even after the procedures of microtechnique were very carefully controlled.

After the first change in stain affinity, development in males and females differs. In the female, the cluster of follicular cells appears to condense from both the inside and the outside. The cells on the inside of the cluster flatten and move toward the periphery of the bundle of follicular tissue, forming the lumen of the alveolus. It is this layer of cells adjacent to the lumen that produces the gametes. As the cells on the inside are condensing, the cells on the outside coalesce and form the outer part of the alveolar wall. This bidirectional condensation results in a new alveolus, or an extension of an old alveolus, which have staining properties similar to that of the surrounding tissue.

In males, the spermatogonia differentiate and begin dividing while the cluster of cells is still small and eosin-
ophilic. For this reason, the complete sequence of the development of the male alveolus is not complete because the later stages were obscured by the developing sperm. Alveolar development appears to be similar to that of females because the wall of the alveolus is composed of a series of membranes. This suggests that a similar flattening and condensation of follicular tissue forms the alveoli in both males and females.

These preliminary observations on the origin of the gametogenic tissue were discovered to be a misinterpretation of the slides of gonadal tissue. While it would appear there is ample evidence, the interconversion of follicular tissue into alveolar tissue does not occur. The follicular tissue does, however, have a nutritive function.
B. Phases of the Reproductive Cycle

Examination of the slides made from gonadal tissue allowed each individual to be placed into one of the following categories representing phases of the reproductive cycle:

1) spent/active
2) active
3) ripe
4) partially spawned
5) spent

Except as noted, the reproductive condition of the gonad was determined by the phase of the majority of the alveoli of the section, were placed.

Phases of the Male Gonad

1) The Spent/active Phase

The spent/active phase is characterized by the presence of alveoli in the spent phase immediately adjacent to alveoli in the active phase (Fig. 13a). Typically, there is a large amount of follicular tissue between the alveoli.

2) The Active Phase

When a majority of the alveoli in a given section show gametogenic activity, the individual is classified as active (Fig. 13b). All stages of spermatogenesis occur during this phase.

3) The Ripe Phase

At this stage, the sperm are no longer arranged in a
reticulate pattern, and are so numerous they fill the lumen of the alveolus (Fig. 13c). Individuals that are ripe may have many of the characteristics of the active phase. This is due to the non-synchronous development of the alveoli.

4) The Partially Spawned Phase

This phase is characterized by a reduction in the amount of sperm in the lumen, compared to the ripe phase (Fig. 13d). However, substantial numbers of sperm remain in the alveolus and further spawning is expected.

5) The Spent Phase

The spent male gonad has alveoli with varying amounts of post-spawning debris in their lumens (Fig. 13e). This debris is characteristic of males, and the animal can be sexed even in the late stages of the spent phase. Phagocytic amoebocytes remove the debris from the lumen and the follicular tissue reforms.

Phases of the Female Gonad

1) The Spent/active Phase

The spent/active phase in females is distinct. Spent alveoli with residual oocytes or other post-spawning debris are adjacent to alveoli in the active phase (Fig. 14a).

2) Active Phase

When a majority of the alveoli in a given section show indications of oogenesis, the individual is classified as active (Fig. 14b). The active phase includes all stages of
oogenesis and ends when the gonad is ripe.

3) Ripe Phase

The gonad is classified as ripe when a majority of the alveoli are in the ripe condition (Fig. 14c). In this phase, the walls of the alveoli are thin and basophilic, and there is very little follicular tissue between the alveoli.

4) Partially Spawned Phase

The gonad has the same physical characteristics as the ripe phase, the difference being a reduction in the number of oocytes in the alveoli (Fig. 14d).

5) Spent Phase

The spent gonad is variable in appearance because alveoli that are in the spent condition vary in appearance. A spent individual usually has alveoli containing residual oocytes (Fig. 14e). Many times there are empty alveoli with alveoli containing residual oocytes in the same section. Rarely will all the alveoli in a spent gonad be empty.

The undeveloped oocytes are sluffed into the lumen of the alveolus, where they are bound by a thin, weblike material. This debris is distinctly characteristic of females. Phagocytic amoebacytes invade the alveoli and remove the oocytes and debris, the shape of the alveolus is usually sufficient to sex the individual.

The amount of follicular tissue also varies during this phase. The follicular tissue is minimal at the beginning of
the spent phase and maximal at the end of the spent phase (Fig. 11).
C. The Reproductive Cycle

When the study began in 1978, the population was already in the process of spawning (Fig. 10). In June 1978, 35% of the population was partially spawned. This indicates that spawning was occurring or had recently occurred. Spawning essentially ceased in July, August, and September as evidenced by the low proportion of the population in the partially spawned and spent phases, and the high proportion of the sample in the pre-spawning phases, the spent/active, active, and ripe phases. The population spawned again in 1978, in October and November. In October, 60% of the population was partially spawned, spawning or spent. The proportion of the November sample in these two phases increased to 75%. Spawning stopped in November, and was followed by a period of gametogenesis which occurred in the winter months of 1978/79. In December, 60% of the population showed gametogenic activity. This proportion increased to 80% in January and peaked in February with 85% of the population in the active phase. This increase in activity culminated in March, with about 80% of the population ripe and ready to spawn. Spawning in 1979 began in April, shown by the appearance of a large population of partially spawned clams. Spawning continued in May, June and July, involving as increasing proportion of the population. Spawning stopped by August. In September, a small percentage of the population showed gametogenic activity.

The proportions in the active phase increases during
the fall months although quite erratically. Gametogenesis increases in the population through March of 1980, and spawning is again initiated in April.

Over the duration of the study, an interesting inverse relationship existed between the pre-spawning and post-spawning phases. If the spent/active, active, and ripe phases are examined as a unit, and compared to the combination of the partially spawned and spent phases, the cyclical pattern exhibited by the gonads is more obvious. For example, when pre-spawning activity is high, there are few individuals in the population that are in the post-spawning phase, and vice-versa. This alternation of dominance of pre-spawning and post spawning phases illustrates the cyclical nature of the condition of the gonads in the population. It also serves to demonstrate that a cycle exists, and again shows the general uniformity of reproductive conditions of the intertidal population.
D. The Relationship Between the Reproductive Cycle and the Amount of Follicular Tissue

There is a strong correlation between the reproductive state and the relative amount of follicular tissue in the gonad (Fig. 11). Two clear trends can be seen from the graph. As gametogenic activity increases, there is a corresponding decrease in the percentage of follicular tissue in the gonad. When the gametogenic activity is low, the amount of follicular tissue increases. In addition, two interesting points can be seen in the graph; gametogenesis is initiated at the seasonal maximum of gonadal tissue, and spawning is initiated at the seasonal minimum.
IV. Discussion:

A. Methods

At no time during the sampling was there a collection which did not fit the existing trends of the reproductive cycle. This indicates a consistency between the reproductive condition of the samples and the entire population. This is noteworthy when considering the wide range of tidal heights over which the specimens were taken. One would expect those animals occupying the higher reaches of the intertidal environment to have a more erratic spawning cycle than those of the lower reaches due to the greater changes in temperature, salinity, etc. Since such variation in the spawning cycle was not observed, it is possible that environmental conditions that would so grossly alter the reproductive pattern would also prove lethal to the animal.
B. The Reticulum in Male Alveoli

In *Tresus nuttallii*, developing sperm form a reticulate pattern in the lumen of the alveolus. This pattern has not been described for other species. In *Mya arenaria*, sperm develop between cells in the interior of the bundle of follicular tissue. Well-nourished individuals of *M. arenaria* will have lumina devoid of follicle cells, crowded out by developing sperm, whereas in poorly-nourished clams, relatively few spermatogonia are formed and the sperm complete development between follicle cells (Coe and Turner, 1938). Sperm developing between the follicle cells in *M. arenaria* would appear to form a reticulate pattern in the alveolus. It is possible that follicle cells are responsible for the reticulate pattern of sperm in *T. nuttallii*, but that is unlikely for two reasons: first, it is improbable that all male specimens collected were poorly nourished, and second, nuclei are absent from the reticulum (Fig. 12). This indicates that the pattern observed in the lumen of spermatogenic alveoli is not cellular in nature, nor is it due to malnutrition. The actual nature of the reticulum has not been determined.
C. The Reproductive Cycle

The reproductive cycle is a continuum and was broken into arbitrary subunits, or phases for convenience. This was not a simple, straightforward process. While it was relatively easy to categorize tissue in the middle of a phase, it was difficult to determine certain limits of a phase. The phases do not represent step-wise gonadal development. Each phase is descriptive of the reproductive state of the gonad at one point in time; it should not be inferred, therefore, that each phase represents the same amount of time.
D. Phases of the Reproductive Cycle

1) The Spent/Active Phase

In most bivalves, a period of inactivity occurs at the end of each reproductive cycle. The inactive phase of *Mya arenaria* in New England referred to the post-spawning phase in which there was no discernable gonadal activity when compared to the previous sampling (Ropes and Stickney, 1965). The definition of the inactive phase was vague; it was unclear whether the inactivity referred exclusively to gametogenesis or whether restoration of the food reserves was included. The authors admitted the difficulty in adequately distinguishing between the spent and inactive phases, and actually, the inactive phase, as defined for *Mya arenaria* described in part, active gametogenesis. The inactive phase for *Mya arenaria* included the stage in which small ovocytes appeared on the alveolar wall (Ropes and Stickney, 1965). This early stage of gametogenesis has been defined as the early active phase in *Protothaca staminea* (Feder et al., 1979). The disagreement in the literature concerning the limits of the inactive phase leads this author to believe that the inactive phase was originally inadequately defined by Ropes and Stickney (1965), and that these authors and Feder (1979), had different concepts of what the phase encompasses.

In *Tresus nuttallii*, no inactive phase occurs. The histological evidence indicates that the reproductive condition of the gonads progress from the spent phase of one
cycle directly into the active phase of the next. This continuous cycling results in an intermediate condition in which the gonad has the characteristics of both the spent condition of the previous cycle and the active phase of the next. The spent/active condition is a distinct indicator that denotes the end of one cycle and the beginning of another.

The inactive condition, while useful in reference to the quiescent reproductive phase in *Mya arenaria* in New England, does not accurately define and describe the similar phase in *Tresus nuttallii*.

The active stage in its late stage, the ripe phase, and the partially spawned phase are difficult to precisely characterize, because they are very similar, and my observations indicate that individuals in the active phase do spawn (Fig. 10). In September of 1978, approximately 65% of the sample was in the active phase. In October, 60% of the sample was partially spawned or spent. The same occurred in March/April of 1980, and to a lesser degree in February/March 1979. Spawning during the active phase was not discussed although it appears to occur in *Pododesmus cepio* (Leonard, 1969) and *Protothaca staminea* (Feder et al., 1979).

An animal in the ripe condition will either spawn or resorb its gametes. The results indicate that the majority of the individuals in the ripe phase will spawn. If resorp-
tion occurred, it would be obvious; the alveoli would contain large quantities of debris in the form of sluffed, degenerating gametes. Resorption of gametes was not observed in *Tresus nuttallii*.

The partially spawned phase implies an extended period of spawning for *T. nuttallii*. In the oyster *Crassostrea virginica* in Long Island Sound, not all individuals in the population spawn at the same time or at the same rate, resulting in an extended spawning period (Loosanoff, 1965). This pattern is common in many bivalves in temperate climates (Sastry, 1979). The animals classified as partially spawned posed a problem similar to that of the late active and ripe phases. Histological evidence indicated that spawning had occurred, but substantial numbers of ripe gametes remained in the alveoli, suggesting that spawning would continue. However, there was no direct evidence that a partially spawned individual would continue to spawn. Evidence for continued spawning must be inferred from the graph of the reproductive cycle (Fig. 10). The changes in the proportions of the partially spawned and spent individuals are followed through time. For example, when spawning occurred in the spring of 1979, the proportion of the population in the partially spawned and spent phases increased steadily from April through July. If the animals that were classified as partially spawned did not spawn again, the portion that spawned once would retain the characteristics of the partially spawned phase and there would be no concurrent
increase in the proportion of spent individuals. An increase in spent individuals was observed in April through July indicating continued spawning from animals classified as partially spawned. In August, however, these proportions did not change appreciably from those of July. This suggests that even though approximately 30% of the sample was classified as partially spawned in July and were expected to continue to spawn, they in fact did not.

During the spent phase, the individual recovers from spawning. Debris is reported to be removed from the alveolus by phagocytic amoebocytes. These phagocytes have been described for the following species, Mercenaria (=Venus) mercenaria (Loosanoff, 1937), Crassostrea virginica (Loosanoff, 1965), Mya arenaria, (Ropes and Stickney, 1965), Pododesmus cepio and Ostrea edulis (Leonard, 1969), Tresus capax (Machell and DeMartini, 1971), Macoma nasuta and Macoma secta (Rae, 1978), and Protothaca staminea (Feder et al., 1979). Phagocytes have not been identified in T. nuttallii, however. Food reserves in the follicular tissue, are also restored during this phase. For gametogenesis, this phase is important. In Crassostrea virginica, the quantity of gametes produced is influenced by the amount of food ingested and the reserves accumulated during the preceding post-spawning period (Loosanoff, 1965). The relationship between food reserves and gametogenesis has been reviewed for Mytilus edulis, Pecten maximum, and Macoma balthica (Sastry, 1979). Nutrients accumulated during the post-spawning period are
used for gametogenesis in these three species, and the same appears to occur in *T. nuttallii*. 
E. Confirming the Functions of the Reproductive Phases

Correlation of the functional aspects of gonadal morphology with the structural aspects is difficult to do based solely on the histology of the alveolar tissue. The names of the reproductive phases are based on the presumed function of these phases. The function of each phase is inferred from the structure of both tissues of the gonad, and how the structure changes over time. Examination of the alveolar tissue alone is inadequate to determine the reproductive cycle, although it has been used. The reproductive cycles of *Spisula solidissima* in New Jersey (Ropes, 1968), *Mya arenaria* in Skagit Bay, Washington (Porter, 1974), and *Tresus nuttallii* in Elkhorn Slough, California (Clark *et al.*, 1975), were investigated by examining the histology of only the alveolar tissue. It is this author's opinion that corroborating evidence is necessary to confirm the assigned functions of the phases, thereby assuring an accurate interpretation of the initiation and duration of the spawning period. The following techniques were used to correlate alveolar structure and function during the phases of the reproductive cycle. The presence of larvae in the plankton was used to determine the time of spawning for *Protothaca (=Paphia) staminea* (Quayle, 1943), *Mulinia lateralis* (Calabrese, 1970), and *Tresus capax* (Bourne and Smith, 1972a), physical changes in the gonad were measured, such as thickness of the gonad of *Crassostrea virginica* (Loosanoff, 1965), weight of the gonad in *Tresus*
capax (Bourne and Smith, 1972a), wet weight versus dry weight in *Protothaca staminea* (Feder *et al.*, 1979), oocyte sizes were measured in *Macoma balthica* (Caddy, 1967), and comparison of reproductive condition with the seasonal changes in the amount of stored glycogen in the gonad of *Tresus capax* (Machell and DeMartini, 1971; Bourne and Smith, 1972a).

In this study of the reproductive cycle of *Tresus nuttallii*, the reproductive condition of the gonad was compared to the relative amount of follicular tissue. The comparison is an indicator of the reliability of the criteria used to determine the reproductive phases of the gonad. The results of the comparison, superimposed on the graph of the reproductive cycle (Fig. 11), indicate a strong negative correlation between the amount of follicular tissue in the gonad and the reproductive condition of the population. When gametogenesis occurs in the winter followed by a spring spawn, gametogenesis is initiated at the seasonal maximum of follicular tissue. As gametogenesis continues during the winter, the amount decreases sharply, with spawning occurring at the seasonal minimum. If gametogenesis is initiated during the summer, as it was in 1978, the amount of follicular tissue remains high throughout gametogenesis and spawning. This may be related to the amount of food available during the summer versus the winter. The results suggest there is sufficient food in the plankton during the summer to support gametogenesis without depleting the food reserves stored in the follicular tissue, while during the
winter there is not. This is supported by the fact that the follicular tissue is restored during the post-spawning phase, which occurred in July, August, September and October of 1979. It is clear, then, that the amount of follicular tissue, measured by the methods used in this study, is a valid means to confirm the results of the reproductive cycle study.
F. The Relationship Between the Alveolar and Follicular Tissues

The conversion of follicular tissue into alveolar tissue was described for *Macoma balthica* in the Thames Estuary, England (Caddy, 1967), and in *Tresus capax* in South Humboldt Bay, California (Machell and DeMartini, 1971). Although observations suggested such a transformation in *Tresus nuttallii*, it probably does not occur. The investigation must go back to the embryonic development of the gonad. The development of gonads in juvenile bivalves was described for *Mya arenaria* (Coe and Turner, 1938), *Protothaca (=Paphia) staminea* (Quayle, 1943) *Mercenaria (=Venus) mercenaria* (Loosanoff, 1937), and *Pecten spp.* (Coe, 1945). The structure and arrangement of the follicular tissue was similar in all species studied. It consisted of a system of anastomosing cords of tissue similar to those of *Tresus nuttallii*. In *Mya arenaria*, the primordial germ cells are distributed randomly along the outer wall of the bundle of follicular tissue as it develops (Coe and Turner, 1938). When the individual matures sexually, the primordial germ cells differentiate into spermatogonia and oogonia in males and females and begin producing gametes. This gives the impression that the gonia and the follicle cells are the same or are derived from a common cell because the gametes simply appear in the follicular tissue.

Primordial germ cells and follicular cells produce only their own kind in *Mya arenaria* (Coe and Turner, 1938). This
contention is in accord with the germ plasm theory stating that the germ cells of one generation transfer a substance directly to the germ cells of the next generation (Eddy, 1975). This substance has been identified and traced in many animals, both vertebrate and invertebrate. The mollusks reported to have germ plasm are the gastropods *Paludina* sp. and *Lymnaea stagnalis*, and the bivalve *Sphaerium striatunum* (Eddy, 1975). It has been noted that the cytoplasm of the fertilized eggs in these mollusks was not homogeneous. A selected region, usually near the animal pole, has characteristic cytoplasmic inclusions that will eventually become part of the cytoplasm of the germ cells. The cells bearing this substance can be traced to the primordial germ cells of the animal. In this way, there is continuity between the germ cells of successive generations (Eddy, 1975). If this theory is correct, that the cells which will produce the germ cells are determined early in embryonic life, then follicular cells do not become gonial cells.

The observations that suggest the conversion, are possibly the result of use of the wrong stains for discerning the primordial germ cells from the follicular cells. The observations, however, describe the decrease in size and flattening of the follicular cells as the energy reserves are utilized during gametogenesis. In the closely related, *Tresus capax*, the energy reserves used in gametogenesis are in the form of glycogen (Reid, 1969). The storage of
glycogen and other carbohydrates in gonadal tissues has been reviewed for many bivalves (Sastry, 1979). Tresus nuttallii also stores glycogen in its gonadal tissues. A comparison of two separate studies, one on the reproductive cycle of T. capax in South Humboldt Bay, California (Machell and DeMartini, 1971), the other on the seasonal changes in the amounts of stored glycogen in the gonad and lipid in the digestive diverticula of T. capax in Southern British Columbia indicate that the amount of stored glycogen in the gonad decreases dramatically during gametogenesis, reaches a minimum at the time of spawning, and increases during the inactive, or post-spawning phase, whereas the lipid level in the gonad remained relatively constant during gametogenesis, decreasing only slightly at the time of spawning (Reid, 1979). The same pattern of cyclical increases and decreases of the follicular tissue with respect to the phase of the reproductive cycle was observed in T. nuttallii in Tomales Bay, as was observed for the glycogen level in T. capax in British Columbia. This leads to the conclusion that a source of energy for gametogenesis is glycogen, and it is stored in the follicular tissue.
V. Summary

The Anatomy of the Reproductive System

The gonads are paired structures that form a superficial layer one to five millimeters thick over the entire visceral mass except the foot. There are no cross-connections between the right and left gonads. The sexes are separate. Gametogenesis occurs in a highly branched system of dorso-ventrally oriented tubules termed alveoli. The alveoli are embedded in a matrix of follicular tissue. Ripe gametes are moved from the alveoli to a dorsally located collecting duct. The duct has an expanded region termed a spawn vesicle. The function of the spawn vesicle is to store ripe gametes awaiting release. The spawn vesicle is connected to the gonopore by a short duct. The gonopores open into the suprabranchial chamber on tiny papillae located immediately anterior of the posterior foot retractor muscle. When spawning occurs, the gametes are shed into the suprabranchial chamber, guided to the excurrent siphon, and shed into the sea where fertilization occurs.

The Reproductive Cycle

The reproductive cycle was studied for a period of 24 months. An average of 20 specimens were collected from the intertidal mudflats at Brazil's Beach, Tomales Bay, California. Gonadal samples were removed from the right side of the visceral mass of each animal and prepared for light microscopy using standard histological techniques.
Examination of the slides of gonadal tissue allowed the placement of each individual into one of five subunits or phases of the reproductive cycle. All animals progress through all five phases in one reproductive cycle. The proportions of the collections in each reproductive phase changes as the reproductive cycle progresses. The major events of the cycle, such as gametogenesis, the initiation of spawning, the duration of spawning period, etc., are inferred from the month-to-month changes in the relative proportions of the samples in each reproductive phase. Four periods of spawning were observed; spring of 1979 and 1980, and in the fall of 1978 and winter of 1979. Spawning appears to occur reliably in the spring, sporadically in the fall and winter.

To confirm the results of the reproductive cycle study, the amount of follicular tissue relative to the amount of gametogenic tissue was measured. The follicular tissue also fluctuates cyclically; the maxima coincided with early gametogenesis, minima with spawning, suggesting that the follicular tissue is the site of storage of food reserves used in gametogenesis.

The possibility of the conversion of follicular tissue was examined. The evidence for the conversion was a misinterpretation of the shrinkage of the follicular tissue as the food reserves were used in gametogenesis.
Literature Cited


Pearce, J.B. On the Distribution of Tresus nuttallii and Tresus capax (Pelecypoda: Mactridae) in the Waters of Puget Sound and the San Juan Archipelago. The Veliger 7(3):166-170.


Figure 1. Adult specimen of *Tresus nuttalii* lying on its right side. The anterior of the animal is to the left, posterior to the right. S, siphon; SEP, siphonal end plate. (0.7x)

Figure 2. Adult specimen of *Tresus capax*, lying on its right side. The dorso-ventral axis of the shell (arrows) is longer than in *T. nuttalii*. (0.6x)
Figure 3. Visceral skirt of *T. capax* shown here supported by two dowels. Mantle has been cut and reflected back to expose visceral mass. VS, visceral skirt; MA, mantle; CT, ctenidium; PAM, posterior shell adductor muscle; S, siphon.

Figure 4. Map of the Study Site
Figure 5. Visceral mass of *T. nuttallii*. Scalpel indicates area of the gonad from where the samples were removed. LP, labial palp; CT, ctenidium; F, foot; MA, mantle.
Figure 6. Phases of the male alveoli (240x)
6a. Active phase
6b. Ripe phase
6c. Partially spawned phase
6d. Spent phase
Figure 7. Phases of the female alveoli (240x)
7a. Active phase
7b. Ripe phase
7c. Partially spawned phase
7d. Spent phase
Figure 8. Diagramatic reconstruction of right collecting duct. Dashed line is dorsal surface of visceral mass. SV, seminal vesicle; GP, gonopore.

Figure 9. Accessory ducting formed by the ctenidium and the visceral mass. The gonopores are located immediately anterior to the posterior foot retractor muscle (PRM) in the inner pair of ducts (arrows). VM, visceral mass; CT, ctenidium; PAM, posterior shell adductor muscle. (3x)
Figure 10. Graph of the reproductive cycle. The percentage of the sample in each gonadal condition is represented by the length of each shaded area.
Figure 11. Graph of the amount of follicular tissue relative to alveolar tissue superimposed on the reproductive cycle graph.
SPENT/ACTIVE  ACTIVE  RIPE  PARTIALLY SPAWNED  SPENT

JUN  JUL  AUG  SEP  OCT  NOV  DEC  JAN  FEB  MAR  APR  MAY  JUN  JUL  AUG  SEP  OCT  NOV  DEC  JAN  FEB  MAR  APR  MAY

1978  1979  1980

PERCENT

100  75  50  25

% COVERAGE

100  75  50  25  0

RELATIVE AMOUNT OF FOLLICULAR TISSUE

CODE

1  2  3  4

100  75  50  25  0
Figure 12. The reticulum in a spent male alveolus. RT, reticulum; RS, residual sperm; FT, follicular tissue. (470x)
Figure 13. Phases of the male gonad (70x)
13a. Spent/active phase
13b. Active phase
13c. Ripe phase
13d. Partially spawned phase
13e. Spent phase
Figure 14. Phases of the female gonad (70x)
14a. Spent/active phase
14b. Active phase
14c. Ripe phase
14d. Partially spawned
14e. Spent phase