A Study of the Sexuality and Gonad Development of the little neck clam, *Paphia staminea* Conrad

by

Daniel B. Quayle

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A STUDY OF THE SEXUALITY AND GONAD DEVELOPMENT
IN THE LITTLE NECK CLAM,

PAPHIA STAMINEA CONRAD
Introduction

Sex in molluscs has been studied by scientists since the end of the 17th. century, but practically all workers have used the genus *Ostrea* as the subject of their studies. More recently, however, careful studies have been made of the sexuality in species of various other genera of pelecypods, namely, *Teredo*, *Venus*, and *Mya*. The object of choosing *Paphia staminea* for this study was chiefly because of the interest being shown in this species by the Fisheries Research Board of Canada, which has begun a program of research into the life history and economics of this and several other species of clams. Fraser and Smith (1928) have already done much of the pioneer work on the ecology of this species and their studies have provided an excellent foundation for this and for future work.

Sex in pelecypods and gastropods seems to be in a very unstable condition and sex changes often occur as a result of age or change in environment. Some species undergo a prolonged and complex series of sexual changes, while in others the change is reduced to a single reversal of sex. Of the former group *Teredo* and the native or Olympia oyster (*O. lurida*) are typical while *Venus mercenaria* is an example of the latter group. Many of the species exhibit partial or complete protandry. The question of protandry in this species along with the development of the primary gonad and gametogenesis were the main objects of this work.

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**Materials and Methods**

Monthly samples of clams were collected from a small beach on the southern point of Woods Island in Ladysmith Harbour, British Columbia. The size of the sample of clams were rather small and for that reason some phases of the work are somewhat incomplete. However, more than 1,000 slides were prepared from nearly 800 clams. With the smaller individuals serial sections were made. Of the larger individuals only several sections were prepared. It must be pointed out that there is a possibility of error in sectioning only a small piece of gonad, for different parts of it may have
attained different degrees of development. It is often found that different tubules in the same section may have reached a different degree of development.

Standard methods of histology were used. Bouin's picro-acetic-formol was used exclusively as fixative. Except for one sample where Ehrlich's acid haematoxylin was tried, Haidenhain's iron haematoxylin with ferric alum mordant was used throughout. A saturated solution of picric acid was used for differentiation after iron haematoxylin in several instances. Excellent differentiation was obtained, the cytoplasm being left quite colourless and the nuclei retaining the stain well. It is difficult, however, to get the correct end point due to the obscuring action of the yellow color from the picric acid.

For the first few months the paraffin technique was used, with cedar oil and xylol as clearing agents. Later the dioxan technique was tried, and it was found to be more satisfactory both from the standpoint of efficiency and time. The following is an outline of the dioxan technique used in this work.

1. Fix in Bouin's 6 to 12 hours.
2. Wash in 50% alcohol 24 hours.
3. Wash in 70% alcohol 24 hours.
4. Wash in dioxan 24 hours.
5. Wash in dioxan 24 hours. (If time is of consequence 6 hours may be substituted for 24 hours, but the latter is safer. Material may be stored in dioxan.)
6. Infiltrate in 1/2 dioxan and 1/2 paraffin one hour.
7. 3 changes of paraffin at hourly intervals.
8. Imbed. (It was found that imbedding several pieces of tissue in a block at least two inches square, gave a better wax matrix with no bubbles such as is the case when imbedding each piece of tissue separately in a small block)

The following is the procedure used with slides.

1. Dissolve off wax in xylol.
2. 2 changes of dioxan.
3. Wash in water.
4. Mordant in 2% iron alum 12 hours.
5. Stain in iron haematoxylin 12 hours.
6. Differentiate in 1% iron alum.
7. Wash in water.
8. 2 changes of dioxan.
9. Xylol.
10. Mount in balsam.

The small clams of which serial sections were to be made were fixed whole in Bouin's and the acid dissolved off the shell which otherwise is difficult to dissect off without injuring the tissue. Sections were cut from 3 to 10 μ. thick.

**Size and Age**

Since *Paphia* spawns from early in the year till late in the summer, there will be considerable variation in the size of a group of clams considered as one year old. It has been found that *Paphia*, if spawned early, may reach a size of .8 millimeters and probably more by the end of the same summer in which it was spawned. Others which have set late, are still very small when growth is considered to cease. Thus clams spawned in the summer
of 1938 may vary from 2 mm. up to 10 mm. in the spring of 1939 just before growth is considered to start. For the purpose of this paper only sizes will be mentioned, for age has probably little connection with time of maturity (Fraser and Smith, 1928). At any rate the age after the time of maturity is of no importance in this work.

Development of the Primary Gonad

The primary gonad begins as two primordia, one on each side of and immediately ventral to the pericardial cavity, in the posterior part of the body. The same general position is maintained throughout the development of the primary gonad and it is only after sexual differentiation that the gonad extends to completely surround the foot and visceral mass of the clam.

The first indications of the gonad are found in individuals slightly less than two millimeters in length. At this time they may be only one or two months old if spawned in the same summer. On the other hand they may be six or eight months old if spawned the previous summer. The primordia at this time consists of a tubular structure of germinal epithelium upon which is laid a single layer of vacuolated follicle cells. The centre of the tubule is vacant. The primary gonad and kidney develop almost simultaneously and the ureters can be traced through the centre of each of the lobes to the approximate middle of the animal where they enter the suprabranchial chamber on each side of the animal. Apparently the ureters serve as gonaducts. The kidney duct is differentiated by the compact layer of ciliated epithelial cells.

In clams 3 millimeters in length the gonad is distributed vertically
between the upper and lower limits of the posterior adductor muscle. Horizontally it is distributed from the visceral ganglia, just in front of the posterior adductor muscle, anterior about one third the length of the body. The lobes are joined at the antero-dorsal region of the gonad.

Gradual extension of the gonad is accomplished by the growth and spread of the germinal epithelium forward and ventrally. Meanwhile the interior of the gonad is becoming more and more compact by the evagination and growth of parts of the germinal epithelium to form what might be called follicle cell "stalks", for the evagination of the epithelium carries with it the layer of follicle cells (plate 1, fig. 3). At the same time the follicle cells are multiplying and a number of layers are formed. Formation of profusely branching tubules is finally brought about by the growth and fusion of the follicle cell "stalks". This is accomplished when the animal reaches a length of between 1.5 centimeters and 3 centimeters; probably depending on environment, inherent tendencies and time of setting. It is not until the complete formation of the tubules that sexual differentiation into the male and female phases takes place. Thus a period of from two to three years is required for the development of the primary gonad.

The follicle cells are purely nutritive in function. The delicate cell wall encloses a thin layer of cytoplasm which is often difficult to observe. The central portion of the cell is occupied by a large vacuole. The comparatively small nucleus is imbedded in the peripheral cytoplasm. The cells differ much in shape and reach a size of 30 μ. As gametogenesis proceeds the follicle cells are gradually replaced by the developing sex cells. After spawning there is a reformation of the follicle cells in the
gonad tubules, although apparently never to the original extent.

**Sexual Differentiation**

It was soon found that this species is not protandric. As soon as the tubules are fully formed, indifferent gonias, the sex of which cannot be determined, arise from the germinal epithelium. Very soon, however, for the process is almost continuous, they become differentiated into ovogonia and spermatogonia. The ovogonia are slightly larger than the spermatogonia, which measure, on the average, 8 μ. The cellular structure and the coarsely granular cytoplasm of the ovogonia is more clearly shown than that of the male cell. The nucleus of the spermatagonium is much larger in proportion and almost completely fills the cell.

The tubules become fully formed when the animals reach a size of between 1.5 cm. and 3.0 cm. in length, there being some variation. Consequently the size at maturity varies, the majority of the clams reaching that stage at a length of about 2.5 centimeters or slightly less, while others reach a length of 3.5 centimeters before spawning. This supports the conclusions of Fraser and Smith (1928).

No hermaphrodites were found.

**Ovogenesis**

After sexual differentiation or in mid-winter after the hibernation period in the case of the adult, the ovocytes begin to grow and enlarge until they are attached to the walls of the follicles only by a slender stalk. When they reach a size of between 55 μ. and 63 μ. they drop off into the lumen and are ready to be spawned. By the time the ova are ripe
and fill the extended tubules there are very few follicle cells remaining, having been used as nutriment for the developing ova.

Besides the numerous ripe ova there are many small ovocytes and gonia still attached to the walls of the follicles. These and the residual ova are retained after the spawning act. Some of these gonia and ovocytes are the forerunners of the next crop of gametes.

**Spermatogenesis**

Due to the extremely small male reproductive cells of this species the exact details were not worked out. However, the process in general can be outlined. After sexual differentiation, or in January and February in the case of mature clams, the spermatogonia lining the tubules grow and divide until a layer of spermatogonia several cells deep is formed. The primary spermatogonia are usually found in close contact with the germinal epithelium, although some are found some distance away from it. They measure approximately 8 μ. The nucleus almost fills the cell and is somewhat oval in shape. The chromatin is dispersed and frequently two nucleoli are found. Often superimposed on the primary spermatogonia are gonia of the second order, whose round nuclei measure about 4 μ. in diameter. Sometimes the secondary spermatogonia are found grouped or dispersed some distance in towards the centre of the lumen. Indeed, this is generally the case with most of the male sex cells. They are seldom arranged in the order of their development, and may be found in almost any position in relation to each other. In some sections definite layers of sex cells in regular order are found. This is usually so in the case of poorly nourished individuals where rather narrow columns of reproductive cells extend radially in towards
the centre of the tubule from the germinal epithelium at various intervals along it. The spaces in between the columns are filled with follicle cells. The nucleolus of the secondary spermatogonium usually occurs close to the nuclear membrane.

Further in towards the centre of the tubule are groups of primary spermatocytes evolved by the division of the secondary spermatogonia. The primary spermatocytes are slightly larger than the secondary gonia. As mentioned above details of the transformation and division of the primary spermatocytes was not observed in detail. The secondary spermatocytes are usually clustered together in large numbers. Their nuclei measure 2μ in diameter. The transformation of the secondary spermatocytes into spermatids takes place after the last division of the spermatocyte by the concentration of the chromatin of the nucleus into a solid, heavily staining mass. This then begins to elongate and assume the form of the mature spermatozoan head. The other details were not observed. The head of the spermatozoan, 6μ in length, is conical in shape and slightly bent. Excepting a single layer around the germinal epithelium, the follicle cells are gradually crowded out and replaced by the sex cells which are finally converted to spermatozoa, which align themselves in various patterns, heads together and tails together, in the lumens of the spermary.

Seasonal Gonad Changes

The author hesitates to draw definite conclusions regarding this phase of the investigation because of the small number of clams studied. Naturally this phase of the investigation requires the examination of adult clams and as the primary purpose of the work dealt with immature individuals
and those just approaching maturity, the major emphasis was placed on these young stages to the neglect of the mature forms.

Only 300 adult individuals were prepared histologically and it is from the study of this rather small sample the following interpretation is made. Also the whole sample was taken from a single beach in Ladysmith Harbour and consequently may not be representative of the typical sexual cycle of the species.

However, the interpretation does fit quite well the results of the plankton investigation and the study of the setting period in Ladysmith Harbour.

At any rate it is thought advisable to give an outline of the seasonal gonad changes as represented by the sample of 300 individuals. It is hoped to extend the investigation to include other areas and so form a representative sample from which more reliable conclusions can be drawn.

September is the last month of the year in which spawning takes place so it would seem logical to start a discussion of seasonal gonad changes at a point immediately after the completion of the sexual cycle.

**Females**

By the end of September the great majority of the clams of this species have spawned or have partly spawned. Even with complete spawning there is usually a considerable number of residual ova left in the tubules of the ovary. In this discussion ova refers to ovarian eggs or more specifically, primary ovocytes. These residual ova may be extruded later; others undergo cytolysis. Still others may be carried right through the winter and spawned the following summer with the new crop of eggs. This
latter event is not of frequent occurrence however. Usually along with the residual ripe ova are a considerable number of residual young ovocytes. After spawning the walls of the tubules, consisting of the fine germinal epithelium and connective tissue are wrinkled and collapsed, with varying quantities of residual ova in the lumens and young ovocytes and undeveloped gonia attached to the germinal epithelium. Soon after spawning proliferation of the follicle cells begins. At the same time growth of new ovogonia is started but ceases when the average size of 10 to 15 μ. is reached. Growth of the follicle cells continues until the tubules are full or nearly so. This is usually completed by December or January. Beyond the short growth period immediately after spawning, the ovogonia show little activity till late in December, when, although water temperatures are low, they begin to show active growth. By February and early March ovogenetic activity is at its peak. In January there is a great deal of variation in the progress of ovogenesis in different individuals. Follicle cells partly fill the lumens of the ovaries in all females. Gonia of various sizes and order have appeared in all while some contain ovocytes in all stages. The condition at the end of January is little different from that at the end of December. During February, however, ovogenesis proceeds rapidly and by early March the majority of animals have gonads in a ripened condition, with the lumens of the tubules distended with mature ova.

During March the first indication of spawning is found. These were partial spawners. It seems that from histological evidence, the spawning of an individual may last over a period of time such as is the case of *Venus mercenaria* (Loosanoff, 1937). It is often found that large numbers of Pacific oysters (*O. gigas*) may discharge only a part of their spawn,
although this is not the general method. It may be similar in the case of *P. staminea*. It does seem, however, that the males expell the whole contents of the spermary at one time.

By the beginning of September the majority, but not all, have spawned, for larvae in considerable numbers are found at the end of September. The samples were not large enough to discover what happens to animals that spawn early in the season. From the degree of gametogenesis discovered throughout the summer it may be that a new crop of gamete is formed and even spawned before the water temperature drops. Indeed Edmondson (1923) claims two spawning periods in a single summer. This is so of the species *Paphia philippinarum*, a Japanese mollusc.

**Males**

The male reproductive cycle follows that of the female quite closely. The recuperative period is longer in the male however, and they may go through the fall and part of the winter in much the same state as they were immediately after spawning. Once spermatogenesis starts, however, it proceeds rapidly and the tubules soon become filled with sex cells in all stages of development.

Immediately after spawning the tubules contain a number of spermatozoae held loosely in the centre of the lumen. The germinal epithelium containing a few inert gonidia is collapsed. This appearance is retained until January when proliferation of both follicle cells and spermatogonia begins almost simultaneously. The residual spermatozoa are maintained up to this time. Their fate is unknown. The follicle cells increase rapidly and soon fill most of the lumen. After the first crop of gametes have been produced
the follicle cells do not seem able to completely fill the tubules. In poorly nourished individual groups spermatogonia appear at intervals along the germinal epithelium. Superimposed upon these are layers of secondary spermatogonia. Then above these again are spermatocytes of both orders and finally spermatids undergoing spermeoteleosis. The centre of the lumens are filled with parallel rows or columns of spermatozoa with the sperm-heads ranged alongside each other, and the tails intertwined in the clear space between the columns or rows of sperm-heads. Well nourished individuals show the tubules tightly packed and distended with reproductive cells in all stages of development and arranged more or less as indicated above.

Spermatogenetic activity reaches its peak in February and March although indications of active spermatogenesis is found in some individuals in each month of the year, especially during the summer. Spawning in the male is completed at one time. Morphologically mature sperms are found in a proportion of the clams in each month of the year. Whether or not they are physiologically mature can not be determined for the greater part of the year at least, due to the lack of mature ova.

**Discussion**

It is interesting to find in *Paphia staminea* a gonad structurally similar to that of *Mya arenaria*, a species in a different family. On the other hand it is also interesting to find that *Venus mercenaria*, which belongs to the family Veneridae, as does *Paphia*, has a gonad structurally different from the latter species. The only way in which the two seem to be allied is in the development of gametes during a time of the year when
the water temperatures are nearly at their lowest.

*Mya arenaria* (Family Myidae), the sexuality of which was investigated by Coe and Turner in 1937, has the same branched tubular structure of the gonad, with the alveoli filled with vacuolated follicle cells, as *Paphia*. *Mya* is also generally unisexual with no evidence of protandry or change of sex. The gonad proliferates by means of a terminal syncytium filled with large and small nuclei, which are destined to become germ nuclei and follicle cell nuclei respectively. This syncytium pushes its way ventrally and anteriorly among the viscera, leaving behind it germ cell and follicle cell nuclei on the walls of the tubules. No such structure or development was found in *Paphia*, where germ cells or their nuclei do not become differentiated as such till the animals are about two or three years old.

In *Mya* and in the majority of pelecypods that have been thoroughly studied, development of the gonad usually takes place in spring when the water temperatures begin to rise. As has already been indicated, gametogenesis in *Paphia* occurs at a time when the water temperatures are nearly at their lowest for the year. This is also true of *Venus mercenaria*, in which the main period of egg and sperm production occurs in the fall and early winter.

Both *Paphia* and *Venus* retain morphologically mature spermatozoa throughout the year. *Venus*, however, shows partial protandry and experiences at least one change of sex.

**Summary**

1. The primary gonad of the little neck clam is different from most
pelecypods in being composed of profusely branched tubules filled with vacuolated follicle cells, whose function is nutritive. It is, however, similar to that of *Mya arenaria*, a species of clam investigated by Coe and Turner (1937).

2. Growth of the primary gonad, which starts from two primordia, one on each side of the body, is by extension and growth of the germinal epithelium. In the growth of the primary gonad of *Mya arenaria*, Coe and Turner found the process was carried out by means of a terminal syncytium composed of large and small nuclei, which were destined to become germ cell nuclei and follicle cell nuclei respectively. No such development was found in *Paphia*. The growth of the gonad in both species is forward and ventral into the tissues surrounding the stomach and intestine.

3. The gonia are not differentiated on the germinal epithelium until the alveoli are fully formed. This takes place when the animals reach a size of between 1.5 and 3.0 centimetres. The indifferent gonia develop immediately into male or female cells, depending on the sex of the clam. The species is generally unisexual; it is not protandric and there is evidently no change of sex.

4. The development of the gametes causes disintegration of the follicle cells until they are completely replaced by sex cells. The follicle cells are reformed after spawning.

5. The spawning season is a long one, lasting from early spring till early in the fall.

6. After spawning, in the female there is slight growth of the gonia, but active ovogenesis does not begin till December and January. Females with ripe ova are found in February. In the male there is very little
spermatogenetic activity till January and February, although some indication of spermatogenesis may be found in some animals in each month of the year. Peak activity is reached in February and March. Morphologically mature sperms can be found throughout the year.


Plate 1.

Fig. 1. Cross section of young Paphia, 4 mm. long, showing the position and extent of the primary gonad. Int.-intestine, l-lumen, f.c.-follicle cells, m-mantle, m.c.-mantle cavity, g-ganglion, h-heart, um-umbo.

Fig. 2. Section of the gonad of young clam 1.24 cm. in length. June. Shows the close connection between the kidney and the gonad. U-ureter, a.b.c.-suprabranchial chamber, gl.-gill, ct.-connective tissue, k-kidney. Other letters as in Fig. 1.

Fig. 3. Enlarged section of the gonad of Paphia 1.26 cm. in length, showing the follicle cells on the stalks and on the germinal epithelium. F.c.s.-follicle cell stalk, n-nucleus.

Fig. 4. Section of the gonad of clam 2 cm. in length, showing the compact tubules separated by connective tissue and containing numerous follicle cells. Primary germ cells (p.g.c.) are seen on the walls.
Plate II.

Fig. 1. Individual tubules showing differentiation of the primary germ cells into female sex cells. c.o.-ovocyte, p.g.c.-primary germ cell, f.c.-follicle cell.

Fig. 2. A more advanced tubule than in Fig. 1. showing the growing ovocytes.

Fig. 3. Section of the gonad of a male clam 2.5 cm. in length. Shows the proliferation of the gonad and the disintegration of the follicle cells. This is the first crop of gametes produced by this clam.

Fig. 4. Enlarged portion of the ovary showing the ovocytes growing between the follicle cells.

Fig. 5. Semi-diagramatic representation of spermatogenesis with the spermatozoa (spz.) in the centre of the tubule. spt.-spermatid, spc.2.-secondary spermatocytes, spc.1.-primary spermatocytes, spg.2.-secondary spermatogonia, spg.1.-primary spermatogonia.
Fig. 1. Section of the gonad of Paphia 1.26 cm. in length, showing the growth and solidification of the primary gonad by the formation and extension of the follicle cell stalks and by multiplication of the follicle cells. f.c.-follicle cells, f.c.s.-follicle cell stalks, int.-intestine. X 100.

Fig. 2. Primary gonad of a clam 2.64 cm. in length showing the structure of the completed tubules. There are a few germ cell nuclei (p.g.c.) along the germinal epithelium. X 100.

Fig. 3. Gonad of adult Paphia 4.16 cm. in length in December. Shows the small oocytes of the new crop of gametes with the alveoli partially filled with follicle cells. X 100.

Fig. 4. Same as Plate 11., Fig. 3.
Plate IV.

Fig.1. Adult clam in August just after spawning and showing the residual spermatozoa (spz.), debris, and the collapsed condition of the walls of the tubules. X 100.

Fig.2. Spermary of a poorly nourished individual in March. X 100.

Fig.3. Gonad of a male clam 3.0 cm. in length showing numerous sex cells in all phases of development, and the relatively few darkly staining spermatozoa in the centre of the tubule. S.c.-sex cells, spz.-spermatozoa.

Fig.4. Spermary of a clam 5.38 cm. in March. Spermatogenesis is almost complete in this individual as shown by the very few developing sex cells and the great number of morphologically mature spermatozoa filling most of the lumens of the tubules. X 100.
Plate V.

Fig. 1. Ovary of a clam 3.56 cm. in length in August. Partial spawning has just occurred and many relict ova are left in the lumens. Some of the ovocyte's are still attached to the walls. X 100.

Fig. 2. Gonad of female Paphia in January. Shows the developing ovocytes and the lumens partly filled with follicle cells. X 100.

Fig. 3. Gonad of adult female in November showing residual ova (r.occ.) Some of the new crop of gonia (ov.) and ovocytes (oc.) can be seen. The walls of the tubules are still somewhat collapsed, but the new follicle cells have begun to proliferate. X 100.

Fig. 4. Rippe ovary of adult Paphia showing the numerous ripe ova distending the walls of the follicles. Very few follicle cells are left. Some of the ovocytes are still quite small. X 100.