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SEASONAL PATTERNS OF GAMETOGENESIS IN A NORTH ATLANTIC BROODING ASTEROID, *LEPTASTERIAS TENERA*

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Brooding behavior has been reported in several species of sea stars, but detailed descriptions of gametogenesis and reproductive behavior are available for relatively few species of *Leptasterias*. Recent valuable contributions to the knowledge of reproductive cycles and brooding behavior in two Northeast Pacific species of *Leptasterias* have been made by Chia (1966, 1968a, b) and Menge (1975) studying *L. hexactis* from Puget Sound, and by Smith (1971) observing *L. pusilla* from Monterey Bay. Stomach brooding behavior in the arctic species, *L. groenlandica,* was described earlier by Lieberkind (1920) and Fisher (1930).

The genus *Leptasterias* is predominently an arctic-boreal group of asteriods (Fisher, 1930; Ekman, 1953). While direct development is characteristic of many arctic invertebrates among many taxonomic groups including echinoderms (Thorson, 1946, 1950), available data indicates that the more southern-occurring species of *Leptasterias,* for example *L. hexactis, L. pusilla* and *L. tenera,* have retained this mode of reproduction even though the majority of sympatric asteroids have planktotrophic development.

Leptasterias tenera, a species of the continental shelf of the Atlantic, occurs from Nova Scotia to Cape Hatteras in the cold-temperate (boreal) marine climatic zone at depths of 18 m to 150 m. This species differs ecologically from the Northeast Pacific species *L. hexactis* and *L. pusilla* in two important respects: first, it is sublittoral as opposed to intertidal; and secondly, it is predominately an inhabitant of soft bottom sediments rather than rocky shores.

Gonads of *L. tenera* collected during a one years period were examined in order to trace the cycles of gametogenesis. Comparisons were made with the available information on the reproductive patterns in *L. hexactis* and *L. pusilla,* and modifications in the various patterns were noted. To evaluate the divergence of the reproductive pattern in a brooding starfish, *L. tenera,* from that of a broadcasting species, brief comparisons were made with the reproductive system of *Asterias vulgaris* as described by Walker (1975). Evidence was sought for a correlation between gametogenesis and the feeding pattern.

An evaluation of features common to the genus *Leptasterias* suggested that the reproductive pattern associated with direct development and brooding is genetically fixed. Since *Leptasterias* apparently lacks the genetic flexibility to "return" to the more primitive broadcasting mode of reproduction, it was suggested that the inherited pattern would be subject to selective modification. Such modifications should be

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discernible among species occurring in divergent ecological and zoogeographic conditions.

Materials and Methods

Samples of *L. tenera* were collected between two locations in Block Island Sound, west of Block Island (41° 11.4′ N, 71° 38.3′ W to 41° 12.1′ N., 71° 36.2′ W) at a depth of about 30 m using a modified scallop dredge. Bottom sediments in this area of Block Island Sound consist of fine to very fine sands mixed with silts and clays. Collections were made on an approximate monthly schedule from October 1971 through September 1972, with the exception of January when no sample was taken.

Within 24 hours of collection, the arms were removed and placed in Bouin's fixative. Gonads were dissected from representative samples of each size group and preserved as above. Observations on the gonads of living animals also were examined at this time. All fixed material was embedded in paraffin and sectioned at 6–10 microns. Longitudinal and transverse sections were stained with Heidenhain's iron hematoxylin and eosin. Due to difficulties encountered in sectioning the extremely yolky oocytes, it was necessary to soak the paraffin blocks in tap water for 12–24 hours prior to sectioning.

Results were based on the study of a minimum of two animals of each sex per month. All measurements were made using an ocular micrometer. The information showing the period of active feeding in *L. tenera* which is presented in Figure 1 was obtained from monthly stomach analyses. These data will be discussed fully in another paper (Hendler and Franz, in preparation).

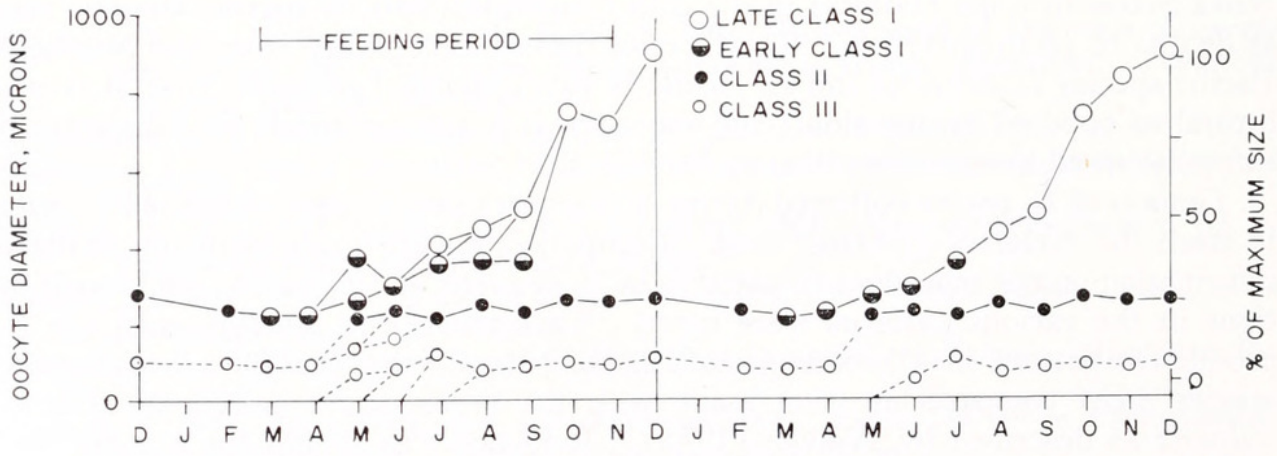


Figure 1. Two-year cycle of oocyte development, as determined by extrapolation from the histological examination of monthly collections during a 12 month period. The information on feeding activity is based on stomach analyses of monthly samples collected over the same period. As shown in the left panel, most oocyte growth including vitellogenesis occurred during the period of most active feeding. The right panel traces the development of a single oocyte, beginning with its first appearance in June. This small (Class III) oocyte would, most likely, become class II the following spring. Active vitellogenesis would begin the following March or April, to produce a mature (Class I) yolk-filled oocyte by November or December, a total developmental period—in this example—of 29–30 months. It is estimated that, in general, oocyte development requires a period of between 24 and 30 months.

Observations and Results

Morphology of the testes

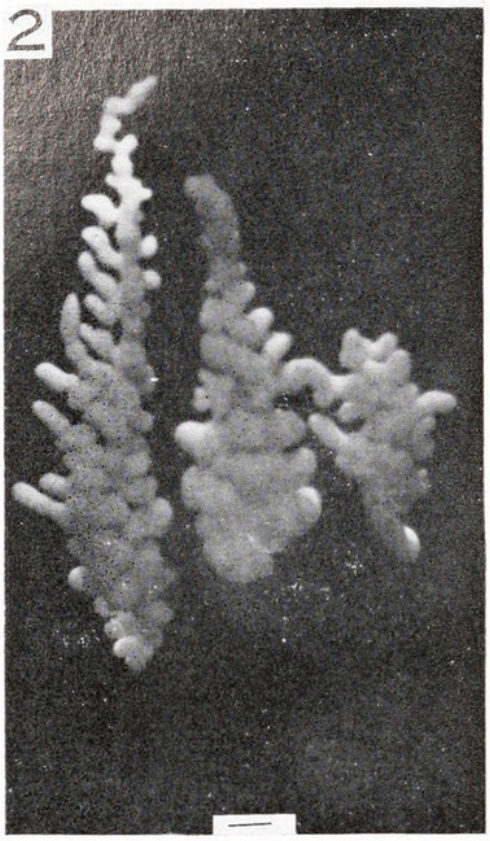
The testes in *L. tenera* exhibit considerable variability in size and shape between different individuals as well as in different arms of the same individual, and also during spermatogenesis. It is not unusual to find two testes of markedly unequal size, only one testis, or even no testis at all in a given arm. Characteristically, each arm contains two sac-like testes with finger-like projections (Fig. 2). Located ventrally in the proximal portion of the arm, they lie on either side of the ambulacral ossicles. The only attachment to the body wall is by means of the sperm duct. Each testis opens independently to the outside through a short sperm duct which leaves the coelom between the dorso-lateral and supramarginal plates and terminates ventrally in a small papilla located near the adambulacral plates. This location of the sperm duct opening is comparable to that described by Fisher (1930) in *L. groenlandica,* and by Smith (1971) in *L. pusilla.*

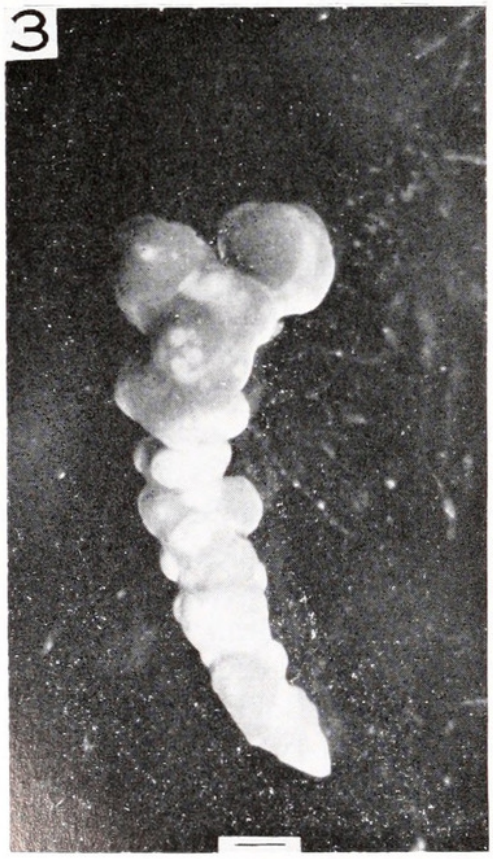
Histology of the testis wall

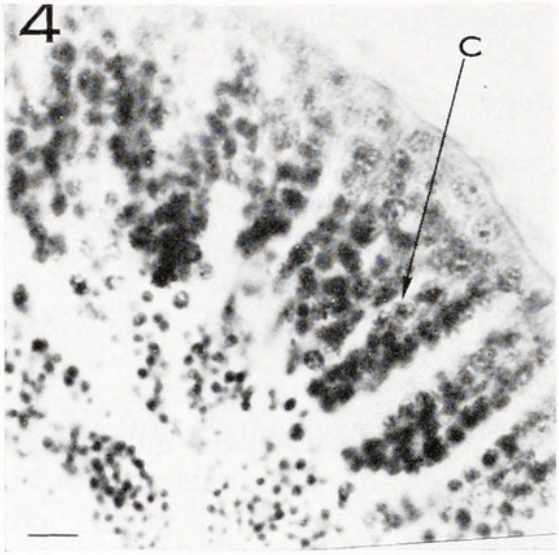
The structure of the testis wall in *L. tenera* is basically comparable to that described by Smith (1971) in *L. pusilla,* and by Walker (1974) in *Asterias vulgaris.* In *L. tenera,* the wall is composed of three parts: an outer, multilayered portion; the haemal sinus; and an inner, germinal epithelial layer which delimits the lumen. The strata which make up the outer portion include a single layer of flagellated, cuboidal, coelomic epithelial cells which become elongated and flattened during the course of gonadal enlargement, and an underlying muscle layer of both longitudinal and cross fibers which becomes thinner as the numbers of sperm increase in the lumen. Closely associated with the muscle layer is the connective tissue stratum which appears to be a syncytium of elongated, kidney-shaped nuclei in a matrix containing numerous longitudinal, radial and criss-cross fibers.

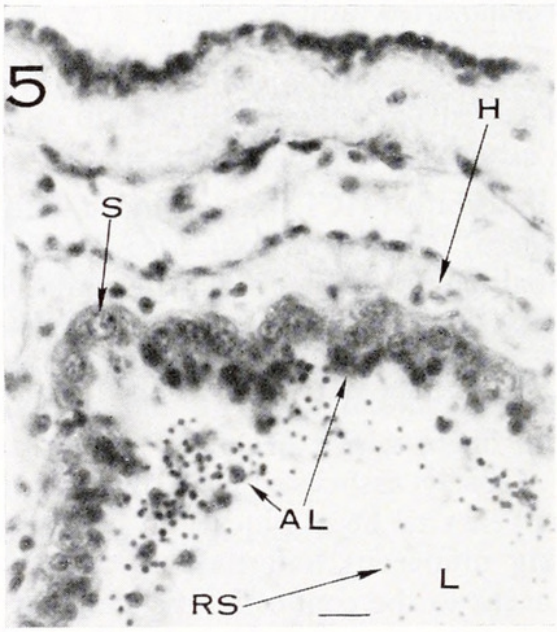
Adjacent to the connective tissue layer is the haemal sinus which is comparable to the haemal space noted by Smith (1971) in *L. pusilla* and the haemal sinus described by Walker (1974) in *Asterias vulgaris.* During the course of a year, the haemal sinus in *L. tenera* varies from an average width of about 10 microns after spawning to not more than one micron when the testis is filled with sperm. Elongated and flattened epithelial cells, widely spaced, line the sinus. Scattered within the sinus cavity are cells (called lymphocytes in this paper) lying in an amorphous, eosinophilic substance. These lymphocytes are of two types, comparable to the asteroid types described by Hyman (1955) and Endean (1966); numerous amoeboid, spindle-shaped cells, 10–16 μ; and round petaloid cells containing balloon-like swellings and very little cytoplasm, 9 μ (Fig. 6). Both Hyman and Endean considered these cells to be two phases of the same cell type, but this relationship has not been demonstrated in *L. tenera,* although both types of lymphocytes occur together.

The basal, germinal epithelium membrane is closely appressed to the haemal sinus (Fig. 5). Where infoldings of the germinal epithelium occur, the sinus also expands and fills the inpushing. A single layer of primordial germ cells appears to be present in the germinal epithelium at all times of year. Sperma-









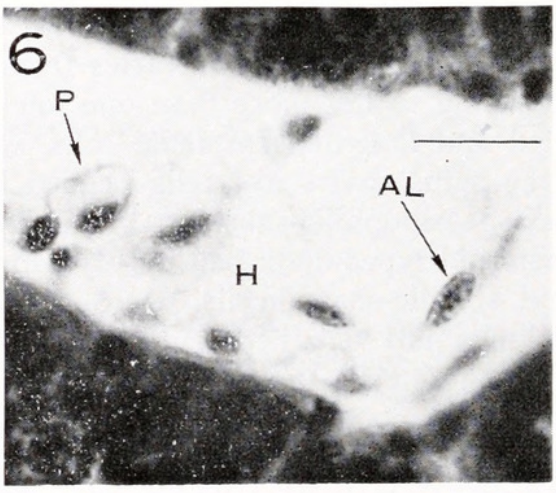


FIGURE 2. Testes from a preserved specimen taken on July 6 showing the typical appearance of the gonads. Note the many finger-like projections arising from the main axis of the testis, and the variation in size; bar is 1 mm.

FIGURE 3. Preserved ovary (November 12) shortly before spawning. Note the massive

togonia are identified by the large nucleus containing a single large nucleolus and reticular chromatin, the relatively small amount of cytoplasm, and their position close to the basal germinal membrane.

Seasonal patterns of spermatogenesis

Based on observations of preserved gonads, it is evident that shedding occurs sometime between the middle of November when the testis is expanded and the lumen is completely filled with sperm and the middle of December when all parts of the testis are greatly reduced and relatively few sperm remain in the lumen (Fig. 5). Occasionally, an otherwise spent testis may have a lobe in the pre-spawning condition indicating that shedding is not complete.

By February, the numbers of spermatogonia have increased noticeably. Differentiation into primary spermatocytes, however, is not apparent until March, which is also the time when feeding is resumed (Fig. 1). In April, primary spermatocytes are numerous and form a layer several cells thick. Spermatogenesis proceeds rapidly throughout the spring and summer months, the period of most active feeding. As the numbers of primary and secondary spermatocytes increase, they become arranged in colonnettes (Cognetti and Delavault, 1962; Smith, 1971) projecting into the lumen (Fig. 4). These formations appear comparable to the spermatogenic columns described by Walker (1976). Spermatozoa begin to break away from the tips of the colonnettes by June, and spermatids continue to mature in ever increasing numbers until the lumen is filled with masses of sperm. Colonnettes disappear by October, although large numbers of secondary spermatocytes and spermatids are still present. Just prior to sperm release, all cells complete spermatogenesis, and the germinal epithelium contains only a single layer of spermatogonia (Fig. 5).

Accessory cells

A major phagocytic function is apparently performed in the testis by the amoeboid lymphocytes. After sperm release, these cells are observed in large numbers in the haemal sinus, within the germinal epithelium squeezed between the spermatogonia, and lined up at the periphery of the lumen where they accumulate in a layer several cells thick as their numbers increase. Many lymphocytes with one or more basophilic sperm heads in the cytoplasm are present in the lumen among the relict sperm. When spermatogenesis begins in March, most of the relict sperm have disappeared. At this time, strongly basophilic, phagocytic cells filled with large basophilic granules appear in the lumen at the edge of the germinal epithelium. Dark staining, elongated cells of comparable size are squeezed between the

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protuberances, representing mature oocytes within the lumen. Light areas are immature (Class II) oocytes attached to the germinal epithelium; bar is 1 mm.

FIGURE 4. Section of testis wall (May 2) showing spermatogenesis and the formation of the colonnettes (C); bar is 10 microns.

FIGURE 5. Section of testis wall after shedding (December 21). Note the presence of relicit sperm (RS) and amoeboid lymphocytes (AL) in the lumen (L), spermatogonia (S) in the germinal epithelium, and haemal sinus (H); bar is 100 microns.

FIGURE 6. Section of testis (June 8) showing petaloid cells (P) and amoeboid lymphocytes (AL) within the haemal sinus (H); bar is 10 microns.

spermatocytes and lined up along the basal membrane. Amoeboid lymphocytes and petaloid cells are both present in the haemal sinus and in addition, cells containing deeply staining basophilic granules are frequently observed.

As the lumen fills with masses of spermatozoa, a few lymphocytes may still be seen scattered among the cells, but there is no evidence of phagocytic activity at this time. The haemal sinus becomes greatly compressed in most regions, and amoeboid and petaloid cells are concentrated in expanded areas associated with infoldings of the germinal epithelium (Fig. 6).

Morphology of the ovary

In *Leptasterias tenera,* two sac-like ovaries with many lumpy protuberances (Fig. 3) are located along the ventral floor of each arm, one on either side of the ambulacral ossicles, extending freely into the coelomic cavity. Some variation was noted in the size and shape of ovaries from different individuals of comparable size collected at the same time, as well as among ovaries in the same individual. It was not unsual to find one large and one very small ovary in the same arm, and infrequently a single ovary in a ray. Variability was less prevalent, however, than in the testes. The ovaries range from an average length of 2.5 mm soon after spawning to a maximum of 6.5 mm just prior to oviposition. Since the average length of an arm in mature specimens is about 20 mm, the ovaries, even at their maximum size, occupy less than twenty per cent of the coelomic cavity.

A single oviduct leaves the gonad on the interradial side 0.5–1.0 mm from its proximal end, extends for about 0.5 mm straight to the lateral wall of the arm and passes to the outside through an opening between the dorso-lateral and supramarginal plates III and IV. After the duct leaves the body wall, it immediately makes a right angle turn and extends under the dermis toward the mouth for 1.0–1.5 mm to open through a small papilla which lies in close proximity to the corresponding papilla of the adjacent arm. These openings are located at the level either of the fifth and sixth adambulacral spines or the sixth and seventh spines. A comparable structure and location for the oviduct openings were described by Lieberkind (1920) in *L. groenlandica,* and by Fisher (1930) in *L. groenlandica* and *L. beringensis.* There are no actino-lateral or inferomarginal spines in this region (designated the interradial platform in this paper), but one or two straight pedicellariae are usually present near the openings.

Histology of the ovarian wall

The basic histological structures of the ovarian wall in *L. tenera* are comparable in most respects to those described above in the testes. The ovarian wall however, is thicker, the connective tissue layer contains more fibers, and the haemal sinus is narrower and without amoeboid or petaloid cells. Whereas in the testis a layer of spermatogonia is present in the germinal epithelium along the basal membrane throughout the year (Figs. 4, 5), the germinal epithelium of the ovary is made up of two types of cells: first, germ cells, represented by scattered oogonia which usually appear in small clusters of a few cells and developing oocytes; and secondly, an almost continuous layer of small, numerous accessory cells which become either follicle cells or amoeboid lymphocytes. Chia (1968a) and Smith

(1971) described a similar construction of the germinal epithelium. When the oogonia differentiate into primary oocytes, they are surrounded by follicle cells and normally remain in the germinal layer, in close proximity to the basal membrane, during the course of growth and development (Figs. 7, 8, 9).

The lumen of the ovary is large and expanded at all times of the year, regardless of the number or size of the oocytes. Although the lumen is reduced in diameter following oviposition, it does not collapse as in the testis, and the walls are not folded since they still contain many developing oocytes.

Seasonal patterns of oogenesis and vitellogenesis

Meiosis in *L. tenera* is not completed until after the oocytes are shed and fertilized, a condition comparable to the pattern of oogenesis in other starfish (Tyler and Tyler, 1966; Chia, 1968a). Therefore, the developmental processes of the oocytes which take place in the ovary are concerned with increase in cell size, changes in chromosomes preparatory to meiotic division, formation of a large germinal vesicle and vacuolated nucleolus, and the development of quantities of yolk. Based on an analysis of development during a one year cycle, it is evident that completion of all these processes requires at least 26–30 months (Fig. 1), a time period similar to that postulated for *L. hexactis* (Chia, 1968a) and *L. pusilla* (Smith, 1971).

Although the developmental process is gradual and more or less continuous, it is convenient for ease of description to designate three developmental categories. These categories correspond, in general, to the three developmental stages specified by Smith (1971) in *L. pusilla,* but differ somewhat from the three descriptive stages indicated by Chia (1968a) in *L. hexactis.*

Class I. In this category are included the large second year oocytes (300–1000 microns) undergoing vitellogenesis from the first appearance of small yolk granules to the final stages when the yolk globules have become large (7–16 microns), round or elliptical, and fill the cytoplasm except for a small area surrounding the germinal vesicle. In living material, these oocytes change from white to yellow-orange to orange during this sequence of development.

Class II. Included in this category are the intermediate-sized oocytes (120–300 microns) which are undergoing cytoplasmic growth and development of a large germinal vesicle, as well as progressive vacuolation of the nucleolus. Cells in this group show no evidence of vitellogenesis and appear white in living material.

Class III. The youngest and smallest oocytes (20–120 microns) make up this category. These cells are distinguishable from the Class II oocytes by their large nucleus containing a small nucleolus and the small amount of cytoplasm. The ratio of nucleus to total cell size is about 0.34 in Class II oocytes and 0.55 in Class III cells. In living material, these small oocytes are transparent.

Seasonal changes in the size and development of oocytes throughout a two-year cycle in *L. tenera* are diagrammed in Figure 1. The feeding period of the Block Island Sound population has been superimposed on this cycle to indicate the relationship between feeding and oogenesis. Although the figure shows the smallest oocytes appearing in May, oogenesis may be initiated at an earlier date. Clusters of oogonia are present in some part of the ovary during all months. May

through July, however, are the months of maximal recruitment of primary oocytes and growth, as shown in Figure 1.

Following the spawning of the fully yolked oocytes between the middle of November and the middle of December, the larger oocytes remaining in the ovary become Class I. They are easily distiguished from the smaller, and younger (now Class II) oocytes by their larger size, large, vacuolated nucleolus in an expanded germinal vesicle, and especially, by the presence of numerous, small basophilic granules scattered throughout their cytoplasm. Class III oocytes are not present, but clusters of oogonia occur in scattered areas of the germinal epithelium. Cells are identified as oocytes when they are surrounded by one or more layers of follicle cells joined in an irregular way to form an enclosing envelope. Large, unshed, fully-yolked oocytes along with some intermediate-sized oocytes are commonly present in the lumen in the process of being broken down by amoeboid phagocytes.

During the months when female *L. tenera* are brooding and feeding ceases (December through March), little visible change takes place in Class II oocytes except for a small increase in nuclear and cell size. Toward the end of this period, Class III oocytes can be identified. Class I oocytes have small yolk globules scattered throughout the cytoplasm and in some cells, a few larger globules are localized at the periphery, usually on the side toward the haemal sinus. Remnants of phagocytized, mature oocytes were found in all ovaries examined from animals collected during February and March.

With the resumption of active feeding in early April, numbers of oogonia increase. All classes of oocytes begin to show growth and development which continue throughout the summer months. As the oocytes enlarge, they bulge into the lumen while retaining their attachment to the basal germinal membrane by the encircling follicle cells. Oocytes appear squeezed together or flattened against the basal membrane (Fig. 7). Enlarging oocytes or growing Class III cells may push other oocytes away from part of their attachment to the membrane or even dislodge them completely.

In fresh, unfixed material, many of the degenerating oocytes found in the lumen during April and May appear orange. In as much as Class I oocytes do not attain even a yellow color until June, it is probable that much of the distintegrating material is still derived from unshed oocytes. However, many more Class I oocytes are undergoing vitellogenesis than will reach maturity, so that a reduction in their number must take place. It is reasonable to assume that at least some of the necrotic cells are current oocytes.

By September, numbers of oogonia decline and Class III oocytes are found in all regions of the ovary. Class II cells have increased the amount of cytoplasm and the size of the germinal vesicle while the nucleolus has become vacuolated. Class I oocytes, which appear orange in living material, are filled with numerous, large yolk globules throughout the cytoplasm, except in the immediate vicinity of the germinal vesicle which is now located at the periphery of the oocyte. Although these cells nearly fill the lumen (Fig. 9), they maintain their attachment to the basal membrane, but the follicle layer is reduced to a single band of flattened cells with elongate nuclei.

Active feeding declines from September to November. During this period,

Class II and III oocytes grow very little. Class I cells, however, continue to enlarge as the yolk globules approximately double in size. Just prior to spawning (November–December), these oocytes have attained their maximum size of 900–1000 microns and lie free in the lumen. All trace of follicle cells has disappeared except for a few scattered, elongate nuclei occasionally observed between two very closely appressed cells. Immature oocytes present in the main body of the ovary are closely pressed and flattened against the basal membrane (Fig. 9), but they retain their rounded shape in the lobes and distal tip which are free of large, mature oocytes.

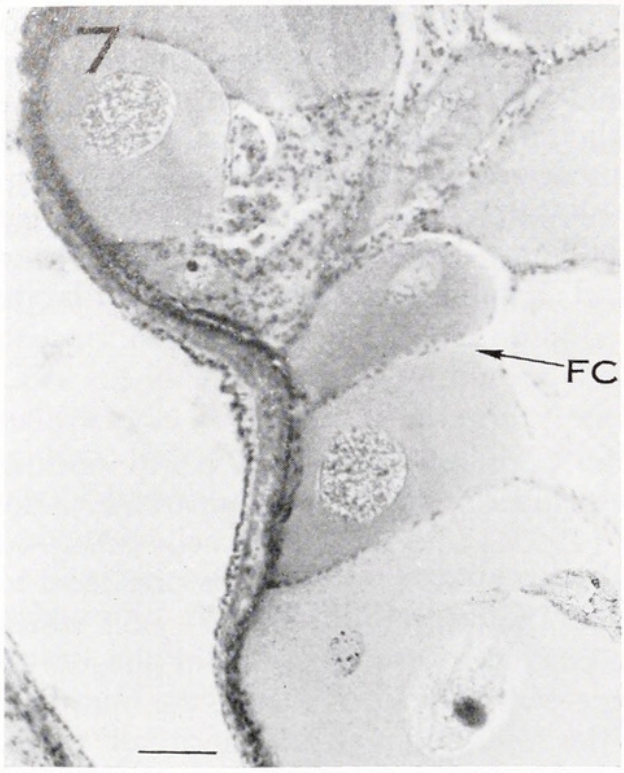
Accessory cells

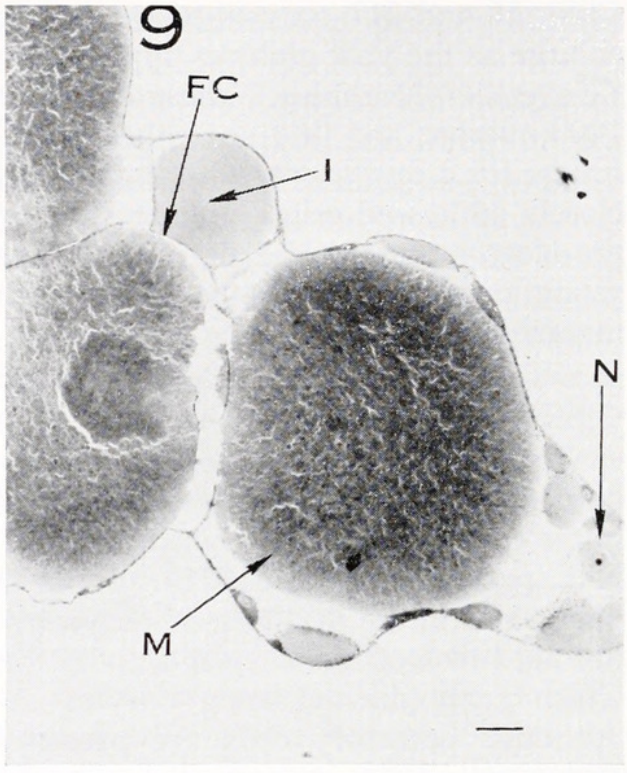
In *L. tenera,* the accessory cells in the germinal epithelium which enclose small oocytes in one or more layers of closely adhering cells, are comparable to the nurse cells observed in *L. hexactis* by Chia (1968a) and the follicle cells described in *L. pusilla* by Smith (1971). In all these species, this type of cell is presumed to play a role in the dynamics of oocyte growth. The remaining accessory cells which are not involved in follicular activity form a potential force of amoeboid phagocytes which enter disintegrating oocytes in large numbers and engulf the material. Although accessory cells are present in the germinal epithelium at all times, their numbers appear to increase markedly when degenerating oocytes are present in the lumen. Petaloid cells of undetermined origin and function were observed in the lumen of the ovary, especially during the summer months, but they were not seen in the haemal sinus at any time.

Vitelline membrane, oviposition and brooding

Both Chia (1968a, *L. hexactis*) and Smith (1971, *L. pusilla*) described localized thickenings associated with follicle cells lying outside the plasma membrane during periods of rapid growth. These thickenings gradually became the continuous, thick vitelline membrane with numerous pores or channels through which passed cytoplasmic bridges connecting follicle cells with the oocyte. Localized clusters of follicle cells around the oocyte were observed in *L. tenera,* but no evidence of their participation in the formation of a membrane was established and no direct interrelationship between follicle cells and the oocyte was observed.

At the time of spawning, a relatively small number (12–18 per gonad) of large, yolk-filled oocytes lie free in the lumen filling the main body of the ovary (Fig. 9). Oviposition undoubtedly is brought about, in part at least, by contractions of the ovarian wall forcing the oocytes into the large, open mouth of the oviduct. The walls lining the lumen of the oviduct are deeply folded which could account for expansion of the lumen to permit passage of the large oocytes. The inner margin of these folds bear great numbers of very long, stout flagella extending into the lumen. Muscle fibers are continuous from the wall of the ovary into the outer wall of the oviduct. A combination of flagellary current and muscular action could propel the oocytes to the outside. During the year, the mouth of the oviduct is small and constricted. However, the presence of amoeboid phagocytes and remnants of degenerating oocytes within the lumen of the oviduct, as well as the appearance of oocytes adjacent to the mouth being drawn into the opening of the oviduct, indicate that the flagellary current is continuously active. Since normal





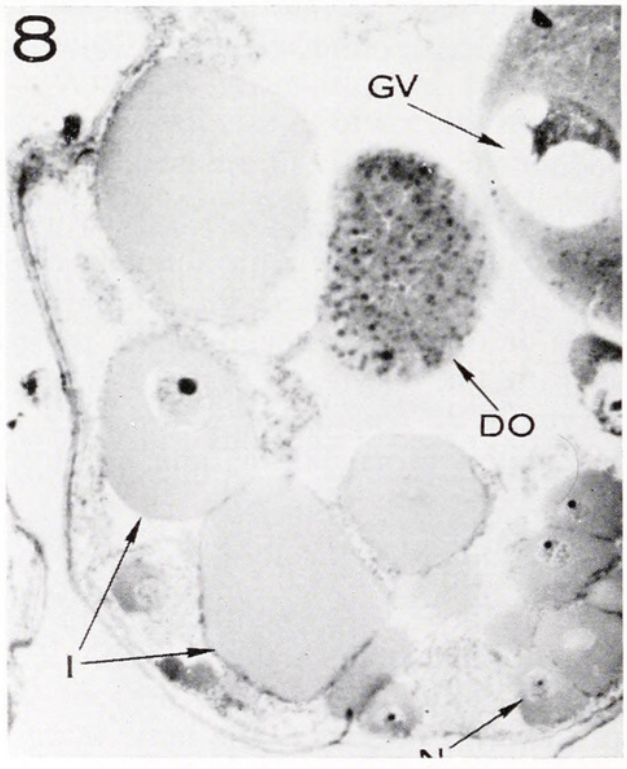


FIGURE 7. Section through the ovary (February 29) showing the attachment of developing oocytes to the germinal epithelium. Oocyte classes II and III are shown, surrounded by follicle cells (FC). Note the crowding of oocytes; bar is 50 microns.

FIGURE 8. Section through the ovary (November 2) showing detached, degenerating oocyte (DO) within the lumen. Note the position of the germinal vesicle (GV) at the periphery of the Class I oocyte. Class II and III oocytes are also shown; bar is 100 microns.

FIGURE 9. Longitudinal section through the ovary (September 1) showing three developmental classes of oocytes. Note that the yolk-filled maturing oocytes (M, Class I) lie in the lumen surrounded by stretched one-cell thick membranes of follicle cells (FC). Note that

oocytes are firmly attached to the germinal epithelial membrane, they would not be drawn into the oviduct.

The time and place of meiosis and fertilization in *L. tenera* are not known, but in a sample of more than 50 animals collected in Block Island Sound on December 21, all females contained embryos in the pyloric stomach (Fig. 10) with the exception of three individuals which had embryos in the brood chamber. Sectioned material showed that the embryos in the pyloric stomach were enclosed in a capsular membrane and had developed to the early gastrula stage, while those outside in the brood chamber were in the very late gastrula stage. Since *L. tenera* retains the embryos in the brood chamber during the later months of brooding, it is assumed that they are held in the pyloric stomach for a relatively short time, then released into the brood chamber where they adhere closely together in a compact mass and complete development in about three months.

Discussion

In general, the male reproductive system of *L. tenera* resembles that of other pentamerous species of *Leptasterias* described by Fisher (1930), as well as reproductive systems of hexamerous species which have been observed (Fisher, 1930; Menge, 1975; Smith, 1971). Some minor differences, however, were noted, especially in the reproductive cycles.

At the time of sperm release in *L. tenera,* spermatogenesis has been completed, no spermatocytes or spermatids are present in the germinal epithelium and the expanded lumen of the testis is filled with masses of mature sperm. In *L. pusilla,* on the other hand, Smith (1971) found that while the mature testis contained great numbers of sperm in the lumen at the time of shedding, colonnettes were still present, and active spermatogenesis of existing spermatocytes continued for 10–14 days after sperm release.

Smith postulated that as a result of spawning, ". . . gonial cells cease dividing until relict spermatozoa are destroyed, and then divide to produce other gonial cells and not primary spermatocytes" (Smith, 1971, p. 104). A comparable delay in the resumption of spermatogenesis was noted in *L. tenera.* Following sperm release (November/December) there was a noticeable increase in the numbers of spermatogonia, but primary spermatocytes did not appear until March. Since the period of active spermatogenesis in *L. tenera* is roughly synchronous with the most active feeding time (March–October), shown in Figure 1, it is possible that initiation of spermatogenesis is correlated with the resumption of active feeding rather than with the removal of an inhibitory factor associated with spawning or sperm destruction, especially since sperm removal continues long after spermatogenesis has begun

Following shedding of the sperm in *L. pusilla,* Smith reported that so many unshed, active, mature sperm remained in the lumen that the ". . . testes were

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they still remain attached to the germinal epithelium. Smaller Class II (I) and Class II (N) oocytes remain tightly bound to the germinal epithelium; bar is 100 microns.

Figure 10. Aboral dissection of female *Leptasterias tenera* showing embryos within the aboral pyloric stomach. Embryos remain within the pyloric stomach for a short time before being expelled into the brood chamber, where they are maintained for the duration of the brooding period; bar is 1 mm.

$\frac{1}{2}$ to $\frac{1}{3}$ the maximal gravid size" (Smith, 1971, p. 95). From this observation, he reasoned that the inefficient release of sperm was due to the limited contractibility of the testis wall. Muscular contraction would appear to be more efficient in *L. tenera,* since relatively few sperm remain unshed and the testes are greatly reduced in size. Although Smith did not indicate that *L. pusilla* released sperm more than once in an annual cycle, the presence of large numbers of unshed, active sperm and the continuation of spermatogenesis following spawning strongly suggest that possibility, especially since partially spawned-out animals were not found after the oocyte spawning period. A second spawning in one season would be most unlikely in *L. tenera* in as much as sperm release is nearly complete. *L. tenera* and *L. pusilla* (Smith, 1971) both produce new populations of sperm each year and unshed spermatozoa are ultimately removed by the phagocytic action of the numerous amoeboid lymphocytes.

In *L. pusilla,* Smith observed an axial core of clear, matrix-like material extending within the fingers of the colonnettes. This core was thought to be a pathway for nutrient transport to the developing germ cells. No such central matrix was noted in *L. tenera,* where the colonnettes tend to be no more than two to four cells in thickness (Fig. 4). The formation of those finger-like extensions results from sequential divisions of spermatocytes which increase the length of the colonnettes as new cells are added. There is no evidence that the developing cells are held together by more than the extended germinal epithelium.

The female reproductive cycle in *L. tenera* follows the basic pattern described for several species of *Leptasterias* (Fisher, 1930; Chia, 1968a; Smith, 1971; Menge, 1975). Production of oogonia in *L. tenera* is a continuous process. Smith (1971) on the other hand, indicated that formation of oogonia in *L. pusilla* was restricted to a five month period. Many more oogonia are produced than differentiate into the primary oocytes which then begin a two-year period of development. Of this number, only 12–18 cells per gonad become mature, fully-yolked oocytes.

During the first year of development, the primary oocyte undergoes nuclear changes resulting in the formation of a large, centrally-located germinal vesicle containing an enlarged, vacuolated nucleolus, and an increase in cytoplasmic material. More oocytes go through this stage than will complete the second year, which is devoted almost entirely to the formation and growth of yolk globules.

In *L. tenera,* as in many other species of starfish (Chia, 1968a; Smith, 1971; Walker, 1974), the basal germinal membrane is closely associated with the haemal sinus. Developing oocytes, surrounded by follicle cells, maintain a close connection with the basal germinal membrane until shortly before oviposition, when the follicle cells disappear and the mature oocytes lie free in the lumen. The location of the oocytes in close proximity to the haemal sinus throughout the developmental period suggests that at least some of the material used by the oocytes for maintenance, growth and vitellogenesis could be supplied from this channel. Chia (1968a) and Endean (1966) suggested that the haemal sinus functioned to store or transmit nutrients to the germ cells, but Smith (1971) and Ferguson (1963) discounted the importance of the haemal systems in the translocation of nutrients.

Competition for maintaining attachment to the basal germinal membrane increases as new oocytes are formed and older ones grow. Cells become tightly

squeezed together, thereby reducing the extent of their attachment, and some cells become dislodged completely to lie free in the lumen (Figures 7, 8). Although the fate of such displaced cells could not be followed, it was observed that degenerating cells are characteristically found in the lumen (Fig. 8), suggesting that detachment from the basal germinal membrane may be related to subsequent breakdown. Such a "position effect", the necessity for developing oocytes to retain a critical contact with the germinal membrane, and the competition for space could account for the observed distortion and the high mortality of developing oocytes. The importance of maintaining contact with the basal germinal membrane has not been discussed in other species of *Leptasterias*.

The presence of yolk in the oocytes of *L. tenera* is recognized first in the form of small, basophilic granules scattered evenly throughout the cytoplasm of the largest oocytes remaining in the ovary after oviposition. Chia (1968a) noted similar granules which appeared at the same stage of oocyte development in *L. hexactis* and called them yolk platelets. Along with the yolk platelets, small yolk globules appear, located initially at the periphery of the cell. As the yolk globules increase in number and size and spread throughout the cytoplasm, they are in close association with large, basophilic granules which are not seen in later stages of development. At no time were these basophilic granules or yolk globules observed in association with or within the follicle cells as noted in *L. hexactis* by Chia (1968a). He suggested that yolk granules or their precursors may be synthesized in the follicle cells and later transferred to the oocytes through cytoplasmic bridges. Smith (1971) reported that in *L. pusilla* entire follicle cells may be incorporated into the cytoplasm of early second-year oocytes. In *L. tenera,* accessory cell nuclei occassionally appear to be located within the cytoplasm of a normal Class II oocyte, but serial sections of such cells show that the nuclei are actually part of the follicle envelope surrounding the oocyte.

Oocytes of all classes in *L. tenera* increase rapidly in size during the active feeding period (April–October) as indicated in Figure 1. Due to the continued growth of the yolk globules, Class I oocytes enlarge even after feeding begins to decline, and maximum size is not attained until the time of spawning. Thus, it would appear that the energy resources for vitellogenesis in *L. tenera* are derived mainly from the current feeding period rather than from reserve energy previously stored in the pyloric caeca. Chia (1968a) reported that in *L. hexactis* a rapid growth of oocytes occurred during the brooding period when the animals were not feeding and that mature oocytes stopped growing in July at a time when the animals were still feeding. The oocytes were thought to remain in a "rest period" until spawning five months later. These findings were contradicted, in part, by Menge (1975) on the basis of his studies of organ indices of several populations of *L. hexactis*. His results indicated that both ovary and storage organ (pyloric caecum) tended to increase in weight during the feeding period until October–January when spawning occurred. Consequently, a positive correlation between ovary growth and energy storage was evident up to a point and was then followed by a leveling-off period in the ovary and decline in the caecum. A preliminary examination of the relationship between annual growth curves of ovaries and pyloric caeca in *L. tenera* indicated a similar positive correlation which in this case continued until the time of spawning (Ander, personal communication).

The major role of the amoeboid, phagocytic, accessory cells is clearly the removal of disintegrating oocytes. While breakdown of oocytes may occur at any time of the year, more degenerating cells were observed late in the reproductive cycle, and following oviposition when relict ova were in the lumen. Smith (1971) suggested that phagocytic follicle cells in *L. pusilla* were instrumental in rapid and selective destruction of second-year oocytes and that nutrient material from the disintegrating oocytes was immediately made available to the remaining oocytes for completion of development. He further postulated that this recycling of nutrients constituted a ". . . mechanism for adjusting the balance between exogenous resources and the number of oocytes such that the maximum number of oocytes can complete maturation" (Smith, 1971, p. 127). Such a mechanism, if it exists would indeed be a noteworthy example of an intraspecific mechanism for density-dependent control of population density. Unfortunately, evidence was not obtained from *L. tenera* to support these hypotheses. The material, however, did indicate that breakdown of oocytes was not a rapid process, as suggested by Smith. Remnants of large, orange, yolk-containing oocytes in varying degrees of phagocytic destruction were still present in the lumen as late as April, a time when large yolk globules have not yet developed in Class I oocytes. While the energy resources presented by disintegrating oocytes may be utilized eventually by the amoeboid accessory cells, and perhaps by other cells within the ovary, there is no evidence that phagocytosis of oocytes in *L. tenera* acts as a regulatory mechanism for oocyte development.

In the description of stomach brooding in *L. groenlandica* (Lieberkind, 1920; Fisher, 1930; Hyman, 1955), the embryos were held in the cardiac portion of the stomach throughout the brooding period. In *L. tenera,* however, the embryos are retained in the pyloric stomach (Fig. 10) for a relatively short time. This difference in the site of stomach brooding is correlated with the stomach structure of the two species. The cardiac stomach of *L. groenlandica* is large and lobed, and the pyloric stomach is small (Fisher, 1930). In *L. tenera,* the pyloric stomach is greatly enlarged, while the cardiac portion is short and unlobed. The temporary retention time for the embryos within the pyloric stomach in *L. tenera* may be associated with the greater difficulty of obtaining oxygen for the growing embryos than would be experienced in the brood chamber, or even in the cardiac stomach as in *L. groenlandica.*

A comparison of the reproductive biology of *L. tenera* with the available information on other brooding species of *Leptasterias* reveals a general reproductive pattern which is especially apparent in females and may be associated, either directly or indirectly, with the brooding habit. The males show little important variation from the general asteroid pattern. The ovaries of *Lepasterias* tend to be small in relation to the size of the arms and consist of a main rachis (Walker, 1974) with small outpushings which may represent vestigial acini (Fig. 3). In some broadcasting species, such as *Asterias vulgaris* (Walker, 1974) and *Pisaster ochraceous* (Menge, 1975), ovaries are large with major acini. Large numbers of primary oocytes are produced, of which very few complete maturation. "Overproduction" of primary oocytes may represent a retention of the ancestral, oogenetic pattern characteristic of broadcasting sea stars. Rather than reduce the number of oocytes produced, a selective process within the ovary provides that only a

limited number of large, fully yolked ova are spawned. In order to produce this yolk-laden oocyte, a growth period of at least two years is required, in contrast to the annual cycle characteristic of broadcasting sea stars. The rate at which vitellogenesis may be accomplished must be limited by the rate at which yolk materials or precursors can be transferred to the cytoplasm of the oocyte. Since the energy which ultimately appears as yolk is derived from food organisms, it is not suprising that the vitellogenic phase is closely associated with the feeding period. Cellular mechanisms exist for the removal and possible recycling of unsuccessful oocytes as well as unshed gametes. Such a cellular disposal mechanism would seem to be a necessity in view of the large number of oocytes which fail to survive and sperm which remain in the testes. In the ovary, cells of the germinal epithelium which develop into accessory cells become amoeboid phagocytes and break down disintegrating oocytes. The removal of relict sperm from the testes is accomplished by the phagocytic activity of amoeboid lymphocytes. It is interesting that the utilization of amoeboid lymphocytes appears to be a general phagocytic mechanism in many invertebrates, whereas the cellular phagocytic mechanism involving amoeboid accessory cells is unique to the ovary in female *Leptasterias.* Some period of parental care or brooding has been found in all species of *Leptasterias* which have been studied. Associated with the brooding pattern is the small number of yolked eggs which undergo direct development (Lieberkind, 1920; Fisher, 1930; Chia, 1955, 1968a; and Smith, 1971). A single oviduct from each gonad which opens to the outside through a ventrally located, interradial papilla is characteristic of all species of *Leptasterias* for which observations have been made. This position contrasts with the multiply-pored, dorsally located openings of the oviduct in the broadcasting starfish, *Asterias vulgaris,* described by Walker (1975).

Assuming that the above traits are genetically fixed in the reproductive strategy of the genus *Leptasterias,* natural selection can and will effect modifications upon this strategy in response to local conditions. This would be true even if the observed similarities resulted from evolutionary convergence. Although the number of species of *Leptasterias* already studied in detail is very small, certain habitat-related modifications are discernible. The number and size of oocytes produced vary significantly among species. Brood protection varies in length of time the embryos remain in the brood chamber as well as in the presence, absence and duration of the stomach phase. The correlation and timing of the reproductive cycle in relation to the feeding period show variations which are probably related to certain specific ecological factors such as feeding specialization and environmental stability.

Until further information is available for more species of *Leptasterias,* general conclusions regarding the importance of ecological factors as modifiers of the reproductive cycle in *Leptasterias* must be tentative.

Summary

The reproductive system of *Leptasterias tenera,* a five-rayed, North Atlantic sea star, was studied throughout an annual cycle. In general, the gross anatomy and histological structure of the gonads were comparable to descriptions for other species of *Leptasterias* (*L. hexactis, L. pusilla, L. groenlandica*). Gametogenesis in

L. tenera followed the characteristic pattern of brooding *Leptasterias,* but certain specific variations were noted.

In *L. tenera,* spermatogenesis was completed prior to shedding, whereas in *L. pusilla,* differentiation of spermatocytes continued for at least 14 days after sperm release. Relict sperm remaining in the testis following shedding in both species were ultimately removed by phagocytic action of amoeboid lymphocytes. Two types of lymphocytes, amoeboid lymphocytes and petaloid cells, were present in the haemal sinus of male *L. tenera.*

In the female reproductive cycle of *L. tenera,* developing oocytes remained close to the basal germinal membrane and haemal sinus throughout the entire period of growth and vitellogenesis, unless squeezed out due to crowding. It was suggested that oocytes which were pushed into the lumen failed to complete development and subsequently were broken down by amoeboid accessory cells derived from the germinal epithelium. The need to maintain contact with the basal membrane was called the "position effect." Other accessory cells in the germinal epithelium become follicle cells.

In *L. tenera,* the timing of the period of greatest activity in both male and female reproductive cycles was positively correlated with the period of active feeding.

The time and place of fertilization and maturation are unknown in *L. tenera.* Sometime following spawning, the ova were placed in the pyloric stomach where the early stages of development occurred. The mass of embryos was then transferred to the brood chamber where development was completed in about three months.

Certain features of the brooding mode of reproduction apparently constitute a genetically fixed pattern within *Leptasterias.* These include a cycle of oogenesis requiring at least two years, resorption of excess oocytes, production of a small number of large, yolky ripe ova, and an extended period of brood-protection within a brood chamber. However, a comparison of reproductive patterns in three species (*L. tenera, L. hexactis* and *L. pusilla*) indicates that modifications of the genetic pattern have occurred.

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