BROODING BEHAVIOR OF A SIX-RAYED STARFISH, LEPTASTERIAS HEXACTIS[1]

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It has been known for a long time that certain starfishes, found in all three orders of the class, exhibit the habit of caring for their young. For example, Sars described the brooding habits of *Leptasterias mulleri* (Order Forcipulata) and *Henricia sanguinolenta* (Order Spinulosa) in 1846. Thomson observed the brooding habit of *Archaster excavatus* (Order Phanerozonia) in 1878.

Since then a number of brooding species have been added to the list. Ludwig (1903), Fisher (1940), and Hyman (1955) have named brooding forms and their distributions. It was pointed out by these authors that most of the brooding species are inhabitants of deep waters of the Antarctic and sub-Antarctic areas. However, some are commonly found on both the Atlantic and Pacific coasts of North America (Verrill, 1914; Fisher, 1911, 1928, 1930). Fell (1959) recorded a New Zealand shallow-water species, *Clavasterias suteri,* which is a brooding form also.

Brooding species usually produce a small number of large, yolky eggs, which undergo the direct type of development. Brooding species of the Orders Forcipulata and Spinulosa usually brood their young at the oral region by arching their arms to form a brooding chamber. However, in *Leptasterias groenlandica* (Lieberkind, 1920; Fisher, 1930), the young actually develop in the cardiac stomach. In the family *Pterasteridae* (Order Spinulosa), it was reported that the young develop in the nidamental chamber, the space between the aboral body wall and the supradorsal membrane (Koren and Danielssen, 1856; Fisher, 1940). Yet the evidence which I have gathered in *Pteraster tesselatus* of the San Juan Archipelago, Washington, indicates that this species does not in fact brood. The development is the direct type in this species, but is planktonic. Brooding species in the Order Phanerozonia, such as *Leptychaster almus* (Fisher, 1917) and *Ctenodiscus australis* (Lieberkind, 1926), usually brood their young among the paxillae on the aboral surface of the body wall. *Leptychaster* of Greenland (Order Phanerozonia), according to Fell (1959), hatches its young in the stomach, as does *Leptasterias groenlandica.*

All the publications of which I am aware that deal with brooding behavior in asteroids are limited to brief descriptions in connection with the studies of classification. No serious study on this subject has been reported. *Leptasterias hexactis* provides ideal material for such a study; it is an abundant intertidal species which

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makes it easier to observe in its natural environment, and when collected, its small size makes it easy to handle and observe in the laboratory. A number of observations on brooding specimens in their natural habitat, as well as in the laboratory, have been made, and a number of simple experiments have also been performed in the Friday Harbor Laboratories.

General Observations

The animals in the Friday Harbor area enter the reproductive season at the end of November and terminate in April. The eggs are yolky and measure about 0.9 mm. in diameter. The number of embryos in each brood varies from 52 to 1491 and is correlated with the size of the female animal.

In order to examine the brooding habits more closely, four to five animals selected at random were kept in a small glass aquarium equipped with running sea water. When the females were ready to spawn, they assumed a typical brooding position (Figs. 1–3). If one was located on the wall of the aquarium, she arched her arms to form a brooding chamber and began to release eggs. During the first few minutes, the eggs were not sticky and did not adhere to each other. They were heavier than the sea water and tended to fall to the bottom of the aquarium. The female had to catch them by means of her tube feet and place them in the brooding chamber. Some eggs, especially those shed from the gonopores of the lower interradii, fell away. The males were spawning prior to the females or at the same time. At some times the spawning of male animals was unobservable and at others the spawning caused the water to become milky for a couple of hours. After the eggs were fertilized, the fertilization membrane became so sticky that the eggs adhered to one another and formed an almost solid mass (Figs. 4, 5). The process of shedding eggs lasted for several hours.

In the natural environment, the animals brood on the undersurfaces or sides of rocks. Attachment of the female to the undersurface has an obvious advantage during spawning, for in this position the eggs can be collected easily in the brooding chamber and the eggs always are provided with some moisture at low tide. In addition, direct exposure to sunlight is avoided. After spawning, the female starfish usually remains in this position until the young have completed metamorphosis. If the female is disturbed, she may move to another place, but does not give up her brooding activities.

In making collections, the animals were often placed in plastic bags containing sea water. These brooding animals usually brought their arms together to form an enclosed chamber, or lay free in the water or attached themselves to the plastic bag with the tube feet at the distal ends of the arms. They often remained in this position overnight without forsaking the embryos when the plastic bag was floated in the aquarium. After being released from the bag, they moved around in the aquarium and finally found a place to attach and resumed the brooding position again.

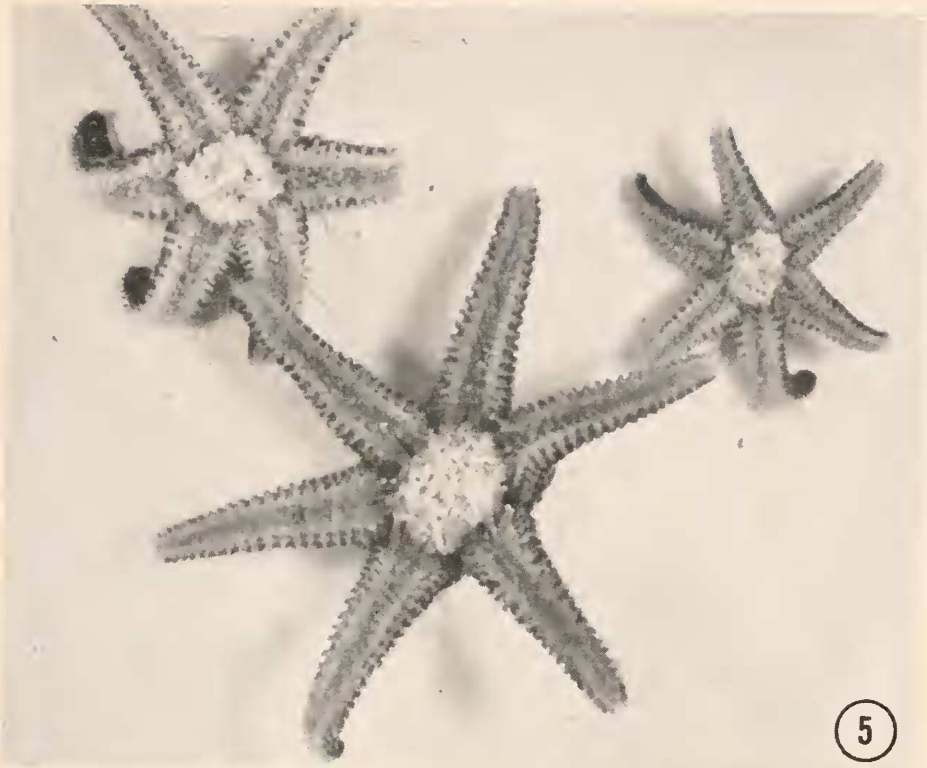
During the first 40 days of brooding, the position remained unchanged. The female attached herself only by the use of the tube feet at the distal end of the arms. The tube feet at the other parts of the arms were used to hold the embryos and orient them by pulling and twisting. The pressure exerted on the embryos by

FIGURE 1. Lateral view of a brooding female on the wall of a Plexiglas tank. Natural size.

FIGURE 2. Oral view of a brooding female on the wall of a Plexiglas tank, showing the completely closed brooding chamber. Natural size.

FIGURE 3. Lateral view of two brooding females on the wall of a Plexiglas tank. Natural size.

FIGURE 4. Cleaving embryos; most are in four-cell stage. Living specimen. 14 ×.



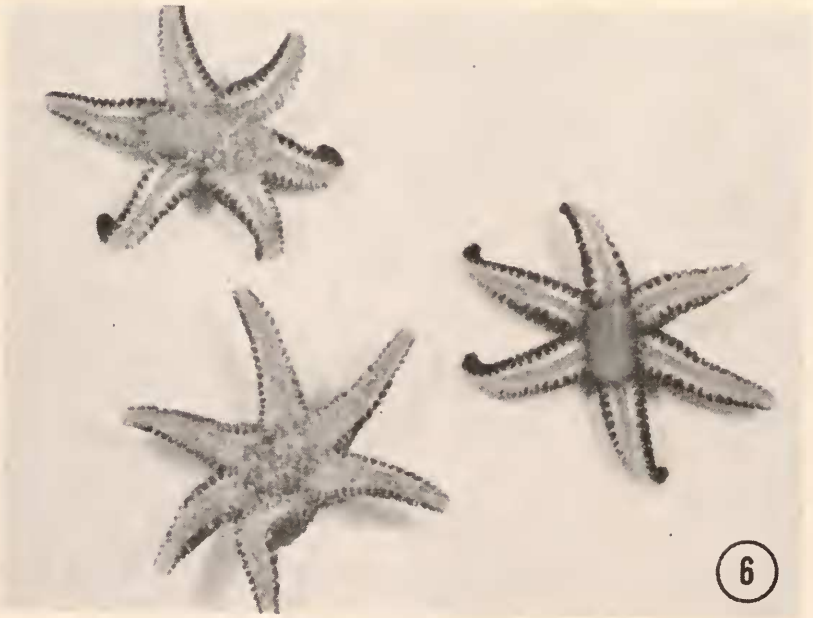


FIGURE 5. Three brooding animals turned oral side up, showing the application of the tube feet on the embryo masses. Living specimens. Natural size.

FIGURE 6. Oral view of three animals shown brooding artificial embryo masses made of gelatin capsules filled with wax. Living specimens. Natural size.

the tube feet may be fairly high and even temporarily distort the embryos. In most cases, from early development through metamorphosis, the embryos did not attach themselves to the substratum; instead, they adhered to each other and to the mother, and remained as a mass within the brooding chamber (Fig. 5). After about 40 days of development, when the tube feet of the young stars started to function, they began to attach themselves to the substratum but did not move away. It was at this stage that the adult resumed the flattened position; that is, she kept her arms straight, but remained with the brood. The whole picture reminds one of a mother hen protecting her chickens under her feathers and wings. After two months of development, when the preoral lobe of the young starfish had been completely absorbed, and the mouth opened and independent existence was possible, the mother animal left.

One female starfish was observed to spawn twice in the tank. This animal just moved a few inches away from her first brooding position and spawned again. The second spawning was about one month after the first and all of the first brood of embryos were metamorphosing and attached to the substratum. However, in most cases the female animals spawn only once per breeding season.

One can remove the embryos from the brooding chamber by a water current from a pipette. There are always small sand granules and debris which accumulate at the peristomial area, but the embryos in all cases observed were completely clean. This observation suggests that one of the functions of brooding is to keep the embryos clean.

Culture in vitro of the Embryos

When the embryo masses were removed from the brooding chambers at the early cleavage stage, two methods of rearing them in the laboratory were repeatedly attempted:

(1) Ten embryo masses were placed in a small aquarium with a continuous flow of sea water. This method would simulate a situation in which the animals would not brood, but only spawn their gametes in the sea water without further care. None of the embryos treated in this manner survived more than five days; due to the sticky nature of the fertilization membrane, they were soon covered by debris and became infected by bacteria and protozoans, and the whole embryo mass decomposed.

(2) Ten embryo masses were placed in separate fingerbowls containing filtered sea water maintained at a temperature of 10° to 15° C. on sea water tables. The sea water in the fingerbowls was changed daily. The embryos seemed to develop satisfactorily until the postgastrula or even the early brachiolarian stage. However, the development of embryos in a mass was not synchronous. In addition, most of the embryos cultured in this way could not break through the fertilization membrane and hatching did not occur. These embryos soon were infected by bacteria or protozoans, and the cytoplasm became liquified and turned tea-brown in color. The embryos swelled to about twice normal size and finally burst. Occasionally, an embryo was able to get one brachiolar arm (usually the dorsal arm) of the preoral lobe out of the fertilization membrane, but only one or two per cent were capable of hatching, and then the process was much delayed.

By way of contrast, the development of the brooded embryos is synchronous and they have no apparent difficulty in hatching. They hatch simultaneously and the post-brachiolaria lasts four or five days until the first sign of metamorphosis. This suggests the possibility that the female may secrete some substances, possibly enzymatic in nature, which break down or weaken the fertilization membrane. To make a preliminary test of this possibility, some tissues of the peristome and cardiac stomach of a brooding animal whose larvae were at prehatching stage or just hatched were homogenized and this preparation was added to the dishes containing embryos at various stages of development. The embryos were observed under a microscope and allowed to develop. In no case was there any positive evidence that this juice weakens the fertilization membrane. It is thought that the hatching processes may be facilitated by the mechanical action on the fertilization membrane of the tube feet of the mother animal.

Selection of Substratum

The brooding animals not only favor the sides and undersurfaces of rocks, as stated above, but are also very sensitive to other characteristics of the rocks. Observations in 1960–1961 demonstrated that the brooding animals show preference for the dark and rough-surfaced rocks. Similar experiments were repeated in 1962–1963 with the same results.

Thirteen brooding animals were put on the floor of a small aquarium supplied with running sea water, in which there were two pieces of rock of comparable size, but one dark and the other pale. The results were recorded as follows:

Time	Dark rock	Pale rock	Wall of aquarium
0 hrs.	0	0	0
4 hrs.	6	0	7
24 hrs.	9	0	4

In another experiment all 13 animals were put on the pale rock and the results were as follows:

Time	Dark rock	Pale rock	Wall of aquarium
0 hrs.	0	13	0
1 hrs.	0	0	13
2 hrs.	2	0	11
5 hrs.	7	1	5
24 hrs.	9	1	3

The first experiment shows that 6 of the animals moved to the dark rock during the first four hours and 7 to the aquarium wall, but none of them moved to the pale rock. Within another 20 hours, about half of the animals originally settled on the aquarium wall had moved to the dark rock, but still none had moved to the pale rock.

The second experiment shows that all the 13 animals migrated to the aquarium wall from the pale rock in the first hour. Twenty-four hours later nine of them moved to the dark rock, one to the white rock and three remained on the aquarium wall.

In another aquarium two pieces of dark rock of similar size, one having a rough surface and the other smooth, were placed in with eight brooding animals. Twenty-

four hours later seven animals were on the rough-surfaced rock and one on the smooth-surfaced one.

The non-brooding animals have been checked by the same method. They may show a preference for the dark and rough-surfaced rocks. However, their reaction is not as definite as that of the brooding animals.

Feeding Activities During Brooding

Since the embryo mass occupies the peristomial region of the brooding animal, whose tube feet are used in orientation of the embryos (Fig. 5), any feeding activity by the brooder would be handicapped. In fact, no feeding activities of brooding animals have been observed, either in the laboratory or in the natural environment. Is this non-feeding situation simply due to the blockage of the food passage or is it controlled by some physiological factors?

To investigate this problem a simple experiment was set up as follows: three small rocks, each covered with about a dozen barnacles (*Balanus glandula*), six limpets (*Acmaea digitalis*), six mussels (*Mytilus edulis*) and one chiton (*Tonicella lineata*), were placed in three separate aquaria, A, B, and C, and supplied with running sea water. In aquarium A five brooding animals were placed, the brood being about 10 days old. There were five brooding animals in aquarium B, but the embryo masses were removed from the brooding chamber. Five non-brooding animals were placed in aquarium C. Throughout a month of observation, no animals in aquarium A fed on the available food items; the animals in aquarium B did not feed in the first two days, but began to feed on barnacles on the third day; the animals in aquarium C started to feed within the first six hours.

This observation clearly indicates that these starfishes stop feeding only when they are actually brooding (aquarium A). This may imply that the cessation of feeding is either a simple matter of interference by the embryo masses or is regulated by physiological factors but a constant stimulation by the embryo is needed. The latent period of two days as shown by the animals in aquarium B may be the time required for readjustment after the embryos were removed.

It is of interest to note that for an average-size star (radius 2.5 cm.), it takes 24 hours to complete the feeding on an *Acmaea digitalis* (1.4 cm. diameter), 30 hours on a small *Tonicella lineata,* and 60 hours on a *Mytilus edulis.*

Brooding on Artificial Embryos

If an embryo mass is removed from a brooding female for five hours and then returned, she will pick up the embryos and continue to brood. A brooding animal also will pick up an extra embryo mass if it is available. These observations lead one to believe that the animals actually recognize embryos of their own species.

To investigate this possibility an experiment was set up by placing 10 embryo masses together with 10 non-brooding animals in an aquarium. Within 8 hours, 9 of the 10 animals had picked up the embryo masses and settled on the aquarium wall to brood. Four of the 9 animals continued to brood until all the embryos completed metamorphosis; the other five ceased brooding activity during the first week. It was proved later by dissection that these five animals included three males (all spawned) and two females (non-spawned). The four which continued

to brood were apparently unspawned females, for they also spawned their own eggs, and therefore possessed embryos of two different stages in the same brood.

The question thus arises as to how the animals recognize the embryo masses. In an attempt to answer this question the following four experiments were performed by using artificial embryo masses.

(1) Artificial embryo masses were made with wax and dyed with orange G. A piece of copper wire was inserted in the center of each of the wax blocks to keep it anchored at the bottom of the aquarium. The size, color, and shape of the artificial embryo masses were somewhat similar to the natural embryo mass. Ten of these artificial embryo masses were placed with 10 non-brooding animals in a small aquarium of running sea water; no response was observed in two days.

(2) The 10 artificial embryo masses prepared for this experiment were similar to those in experiment 1 except that they were punched with numerous holes by using a small needle and then soaked overnight in an homogenate of embryos of *Leptasterias*. It was hoped that some kind of substances from the embryos might be a factor in attracting the animals to brood. But after these artificial embryo masses were given to 10 non-brooding animals again, no reaction was observed in a two-day period.

(3) In a third experiment, 10 artificial embryo masses were made by filling gelatin capsules with colored wax and pieces of copper wire. After being placed in sea water, the gelatin swelled a little and became sticky; this condition is similar to that of the fertilization membrane. Two hours later, three of the ten non-brooding animals each picked up an artificial embryo mass to brood (Fig. 6). Twelve hours later, 8 of the artificial embryo masses had been picked up by 7 animals; one animal picked up two. The brooding of the artificial embryos, however, did not last more than two days. After 24 hours the gelatin capsules had swollen so much as to become loose from the wax blocks, and were discarded by the starfishes.

(4) For this experiment 10 non-brooding animals were given 10 artificial embryo masses which included five with gelatin capsules and five without gelatin capsules. Twelve hours later, three of those artificial embryo masses with gelatin capsules were picked up; none of the artificial embryo masses without gelatin capsules were picked up. The possibility that the animals were actually feeding on the gelatin capsules instead of brooding was ruled out by the fact that no extrusion of the stomach was ever observed in any of the cases.

These four experiments suggest that the animal's acceptance of a "mass" for brooding has little to do with its size, color, or shape. It also failed to provide evidence that chemotaxis plays a significant role in stimulating the recognition. Rather, the physical character of the surface layer of the embryo is possibly the main factor in its acceptance.

Conclusion and Discussion

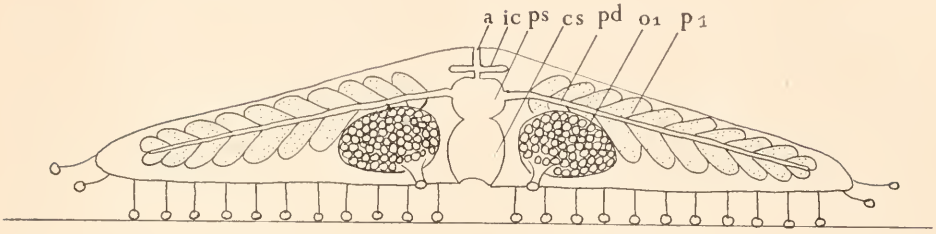
In a recent study Arnold (1962) demonstrated that in the cephalopod, *Loligo pealii,* an artificial egg mass can stimulate sexual behavior, which is followed by the establishment of a social hierarchy. The stimulus, according to the author, is

a visual response. On the other hand, Fell (1940), reported that in *Amphipholis squamata*, a brooding brittle star, there is actually a trophic relationship between the mother and the young. This is to say that the female animal secretes some nourishing substance(s) necessary for development of the embryos. In the present species, as it has been demonstrated, the animal will pick up an artificial embryo mass to brood, but this is apparently a response to the physical contact between the mother and the embryos. Meanwhile, no trophic relationship between the mother and young was indicated, because the embryos, after being removed from the mother, develop normally up to pre-hatching larval stage, and, if the fertilization membrane is removed manually, they will develop normally up to the young adult stage without any sign of morphological abnormality. In other words, the stored yolk in the ooplasm is sufficient to provide all necessary nutrients for development to a young adult. Thus, the major functions provided by brooding are protection, cleaning, maintenance of a uniform environment, and initiation of the hatching process.

Compared with all the other intertidal sea-stars at the San Juan Island area, *Leptasterias hexactis* has been very successful in spite of the fact that it produces only a small number of eggs and lacks the pelagic larvae to disperse the progeny. Embryos protected in the brooding chamber not only avoid exposure to severe effects of some environmental factors such as desiccation but also are safe from predators. The only predators for adult *Leptasterias* I have observed are other starfishes such as *Solaster, Crossaster*, and *Pycnopodia*. These were observed to prey on *L. hexactis* in the laboratory; however, species of these genera rarely exist together in the natural habitat.

The movement of cilia and tube feet is responsible for removing dirt and debris which become attached to the embryos. It is also responsible for maintaining a water current which insures a uniform environment and facilitates synchronous development. As for breaking through the fertilization membrane, there are two possible ways by which this may be achieved: (1) the embryo itself may secrete a digestive enzyme to dissolve the membrane; (2) the brooding animal may provide other chemical or mechanical means to rupture the membrane; possibly, a combination of these is involved. The failure of isolated embryos to hatch, as demonstrated in this study, may imply that the brooding mother is fully responsible for this process, but the possibility of the failure of embryos to secrete such a digestive enzyme because of the isolation from the mother is not excluded.

One of the most interesting problems, of course, is the control mechanism of brooding behavior. When an animal assumes a brooding position (Fig. 8) from a pre-brooding position (Fig. 9), one can easily see that at least three obvious changes occur in functional morphology. (1) Muscular system: the contraction of some muscles (longitudinal adambulacral muscles, for example) enables the animal to form a brooding chamber to house the embryos. This position lasts for at least 30 days. (2) Ovary: it is filled at the pre-brooding stage and emptied at the brooding stage, during which all the eggs are fertilized in the brooding chamber; they then form the embryo mass. (3) Pyloric caeca: materials such as lipids, polysaccharides, acidophilic granules, and zymogen granules, which are abundant in the epithelial cells of the pre-brooding animal, disappear after 30 days of brooding. Details of this phenomenon will be reported in a separate paper.



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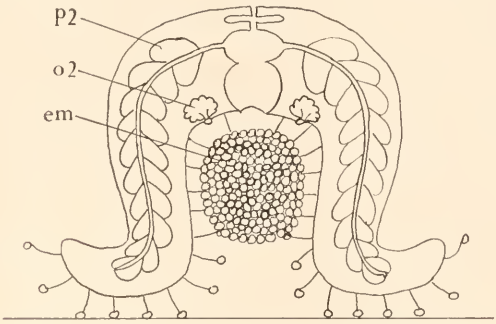

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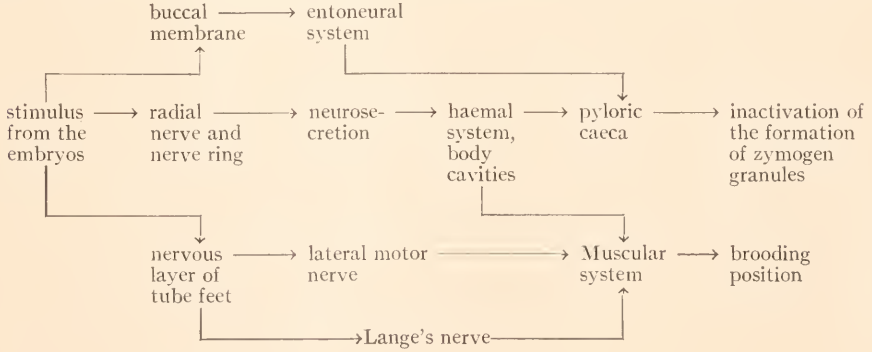
FIGURE 7. Diagrammatic drawing to illustrate a prebrooding animal (feeding). a. anus; ic, intestinal caecum; ps, pyloric stomach; cs, cardiac stomach; pd, pyloric duct; O_1, ovary before spawning; P_1, pyloric caecum of a feeding animal.

FIGURE 8. Diagrammatic drawing to illustrate a brooding animal. em, embryos; O_2, ovary after spawning; P_2, pyloric caecum of a brooding animal.

A number of other behavioral changes also occur during brooding, such as increased sensitivity to the substratum and cessation of feeding.

To maintain the brooding position, as shown in this study, a direct contact between the mother and the embryos is necessary, since the animal straightens her arms to a normal position and resumes feeding if the embryos are removed. Furthermore, the stimulus resulting from the contact between the embryos and the mother is thought to be physical rather than chemical in nature. But how does the stimulus pass from the contact area to the target organs? In other words, what is the coordination system? Taking the pyloric caeca and the muscular system as target organs, a hypothetic coordination process can be presented as follows:



In support of this hypothesis, a number of facts, such as the nature of the stimulus and the changes in neurosecretion during brooding, have to be substantiated and studies along this line are being pursued.

Recent studies of Chaet (1964), Kanatani and Nounura (1962) and Noumura and Kanatani (1962) have shown that an intracoelomic injection of water extract from the radial nerve cord induces spawning in several species of asteroids. Furthermore, Unger (1960, 1962) has demonstrated the presence of neurosecretory products in the radial nerve of *Asterias glacialis* and their effect on motor activity, osmoregulation and pigmentation; therefore, it is reasonable to assume that neurosecretion may be involved in controlling the brooding behavior in the present species. A preliminary study on neurosecretion by histological methods in the present species revealed a scant number of neurons with secretory granules in the radial nerve cords, but it failed to demonstrate any substantial differences in quality between the brooding and non-brooding animals. However, the possibility that the brooding behavior is influenced by neurosecretion is far from exhausted.

Summary

1. The brooding habit is apparently necessary for these animals; none of the embryos survived without brooding.

2. The main functions provided by brooding are protection, cleaning, maintenance of a uniform environment, and initiating the hatching process.

3. During brooding, the animals are particularly sensitive to the substratum. They not only favor the under and vertical surface of rocks, but also prefer the darker-colored and rougher-surfaced rocks.

4. At the time of brooding, *Leptasterias hexactis* stops feeding completely, but the animals will take food after a latent period of two days if the embryos are removed from the brooding chamber. Thus, a constant stimulation by the embryos is apparently necessary to prevent the animals from feeding.

5. During the breeding season, all the adult animals (female, male, spawned and non-spawned) are able to recognize their embryos and will pick them up to brood. This recognition is not due to the size, color, or shape of the embryo masses but rather to the physical nature of the fertilization membrane.

6. The significance and possible control mechanism of brooding were discussed.

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