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THE REPRODUCTIVE CYCLE OF *GORGONOCEPHALUS CARYI* (ECHINODERMATA; OPHIUROIDEA) [1]

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To the present time, there has been no detailed study of the reproductive cycle of any ophiuroid. Thorson (on *Ophiocten sericeum*, 1934) and Smith (on *Ophiothrix fragilis*, 1940) made brief observations on the ovarian histology at different times of the year and determined the breeding seasons for their respective species. All other determinations of breeding seasons for ophiuroids are based primarily on the times of the year when larvae appeared in the plankton or on direct observations of spawning. Boolootian (1966) prepared a summary graph of the known breeding seasons of ophiuroids; however, no references to Smith or Thorson are made. Most ophiuroids have a spawning season of from one to three months. The duration of known spawning seasons for ophiuroids ranges from one month (*e.g.*, *Ophiothrix texturata*, Olsen, 1942; Mortensen and Lieberkind, 1928) to six months (*Amphiura filiformis*, Olsen, 1942; Mortensen and Lieberkind, 1928; and *Ophiothrix fragilis*, Smith, 1940). *Ophiocten sericeum* spawns for 4 to 5 months (Thorson, 1934). The viviparous ophiuroid *Amphipholis squamata* appears to breed throughout the year (Fell, 1946).

Most studies of echinoderm reproductive cycles utilize the gonad index as the measurement of reproductive condition. The gonad index is defined as the ratio of the gonad volume to total wet weight × 100 (Lasker and Giese, 1954). This gives a useful measure of the relative size of the gonads, but it gives no indication of the actual condition of the gametes. Moreover, a gonad index cannot be determined on animals such as *Gorgonocephalus* where the numerous gonads fill most of the body cavity, and their attachments are such that they cannot be removed in their entirety from the animal. Only a few studies have employed periodic histological examination of the gonads as a means of determining the reproductive cycle in echinoderms (Yoshida, on *Diadema*, 1952; Tanaka, on *Stichopus*, 1958; Fuji, 1960a, 1960b, on *Strongylocentrotus nudus* and *S. intermedius*; Pearse, on *Odontaster*, 1965; Chia, on *Leptasterias*, 1964, 1968; Holland, on *Stylocidaris*, 1967). The need for information concerning the reproductive cycles of ophiuroids and the paucity of histological data on the reproductive cycles of echinoderms prompted this study.

Materials and Methods

Monthly collections of *Gorgonocephalus caryi* were made for 13 months (from August 23, 1965, to August 26, 1966) from Boundary Pass in the San Juan Islands

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48° 44.4′ N., 23° 1.7′ W) from depths of 20–70 fathoms. Samples of the ventral gonadal lobes (Patent, 1968) were taken from 8 to 28 animals each month. The proportion of males to females varied from month to month, but the overall number of animals of each sex examined was about equal. The tissues were fixed in Heidenhain's susa fixative made up with sea water, dehydrated by the tertiary butyl alcohol technique (Johansen, 1940), embedded in Paraplast, and sectioned at 5 μ–7 μ. At least 20 gonad sections were made from each animal, and these were stained with a modified Masson's trichrome (Patent, 1968). The mean diameters of twenty of the largest oocytes (sectioned through the nucleolus) from each female were measured by averaging two diameters taken perpendicular to each other. The twenty oocyte diameters from each animal were averaged and the standard error determined. The standard errors were very small (from 0.8 μ to 2.9 μ). The average values for the animals collected each month were then averaged, giving a mean size for the largest oocytes for each month.

After examination of slides of gonads from all times of the year, the reproductive cycles of the female and the male were divided into five stages. These stages were derived independently for the male and the female.

The Maturity Index (M.I.) of Yoshida (1952) was calculated for both males and females for each month. The formula used is as follows:

$$\text{M.I.} = \frac{\Sigma[1\ (\#\text{ animals in Stage 1}) + 2\ (\#\text{ animals in Stage 2}) \cdots + n\ (\#\text{ animals in Stage n})]}{\text{Total number of animals staged that month}}$$

Results and Discussion

The stages of the reproductive cycle

Examination of the histological material showed that the reproductive cycle of the female and that of the male could be divided into five fairly distinct stages. The key criteria used are as follows:

Stage 1: Post-spawning. Females: The lumen of the ovary is filled with degenerating oocytes (Fig. 1A). Males: Spermatogenesis has stopped, and only spermatogonia and spermatozoa are present. There are often areas of the testicular lining which are devoid of spermatogonia (Fig. 2A).

Stage 2: Early growth. Females: Little or no post-spawning debris is present. The ooplasm stains predominantly red with Masson's trichrome (Fig. 1B). Males: the testes are small and the distribution of the spermatogonia is uneven. There are more spermatogonia near the end of the gonad where the germ cells enter and/or the gonads are very small with very few sperm present (Fig. 2B).

Stage 3: Resting. Females: Green granules can be seen as clumps in the ooplasm in stained material. The oocytes are under 121 μ in diameter (Fig. 1C). Males: The spermatogonia are evenly distributed, and there are deep indentations in the wall of the testis. In some testes, spermatogenesis has begun (Fig. 2C).

Stage 4: Late growth. Females: The largest oocytes are over 121 μ in diameter, and the outer membrane (Patent, 1968) has begun to form (Fig. 1D). Males: The layer of spermatogenic tissue is very thick and spermatogenesis is well under way. The spermatozoa do not yet form a separate mass in the testicular lumen (Fig. 2D).

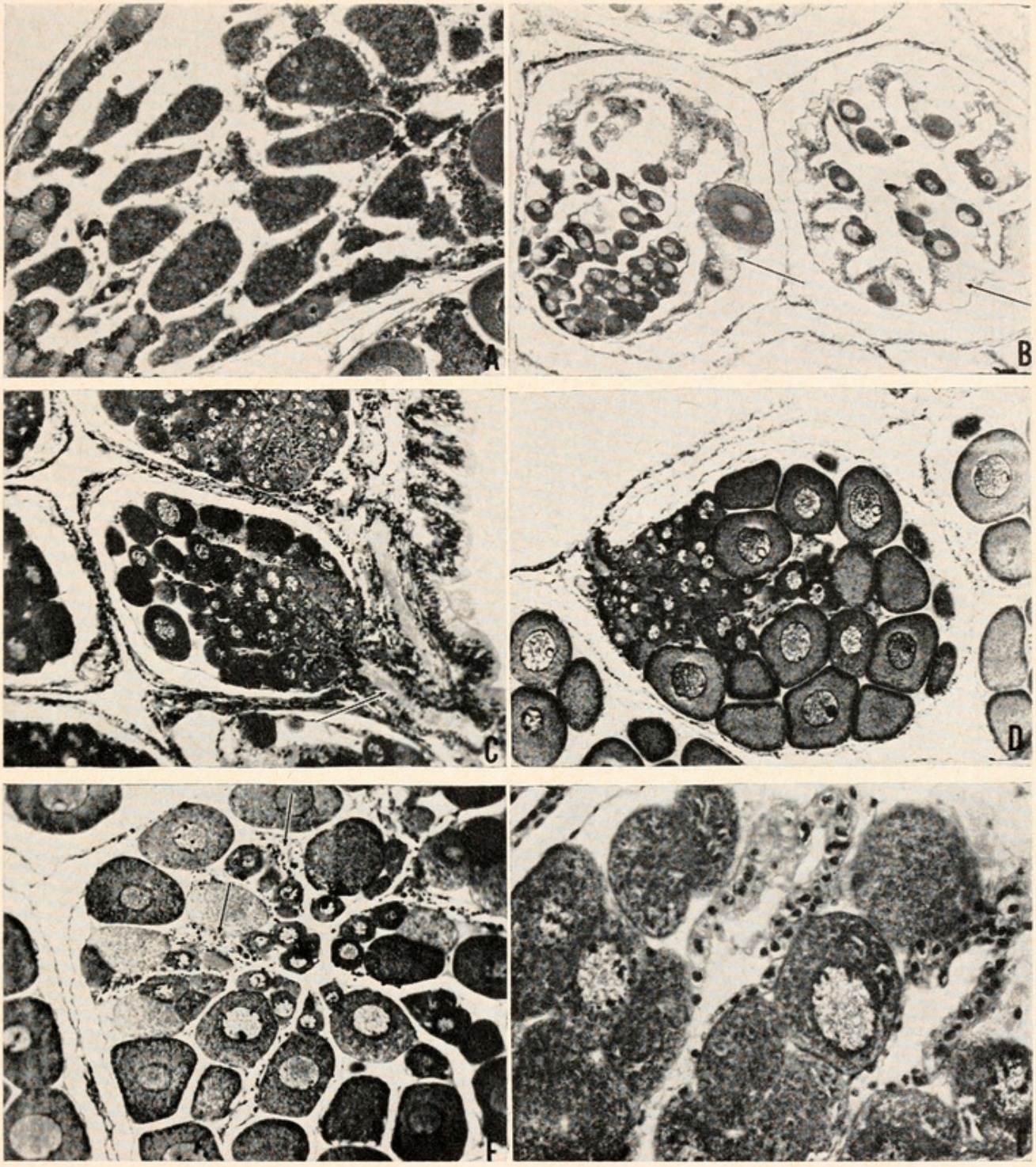

FIGURE 1. Histological sections of gonads of *Gorgonocephalus*. (A) Stage 1 ovary from animal collected in November. The lumen of the ovary is filled with degenerating oocytes. (B) Stage 2 ovaries from animal collected in December. The walls of the ovaries are still stretched, and the hemal fluid can be seen (arrows). Note the one large oocyte which was not released into the lumen when the animal spawned. (C) Stage 3 ovary. Note the uniform staining of the oocytes. The ovarian duct can also be seen (arrow). (D) Stage 4 ovary. The largest oocytes are located in the distal portion of the gonad. Note the lighter staining of the large oocytes (green with Masson's trichrome) and the dark staining of the periphery of the large oocytes (red with Masson's). (E) Stage 5 ovary. The oocytes are large, and debris left over from unspawned oocytes can be seen in the ovarian lumen (arrows). (F) Portion of an ovary from an animal collected in December. The layer of nurse cells lining the lumen is prominent. Clumps of material, some of which look like pycnotic nuclei of nurse or follicle cells can be seen inside the oocytes. Photographs 1A–1E are all 78 ×. Photograph 1F is 250 ×.

Stage 5: Spawning. Animals in this stage are assumed to have spawned at least once and will spawn at least once more before the spawning period is over (see below). Females: Cellular debris from the breakdown of unspawned oocytes is seen in the ovarian lumen (Fig. 1E). Males: A large number of sperm have been produced and are seen as a mass in the center of the gonad, separate from the layer of spermatogenic tissue (Fig. 2E).

The above criteria were used to classify all the animals from which gonadal tissue had been collected. However, much more can be said about the histology of the gonads, especially of the ovaries, at the different stages.

The ovarian cycle

In early Stage 1 ovaries, the degenerating oocytes are seen as distinct masses of material in the ovarian lumen (Fig. 1A). Later, the degenerating cytoplasm coalesces. Throughout Stage 1, spheres from degenerating oocytes are seen in the cytoplasm of the nurse cells which border the lumen and are attached to the young oocytes (Patent, 1968). It is difficult to measure oocytes in Stage 1 ovaries because the ovarian wall is still distended and very few oocytes are seen in a given section. In addition, the oocyte boundaries are indistinct.

In early Stage 2 ovaries, the walls are still stretched, so that the lumen and the fluid in the hemal space surrounding the gonad (Patent, 1968) are easily seen (Fig. 1B). Later, the walls contract and the gonad is more compact. The nurse cells are abundant and are frequently arranged as an epithelium lining the ovarian lumen (Fig. 1F). The oocytes often contain ingested cellular debris (Fig. 1F) (Patent, 1968). All oocytes in Stage 2 ovaries are under 80 μ in diameter.

The nurse cells in Stage 3 ovaries are more clumped. The largest oocytes have begun vitellogenesis. The cortical granules have also begun to form (Patent, 1968). The oocytes are more crowded and, instead of having a more or less oval outline as in Stage 2, they are developing a more polygonal form. The largest oocytes in Stage 3 ovaries range from 84 μ to 121 μ in diameter.

In the stage of late growth (Stage 4), the largest oocytes are from 122 μ to 150 μ in diameter. The outer membrane has not formed around all the large oocytes. In some ovaries, many oocytes will be developing the membrane, whereas it may not be present on any of the oocytes in other ovaries. The oocytes are packed tightly, and their shape is variable (Fig. 1D). The stalk attaching the oocyte to the nurse cells (Patent, 1968) is very evident. The cortical granules lie mostly at the periphery of the oocyte.

The size of the oocytes in Stage 5 ovaries is variable, depending on when the animals spawned and also probably on the size of the animal. In most Stage 5 animals, the largest oocytes were between 150 μ and 160 μ in diameter, with a range of 115 μ to 177 μ. Since fresh, living fertilized eggs measure 220 μ, it seems probable that fixation (in susa fixative) and dehydration (through the tertiary butyl alcohol series) cause about 20% shrinkage of the oocytes.

The histological condition of the largest oocytes is variable in Stage 5 ovaries. Usually, the germinal vesicle is similar to that of Stage 4 oocytes in size, but sometimes it has swollen (Fig. 2F). In two females collected in November, 1965, there were secondary oocytes present. The follicle cells around them were disintegrating,

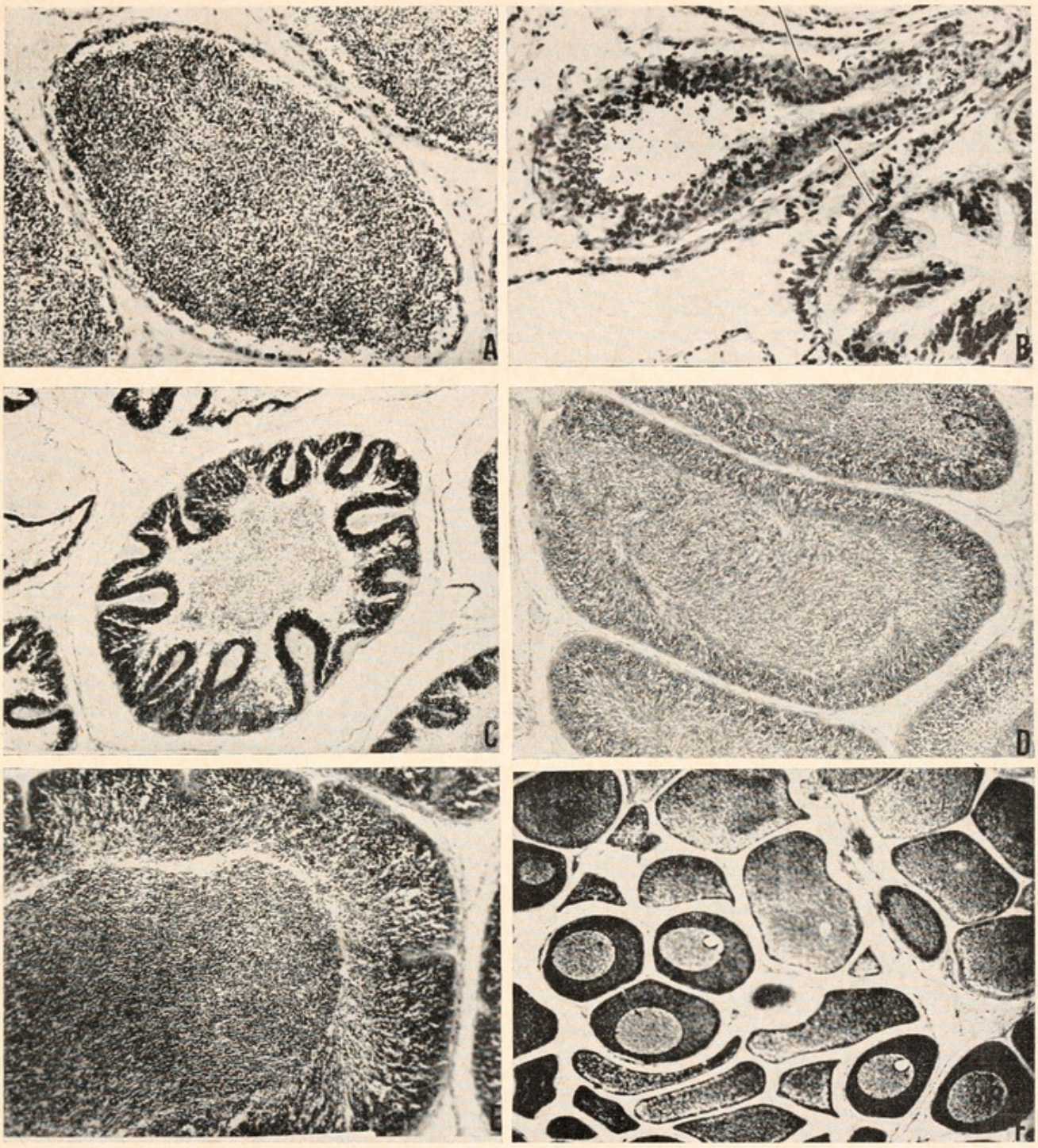

FIGURE 2. Histological sections of gonads of *Gorgonocephalus*. (A) Stage 1 testis. Spermatogenesis has stopped. Areas without spermatogonia can be seen along the testicular wall. 156 ×. (B) Stage 2 testis. Note the thick layer of spermatogonia at the duct end of the testis (arrows). 156 ×. (C) Stage 3 testis. The layer of spermatogenic tissue is thickened. The loops of germinal tissue underlain by hemal fluid in the hemal space are easily seen. 78 ×. (D) Stage 4 testis. The spermatozoa do not form a separate mass in the lumen. 62 ×. (E) Stage 5 testis, showing the separate mass of spermatozoa in the lumen. 78 ×. (F) Section of ovaries just before spawning, showing the shape of the eggs and their small nuclei. Primary oocytes with swollen germinal vesicles can also be seen, and the degeneration of the follicle cells is evident. 78 ×.

and their shape was distorted. The nuclei were small, and the cortical granules were all at the periphery of the oocytes (Fig. 2F). Since only two such animals were collected, it is probable that the oocytes undergo at least the first maturation division within hours of spawning. The gonads of these animals were fixed in the afternoon. When spawning occurred in the laboratory in the winter of 1966, it always occurred between 7:00 and 9:00 p.m. It is probable that the animals which contained secondary oocytes would have spawned the evening

TABLE I

*Numbers of animals in each gonadal stage collected each month**

Females Stage no.	Year												
	1965					1966							
	Month												
	A	S	O	N	D	J	F	M	A	M	J	J	A
1			1	3	1								
2					3	1	1			1			
3						6	9	8	7	2	1		
4	4									8	3	2	3
5	9	10	12	3							1	1	4
Total	13	10	13	6	4	7	10	8	7	11	5	3	7
M.I.	4.7	5	4.7	3	1.8	2.9	2.9	3	3	3.6	4	4.3	4.6
Males Stage no.													
1				9	2								
2					7	2	3	3	1	2			
3					1	3	6	8	3	3			
4										3	1		
5	9	12	11	5						2	5	4	4
Total	9	12	11	14	10	5	9	11	4	10	6	4	4
M.I.	5	5	5	2.4	1.9	2.6	2.7	2.7	2.7	3.5	4	5	5

* M.I. = Maturity Index. The collections were made from August, 1965 to August, 1966.

of the day they were collected, a few hours after the oocytes underwent maturation division. This is in keeping with the pattern in other echinoderms which have been studied, with the exception of euechinoids. Holland (1967) has concluded that the lack of accumulation of ripe eggs is a primitive echinoderm trait which has been altered in the euechinoids. In asteroids and some ophiuroids, maturation divisions do not occur until after spawning (Costello, Davidson, Eggers, Fox and Henley, 1957). In the cidaroid sea urchin *Stylocidaris affinis* (Holland, 1967) and in the crinoid *Comanthus japonica* (Dan, 1952), maturation divisions occur a short time

before spawning. In *Comanthus,* all the animals spawn within a few minutes of one another during late afternoon in early October, and the oocytes undergo maturation division almost simultaneously throughout the population. Thus, it was possible for Dan to study the changes in the oocytes which precede and accompany maturation division. In *Gorgonocephalus,* the oocytes do not undergo maturation division simultaneously; rather, different stages can be seen at once in an ovary. As in *Comanthus,* the germinal vesicle is first drawn out towards the stalk connecting the oocyte with the other ovarian tissue. The nucleoli at this stage are fragmenting. It was not possible to see in *Gorgonocephalus* just how the oocytes broke away from their attachments, but the follicle cells are seen to be disintegrating. The oocytes of *Comanthus* and *Gorgonocephalus* both lose their shape after becoming free (Fig. 2F). The membrane of the germinal vesicle disappears and the spindle-forming bodies migrate to one pole. Dan wrote that this was the stalk end in *Comanthus,* but this could not be determined for *Gorgonocephalus.* After the spindle-forming bodies migrate, maturation division begins.

As is seen in Table I, some animals of both sexes were in the spawning stage from August to November, 1965, and from June to August, 1966. Assuming that the spawning season at a given locality is fairly constant from year to year, *Gorgonocephalus* has a spawning season of six months. This also assumes that the criteria used for determining when spawning is taking place are valid. The key criterion for determining the spawning season of the females was the presence of cellular debris in the lumen of the gonad. It was assumed that this debris represented the remains of oocytes which had matured but which remained in the ovary and were not spawned. Pearse (1965) and Delavault (1961) noted that degenerating oocytes were found in some asteroid ovaries throughout the year. Pearse postulated that these oocytes serve as storage organs for the nutrition of other oocytes. The debris in the *Gorgonocephalus* ovary (assumed to be the remains of unspawned oocytes) could represent the remains of such storage oocytes which had been broken down. However, the evidence is to the contrary. In *Gorgonocephalus,* a few large residual oocytes occasionally remain in the ovary after the spawning season (Fig. 1B). These are attacked by phagocytes *in situ* some time prior to the next spawning season, and no fragments are released into the ovarian lumen. Secondly, the debris in the lumen of the gonad is found only during the presumed spawning period and in the post-spawning period, not year around. Thirdly, the first appearance of the cellular debris coincides with the first time that oocytes over 150 μ in diameter are seen in the ovaries. These large oocytes do not appear in the one animal collected that month which is assumed to have spawned (*i.e.,* the ovarian lumina contained cellular debris). Lastly, the criteria for determining the stages of the reproductive cycle for males and females were arrived at independently, yet the data from the males also indicates that the animals spawn from June through November. Thus, it seems reasonable to conclude that *Gorgonocephalus* spawns for six months of the year and that the debris in the ovarian lumina is derived from unspawned oocytes.

Other workers (Tanaka, 1958; Chia, 1964, 1968) who have divided the reproductive cycle of echinoderms into stages have included a resting stage. In *Stichopus,* most of the females appear to have a post-spawning rest period of three months (Tanaka, 1958). This is usually assumed to be the typical echinoderm pattern

(Boolootian, 1966). Chia (1964, 1968) has shown that in *Leptasterias* there is a resting period of several months after the largest oocytes have reached their terminal size. During this time there is very slow growth of the small oocytes. In *Gorgonocephalus,* there is a resting period in the females from January through March. During this time, the average diameter of the largest oocytes remains

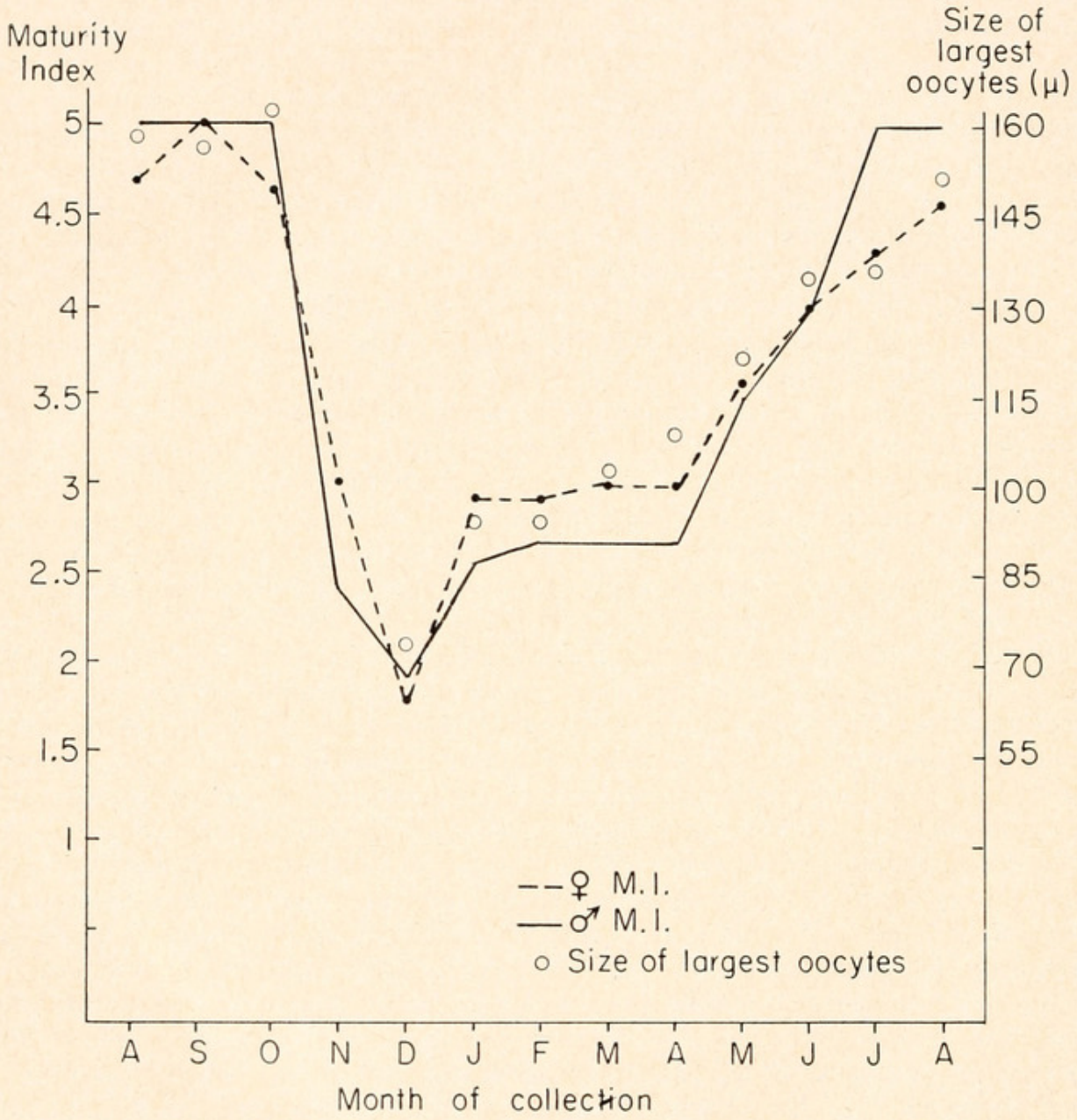

FIGURE 3. Graph showing the Maturity Index (left scale) and the size of the largest oocytes (right scale) for the animals sampled in this study. The first month of collection was August, 1965; the last month was August, 1966. All collections were made in Boundary Pass in the San Juan Islands.

relatively constant, and the ovaries of animals collected in January show no striking differences from those collected in March. However, it is possible that more oocytes are growing and reaching the maximum size for this stage (under 121 μ). The resting period occurs after the oocytes have undergone considerable growth (from a maximum diameter of 78 μ in December to a maximum of 104 μ in Jan-

uary). Probably the growth from December to January and what little growth may occur during the resting period result from the utilization of the nutrients derived from the degenerating unspawned oocytes at the end of the spawning season. The rest period from January through March then corresponds to the period of least availability of nutrients. During the winter months in the San Juan Islands, there is very little plankton. By April, the daylight hours have increased considerably, and the plankton becomes more abundant. In April, the oocytes resume growth. Holland (1964) has shown that in *Strongylocentrotus purpuratus* continuous feeding stimulates, and starvation inhibits, production of large cells in the ovaries (and of new cells in the males). Thus, at least in a regular echinoid, the availability of nutrients can be reflected in the development of the gonads. However, whether the rest period in *Gorgonocephalus* results directly from a lack of food or whether it is controlled in some other way and evolved in response to the period of minimum food availability cannot be stated.

It can be seen in Figure 3 that the diameters of the largest oocytes correlate well with the Maturity Indices for the different months. The only time that oocyte diameters were used to determine the stage of an animal's ovaries was when there was some question as to whether an animal was in Stage 3 or 4; occasionally it was difficult to determine if the outer membrane had begun to form. In these cases, the animals were placed in Stage 3 if the oocytes were under 121 μ in diameter and in Stage 4 if they were over 121 μ. This was necessary in only a few cases; so in general, the average diameter of the largest oocytes is a good measure of gonadal maturity.

The testicular cycle

Although the ovarian stages used in this study were determined independently of other studies, the testicular stages are very similar to those reported by Tanaka (1958). He divided the reproductive cycle of *Stichopus* into five stages: Resting, recovery, growing, mature, and shedding. Histologically, the resting stage of *Stichopus* testes resembles the post-spawning stage of *Gorgonocephalus*. However, as is seen in Table I, this post-spawning stage is not a resting stage for male *Gorgonocephalus*, as it lasts no more than a month. The recovery stage of *Stichopus* is similar to the early growth stage of *Gorgonocephalus* males, and the growth stage of *Stichopus* is almost identical histologically to what is designated as the resting stage (Stage 3) in *Gorgonocephalus*. However, some male specimens of *Gorgonocephalus* appear to rest in Stage 2. At least, during the months of January through April, the proportion of animals in Stages 2 and 3 remain stable for the population, as is shown by the Maturity Index (Table I, Fig. 3). One could conclude that the population shows a resting period from January to April, but that one cannot define a resting period precisely for individual animals.

General comments

The Maturity Indices for the monthly collections are plotted in Figure 3. The Maturity Index (Yoshida, 1952) is useful when the sample size from different months is different, and it provides a way to compare the data from males and females. It can be seen (Fig. 3) that the criteria used for determining the male

and female cycles are almost equivalent. The differences between the males and females could be due to imperfections in the method of estimating the stage of development or to differences in the ways in which gametogenesis proceeds in males and females. If one assumes no defect in the method, some comments can be made about the differences. Table I shows that males in Stages 2 and 3 are present from January through May, whereas Stage 2 females are no longer present after February, except for one aberrant individual in May. Stage 4 males are present for a short time only, in May and June, whereas there are Stage 4 females present from May through August. This could indicate that intensive spermatogenesis does not begin until just before the spawning season and that once it has begun, all the males are spawning. In contrast, oogenesis proceeds for a longer time before the oocytes reach spawning size. Not all the females spawn throughout the season, but it cannot be determined how frequently a particular female spawns during a season. Females spawn more than once, most of them through October, some into November.

In the winter of 1966–67, specimens of *Gorgonocephalus* collected southwest of the monthly collection site spawned many times in the laboratory. All spawnings took place between 7 and 9 p.m. Spawning between 8 and 10 p.m. has been noted in *Ophiura brevispina, Ophiopholis aculeata,* and *Ophiocoma echinata* (Grave, 1899). Only once was the spawning of *Gorgonocephalus* observed closely (by Dr. Gregory Patent). The animal observed was a male which spawned for about ½ hour, pumping the disk up and down. Sperm were released on the downward push. The dates on which spawning occurred were as follows: November 21, 1966; December 20, 1966; January 7, 15, 16, 17, and 21, 1967; February 10 and 21, 1967; and March 10, 1967. It is not known whether the same or different animals spawned on the different days. Sometimes only males spawned; at other times both males and females spawned. No pattern can be seen in the days on which spawning occurred. February 10 was the last day on which females single males which had been in the laboratory for at least two months would not spawned, so it is possible that the later February and the March spawnings by have occurred in the field. Even so, the animals from this location spawned three months later than the animals from Boundary Pass would have spawned the previous year. It is possible that the spawning season of *Gorgonocephalus* varies from year to year. Giese (1959) followed the reproductive cycle of *S. purpuratus* from one location for many years and found that the gonad index curve and the time of spawning were often very different from one year to the next. The factors causing this variation are unknown. It is also possible that specimens of *Gorgonocephalus* from different locations have different spawning seasons. Thorson (1934) found that *Ophiocten sericeum* from different locations around East Greenland varied in the stage of ovarian development. Further study is required to determine the cause of the observed differences in spawning season of *Gorgonocephalus* from different localities.

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Summary

1. Samples of gonads from *Gorgonocephalus caryi* collected monthly over a 13-month period were examined histologically to determine the course of the reproductive cycle.

2. The reproductive cycle of both sexes was divided into 5 stages: Post-spawning, Early Growth, Resting, Late Growth, and Spawning. The Maturity Index (Yoshida, 1952) was calculated separately for males and females for each month.

3. The histology of the ovaries in the various stages is described.

4. Maturation divisions in females probably occur on the day of spawning and proceed very much as in the crinoid, *Comanthus japonica* (Dan, 1952).

5. *Gorgonocephalus* has an annual reproductive cycle and spawns for six months of the year, from June through November in the population studied in 1965–1966. In the winter of 1966–1967, animals collected from a different location spawned in the laboratory from November through March.

6. Each animal spawns more than once during the spawning season.

7. Gonad development is arrested after the initial stages of gametogenesis have commenced rather than immediately after spawning, as occurs in most other echinoderms.

8. The curve for growth of the largest oocytes corresponds well with the female Maturity Index, indicating that oocyte size is a good measure of gonadal maturity.

9. The male and female Maturity Indices correspond well, and the differences between them can be explained by differences between oogenesis and spermatogenesis.

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