

**ANNUAL REPRODUCTIVE CYCLE OF THE BRITTLE-STAR
AMPHIPHOLIS KOCHII (ECHINODERMATA: OPHIUROIDEA),
WITH SPECIAL REFERENCE TO THE GROWTH
PATTERN OF OOCYTES**

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With Text-figures 1-6 and Tables 1-2

Introduction

In the Echinodermata, detailed studies on the reproductive cycle have been made for echinoids by many authors, but have been scarcely done for asteroids, holothuroids, crinoids and ophiuroids (Holland *et al.*, 1975). Concerning the reproductive cycle of the ophiuroids, several authors report their brief observations on the gonadal state made at a few different times of year for determining the breeding season of the species used for developmental studies (Smith, 1940; Olsen, 1942; Fell, 1946; Boolootian, 1966). A complete observation of the annual reproductive cycle of the ophiuroids has been published only in four papers for the following five species, *Gorgonocephalus caryi* Lyman (Patent, 1969), *Amphiura chiajei* Forbes (Fenaux, 1970), *Ophioderma longicauda* Retzius (Fenaux, 1972), *Ophiura albida* Forbes and *O. texturata* Lamarck (Tyler, 1977).

The present paper deals with the annual reproductive cycle of the brittle-star *Amphipholis kochii* Lütken, 1872 (Echinodermata: Ophiuroidea), with special regards to the oocyte growth pattern.

Materials and Methods

The brittle-star *Amphipholis kochii* used in this study was collected from under stones between the tidemarks at Abuta along the Funka Bay on the Pacific side of south-western Hokkaido, North Japan. Sampling of the animals for the study was made once a month during a period from June 1979 through July 1980, and at each collection, 9-24 specimens which were more than 5 mm in disk diameter were selected (Table 1). Within 6 hours after collection, arms were cut off from each disk, and the disks were fixed in Bouin's solution for 3 hours or more. After fixation, the disks were preserved in 70% ethanol until they were examined.

For histological observation, fixed gonads removed from the disk were dehydrated with ethanol and embedded in paraffin (Tissue-Prep; Fischer Scientific Company,

Table 1. Distribution of males, females and unsexuable individuals in each collection.

Date	Males	Females	Unsexuable individuals	Total
'79 12/June	6	14		20
25/July	4	8	2	14
9/Aug.	3	3	3	9
6/Sep.	12	2		14
22/Oct.	6	15		21
21/Nov.	11	8		19
21/Dec.	5	12		17
'80 18/Jan.	17	7		24
18/Feb.	12	8		20
20/Mar.	7	13		20
17/Apr.	6	9		15
16/May	9	12		21
13/June	8	7		15
13/July	3	6	5	14
Total	109	124	10	243

Fair Lawn, New Jersey). The serial sections of 3–5 μm were stained with Delafield's hematoxylin and eosin.

The gonad index for the gross analysis of the annual reproductive cycle was calculated by the following formula:

$$\text{Gonad Index} = (\text{Weight of Gonads} / \text{Weight of Disk}) \times 100$$

For the examination of the growth pattern of the oocytes, the frequency polygon method of Pearse (1965) was adopted as follows: All those oocytes with a nucleolus found in three longitudinal sections were chosen to measure the diameter with an ocular micrometer. The diameter of each oocyte was represented by a mean of the lengths along long and short axes, because the most oocytes were somewhat elliptical in outline. The obtained diameters were separated into 10 μm size classes for examining the size-frequency distribution of the samples.

The growth rate of the largest oocytes was examined by calculating the instantaneous relative growth rate according to the following formula by Brody (1945):

$$k = \frac{\ln V_2 - \ln V_1}{t_2 - t_1},$$

where k represents the instantaneous relative growth rate, V_1 is the largest oocyte volume at time t_1 , and V_2 is the largest oocyte volume at time t_2 . From the mean diameter of oocyte, i.e., the mean length of long and short axes of oocyte, the volume of oocyte was calculated on assumption that the oocyte was adequately represented as a sphere. The largest oocyte volume in each female was gained by building the intermediate volume of largest five oocytes in each female. The largest oocyte volume in each month was then obtained by averaging the largest oocyte volume of each female in a month. The resulting volume was plotted on a graph with

semi-logarithmic scale against time starting on July 26th, 1979, after the spawning had occurred.

Results

Annual change of the gonad index.

The annual reproductive cycle is well defined in the population of *Amphipholis kochii* at Abuta as shown in Fig. 1. The gonad indexes in both sexes are higher in May-June and lower in July-October, being decreased suddenly between June and July. The spawning of this species is presumed to occur in June and July. No significant change of the gonad index is found from July to October, but from November

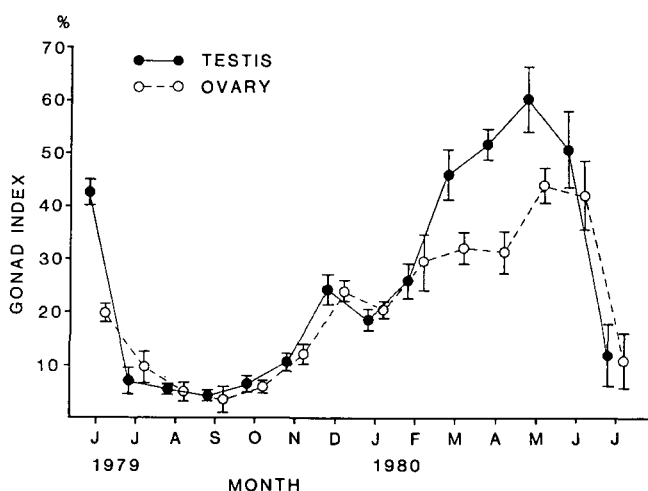


Fig. 1. Monthly variations in the gonad index from June 1979 through July 1980. Average \pm standard error.

Table 2. Characteristics of gonadal stages.

STAGE	OVARY			TESTIS		
	Color	Internal feature	The largest oocyte diameter	Color	Internal feature	% of sperm
Stage 0 (Unsexuable)	Brown	Post-spawning debris	—	Brown	Post-spawning debris	—
Stage 1 (Recovering spent)	Orange	Previtellogenic oocytes	<30 μ m	Light yellow	Arrest of spermatogenesis	—
Stage 2 (Growing)	Dark orange	Vitellogenic oocytes	<60 μ m	Cream	Beginning of spermatogenesis	<25%
Stage 3 (Premature)	Dark orange	Cytoplasmic globules	<80 μ m	Milky white	Active spermatogenesis	25-50%
Stage 4 (Mature)	Brownish red	Cytoplasmic globules	80 μ m \leq	Milky white	Large sperm mass	50% \leq
Stage 5 (Spent)	Dark brown	Relict oocytes	—	Brown	Relict sperm	—

it increases constantly. Significant differences are found between the index values of the female and the male from March through May, with a confidence limit of 95% (*t*-test).

Gametogenesis in annual reproductive cycle.

For observing the annual cycle of the gonad, gonad condition is classified into the following six stages (Table 2):

Stage 0 (unsexuable): In this stage, the gametogenesis in both sexes rests after the shedding of the gametes, and the relict gametes degenerate into post-spawning debris in a short phagocytic period (Fig. 2 a). The gonads are very small, and the

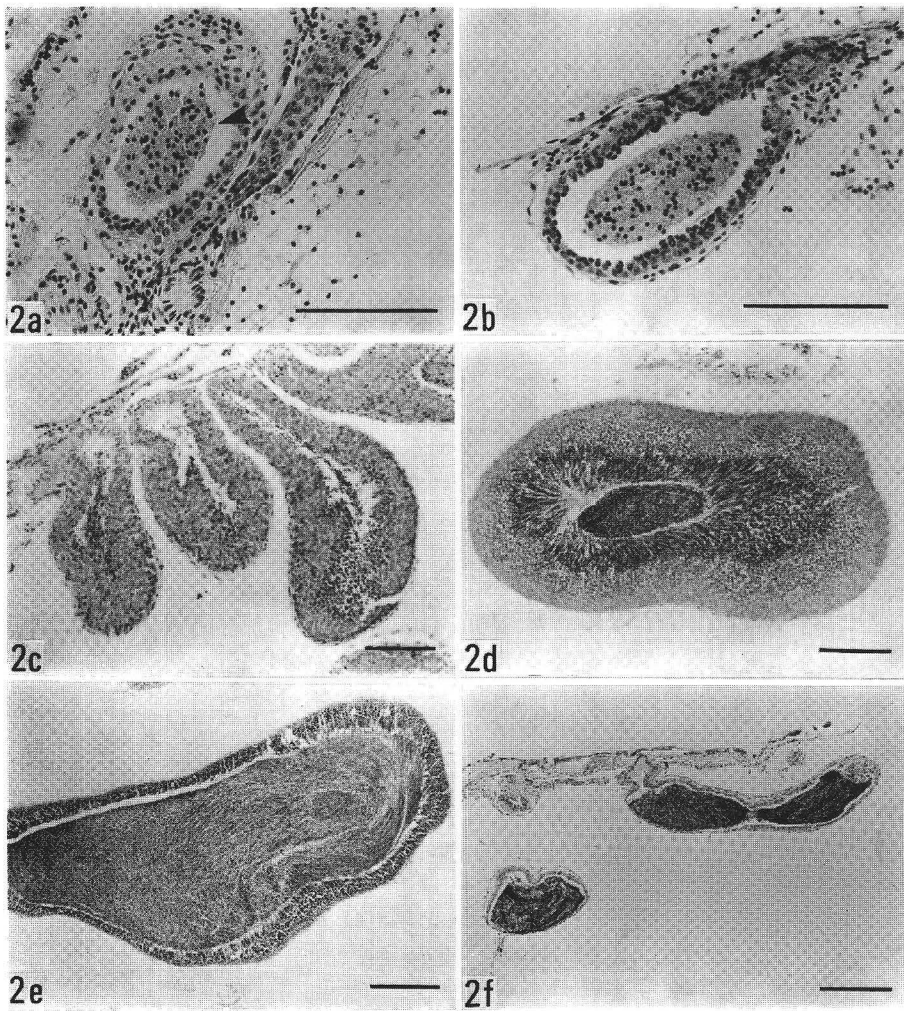


Fig. 2. Sections of the gonad in stage 0 (unsexuable) and the testes in each gonadal stage. a: stage 0, arrowhead showing the post-spawning debris, scale 100 μ m. b: stage 1, scale 100 μ m. c: stage 2, scale 100 μ m. d: stage 3, scale 100 μ m. e: stage 4, scale 200 μ m. f: stage 5, scale 200 μ m.

sex cannot be determined even by histological observation. Along the inner side of the gonadal wall, occasionally some gonial cells without any sign of oogonia or spermatogonia can be seen (Fig. 2 a).

Stage 1 (recovering spent): The gonads are still small, but their color differs according to the sex, orange in the female and light yellow in the male. The lumen of the gonad still has a small amount of the post-spawning debris (Figs. 2 b and 3 a). The testis has a spermatogonial layer without showing spermatogenesis in progress (Fig. 2 b). Along the ovarian wall, previtellogenic oocyte being less than $30\ \mu\text{m}$ in diameter can be found (Fig. 3 a).

Stage 2 (growing): The cream-colored testis slowly starts spermatogenesis and

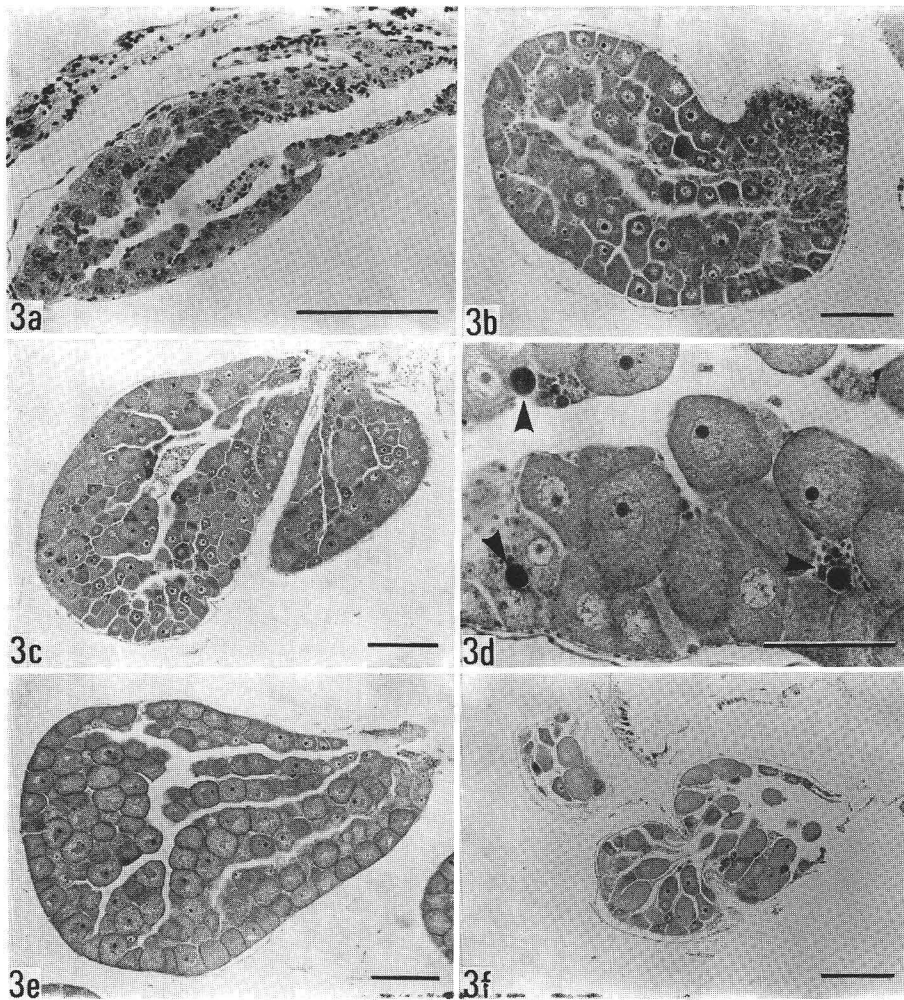


Fig. 3. Sections of the ovaries of five gonadal stages. a: stage 1, scale $100\ \mu\text{m}$. b: stage 2, scale $100\ \mu\text{m}$. c: stage 3, scale $200\ \mu\text{m}$. d: cytoplasmic globules (arrowheads) in ovary of stage 3, scale $100\ \mu\text{m}$. e: stage 4, scale $200\ \mu\text{m}$. f: stage 5, scale $200\ \mu\text{m}$.

the percentage of sperm in a cross section is under 25% (Fig. 2 c). The ovary, dark orange in color, develops vitellogenic oocytes which attain $60\ \mu\text{m}$ in diameter (Fig. 3 b).

Stage 3 (premature); The testis is milky white in color and spermatogenesis is actively proceeded. Layers of spermatogonia and spermatocytes become thicker, and sperm mass is formed in the central region of the testis, with 25–50% sperm range in a cross section (Fig. 2 d). The ovary is of a dark orange color, and the largest oocyte reaches $80\ \mu\text{m}$ in diameter (Fig. 3 c). Among the developing oocytes, cytoplasmic globules stained with both hematoxylin and eosin are found (Fig. 3 d).

Stage 4 (mature): The gonads in both sexes attain their maximum volume. The testis is milky white in color, and has a large sperm mass. The percentage of sperm in a cross section reaches over 50%. On the contrary, the layers of spermatogonia and spermatocytes become thinner than those in the previous stage (Fig. 2 e). The ovary now has turned brownish red, and its lumen is almost occupied by fully grown primary oocytes which are more than $80\ \mu\text{m}$ in diameter (Fig. 3 e). The cytoplasmic globules found in the premature stage are still observable.

Stage 5 (spent): The gonads are dark brown in the female and brown in the male, and have a small number of relics of oocytes or sperms in the lumen (Figs. 2 f and 3 f).

Fig. 4 shows monthly changes of the relative abundance of the animals with

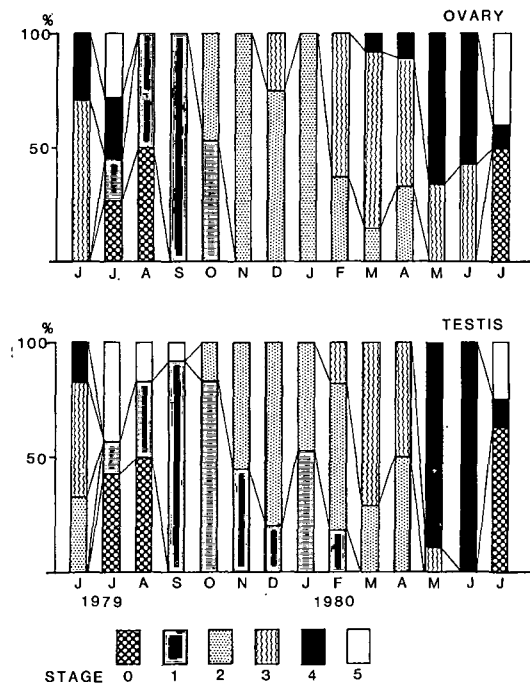


Fig. 4. Monthly changes in relative proportion of the animals in each gonadal stage.

each gonadal stage. In May-June, the number of the animals of stage 4 (mature) increases in both sexes, and in July those of stage 5 (spent) come to appear. These results indicate that the spawning period of *Amphipholis kochii* at Abuta is in June-July and occurs once a year. The period of stage 4 (mature) is shorter than that of other stages: this fact suggests that this species matures relatively acutely. The post-spawning resting period (stage 0) of the gonads lasts about one month around July and August.

Growth pattern of oocytes.

Monthly changes in the size-frequency distribution of the oocytes have been demonstrated by a frequency polygon method as shown in Fig. 5. From these data, the following three particular features can be recognized on the oocyte growth in this species: 1) the entire population of oocytes does not grow as a single generation but grows as multiple generations. This is clearly indicated by the shape of polygon, which does not show a single peak but a diffused figure. 2) small oocytes, under $20\ \mu\text{m}$ in diameter, are present every month. 3) as maturation proceeds, the large oocyte population and the small oocyte population separate distinctly from each other: in other words, the number of medium-sized oocytes, $40\text{--}60\ \mu\text{m}$ in diameter, is reduced.

The fact that small oocytes are present throughout the year suggests a constant production of oocytes through an annual reproductive cycle. This suggestion is justified by the observation that mitotic figures of oogonia are found through all the seasons (unpublished). This suggestion also explains why the size-frequency polygon does not show a single generation of oocytes. The reduction of the number of medium-sized oocytes is probably caused by the following two events: First, the growth of small oocytes is arrested and medium-sized oocytes become large ones. Second, the medium-sized oocytes are selectively destroyed by somatic cells in the ovary or by autolysis. The cytoplasmic globules found in the premature or mature ovary

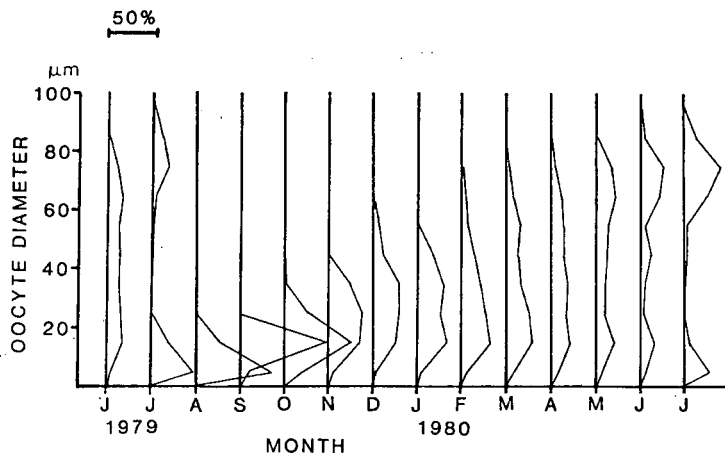


Fig. 5. Monthly variations in the size-frequency distribution of oocytes.

probably originate from these destroyed oocytes.

Growth rate of the largest oocytes.

Fig. 6 shows the growth curve of the largest oocytes. Three stages are distinguishable on the growth pattern of the largest oocytes: a resting stage for about one month immediately after spawning, a fast growth stage from late August to December and a slow growth stage from December to June. The instantaneous relative growth rates of the oocytes in each stage are 0 in the resting stage, 0.031 in the fast growth stage, and 0.007 in the slow growth stage. These results indicate that the volumes of the oocytes increase about 3.1% per day in the fast growth stage and about 0.7% per day in the slow growth stage.

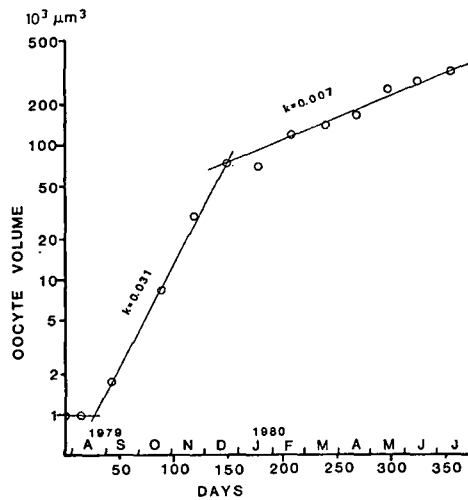


Fig. 6. The growth curve for the largest oocytes. The instantaneous relative growth rate is shown as *k*.

Discussion

The characteristics of the gametogenesis in *Amphipholis kochii* are somewhat different from most of those found in echinoids. It has already been known that, in the sequential changes of the gametogenesis in the echinoids, two distinct stages were recognizable in the annual reproductive cycle (Yoshida, 1952; Fuji, 1960; Pearse, 1970): In the first stage, the nutrient accumulation, accompanied by an increase of the gonadal size and the gonad index, takes place, and nutrients are reserved in the cytoplasm of the nutritive phagocytes (somatic cells in echinoid gonads) as spherical bodies of several types; in the second stage following the nutritive build-up, gametogenic cells begin to grow, consuming the nutrient reserves in the nutritive phagocytes. However, in the annual reproductive cycle of the present species, we detected none of the two stages mentioned above either by examination of gonad indexes or by histological observation. This is presumably due to the fact

that the gonad of the ophiuroids does not have any nutritive reservoir comparable to the nutritive phagocytes of the echinoids (Smith, 1940; Patent, 1976), and in this species, unlike the echinoid, the nutrients are seemingly thought to be transferred continuously to the gonads.

Resting periods of the annual reproductive cycle in echinoderms have been found by many authors, and the periods are usually assumed to be a typical echinoderm pattern (Booolootian, 1966). In ophiuroids, a clear resting period for about three months after spawning was described by Patent (1969) for the basket-star *Gorgonocephalus caryi* Lyman. The post-spawning resting period can be observed in the present species too, but it is relatively short as compared with *G. caryi*. The resting periods of *A. kochii* and of the crinoid *Comanthus japonica* Müller are very similar in that after spawning the gonad passes through a brief phagocytic stage and enters an unsexable stage (post-spawning resting period) (Holland *et al.*, 1975).

In the present species, a significant difference in the gonad indexes is present between the female and the male. But in other echinoderm gonad indexes, no apparent sexual difference has been observed, although Tyler (1977) found significant difference in the maturation index of the brittle-star *Ophiura texturata* Lamarck. He suggested that this difference was due to the fluctuation of sampling. However, in the present species, the difference may depend not on the fluctuation of sampling but on the difference in gametogenesis of the female and the male.

The ovary of most echinoderms contains more than one oocyte generation, although several species produce only single generation of oocytes; e.g., the asteroid *Asterias rubens* Linné (Schlieper, 1957) and *Patiriella regularis* Verrill (Crump, 1971), and the crinoid *Comanthus japonica* Müller (Holland *et al.*, 1975). Up to now, the fact that oocytes are produced throughout the year has been poorly known in echinoderms with the exception of the echinoid *Stylocidaris affinis* Philippi, which has a similar size-frequency polygon to that in the present species (Holland, 1967). In *S. affinis*, oogonia are giving rise to primary oocytes throughout the annual reproductive cycle and, when the small oocytes grow to a diameter of 25–30 μm , they are destroyed if large oocytes are present in the same ovary. On the other hand, breaking down of oocytes occurs only near the breeding season in the present species.

Up to the present time, two reports on oocyte growth rate in echinoderms exist; for the echinoid *Strongylocentrotus purpuratus* Stimpson by Gonor, 1973, and for the crinoid *Comanthus japonica* Müller by Holland *et al.*, 1975. In *S. purpuratus*, the instantaneous relative growth rate is highest for medium-sized oocytes and not for small ones. In *C. japonica*, the oocytes grow rapidly at first and more slowly later on. The pattern of oocyte growth in the present species is contrary to that of *S. purpuratus*; here the growth rate is highest in small oocytes. In comparison with *C. japonica*, the following point differs: Oocytes in the present species show clearly two successive stages in their growth pattern, the one called the fast stage and the other called the slow stage, while the oocyte growth rate in *C. japonica* changes gradually. Such a pattern of oocyte growth as for the present species is unknown in other echinoderms.

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This paper is dedicated to the late Dr. Jean Clark Dan.

Summary

The annual reproductive cycle and the oocyte growth pattern of the brittle-star *Amphipholis kochii* have been described. The annual reproductive cycle was studied by the gonad index method and by histological observations. *A. kochii* shows a well defined annual reproductive cycle. The spawning period is in June and July and takes place once a year. The male and female gonad indexes differ from each other significantly from March through May. The growth pattern of oocytes was studied by the frequency polygon method and by the instantaneous relative growth rate. Oocytes are produced in all seasons, and as maturation proceeds, the number of medium-sized oocytes is reduced. Following the post-spawning resting period of about one month, the growth pattern of the largest oocytes clearly shows two successive stages, the fast and the slow growth stages.

REFERENCES

- Booolootian, R.A. 1966. Reproductive physiology, 561-613 pp. In: R.A. Booolootian, Ed., Physiology of Echinodermata. Interscientific Publishers, New York.
- Brody, S. 1945. Bioenergetics and growth. 1023 pp. Reinholdt Publishing Co., New York.
- Crump, G.G. 1971. Annual reproductive cycles in three geographically separated populations of *Patiriella regularis* (Verrill), a common New Zealand asteroid. J. exp. mar. Biol. Ecol., 7: 137-162.
- Fell, H.B. 1946. The embryology of the viviparous ophiuroid *Amphipholis squamata* Delle Chiajei. Trans. Roy. Soc. New Zealand, 75: 419-464.
- Fenaux, L. 1970. Maturation of the gonads and seasonal cycle of the planktonic larvae of the ophiuroid *Amphiura chiajei* Forbes. Biol. Bull., 138: 262-271.
- 1972. Evolution saisonnière des gonades chez l'Ophiure *Ophioderma longicauda* (Retzius), Ophiuroidea. Int. Rev. ges. Hydrobiol., 57: 257-262.
- Fuji, A. 1960. Studies on the biology of the sea urchin. I. Superficial and histological gonadal changes in gametogenic process of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius*. Bull. Fac. Fish. Hokkaido Univ., 11: 1-14.
- Gonor, J.J. 1973. Reproductive cycles in Oregon populations of the echinoid, *Strongylocentrotus purpuratus* (Stimpson). II. Seasonal changes in oocyte growth and in abundance of gametogenic stages in the ovary. J. exp. mar. Biol. Ecol., 12: 65-78.
- Holland, N.D. 1967. Gametogenesis during the annual reproductive cycle in a cidaroid sea urchin (*Styllocidaris affinis*). Biol. Bull., 133: 578-590.
- J.D. Grimmer and H. Kubota 1975. Gonadal development during the annual reproductive cycle of *Comanthus japonica* (Echinodermata: Crinoidea). Biol. Bull., 148: 219-242.
- Olsen, H. 1942. The development of the brittle-star *Ophiopholis aculeata* (O. Fr. Müller), with a short report on the outer hyaline layer. Bergens Mus. Arbok. Naturvid., 6: 1-107.
- Patent, D.H. 1969. The reproductive cycle of *Gorgonocephalus caryi* (Echinodermata; Ophiuroidea). Biol. Bull., 136: 241-252.
- 1976. Gonadal histology of the basket star, *Gorgonocephalus eucnemis*. Thalassia Jugoslavica, 12: 269-276.
- Pearse, J.S. 1965. Reproductive periodicities in several contrasting populations of *Odontaster validus*

- Koehler, a common antarctic asteroid. *Biology of Antarctic seas. II. Antarctic Res. Ser.*, 5: 39-85.
- Pearse, J.S. 1970. Reproductive periodicities of Indo-Pacific invertebrates in the Gulf of Suez. III. The echinoid *Diadema setosum* (Leske). *Bull. mar. Sci.*, 20: 697-720.
- Schlieper, C. 1957. Comparative study of *Asterias rubens* and *Mytilus edulis* from the North Sea (30 per 1,000 S) and the Western Baltic Sea (15 per 1,000 S). *Ann. Biol.*, 33: 117-127.
- Smith, J.E. 1940. The reproductive system and associated organs of the brittle-star *Ophiothrix fragilis*. *Quart. J. micro. Sci.*, 82: 267-309.
- Tyler, P.A. 1977. Seasonal variation and ecology of gametogenesis in the genus *Ophiura* (Ophiuroidea: Echinodermata) from the Bristol Channel. *J. exp. mar. Biol. Ecol.*, 30: 185-197.
- Yoshida, M. 1952. Some observations on the maturation of the sea urchin, *Diadema setosum*. *Annot. Zool. Jap.*, 25: 265-271.