REPRODUCTIVE CYCLE IN A ZOANTHID *PALYTHOA TUBERCULOSA* ESPER[1)]

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With 4 Text-figures

Introduction

Very little is known on reproduction of the genus *Palythoa* or even of the entire group of the Order Zoanthidea. Some accounts were made along with the systematic descriptions of the genus by such workers as HADDON and SHACKLETON (1891), HADDON and DUERDEN (1896), DUERDEN (1898), and PAX and MÜLLER (1956, 1957). According to these studies the genus *Palythoa* including the former genus *Gemmaria* have both dioecious and hermaphroditic forms.

Recently in the course of a toxicity study of *Palythoa tuberculosa* ESPER in which the toxicity was found to be closely connected with the presence of mature ova, KIMURA *et al.* (1972) found that the species have both dioecious and hermaphroditic polyps and suggested the species to be a protogynous hermaphrodite. However, since their study was made on the samples collected at random, what was revealed thereby are applicable only to a population of polyps and a limitted information was obtained as to the changes which might take place within a given colony and the differences or similarities which might exist among different colonies. The latter information can be best obtained from a study in which sampling is made successively from a certain number of tagged colonies.

This paper deals with the results of such a study, and it is here reported that not all of the colonies exhibit protogynous hermaphroditism, and that among the hermaphroditic colonies there are two types, one with both functional female and hermaphroditic polyps and the other with non-functional female polyps but without hermaphroditic polyps.

Materials and Methods

Thirteen colonies of *P. tuberculosa* were selected from those growing in the tide pools behind the margin of a reef near Naha Air Port, Okinawa. It is the same site

1) Contributions from the Sesoko Marine Science Laboratory, No. 2.

where some of the materials for the previous study (KIMURA *et al.*, 1972) were collected. These colonies ranged in size from 15 to 20 cm across. Each colony was marked with a piece of plastic plate bearing a letter from alphabet (A to M). From each colony a portion containing from 10 to 30 polyps was removed by cutting with a knife once a month from March, 1971 to April, 1972. The samples were fixed and preserved in 10% formalin before they were analyzed. On some collecting dates, sampling from some of the colonies was prevented by rough weather.

The identification of gonads was made by examining the mesenteries of the preserved materials under a stereoscopic microscope. A polyp was designated as either female or male when it contained either ova or testes, respectively, within the mesenteries. It was called a hermaphrodite when both ova and testes were contained in a single polyp, and a sterile when neither ova nor testes were contained. In some instances, the gonads were too small to be identified under a stereoscopic microscope. In such instances the histological sections were prepared from the isolated mesenteries which were fixed in 10% formalin, dehydrated via a graded series of alcohol, cleared in xylene, and embedded in paraffin. The sections were stained with hematoxylin and eosin.

The size of gonads was estimated by micrometers. A mesentery was picked up at random from each polyp, spread on a microscope slide glass, covered gently with a cover glass, and the longer axis of one representative gonad was determined. Ten to thirty measurements were made for a sample colony, and the size of gonads of each colony was estimated in terms of the mean of these values and their standard deviations from the mean.

In order to determine more precisely the sequence of changes in the gonads, samples were taken at shorter intervals from four colonies (E, G, H, and J). The sampling was made on the following dates: June 25, July 2, 10, 28, August 6, 12, and 20, 1972. Examinations and measurements on gonads were made as described above.

Results

Seasonal Change in the Appearance of Sexually Different Polyps

The relative abundance of polyps with different sex of each colony and their seasonal changes are shown in Figs. 1 and 3. The female, male, hermaphroditic, and sterile polyps constitute 21, 32, 3, and 44%, respectively, of the total number of polyps examined (7,945) during the entire study period, from March, 1971 to April, 1972. The corresponding figures for the samples reported by KIMURA *et al.* (Fig. 8, 1972) are 8, 36, 4, and 52 (5,668). The differences between the two sets of results may be due to the difference in the sampling methods. The data in Figs. 1 and 3 clearly indicate that a colony of *P. tuberculosa* belongs to either one of the following three types: Type 1, those colonies which are composed of all the four forms of polyps, that is, the female, male, hermaphroditic, and sterile polyps. Type 2, those lacking hermaphroditic

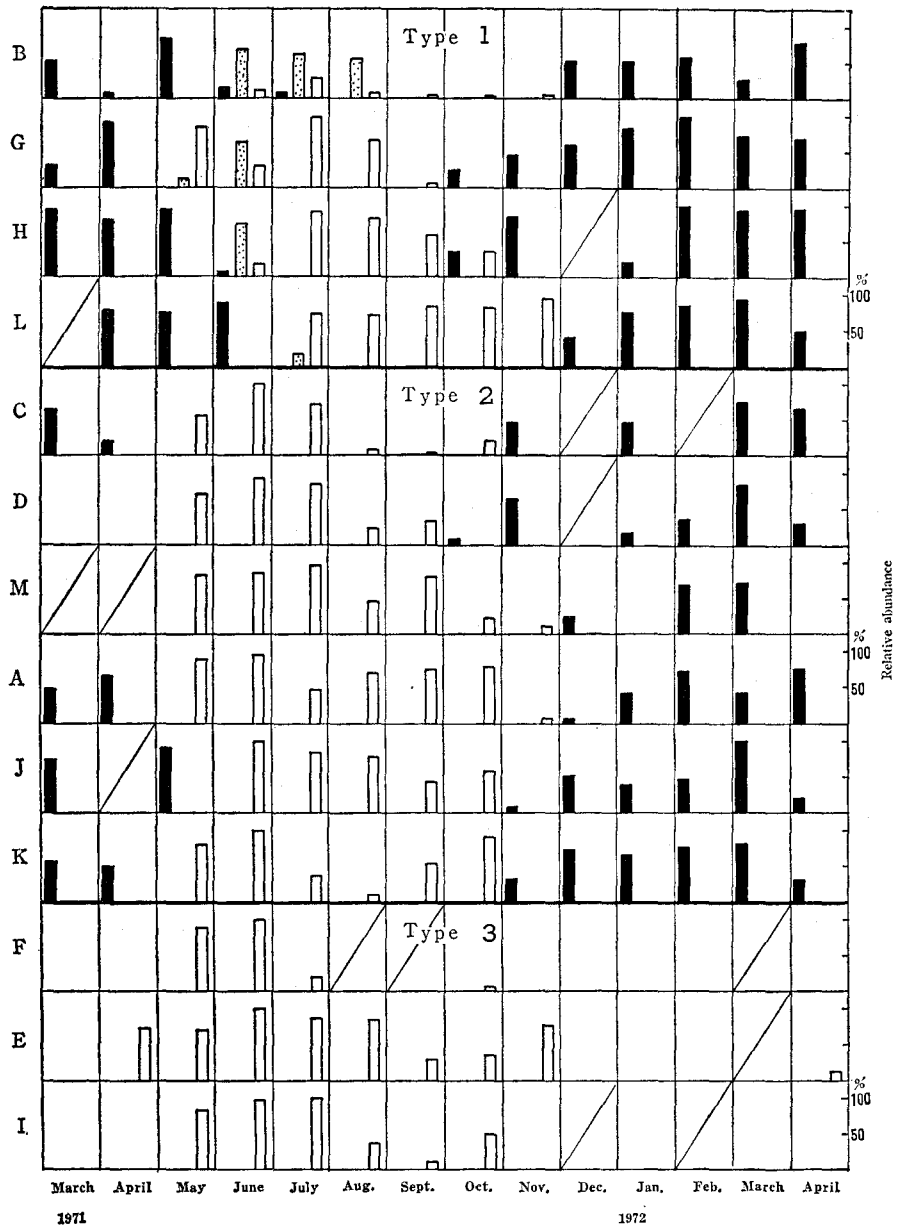

Fig. 1. Seasonal change in the relative abundance of sexually different polyps in thirteen colonies of *P. tuberculosa*. The relative abundance of the female (■), male (□), and hermaphroditic polyps (▩) are expressed as the percentage of the total number of polyps contained in each sample collected every month from March, 1971 to April, 1972. The relative abundance of the sterile polyps can be obtained by subtracting the sum of the other three polyps from 100%.

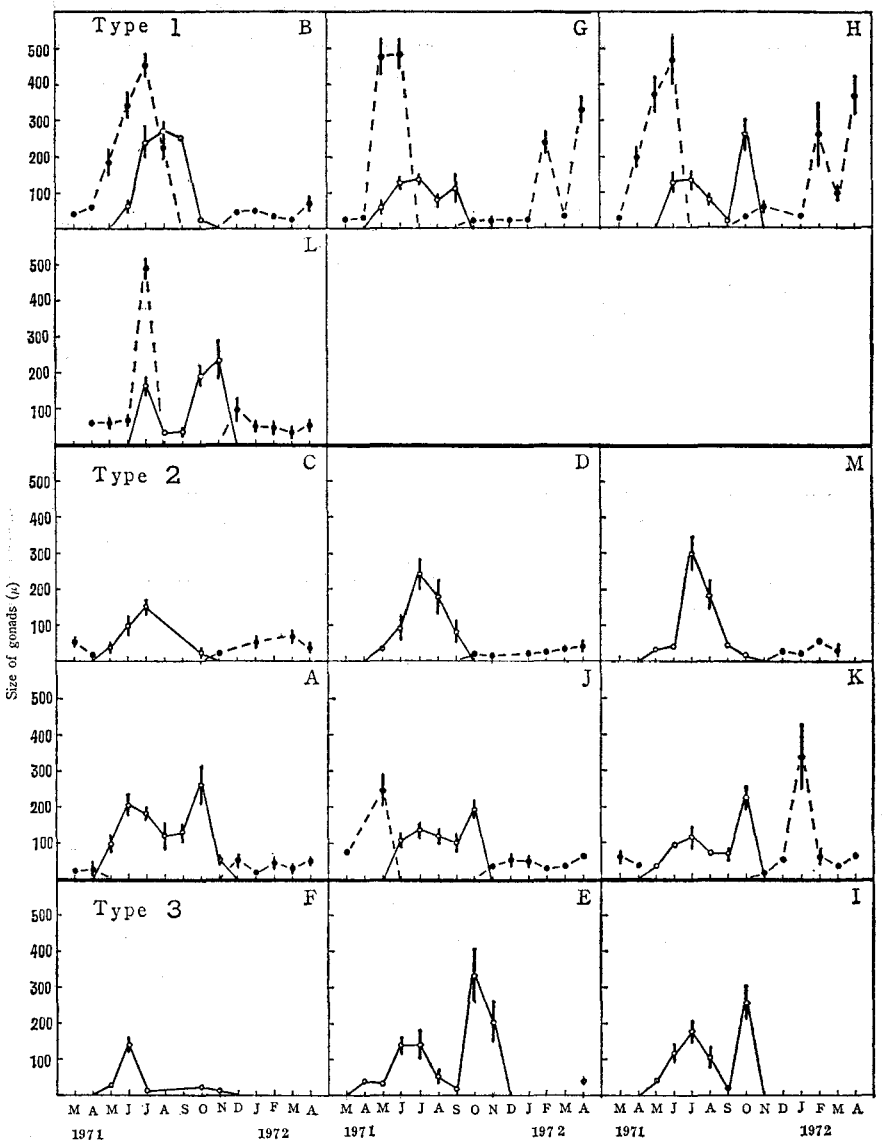

Fig. 2. Seasonal change in the size of gonads in thirteen colonies of *P. tuberculosa*. The size of ova (···●···) and testes (—○—) for each colony (A to M) are shown by a monthly mean and the standard deviation (vertical bars through each point), and expressed in microns.

polyps. Type 3, those composed of only male and sterile polyps.

In the Type 1 colonies (B, G, H, and L), female polyps appear from October to July, male polyps from May to November, and hermaphroditic polyps from May to August. The colonies belonging to Type 2 (C, D, M, A, J, and K) contain female polyps from October to May and male polyps from May to November. The colonies belonging to Type 3 (F, E, and I) contain male polyps from April to November. Although there are some individual variations, the male polyps, in general, occur at a definite season, that is, from May to November regardless of the type of colony. In all of the six colonies belonging to Type 2, male and female polyps do not occur together within a given colony at any given time. This is not the case with the Type 1 colonies in which male and female polyps do co-exist within a single colony at certain months, June, July, and October. During these months except October, hermaphroditic polyps also occur together with male and female polyps.

Seasonal Change in the Size of Gonads

Seasonal change in the size of ova and testes is shown in Figs. 2 and 4. It was previously reported that mature ova range in size between 300 and 500 μ in longer axes, and mature testes between 170 and 300 μ (KIMURA *et al.*, 1972). When these values are applied to the data given in Fig. 2, the period when the polyps reach maturity can be predicted. In the colonies belonging to Type 1, the female polyps become mature between May and July. This period may be extended to April as is the case with Colonies G and H in April, 1972. None of the colonies belonging to Type 2 contain mature female polyps at any period of year except Colony K which contained mature female polyps in January, 1972. Colony J may also have contained some mature female polyps in May, 1971.

Regarding the period of maturation of the male polyps, the colonies can be divided into two groups, the first having one period and the other having two periods of maturation. Colonies B, G (both Type 1), C, D, M (all three Type 2), and F (Type 3) have one period of maturation from June to September. Colonies L (Type 1), A, J (both Type 2), E, and I (both Type 3), and possibly H (Type 1) and K (Type 2) have two periods, one in June and July and the other in October and November. Comparisons of Fig. 2 with Fig. 1 reveal that there is some correlation between the size of testes and the relative abundance of male polyps. Those colonies in which testes mature in October or in October and November have relatively large number of male polyps during these months. Although the data presented in Fig. 4 are not sufficient enough, there is some indication that the size of testes changes periodically with the period ranging from 33 to 48 days. It may be possible that the polyps of the colonies belonging to the first group remain mature throughout the period extending from June to November and what appears to have two separate periods of maturation is only a matter of sampling. This possibility is shown for Colony E in which the polyps appeared immature in July and August, 1971 (Fig. 2) but mature in the same

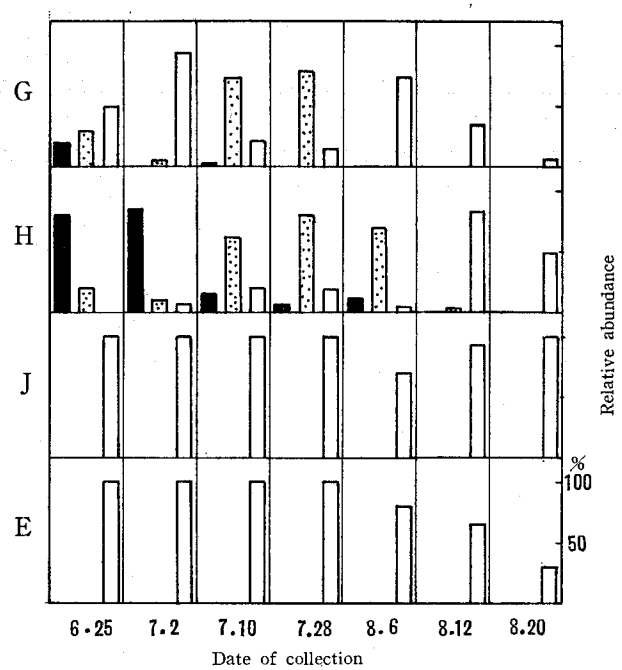

Fig. 3. A short period change in the relative abundance of sexually different polyps in 4 colonies (G, H, J, and E) of *P. tuberculosa*. The samples were collected from June 25 to Aug. 20, 1972. (See Fig. 1 for explanation).

months in 1972 (Fig. 4), except on July 2 and August 12. The same is also observed for Colony J.

Discussion

Sexual Change in the Polyps

There seems to be enough evidence which shows that the polyps of the colonies belonging to Type 2 would change their sex from female to male sometime during April and May and then from male to female sometime during October and November. Similarly the same change may also take place in the polyps of the Type 1 colonies, but here is an intervening period of hermaphroditism between the female and male phases. From the data presented in Figs. 1 to 4, the following scheme is proposed as a possible sequence of changes that might take place in the polyps of the Type 1 colonies. The ova appear in the mesenteries sometime during October to December, and become mature sometime during May and June of the following year. At about the same time as the ova become mature testes develop in the same mesenteries making the polyps hermaphroditic. Within a short period the ova are released possibly by the end of August, while the testes remain until November. Since these changes

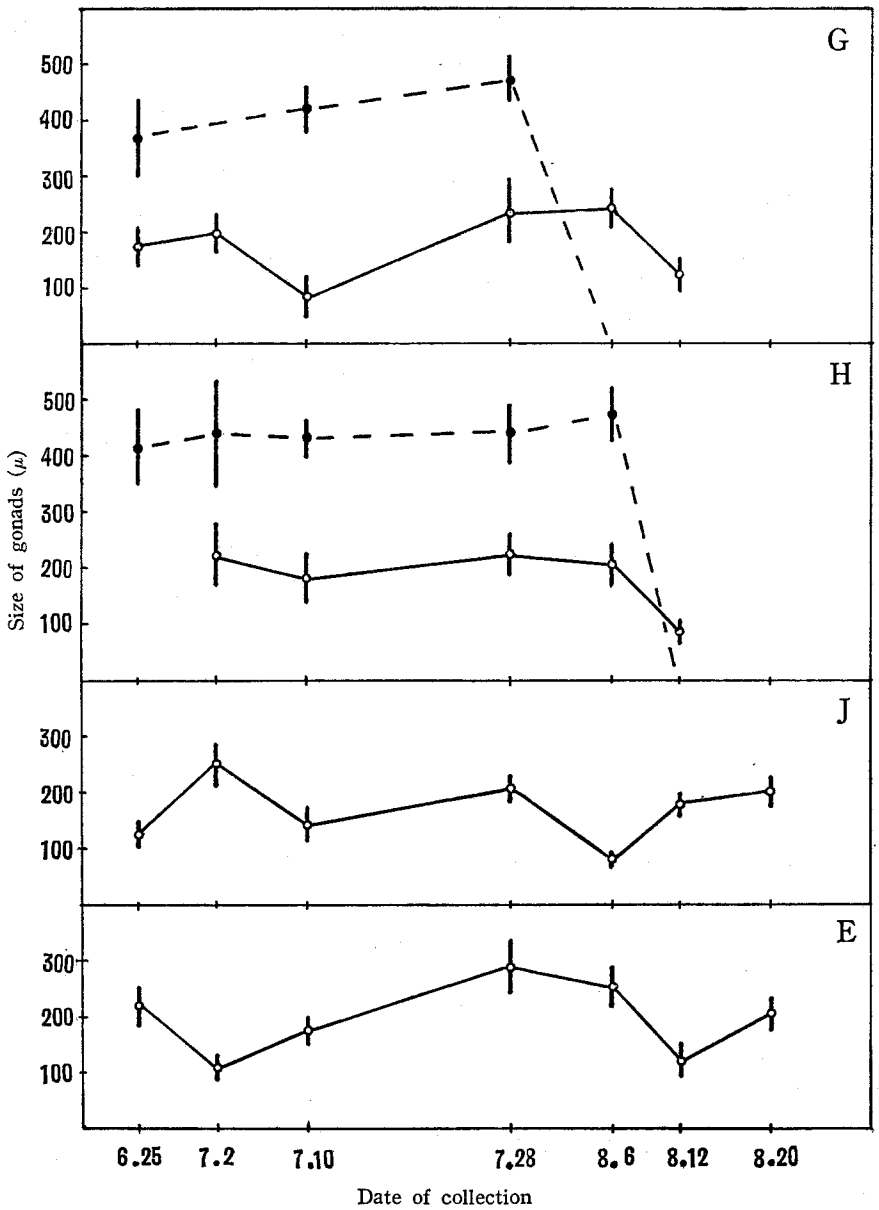

Fig. 4. A short period change in the size of gonads in 4 colonies (G, H, J, and E) of *P. tuberculosa*. (See Figs. 2 and 3 for explanation).

may take place asynchronously among different polyps, and testes become mature starting sometime in June, fertilization of ova is secured even though there is a certain time lag in maturation of gonads of both sexes. The cycle starts again with the appearance of ova in the period from October to December. Not quite ideal but the best available examples of the proposed scheme are seen in Colony B in Fig. 1 and Colony H in Fig. 3.

Nature of the Sexually Different Types of Colonies

Besides the three types of colonies described above, KIMURA *et al.* (1972) reported on two other types, the female colonies which have female polyps along with the sterile and the sterile colonies which have only sterile polyps. A question may be asked why these types are not represented by the colonies studied in the present study. There are two possible answers to this question, one being that these two types of colonies may have simply not been sampled, and the other that there are no such colonies but the colonies which were regarded as the female in the previous report are merely the female phases of those which belong to either Type 1 or Type 2, and those which were regarded as the sterile, the sterile phases of the Type 3 colonies.

Examination of Fig. 1 reveals that in any month except July to September there is always a possibility of picking up one or more colonies which might be regarded as the female colonies if they are not sampled successively but at random, as was the case with the previous study. In addition the percentage of the female polyps of the previous study is much less than that of the present (8 vs. 21%), though this is against what could be expected from the fact that it is the previous study in which the presence of the female colonies was proposed. Although these alone may not be enough, it may be permissible to make a tentative conclusion that this species has no female colony. By similar arguments the presence of the colonies which are exclusively made up of sterile polyps throughout a year should be doubted. Relatively high percentage of sterile polyps of the present and previous studies, 44 and 52% respectively, could be explained by the fact that every colony has some or many sterile polyps along with the other forms throughout a year.

If these two types of colonies are not accepted, then we can recognize the following three types of colonies within this species:

Type 1. *Functional protogynous hermaphrodites* in which the individual polyps change their reproductive phases from functional female to functional hermaphrodite, and then to functional male.

Type 2. *Consecutive protogynous hermaphrodites* in which the polyps change from non-functional female to functional male without an intervening period of hermaphroditism.

Type 3. *Male colonies* which have only male polyps along with sterile ones.

This scheme explains an unusually high male to female ratio of 4 to 1 reported in the previous study. Since the population of the Type 1 colonies alone has both sexes

functional, it is natural to regard this to be the basic type of this species. Type 2 and 3 can be viewed as derived from Type 1 by a simple, successive loss of significance exhibited by the female phase.

Summary

From the study on the reproductive cycle exhibited by thirteen colonies of *P. tuberculosa* a previous proposal by KIMURA *et al.* (1972) that this species is a protogynous hermaphrodite was confirmed. It was further elucidated that there are two types of hermaphroditic colonies, one with both functional female and hermaphroditic polyps along with functional males and the other with non-functional female and without hermaphroditic polyps. In addition the third type of colony, the male was also recognized. Among the three types of colonies, the first was regarded as the basic while the other two as derived from the first by a successive loss of the female phase.

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DISCUSSION

ROSS: Did the speaker find any differences within a colony in the distribution of the different sexual types, say, at the periphery where one would find the more recently added individuals?

YAMAZATO: In some colonies you may have clumps of polyps of the same sex, but in others the distribution of a particular sex is random. However, there is a tendency of increase of sterile polyps towards periphery of the colony.