ZOOLOGICAL SCIENCE **22**: 247–255 (2005)

Reproduction of *Zoanthus sansibaricus* in the Infra-Littoral Zone at Taisho Lava Field, Sakurajima, Kagoshima, Japan

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ABSTRACT—In order to obtain data on the reproductive pattern of the clonal cnidarian *Zoanthus sansibaricus*, polyps were sampled by scuba gear at Taisho Lava Field, Sakurajima, for 24 months between April 2000 and March 2002 (polyps collected weekly for breeding season). According to cross-sections, *Zoanthus* polyps were divided into three sexual types; male, female and asexual, and were found in the same colony. At Sakurajima, *Zoanthus sansibaricus* spawned in the middle of July, releasing oocytes and sperm. These spawning events occur synchronously with moon phase. In gametogenesis of *Zoanthus sansibaricus*, oocytes became recognizable in February and grew rapidly from the end of June onward. Spermatocytes became recognizable in June and matured rapidly in the middle of July. After spawning events, oocytes still remaining in the endoderm were absorbed into *Zoanthus* tissue quickly.

Key words: *Zoanthus*, Sakurajima, reproduction, gametogenesis, oocyte

INTRODUCTION

The encrusting anemone genus *Zoanthus* (suborder Brachycnemina, family Zoanthidae) is commonly found in warm temperate and tropical waters along rocky and coral reef shorelines. In Japan, *Zoanthus* is found from mid-Honshu south at depths below the low tide line. *Zoanthus* is a clonal cnidarian, and thus all polyps in one interconnected colony can be considered clonemates.

Currently, little information of the sexual reproduction of suborder Brachycnemina is known. Kimura *et al.* (1972) investigated the reproductive cycle and toxicity of the zoanthid *Palythoa tuberculosa* in Okinawa. Sexual reproduction was found to occur during the 4 months' time between June and September, with female polyps having eggs between March and September, while male polyps were found to contain sperm between June and November. Cooke (1976) investigated *Zoanthus pacificus* in Hawaii, and found that sexual reproduction occurred in May to October, and in *Palythoa vestitus* in September to October. *Zoanthus* spp. and *Palythoa caribaeorum* in Jamaica have been shown to breed between June and July (Karlson, 1981). In Belize, Fadlallah *et al.* (1984) found that *Zoanthus sociatus* and *Palythoa caribaeorum* showed seasonal breeding from December to June, while *Zoanthus solonderi* did not exhibit any seasonal breeding pattern. In a review of the reproductive biology of Zoanthidea, *Parazoanthus parasiticus* started oocyte formation in March and April, and spawned at night during full moons in September (Ryland, 1997). On the Great Barrier Reef (GBR), *Protopalythoa* sp. produced both egg and sperm in the same polyp, and released these simultaneously (Babcock and Ryland, 1990). It is believed the suborder Brachycnemina is composed of such "spawners".

In Kagoshima Prefecture, Japan, many colonies of *Zoanthus* spp. exist in the infra-littoral zone at the Taisho Lava Field on Sakurajima (Ono *et al*., 2002, 2003), but information and research on their reproductive biology remains sparse. The purpose of this paper is to investigate the patterns and timing of gametogenesis and maturation of *Zoanthus sansibaricus* at Sakurajima (Fig. 1).

MATERIALS AND METHODS

For 24 months between April 2000 and March 2002, samples of *Zoanthus sansibaricus* were collected from the infra-littoral zone of a single field site at the Taisho Lava Field coastline, Sakurajima, Kagoshima Prefecture, Japan (31°35'N, 130°35'E) (Fig. 2). Samples

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Fig. 1. *Zoanthus sansibaricus* colony at Taisho Lava Field, Sakurajima, Kagoshima, Japan. Depth=3m. Scale bar=1cm

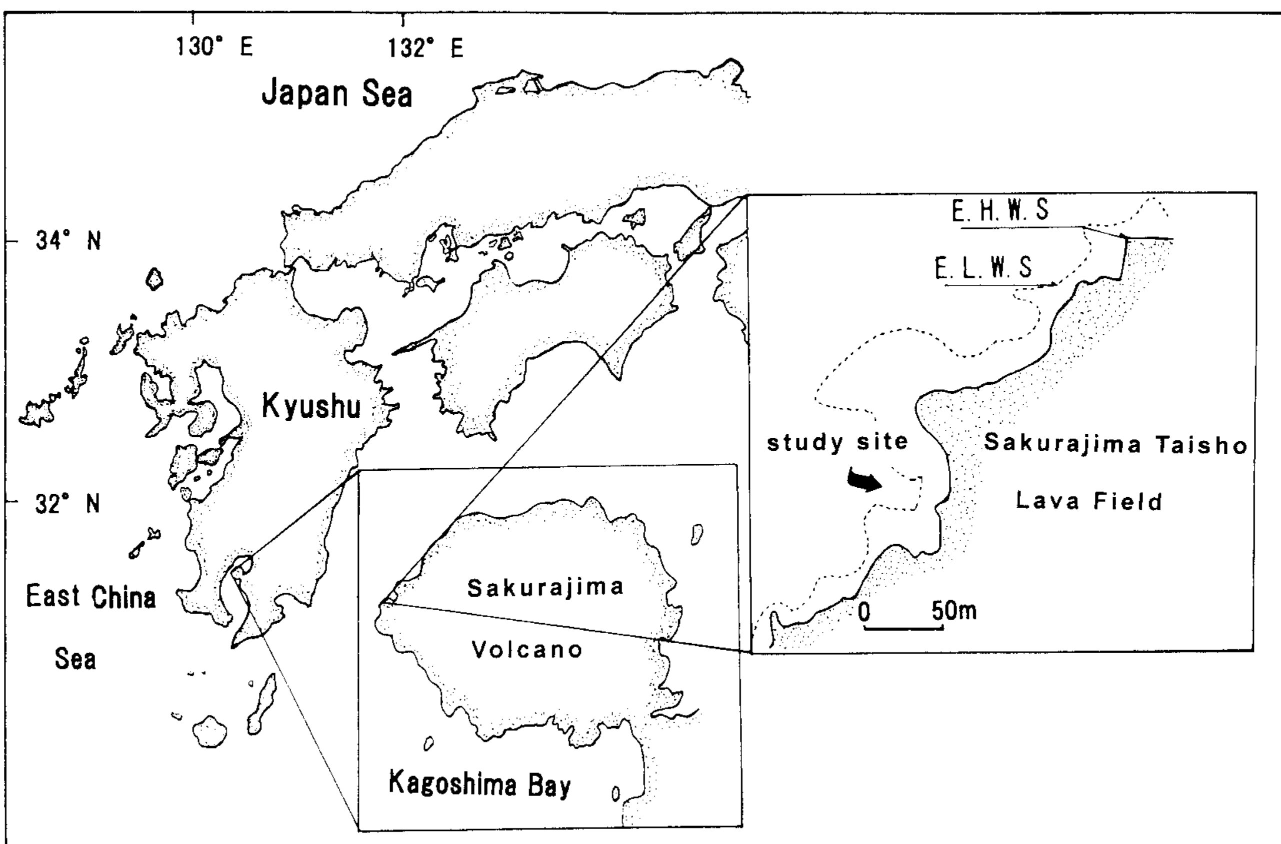

Fig. 2. Map of the study site, Taisho Lava Field, Sakurajima, Kagoshima, Japan. E.L.W.S=Extreme Low Water Spring (dotted line), E.H.W.S=Extreme High Water Spring (solid line).

were always taken from the same colony, consisting of approximately 300–400 polyps, situated on top of a dead *Porites* sp. colony at a depth of 3 m. Samples were collected monthly, except for the period between May and July, when samples were collected weekly. For each sampling, 5 samples of 2–3 polyps each were collected, roughly corresponding to the center of the colony, and the 3, 6, 9, and 12 o'clock positions (Fig. 3). During sampling, water temperature at the surface and a depth of 3 m was noted. After collection, samples were put in Boum's fluid and embedded in paraffin. Samples were then cross-sectioned into 8 μm thick sections, and stained with Azan and observed under a microscope. Cross sections were made continuously along the length of the sampled polyps, and oocyte diameter (i.e. equatorial plane diameter) was determined as the largest measurement observed for each individual oocyte.

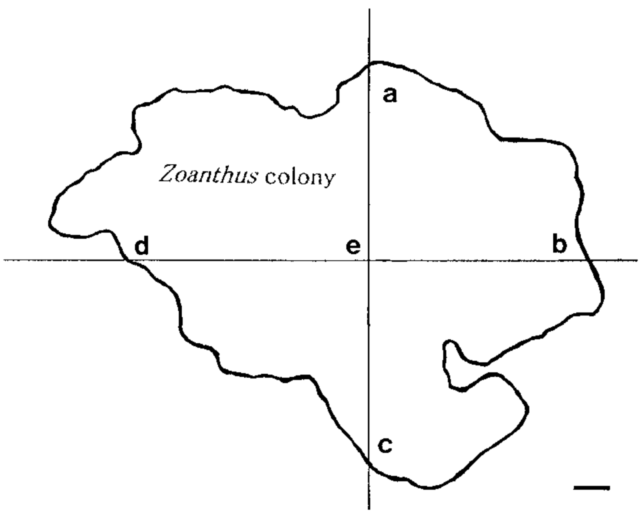

Fig. 3. Five sampling points of polyps on *Zoanthus sansibaricus* colony, corresponding to 12 (location a), 3 (b), 6 (c), and 9 o'clock (d), and the middle of the colony (e). Scale bar=1cm

RESULTS

Sexual types and spawning patterns

The sampled *Zoanthus sansibaricus* was found to be hermaphroditic, but individual polyps were classified as males bearing sperm, females with only oocytes, or polyps without gametes (unclassifiable). Thus while colonies can be considered to be hermaphroditic, individual polyps are not hermaphroditic. Our *Zoanthus sansibaricus* samples were not internal brooders, but instead spawners, releasing their unfertilized eggs and sperm into the external environment. Our results show that within individual polyps gamete numbers dropped rapidly over the span of just one week, approximately in the middle of July (Fig. 6). These results taken at the colony level show *Zoanthus sansibaricus* at Sakurajima is a hermaphroditic spawner.

For the period between May 26 and August 11 for both 2000 and 2001, polyps' sex classifications were determinable (74 polyps, 22 male + 52 female) female more often than not (26 polyps indeterminable) (Table 1). Female polyps were observed more often than male polyps. No clear relationships regarding sampling location of colony and polyp sex were seen for 2000~2001 (Table 1).

Table 1. Changes of *Zoanthus sansibaricus* oocyte numbers and of average diameter of oocytes from April 2000 to March 2002

	Sex of *Zoanthus* polyps							
	a	b	c	d	e	AOD±SD(μm)	N	SWT(°C)
2000.4.24	I	I	I	I	I			17.5
5.03	I	I	I	I	I			18.0
5.15	I	I	I	I	I			21.2
5.20	I	I	I	I	I			21.0
5.28	I	I	I	I	I			22.2
6.04	F	F	I	I	F	62.8±13.9	65	21.2
6.11	I	F	I	F	I	79.5±19.1	42	22.8
6.18	I	F	M	M	M			23.6
6.25	F	F	F	I	F	87.8±14.7	66	25.8
7.02	F	F	M	M	M	116.3±16.5	20	27.3
7.09	M	F	I	M	M	155.4±34.2	23	26.1
7.16	M	M	I	M	M			28.5
7.22	M	M	M	I	M			28.2
7.31	I	I	I	I	M			28.2
8.29	I	I	I	I	I			30.0
9.28	I	I	I	I	I			27.0
10.21	I	I	I	I	I			24.8
11.25	I	I	I	I	I			21.3
12.26	I	I	I	I	I			18.9
2001.1.21	I	I	I	I	I			17.0
2.24	I	I	I	I	I			16.8
3.02	I	I	I	I	I			16.0
4.22	I	F	I	I	I	39.9±11.5	17	16.2
5.26	I	F	F	I	F	70.1±14.3	57	20.1
6.09	F	F	F	F	F	90.3±15.2	103	21.0
6.17	M	F	F	F	F	115.5±21.0	66	23.0
6.24	F	F	F	F	F	118.2±18.3	101	23.0
7.05	M	F	F	F	F	179.4±32.0	107	26.2
7.08	M	F	F	F	F	154.0±29.9	67	26.0
7.14	F	F	M	F	F	205.0±23.0	58	26.0
7.21	F	I	I	F	F			27.1
7.31	I	F	I	F	F	146.7±57.5	3	29.1
8.11	I	F	F	F	F	220.1±21.4	28	30.1
8.30	I	I	I	I	I			27.5
9.30	I	I	I	I	I			25.8
10.27	I	I	I	F	I		1	24.0
11.24	I	I	I	I	I			21.3
12.24	I	I	I	I	I			18.3
2002.1.20	I	I	I	I	I			16.9
2.17	I	F	F	I	F	35.9±9.9	23	15.8
3.21	I	F	I	I	I	56.0±20.5	23	16.8

I=indeterminable, F=female, M=male, AOD=average oocyte diamater, N=number, SWT=seawater temperature

Gametogenesis

Fig. 4 shows a cross-section of *Zoanthus sansibaricus* from March to September as eggs are produced. One single

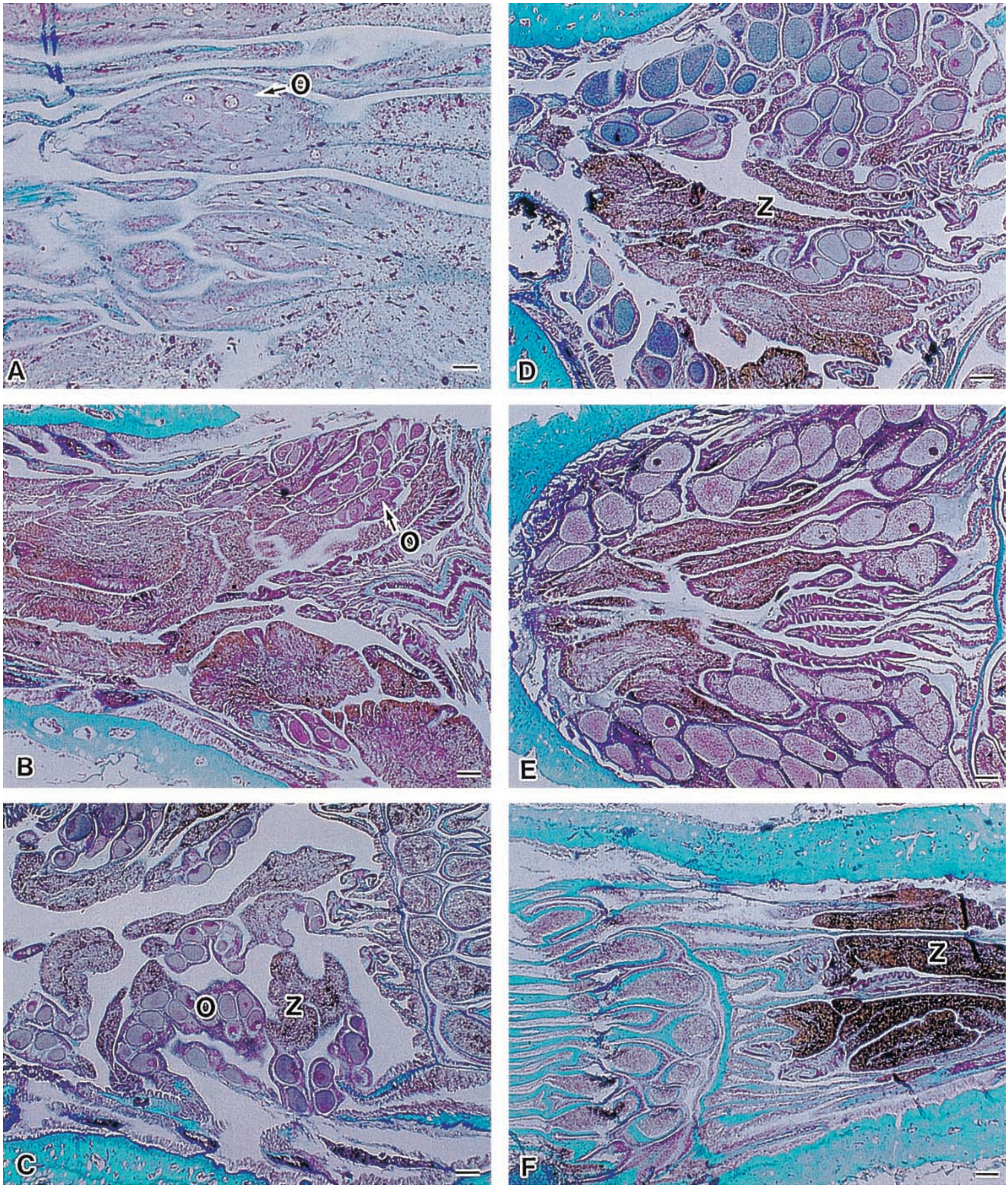

Fig. 4. Process of gametogenesis in *Zoanthus sansibaricus* polyps. All scale bars=100 μm O: oocyte Z: zooxanthellae. A) April 22, 2001, B) June 9, 2001, C) June 24, 2001, D) July 5, 2001, E) July 15, 2001, F) September 30, 2001.

polyp produces up to 100 eggs that are globular in shape, and are situated along the mesentery filaments. Generally egg production is completed by early July. Eggs at this time are approximately 200–300 μm in diameter, creamy white in color, and contain a germinal vesicle.

In 2000, eggs were first seen on June 4, and had a Average Oocyte Diameter (AOD) of 62.8±13.9 μm (range 30~100 μm), when water temperature at 3 m depth was 21.2°C. In 2001 eggs were first seen on April 22, with a AOD of 35.9±11.5 μm (range 25–55 μm), while ocean temperature was 16.2°C. In 2002, eggs were first seen very early, on February 17. At this time AOD was 35.9±9.9mm, and ocean temperature was 15.8°C.

In 2000, eggs reached a maximum AOD of 155.0±34.2 μm on July 9 (ocean temperature=26.1°C). The maximum AOD figure for 2000 should be interpreted with caution, however, as no female polyps were observed after July 9, 2001, and it is likely that oocytes continued to grow after this date. In 2001, a maximum AOD of 205.0±23.0 μm was seen on July 14 (ocean temperature=26.0°C), and in 2002, AOD was largest on August 11 (220.0±21.4 μm). It should be noted that in 2002, the maximum AOD result was from only one polyp that had seven eggs, which we presume to be left over from the spawning event earlier in the same year.

Spermatogenesis was first observed in 2000 on June 18, when ocean temperature was 23.6°C. Spermatids of 2~4 μm in diameter were seen on the testiculus. At this time testis vesicles were approximately 20~40 μm in diameter. Spermatids were easily distinguished by Azan blue staining, appearing dark blue, while eggs were dark purple and zooxanthellae appeared dark brown-green. At the beginning of spermatogenesis, flagella were not seen, but by the begin-

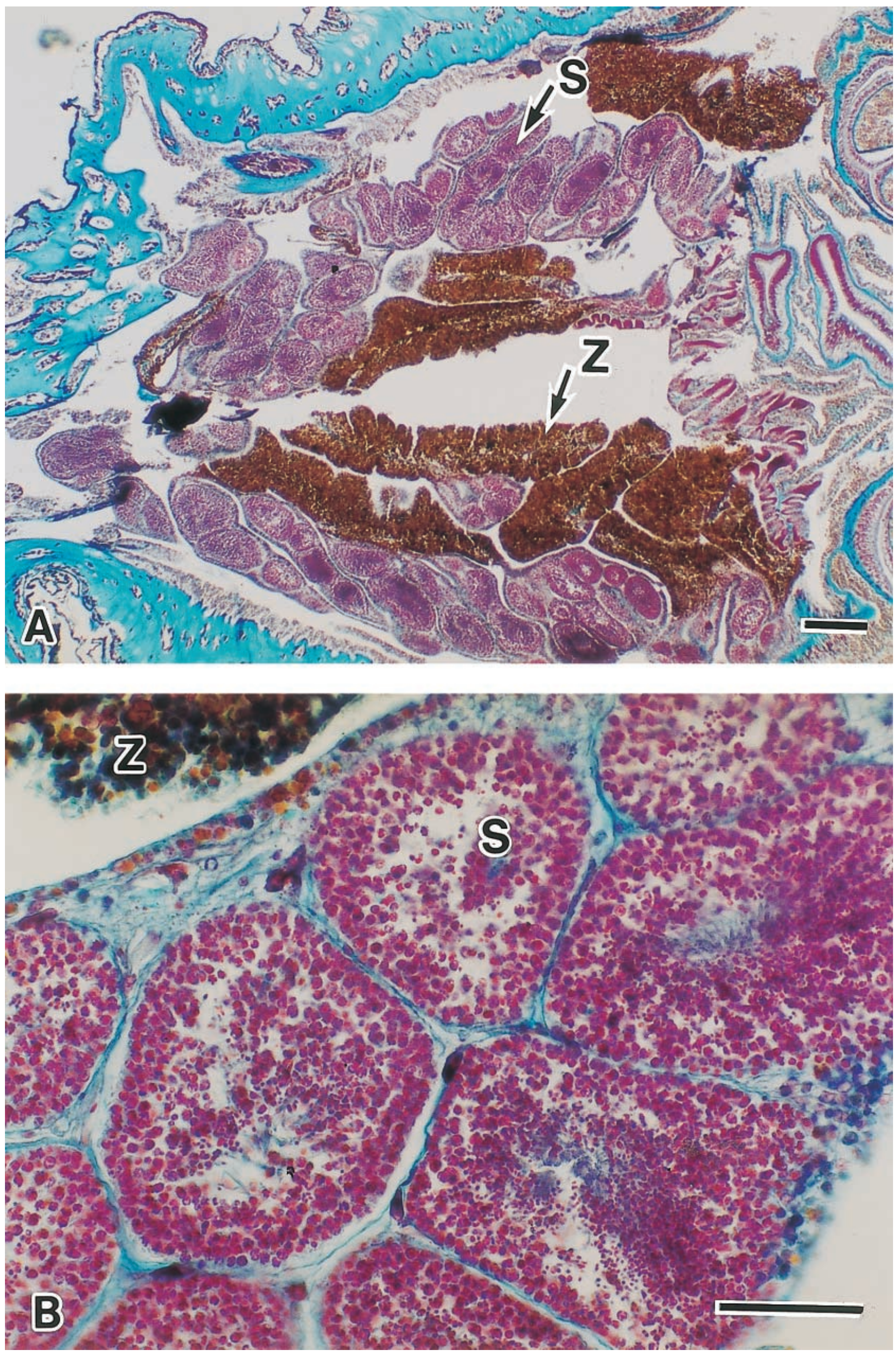

Fig. 5. *Zoanthus sansibaricus* male polyp cross sections with Azan stain. S: Sperm Z: zooxanthellae. A) Scale bar=100 μm, B) Scale bar=100 μm. Both images taken 2001.

ning of July flagella became visible. By the middle of July the testis vesicles had swollen to approximately 200 µm in diameter and flagella (length 30~50 µm) were plainly visible on individual spermatids.

In 2001, spermatids were first recorded on June 17 (temperature=23.0°C), and flagella first seen on July 5 (temperature=26.2°C). Upon removal from the testis vesicles, spermatids swam. Fig. 5 shows a cross-section of a July 5 polyp with spermatid (dark blue) and sperm visible.

Gametogenesis period

Our results show that the rate of gametogenesis in *Zoanthus sansibaricus* increases drastically once ocean temperatures (depth=3 m) reach 25.0°C, usually around the end of June. For example, on June 25, 2000, AOD of eggs was 87.8±14.7 µm, and by July 9, 2000 the AOD had increased to 155.4±34.0 µm. Over the same time period ocean temperature at 3 m depth increased from 25.8°C to 27.3°C. Similarly, in 2001, AOD on June 24 was 118.2±18.3 µm, and on July 5 it had increased to 179.4±32.1 µm. Temperature over the same period again increased, from 23.0°C to 26.2°C (Table 1).

Fig. 6 shows the change in size classes of eggs over time. On July 9, 2000, eggs in the size class of 51~100 µm

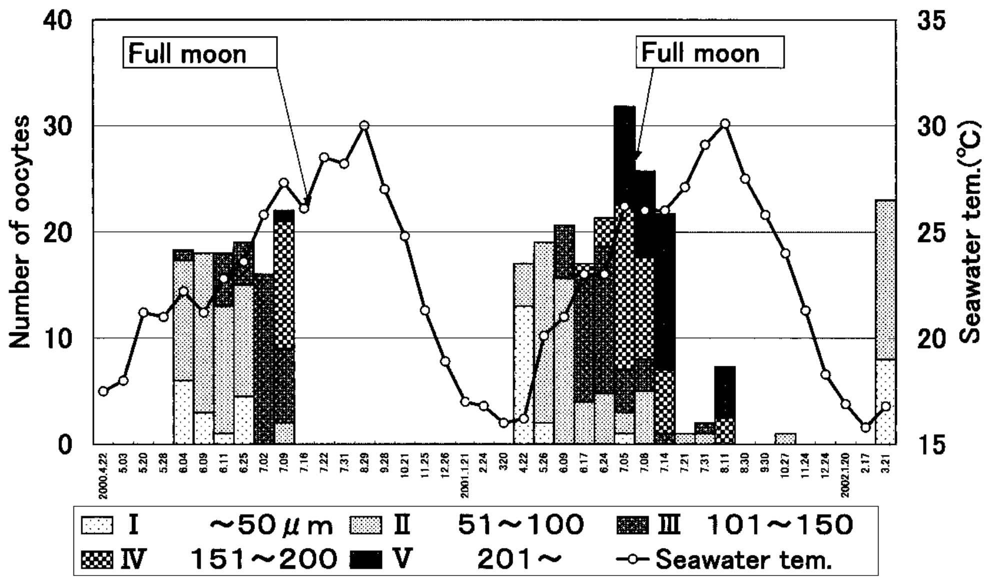

Fig. 6. Temporal changes in number and size class of oocytes in *Zoanthus sansibaricus* polyps. Legend=size class (diameter of oocytes, µm)

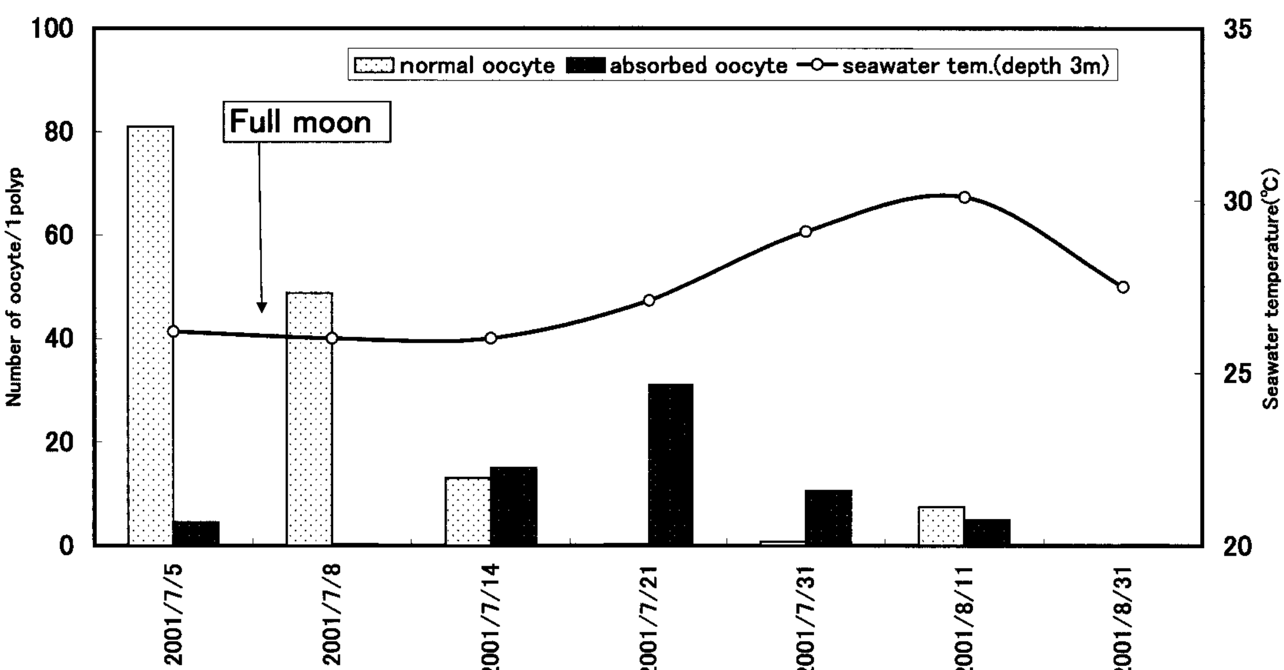

Fig. 7. Temporal changes of number of absorbed oocytes and normal oocytes per *Zoanthus sansibaricus* polyp with seawater temperature.

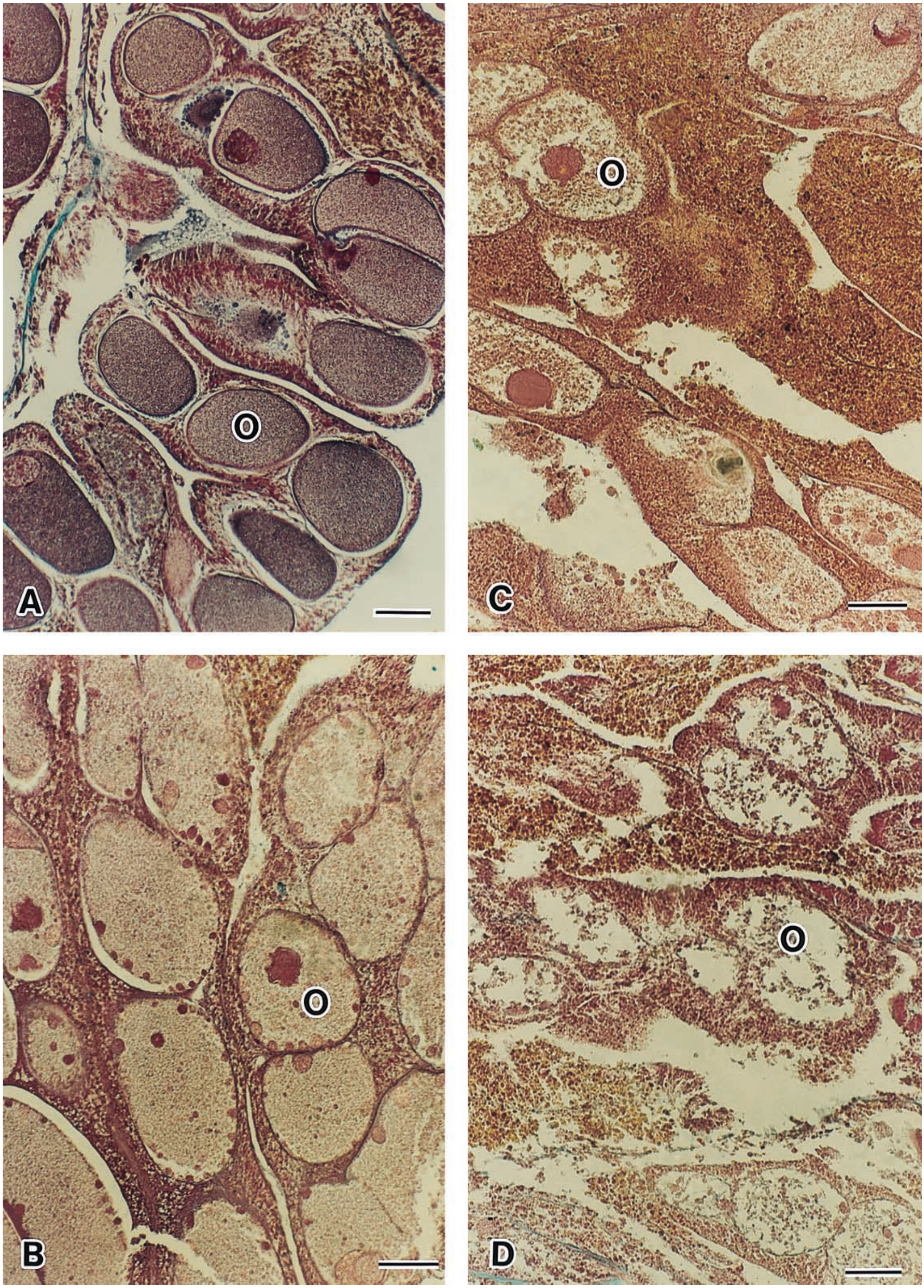

Fig. 8. Process of oocyte re-absorption in *Zoanthus sansibaricus* tissue. All scale bars=100 µm. O: oocyte. A) July 8, 2001 B) July 14, 2001, C) July 21, 2001, D) July 31, 2001.

diameter amounted to 9.1% of total eggs, eggs 101~150 μm 31.8%, 151~200 μm 54.5%, and eggs equal to or above 201 μm 4.5%. It should be noted that after this sampling date no observed polyps contained any eggs at all.

In the same manner, on July 8, 2001 eggs 51~100 μm amounted to 19.5%, eggs 101~150 mm 11.7%, 151~200 μm 37.7%, and eggs equal to or above 201 μm 31.1%. On July 14 the same year, eggs 101~150 μm made up 4.6% of total eggs, 151~200 μm 27.6%, and eggs equal to or above 201 μm jumped to 67.7% of all eggs counted. Polyps sampled after this date showed few or no eggs present.

A full moon occurred on July 16, 2000 and on July 6 in 2001. Based on this information and our observed results, we can speculate that gamete formation in *Zoanthus sansibaricus* at Sakurajima's Taisho Lava Field is completed by the beginning of July, and spawning occurs around the next full moon. It appears that not all eggs and sperm are released during spawning events, and that remaining gametes inside *Zoanthus sansibaricus* are digested and nutrients returned to the polyp (Fig. 7). Reabsorption of oocytes between July 8~July 31, 2001 is shown in Fig. 8.

DISCUSSION

Sexual types

Up until now several different cases of hermaphroditism in polyps in Brachynemina and Zoanthidae have been reported, *Palythoa tuberculosa* in Okinawa (Kimura et al., 1972; Yamazato *et al*., 1973), *Zoanthus pulchellus* in Jamaica (Karlson, 1981), *Zoanthus solanderi* and *Zoanthus sociatus* in Panama (Fadlallah *et al*., 1984). However, whether these species are brooders or spawners has not yet been determined. Babcock and Ryland (1990) alone have reported that *Protopalythoa* sp., which is closely related to *Zoanthus*, are hermaphroditic spawners.

Other, more detailed information exists for scleractinian corals of the Indian and Pacific Oceans. Richmond and Hunter (1990) investigated numerous species, and found that 126 species were hermaphroditic spawners, 6 species were hermaphroditic brooders, 34 species were non-hermaphroditic spawners, and 9 species were non-hermaphroditic brooders. In other words most stony corals, which are closely related to *Zoanthus,* appear to be hermaphroditic spawners. While this report has ascertained that Sakurajima *Zoanthus sansibaricus* is a hermaphroditic spawner, no other information on Japanese *Zoanthus* spp. exists, and this would be an excellent future research project.

Gametogenesis period

Table 2 displays research up until now detailing gametogenesis periods and season of maturity and locality, spawning time in Zoanthiniaria.

Zoanthus sansibaricus at Sakurajima's Taisho Lava Fields produces gametes by July. This compares with periods of May to October for Hawaiian *Zoanthus pacificus* (Cooke, 1976), September to October for Hawaiian *Palythoa vestitus* (Cooke, 1976), June to July for Panamanian *Zoanthus sociatus* and *Z. solenderi* (Fadlallah, 1984), and November to December for *Protopalythoa* sp. of the Great Barrier Reef (GBR) in the Southern Hemisphere (Ryland, 1997).

Our results also showed the lunar periodicity of *Z. sansibaricus* spawning events. In 2000 and 2001 spawning occurred 3 to 5 days after the full moon of early July. Similar examples have been reported in the majority of scleractinian corals (Babcock *et al*., 1997). Further evidence supporting our findings can be seen in Floridian *Protopalythoa* sp., which spawn 3 days after the full moon (Babcock, 1990), *Protopalythoa* sp. of the GBR which spawn 3 to 5 days after a full moon (Babcock, 1990), and *Parazoanthus parasiticus* in Bermuda, which spawn during the full moon (Ryland, 1997).

Sex changes

An interesting paper published by Yamazato *et al.*

Table 2. Data on reproduction in some zoanthid species from previous literature.

Species	Season of maturity	Locality	Spawning time	Reference
Zoanthus pacificus	May-October	Hawaii		Cooke, 1976
Zoanthus **spp**.	June-July	Caribbean		Karlson, 1981
Zoanthus sansibaricus	February-July	Kagoshima, Japan	3-5 nights after full moon	Ono *et al*., present study
Palythoa **spp**.	October-January	Madagascar		Herberts, 1972
Palythoa tuberculosa	November-May (Female)	Okinawa, Japan		Yamazato *et al*., 1973
Palythoa caribaeorum	June-July	Caribbean		Fadlallah *et al*., 1984
Palythoa tuberculosa	May-October (Male)	GBR		Ryland and Muirhead, 1994
Sphenopus marsupialis	December-February	GBR		Burnett *et al*., 1996
Protopalythoa vestitus	September-October	Hawaii		Cooke, 1976
Protopalythoa **sp**.	November-December	GBR	3-5 nights after full moon	Babcok and Ryland, 1990
Protopalythoa **sp**.	July	GBR		Burnett *et al*., 1996
Parazoanthus axinella	July-October	Western Mediterranean		Herberts, 1972
Parazoanthus parasiticus	March-September	Bermuda	full moon	Ryland and Westphalen unpubl

(1973) on Okinawan *Palythoa tuberculosa* showed that among 13 colonies investigated 4 colonies and their polyps were female in spring (March to May), were hermaphroditic in summer (May to September), and male in fall (August to October), reverting to non-sexual polyps in winter. In other words, it appears that at least this species may provide an example of protogynous hermaphroditism.

At Sakurajima, individual polyps in the same *Zoanthus sansibaricus* colony were shown to be male, female, or unclassifiable. No hermaphroditic polyps were seen, but as both male and female polyps were seen in the same interconnected clonal colony we can consider *Z. sansibaricus* at Sakurajima to be hermaphroditic at the colony level. However, it should be noted that in 2000 40% of all polyps sampled possessed sperm and were male, and 28.9% of polyps were females possessing eggs. In 2001, 72.7% of all polyps were female, and only 7.3% of polyps male. Similar seasonal changes have been observed in 15 species of echinoderms (Policansky, 1982) and in apodid sea cucumbers (*Patinapta ooplax*) in Kagoshima (Kubota, 2000). These figures show that sex changes or gender shift inside *Z. sansibaricus* colonies must be occurring. Further investigation into these phenomena is warranted.

REFERENCES

Babcock RC,Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986) Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90: 379–394

Babcock RC, Ryland JS (1990) Larval development of a tropical zoanthid (*Protopalythoa* sp.). Invert Repro Develop 17: 229–236

Cooke WJ (1976) Reproduction, growth and some tolerance of *Zoanthus pacificus* and *Palythoa vesititus* in Kaneohe Bay, Hawaii. In “Coelenterate Ecology and Behaviour” Ed by GO Mackie, Plenum Press, New York, pp 281–288

Fadlallah YH, Karlson RH, Sebens KP (1984) A comparative study of sexual reproduction in three species of Panamanian zoanthids (Coelenterata: Anthozoa). Bull Mar Sci 35: 80–89

Karlson RH (1981) Reproductive patterns in *Zoanthus* spp. from Discovery Bay, Jamaica. Proceeding of Fourth International Coral Reef Symposium, Manila Vol.2, pp 699–704

Karlson RH (1988) Growth and survivorship of clonal fragments in *Zoanthus solanderi* Lesueur. J Exp Mar Biol Ecol 123: 31–39

Kimura S, Hashimoto Y, Yamazato K (1972) Toxicity of the zoanthid *Palythoa tuberculosa.* Toxicon 10: 611–617

Kubota T (2000) Reproduction in the apodid sea cucumber *Patinapta ooplax*: semilunar spawning cycle and sex change. Zool Sci 17: 75–81

Ono S, Reimer JD, Tsukahara J (2002) Seasonal changes in *Zoanthus* spp. in the infra-littoral zone at Taisho Lava Field, Sakurajima, Kagoshima, Japan. South Pacific Study 22: 41–52

Ono S, Reimer JD, Tsukahara J (2003) Long-term changes of *Zoanthus* spp. in the infra-littoral zone at Taisho Lava Field, Sakurajima, Kagoshima, Japan. Galaxea JCRS 5: 21–31

Policansky D (1982) Sex changes in plants and animals. Annu Rev Eco Syst 13: 471–495

Richmond RH, Hunter CL (1990) Reproduction and recruitment of coral: comparisons among the Caribbean, the tropical Pacific and the Red Sea. Mar Eco Prog Ser 60: 185–203

Ryland, JS (1997) Reproduction in Zoanthidea (Anthozoa:Hexacorallia). Invert Repro Develop 31 (1–3): 177–188

Yamazato K, Yoshimoto F, Yoshihara N (1973) Reproduction cycle in a zoanthid *Palythoa tuberculosa* Esper. (Proc. 2nd Int Symp on Cnidaria) Publ Sesoko Mar Biol 20: 275–283

(Received September 22, 2004 / Accepted November 22, 2004)