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Seasonal Changes in *Zoanthus* spp. in the Infra-Littoral Zone at Taisho Lava Field, Sakurajima, Kagoshima, Japan

Shusuke ONO, James Davis REIMER and Junzo TSUKAHARA

Abstract

Changes in *Zoanthus* spp. cover and polyp number were recorded by transect survey at Taisho Lava Field, Sakurajima, Kagoshima, Japan (N31°35', E135°35') for 30 months between May 1980 to October 1982. In addition, lab experiments were conducted to investigate changes in polyps, *Zoanthus*' gonads and zooxanthellae number at different seawater temperatures. Our results indicate that *Zoanthus* polyps grow during summer and autumn, and experience a decrease in size during winter and spring. Polyps close during periods with low winter temperatures, and zooxanthellae activity and concentration also decrease, perhaps due to the winter growth in many types of algae above polyps and the appearance of diatoms on polyps themselves. In addition, volcanic ash from the nearby active volcanic cone of Sakurajima also has a negative effect on *Zoanthus* polyps.

Key Words: *Zoanthus*, seawater temperature, seasonal changes, volcanic ash, diatom

Introduction

Zoanthus spp. (Brachcnemia, Zoanthidae) are an encrusting anemone-like group that form colonies of polyps, and are found in Japan from mid-Honshu south at depths from the low tide line and below. To investigate changes in the infra-littoral zone in corals and Cnidarians at Sakurajima's Taisho study site, *Zoanthus* was selected as the subject species for an on-going transect survey, changes in the environment are easily seen in *Zoanthus* polyps, although until now research has been scarce (BABCOCK and RYLAND, 1990).

Besides *Zoanthus* spp., *Stereonephthya*, *Dendronephthya*, *Acropora*, *Favia*, *Porites*, *Pavona*, *Montipora*, and *Hydnophora* species are all readily visible at the rocky Taisho Lava Field site, located in Sakurajima, Kagoshima, Japan (HIRATA and OSAKO, 1969). Previous research has shown that these and other species are influenced not only by expected environmental factors, such as seawater temperature, but that volcanic ash-fall from the nearby Sakurajima volcanic cone also impacts the Taisho Lava Field infra-littoral environment (ONO and TSUKUHARA, 2000).

Data collected from transect surveys for this study showed that of all the species at the Taisho site, *Zoanthus* especially displayed large variability from season to season. Laboratory experiments were carried out to investigate possible reasons behind this variability. Identification of *Zoanthus* to the species level is extremely difficult (BORNEMANN, 1998),

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and therefore identification was made only to the genus level. Further research investigating what species of *Zoanthus* are present at the Taisho site is necessary.

Materials and Methods

The subject study species *Zoanthus* forms colonies consisting of many individual polyps (Fig. 1). Polyps are on average 5~7 mm in diameter across the oral disk, and have a length of 10~30 mm. Inside the polyp in the internal gastro-intestinal cavity innumerable endosymbiotic *Symbiodinium* spp. zooxanthellae are present.

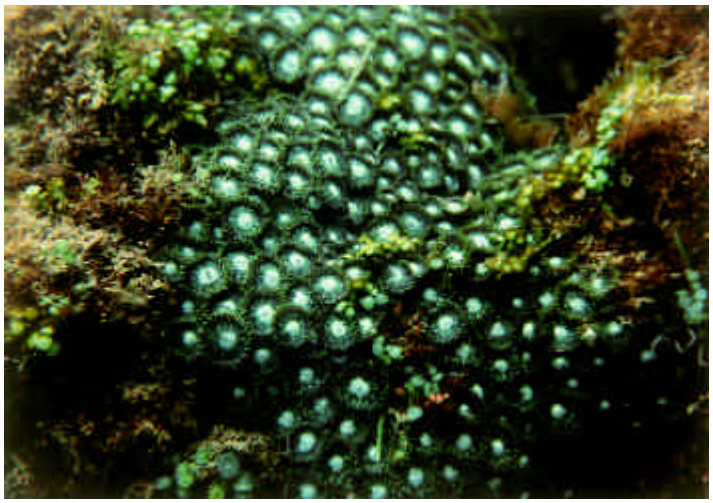

Fig. 1 Colony of *Zoanthus* aff. *pacificus* in natural condition in the infra-littoral zone at Taisho Lava Fields, Sakurajima. *Zoanthus* polyps are 5~7 mm in diameter when open, and have a length of 10-30 mm, containing innumerable zooxanthellae in the gastro-intestinal cavity.

The study site at the Taisho Lava Field is located on the western shore of Sakurajima, Kagoshima (Fig. 2). The site was formed during the Taisho Eruption of Sakurajima in 1914, and the area still receives regular dustings of volcanic ash from the active Sakurajima volcano's eruptions (FUKUYAMA and ONO, 1981). The study site is in a small bay and is 20 × 50 m. Four stations (Stations 1~4) were chosen at depths of 1~3 m, and every month from May 1980 to October 1982 (30 months total) these areas were surveyed using a 50 × 50 cm transect. Coverage of *Zoanthus* and other species were noted. At the same time, samples of *Zoanthus* were collected from depths of 1~3 m near the transect stations during each survey, and stored in the laboratory at -60℃. The samples were used to examine polyp size and zooxanthellae condition.

To investigate polyp growth and shape, samples were collected during August from various places in the study site as entire portions of a colony (still connected with stolons) to prevent unwanted mortality. Individual polyps were taken from the colony sample, and

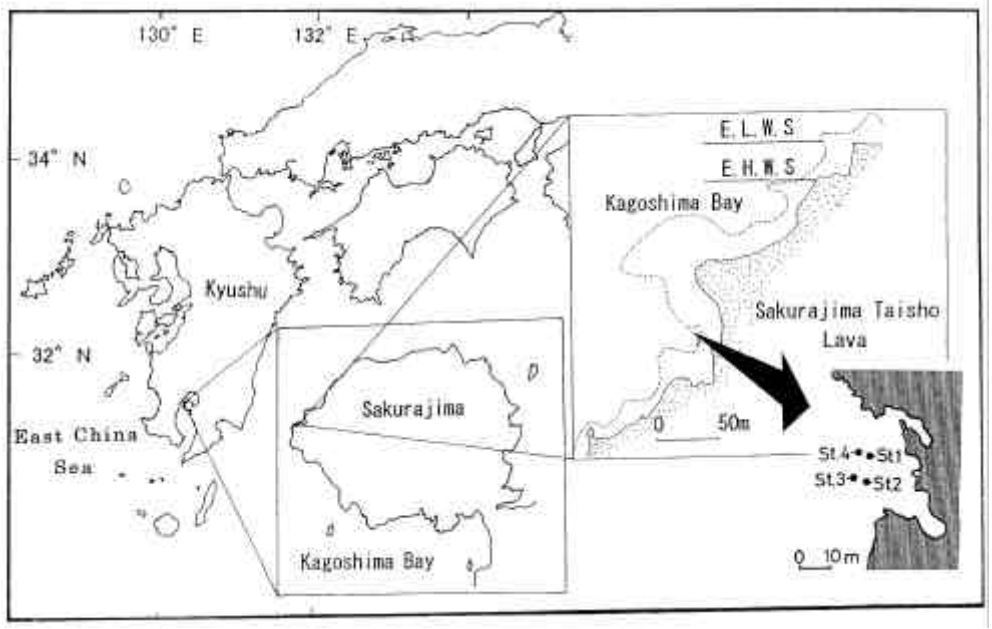

Fig. 2 Map of study site: Taisho Lava Field, Sakurajima, Kagoshima, Japan. E.L.W.S. = Extreme Low Water Spring tide line, E.H.W.S. = Extreme High Water Spring tide line, St. = Station.

the samples were placed in salt water tanks on glass plates in a laboratory under two different controlled conditions; (1) L:D = 12:12 hrs, 4000 lux, 22℃- corresponding to the study site average spring and autumn temperature, and (2) same as (1) with a temperature of 16℃, which is the average seawater temperature in winter. Ten individual polyps were placed in each temperature regime tank. Individual polyps were also examined, and oral disk diameter, length, stolon count, and polyp count were recorded. No food was given to samples before, during, and after the experiments were conducted.

Further, to investigate gonad changes, every month from March 2000 to April 2001 (every week between April and July 2000) several polyps were collected from near Station 2 and placed in Bouin's fixative solution. Samples were then mounted in paraffin and stained with Azan's staining solution. For the scanning electron microscopic study of the ultrastructural surface of the polyps samples were fixed in glutaraldehyde and OsO_4, dehydrated, dried, and coated with platinum. A Hitachi S-4100 scanning electron microscope was used.

Results

Seasonal changes in *Zoanthus* cover

Station 1 is located 10 m from shore, at a depth of 1 m. The bottom consists mainly of a large volcanic rock approximately 3 m in diameter. Compared with stations 2 and 3, station

1's water temperature and wave height, are more variable. Compared with other stations, *Zoanthus* cover at Station 1 was consistently higher throughout the course of the transect survey. Stations 2, 3, and 4 are located approximately at depths of 2.5, 3, and 3 m, and 15, 17, and 18 m from infra-littoral line, respectively. *Zoanthus* cover at Stations 2, 3, and 4 was generally below 40%..

As shown in Figure 3, *Zoanthus* cover increased between August and December, and decreased from December to August. During the last part of August in 1982 Sakurajima erupted heavily several times, resulting in extremely heavy ash-fall (5.5 kg/m^2/day) (KAGOSHIMA METEOROLOGICAL STATION: PERSONAL COMMUNICATION). At stations 2 and 3, where wave influence is low, up to 3~5 cm of ash covered *Zoanthus*. This resulted in the cover of *Zoanthus* decreasing from 45.5 % in July 1982 to 10.1% in October 1982.

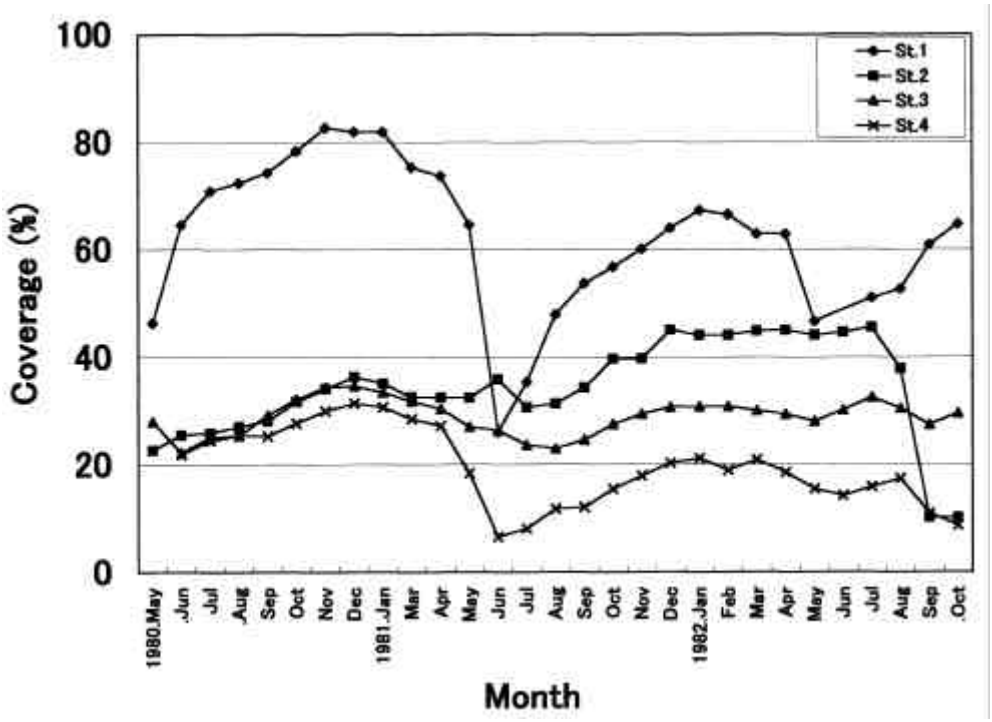

Fig. 3 Changes in *Zoanthus* cover, May 1980~Oct. 1982.

Figure 4 shows colony numbers and size data from Stations 1~4. Colony size was divided into five sizes; less than 0.25 cm^2, 0.25~6.25 cm^2, 6.25~25.0 cm^2, 25.0~100 cm^2, and over 100 cm^2. The number of small (0.25 cm^2 or less) colonies (i.e. new colonies) increased between May and September. From September to November the number of colonies less than 0.25 cm^2 decreased, and the number of colonies in the range of 0.25~6.25 cm^2 increased. From November to April the number of all colonies decreased.

The length of polyps was investigated (Fig. 5) by counting approximately 200~300 polyps per month. Small polyps with a length of less than 5 mm were very common

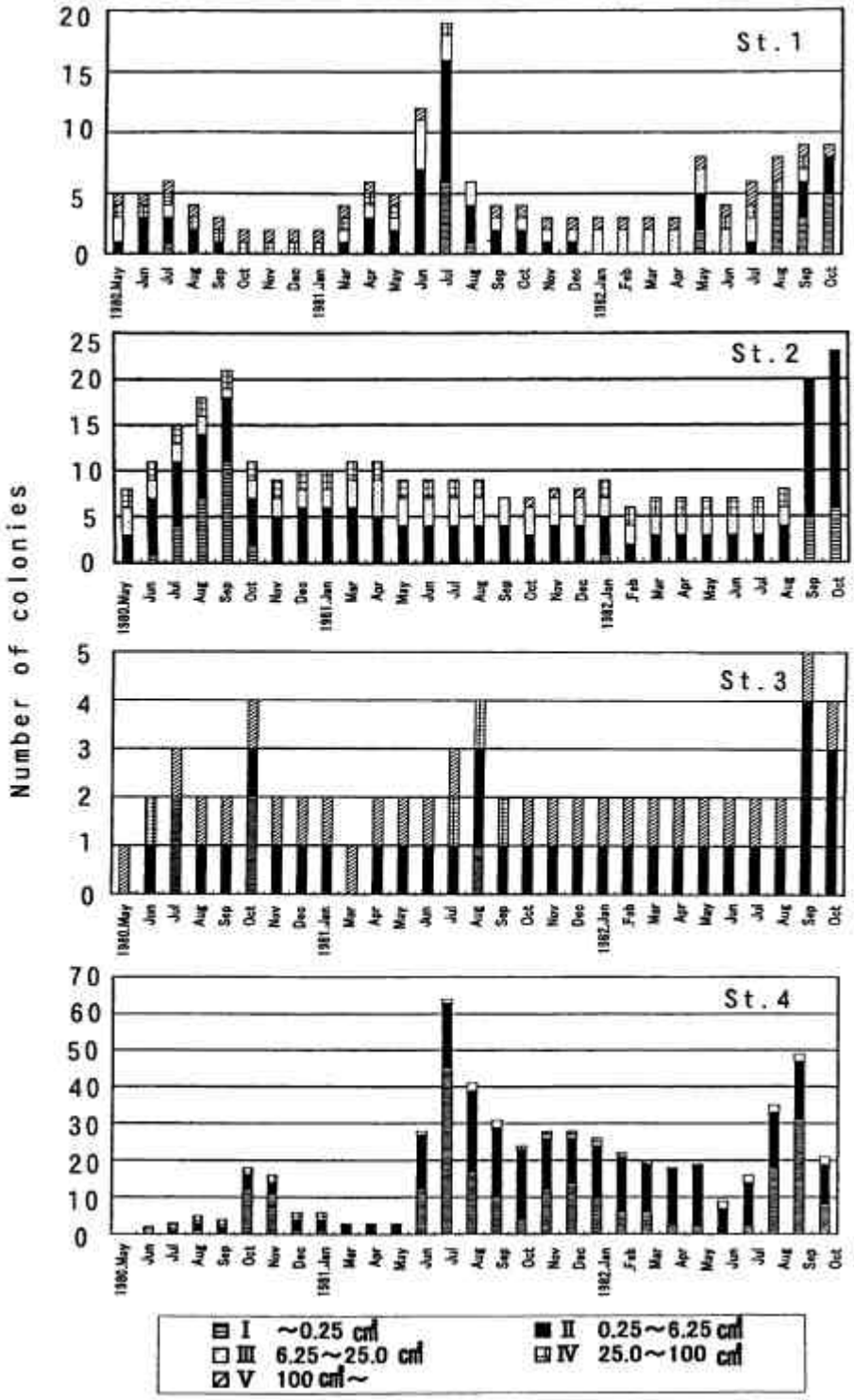

Fig. 4 Seasonal changes of *Zoanthus* colonies at Stations 1~4 by number and colony size, May 1980~Oct. 1982.

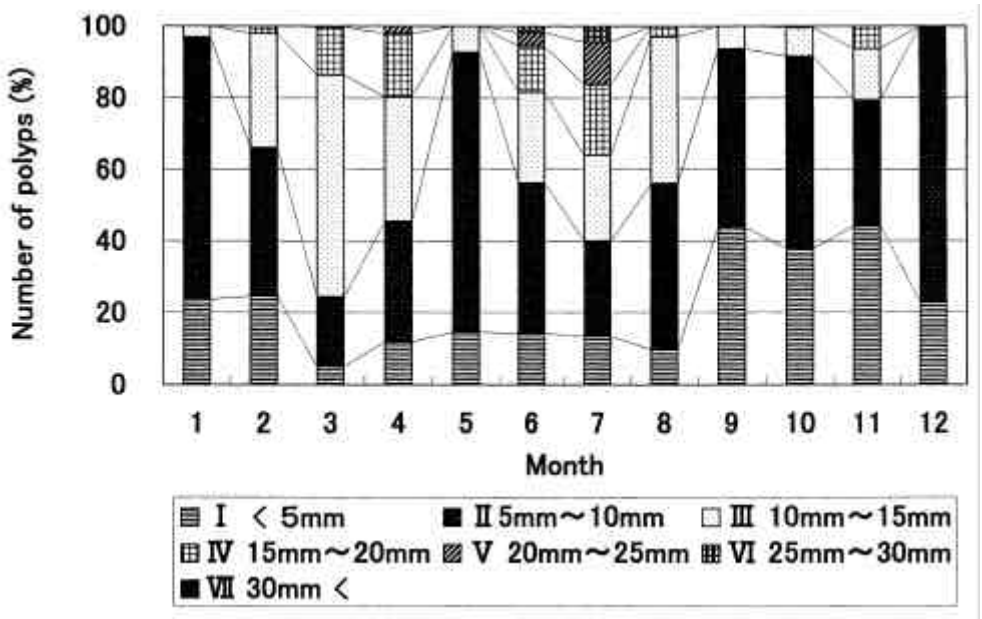

Fig. 5 Changes in *Zoanthus* polyp length (mm) by month, 1981.

between September and February.

Seasonal frequency of macro-algae and diatoms

A strong seasonal variation was seen in macro-algae and diatom (Fig. 6a, b, c) cover. *Sargassum*, *Colpomenia sinuosa*, and other species of macro-algae grow to approach 100% cover during the winter to spring period (Fig. 7). In areas above *Zoanthus* polyps this macro-algae cover appears to be especially dense. Diatoms appeared during winter and spring covering *Zoanthus* polyps.

Seasonal frequency of inactive zooxanthellae

Winter sea water temperatures at the Taisho Lava Field reach a low of approximately 15 ~16℃, and at this temperature *Zoanthus*' oral disks close, which was observed both in the field and in the lab. As seen in Figure 8, the ratio of inactive zooxanthellae generally increased from winter (January) to summer (June). This is reflected in the decreased healthiness in the zooxanthellae, and lower chlorophyll concentrations (personal data). When macro-algae cover is 100% during May (personal data), chlorophyll-less zooxanthellae account for 79% of all zooxanthellae, while in September this number decreases to 12.2% .

***Zoanthus* temperature and polyp growth experiments**

Figure 9 shows the number of stolons of Zoanthus kept in tanks at results from *Zoanthus* polyps kept in lab tanks at 16℃ and 22℃ over 10 weeks. At 16℃, no new stolons developed, and all polyps closed their oral disks. No polyps were seen to attach themselves

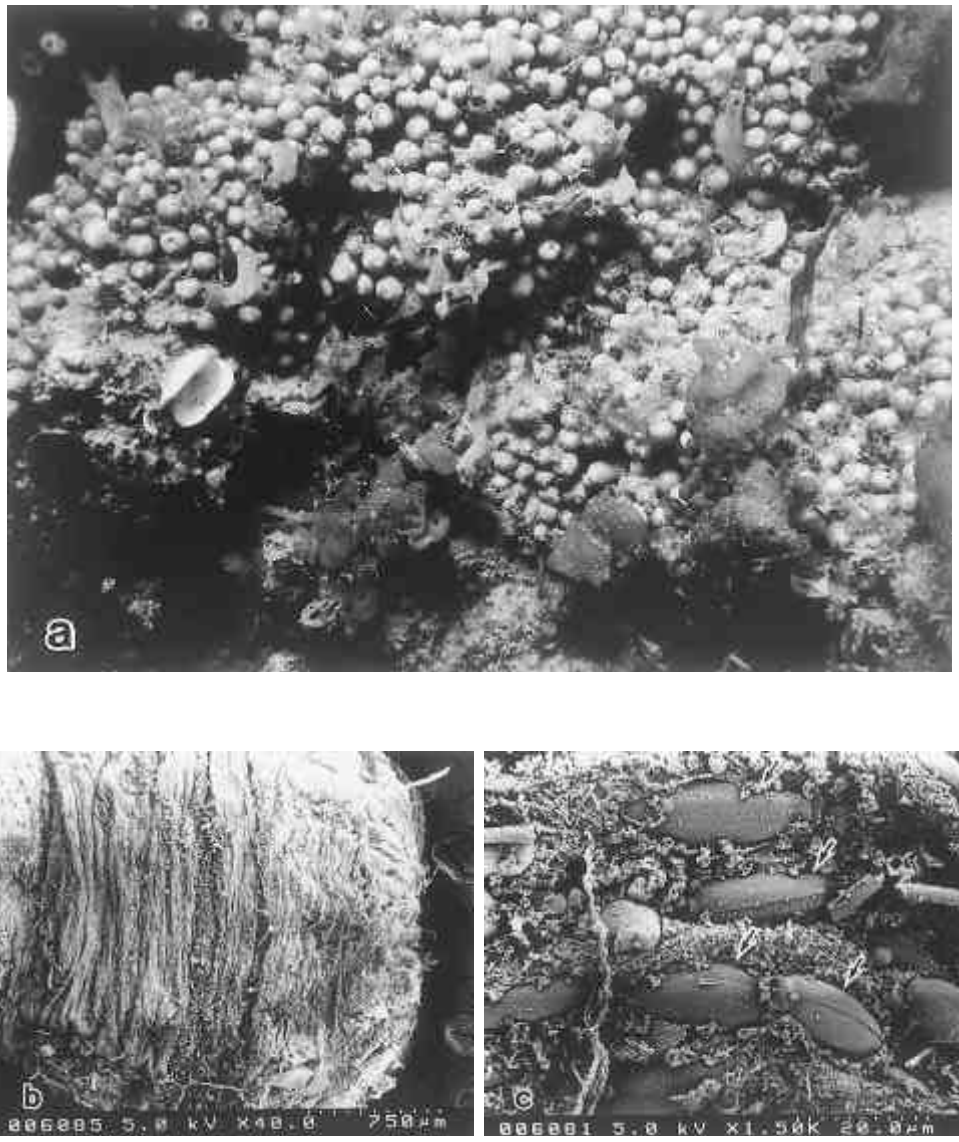

Fig. 6 *Zoanthus* polyps in winter

(a) *Zoanthus* polyps in the infra-littoral zone, February 2000. Polyps are in typical winter condition, with oral disks closed.

(b) *Zoanthus* polyp epidermis under electron microscope, February 2000. Small disk-like objects are diatoms.

(c) Detail of (b). The presence of many diatoms on the surface of the *Zoanthus* polyps is clearly evident.

Both (b) and (c) were taken with a scanning electron microscope. Scale is shown on the photographs.

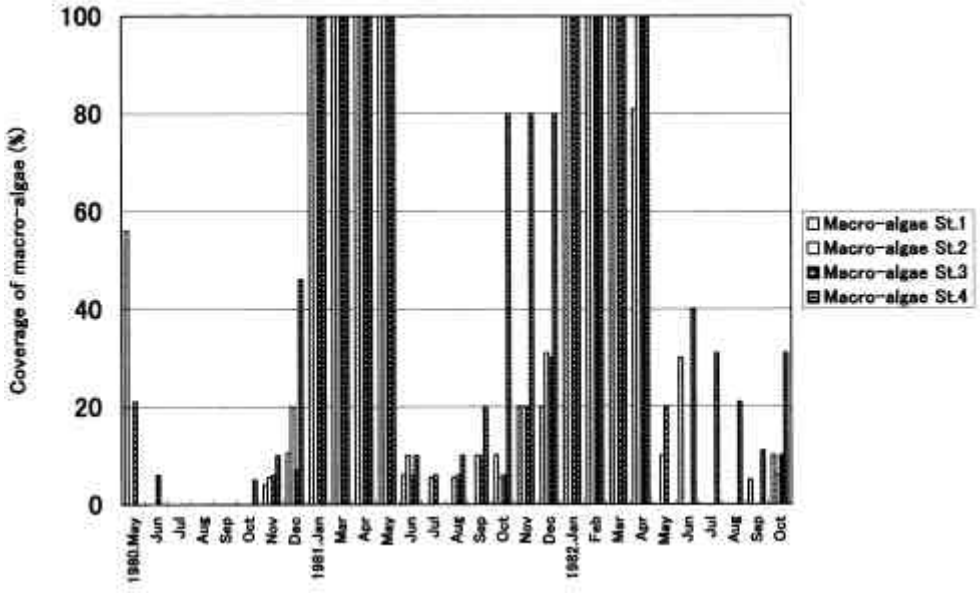

Fig. 7 Changes in macro-algae cover at Stations 1~4, May 1980 to Oct. 1982.

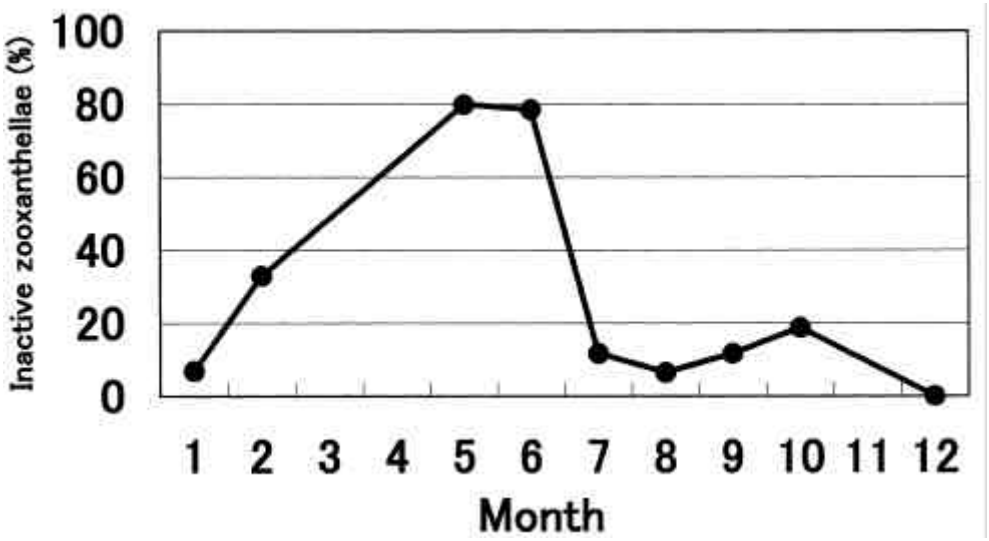

Fig. 8 Changes in inactive zooxanthellae over 1 year (1981) at the study site.

to the glass plate substrate, while at 22℃ polyps attached themselves to the substrate. In addition, at 22℃, stolons were seen to develop at 28 days from the start of the experiment, and after approximately 6 weeks all polyps showed division of the stolon. However, even after 200 days, no new formation or division of polyps was seen.

***Zoanthus* reproduction**

Based on the monthly (and weekly from April to July 2000) cross-sections of *Zoanthus*,

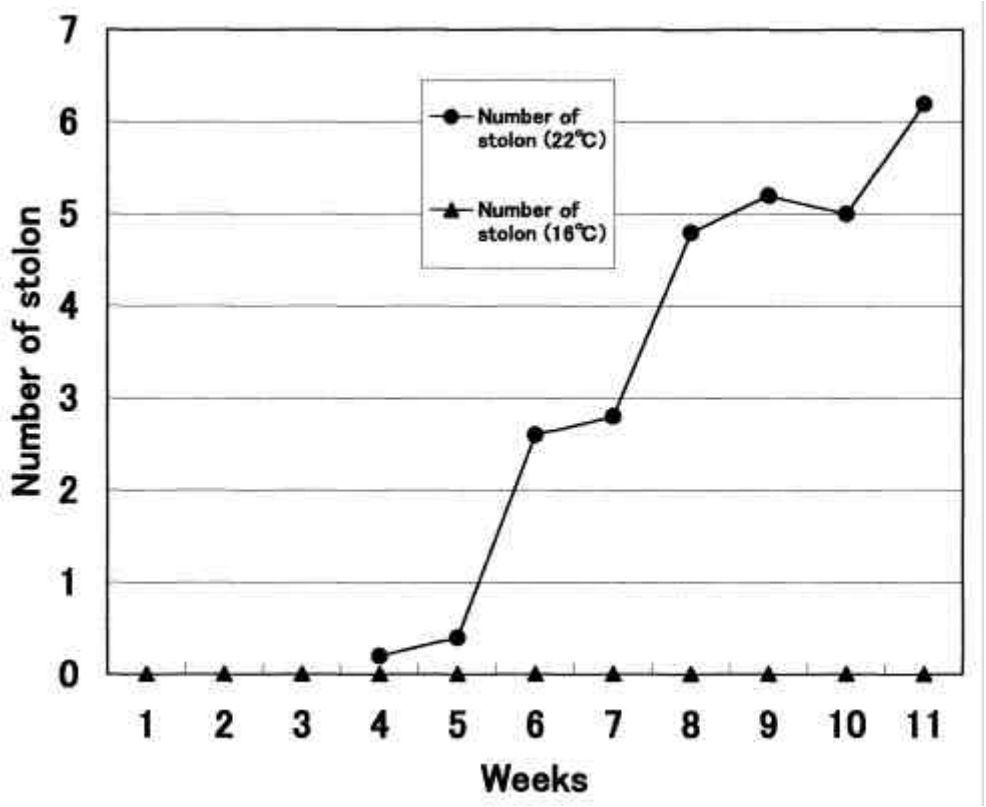

Fig. 9 Changes in *Zoanthus* stolon number L:D =12:12 hr, 4000 lux, 16℃ and 22℃, over 10 weeks.

eggs appear inside polyps from June, and grow bigger until being released during the largest tides of July (Fig. 10a). After spawning no eggs were to be seen in the cross section until the following June. Spermatogenesis began at the beginning of July, when early spermatogenia were seen to develop into spermatocytes, followed by spermatids, and finally developed into sperm (Fig. 10b). Sperm were released at the same time as the eggs during the big summer tides. After spawning, vesicles shrank until they were not visible. Based on these observations it is likely that fertilized embryos appear after the July spawning, leading to the increase in small <0.25 cm^2 polyps seen from September.

Discussion

The Effect of Light and Volcanic Ash

The growth cycle of *Zoanthus* polyps can be divided into two different phases, an expansion phase from summer to autumn, and a contraction phase from late autumn to early summer. *Sargassum*, *Colpomenia sinuosa*, and other species of macro-algae grow to approach 100% cover from winter to spring. Diatoms were seen to grow on individual *Zoanthus* polyps.

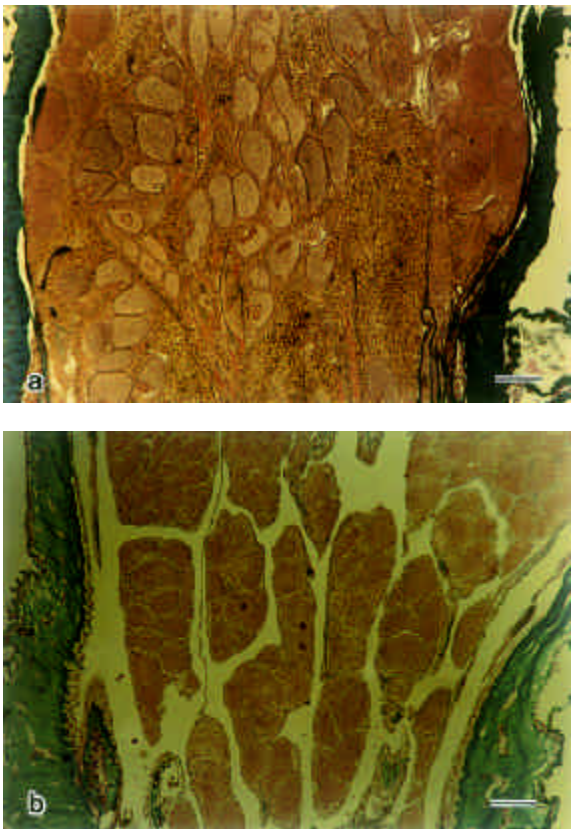

Fig. 10 *Zoanthus* polyps' gonads
(a) Ovaries of a sample polyp one week before release of eggs and sperm, July 2000. Diameter at widest point of ovaries, approximately 100~150 μm; diameter at narrowest point, 70~100 μm. Scale bar: 100 μm.
(b) Sperm bundles one week before release of eggs and sperm, July 2000. Each bundle is approximately 100 μm in diameter.
Both photos were taken with a light microscope at 100 ×. Scale bar: 100 μm.

Undoubtedly this cover influences the growth and fitness of *Zoanthus*, both on the colony and polyp level.

Based on the results, it seems safe to say that at low sea water temperatures *Zoanthus* polyps close their oral disks, decrease in size and number, and that endosymbiotic zooxanthellae decrease their activity, while macro-algae and diatom cover increase. At higher temperatures, polyps enter a state of growth and activity, characterized by an opening of oral disks, development and growth of stolons and polyps, and the appearance of small, new polyps, and spawning may occur during extreme summer tides. As well, these summer conditions correspond with a decrease in macro-algae and diatom cover. In addition, *Zoanthus*' reliance on light may be further demonstrated by the fact that very few plankton and other types of potential food sources were seen inside the gastro-intestinal

cavity during the course of the experiment. In fact, it is easy to suggest that *Zoanthus* almost completely relies upon its zooxanthellae for energy. Thus, in times when light levels are not sufficient, *Zoanthus* cannot grow nor expand.

Due to the fact that the study site is located in a small bay removed somewhat from currents and other influences, volcanic ash should have a strong effect on organisms living in the infra-littoral zone of the study area (ONO *et al*., 2002, in preparation). During the last part of August 1982 there were several large eruptions from Sakurajima volcano. 3~5 cm of ash covered *Zoanthus* and other encrusting species. In the months after the eruption dead *Zoanthus* (as well as other species) were seen at the study site. Figure 11 shows the ash-fall at sites near the Taisho field site (ANNUAL REPORT OF KAGOSHIMA PREFECTURE EMERGENCY OFFICE, 1998). Based on our observations and results, it is safe to say that volcanic ash has a harsh and debilitating effect on *Zoanthus*.

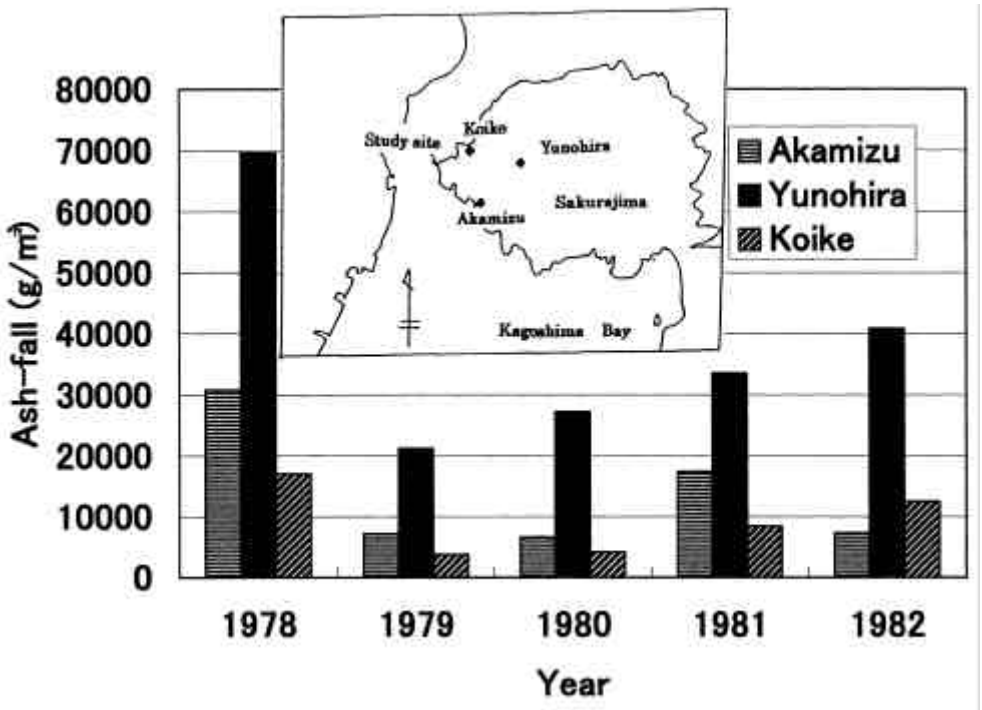

Fig. 11 Changes in volcanic ash-fall from 1978 to 1982 at three sites near the study site at Sakurajima, Kagoshima.

The Effect of Seawater Temperature

Low seawater temperatures result in the closure of polyps' oral disks, as well as the growth of macro-algae and diatoms. This causes a decrease in the activity of endosymbiotic zooxanthellae, and a stoppage and/or reversal in *Zoanthus* growth. Based on these results, one could expect to find *Zoanthus* in areas where winter sea temperatures do not go much below 16℃. This hypothesis merits further investigation.

Worldwide, widespread coral bleaching was observed in 1998, likely due high seawater temperatures (TSUCHIYA, 1999). In Japan, Okinawan coral reefs suffered heavy bleaching damage (SUGIYAMA *et al*., 1999; YAMAZATO, 1999). As noted in separate research (ONO *et al*., 2001) Sakurajima also showed signs of bleaching.

As worldwide temperature increases are expected in the coming years, one can expect that the frequency of coral bleaching will also become more intense, and therefore further research into the effect of temperature and the environment on *Zoanthus* and other species of encrusting corals has become more necessary than ever.

Acknowledgments

The authors wish to thank Professor Emeritus Yoshiko Kakinuma and Professor Toshio Ichikawa for their help and advice during the course of the research. For gracefully providing volcanic ash fall data, our sincere thanks go to the Kagoshima Prefectural Emergency Office.

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Olfactory Organs of Two Pelagic Teleost Fish—Opah (*Lampris guttatus*) and Dolphin fish (*Coryphaena hippurus*)

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Abstract

Olfactory organs of two pelagic teleost species—opah (*Lampris guttatus*) and dolphin fish (*Coryphaena hippurus*) were investigated with scanning electron microscope. Gross morphological observation showed that in both fish the paired olfactory organ is situated on the snout. Anterior and posterior openings are present in both fish. Numerous number of lamellae radiate around a short raphe. Olfactory ventilation sac is present in both fish but is more developed in opah. Olfactory sensory epithelium is found intermingled as islets or patches within the nonsensory epithelium. Ciliated olfactory receptor neuron and microvillous olfactory receptor neuron are observed in both fish with the former being more abundant. The population of receptor neurons is estimated to be ~3.0 and ~7.7 million in opah and dolphin fish respectively. Ciliated nonsensory cell is rare or absent in all lamellae examined while goblet cells are observed in both sensory and nonsensory epithelia. Epidermal cells forming microridge of finger-print like patterns are the primary cells forming the nonsensory epithelium.

Keywords: *Coryphaena hippurus*, *Lampris guttatus*, olfactory organ, pelagic fish

Introduction

Vision and chemoreception are probably the most important sensory systems used in oceanic fish in search of food in vast pelagic environment. Olfaction in particular has shown to induce prey-searching behaviors and feeding responses in little tuna (*Euthynnus affinis*) and yellowfin tuna (*Thunnus albacares*) (VAN WEEL, 1952). ATEMA *et al*. (1980) demonstrated that the yellowfin tuna can form chemical (olfactory) search image in procurement of food as a convenient system that enables the fish to switch to a major food source while ignoring less abundant food source. As a means to delay dilution of potent cues in open ocean, prey odors and other chemical cues are being entrained in lipid components of liposomes so as to provide persistent arousal and search cues for tunas (WILLIAMS *et al*., 1992) and other pelagic critters. Recently, similar chemosensory information carriers are found in land animals (LAZAR *et al*., 2001).

Olfactory cues are detected by the olfactory organ and relevant behaviors are released in any given organism. Literature showed that structures of olfactory organ of Genus *Thunnus* were studied especially its relevance to Scombridae taxonomy (IWAI and NAKAMURA, 1964). GOODING (1963) revealed that the skipjack (*Katsuwonus pelamis*) has a well-developed olfactory organ and showed that the olfactory ventilation sac may function

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as a pumping device to draw in water into the olfactory chamber during swimming. By scanning electron microscopy YAMAMOTO and UEDA (1979) first studied the olfactory organs of bluefin tuna (*Thunnus thynnus*) and other small pelagic fish. MANA *et al*. (1998) also revealed that the olfactory organs of some large pelagic species possess two types of olfactory receptor neurons—ciliated and microvillous olfactory receptor neurons on the lamellar surface, both of which are comparable to the receptor neurons found in red sea bream (*Pagrus major*) (MANA, 2001). Further the olfactory system in bigeye tuna (*Thunnus obesus*) and striped marlin (*Tetrapturus audax*) not only possess an olfactory ventilation sac but the density of olfactory neurons ranged from 40 000–68 000/mm^2 (MANA, 2000).

To reveal the diversity of olfactory systems in pelagic fish, the olfactory organs of opah (*Lampris guttatus*) and dolphin fish (*Coryphaena hippurus*) were investigated with scanning electron microscopy. Results indicated that opah has a well-developed olfactory ventilation sac with ~3.0 million olfactory receptor neurons in one rosette while dolphin fish has ~7.7 million olfactory receptor neurons per rosette. Adaptive morphological features of the olfactory systems of pelagic fish are discussed in relation to pelagic mode of life.

Materials and methods

Source of Materials

Specimens used in this study were caught by tuna longline on board Kagoshima University training vessel, Keiten Maru during ocean cruise at Northern Pacific and South of Okinawa in 1996–1997. Table 1 showed the localities where fish were sampled, standard body length and number of lamellae per olfactory organ.

Ultrastructures

For scanning electron microscopic (SEM) observation, specimen was sacrificed by decapitation. Immediately each nasal sac was flooded with 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) through anterior nasal opening for ~10 min as suggested by MORAN *et al*. (1992). Then the rosettes were surgically removed and fixed in the same fixative for 12 hr. The lamellae were post-fixed in 1% OsO_4 for 2 hr. After dehydrated through a gradient series of ethanol, the lamellae were dried in liquid CO_2 critical-point apparatus Hitachi HCP-2, coated with platinum-palladium in a Hitachi E-1030 ion sputter and viewed with a Hitachi S-430 scanning electron microscope.

In density analysis of olfactory receptor neurons (ORNs) we estimated the number of ORNs based on the SEM micrographs that included both nonsensory and sensory regions to minimize the effect of the unique sensory pattern in both fish. Micrographs were taken randomly on lamellar surface at a magnification of 2000 depicting an area of 750 μm^2. The counts were then converted to density/mm^2. Lamellar areas were determined by cutting and weighing of the well-preserved lamellae. A total of 16–24 micrographs from the lamellae of 3–5 rosettes in each fish species were examined.

Results

Gross Morphology of Olfactory Organ

The opah and dolphin fish possess a pair of olfactory organs situated on the dorsolateral side of the head just anterior to the eye (Fig. 1 A, B). The olfactory chambers are not

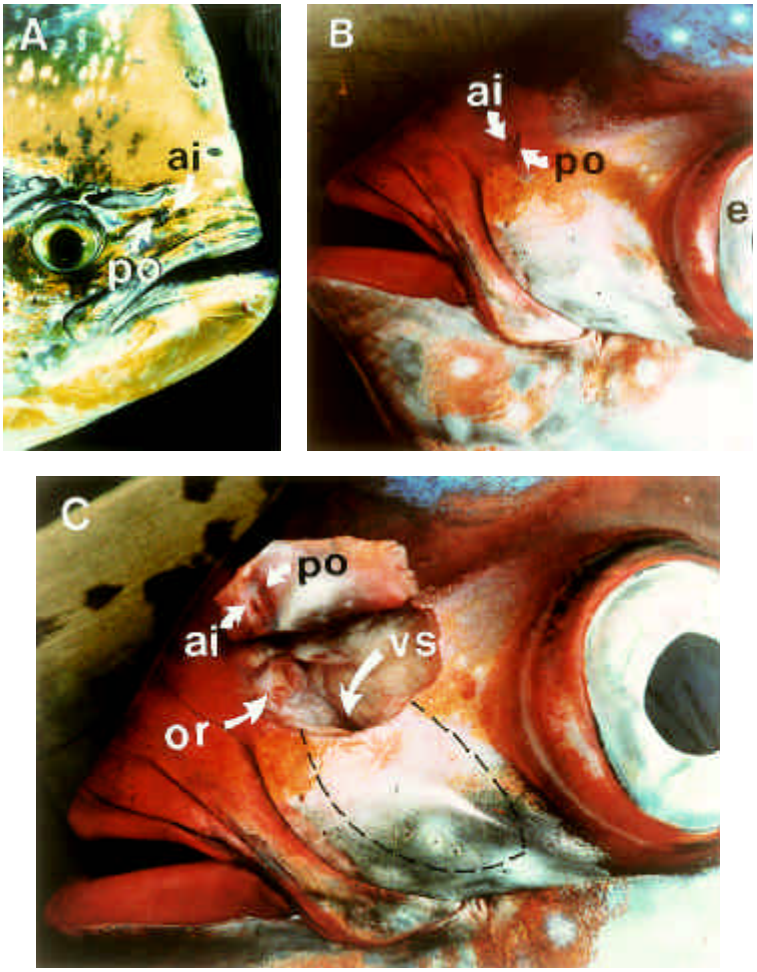

Fig. 1 Head region showing the position of olfactory nostrils and olfactory organ in (A) dolphin fish and (B) opah. (C) Olfactory chamber of opah exposing the olfactory rosette (or) and an opening leading to a ventilation sac (vs) as shown by the hatched lines. (ai) anterior opening, (po) posterior opening, (e) eye.

connected to the respiratory system in both species. When a pelagic fish swims in open seas/oceans the water containing odorants enters into the olfactory chamber via an anterior inlet and exits via a posterior outlet. In both species, the inlet and outlet are separated by a nasal bridge of epidermal tissue which is ~1–2 mm wide in both species and the inlet is smaller (~1 mm diameter) than the outlet (~2 mm diameter). In the dolphin fish an

upstanding nasal flap around the inlet probably serves to catch the water into the nasal cavity from the faster moving layers not immediately in contact with the body when the fish is swimming. Ventilation sac is present in both species and well-developed in the opah (Fig. 1c) but it was not thoroughly investigated in the dolphin fish. The ventilation sac in the opah connects to the olfactory chamber by an opening and is situated beneath the lachrymal bone. There are no muscles attached to the ventilation sac. It was demonstrated that as the mouth closed the ventilation sac is compressed and expanded when the mouth is opened. Olfactory rosette, an outgrowth of the floor of the olfactory chamber comprised numerous lamellae radiating from a short midline raphe (Fig. 2) and it (olfactory rosette) is seen

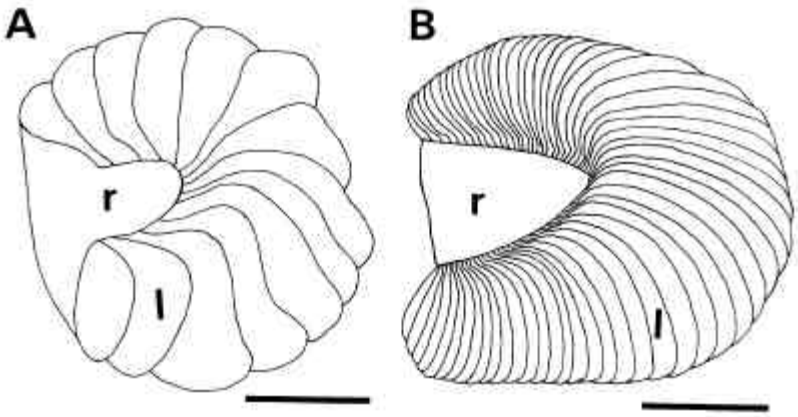

Fig. 2 Semischematic diagrams of olfactory rosettes of (A) opah and (B) dolphin fish. (r) midline raphe, (l) lamellae. Scale bar = 3 mm.

directly through the anterior opening in both species. The olfactory lamellae contain the olfactory mucosa. The number of lamellae varies between fish in each species and between the pair of organs in the same specimen. There are 14–16 and 61–64 lamellae in the opah and dolphin fish respectively (Table 1).

Table 1. Sampling area and localities, standard length and number of olfactory lamallae of two pelagic fish

Species name (Common name) [Japanese name]	Area of fish sampling	Localities of fish sampling	Standard length in cm	Number of lamellae	
				in right rosette	in left rosette
Lampris guttatus (Opah) [Aka mambou]	Northern Pacific South of Okinawa	18°34' N 132°57' E 22-25°N 127-131°E	101.1 104.9	14	16
Coryphaena hippurus (Dolphin fish) [Shiira]	Northern Pacific South of Okinawa " "	27°43' N 130°59' E 22-25°N 127-131°E " "	104.8 101.5 106.6	61	64

Fine Structures of Olfactory Epithelium

In the opah and dolphin fish the lamellar faces were lined with sensory and nonsensory epithelia. The sensory epithelium was found separated into patches or intermingled as islets within the nonsensory epithelia in all lamellae examined (Fig. 3). Both species sensory

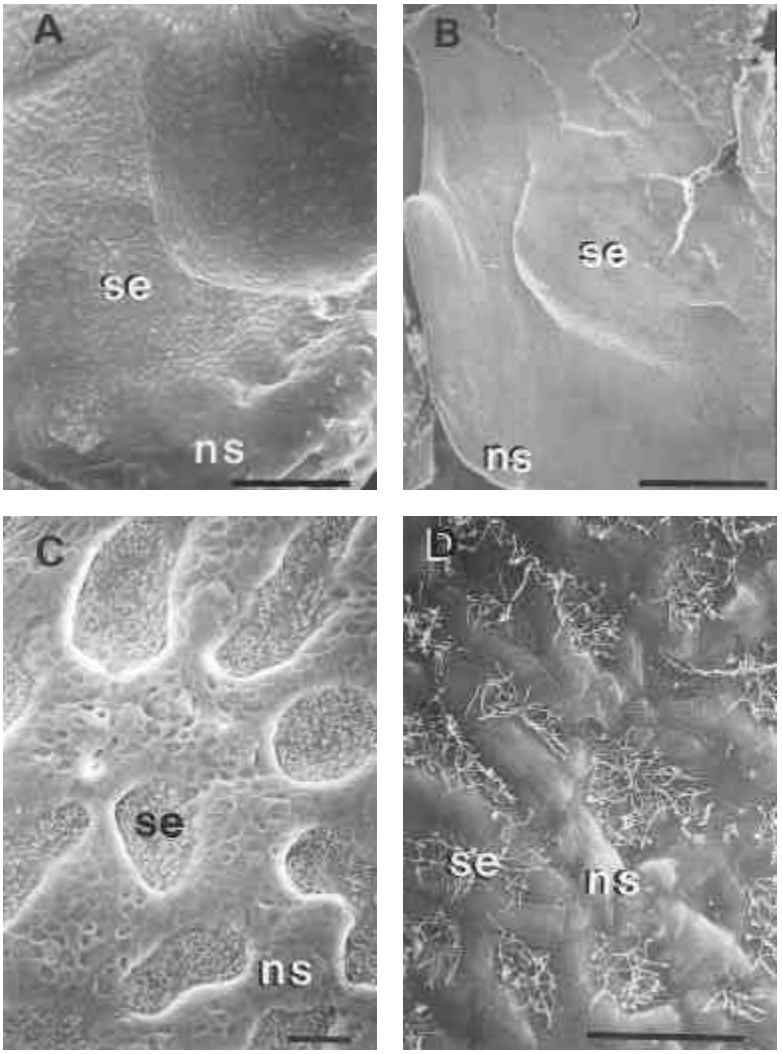

Fig. 3 Topographic distribution of sensory epithelium in (A) opah and (B) dolphin fish. In both species sensory region (se) covers the lamellar face except for the lamellar margins which consist mainly of nonsensory epithelium (ns). Sensory epithelia which are thrown into islets or patches are surrounded by nonsensory epithelium in (C) opah and (D) dolphin fish. Scale bar = 0.6 mm for A and B, 30 μm for C and D.

epithelia were composed of olfactory receptor neurons (ORNs), sustentacular and goblet cells. Ciliated olfactory receptor neuron (cORN) and microvillous olfactory receptor neuron

(mORN) which send their axons directly to the sessile type of olfactory bulb were observed in both species. cORN and mORN were confirmed to possess axon-like processes in a marine species (MANA, 2001). The dendrites of cORN are consisted of a protruding knob (1.0–1.3 μm in diameter) which bears 3–8 cilia radiating around it. These apical dendritic knobs tend to extend further to the surface of the adjacent epithelium in opah than the ones observed in dolphin fish (Figs. 4–5). This suggests of species specificity since all ORNs

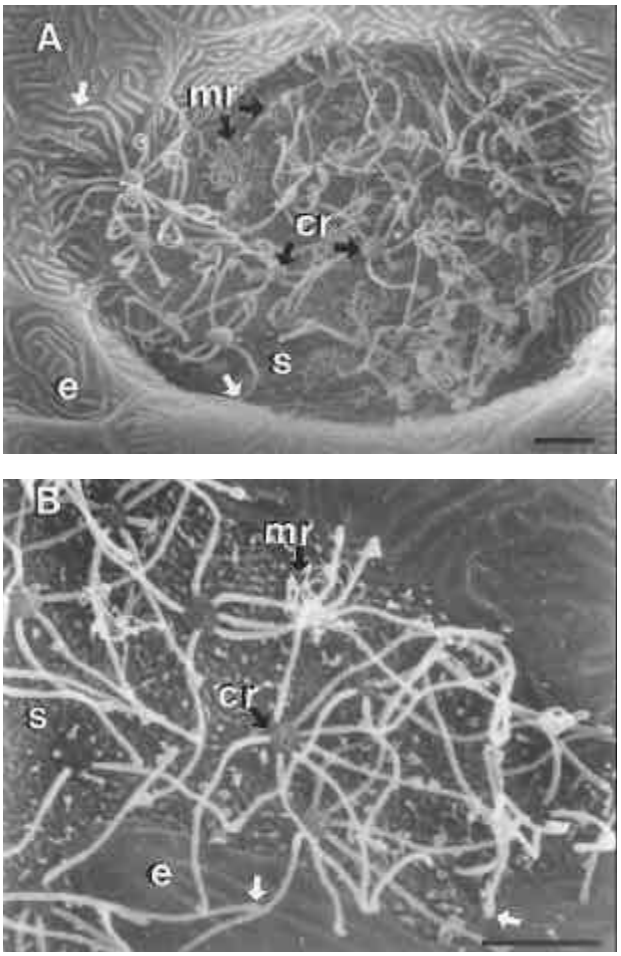

Fig. 4 Sensory epithelium of (A) opah and (B) dolphin fish which bear the ciliated olfactory receptor receptor neuron (cr) and microvillous olfactory receptor neuron (mr). (s), sustentacular cell, (e) epidermal cell. White arrows indicate tapering cilia of cr in (A) which contrast to bloated apical processes of the same type of neuron in (B). Scale bar = 3.0 μm.

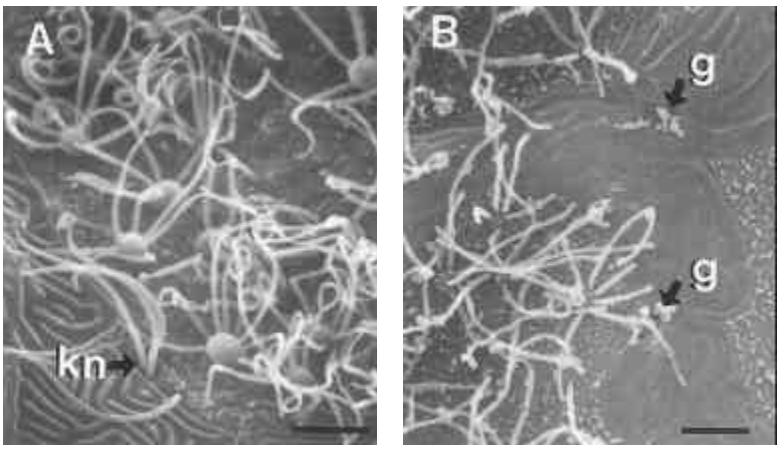

Fig. 5 Ciliated nonsensory cell (kn) in (A) opa and goblet cell (g) in (B) dolphin fish. Note the apical knobs of ciliated receptor neuron are more extended to the neighboring epithelial surface in (A) than in (B). Scale bar = 3.0 μm.

observed displayed the similar dendritic knob form. Those cilia were much longer in opah (5.6 μm) with bulbous apical processes than the tapering cilia in dolphin fish (5.0 μm) (Fig. 4). The second type of ORN comprised 20–80 microvilli projected from an olfactory knob which is usually buried in the sensory epithelium in both species. Opah possesses more number of microvilli per olfactory knob than dolphin fish. Microvilli of mORN are 1.4 μm long and 0.1 μm in diameter. There is no evidence so far to suggest motility in the cilia and microvilli of the ORNs. Another cell type found in the sensory epithelium is the sustentacular cell. They have microvilli-like protrusions on their most apical surface (Figs. 4–5). Ciliated nonsensory cell is rare in both species (Fig. 5). Goblet cells were observed in both sensory and nonsensory epithelia (Fig. 5) of both species. Nonsensory epithelium consists mainly of epidermal cell with a finger-print like pattern of microridges (Figs. 4–5).

Density and Population of Olfactory Receptor Neurons

The mean density of ORNs in opah and dolphin fish are 55 000/mm^2 and 32 000/mm^2 respectively. cORNs are the most abundant in both species while mORNs are occasionally observed especially in dolphin fish. Calculation of lamellar area showed that the exact sensory area in opah is 1.82±0.49 mm^2 (mean±SD, n = 4) while the nonsensory area is 2.36±0.50 mm^2 (n = 4). In dolphin fish the sensory and nonsensory areas are 1.92±0.37 mm^2 (mean±SD, n = 6) and 2.49±0.46 mm^2 (n = 6) respectively. Therefore the number/-lamellae in both species are much higher than density/mm^2 (Fig. 6). ORNs found in patches or islets in dolphin fish tend to be more scattered on lamellar surface than in opah as indicated from high standard deviation in mean density/lamellar area (Fig. 6). Opah with 15 lamellae would yield ~3.0 million ORNs in one olfactory rosette. Similarly dolphin fish with 63 lamellae would yield ~7.7 million ORNs in one olfactory rosette.

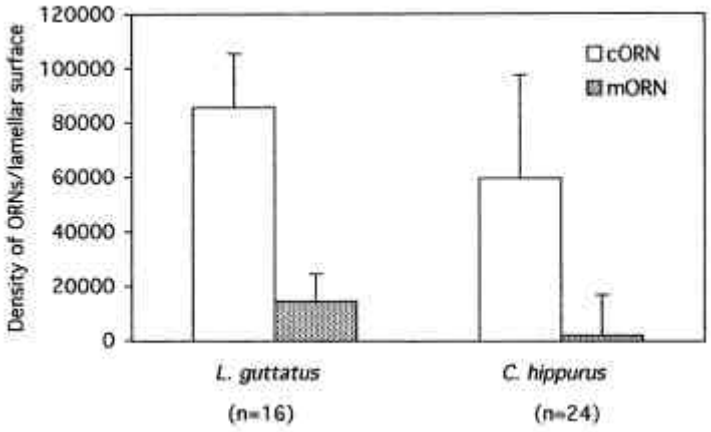

Fig. 6 Density of olfactory receptor neurons (ORNs) in two pelagic fish. Bar represents means ± SD of microvillous (mORN) and ciliated (cORN) receptor neurons on a lamellar surface.

Discussion

This report describes the macromorphology and microstructures of the olfactory organ of two pelagic species—opah and dolphin fish. Both fish possess an olfactory ventilation sac into which the seawater containing odorants is drawn into the olfactory chamber via an anterior inlet when fish opens its jaws and subsequently, the incurrent olfactory water leaves the olfactory chamber via the posterior outlet when the fish closes its mouth. The same ventilation system is observed in skipjack (GOODING, 1964), bigeye tuna and striped marlin (MANA, 2000), indicating that even at lower cruising speed the olfactory organ is continuously sampling olfactory water in the fast-swimming pelagic teleosts. At higher swimming speed it is most likely that water current through olfactory chamber is produced by the forward motion of the fish. Further almost all pelagic species studied so far tend to possess a round rosette or similar form with numerous lamellae radiating around a short midline raphe (GOODING, 1964; IWAI and NAKAMURA, 1964; YAMAMOTO and UEDA, 1979; MANA *et al*., 1998; MANA, 2000) and the most rostral part of the rosette reach the anterior opening. These intrinsic features of olfactory organs display not only an ideal arrangement for fish that inhabit vast deserts of open oceans but also a central design in pelagic forms. Another common feature in pelagic fish insofar, is the distribution pattern of the sensory epithelium on lamellar surface—sensory epithelium is found intermingled as islets or patches within the nonsensory epithelium which may be regarded as a manifestation in fast-swimming pelagic species. Other types of lamellar topography in YAMAMOTO and UEDA's (1979) classification would not be an ideal form for fast-swimming fish especially the scombroids (Families Scombridae, Istiophoridae and Xiphiidae) because the olfactory seawater entering the anterior inlet is of high pressure and any shearing force acting upon the delicate olfactory mucosa is perhaps reduced by the unique structural pattern of the sensory

epithelium on the lamellar surface to ensure strictly laminar flow 0.2<Re<2.0 (ATEMA, 1988) over and in between the olfactory lamellae; a smooth water flow over the olfactory mucosa is prerequisite for the olfactory system to detect and encode biologically relevant cues while avoiding adaptation effect on receptors (HARA, and LAW, 1972). The nonsensory epithelium is comprised mainly of epidermal cells which form a characteristic microridge of finger-print like pattern and these cells are thought to play a role in supporting tissues exposed to abrasive forces (UEHARA *et al*., 1991). Adaptational forces to pelagic way of life have rendered the kinocilia of nonsensory ciliated cells redundant in pelagic forms. This cell has a motility function (SLEIGH, 1989) and it is thought to draw in water into the olfactory cavity and propel olfactory water/mucus over the lamellar surface in fish that possess nonsensory ciliated cells in high density such as red sea bream (MANA and KAWAMURA, 2002). In planktonic life-form of pelagic fish larvae, kinocilia might be present to aid in larval olfaction and these kinocilia are becoming redundant as the fish grows. This could explain the rarity or absence of these cells in all pelagic fish studied so far.

In fish two olfactory receptor neurons are commonly present—cORN and mORN. Both of these receptor neurons are found in opah and dolphin. However, cORN is more dominant in both species (Fig. 6). Similarly, cORN is also dominant in bigeye tuna and striped marlin (MANA, 2000) and in other scombroid fish—yellowfin tuna and albacore tuna (*Thunnus alalunga*) (MANA, unpublished). The reason as to why one receptor neuron type is dominant over another is poorly understood in pelagic fish olfaction. However since most scombroids and other pelagic species are highly migratory, abundant cORN might be related to migratory behavior in fish, as postulated by PYATKINA (1976). In non pelagic forms, recent biomolecular studies in goldfish have revealed that mORN express amino acids receptors (CAO *et al*., 1998; SPECA *et al*., 1999). This evidence perhaps strengthened THOMMESEN's (1982) suggestion in salmonids that, cORN is more specific to bile salts while amino acids are detected by mORN. On the contrary, ZIELINSKI and HARA (1988) showed that only cORN was present in developing rainbow trout and responded to amino acid stimulation. Further, the sea lamprey (*Petromyzon marinus*) possesses only cORN and it's amino acid receptors are not only restricted to arginine (LI and SORENSEN, 1992) but the fish can also detect bile acids at a threshold concentration of 10^{-13} M (LI and SORENSEN, 1993). In light of the current evidences on olfactory receptors specificity in fish it can be said that the responsiveness of olfactory receptor neurons is species-specific. Although little is known about the olfactory sensitivity in many pelagic fish, yellowfin tuna can detect free amino acids at threshold 1×10^{-11} M (ATEMA *et al*., 1980). This threshold concentration is similar to free amino acids present in open seas (GARRASI *et al*., 1979). Although the population of olfactory receptor neurons in the opah (~3.0 million) and dolphin fish (~7.7 million) are much lesser than the adult red sea bream (~13.3 million) (MANA, 2001), it would be grossly unrealistic to make any kind of comparison between a macrosomatic fish and the pelagic fish at this stage. Moreover, THOMMESEN (1983) found that adult salmonids (arctic char *Salmo alpinus*) possess less density of receptor neurons (24 000/mm^2) and a fewer number (~12) of olfactory lamellae which yielded 0.5–1.0 million receptor neurons per olfactory organ nonetheless, all current evidences point to olfaction as the major sensory

system that guides those long distance migratory salmonid fish to their natal waters to spawn. Thus, all we can say is the pelagic fish have evolved to inhabit the vast oceanic environment. Evolution of olfactory system in pelagic forms not only has overcome hydrodynamic constrains in time but this distance chemosensory system is perfectly designed and tuned to the survival and success of those organisms.

In conclusion, macromorphology and micromorphology of olfactory organs of the opah and dolphin fish were studied. The results indicated that both species have functional olfactory systems best evolved for pelagic way of life. Further studies on other pelagic species olfaction will make us understand the central design of the olfactory system and it's function in pelagic fish behavior.

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