Distribution and habitat preferences of sea urchin species in Shirikawa Bay, Yamaguchi, during the period from 2005 to 2007

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Abstract

We studied habitats of sea urchin species in Shirikawa Bay in Yamaguchi City, Yamaguchi Prefecture, during the period from June 2005 to June 2007. Nine species were identified from Shirikawa Bay, which included 6 families. Several hundreds of *Scaphechinus mirabilis* appeared in a sandy beach in only early spring, while some regular sea urchin species, the mature *Hemicentrotus pulcherrimus* and the smaller *Anthocidaris crassispina* than ones in a shore out of the bay, were found often under the rocks in a shore in the bay through these survey periods. Additionally, *Pseudocentrotus depressus* were always found in the subtidal zone in a shore in/out of the bay. In a muddy beach, the only mature *Temnopleurus toreumaticus* inhabited. These results suggested that each sea urchin species in Shirikawa Bay segregates the own habitat and that *S. mirabilis* may change their habitats for spawning. We also investigated the breeding seasons of *H. pulcherrimus* and *T. toreumaticus* in this area by chemical induction of spawning. *H. pulcherrimus* spawned mature gametes from the middle of December to the middle of April and *T. toreumaticus* did from June to the middle of November. These results indicate the possibility that the provision of mature gametes by these species for a whole year contributes to the developmental biology with sea urchins in this area.

Key words: sea urchins, Shirikawa Bay, habitat, breading season

Introduction

Phylum Echinodermata in marine invertebrates includes five classes, Crinoidea (sea lilies), Asteroidea (sea stars), Ophiuroidea (brittle stars), Echinoidea (sea urchins), and Holothuroidea (sea cucumbers). Common features in this phylum are that the species show five-radial symmetry and they have a water vascular system to locomotion, respiration, feeding and many other things. This phylum appeared around the Cambrian period and

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now about 6,000 resent species were known (reviewed by Beaver *et al.*, 1967; Ruppert and Barnes, 1994).

Echinoidea has been important as a useful organism for modern developmental biology. Firstly, Echinoidea are available very easily because that many sea urchin species live in shallow shore and sandy beach of the tidal zones in the world. Secondly, they have abundant gametes and their eggs are spawned after complication of the meiosis, and so the artificial insemination is performed easily. Thirdly, they develop transparent simple embryos and larvae. Therefore, they have been used as really good materials for biochemical and molecular characterizations in the experimental embryology of fertilization, cell-cell interaction, and morphogenesis (reviewed by Wray, 1997).

Class Echinoidea that has been described about 950 species has been thought to consist of two subclasses: the regular (radial) echinoids are spherical and covered with relatively long and movable spines (sea urchins), and the irregular (bilateral) ones are flattened and covered with small spines (sand dollars, heart urchins, and cake urchins). The majority of the regular urchins adapt around rocky shore as grazers, whereas the irregular ones adapt to burrowing shallowly under sand as detritus feeders (reviewed by Ruppert and Barnes, 1994).

Yamaguchi Prefecture faces the Sea of Japan in the north part and the Inland Sea (Setonai) in the south part, and the sea area of the north parts of Yamaguchi Prefecture is one of the most famous fishery places of sea urchins in Japan. However, ecological studies, such as the distribution and habitat preferences, of sea urchin species including inedible ones in both sea areas of Yamaguchi Prefecture remain to be clear in detail. In this study, we surveyed the habitats of sea urchin species around the tidal zones of Shirikawa Bay in the Inland Sea (Setonai) side, Yamaguchi City, Yamaguchi Prefecture, on a day of the spring tide once or twice a month from June 2005 to June 2007, and also investigated the breeding seasons of two sea urchin species, *Hemicentrotus pulcherrimus* and *Temnopleurus toreumaticus*, of this survey area.

Materials and Methods

Survey sites

Shirikawa Bay is positioned in the Inland Sea (Setonai) side, Aio, Yamaguchi City, Yamaguchi Prefecture (Figs. 1A and 2A). The intertidal zone of Shirikawa Bay was divided into four typical areas by its geological and vegetation features (Figs. 1B and 2): survey area A where is a sandy beach in front of Take Island in the most northern area of this bay (Fig. 2B), survey area B where is a shore with growing of a large amount of algae (Fig. 2C), survey area C where is a muddy beach (Fig. 2D), and survey area D where includes the most southeastern part of the bay and a shore out of the bay (Fig. 2E).

Survey procedure

A survey of sea urchin species was conducted in the intertidal and subtidal zones of

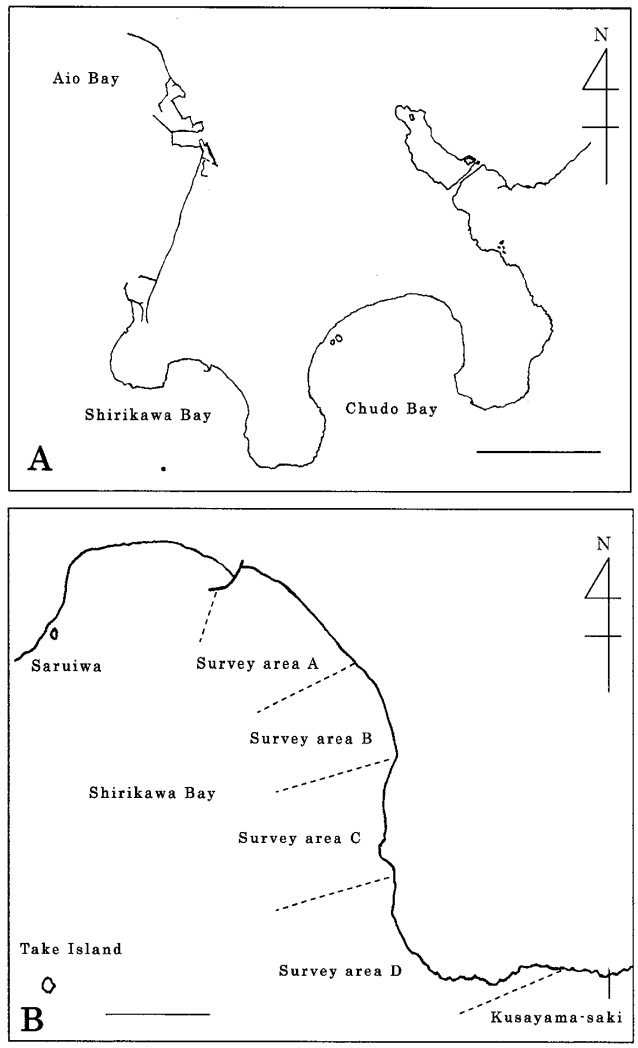

Fig. 1. Location of survey areas. A) A map shows the area of Aio in Yamaguchi City. B) The map is a higher magnification of (A). It indicates the survey area A-D in Shirikawa Bay. Scale bars: 1 km for (A), 250 m for (B), respectively.

Shirikawa Bay (Fig. 2) on a day of the spring tide (the tidal level is around -7 to 46 cm) once or twice a month from June 2005 to June 2007 by walking, except for once by snorkeling. Sea urchins were collected by hand, photographed with digital cameras (Finepix F710, Fujifilm, Tokyo; μ 810, Olympus, Tokyo), identified to species, measured to the body length and immediately released back into sea. The identification of species was according to Nishimura (1995) and Shigei (1986).

Gametes

Hemicentrotus pulcherrimus and *Temnopleurus toreumaticus* sea urchins were collected from around the Shirikawa Bay in Yamaguchi City. Spawning of eggs and sperm was induced by injecting of a small amount of 0.5 M KCl solution into the body cavity several times. The eggs were washed by filtered sea water three times and then fertilized. The fertilized eggs were observed under the microscope (OPTIPHOT-2, Nikon, Tokyo) and photographed with the digital camera (Finepix F710).

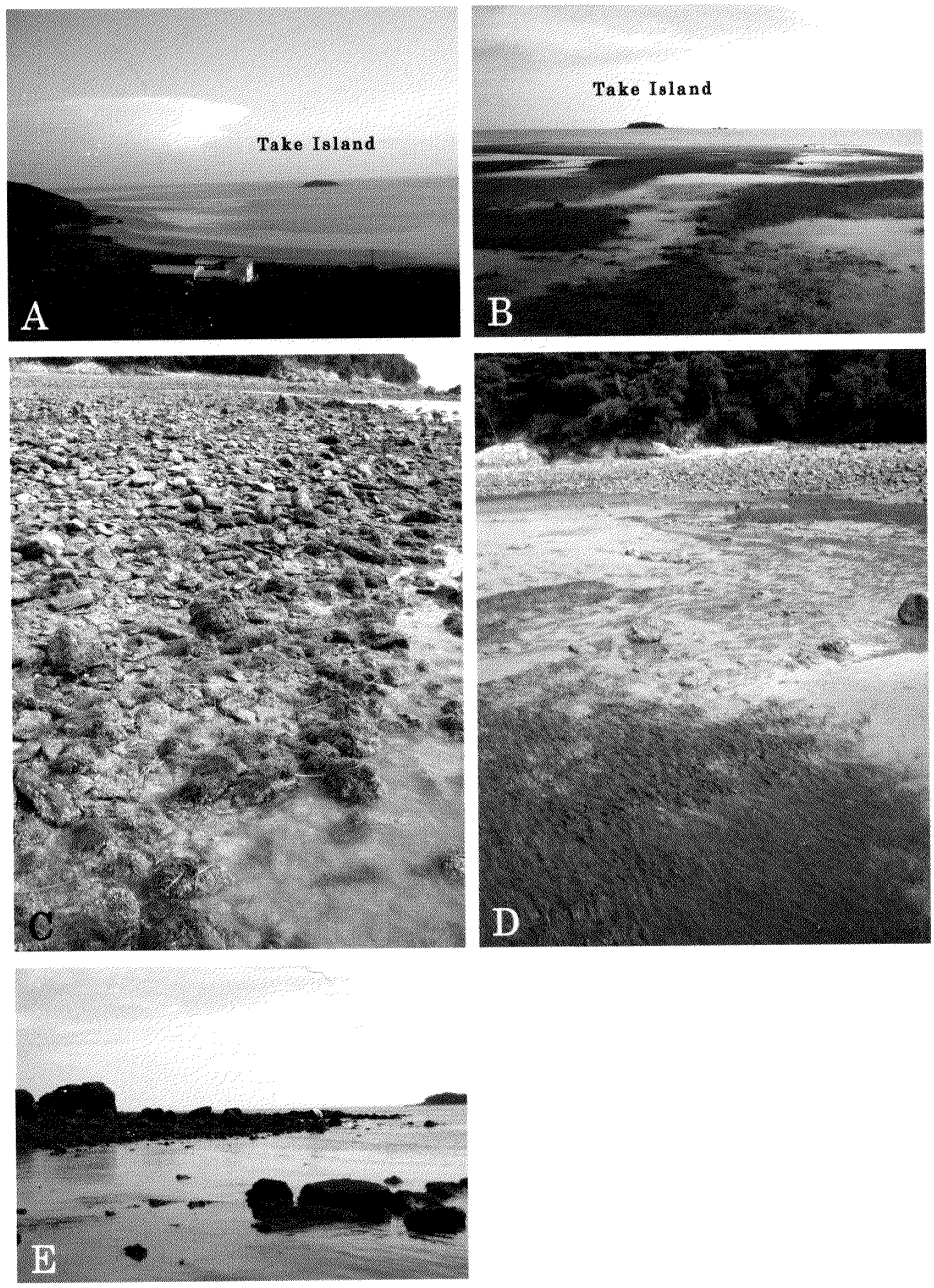

Fig. 2. Views of survey areas. A) East side view of Shirikawa Bay. B) Survey area A where is a sandy beach in front of Take Island. C) Survey area B where is a shore with growing of a large amount of algae including *S. fusiformis*. D) Survey area C where is a muddy beach with growing of *Z. marina*. E) Survey area D where consists of shores in/out of the bay. The shore out of the bay is beyond rocks on the left side in this photograph, there is a shore out of the bay.

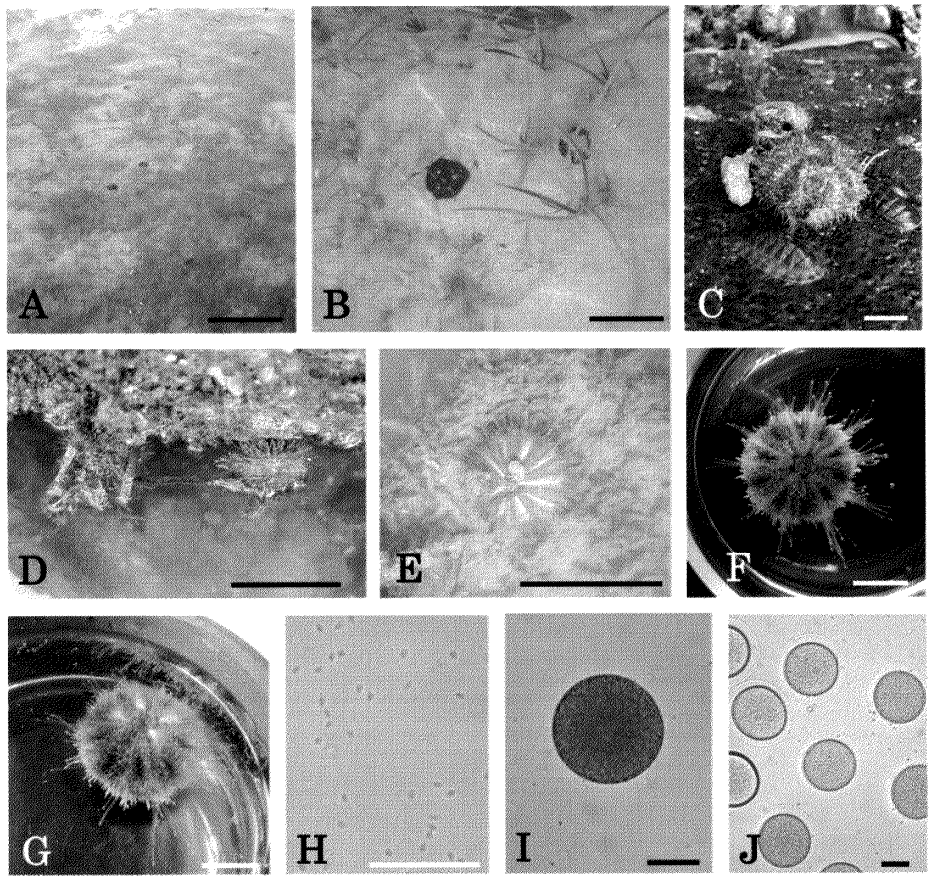

Fig. 3. Sea urchin species in Shirikawa Bay. A) *S. mirabilis* sand dollars in the survey area A in the spring. B) A close-up view of (A). C) A mature *H. pulcherrimus* in the survey area B. After rocks were turned upside down, we could find this species. D) *T. toreumaticus* in the survey area B. They stuck loosely on the rocks. E) *T. toreumaticus* in the survey area C. F) *T. sculptum* from the survey area D. G) Spawning of (F). After transferred from the laboratorial aquarium to the glass dish, it started to spawn sperms. H) A higher magnification of sperms from (G). I) A mature unfertilized egg of *H. pulcherrimus* obtained in December. J) The fertilized eggs of *T. toreumaticus* obtained in September. Scale bars: 50 cm for (A), 10 cm for (B), 1 cm for (C, F and G), 5 cm for (D and E), and 50 μm for (H-J), respectively.

Results

Survey area A

This area, where is used as a beach resort place every summer, is covered the surface with small white sand (the diameters of particles were 25.2% of 0-250 μm, 72.8% of 250-1,000 μm, and 2.0% of >1,000 μm)(Fig. 2B). Colonies of *Zostera marina* were found in places in the intertidal zone. In the whole year, several to some hundreds of adult tests of sand dollars like *Scaphechinus mirabilis* and *Peronella japonica* and a few ones of *Astriclypeus manni*, were found. Moreover, a lot of peanut worms were found in a dark grayish sandy layer with organic mater under a few centimeters in depth of white sandy layer.

At the subtidal zone in this area, three living species of irregular sea urchins like sand dollars (one Scutellidae and one Astriclypeidae) and a heart urchin (Loveniidae) inhabited (Table 1). Several hundreds of living individuals of *S. mirabilis* only appeared on the sand

Table 1. Inhabiting sea urchin species list with appearance month and sites in Shirikawa Bay during the period from June 2005 to June 2007

Area	Families and Species	Month of Identification*
Survey area A (sandy beach)		
	LOVENIIDAE	
	Echinocardium cordatum	Jun. '05
	SCUTELLIDAE	
	Scaphechinus mirabilis	Aug. '05** / Apr. '06 / Mar. '07
	ASTRICLYPEIDAE	
	Astriclypeus manni	Aug. '06**
Survey area B (shore)		
	STRONGYLOCENTROTIDAE	
	Hemicentrotus pulcherrimus	Jun. '05 / Jul. '05 / Sep. '05 / Nov. '05 / Jun. '06 / Jun. '07
	Pseudocentrotus depressus	Jun. '05 / Sep. '05
	TEMNOPLEURIDAE	
	Temnopleurus toreumaticus	Jun. '05 / Jul. '05 / Sep. '05 / Jun. '06 / Jun. '07
	ECHINOMETRIDAE	
	Anthocidaris crassispina	Jun. '05 / Jun. '06
Survey area C (muddy beach)		
	TEMNOPLEURIDAE	
	Temnopleurus toreumaticus	Jun. '05 / Sep. '05 / Oct. '05 / Jun. '06 / Aug. '06
Survey area D (shore out of the bay)		
	STRONGYLOCENTROTIDAE	
	Hemicentrotus pulcherrimus	Oct. '05 / Feb. '06 / Mar. '06 / Apr. '06 / Jun. '06 / Oct. '06 / Nov. '06 / Dec. '06 / Mar. '07 / Apr. '07 / May '07 / Jun. '07
	Pseudocentrotus depressus	Oct. '05 / Feb. '06 / Jun. '06 / Oct. '06 / Nov. '06 / Dec. '06 / Mar. '07 / Apr. '07 / Jun. '07
	ECHINOMETRIDAE	
	Anthocidaris crassispina	Oct. '05 / Feb. '06 / Mar. '06 / Apr. '06 / Jun. '06 / Oct. '06 / Nov. '06 / Dec. '06 / Mar. '07 / Apr. '07/ May '07 / Jun. '07
	TEMNOPLEURIDAE	
	Temnopleurus hardwickii	Jun. '06 / Oct. '06
	Temnopleurus toreumaticus	Mar. '06 / Apr. '06 / Oct. '06 / Nov. '06 / Dec. '06 / Mar. '07 / Apr. '07 / May '07 / Jun. '07
	Temnotrema sculptum	May '07

*We did not survey at this bay in Dec. '05, Jan. '06, Jul. '06, Sep. '06, Jan. '07, and Feb. '07.
** Snorkeling.

in early spring (Fig. 3A, B), but two individuals were found by snorkeling in August 2005. The mean of the diameter (on the perpendicular axis to the one including the anus) of the adult specimens which were found in the spring in 2007 was 6.2 cm (n=41). In the spring in 2006, young individuals which diameters were around 1 to 3 cm were found. The test of the smaller sand dollars in them was still transparent their inside and white but the test of bigger ones became thick and purple.

Survey area B

This area is a rocky shore, and algae including *Sargassum fusiformis* grow on the surface of many rocks (Fig. 2C). In this area, four regular sea urchin species, including of two Strongylocentrotidae, one Temnopleuridae, and one Echinometridae, were found (Table 1). Basically, it was found that the mature individuals of *Hemicentrotus pulcherrimus* (Fig. 3C, the diameter of the test is > 1.7 cm) and the smaller ones of *Anthocidaris crassispina* (the diameter of the test is < about 5 cm) than ones that were found in survey area D adhered under the rocks in the both tidal zones. However, *Pseudocentrotus depressus* sea urchins were found only in the subtidal zone. The mature individuals of *Temnopleurus toreumaticus* (the diameter of the test is > 2.6 cm) were sometimes found in the muddy shore of the intertidal zone in close proximately to the survey area C (Fig. 3D).

Survey area C

This area is a muddy beach with *Z. marina* (Fig. 2D). There were only mature individuals of *T. toreumaticus* in the intertidal and subtidal zones (Fig. E, Table 1). We usually found them lying on the ground.

Survey area D

This area consists of the most southeastern shore of the bay and the shore out of the bay always suffering hard wave attack (Figs. 1B and 2E). Six regular sea urchin species, including of two Strongylocentrotidae, one Echinometridae, and three Temnopleuridae, were found in this area (Table 1). Basically, in the muddy area in close proximately to the survey area C, *T. toreumaticus* were found in the both tidal zones and *P. depressus* were done in the subtidal zone. However, in the both tidal zones of the outer bay many mature individuals of *H. pulcherrimus* and the bigger individuals of *A. crassispina* than ones that were found in the survey area B were found. In this area, a few individuals of *Temnopleurus hardwickii* and one of *Temnotrema sculptum* were also found (Fig. 3F).

Breading seasons of two regular sea urchins

The breading seasons of two regular sea urchin species, *H. pulcherrimus* and *T. toreumaticus*, in Shirikawa Bay were examined by chemical induction of spawning. *H. pulcherrimus* sea urchins spawned mature gametes without germinal vesicle from the middle of December 2005 to the middle of April 2006 and then started to spawn again from the middle of December 2006 (Fig. 3I) but they did not spawn any longer at the end of April 2007. They never spawned in other seasons. On the other hand, it was identified that in 2005, *T. toreumaticus* sea urchins had mature gamete in September and October (Fig. 3J) but they did not in November. They started to spawn again from June 2006 and kept mature gametes until the middle of November. After this period, they never spawned. In 2007, we identified that they had mature gametes from June and their embryos developed after fertilization (Fig. 3J, data not shown).

Additionally, an individual of *T. sculptum* which was found and collected in the survey area D spawned sperms on June 6, 2007 after a few weeks culture in the laboratorial aquarium (Fig. 3G, H).

Discussion

Habitat segregations of sea urchin species

In the present study, it was indicated that the Shirikawa Bay equips general coastal environments in Japan for sea urchins. However, the results to the distribution and habitat preferences of sea urchin species in detail suggested that sea urchin species in Shirikawa Bay segregate their own habitats showing below; irregular sea urchin species inhabit in the white sandy beach, *H. pulcherrimus* does in the shore in the tidal zones, *T. toreumaticus* does in the muddy shore, *P. depressus* does in subtidal zone in close proximately to the muddy shore, and the bigger individuals of *A. crassispina* does in the shore out of the bay always suffering hard wave attack. These results are supported by the reports for the habitats of these species in Japan shown by Ishikawa and Noguchi (1988); *S. mirabilis* or *A. manni* inhabits underground in shallow sandy layer in the neritic zone from Otaru, Hokkaido, to the south end of Kyushu or from the central part of the Main Island of Japan to Kyushu; *Echinocardium cordatum* inhabits in the muddy area in the world; *H. pulcherrimus* inhabits under rocks in a comparatively shallow neirtic shore from the north end of the Main Island of Japan to the south end of Kyushu; *T. toreumaticus* inhabits on the sandy bottom in bays from the central to south part of Japan; *P. depressus* inhabits under 0-5 m in depth of water from Tokyo Bay to around the coast of Kyushu; *A. crassispina* inhabits between rocks from the central part of Japan to the south end of Kyushu.

One of reasons of differences of habitats among sea urchin species is that each species may eat different food. Imai (1980b) reported that *H. pulcherrimus* and young *A. crassispina* inhabit around the shallow zone with *S. fusiformis* and older *A. crassispina* than four years old may migrate to the deeper zone with growing of *Ecklonia cava* in Miura City, Kanagawa Prefecture, where is in front of Sagami Bay in the east of Japan. Moreover, Imai (1980a) described that both *H. pulcherrimus* and *P. depressus* inhabit in the different areas in Miura City but not in Waku, Shimonoseki City, Yamaguchi Prefecture (Japan Sea Research Division of Yamaguchi Prefectural Fisheries Research Center, 1967). From these reports, Imai (1980a) also discussed necessity to study for the competition for food and space between these species. These results have a similar tendency of habitat segregation among *H. pulcherrimus, A. crassispina*, and *P. depressus* to Miura City (Imai, 1980a, b). The habitat segregation among these species will be clear by further surveys in many areas with different geological and vegetation features.

Another reason will be considered that these species have different morphologies of tube feet, external extensions of the water vascular system, to cause different adhesion on the bottom substrate. The tube feet of sea urchins mainly consist of the sucker on the tip and

connective tissues in the stem. The tube feet have some skeletons such as rossetes and frames in the sucker (reviewed by Beaver *et al.*, 1967; Nichols, 1967) and spicules in the connective tissues (Ocaña Martín *et al.*, 2006). The spicules may be thought to reinforce the connective tissue (Koehl, 1982) and the adhesion of the sucker depends on its size. Among the surveyed species in the present study, differences in skeletal morphologies (Higashide, 2002) and amount of spicules in the tube feet (unpublished data) are recognized. Santos and Flammang (2005) also suggested that the difference in local distribution among sea urchin species is not only explained by the mechanical properties of their tube feet but may involve other factors such as tube foot number and arrangement, tube foot disc tenacity or sea urchin size by comparison of tube foot stems. These may be suggested that morphological changes in the tube feet had caused sea urchin species to segregate each habitat.

Furthermore, three Temnopleuridae were identified in this bay (Table 1). It is reported that their habitats are the tidal zones from Sagami Bay to Kagoshima Bay, at Korean Peninsula, and around the coast of the Chinese Continent for *T. toreumaticus*, the subtidal zone from the south part of Hokkaido to the north part of Kyushu, at Korean Peninsula, around the coast of north China, and in the East China Sea for *T. hardwickii*, and the subtidal zone from Mutsu Bay to Kyushu and in the East China Sea for *T. sculptum*, respectively (Nishimura, 1995). In the present study, *T. toreumaticus* were mainly identified in the intertidal zone in the survey area B-D. In the laboratorial culture of sea urchins in aquariums, this species usually remains at the shoreline (personal data). These suggest that this species has been adapted to the shallow environment. Further surveys of the lower subtidal zone in Shirikawa Bay will also reveal habitats of both *T. hardwickii* and *T. sculptum* in detail.

Additionally, to clear the daily changes of habitats of sea urchin species according to the tidal level will be important to understand their life histories.

Seasonal changes of habitats of sea urchin species

Many *S. mirabilis* sand dollars were mainly identified in the subtidal zone of Shirikawa Bay on a day of the spring tide in spring. However, no living *S. mirabilis* individuals were identified in the same zone in summer-winter of this survey periods (Table 1). Interestingly, Yamaguchi Fisheries Cooperative Association sweeps away some tons of mature *S. mirabilis* from the offshore sandy bottom of Chudo Bay (Fig. 1A) adjoining Shirikawa Bay by using draft nets to protect the flounder fishery in the end of March every year (personal communication). Hence, this population of Shirikawa Bay mainly inhabits at the offshore, and may change their habitats seasonally to adapt their spawning or growth periods. On the other hand, *S. mirabilis* population of Naka Island in the Inland Sea (Setonai), Ehime Prefecture, exhibits the spawning periods twice a year, spring and late autumn (Ishikawa and Noguchi, 1988), suggesting that *S. mirabilis* population of Shirikawa Bay migrate toward to subtidal zone from the offshore sandy area for spawning in spring only unlike *S. mirabilis* population of Naka Island.

Breading seasons of two regular sea urchins

Our results revealed that two regular sea urchins, *H. pulcherrimus* and *T. toreumaticus,* in Shirikawa Bay exhibit the ability to spawn by chemical induction of spawning from the middle of December to the middle of April and from June to the middle of November, respectively. Ishikawa and Noguchi (1988) showed that *H. pulcherrimus* populations of Naka Island and Mukai Island (Hiroshima Prefecture) spawn from January to early April and from January to the middle of March, respectively, while *T. toreumaticus* populations of Mukai Island and Shirahama (Wakayama Prefecture) where fronts on the Pacific Ocean spawn from the end of May to early August and from the middle of June to early October, respectively. The present results indicate that the both species can obtain mature gametes in Shirikawa Bay for the longer period than other three places. As one of reasons, it will be considered that Shirikawa Bay has enough algae as food to maintain gametes for long period. The provision of mature gametes by these species in almost whole year will contribute to study sea urchin development around this area. During about two decades, a survey for habitat preference and life histories of echinoids has not been undertaken using sea urchin species collected from the Inland Sea (Setonai) coastal areas. Therefore, our results suggest that the environment in the Inland Sea (Setonai) has been changing to cause breading seasons of sea urchin species longer from some reason such as climatic warning, rising sea temperatures and so on.

Further studies will reveal the breeding cycles and seasonal migrations of the Yamaguchi populations of sea urchin species.

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