Relation between Expanding Range of Bivalve-Inhabiting Hydrozoans and Water Temperature

Arei Kobayashi and Shin Kubota

Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University, Shirahama, Nishimuro, Wakayama, 649-2211 Japan; e-mail: kobayashi.arei@gmail.com (A. Kobayashi)

Abstract. After the first find, in 2002, of polyps of *Eugymnanthea japonica* Kubota and *Eutima japonica* Uchida on the Sea of Japan coast of western Japan, colonization patterns of these two hydrozoans were studied at six sites in this region between October, 2003, and October, 2004. The prevalence of polyps in bivalves was analyzed with respect to the annual fluctuation in seawater temperature. Presence of polyps was confirmed at four of the sites, and their prevalence was higher in October, 2004, than in October, 2003. This indicates that settlement of these polyps is progressing. Overwintering populations were observed at three of the four sites, but not at least at the two eastern sites. Their absence there in winter was evidently due not only to the low water temperature in that season, but also to the distance from presumptive source populations at Tsushima Island and Iki Island. At Tsushima Island and Hamada, polyps of both species coexisted in the same mussel, and there was a clear difference in the peak time of sexual reproduction of the two hydrozoan species, a case of allochronic reproduction. At the four sites where polyps were observed, a time-lagged correlation existed between the prevalence of polyps and water temperature fluctuations. The distributional range expansion of these hydrozoans in the Sea of Japan may be the result of the reproductive success of a settled population, supported by the recent climatic regime shift.

Key words: Bivalve-inhabiting hydrozoans, *Eugymnanthea japonica*, *Eutima japonica*, Geographical distribution, regime shift, Water temperature, Sea of Japan.

Introduction

The polyps of Eugymnanthea japonica Kubota and Eutima japonica Uchida attach to the soft body parts of such bivalves as Mytilus galloprovincialis Lamarck, Barbatia virescens Reeve, Crassostrea gigas Thunberg, C. vitrefacta Sowerby, among others, and reproduce asexually by budding of polyps (Kubota, 1987, 1992). In the breeding season, Eugymnanthea polyps release mature medusae with an atrophic feeding apparatus, no tentacles, and a considerably reduced manubrium (Kubota, 1983; Piraino *et al.*, 1994). The released medusae spawn within a day after release (Kubota, 1979). On the other hand, *Eutima* polyps release medusae with immature gonads, long tentacles and a well-developed manubrium (Kubota, 1983). The released medusae of this species mature in 1–4 weeks, and then spawn repeatedly until death. Following fertilization, embryos metamorphose into planula larvae, which settle in host bivalves and then develop into primary polyps. Recently, polyps of *Eutima japonica* were found in the scallop *Mizuhopecten yessoensis* Jay, a new host, and their presence was shown to affect the growth of the shell (Baba *et al.*, 2007). In juvenile scallops, inhabitation with polyps reduced the shell length growth by 43%, so inhabitation of

^{*}Corresponding author: shkubota@medusanpolyp.mbox. media.kyoto-u.ac.jp

Eutima on juvenile scallop is regarded as parasitism, rather than inquilinism or commensalism. In other host species, the association has been assumed to be commensal in nature (Kubota, 1983; Piraino *et al.*, 1994).

In Japan, Eugymnanthea japonica is distributed along the Pacific Ocean coasts from Okinawa Prefecture (Okinawa Island) to Kanagawa Prefecture, and four forms of Eutima japonica are distributed sequentially along the Pacific Ocean coasts from Miyazaki Prefecture to Hokkaido (Kubota, 1992; Kubota et al., 2003). The species ranges thus overlap along the southern coast of the Japanese main islands, but only rarely do they co-occur. Until recently, excluding Tsushima Island and Iki Island off Kyushu, there was no record of occurrence of these hydrozoans along the coast of the Sea of Japan from either Kyushu or Honshu, nor along the Korean coasts (Kubota et al., 2006). However, in 2002, these polyps were found for the first time along northern coasts of Yamaguchi Prefecture and Shimane Prefecture, facing the Sea of Japan (Kobayashi et al., 2004). We speculated that both species had dispersed to there from Tsushima Island and/or Iki Island via the Tsushima Current. But such a range expansion may not be explainable solely by current patterns, and other primary factors may be involved. One possibility is water temperature, since the mortality of two hydrozoan species, *Moerisia lyonsi* Boulenger and *Eucheilota paradoxica* Mayer, is controlled by it (Ma and Purcell, 2005; Carré and Carré, 1990). Indeed, a recent rise in mean annual water temperature has been reported in the southwestern Sea of Japan (Senjyu, 2004). In this study, therefore, we investigated the settlement of two hydrozoans, *Eugymnanthea japonica* and *Eutima japonica*, at several selected sites in Honshu, facing the southwestern Sea of Japan. We also analyzed the correlation of the prevalence of polyps in mussels with the annual seawater temperature fluctuation.

Materials and Methods

We collected *Mytilus galloprovincialis* at six sampling sites located along the Sea of Japan coast of southwestern Japan (Fig. 1). The selected sites were (1) Mikata, Tsushima Island, Nagasaki Prefecture ($34^{\circ}17'N$, 129°15′E), where both species of bivalveinhabiting hydrozoan have been present at least since the 1970s; (2) Murotsu, Yamaguchi Prefecture ($34^{\circ}08'N$, 130°53′E) and (3) Senzaki, Yamaguchi Prefecture ($34^{\circ}23'N$, 131°11′E), at both of which



Fig. 1. Sampling sites (•) in southwestern coasts of the Sea of Japan.

Table 1. Monthly changes in prevalence of polyps of *Eugymnanthea japonica* and/or *Eutima japonica* associated with *Mytilus galloprovincialis* at six sites along the Sea of Japan coast of western Japan.

Sampling	2003			2004					-				
site	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.
Tsushima	78		79	80		71	35		78	87			95
Island*	(100)		(100)	(100)	-	(100)	(100)		(100)	(100)		_	(22)
Hamada*	31	34	31	15	12	8	5	6	8	8	9	29	59
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Murotsu	8		20	2	2	2	1		5	12	16	19	72
	(100)	~	(100)	(100)	(100)	(100)	(100)	-	(95)	(100)	(100)	(100)	(100)
Senzaki	23	58	43	8	0	0	0	0	0	6	11	14	85
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(80)	(100)	(57)	(100)	(100)
Kasumi	0	0	0	0	0	0	0	0	0	0	0	0	0
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(75)	(100)
Maizuru	0				0	0	0	0	0	0	0	0	0
	(100)	_			(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)

Upper figure: prevalence (%) of polyps; lower figure (in parentheses): number of mussels examined; -: not examined; *: two species combined.

Eugymnanthea japonica was found for the first time in 2002; (4) Hamada, Shimane Prefecture (34°54'N, 132°03'E), where both species were found for the first time in 2002; and (5) Kasumi, Hyogo Prefecture (35°38'N, 134°38'E) and (6) Maizuru, Kyoto Prefecture (35°29'N, 135°22'E), at both of which polyps had not yet been reported (Kubota, 1992; Kubota et al. 2003; Kobayashi et al. 2004). In principle, we collected maximally 100 individuals of mussels at each site every month from October, 2003, to October, 2004. At each site an abundant mussel population was found on all sampling occasions throughout the year, except for unexpected decreases in the mussel populations at Tsushima Island in October, 2004, and Senzaki in August, 2004 (Table 1). To reveal seasonal changes in the prevalence of polyps, we selected mature mussels with antero-posterior axes of more than 3 cm.

Mussels were brought alive to the laboratory at each station and preserved in buffered 100% formalin. The soft body of each was removed from the shell and cut into seven pieces with a knife: left mantle, right mantle, left gill, right gill, left labial palp, right labial palp, and visceral mass (no polyps were found on foot). Under a stereomicroscope, all the polyps attached to each body part were scraped off with a needle and transferred into a petri-dish. The number of polyps attached to each body part was counted and the polyps were classified into three developmental stages: those with no medusa buds, those with one or more immature medusa buds, and those with one or more well-developed medusa buds. The appearance of well-developed medusa buds can be used as an indicator of a period of sexual reproduction, and it also can be used to distinguish these two species. Polyps whose medusa buds have gonads are Eugymnanthea japonica, while those with medusa buds lacking gonads are Eutima japonica. As for prevalence, the proportion of mussels with any number of polyps was noted for each of the two species. The statistical significance of the correlation between the prevalence of polyps and annual water temperature fluctuation was tested based on the Pearson product-moment correlation coefficient.

Seawater temperature data were obtained from the following six sources: the National Federation of Pearl Cultivators Cooperative Association, the Shimonoseki City Fish Farming Center, the Yamaguchi Prefectural Fisheries Research Center, the Shimane Prefectural Fisheries Experimental Station, the Hyogo Tajima Fisheries Technology Institute, and the Maizuru Fisheries Research Station of Kyoto University.



Fig. 2. Monthly changes in water temperature and prevalence of polyps of *Eugymnanthea japonica* and/or *Eutima japonica* associated with *Mytilus galloprovincialis* at six sites along the Sea of Japan coast of western Japan.

Results

Incidence of polyps on bivalves and monthly changes in water temperature

The incidence of polyps of Eugymnanthea japonica

and/or *Eutima japonica* in mussels over the course of the surveyed year is shown in Table 1. At Tsushima Island, Hamada, and Murotsu, polyps of one or both species were found with evidence of overwintering, while at Senzaki, polyps of *Eugymnanthea japonica*



Fig. 3. Monthly changes in the number of polyps with medusa buds at four sites along the Sea of Japan coast of western Japan. Vertical bars indicate SD.

were found in the warm and hot seasons only and they cannot overwinter there (Table 1, Figs 2A–D). At Kasumi and Maizuru, no polyps of any species appeared during the year (Table1, Figs 2E, F).

At Tsushima Island, the prevalence of polyps in mussels was the highest among the six sites (maximally 95%, Table 1). Moreover, the lowest water temperature at Tsushima was 13.3°C, and the minimum prevalence was 35%, and both of these values were the highest among the six sites (Table 1, Fig. 2). At Hamada, the maximum prevalence was recorded as 59% in this study, in October, 2004. The overwintering rate in February at Hamada increased from 5% in 2003 (Kobayashi *et al.*, unpublished data) to 12% in 2004, indicating progressive settlement of polyps. At Murotsu and Senzaki, only *Eugymnanthea japonica* appeared, and *Eutima japonica* was not found during

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3, 'a the course of the study (Table 1, Figs 2C, D). At Murotsu, until 2003 these polyps had never appeared during winter (Kobayashi et al., unpublished data), but in February, 2004, the prevalence of polyps there was 2% (Table 1), which clearly suggests that polyps now succeed in surviving the winter there. On the other hand, at Senzaki, located northeast of Murotsu, no polyps were found from February to June, 2004. The minimum water temperature was very low at both sites, 10.5°C at Murotsu and 8.5°C at Senzaki (Figs 2C, D). At Maizuru, where no polyps appeared, the water temperature in winter was the lowest among the six sites, reaching a minimum of 1.2°C during the study period. However, in March, 2004, the water temperature rose to the level inhabited in winter by polyps at other sites (about 10°C; Fig. 2F). Indeed, there was no remarkable difference in annual seawater temperature fluctuation between Kasumi, where no polyps occurred, and Murotsu, where polyps were found throughout the year (r=0.9898, p<0.001). At all four sites where polyps were found, the prevalence in October, 2004, was greater than in October, 2003.

Season of sexual reproduction

Figure 3 displays the fluctuation in the proportion of polyps with medusa buds during the year of the study. At the sites where *Eugymnanthea japonica* occurred (Tsushima Island, Murotsu, Senzaki, and Hamada), sexual reproduction took place from at least July to October. At Tsushima Island and Murotsu, it was most prevalent in July (Figs 3A, C), and at Hamada and Senzaki, in October (Figs 3B, D). At all four sites, polyps with medusa buds never appeared during winter and spring (March-April at Tsushima Island; December-April at Hamada and Murotsu; and February-June at Senzaki).

Polyps of *Eutima japonica* were observed at Tsushima Island and Hamada (Fig. 3). At Tsushima Island, the sexual reproduction took place throughout the year, with two peaks in December, 2003, and April–June, 2004. At Hamada, sexual reproduction was also observed throughout the year except in October, 2004, with a peak in June, 2004. In October, 2004, medusa buds were not observed at Hamada, and their proportion of polyps was the fewest for the year at Tsushima Island. October corresponds to the period when sexual reproduction of *Eugymnanthea japonica* was at its peak.

Co-occurrence

At Tsushima Island and Hamada, polyps of the two species were distributed sympatrically, and symbiosis of both species simultaneously in the same host was observed at these two sites. This is the third record of such a joint symbiosis, it having been previously reported as a rare case at Atami, Shizuoka Prefecture, and at Zagashima Island, Mie Prefecture (Kubota, 1992). The coexistence rate of polyps of the two species was 4% (*N*=4, October, 2003) at Hamada and 31% (*N*=31, October 2003) at Tsushima Island. In all the part of *Mytilus* (left mantle, right

mantle, left gill, right gill, left labial palp, right labial palp, and visceral mass), coexistence of the two species was observed, and there was no habitat segregation of polyps of the two species among the soft body parts of bivalves.

Discussion

Correlation between polyp prevalence and water temperature

At four sites where the polyps were observed, a time lag was noted in the correlation between polyp prevalence and water temperature. The lag at the northeastern sites was longer than at the southwestern ones: two months at Murotsu (r=0.6735, p<0.05), two months and two weeks at Senzaki (r=0.6984, p < 0.01), and three months at Hamada (r=0.8566, p < 0.001). There was a lag of one month and two weeks at Tsushima Island (r=0.7227, p<0.05). Depending on the distance of the sites on Honshu from Tsushima Island, greater or lesser dispersal from Tsushima in the medusa stage might be expected via the Tsushima warm current. At Hamada, since the influence of the Tsushima warm current is weaker than the two sites in Yamaguchi Prefecture, it is susceptible to the effect by the decrease of water temperature in winter. Therefore, it supposed there was a strong association between the prevalence of polyps and water temperature.

At Hamada and Murotsu, where polyps overwintered, the prevalence increased between February, 2003, and February, 2004. It has been reported that there is a 20-year cycle of changes in the hydrographic regime of the southwestern part of the Sea of Japan, with the water temperature and the tidal level changing greatly in recent years (Senjyu *et al.*, 1999). Regime shifts are defined as rapid reorganizations of ecosystems from one relatively stable state to another. In the marine environment, regime shifts may involve variabilities in such parameters as the sea surface temperature, upper ocean heat content, and sea level, and they particularly affect the water temperature in winter (Senjyu & Watanabe, 2004). Indeed, the mean annual water temperature has continued to rise since 1985 in this area (Senjyu, 2004). Such rise in water temperature enables the polyps to overwinter, as the present study demonstrates.

At Kasumi and Maizuru, polyps of both species never appeared throughout the year (Figs 2E, F). If one compares the circumstances at Kasumi with those at Murotsu, one of the causative factors for the absence of polyps at the former site is clearly not fluctuation in water temperature, but the greater distance from Tsushima or Iki Island. Namely, there is no significant difference in the annual pattern of water temperature between Kasumi and Murotsu. It is reasonable to conclude that Kasumi and Maizuru may be simply too far from a medusa source to have been colonized yet via the Tsushima Current.

The allochronic reproductive isolation

Polyps of both hydroid species sometimes coexisted in the same mussel specimen, but strong habitat segregation was not observed between them and both species coexisted on the same body parts such as mantle, visceral mass, gill and labial palp. Nontheless, allochronic reproductive isolation is functioning between the two species, as is shown by the clear differences in the peak time of sexual reproduction (Fig. 3). By allochronic reproductive isolation, gene flow between members of different but closely related species is impeded. In animals, this may involve differences in mating period or season, spawning time, and so on, and in free-spawning marine organisms a difference of only a few hours can be sufficient (Knowlton et al., 1997; Clifton & Clifton, 1999). In related taxa of fish, breeding may take place in alternate years (Aspinwall, 1974), and some species pairs of periodical cicadas breed simultaneously only every two centuries (Lloyd & Dybas, 1966). In the case of bivalve-inhabiting hydrozoans, different peak period of medusa bud production, and thus also sexual reproduction, were recognized between the species in this study.

Process of expansion of distribution

The process of range expansion of populations of bivalve-inhabiting hydrozoans along the Sea of

Japan coast of western Japan likely occurred in two steps. First, some new populations in this area were temporarily established from a source population (at Iki Island and/or Tsushima Island or elsewhere by chance in spring and autumn, but could not overwinter due to the low water temperature. These populations likely died out each year after having been reestablished each year by migration of planulae or medusae drifting from a distant source. We may call this "abortive migration".

The second stage of permanent establishment occurred when, for one reason or another, overwintering at the new site became possible and reproduction during the breeding season and local settlement could take place. At Murotsu and Hamada, where overwintering polyps were detected, these polyps may be able to reproduce there (Figs 2B, C). On the other hand, the Senzaki population is likely to have originated by abortive migration, because no polyps were observed there from February to June (Fig. 2D), probably due to the low minimal water temperature as was noted above (2°C lower than at Murotsu, 10.5°C: Fig. 2C). Similar patterns of repeated but abortive colonization have been reported in other marine animals: Coenobita purpureus Stimpson (Ikeda & Imafuku, 1987), Macrophthalmus boscii Audouin (Wada, 1985), Chaetodon auripes Jordan and Snyder (Suzuki & Hioki, 1985). It is necessary to conduct repeated and more extensive distributional surveys to confirm our hypothesis. In order to reveal the root of the range expansion, it is also important to analyze the intraspecific and interspecific relations of various populations of these two species of bivalve-inhabiting hydrozoans at the molecular level.

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