Utility of artificial settlement collector using crustose coralline algae for sampling abalone larvae in southwestern Japan.

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Abstract To assess the utility of artificial settlement collectors in southwestern Japan, collecting trials for abalone were carried out at Hirado and Iki, northwestern Kyushu, from Oct. 2001 to Feb. 2002 using settlement plates with different algal species. Corrugated polyvinyl chloride plates were conditioned as settlement plates with four treatments: high cover (30-80%) with crustose coralline algae (H-CCA), low cover (5-40%) with crustose coralline algae (L-CCA), cover with *Myrionema* sp. (MY), and no conditioning (NC). Most of the postlarvae (440 to 1250 μm) were collected from mid November to early December. The largest number of postlarvae were collected by H-CCA plates, followed by L-CCA and MY plates, and no postlarvae was collected by NC plates. These results suggested that the H-CCA conditioning was the most effective among the four treatments, and some conditioning of settlement collectors is necessary to optimize settlement of abalone larvae.

Key words: abalone, settlement collector, crustose coralline algae, *Myrionema*

The recruitment of marine benthic invertebrates with planktonic larvae is determined by two different factors, settlement strength of planktonic larvae and post-settlement mortality (Connell, 1985). Abalones have two planktonic larval stages, the trochophre and veliger stages, and the settlement strength of the veliger larvae is an important factor determining the recruitment as well as the post-settlement mortality of postlarval juveniles (McShane, 1995). Therefore, surveys of the settlement strength have been carried out by means of sampling postlarvae on natural boulders (Shepherd and Turner, 1985), collectors with intact settlement materials (Tomita *et al.*, 1977), or collectors with diatom plates that were pre-grazed in the laboratory by juvenile abalone (Nash *et al.*, 1995).

Many abalone postlarvae were collected in eastern Japan on natural boulders covered with crustose coralline algae (CCA) (Tanaka *et al.*, 1986; Sasaki, 2001) and intact settlement materials (Tomita *et al.*, 1977; Tanaka and Kasai, 2000), and inter-annual variations of the spawning period and the settlement strength were studied (Tanaka and Kasai, 2000; Sasaki, 2001). However, in southwestern Japan, few postlarvae were collected by intact settlement materials (Yamashita *et al.*, 1977). Although the cause for collecting few postlarvae in south-

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western Japan is unknown, whether it depended on the suitability of the substratum used or if only few larvae were present (Connell, 1985), potentially more postlarvae would be collected using more appropriately conditioned substratum.

In field observations, abalone recruits are found almost exclusively on crustose coralline algae (Shepherd and Turner, 1985; Kojima, 2005) and in laboratory experiments CCA have been shown to be one of the most effective substrate to induce larval settlement for all species of abalone (Roberts, 2001). Therefore, the object of this study is to assess the utility of artificial settlement collectors in southwestern Japan with high and low cover of CCA and *Myrionema* sp., which is used as a food item for early juvenile abalone in seed production (Yotsui, 1978a).

Materials and Methods

The artificial collector consisted of settlement plates, a plate holder, a stainless steel frame, and six sinkers, and it weighed about 18 kg (Fig. 1). The settlement plates were made of corrugated polyvinyl chloride (PVC), 35 cm × 40 cm, which is used in hatcheries as a substrate for rearing juvenile abalone. The PVC plates were conditioned with the following treatments: high cover with CCA (H-CCA), low cover with CCA (L-CCA), cover with *Myrionema* sp. (MY), and no conditioning (NC). In H-CCA, PVC plates were vertically set in holders, and maintained in a tank with flowing seawater and aeration supplied for more than three months (Fig. 2). To remove other algae or attached organisms, the surfaces of the PVC plates was cleaned once a month using a cloth. CCA spontaneously attached onto the PVC plates but they were dense at the upper part of each plate and the plates at both ends of each holder (Fig. 2). Therefore, every holder with PVC plates was turned upside down and the PVC plates at the ends were changed to the middle of each holder to attach CCA equally on PVC plates. The treatment of L-CCA was similar to that of H-CCA, except for lower frequency of the cleaning and the lack of replacement of the PVC plates. MY treatment was done on PVC plates by introducing small plastic sheets attached with *Myrionema* sp. in advance so that natural seeding of the PVC plates occurred (Yotsui, 1978b).

The study sites were Hirado and Iki, Nagasaki Prefecture, northwestern Kyushu, Japan (Fig. 3). At Hirado, four collectors each containing four PVC plates were set from Oct. 23, 2001 to Feb. 18, 2002, and at Iki from Oct. 31, 2001 to Feb. 28, 2002. The settlement plates of each postlarval collector were composed of 1 H-CCA, 2 L-CCA, 1 NC at Hirado, and 1 L-CCA or H-CCA, 2 MY, 1 NC from Oct. 31 to Dec. 31, and 1 H-CCA, 1 L-CCA, 1 MY, 1 NC from Dec. 31 to Feb. 28 at Iki.

The collectors were pulled up and the settle-

Fig. 1. An artificial collector with three settlement plates

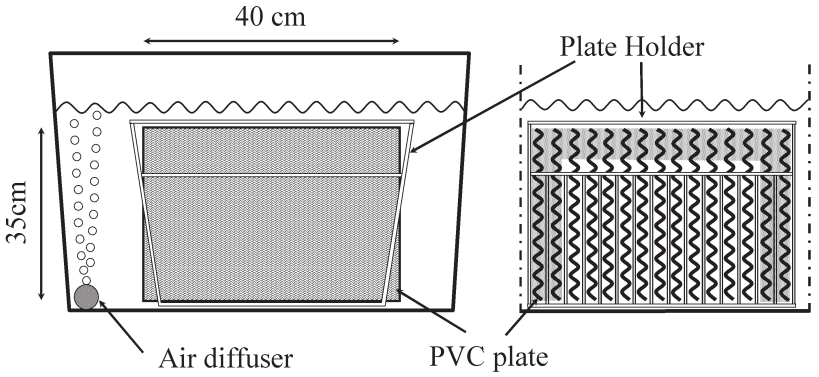

Fig. 2. Schematic drawing on crustose coralline algae conditioning of the polyvinyl chloride (PVC) plates. PVC plates were vertically set in plate holders. Hatched areas of PVC plates showed the area that CCA occurred densely.

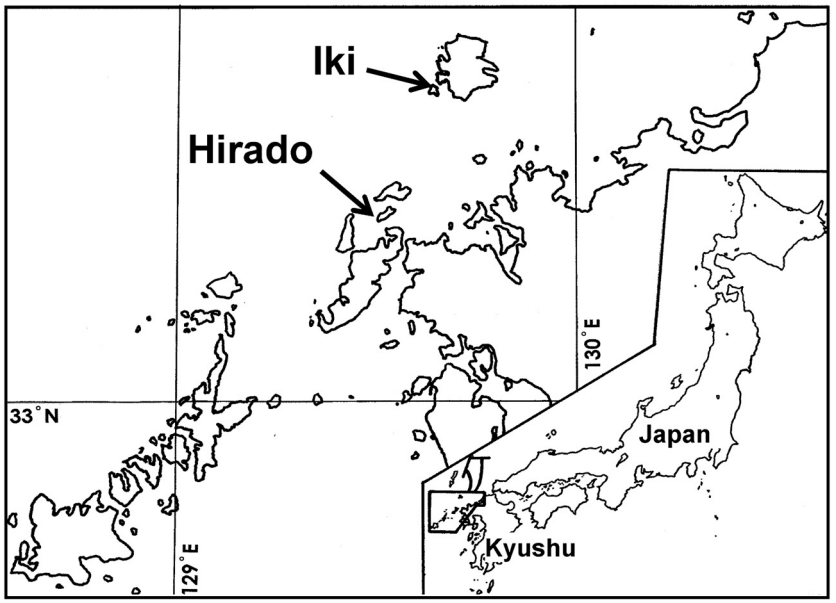

Fig. 3. Map of the study sites.

ment plates were replaced with conditioned plates from the laboratory approximately every two weeks. After treatment with 10% ethanol or freezing, organisms on the settlement plates were washed off and filtered by 100 μm mesh net. After washing the plate, the percentage cover of CCA and *Myrionema* sp. on the each plate was visually estimated. The number of abalone was counted and the shell length was measured using an optical microscope equipped with a micrometer.

Results

The percent cover of CCA on the retrieved settlement plates was 30-80% (mean 64.1%) in H-CCA, 5-40% (13.4%) in L-CCA, 0-5% (4.0%) in MY, and 0-5% (0.5%) in NC. The percent cover of *Myrionema* sp. was 10-100% (mean 64.0%) in MY.

In Hirado, more than ten individuals of abalone postlarvae were collected on the H-CCA settlement plates during two setting terms from Nov. 9 to Dec. 8 (Table 1). During the

Table 1. Number of postlarvae on each treatment of plates at Hirado. Numbers in parenthesis are the number of plates retrieved.

Setting term	Duration (Days)	Treatment of plates		
		H-CCA	L-CCA	NC
Oct.23 - Nov. 9	17	0 (4)	0 (8)	0 (4)
Nov. 9 - Nov.20	11	12^{a} (4)	1^{b} (4)	0^{b} (4)
Nov.20 - Dec. 8	18	11^{c} (4)	2^{d} (8)	0^{d} (4)
Dec. 8 - Dec.25	17	0 (4)	1 (8)	0 (4)
Dec.25 - Jan. 7	13	0 (4)	0 (8)	0 (4)
Jan. 7 - Jan.18	11	0 (4)	0 (8)	0 (4)
Jan.18 - Feb. 2	15	0 (4)	0 (8)	0 (4)
Feb.2 - Feb.18	16	0 (4)	0 (8)	0 (4)

Significantly different between values with the following letters; a-b: $p < 0.05$, c-d: $p < 0.01$ using Tukey's method among conditions of plates at each setting term.

Table 2. Number of postlarvae on each treatment of plates at Iki. Numbers in parenthesis were the numbers of plates retrieved. -: not done.

Setting term	Duration (Days)	Treatment of plates			
		H-CCA	L-CCA	MY	NC
Oct.31 - Nov.17	17	4 (4)	- (-)	2 (8)	0 (4)
Nov.17 - Dec. 1	14	- (-)	1 (4)	1 (8)	0 (4)
Dce. 1 - Dec.17	16	- (-)	0 (4)	0 (8)	0 (4)
Dec.17 - Dec.31	14	- (-)	0 (4)	0 (8)	0 (4)
Dec.31 - Jan.12	12	0 (2)	1 (3)	0 (3)	0 (2)
Jan.12 - Jan.25	13	0 (2)	0 (2)	0 (2)	0 (2)
Jan.25 - Feb.13/14	19/20	0 (2)	0 (2)	0 (2)	0 (2)
Feb.13/14 - Feb.28	14/15	0 (2)	0 (2)	0 (2)	0 (2)

same period, one or two postlarvae were collected on the L-CCA plates, but no postlarva was collected on the NC plates. After that, only one postlarva was collected on the L-CCA plates on Dec. 25. There were significant differences in the number of postlarvae between H-CCA and L-CCA plates ($p < 0.05$ retrieved on Nov. 20, $p < 0.01$ retrieved on Dec. 8), and between H-CCA and NC plates ($p < 0.05$, $p < 0.01$, respectively), however the numbers of postlarvae on L-CCA were not significantly different from those on NC during the same two terms. The shell lengths of postlarvae on these plates were from 440 to 1240 μm.

In Iki, the collected postlarvae were fewer than those in Hirado. The number of postlarvae collected from Oct. 31 to Nov. 17 were four on four H-CCA plates and two on eight MY plates (Table 2). During the next setting term, from Nov. 17 to Dec. 1, the numbers of postlarvae were one on four L-CCA plates and one on eight MY plates. Since then, one postlarva was collected on L-CCA plate on Jan. 12. No postlarva was collected on NC plates throughout this survey. The shell lengths of postlarvae collected on these plates were from 450 to 1250 μm.

Discussion

In this study, abalone postlarvae were sampled from mid-November to early-December in Hirado and Iki. These results were consistent with those in Uku Island, Nagasaki Prefecture

(Ichiki *et al.*, 1977).

The numbers of collected postlarvae were different in the four conditions. In Hirado, the number of postlarvae on the H-CCA plates was significantly more than that on the L-CCA plates. In Iki, the number of postlarvae on the L-CCA plates were similar to that on the MY plates. No postlarva was collected on NC plates either at Hirado or at Iki. Therefore, H-CCA plates were most effective in collecting postlarvae among the four different treatments for plates.

One of the causes of high efficiency in H-CCA plates is considered that CCA induces settlement of abalone larvae (reviewed Roberts, 2001). Morse *et al.* (1979) showed that *Haliotis rufescens* larvae selectively settled on CCA. Shepherd and Turner (1985) showed that *H. scalaris* and *H. laevigata* larvae settled only on CCA in the field by detailed observations and experimental collections of boulders.

Diatom plates grazed by juvenile abalone are used (Suzuki *et al.*, 1987) as settlement substratum for abalone larvae in seed production systems, and also have been used as settlement substratum for abalone collectors in the field (Nash *et al.*, 1995; Horii *et al.* unpublished data). It will be needed to compare the properties of the CCA plates with the diatom plates (efficiency, time and effort for preparation, and so on) in order to determine the most suitable method.

The maximum number of collected postlarvae per square meter in eastern Japan from boulders covered with CCA on the sea bottom was 52 (Tomita *et al.*, 1977), 1800 (Tanaka *et al.*, 1986), 220 (Sasaki, 2001), and that from intact concrete blocks was 217 (Tanaka and Kasai, 2000). The numbers of postlarvae in these studies were much more than that in our study, but Tanaka and Kasai (2000) reported that marked inter-annual variations were observed in the number of collecting postlarvae. Therefore it is necessary to monitor the number of postlarvae in southwestern Japan.

The results of this study showed that abalone postlarvae could be collected using collectors with H-CCA plates even in southwestern Japan. More research is needed to assess the suitable conditions of collectors other than settlement materials.

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