Effect of Silt and Clay on the Settlement of Short-necked Clam *Ruditapes philippinarum* Larvae

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ABSTRACT
In this study, we investigated the effect of silt and clay in the sediment on the settlement of *Ruditapes philippinarum* larvae. Mountain, river and sea sands of which silt and clay (< 0.075 mm) content was adjusted using silt and clay originating from these sands or dredged sediment (DS) were examined for settlement of *R. philippinarum* larvae. The addition of DS and Otagawa river sand-originated silt and clay did not accelerate the larval settlement, whereas the mountain and Jigozen sea sands-originated silt and clay promoted larval settlement at 5%. The negative impact of Otagawa river sand-originated silt and clay might be due to its high leachability of Mn, while the accelerated larval settlement by Jigozen sea sands-originated silt and clay could be attributed to its organic contents. The provided results indicate that a suitable ratio of the fine particles to coarse particles exists for the acceleration of the larval settlement, though the effect might be dependent on their chemical properties.

Keywords: larval settlement, restoration, *Ruditapes philippinarum*, silt and clay, tidal flat

INTRODUCTION

Short-necked clam (*Ruditapes philippinarum*) resources in Japan have been decreasing for decades. To maintain the clam production, cultivated young short-necked clams have been transplanted to clam fishery areas. As for the causes of decrease in short-necked clam resources, deterioration of the sediment environment and decrease in number of their larvae in seawater were reported (Ishii *et al*., 2001; Sekiguchi and Ishii, 2003). Therefore, restoration of the sediment environment is an important task to reestablish clam habitats for the natural recovery of short-necked clam resources.

Throughout the life cycle of a short-necked clam, the larval stage is the most sensitive to environmental deterioration (Yamamoto *et al*., 1995; Yamamoto, 2004). For the promotion of larval settlement, sand cover on the sediment of clam fishery area has been recognized as the practical method (Ishii *et al*., 2001; Ueda and Yamashita, 1997). Although mechanisms on the larval settlement promotion are not fully understood, the particle size of sediment has been reported as one of the key factors (Fujimoto *et al*., 1985; Yanagihashi, 1992; Ueda and Yamashita, 1997). Ueda and Yamashita (1997) surveyed the clam fishery areas in Japan, and reported that biomass of clam was relatively small in the area with sand less than 0.25 mm of a median particle diameter (MPD) and that the area with sand more than 2 mm MPD was suitable for settlement of clam larvae. In the other survey of 316 monitoring points in tidal flats, the settlers were the most abundant in the area with 0.4 - 0.6 mm of MPD, whereas the lowest number

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Received May 10, 2011, Accepted October 20, 2011.

was found in the area with sand less than 0.2 mm of MPD (Fujimoto *et al*., 1985). Furthermore, the laboratory investigation of the size effects on the larval settlement showed that particles with a diameter of more than 0.5 mm were required for the larval settlement and that the optimal size ranged from 1.0 to 2.0 mm (Yanagihashi, 1992).

It may be true that the coarse particles in the sand cover accelerate short-necked clam larval settlement in the real fishery area; however, the coarse particles are just components of sand. Since fine particle content in the sediment was reported to affect the benthos community and bacterial population emerging in the artificial tidal flat (Lee *et al*., 1998), the size distribution was also expected to affect the larval settlements. Although the previous surveys (Fujimoto *et al.*, 1985; Ueda and Yamashita, 1997) indicated the possible occurrence of the effect of fine particles on the clam larval settlement, conclusions cannot be derived yet due to the containment of particles with various sizes in the real sediment.

Therefore, we focused on the silt and clay in the sediment and investigated the effect of these fine particles on the settlement of *R. philippinarum* larvae using mountain, river, and sea sands as well as dredged sediment (DS). The reason for use of these sandy media is their possible application for the establishment of clam fishery area. As for DS, it may be used as an additive to adjust the silt and clay content in practical use, because DS mainly consists of silt and clay (van Dolah *et al*., 1984; Bolam and Whomersley, 2005).

MATERIALS AND METHODS

Sediment media for larval settlement

Mountain, river, and sea sands (MS, RS, and SS) were used as basal media. Mountain sand was commercially obtained, while RS and SS were collected from Otagawa river mouth and Jigozen tidal flat in Hiroshima Pref., Japan. Prior to use, both RS and SS were dried for a month at room temperature. To adjust the silt and clay content of these sands, MS, Otagawa RS, and Jigozen SS-originated silt and clay (MSC, RSC, and SSC, respectively) obtained by sieving MS, RS, and SS at 0.075 mm were used. Briefly, silt and clay-free MS, Otagawa RS, and Jigozen SS were prepared by sieving at 0.075 mm, and MSC, RSC, SSC were respectively added to these coarse sands to adjust the silt and clay content up to 20%.

In the tests for DS, MS was used as the basal medium without removal of its silt and clay. Dredged sediment was collected from Ago Bay, Mie Pref., Japan. In this study, we used DS which was granulated using poly-aluminum chloride (PAC) or the argent which originated from paper sludge ash (PS) (Imai *et al*., 2006). The granulation treatment was carried out for the improvement of handling DS.

Since the addition of MSC and SSC accelerated the settlement of *R. philippinarum* larvae as mentioned later, a fine glass powder (FGP, < 0.075 mm) and organic matter-removed SSC at 700˚C for 60 min were tested for the larval settlement to understand the acceleration effect by the silt and clay. The reason for use of FGP was to confirm the effect of fine particle existence on the larval settlement. The fine glass powder was obtained by milling glass beads followed by sieving at 0.075 mm. In addition, non-granulated, organic matter- and sulfide-removed DSs were also used. Organic matter- and sulfide-removed DSs were respectively obtained by hydrogen peroxide oxidation or drying for a month at room temperature, while the heating treatment at 700˚C was carried out to prepare for both organic matter- and sulfide-removed DS. These DSs were prepared to investigate whether the organic matter and sulfide of DS gave a negative impact on the settlement of *R. philippinarum* larvae.

Larval settlement

About 10 g of the sediment media were flatly put in 28 mmø Petri dishes, and the Petri dishes were placed at the bottom of a 30 L polycarbonate tank (Fig. 1). After pouring 10 L of sand-filtered seawater without disturbance of the sediment media, full-grown larvae of *R. philippinarum* with 0.2 mm of the averaged length were inoculated in seawater and allowed to settle for 4 d at 22˚C. The time allowance was set according to the preliminary experiment result where about 90% of larvae vanished from the seawater in the experimental tank. The number of the settled larvae was counted by microscope observation after dyeing by Rose Bengal in 10% of formalin solution, and the results were statistically analyzed with one-way ANOVA, followed by Tukey tests.

Analyses

Silt and clay content and ignition loss of the sediment media were analyzed according to Japanese Industrial Standards (JIS)-A1204 and A1226, respectively (Japanese Standards Association, 2000a, 2000b). According to JIS-A1204, sediment media samples analyzed for particle diameter distribution were pretreated with 30% hydrogen peroxide to break agglomeration by the sediment matrices, and then the samples were wet-sieved at 75 μm. Sulfide content was measured as acid volatile sulfide using a hydrogen sulfide gas detector tube (Hedrotec-S, Gastech, Japan). The characteristics of MS, Otagawa RS, Jigozen SS, MSC, SSC, and DS are summarized in Table 1.

Fig. 1 - Larval settlement on the silt and clay-adjusted sediment.

Table 1 - Characteristics of the materials used for sediment media.

	MS	Otagawa RS	Jigozen SS	MSC	Jigozen SSC	DS
Silt and clay content [%]	1.4	11	3.1	100	100	>96
Sulfide [mg/kg]	—[a]	—	—	—	—	32
Ignition loss [%]	0.95	2.1	1.7	5.9	6.3	14

[a]Not measured

As for the measurement of heavy metals such as Cu, Zn, Cd, Mn, and Cr (VI), elution tests were carried out according to the notification 46 of Environmental Agency of Japan (1991) with an exception that sand-filtered sea water was used as a solvent. These heavy metals in the eluate were analyzed by an inductively coupled plasma-optical emission spectrometry (ICAP-575II, Nippon Jarrell-Ash, Japan).

RESULTS AND DISCUSSION

Effect of MSC, RSC, and SSC on the larval settlement

The number of settlers onto the Jigozen SS of which silt and clay content was adjusted by adding its originated SSC is summarized in Fig. 2, where the addition of SSC at 5% showed the highest value. Since the Tukey test showed a significant difference in the number of settlers between 5% and other ratios ($p < 0.05$), the result confirmed that the addition of SSC accelerated the larval settlement. At a ratio of more than 10%, no acceleration was observed as compared to 0%, thereby indicating that a suitable ratio of the fine particles to coarse particles exists for the acceleration of the larval settlement. The reason why the accelerated settlement did not occur at a ratio more than 10% might be that too much silt and clay is unfavorable for the larval settlement (Fujimoto *et al.*, 1985).

In Fig. 3, the effect of silt and clay addition is compared using the normalized number of settlers of which the value was obtained by dividing the number of settlers by that observed at 0%. The mixture of MS and MSC showed similar increase in the number of settlers at a ratio of 5% as that of Jigozen SS and SSC. The Tukey test confirmed a significant difference in the number of settlers onto the mixtures of MS and MSC, Jigozen SS and SSC between 0% and 5%, 5% and 20%, respectively, although no statistical difference was observed between 5% and 10% due to the wide variance in the number of settlers. The result confirmed that SSC, as well as MSC, accelerated the larval settlement.

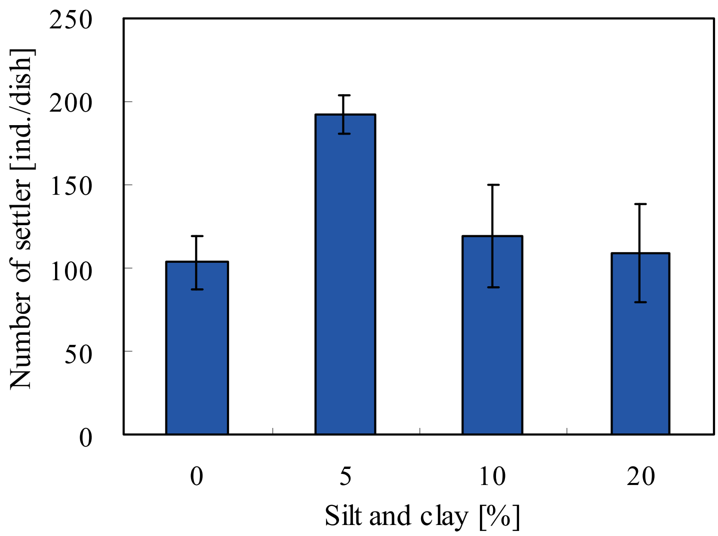

Fig. 2 - Larval settlement onto the Jigozen SS of which silt and clay content was adjusted by adding SSC. Bars indicate standard deviation (n = 3).

In contrast, no accelerated settlement was observed for the mixture of Otagawa RS and RSC. When the leachable heavy metals were compared, the mixture of Otagawa RS and RSC showed the highest concentration of Mn ions in the eluate (Fig. 4). Tsukuda *et al.* (2008) observed the mortality of *R. philippinarum* larvae in sea water spiked with Mn ions to 5.4 mg/L. Although the reported value was higher than that eluted from the Otagawa RS and RSC mixture, the most plausible explanation for the inactivity of RSC in the larval settlement might be the release of Mn ions.

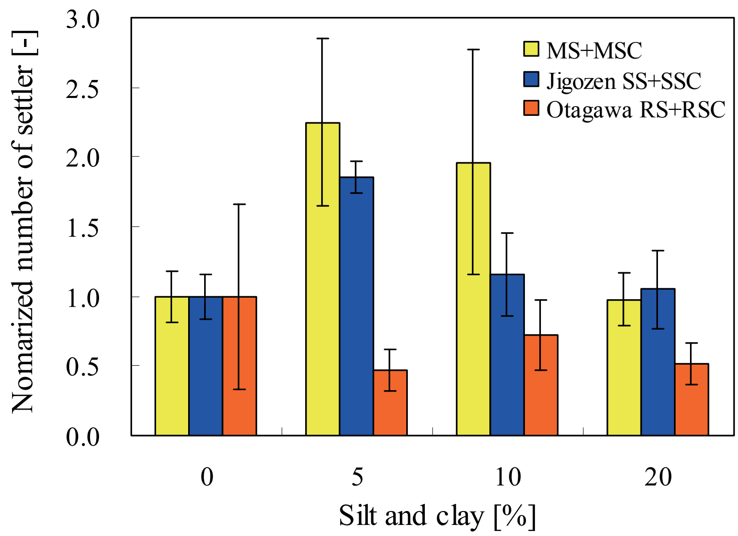

Fig. 3 - Normalized number of settlers onto the mixtures of (i) MS and MSC, (ii) Jigozen SS and SSC, and (iii) Otagawa RS and RSC. Bars indicate standard deviation (n = 3 for 10% of MS + MSC, Jigozen SS + SSC, and Otagawa RS + RSC; n = 5 for 0%, 5% and 20% of MS + MSC).

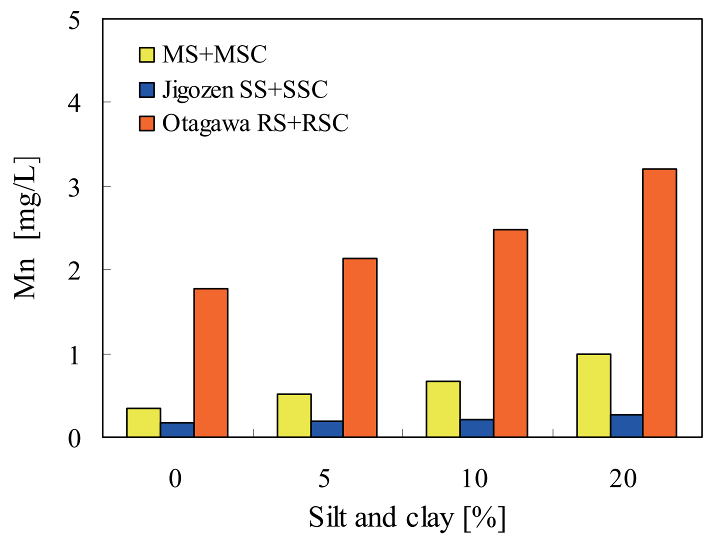

Fig. 4 - Mn ion concentration in the eluates of the mixtures of (i) MS and MSC, (ii) Jigozen SS and SSC, and (iii) Otagawa RS and RSC.

As for the causes of the acceleration of larval settlement by the MSC and SSC, (i) change of physical characteristics of sand such as interstitial volume by the existence of fine particles and/or (ii) inorganic or organic components of MSC and SSC are possible. Montserrat *et al*. (2005) reported that γ-amino butyric acid and epinephrine promoted metamorphosis and settlement of bivalve larvae such as mussel, and this supported the second hypothesis.

To verify these, FGP and the organic removed SSC were respectively added to the silt and clay-free Jigozen SS, and the resultant mixtures were tested for larval settlement. The organic-removed SSC showed no accelerated settlement (Fig. 5), while the heating did not cause an increase in the leachability of the heavy metals such as Cu, Zn, Cd, Mn, and Cr (VI) from the mixture of Jigozen SS and organic-removed SSC. The results showed that the accelerated settlement by SSC could be attributed to certain organic compounds attracting *R. philippinarum* larvae. As for FGP, significant difference was not confirmed due to the wide variance at 0%; however, on the average the number of settlers was high at 5% (Fig. 5). Therefore, the possible improvement of the physical characteristics of sand by the existence of fine particles still remains. Future research must focus on the chemical and physical characteristics which attract *R. philippinarum* larvae to accelerate their settlement.

Effect of DS on the larval settlement

When DS was added to MS, the larval settlement was clearly suppressed (Fig. 6). Even though PAC or PS was used for granulation of DS, no change was observed in the settlement on DS-added MS. These results indicate that the addition of DS does not accelerate the larval settlement.

Dredged sediment contained organic matters corresponding to about 14% of ignition loss and 31.7 mg/kg of sulfides as shown in Table 1, and this allowed us to expect that the negative impact of DS on the larval settlement was due to such characteristics.

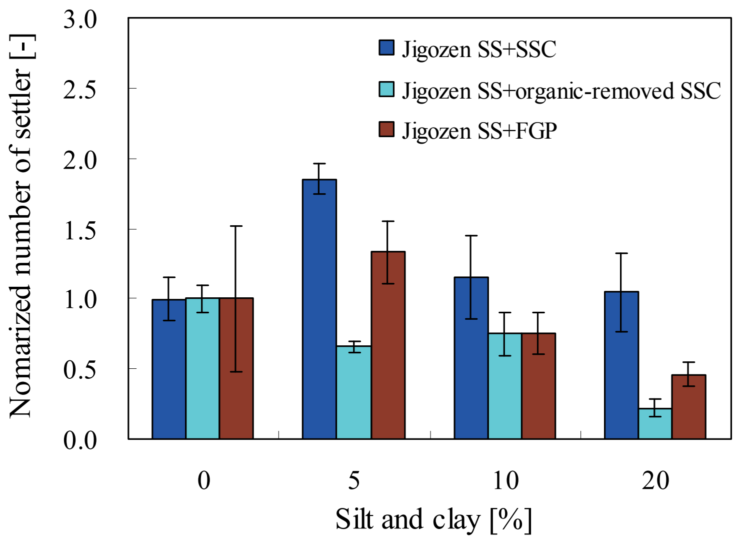

Fig. 5 - Normalized number of settlers onto each mixture of Jigozen SS and SSC, organic removed SSC, FGP. Bars indicate standard deviation (n = 3).

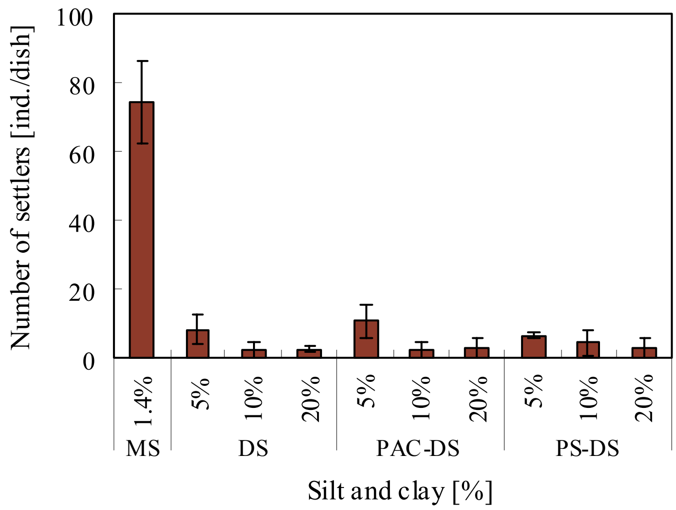

Fig. 6 - Settlement density of *R. philippinarum* larvae onto MS and DS + MS. Bars indicate the difference between average and observed numbers.

Therefore, organic matter-removed MSC, and organic matter- and/or sulfide- removed DSs were used as additives for MS. Since any treatment did not improve the larval settlement, the causes for the negative impact of DS are still lacking. Since the chemical and physical characteristics of DS are dependent on the dredging site, further tests are necessary to investigate the usability of DS for the promotion of larval settlement.

CONCLUSIONS

An effect of silt and clay (< 0.075 mm) in the sediment on the settlement of *Ruditapes philippinarum* larvae was investigated. The settlement experiments using the mixtures of (i) Jigozen sea sand (SS) and SS-originated silt and clay (SSC) and (ii) mountain sand (MS) and MS-originated silt and clay (MSC) showed that the SSC and MSC have promoting effect on the larval settlement at a ratio of 5%; however at a ratio of more than 10%, the accelerated settlement was not observed. In contrast, dredged sediment and Otagawa river sand-originated silt and clay (RSC) did not accelerate the larval settlement. The negative impact of Otagawa RSC might be due to its high leachability of Mn, while the accelerated larval settlement by SSC could be attributed to its organic contents. These results indicate that a suitable ratio of the fine particles to coarse particles exists for the acceleration of the larval settlement, though the effect might be dependent on their chemical properties. Future research must focus on the chemical and physical characteristics which attract *R. philippinarum* larvae to accelerate their settlement.

ACKNOWLEDGMENTS

We thank Dr. M. Hamaguchi, Ms. M. Sasaki and Mr. M. Kanematsu of National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, for the technical advice and assistance. This study was supported by the Collaboration of Regional Entities for the Advancement of Technological Excellence (CREATE), Japan Science and Technology Agency.

REFFERENCES

Bolam S. G. and Whomersley P. (2005) Development of macrofaunal communities on dredged material used for mudflat enhancement: a comparison of three beneficial use schemes after one year. *Mar. Poll. Bull.*, **50**, 40-47.

Environmental Agency of Japan (1991) Notification 46 (*http://www.env.go.jp/hourei/syousai.php?id=01000057*) (in Japanese) (August 23, 1991)

Fujimoto T., Nanamura M., Kobayashi M., Hayashi I., Takiguchi K., Oda K. and Ujima H. (1985) The fishery formation of short-neck clam *Ruditapes philippinarum*. *Fukuoka Pref. Buzen Sea Fish. Exp. Stn. Res. Rep.*, 34-106. (in Japanese)

Imai D., Kato T., Dabwan A. and Harada I. (2006) Development of technology for reproducing of marine environment from dredged sediments by using paper sludge ash and its beneficial applications for cleaning Ago Bay. *Kami Pa Gikyoshi/Japan Tappi J.*, **60**, 80-88. (in Japanese)

Ishii R., Sekiguchi H., Nakahara Y. and Jinnai Y. (2001) Larval recruitment of the manila clam *Ruditapes philippinarum* in Ariake Sound, southern Japan. *Fish. sci.,* **67**, 579-591.

Japanese Standards Association (2000a) JIS A1204: 2000 Test method for particle size of soils, Japanese Standards Association, Tokyo, Japan. (in Japanese)

Japanese Standards Association (2000b) JIS A1226: 2000 Test method for ignition loss of soils, Japanese Standards Association, Tokyo, Japan. (in Japanese)

Lee J. G., Nishijima W., Mukai T., Takimoto K., Seiki T., Hiraoka K. and Okada M. (1998) Factors to determine the functions and structures in natural and constructed tidal Flats. *Water Res.,* **3**, 2601-2606.

Montserrat G.-L. A., Silva M., Abad A., Pazos J. and Sanchez L. (2005) Effects of GABA and epinephrine on the settlement and metamorphosis of the larvae of four species of bivalve mollusks. *J. Exp. Marine Biol. Ecol.*, **316**, 149-156.

Sekiguchi H. and Ishii R. (2003) Drastic decreasing of annual catch yields of manila clam *Ruditapes philippinarum* in Ariake Sound, southern Japan. *Oceanogr. Japan,* **12**, 21-36. (in Japanese)

Tsukuda M., Yamaguchi H., Takahashi T. and Tsutsumi H. (2008) Impact of high concentrations of manganese on the survival of short neck clam Ruditapes philippinarum juveniles in sandy tidal flat sediment in Ariake Bay, Kyushu, Japan. *Plankton Benthos Res.,* **3**, 1-9.

Ueda T. and Yamashita T. (1997) The case of development of Japanese littleneck clam *Ruditapes philippinarum* culture ground. *Fish. Engineer.*, **33**, 213-218. (in Japanese)

van Dolah R. F., Calder D. R. and Knott D. M. (1984) Effects of dredging and open-water disposal on benthic macroinvertebrates in a South Carolina estuary. *Estuaries,* **7**, 28-37.

Yamamoto M. (2004) Sediment environment and the characteristics on the fishing ground of *Venerupis philippinarum*. *Bull. Fish. Res. Agency,* **Suppl. 3,** 17-25. (in Japanese)

Yamamoto M., Hagino S. and Ishida K. (1995) Study on physical factors to design of aquaculture grounds for short-necked clams. *Bull. Natl. Res. Inst. Fish. Engineer.,* **16,** 1-28. (in Japanese)

Yanagihashi S. (1992) Selective settlement of Japanese littleneck larvae and their distribution in Mikawa Bay. *Fish. Engineer.,* **29**, 55-59. (in Japanese)