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Effects of turbation of the Japanese common lancelet *Branchiostoma japonicum* (Cephalochordata) on sediment condition: laboratory observation

Hiroshi Ueda[1]* & Kanako Sakaki[2]

[1] *Usa Marine Biological Institute, Kochi University, Inoshiri 194, Usa, Tosa, Kochi 781–1164, Japan*
[2] *Center for Marine Environmental Studies, Ehime University, Bunkyo-cho 3, Matsuyama, Ehime 790–8577, Japan*

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Abstract: Behavior of the common lancelet *Branchiostoma japonicum* in sediment and effects of their turbation on sediment were observed in a tank to discover their ecological significance in a sandbank with a dense population of the animals. Direct observation using a video camera revealed that a lancelet frequently moves in the sediment only a short distance (a few centimeters at most) over the course of a day and rarely swims out from the sediment, and only for a few seconds. Sand ridges 4 cm high formed in the tank with lancelets at a density of 560 animals m^{-2} became almost level during an incubation period of 10 days, in contrast to a tank without lancelets, where there was no change. Dissolved O_2 concentration in the interstitial water of the sediment with lancelets was 2–5 times higher than that without lancelets at the end of incubation for 7 or 12 days. These results indicate that bioturbation by a dense population of lancelets has significant effects on the physical and chemical conditions of the sediment.

Key words: bioturbation, *Branchiostoma japonicum*, lancelet, sandbank, sediment condition

Introduction

A lancelet (=amphioxus) of the subphylum Cephalochordata is known as a biologically important invertebrate because of its phylogenetic position close to vertebrates. The lancelet *Branchiostoma japonicum* (Willey), formerly known as *Branchiostoma belcheri* (Gray) (Zhang et al. 2006, T. Nishikawa, personal communication), has been observed frequently in the shallow sandy bottom along the temperate coasts of Japan. However, its population in Japan has been greatly diminished as in the Chinese lancelet populations (Fang 1987). The main cause of the population reduction is environmental degradation of their habitats, especially an increase in the mud content of the sediment and a diminishment of their favorite sandy bottoms due to reclamation of shallow waters and sand extraction from the sea bottom since the 1960's (Nishikawa & Mizuoka 1990). In spite of this situation, our preliminary surveys in the shallow waters of the western Seto Inland Sea revealed that a dense population of *B. japonicum* still inhabits the remaining small sandbanks. The highest density of lancelets obtained by a Smith-McIntire grab sampler in a sandbank of our study site was over 4,000 animals m^{-2} (Ueda, unpublished data).

Bioturbation of marine sediments by benthic animals has been studied for more than a hundred years (see Cadée 2001). Ricketts et al. (1985) noted that the Californian lancelet *Branchiostoma californiense* Andrews "can burrow through packed sand as rapidly as most fish can swim." It is expected that such vigorous movements of lancelets have significant impacts on sediment conditions where dense lancelet populations exist. As for studies on bioturbation of lancelets, however, there have been only a few simple observations in the laboratory (Hagmeier & Hinrichs 1931, Schäfer 1962) and no quantitative studies on its effects on sediment. In the present study, we observed behavior of a lancelet in sediment using a video camera, and made two kinds of experiments in a small tank to evaluate the effects of their turbation on the sediment. One experiment was focused on physical effects, in which we measured degradation of sand ridges in a tank as an index of their turbation. The other concerned their effects on chemical conditions in the sediment, in which dissolved oxygen (DO) concentration in the sediment was measured. We present these results and discuss the ecological role of lancelets in a sandbank.

*Corresponding author: Hiroshi Ueda; E-mail, hueda@kochi-u.ac.jp

Materials and Methods

Lancelets and sediment used in the experiments were collected from a small sandbank in the western part of the Seto Inland Sea, about 1.5 km off the northwest coast of the mainland of Shikoku, Japan, using a Smith–McIntyre grab. The depth of the sampling site was about 12–13 m. Our monthly field surveys at the sandbank in 2003 revealed that sediment is composed mainly of very coarse sand, the median grain size of which is -1 to 0 in ϕ scale or 1 to 2 mm in diameter, and the larger part of the gravel-sized fraction consists of fragments of mollusk and barnacle shells.

All experiments and observations were carried out using plastic-framed glass tanks with a base area of 540 cm^2 (18 cm$\times$30 cm) and a height of 24 cm placed in a thermostatic room at 20°C. Sediment, from which lancelets were carefully removed by hand, was used without rinsing because Webb (1958) found that lancelets preferred unsieved natural sand with a fair organic content.

The direct observation on lancelet behavior in sediment was carried out in a transparent CD case (12 cm$\times$14 cm$\times$0.9 cm) filled with sediment and attached inside the glass of a tank. Many small holes were drilled on the lid of the case to pass water. A lancelet, captured on 13 June 2004 and preserved in a tank, was introduced into the case at 9:00 on 23 June. Its movement was recorded under constant darkness for 48 hours with a noctovision digital video camera (Sharp, VL-PD7), an infrared lamp (Sony, HVL-IRM), and a digital video recorder. In addition to the behavior in sediment, the number of lancelets swimming out from the sediment was recorded using the same video set. At 20:30 on 27 October 2005, fifty lancelets captured in the daytime of the same day were introduced into a tank with sediment more than 5 cm deep and equipped with an undergravel filtering system (UFS) to circulate water and maintain the water quality, and recorded until 6:00 on 30 October under the light condition of 12 hours of (about 6:00–18:00) dim light and 12 hours of dark. Movements of lancelets were observed in detail and counted on the video.

The experiment for physical effects of turbation was carried out twice. In the first experiment, thirty lancelets of about 4 cm in body length, which were collected on 30 July 2003, were put in a tank; the density of lancelets in the tank was 560 animals m^{-2}, which was within the range of those observed at the sandbank. The bottom of the tank was covered with sediment more than 5 cm deep and equipped with UFS. Light condition was set as a cycle of 12 hours of dim light and 12 hours of dark by covering tanks with a cardboard box during the night. After incubation for 19 days for acclimation to the tank, the experiment was started by making two sand ridges on the sediment at night on 18 August. Another tank with the same conditions but without lancelets was placed next to the tank with lancelets. Photographs of the ridges were taken with a digital camera fixed in front of the tanks twice a day without a flash light, soon after the cover was removed and soon before the cover was set. After 10 days, the levels of the top of each ridge and the lowest sediment surface (trough) between the ridges were measured to the nearest 0.5 mm on a display screen of a computer by comparing a ruler attached to the front glass of the tank. The height of a ridge was expressed as distance from the level of the trough to the top of the ridge. The initial ridge heights were 41 and 42 mm in the tank with lancelets and 23 and 27 cm in the tank without lancelets.

The second experiment was made in two tanks to know how long lancelets were active in a tank without UFS, as well as to confirm the result of the first experiment, because UFS could not be used for the experiment for chemical effect on sediment condition. In one tank, water was circulated by aeration with an airstone fixed at the water surface instead of UFS. Another tank was equipped with UFS as in the first experiment. Lancelets were captured on 22 May 2004 and incubated in a tank with UFS. The experiment was started in the evening of 24 May by making sand ridges and then introducing lancelets into both tanks, and continued for 12 days. The initial ridge heights were 25 and 28 mm in the tank without UFS, and 29 and 30 mm in the tank with UFS. Light condition was set as a cycle of 10 hours of

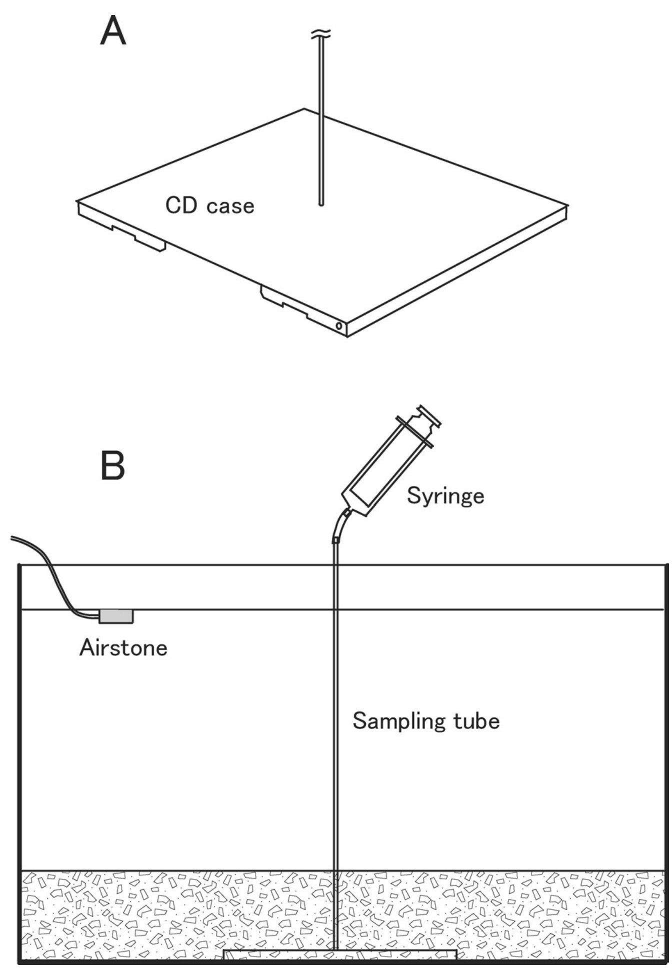

Fig. 1. Device to sample interstitial water in sediment (A) and its usage in the tank (B).

(about 9:00–19:00) dim light and 14 hours of dark. Other methods were as in the first experiment.

The experiment for chemical effects was performed three times in 2004. To measure DO concentration in sediment, a device to sample interstitial water of sediment was made using the body of a CD case (Fig. 1). A sampling tube of 35 cm long and 4 mm in inside diameter was attached to a hole bored at the center of the case. Interstitial water in sediment was introduced to the case through breaks in its side walls by suction with a syringe through the tube. Two tanks were set by placing the device on the bottom of the tank, adding sediment with a thickness of about 5 cm, and then filling with seawater gently. The water in the tanks was fairly turbid first due to resuspension of mud from the sediment; the mud content of sediment at the sampling station obtained in our 2003 survey ranged 0.9–2.6%. The first to third experiments were started on 26 June, 11 July and 29 July, respectively, by introducing 20 large (about 4 cm long) and 30 small (about 2 cm long) lancelets to one of two tanks; another one without lancelets was for the control. The lancelets used were collected on 13 June in the first experiment and 11 July in the second and third experiments, and preserved in a tank with UFS for 13, 0, and 18 days, respectively, until the experiment. During the experiment, water was gently aerated using an airstone fixed at the water surface level; strength of aeration in both tanks was checked every day. Light condition of the first experiment was set 24 hours dark, and those of the second and third experiment were as a cycle of 12 hours of dim light and 12 hours of dark. After 12 days (the first and second experiments) or 7 days (the third experiment) incubation, 30-ml waters were sampled from mid-water and sediment of each tank. The DO concentration was measured with a portable DO meter (YSI Inc., Model 58).

Results

Behavior of lancelets

The lancelet observed was completely buried in sediment and almost remained stationary except for momentary movements, the frequency of which therefore was easily counted. The movements were usually limited to a small distance (a few centimeters at most), and active swimming behavior in sediment as described by Ricketts et al. (1985) was never observed. The mean frequency of the movements for 48 hours was 9.6 times hour^{-1} (=230 times day^{-1}) (Fig. 2). The lancelet moved more frequently in the first 24 hours (380 times) than in the next 24 hours (181 times), and most frequently (33 times hour^{-1}) from 1:00 to 2:00 on 23 June.

The observation on lancelets swimming out from the sediment showed that no lancelets came out from the sediment during the first night after they were introduced. The total numbers of swimming-out behavior in the following day (6:00–18:00) and night (18:00–6:00) were 5 (day), 2 (night), 11 (day), and 8 (night), respectively. The mean

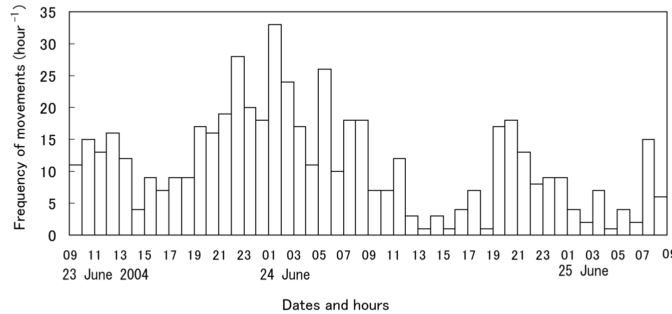

Fig. 2. Frequency of movements of *Branchiostoma japonicum* over 48 hours in sediment.

number of this behavior except for the first night was 0.26 times lancetet^{-1} day^{-1}. Lancelets always showed sudden appearance from the sediment as if they jumped to the water. They swum vigorously in the water in a moment and then dashed into the sediment.

Physical effects on sediment

In the first experiment, the surface of sediment in the tank with lancelets became almost level during the experiment, in contrast to the tank without lancelets (Fig. 3) where there was no significant change. Ridge heights in the tank without lancelets varied within a range of 1 mm except for the first night (Fig. 4). The increase in ridge height during the first night was due to the falling down of an unstable piece of gravel at the deepest point of the trough. In the tank with lancelets, the heights of the left and right ridges during the first night decreased sharply by 14 and 20 mm, respectively. Then the two ridges were lowered by 19 and 17 mm to the end of the experiment. The decrease was not linear, with relatively large drops on the second night (4 and 5 mm), the third day (4 mm in the left ridge), the fourth night (2.5 mm in the left ridge) and the ninth day (2 mm in the left ridge). Comparison of the successive photos showed that these intermittent drops resulted from movement of a piece of gravel at top of the ridges or depths of the trough.

The ridge heights in the second experiment continued to decrease in both tanks during the experiment (Fig. 5), indicating that lancelets were active in the tank without UFS until the end of the experiment. However, the decreasing pattern of the heights in the tank without UFS was somewhat different from the tank with UFS. In the former tank, the left ridge did not change for the first four days and then decreased its height gradually. The right ridge also did not show a significant change during four days from the second to seventh morning, though the ridge was apparently lowered for a day between the first and second mornings. The heights of both ridges decreased similarly from the sixth morning (30 May) to the end of the experiment. These data show that lancelets in the tank without UFS were inactive for the first five days.

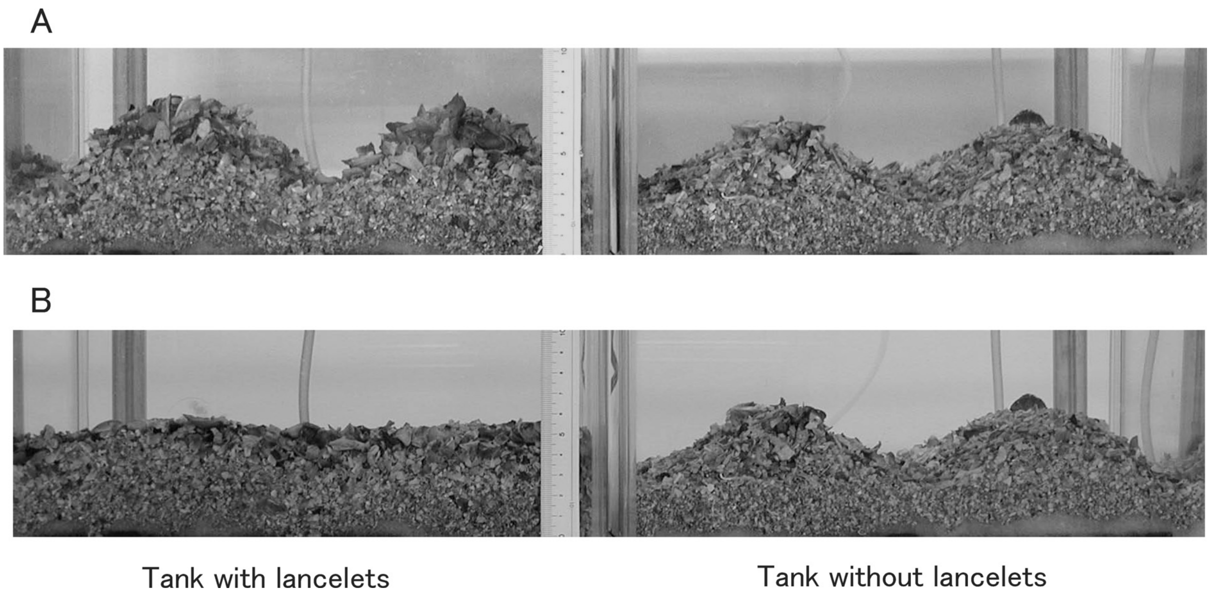

Fig. 3. Photographs of the sand ridges on the first day (18 August 2003) (A) and the last day (28 August 2003) (B) of the first experiment for physical effects of *Branchiostoma japonicum*.

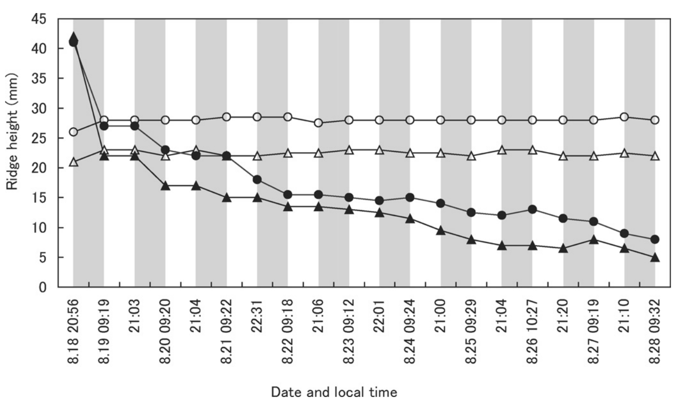

Fig. 4. Changes in ridge heights in the first experiment for physical effects of *Branchiostoma japonicum*. Filled and open symbols indicate the tanks with and without lancelets, respectively. Circles and triangles represent the left and right ridges, respectively. Shaded parts indicate the dark periods.

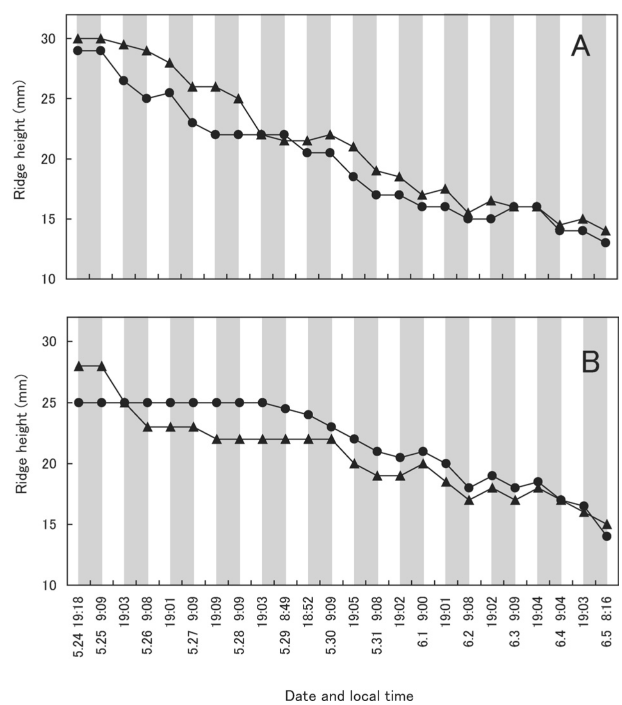

Fig. 5. Changes in ridge heights in the tank with (A) and without (B) an undergravel filtering system in the second experiment on physical effects of *Branchiostoma japonicum*. Circles and triangles represent the left and right ridges, respectively. Shaded parts indicate the dark periods.

Chemical effects on sediment

The DO concentration in sediment at the ends of the experiments for chemical effects was 2–5 times higher in the tank with lancelets than in the tank without lancelets (=control tank) (Fig. 6). It was also higher in water above the sediment in the tank with lancelets than in the control tank, but the difference was much smaller than that in the sediment except for the first experiment, in which the concentration was lower by 31% in the control tank than in the tank with lancelets. The DO concentration in the sediment in the tank with lancelets was higher in the second and third experiments than in the first. This is probably due to the fact that the lancelets used in the second experiment were caught only several hours before the experiment and therefore might be more active than those in the first experiment and that the incubation time was shorter in the third experiment than in the first. At the end of each experiment, a number of bacterial flocs were observed to cover the sediment in the tank without lancelets, but no flocs were seen in the tank with lancelets.

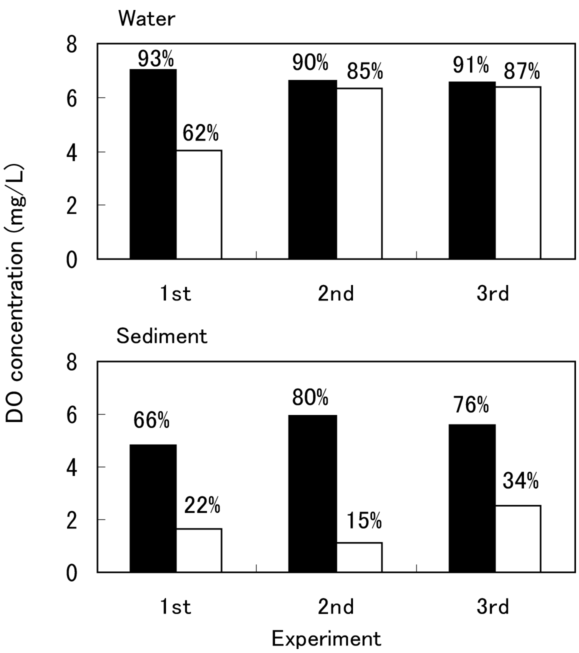

Fig. 6. DO concentration in water above sediment (top) and interstitial water of the sediment (bottom) at the ends of the experiments for chemical effects of *Branchiostoma japonicum*. Incubation times of the 1st to 3rd experiments were 12, 12, and 7 days, respectively. Filled and open bars indicate the tanks with and without lancelets, respectively. The DO saturation value is presented above each bar.

Discussion

The present study obviously revealed that sand ridges in a tank with lancelets became flattened day by day. This indicates that the physiological effect of their turbation is so significant that it makes a rugged surface of the sediment smooth in a short period of time. The observation of lancelet behavior suggests that ridge degradation is caused by two kinds of lancelet movements, i.e. small-distance movement in sediment and darts from/into the sediment when a lancelet migrates to water above in a moment. Short-time migration of lancelets into water has been known as spawning behavior at night (Wickstead 1975, Mizuta & Kubokawa 2004). However it is unknown for what purpose lancelets migrate into water in the tank, because they darted not only at night but during the day. As a single movement, the movement of dart from/into the sediment apparently gives much more impact than the small-distance movement in sediment. However, the contribution of the latter movement to turbation is probably greater than the former, because our data showed that frequency of the small-distance movement was three orders greater.

It was also obvious that DO concentration in sediment is maintained at a significantly higher level by the presence of lancelets. The most likely mechanism for this is turbation by lancelets, which enhanced exchange of interstitial water in sediment with water above the sediment. After the tanks were set, suspended mud was settled and covered the sediment several hours later. The settled mud, which is an obstacle to exchange of water through the sediment surface, disappeared in the tank with lancelets during the course of the experiment but remained in the tank without lancelets until the end of the experiment. Hagmeier & Hinrichs (1931) observed that mixed sand in a tank with lancelets became stratified with coarse sand moving upward and fine sand downward after 14-day incubation. Water movement in sediment may also be enhanced by breathing of lancelets, which take water through the mouth at the forehead and discharge it through the atriopore in the posterior body (Schäfer 1962).

The low DO concentrations in the tank without lancelets may be partly attributed to DO consumption by bacterial flocs on the sediment. The absence of bacterial flocs in the tank with lancelets is presumably due to their physical turbation, which disperses the flocs, and feeding on bacteria. Knowledge of lancelets' feeding habits is very limited. Jin & Guo (1953) examined gut contents of adult lancelets and identified benthic and planktonic diatoms, such as *Coscinodiscus* spp. and *Navicula* spp., and partly digested small zooplankton. However, it is unlikely that plankton is a major food for lancelets because they are completely buried in sediment where sand is suitable for them (Webb & Hill 1958). Planktonic forms found in guts of lancelets are seemingly those settled and fed with other benthic foods. Ruppert's (1997) electron microscopic observation on epipharyngeal food cord of lancelets indicated that it contained primarily bacteria. Although bactivorous feeding of lancelets is still hypothetic, the absence of bacterial flocs and higher DO concentration in sediment in the tank with lancelets indicate that turbation by lancelets has the ability to change and maintain the chemical condition of sediment better for them.

Webb (1969) thought that sorting grains into layers by turbation of lancelets changes the characteristics of sand, and that alternation of sand properties through sorting has been a contributory factor in the disappearance of the majority of *Branchiostoma nigeriense* population from Lagos Lagoon, Nigeria, because any change of sand under nearly optimal conditions is deleterious. Patchy microdistribution of lancelets in sands was also considered by Webb due to sorting of grains by turbation of lancelets. In our study, however, improvement of conditions in sediment appears to be properly evaluated as turbation of lancelets rather than deleterious effects on sediment. The sand ridges in the experiment for physical effects continued to be lowered by completely buried lancelets until the end of the experiment. This indicates that lancelets maintained their activity without environmental degradation throughout the experiment. We have had experience of a long (more than a month) incubation of lancelets in a tank with a large volume of mixed sand and UFS, and observed no noticeable consolidation of the sediment after the incubation. The clearest evidence is the present results that DO concentration in sediment with lancelets was kept higher than that without lancelets. Nara et al. (2002) studied tolerance of lancelets for covering with mud on sediment and found that all lancelets died in coarse sand covered with sandy mud more than 3 cm thick, suggesting that low DO concentration is fatal to lancelets. To maintain DO concentration in their habitat may be the most important evolutionary advantage of their activity in sediment.

The present study revealed that bioturbation by a dense population of lancelets affects noticeably both physical and chemical conditions of sediment even on a day scale. Although the population of lancelets has declined in Japan, it is observed in our study site that the dense population still remains at sandbanks as the dominant member of macrobenthic animals. The Seto Inland Sea has numerous islands and large tidal range, which is about 4 m at the maximum spring tide and creates strong tidal currents in channels between these islands. The channels usually have sandbanks nearby, which have been formed by erosion of the bottom and transportation of coarse sands and shell fragments due to the strong currents (Inouchi 1990). These sandbanks are the most suitable habitat for lancelets and expected to play a role in seawater purification because the swift water current above sandbanks and their coarse sand would allow water to pass through sands easily and therefore enhance decomposition of organic matters by aerobic bacteria in sediment. Bioturbation by lancelets would help the activity of seawater purification of sandbanks by sorting sands and maintaining DO concentration in sediment at a higher level. Thus the present study indicates that a lancelet is an important animal not only for its phylogenetic position but for its ecological role, similar to that of an earthworm under the ground.

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References

Cadée GC (2001) Sediment dynamics by bioturbating organisms. In: Ecological Comparisons of Sedimentary Shores (ed K Reise K). Springer, Berlin, pp 127–147.

Fang YQ (1987) Ecological habits and conservation of lancelet. Chin J Zool 22: 41–45. (in Chinese)

Hagmeier A, Hinrichs J (1931) Bemerkungen uber die Ökologie von *Branchiostoma lanceolatum* (Pallas) und das Sediment seines Wohnortes. Senckenbergiana 13: 255–267.

Inouchi Y (1990) Origin of sand and its distribution pattern in the Seto Inland Sea, Southwest Japan. Bull Geol Surv Jpn 41: 49–86.

Jin D, Guo R (1953) Lancelets in Amoy. Acta Zool Sin 5: 65–78. (in Chinese)

Mizuta T, Kubokawa K (2004) Non-synchronous spawning behavior in laboratory reared amphioxus *Branchiostoma belcheri* Gray. J Exp Mar Biol Ecol 309: 23–251.

Nishikawa T, Mizuoka S (1990) Past, present and future of two localities of the lancelet *Branchiostoma belcheri* Gray, designated to natural monuments in Japan. Coll Breed 52: 152–155. (in Japanese)

Nara M, Watanabe H, Inouchi Y, Kutsuna S, Yanagisawa A (2002) Tolerance of the lancelet *Branchiostoma belcheri* to rapid burial with coarse- and fine-grained clastics. Jpn J Benthol 57: 97–105.

Ricketts EF, Calvin J, Hedgpeth, JW (1985) Between Pacific Tides (5th edition). Stanford University Press, Stanford, 652 pp.

Ruppert EE (1997) Cephalochordata (Acrania). In: Microscopic Anotomy of Invertebrates, Vol. 15 (ed Harrison FW, Ruppert EE). Wiley-Liss, New York, pp. 249–504.

Schäfer W (1962) Ecology and palaeoecology of marine environments. 568 pp. (English translation, University of Chicago Press, Chicago, 1972)

Webb JE (1958) The ecology of Lagos Lagoon V. Some physical properties of lagoon deposits. Philos Trans R Soc Lond B 241: 393–419.

Webb JE (1969) Biologically significant properties of submerged marine sands. Proc Roy Soc Lond 174: 355–402.

Webb JE, Hill MB (1958) The ecology of Lagos Lagoon IV. On the reactions of *Branchiostoma nigeriense* Webb to its environment. Philos Trans R Soc Lond B 241: 355–391.

Wickstead JH (1975) Chordata: Acrania (Cephalochordata). In: Reproduction of marine invertebrates. Vol. 2. Entoprocts and lesser coelomates (ed Giese AC, Pearse JS). Academic Press, New York, pp. 283–319.

Zhang QJ, Zhong J, Fang SH, Wang YQ (2006) *Branchiostoma japonicum* and *B. belcheri* are distinct lancelets (Cephalochordata) in Xiamen waters in China. Zool Sci 23: 573–579.