# Preliminary Study on the Bioturbation Activity of the Subcrenated Ark Shells, *Scapharca kagoshimensis*

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*Abstract:* The physical and chemical changes of sediment after rearing of the subcrenated ark shell *Scapharca kagoshimensis* for one week in a laboratory were examined. Surface sediments (layer 0-2 cm in depth) were used for the analysis of moisture content, loss on ignition, acid-volatile sulphide and redox potential. It was found that moisture content and loss on ignition did not show significant differences between the controls (initial and control groups) and the experimental groups (ark shell groups). The levels of acid-volatile sulphide in the experimental samples were not lower than those in the controls, and the redox potentials of the groups were not higher than those of the controls. These results suggest that the bioturbation activity of subcrenated ark shells has limited effect on bottom sediments.

**Keywords:** *Scapharca kagoshimensis*, subcrenated ark shell, bioturbation

*Abstrak*: Perubahan fizikal dan kimia sedimen selepas seminggu kerang bulu, *Scapharca kagoshimensis* diternak dalam makmal telah diperiksa. Permukaan sedimen (kedalaman lapisan 0-2 cm) telah digunakan untuk analisis kandungan kelembapan, *loss on ignition*, asid meruap sulfida dan potensi redoks. Keputusan yang didapati menunjukkan kandungan kelembapan dan *loss on ignition* tidak menunjukkan perubahan yang signifikan antara kawalan (permulaan dan kumpulan kawalan) dan kumpulan ujikaji (kerang bulu). Paras asid meruap sulfida dalam sampel-sampel ujikaji tidak rendah daripada kumpulan kawalan dan potensi redoks dalam sampel-sampel ujikaji tidak melebihi daripada kumpulan kawalan. Keputusan ini mencadangkan *bioturbation activity* kerang bulu mempunyai kesan yang terhad terhadap sedimen dasar.

## Introduction

Feeding, nesting and burrowing of benthic organisms, such as shellfish, polychaetes and crustaceans, in tidal flats involve bioturbation of sediment. These activities affect both biota and non-living features of the environment (Kikuchi and Mukai, 1994; Widdicome and Austin, 1999; Widdicome *et al.*, 2000; Tamaki, 2005; Kogure and Wada, 2005; Kawaguchi and Baba, 2007; Otani *et al.*, 2009). There have been many case studies on bioturbation in benthic animals; in the case of the ocypodid crab *Macrophthalmus japonicus*, the burrowing volume of sediment has been estimated from the nest hole diameter and the volume of expelled mud (Otani *et al.*, 2009). Additionally, in the case of the soldier crab *Mictyris longicarpus* var. *brevidactylus*, the turbation activity was found to have a reducing effect on silt clay particles and an oxidating effect in terms of the redox potential in sediment via its feeding behavior (Kawaguchi and Baba, 2007). On the other hand, the polychaete *Capitella* sp. I is released to organic mud was found to suppress the levels of organic carbon, organic nitrogen and acid-volatile sulphide in the mud by its feeding activity (Montani and Tsutsumi, 1996).

In terms of the turbation effect of bivalves, there have been a few case studies on the soft-shell clams *Mya arenaria* and *Macoma balthica*. The burrowing activity by these clams to produce their holes was found to affect the nitrate concentration in interstitial water (Henriksen *et al.*, 1983). In the case of *Mya arenaria*, this clam burrows into bottom sediment to a depth of about 18 cm, and the burrowing activity increases the nitrate ion concentration and nitrification rate in the interstitial water in pores around the hole.

Additionally, in the case of *Macoma balthica*, which burrows to a depth of about 3 cm, the nitrification rate was found to increase in the surface layer to a depth of about 2 cm (Henriksen *et al.*, 1983). These results suggest that the production of a hole leads to the permeation of oxygen into the sediment and an increase in the nitrification rate due to the activation of aerobic nitrifying bacteria in this newly oxidative environment (Uchiyama *et al.*, 1999).

The annual catches of the subcrenated ark shell *Scapharca kagoshimensis* and the Manila clam *Ruditapes philippinarum* in Ariake Bay, Kyushu, west Japan have each dropped below 10,000 tons in recent years (Sekiguchi and Ishii, 2003; Masaki and Onohara, 2003). For this reason, sustainable production of these bivalves is a very important issue in this region. The decrease in the size of these catches was suspected to have been due to several types of environmental degradation (Ito, 2004; Fujita *et al.*, 2007; Higano *et al.*, 2010) such as red tide (Tsutsumi *et al.*, 2005; Yamatogi *et al.*, 2006), hypoxic water mass (Kimoto *et al.*, 2003; Matsuoka *et al.*, 2005) and reduction of sediment (Okamura *et al.*, 2006) in summer. However, these bivalves in the interior part of this bay have an important ecological position in terms of the circulation of material in the water column, and play a role in maintaining the environment at this site (Tanaka and Ohtsuka, 2000; Nakamura, 2004). In particular, the feeding activity of bivalves has drawn attention from researchers as a potential environmental remediation method (Matsuda, 2010).

In recent years, to focus on the environmental remediation ability of benthic animals in the interior of Ariake Bay, several experiments have been undertaken, such as estimation of the water-filtering activity of subcrenated ark shells under various conditions (Nakamura, 2005), a study on the creation of a bivalve fishing ground (Ohkuma *et al.*, 2004) and efforts to improve the environment using oysters of *Crassostrea* spp. (Ogawa *et al.*, 2007; Iyo-oka *et al.*, 2008). In addition, Tsujo and Ariyoshi (2009) focused on the bioturbation activity of the jackknife clam, *Sinonovacula constricta*, in terms of its potential for environmental improvement in Ariake Bay; they estimated its bioturbation activity by measuring the redox potential on the tested sediment. Redox potential was found to have been clearly changed by the bioturbation after one week; it was increased from lower than -100 mV in the original reduced sediment to oxidized sediment at over 300 mV. This result suggests that bioturbation by the jackknife clam has an oxidative effect on reduced sediment. However, the catch of jackknife clams in Ariake Bay, including along the previous major fishing grounds of the Saga Prefecture coast, has rapidly decreased from 500 tons in the late 1980s to almost zero in recent years (Nakano, 2008). Against this background, if there is a possibility that sediment could be improved by bivalves in a wide area across Ariake Bay, we need to have information about the bioturbation activities among other bivalves. In this paper, we focus on subcrenated ark shells, which are important for fisheries on the muddy bottom of Ariake Bay. The bioturbation activities of these shells were estimated using several sediment-related indicators, such as moisture content, loss on ignition, acid-volatile sulphide and redox potential.

## Materials and methods

*Bivalves and sediments*

Subcrenated ark shells (shell length: 3.0-3.7 cm), collected from Ariake Bay in late fall, were used for this study. These specimens were transported to an indoor tank and reared in small cases filled with anthracite grains (diameter 1.5 mm, Tochemi Co. Ltd., Japan). During the rearing, these specimens were provided with alga-free seawater at a temperature of 26.5°C (salinity, 34 PSU) and aerated for a week. This initial preparation was intended to prevent the influence of excretion from the shells during the experiment and to screen for healthy specimens that could burrow into the bottom.

Sediments were collected as medium-grained sand (sandy mud: mud content about 20%; Suzuki *et al.*, 2009) and silty clay (mud: mud content over 80%; Suzuki *et al.*, 2009) using a handy bottom sampler in offshore areas near Arao City, Kumamoto Prefecture, and Kashima City, Saga Prefecture, in Ariake Bay at almost the same time of year as the bivalve sampling. The collected sediments from near Arao and Kashima were filtered through a sieve with mesh size 5 mm and a sieve with mesh size 1 mm, respectively. The treated sediments were kept in a closed vessel at room temperature for over a week until the examination.

*Observation of specimens under experimental conditions*

The survival status of the subcrenated ark shells during the experiment was estimated by observation of their breathing hole formation and reaction to needle stimulation. If mortality occurred, the number of dead individuals was counted but the examination was continued without the removal of dead individuals.

*Experimental method*

Cylindrical containers with a diameter of 16 cm and a height of 11 cm were filled with various sediments to a height of 6 cm (Fig. 1A). These containers were immersed in a water bath (92 × 62 × 21 [height] cm, volume 78 l) and kept in alga-free seawater maintained at a set temperature by heating (Fig. 1B). The temperature and salinity of the water bath were shown to have the ranges of 26.4 ± 0.2°C and 34.1 ± 0.1 PSU, respectively, by measurements using a sensor (sensION5; Hach Co. Ltd., USA) at the beginning and the end of the experiment. The flow of seawater into the bath was set at approximately 1 l/min, and an airstone was placed at the feedwater inlet along with a net to avoid bubbles interfering with the observation of the bivalves. The next day, the weeklong bioturbation experiment was started, at which point the subcrenated ark shells were placed at equal density inside the cylindrical containers. For each sediment type, the test sediment for the subcrenated ark shells was arranged into two groups: one was a control group into which shells were not placed, while the second group was a test group into which subcrenated ark shells were placed (n = 18). The total volume of the bivalves was adjusted to 120 ml in a test group.

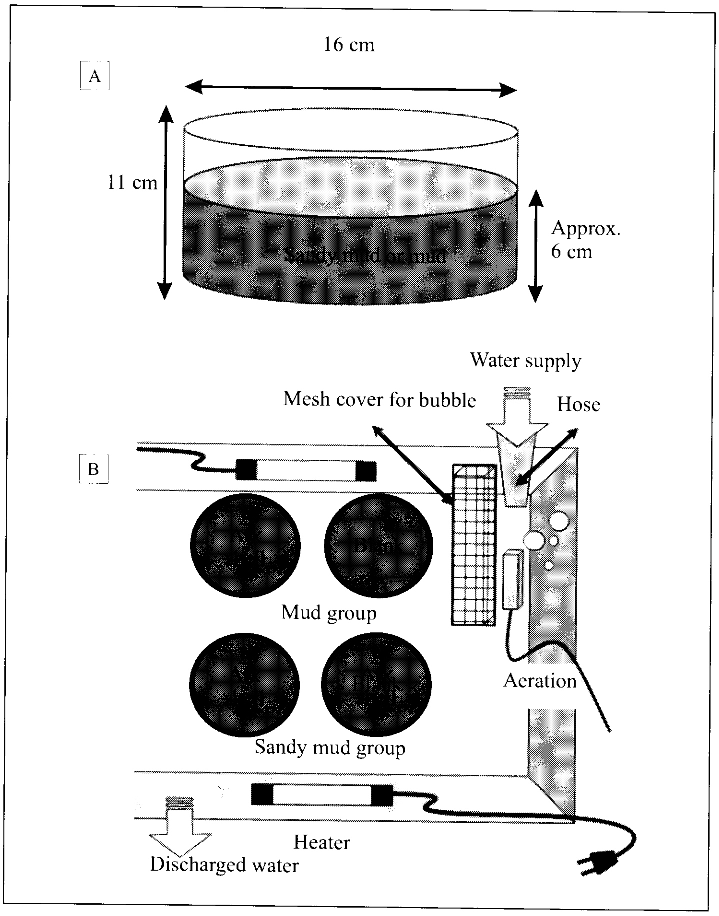


**Figure 1:** Schemes of the bioturbation effect experiment used for the subcrenated ark shell *Scapharca kagoshimensis* as a bioturbator. Scheme A is a cylindrical container filled with sediments. Scheme B is a placement of the instruments for the experiment

*Sediment sampling in the experiment*

Samples for the analysis of moisture content, loss on ignition and acid-volatile sulphide were collected from sediment up to a depth of 2 cm from the surface bottom using a plastic syringe (1.5 cm in diameter) removed the tip. In each individual experiment, the core samples were randomly collected at both the start and the end of the weeklong experimental period. In addition, the initial measured values at the start of the experiment were pooled together for all individual experiments (mean ± standard deviation). An electrode for measuring redox potential was directly inserted into the mud or sandy mud bottom in the container, and the potential at a depth of 1 cm from the surface was measured. The measurement was randomly carried out in the same way in each group. In addition, the values at the start time from all groups were pooled together (mean ± standard deviation).

*Analysis of sediment quality*

A part of the sediment sample was used for measurements of fresh wet weight and dry weight, the weighed fresh sediment was dried in an oven at 60°C overnight and the two weights were used to calculate moisture content (%). The dried sediment was then ignited in an electric furnace at 550°C for six hours, and the decrease of weight of the sample was used as the loss on ignition (%). The content of acid-volatile sulphide in sediment was measured by a glass tube 201H or 201L (Gastec Co. Ltd., Japan; Montani, 2003). A part of the same sediment was used for the measurement of fresh wet weight and dried weight in the same manner as described above, and then the amount of sulphide contained in 1 g (dry weight) of sediment was determined (mg/g-dry). Redox potential in 1 cm layer of surface sediment was measured using an Oxidation-Reduction Potential (ORP) sensor (RM-20P; TOA-DKK, Japan). Additionally, the redox potential was determined using the electric potential of hydrogen by the calculation of measurement potential and mud temperature

*Statistical analysis*

Comparisons of moisture content, loss on ignition, acid-volatile sulphide and redox potential in sediments of the initial and examined groups (control and bivalve groups) were performed using one-way ANOVA and/or multiple Tukey–Kramer test with significance set at $P < 0.05$.

## Results

*Survival status of bivalves*

Mortality among the subcrenated ark shells was observed in 6 out of 80 individuals in the control group reared with anthracite grains in the water tank. On the other hand, in the experimental samples, no dead individuals were observed in the Sandy mud group and one was observed in the Mud group out of a total of 18 individuals in each. Therefore, the mortality rate in each experimental group was lower than that in the control.

*Moisture content*

As shown in Fig. 2, moisture contents before the experiment in the initial sediments of sandy mud and mud groups were 44 ± 2% ($n = 9$) and 70 ± 2% ($n = 9$), respectively. The levels in the examined control samples lacking bivalves were 43 ± 1% ($n = 3$) and 69 ± 2% ($n = 3$) in the Sandy mud and Mud groups, respectively. These results showed no significant differences between the examined control and the initial sediments. In addition, there were no significant differences between the experiments with bivalves (sandy mud 43 ± 4% [$n = 3$], mud 69 ± 2% [$n = 3$]) and the control samples.

In the experimental group, the subcrenated ark shell *Scapharca kagoshimensis* was used as a bioturbator. Graph A is for the group with sandy mud. Graph B is for the group with mud. Means (large bars) and standard deviations (small bars) without common letters above the bars show significant differences between treatments, as detected using ANOVA; treatments with the same letter are not significantly different ($P < 0.05$) from each other. Numbers in parentheses indicate the number of repetitions.

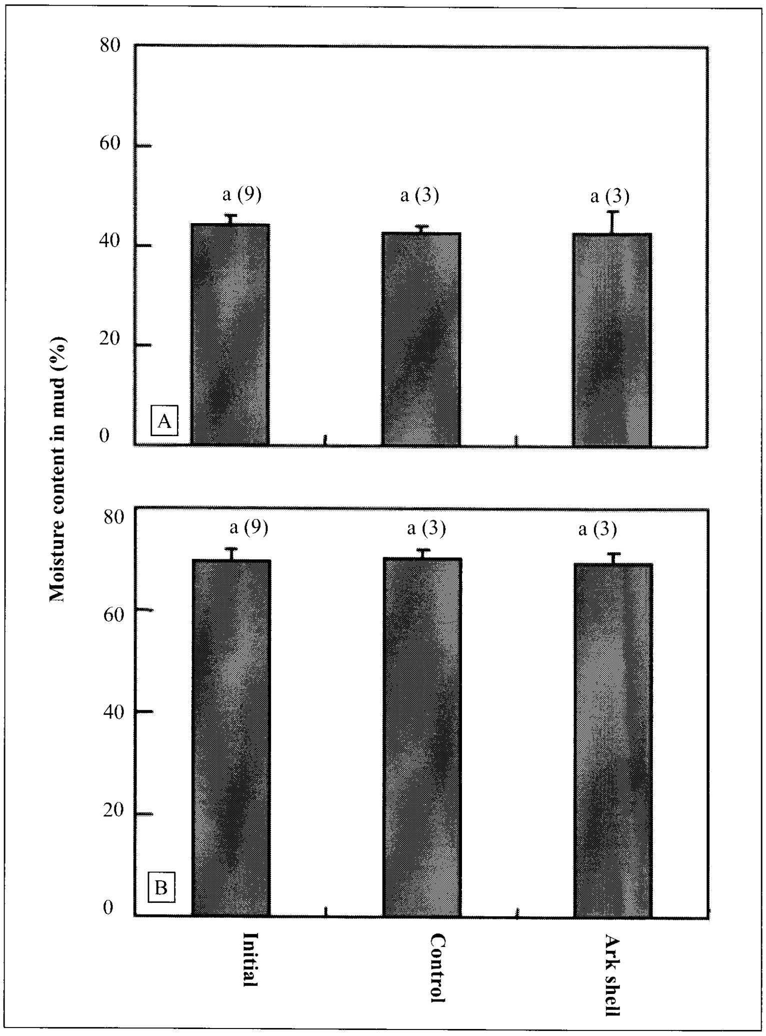


**Figure 2**: Moisture content (%) of the surface sediments in control and experimental samples as well as in initial samples

*Loss on ignition*

As shown in Fig. 3, losses on ignition in the initial and examined control sediments of sandy mud and mud groups were 6.5 ± 0.3% ($n$ = 9) and 13.0 ± 1.6% ($n$ = 9), respectively. The values in the examined control samples were 7.1 ± 0.2% ($n$ = 3) and 12.8 ± 0.2% ($n$ = 3) in the sandy mud and mud groups, respectively. These results showed no significant differences between the initial and control sediments. Furthermore, there were no significant differences between the experimental samples (sandy mud 6.8 ± 0.4% [$n$ = 3], mud 12.5 ± 0.4% [$n$ = 3]) and the examined control samples.

Graph A is for the group with sandy mud. Graph B is for the group with mud. Means (large bars) and standard deviations (small bars) without common letters above the bars show significant differences between treatments detected using ANOVA; treatments with the same letter are not significantly different ($P < 0.05$) from each other. Numbers in parentheses indicate the number of repetitions.

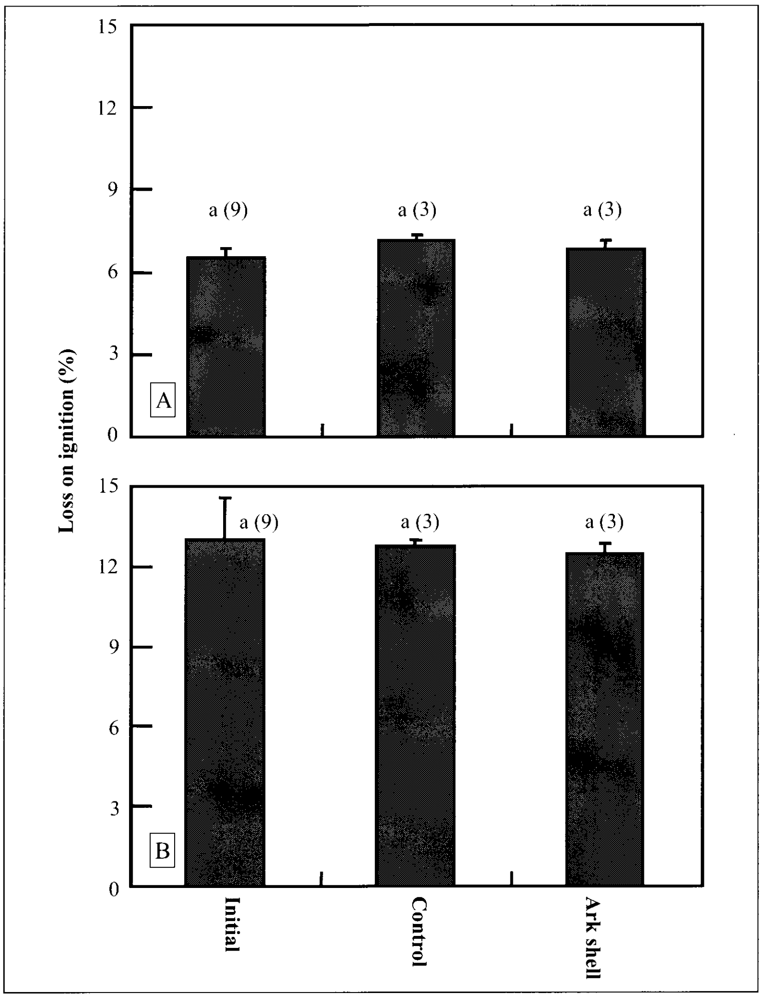


**Figure 3**: Loss on Ignition (%) of the surface sediments in control and experimental samples as well as in initial samples

*Acid-volatile sulphide*

As shown in Fig. 4, acid-volatile sulphide levels in the initial sediments of Sandy mud and Mud groups were 0.10 ± 0.02 mg/g-dry ($n$ = 9) and 0.57 ± 0.06 mg/g-dry ($n$ = 9), respectively. The values in the control samples were 0.11 ± 0.01 mg/g-dry ($n$ = 3) and 0.53 ± 0.15 mg/g-dry ($n$ = 3) in the sandy mud and mud groups, respectively. These results showed no significant differences between the initial and control sediments. In addition, there were no significant differences between the experimental samples (sandy mud 0.11 ± 0.03 mg/g-dry [$n$ = 3], mud 0.63 ± 0.07 mg/g-dry [$n$ = 3]) and the control samples.

In the experimental group, the subcrenated ark shell *Scapharca kagoshimensis* was used as a bioturbator. Graph A is for the group with sandy mud. Graph B is for the group with mud. Means (large bars) and standard deviations (small bars) without common letters above the bars show significant differences between treatments detected using ANOVA; treatments with the same letter are not significantly different ($P < 0.05$) from each other. Numbers in parentheses indicate the number of repetitions.

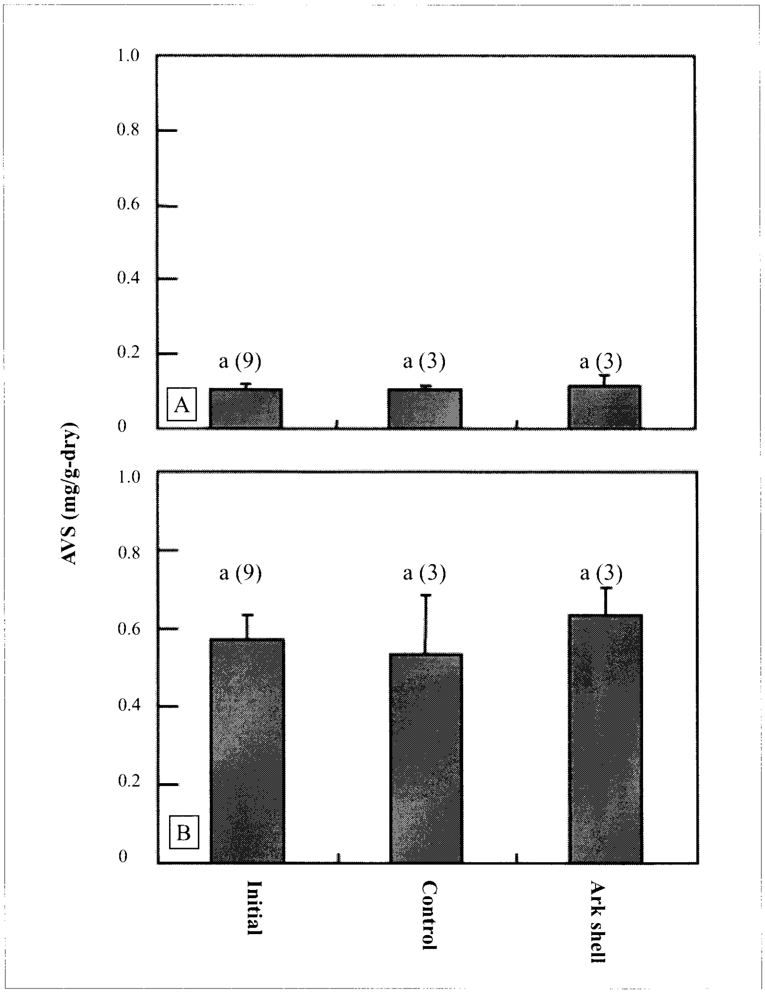


**Figure 4**: Acid-volatile sulphide content (mg/g-dry) of surface sediments in control and experimental samples as well as in initial samples

*Redox potential*

As shown in Fig. 5, redox potential in the initial sediments of sandy mud and mud groups were -28 ± 38 mV ($n = 9$) and 4 ± 62 mV ($n = 9$), respectively. The values in the control samples were 24 ± 44 mV ($n = 3$) and -30 ± 23 mV ($n = 3$) in the sandy mud and mud groups, respectively. These results showed no significant differences between the initial and control sediments. In addition, there were no significant differences between the experimental samples (sandy mud -23 ± 28 mV [$n = 3$], mud -69 ± 17 mV [$n = 3$]) and the control samples.

In the experimental group, the subcrenated ark shell *Scapharca kagoshimensis* was used as a bioturbator. Graph A is for the group with sandy mud. Graph B is for the group with mud. Means (large bars) and standard deviations (small bars) without common letters above the bars show significant differences between treatments detected using ANOVA; treatments with the same letter are not significantly different ($P < 0.05$) from each other. Numbers in parentheses indicate the number of repetitions

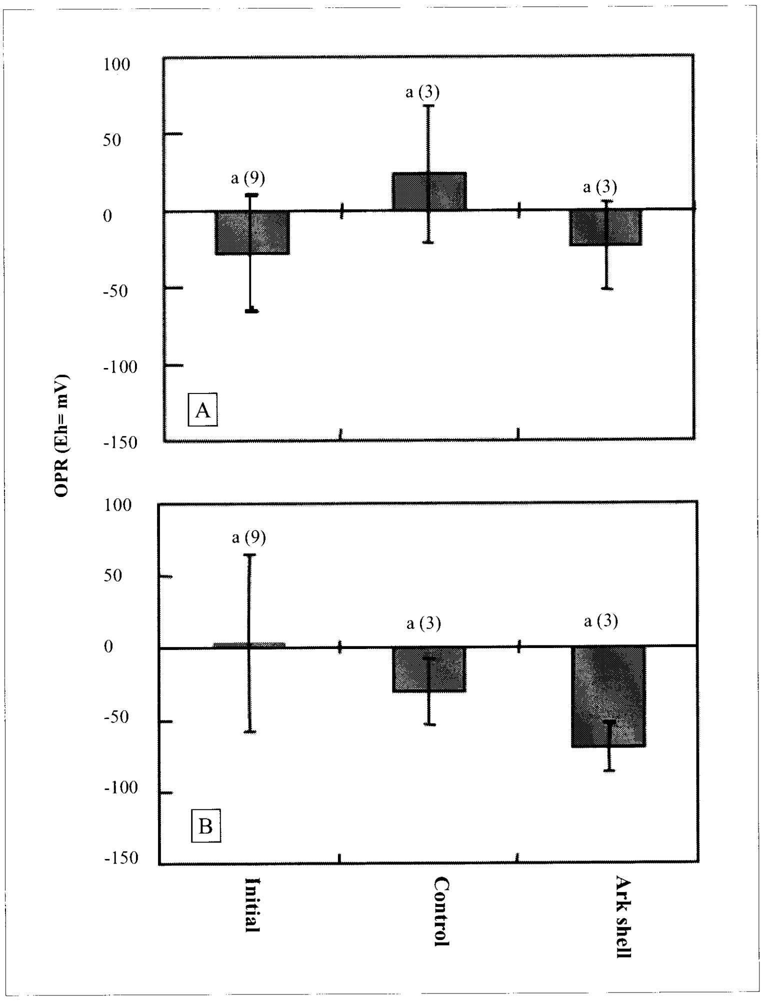


**Figure 5**: Redox potential (Eh = mV) of surface sediments in control and experimental samples as well as in initial samples

## Discussion

On the observation of specimens under experimental conditions, all mortality rates were lower than 8% and mortality was observed in 6 out of 80 individuals in the control group reared in a water tank. None of 18 individuals in the sandy mud group and 1 out of 18 individuals in the mud group died. These results indicate that the conditions applied in this experiment, namely, sandy mud and mud sediments, had little impact on the survival of the bivalves.

In the cases of the polychaetes and soldier crab, their turbation activities on sediment were previously shown to have a number of beneficial effects, in terms of the contents of acid-volatile sulphide, organic carbon and organic nitrogen, as well as the grain size distribution of sediment and the oxidation level in terms of redox potential (Kawaguchi and Baba, 2007; Ohtani *et al.*, 2009). In this experiment, moisture content and loss on ignition were employed as useful indices of sediment quality to reflect bioturbation activity because these variables are closely related to the consolidation of sediment and the elution of organic matter from sediment. However, neither of these indices showed a significant difference between the control and experimental groups, and the bioturbation effect on sediment by the subcrenated ark shells was very weak (Fig. 2 and 3). In addition, observation of the bivalves showed that many individuals burrowed into the surface sediment and then maintained the same position for several days. From this finding, if we expect changes on values of moisture content and loss on ignition, it is more intensive or widespread bioturbation might be required in order to see changes in moisture content and loss on ignition

The presence of a high level of acid-volatile sulphide in sediment, which mainly binds to minerals like iron, reflects the generation of hydrogen sulphide gas by reducing bacteria under a reducing environment (Hibino and Matsumoto, 2006). This substance is susceptible to oxidative decomposition in air (Satoh *et al.*, 1998). However, in this experiment, the levels in the sandy mud or mud groups were not significantly decreased compared with other samples (Fig. 4). Furthermore, only one or no cases of mortality occurred during the experiment. This indicates that the breakdown of a dead individual was unlikely to have affected the volume of acid-volatile sulphide in the sediment. This finding may explain why the subcrenated ark shells did not exhibit significant bioturbation activity on sediments, such as in terms of oxidation of acid-volatile sulphide.

Redox potential, reflecting the potential difference between oxidative and reduced substances, is a useful index with which to evaluate sediment (Hibino and Matsumoto, 2006). In the present study, the experimental groups in sandy mud and mud did not show significant differences with the control groups (Fig. 5). Additionally, the both groups showed low values of redox potential compared with the control samples. In the case of the jackknife clam, the redox potential in a similar experiment showed significant changes of more than 300 mV from reduced sediment as a result of bioturbation for a week (Tsujo and Ariyoshi, 2009). This may be because, in the wild, the jackknife clam burrows down to form a clam hole of over 30 cm in depth in muddy sediment. In contrast, in the case of subcrenated ark shells, the holes they form to live in are unclear and very shallow (Tsujo and Ariyoshi, 2009). For this reason, the oxidating effect on reducing sediment is small in subcrenated ark shells.

To summarize the findings on the bioturbation activity of subcrenated ark shells, this study did not show a clear improvement in terms of reducing sediment. In particular, the analysis results on redox potential and acid-volatile sulphide content suggested that the oxidating effects of ark shells are very small.

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