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Effects of Clamworm, *Perinereis aibuhiteusis*, Density on Acid Volatile Sulfide, Chemical Oxygen Demand, and Loss of Ignition in Benthic Sediment, and on Survival of Scallop, *Chlamys farreri*, Spat

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Bivalve aquaculture provides more and more food for global demand (FAO 2002). Influences of bivalve farming on sediment eutrophication are increasingly being investigated. Although benthic effects of bivalve farming were considered to be less than aquaculture of fish or crustaceans, due to the fact that diets are not fed, studies have shown that the effects cannot be neglected and sometimes can cause serious impacts on local environment (Christensen et al. 2003; Giles et al. 2006).

Sites where bivalve farms are located may have increased sedimentation of organic materials (such as feces), due to the concentration of organisms (Christensen et al. 2003). Previous studies have shown that the sedimentation rate underneath bivalve farms was two to three times higher than that of the surrounding area (Dahlbäck and Gunnarson 1981; Grant et al. 1995). The increased organic sedimentation results in benthic eutrophication. Benthic eutrophication can alter the oxygen dynamic, nutrient cycles, and benthic communities of microbiota and macrobiota (Grall and Chauvaud 2002), while in extreme circumstances, hypoxia and mortality of benthic organisms could occur (Gray 1992).

Increased organic input has been reported to increase nutrient turnover in bottom sediments because of increased oxygen consumption in the soil, with a concomitant increase in nitrogen recycling rates. The rates of these processes, which are mainly due to bacterial activities, are dependent upon many factors including temperature, light conditions, and currents.

There have been several reports on the effects of exchange and vertical mixing of seawater, and the addition of microbiological and benthic macrofauna to the ocean sediment to improve and/or restore the biodiversity (Chareonpanich et al. 1994; Snelgrove and Butman 1994; Michio et al. 2003). This could prevent an incident where the benthic substrate environment could become anoxic.

Among these methods, the effects of deposit feeders have attracted more and more attention due to the long effect and less damage for natural habitats. Deposit feeders can reduce the organic matter and improve sediment quality by bioturbation and feeding activity. Bioturbation can greatly influence the oxic conditions of surface sediments through transporting oxygen and reworking sediment (Aller 1994). Moreover, feeding activity results in the direct ingestion of benthic sediments and its associated organic matter. This organic material is transformed during the digestion process to other materials

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that can be utilized by other benthic organisms. Careful selection of deposit-feeding organisms for use in bioremediation of eutrophic ocean sediments needs to be conducted because the distributions of deposit-feeding organisms are commonly area specific.

There are parameters that can be measured to determine whether organic compounds are accumulating in bottom sediments. Acid volatile sulfide (AVS) is an important indicator of bioavailable toxic trace metals and is used in sediment studies to predict the toxicity of metal-contaminated sediments. The bioavailability of metals (such as cadmium, copper, nickel, lead, and zinc) in sediments is largely controlled by the available amount of reactive sulfide or AVS. Metal-contaminated sediments that have high AVS levels accumulate trace metals as sulfides, which are not readily available. Chemical oxygen demand (COD) is used to indirectly measure the amount of organic compounds in water or sediments and indicates the oxygen consumed. Loss of ignition (LOI) is commonly used to estimate the organic and carbonate content of sediments.

The clamworm, *Perinereis aibuhiteusis*, is a deposit-feeding species, commonly found in marine sediments, both in intertidal and subtidal zones. As a native species, use of *P. aibuhiteusis* for bioremediation in this region will not cause the misgivings regarding the use of a foreign species. Scallops, *Chlamys farreri*, are produced offshore in Namhae Island, South Korea. Below the scallop farms, sediments have been subjected to a high degree of eutrophication due to the scallop fecal and pseudofecal materials, as well as scallop and epibiota mortality. Thus, research into remediation methods is essential. There is no previous study determining the ability of *P. aibuhiteusis* to decompose organic matter caused as a result of *C. farreri* farming.

In the present study, an evaluation of the bioremediation effect of *P. aibuhiteusis* on benthic sediments and the effect on survival of cultured scallop, *C. farreri* were discussed. Furthermore, the relationship between decreasing tendency of organic matters in sediments and the density of clamworm and elapsed time were estimated under laboratory conditions.

Materials and Methods

Sediment Collection and Disposal

Eutrophic sediments were collected from Namhae Island, South Korea. An Eijkelkamp core sampler was used to collect the sediment samples. After collection, the sediment cores were immediately placed in an insulated box and transported to the laboratory. Upon arrival, sediment samples were sieved so as to pass through a 1-mm mesh screen, thoroughly mixed to homogenize the sample, and frozen at −50 C for 48 h to kill infaunal organisms. Frozen sediments were laid into polystyrene tanks (100 cm × 50 cm × 40 cm) with a depth of 12 cm, and then 150-L filtered seawater (salinity 32 ppt; pH 7.8) was added. The temperature of the laboratory was maintained constant at 20 C.

Collection of Scallop and Clamworm

Scallop spats (shell width 13.6 ± 2.3 mm; tissue weight 1.1 ± 0.2 g) were collected from the same location as sediment described previously. After collection, scallop spats were transported to the laboratory. Upon arrival, spats were acclimated for 7 d in filtered sea water. Spats were maintained in suspension and fed every 2 d a mixture of microalgae consisting of *Chaetoceros* sp. and *Isochrysis* sp. at an approximate concentration of 3×10^4 cells/mL. Water from tanks was replaced every 2 d prior to the addition of microalgae. Clamworms (body length 16.0 ± 1.3 mm; body weight 0.4 ± 0.1 g) were collected from Wonpo, in Jeonnam, South Korea. After collection, the clamworms were immediately placed in an insulated box and transported to the laboratory. Upon arrival, worms were acclimated for 5 d in noneutrophic sediment before being transferred to sediment microcosms.

Experiment Design

In each tank, 50 scallop spats were maintained in suspension, while clamworms were added to the sediment at one of four densities:

100, 200, 300, and 400 individuals. Microalgae (*Chaetoceros* sp. and *Isochrysis* sp.) at a concentration of 5×10^3 cells/mL was added every 3 d. For each treatment, three replicates were used, while two tanks without clamworms were used as a control. Three subsamples of sediment were taken with a core sampler (2 cm in diameter, 10 cm in height) from each tank every 15 d. The experiment was conducted for 75 d.

Chemical Analysis

The sediment subsamples were analyzed to determine the concentration of AVS, COD, and LOI. Prior to the chemical analysis, the sediment sample was mixed well. The AVS level was determined according to Wu et al. (2003) by using a test column (model 201H; Gastec, Co., Kanagawa, Japan). A sample of sediment was dried at 105 C for 24 h to determine dry weight. The COD analyses were performed on diluted sediment subsamples according to McIntosh et al. (2001) based on the U.S. Environmental Protection Agency methodology (American Public Health Association 1995). The organic matter of the sediments was evaluated as LOI according to Mendiguchía et al. (2006) by calculating the weight difference before and after combustion at 500 C during 3 h.

Rates of AVS, COD, and LOI were calculated using the following equation:

$$R = \frac{C_t - C_0}{t}$$

where C_0 and C_t are the concentration of AVS, COD, and LOI at day 0 and day t.

Estimation of Survival Rate of Scallop Spat

Their viability was determined by carefully prodding their foot or mantle with the blunted tip of dissection tweezers in the test water. Those spats which failed to close their valves upon stimulation or were gaped beyond normal valve activity were considered dead (Loayza-Muro and Elías-Letts 2007).

Statistical Analysis

Two-way ANOVA was used to test the significance of difference between the treatment and control set, and multiple comparisons were used to determine which treatments were significantly different using SPSS for Windows. Multiple linear regression analysis was used to determine the relationship among levels of AVS, COD, LOI, and survival rate against elapsed time and individual number of clamworms. Significant difference was defined as $P < 0.05$ in all cases. Statistical analyses were performed using the statistical software SPSS for Windows.

Results

The concentration of AVS in all sediment samples decreased with the addition of clamworms during the 75-d experiment; however, there was a significant difference ($P < 0.05$) among all densities of clamworms used in the present study with the treatment with 400 clamworms/m^2 having a significantly lower AVS compared to all other clamworm densities (Fig. 1A). The AVS of sediment with 400 clamworms/m^2 was approximately four times lower compared to AVS of sediment with 100 clamworms/m^2. Regression analysis showed the dependent relationship between the concentration of AVS and clamworm density (Fig. 2A) and was expressed by the equation $y = 0.001 + 0.0011x$ ($r^2 = 0.973$).

The decrease in COD as the density of clamworms increased is shown in Figure 1B. The concentration of COD in the sediment samples with 400 clamworms/m^2 was significantly lower ($P < 0.05$) than in sediment with lower clamworm densities. Sediment with 100 clamworms/m^2 had significantly higher ($P <$ 0.05) COD than all other clamworm densities. Regression analysis showed the dependent relationship between COD and clamworm density (Fig. 2B) and was expressed by the equation $y = 0.0388 + 0.0392x$ ($r^2 = 0.986$).

As in COD, LOI was significantly lower ($P < 0.05$) in sediment with 400 clamworms/m^2 than in sediment with lower densities of clamworms (Fig. 1C). Sediment with 100

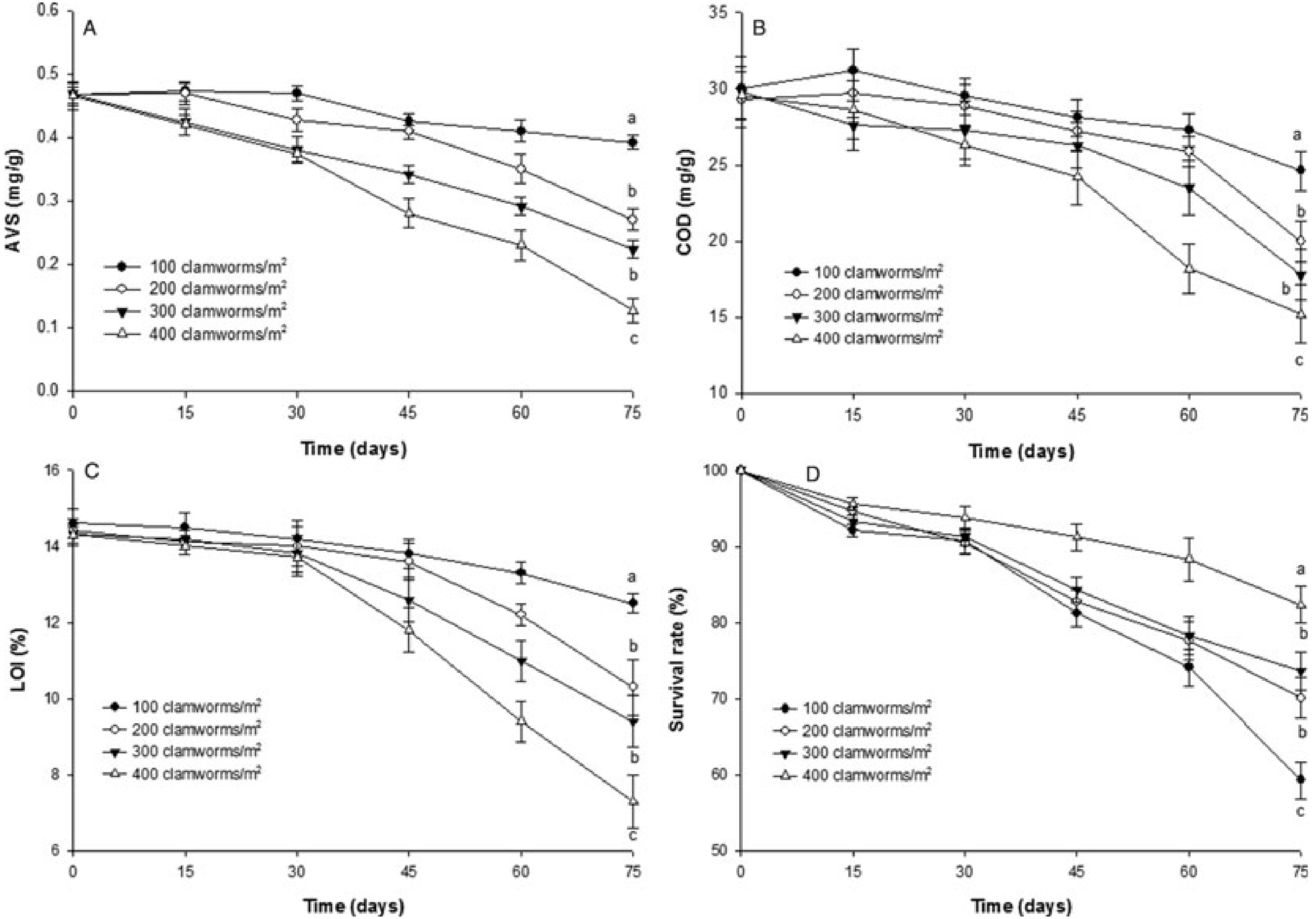

FIGURE 1. *Measurements of (A) acid volatile sulfide (AVS), (B) chemical oxygen demand (COD), and (C) loss of ignition (LOI) of benthic sediments, and (D) survival of scallop spat at the end of day 75 with* Perinereis aibuthtensis *at different densities (100, 200, 300, and 400 clamworms/m^2). Final means with different letters were significantly different ($P < 0.05$).*

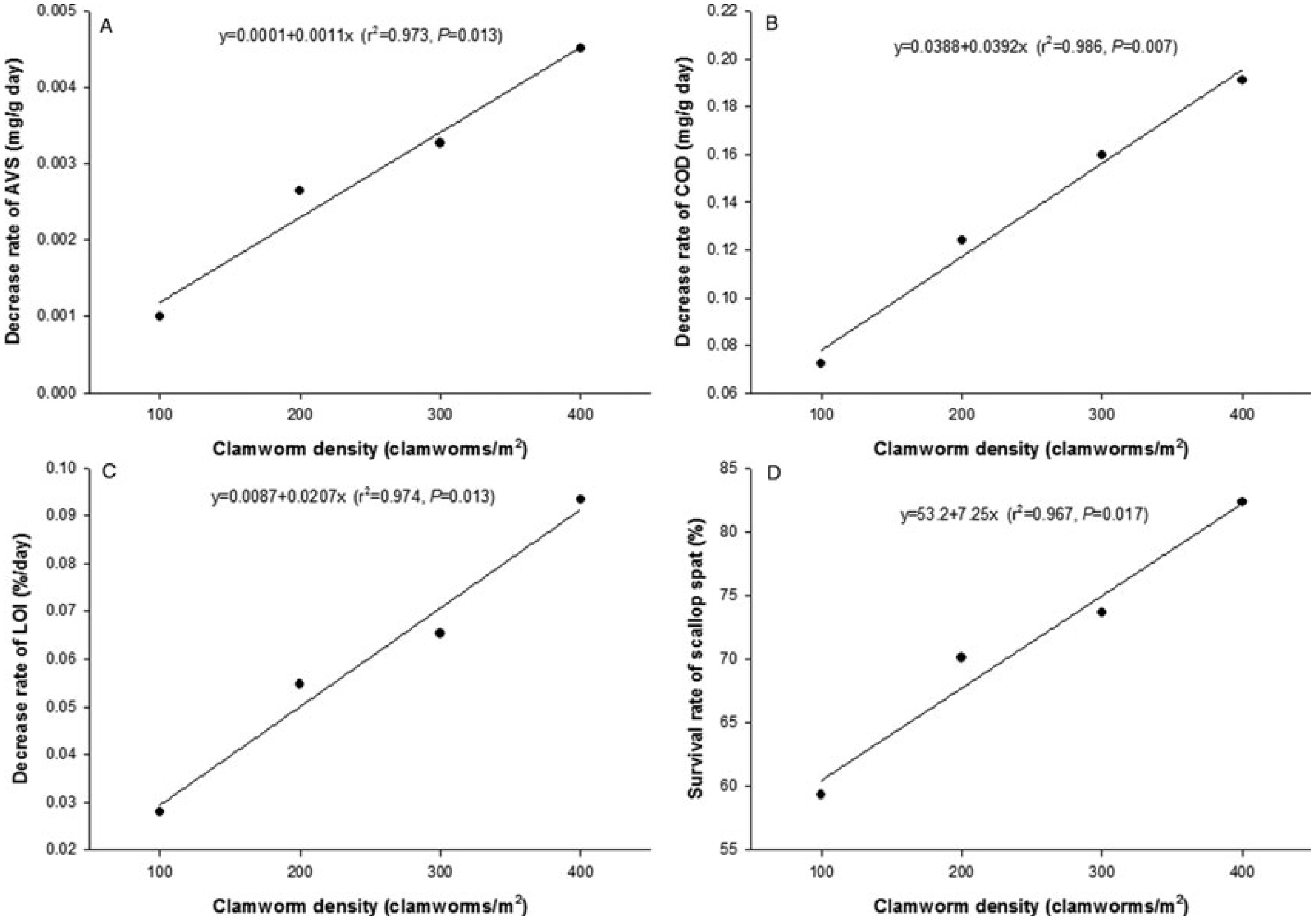

FIGURE 2. *Regression curves of (A) acid volatile sulfide (AVS), (B) chemical oxygen demand (COD), and (C) loss of ignition (LOI) of benthic sediments, and (D) survival of scallop at the end of day 75 with* Perinereis aibuthtensis *at different densities (100, 200, 300, and 400 clamworms/m^2).*

clamworms/m^2 had the highest ($P < 0.05$) final LOI of all clamworm densities evaluated. The LOI for sediment with 400 clamworms/m^2 was approximately 50% lower than the LOI for sediment with 100 clamworms/m^2. Regression analysis showed the dependent relationship between LOI and clamworm density (Fig. 2C) and was expressed by the equation $y = 0.0087 + 0.0207x$ ($r^2 = 0.974$).

Survival of scallop spat was significantly higher ($P < 0.05$) when 400 clamworms/m^2 was used compared to all other lower clamworm densities (Fig. 1D). Survival of spat in the treatment with 400 clamworms/m^2 was approximately 83%, whereas survival of spat in sediment with 100 clamworms/m^2 was approximately 59%. Regression analysis showed the dependent relationship between survival rate of scallop spat and clamworm density and was expressed by the equation $y = 53.2 + 7.25x$ ($r^2 = 0.967$).

Discussion

Shellfish aquaculture can cause adverse environmental effects that need to be monitored. High levels of nutrients, suspended solids, and organic matter from shellfish farming can result in increased oxygen demand, low dissolved oxygen levels, increased algae growth, and poor water quality. The detrimental effects can range from limited (Danovaro et al. 2004) to significant (Newell 2004). An increase in organic materials can alter the sediment composition, both physical and chemical, and can result in the bottom sediment becoming anoxic and metal traps (Christensen et al. 2003), reduction in biodiversity (Stenten-Dozey et al. 2001), and altering carbon, oxygen, and nitrogen cycles in the sediments underlying the farms. While organic matter from shellfish operations increase sedimentation, due to an increase in production of feces and pseudofeces, detrital material can also be created from epibiota attached to culture structures associated with shellfish culture (Kaiser et al. 1998). This further increases the rate of sedimentation.

Data from the present study indicate that sediment quality for the parameters tested was improved, as higher densities of clamworms were added to the sediment. This suggests that the use of deposit-feeding organisms, such as the clamworm, could have potential to improve sediment conditions where scallop culture exists. The serious impacts of marine aquaculture on local environment, due to release of residual fish food and excreta, have been stated in previous studies (Kaspar et al. 1988; Hall et al. 1992; Christensen et al. 2000), especially the impacts on macrofaunal assemblages.

Use of benthic organisms to bioremediate the bottom sediment has shown promise. It has been reported that the lugworm, *Arenicola marina*, at a density of 30 worms/m^2 replaces a layer of 15 cm of bottom sediment annually, while 3 L/m^2 of seawater is concomitantly infused into the sediment, reducing or eliminating anoxic conditions in this soil layer (Cadée 1976; Riisgård and Banta 1998). Kristensen and Mikkelsen (2003) indicated that the worm, *Nereis diversicolor*, increased the degradation of buried organic matter through feeding and irrigation-associated stimulation of microbial decomposition. Chareonpanich et al. (1994) indicated that the worm, *Capitella* sp., also had success at decomposing organic matter through its normal activities. Data from the present study suggest that 400 clamworms/m^2 achieve best results, compared to lower densities. However, a problem could be obtaining enough clamworms to remediate the benthic sediment of affected areas.

Deposit-feeding benthic organisms may improve benthic sediment quality by two mechanisms. In the first mechanism, clamworms ingest sediment, including the micro-organisms and organics contained in the sediment, and after digestion, the sediment (minus some nutrients but possibly containing microbiota from the organism's digestive tract) is defecated back. Ahrens et al. (2001) indicated that digestive fluids of deposit feeders were far more effective than seawater in solubilization of organic materials associated with sediments.

The second mechanism involves bioturbation. A major part of the carbon reaching the ocean floor becomes mineralized due to the activity of various micro-organisms

(Canfield 1989). Bioturbations caused by sediment-dwelling polychaetes could affect microbial populations either directly or indirectly. Bioturbation greatly influences the oxic conditions of surface sediments and alters oxic/anoxic boundaries by transporting oxygen into anoxic zones and improving the movements of substances across the boundaries (Aller 1994; Riisgård and Banta 1998). Further, the presence of clamworms could favor the growth of specific bacterial groups, which breakdown, utilize, and enrich specific types of organic matter (Cuny et al. 2007). Based upon the limited scope of the present study, it is not possible to determine which one, if either, of these mechanisms was active in the present study. It could be that both mechanisms occur and operate synergistically.

Data from this study indicate that clamworms have an indirect effect on the survival of scallop spat. This could be due to an improvement in the benthic sediments and/or water exchanged by vertical mixing of the sediment. In this study, the positive effect of clamworm on survival of scallop spat was significant. It may be partly attributed to the thoroughly vertical mixing by continual aeration which improved the exchange of sediment and water. Therefore, the application of clamworm may have a better effect in a shallow bay where vertical mixing is thorough. Further research should be conducted in this area to determine if the data from the present laboratory study can be applied to real environmental situations.

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