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# **The Effect of Bioturbation Activity of the Ark Clam**  *Scapharca subcrenata* **on the Fluxes of Nutrient Exchange at the Sediment–Water Interface**

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**Abstract** Filter-feeding shellfish are common benthos and significantly affect the biogeochemical cycle in the shallow coastal ecosystems. Ark clam *Scapharca subcrenata* is one of the widely cultured bivalve species in many coastal areas owing to its tremendous economic value. However, there is little information regarding the effects of the bioturbation of *S. subcrenata* on the fluxes of nutrient exchange in the sediment-water interface (SWI). In this regard, *S. subcrenata* was sampled during October 2016 to determine the effects of its bioturbation activity on the nutrient exchange flux of the SWI. The results showed that the biological activity of *S. subcrenata* could increase the diffusion depth and the rate of the nutrients exchange in the sediments. The bioturbation of *S. subcrenata* could allow the nutrients to permeate into the surface sediments at 6–10 cm and increase the release rate of nutrients at the SWI. The releasing fluxes of DIN and  $PO<sub>4</sub><sup>3−</sup>P$  in the culture area were found to be around three times higher than that in the non-cultured region. The culture of *S. subcrenata* has been proved to be an important contributor to nutrient exchange across the SWI in the farming area of Haizhou Bay. Nutrients exchange in the SWI contributes a part of 86% DIN, 71%  $PO_4^{3-}$ -P and 18%  $SiO_3^{2-}$ -Si for the aquaculture farm.

**Key words** bioturbation; nutrients; exchange flux; ark clam; sediment-water interface

# **1 Introduction**

The behaviors of benthic organisms, such as feeding, excreting, digging, and creeping species, are capable of changing the physicochemical properties of sediments and the biogeochemical processes at the sediment-water interface (SWI) (Widdows *et al*., 1998). The biogeochemical effect of these behaviors, namely, bioturbation, has been recognized for the redistribution of particulate organic matter at the surface of the sediments. The activities of caving benthos increase the porosity of the sediments and its permeability, thereby changing the physical properties of the sediments (Jones and Jago, 1993; Creed *et al*., 2010). Bioturbation also significantly affects the

nitrification and denitrification of nitrogen in the sediments and promotes coupling between the two processes (Pelegrí and Blackburn, 1994; Hulth *et al*., 2005). Further, due to bioturbation and biological irrigation of macrobenthos, the nutrient exchange of SWI is affected by the destroyed vertical structure of the sediments and their altered physical and chemical environmental conditions (Volkenborn *et al*., 2007).

Filter-feeding shellfish are common benthos in the shallow coastal sea, significantly affecting the biogeochemical cycle of the shallow coastal sea. They ingest nutrients from water columns through their filtration activities and egest the particles into the water. The resuspension rate of the sediment could be increased by four times due to the bioturbation of Baltic clam *Macoma balthica*, and also the culture of oyster could increase the deposition rate of inorganic and organic matter by three

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times (Mortimer *et al*., 1999; Forrest and Creese, 2006). It has been found that the resuspension of the sediment bed could increase the concentrations of dissolved silicon by 125%, nitrate by 67% and phosphate by 66% in the water columns (Couceiro *et al*., 2013). Oxygen consumption and exchange flux of nutrients in sediments could be largely increased owing to the activity of the venus clam *Cyclina sinensis*, which may clearly increase the marine primary productivity (Nicholaus and Zheng, 2014). In addition, the burrowing benthic animals can speed up the flow of water in the cave due to their respiratory or other activities (such as feed, defecate, burrow, and respire); therefore, the overlying water flows through the cave to complete the material diffusion between the pore water of deeper sediments, the process of which is called bioirrigation (Peter and Dirk, 2006). Bioirrigation accelerates the exchange of solutes between the pore water and the overlying water in the sediments. On the one hand, it increases the diffusion rate of the dissolved oxygen into the sediments, and on the another hand, it also promotes the dissipation of biological metabolites into the sediments, thus altering the balance of the biogeochemical process in the sediments (Aller and Aller, 1998).

The ark clam *Scapharca subcrenata*, belonging to the phylum Mollusca, class Bivalvia, is an important species in the benthic system, especially in Haizhou Bay, China. Currently, most of the research on the biological effects of *S. subcrenata* is based on the monoculture of *S. subcrenata* or its co-culture with other aquatic organisms (*e*.*g*., sea cucumber) (Niu, 2006). However, the understanding of of the bioturbation activity of *S. subcrenata* affecting nutrient exchange fluxes at the SWI is still limited.

To reveal the vertical distribution of nutrients and the mechanisms of its effect on the exchange fluxes of nutrients at the SWI, and to provide the basic information on restoring and building the nutrients exchange model to estimate the exchange flux, this study investigated the effects of bioturbation and the density effects of *S. subcrenata* on the nutrient exchange at the SWI based on laboratory experiments.

# **2 Materials and Methods**

#### **2.1 Sample Collection and Treatment**

The sediment samples were collected from the culture area of *S. subcrenata* at 34˚49´58´´N and 119˚17´30´´E in the Haizhou bay of the Jiangsu Province of China, by the improved Gray-O'Hara box corer on October 10th, 2016. The samples were collected stochastically from the center of the culture area. The surface area of each sample was about 0.1 m<sup>2</sup>, and the depth was  $20-25$  cm and  $0.36$  m<sup>3</sup> sediment was sampled in total. Meanwhile, the bottom seawater above the sediments was also collected and filtered by a microfiltration membrane with a pore size of  $0.45 \,\mu$ m (Φ50×0.45 $\mu$ , Sinopharm Chemical Reagent Co., Ltd.). The bottom water was collected with the help of a water sampler. The collected sediments and water samples were refrigerated at 0–4℃ until laboratory treatment. The clams were directly collected from the culture area using the bottom trawling and brought to the laboratory. The sediments were sieved through a 0.5mm mesh screen to remove macrobenthic organisms, sand and other impurities, after which the clams were transferred into a water tank for acclimation (Deng *et al*., 2012; Nicholaus *et al*., 2014). Healthy *S. subcrenata* with shell sizes ranging from 28.2 to 28.6 mm in length were selected and cultured for seven days in the water tank. About 200 clams were cultured in two 90-liter capacity tanks. During the culture period, 10 liters of seawater was changed daily and the dissolved oxygen was mainatained at  $8.5 \pm 1$  mg  $L^{-1}$ .

#### **2.2 Experimental Design**

An opaque PVC cylinder with a diameter of 50cm and a height of 40cm was designed as the experimental container. The sediments treated as described hereinbefore were added into the device with a depth of 15cm, and the sampled and filtered bottom seawater was slowly added above the sediment with a depth of 20 cm (Fig.1). The experiment was segregated into four groups: A0, A5, A10



Fig.1 The experimental incubation apparatus specifically designed for this study.

and A20. Group A0 was the control group, which only contained sediments and the bottom seawater; A5 consisted of the low density bioturbation group with sediments, bottom seawater, and 5 clams  $(25 \text{ ind m}^{-2})$ . A10 group comprised the bioturbation group with sediments, bottom seawater and 10 *S. subcrenata* (51 ind m<sup>-2</sup>), wherein the biological density was consistent with that in the field; A20 included the highest density bioturbation group with sediments, bottom seawater and 20 *S. subcrenata* individuals  $(102 \text{ ind m}^{-2})$ . Triplicate samples were set for each group, and the culture period lasted for 15 days without feeding. The temperature, dissolved oxygen and pH of the overlying water were measured daily. During the culture period, the stability of dissolved oxygen being maintained by using a pump. The data on the water quality throughout the culture period are shown in Table 1. The overlying water was replaced every 24 h to avoid resuspension of the sediment. With the help of a syringe,  $50 \text{ mL}$  seawater was collected at  $1-3 \text{ cm}$  above the sediment before and after the water replacement, then filtered using a cellulose acetate membrane with a pore size of 0.45 μm and cryopreserved at −80℃ with chloroform before measuring the nutrients. After 15d of the culture period, the pore water in the sediments was sampled at a 1 cm interval by the Rhizon soil moisture sampler (Rhizon SMS 10 cm porous, male luer-19.01.01, Rhizonsphere research products B.V., The Netherlands).

Table 1 Water quality parameters (temperature, dissolved oxygen [DO] and pH) for the mesocosms during the incubation experiment

Group		Temperature $(\mathcal{C})$	$DO(mgL^{-1})$	pН			
	A0	$18.10\pm0.23$	$8.98 \pm 0.41$	$8.05 \pm 0.03$			
	A5	$18.08 \pm 0.20$	$8.71 \pm 0.57$	$8.01 \pm 0.08$			
	A10	$18.11 \pm 0.18$	$8.72 \pm 0.69$	$7.98 \pm 0.06$			
	A20	$18.06 \pm 0.29$	$8.57 \pm 0.65$	$7.96 \pm 0.07$			

#### **2.3 Methods of Measurement and Calculation**

Measurement of water samples was performed by using the DeChem-Tech CleverChem 380 (DeChem-Tech, Germany) automatic discontinuous analyzer.  $PO<sub>4</sub><sup>3-</sup>$ ,  $SiO<sub>3</sub><sup>2-</sup>$ ,  $NH_4^+$ ,  $NO_3^-$  and  $NO_2^-$  were measured by using molybdenum blue spectrophotometry, silicon molybdenum blue method, phenol-hypochlorite colorimetry, cadmium column reduction method and Diazo-azo method, respectively. The SWI nutrient exchange flux was calculated based on the following formula (Michaud *et al*., 2006),

$$
F = \frac{M(t)}{A \cdot \Delta t},\tag{1}
$$

where *F* is the exchange fluxes of nutrients at the SWI  $\text{mmod } m^{-2}d^{-1}$ ,  $M(t)$  is the variation of nutrient concentration in the overlying water (mmol), *A* is the sectional area of the culture column  $(m^2)$ , and  $\Delta t$  is the variation of the culturing time (d).

The diffusion rate of the nutrient was calculated based on the Fick's first law (Boudreau, 1997), which is as follows:

$$
J = -\Phi D_{\rm S} \frac{\partial C}{\partial X},\tag{2}
$$

$$
D_{\rm S} = D_0 \cdot \Phi^{m-1},\tag{3}
$$

$$
\Phi \le 0.7, \ m = 2; \ \Phi \ge 0.7, \ m = 2.5,
$$
 (4)

where  $J$  is the SWI diffusion flux,  $\Phi$  is the porosity of sediments surface,  $D<sub>S</sub>$  is the diffusion coefficient of the molecule in sediments,  $\partial C_{\partial X}$  is the concentration gradient of SWI, and  $D_0$  is the molecular diffusion coefficient in water.

Determination of Porosity: a known volume of a graduated cylinder (*V*) is taken and its weight is measured as  $W_1$ . Then the cylinder is filled with the sediment and its total weight was measured as  $W_2$ . Afterward,  $W_3$  is measured after soaking the sediment sample in water for 24h. The porosity is calculated according to the following equation:

$$
\Phi = \frac{(W_3 - W_1) - (W_2 - W_1)}{V},\tag{5}
$$

where Φ is the porosity of sediment's surface, *W* is the weight (g),  $V$  is the volume (cm<sup>3</sup>).

#### **2.4 Statistical Analysis**

Data analysis was conducted using SPSS (SPSS 20.0. 0). Single sample Kolmogorov-Smirnov (K-S) test was performed to verify whether the data obeyed the normal distribution, and the independent sample *t* test was performed for the comparison of nutrient diffusion and exchange fluxes.

#### **3 Results**

### **3.1 The Exchange Fluxes of the Nutrients at the SWI**

Fig.2 shows the exchange fluxes of the nutrients at the SWI. In the  $NH_4^+$ -N experiment (Fig.2a), the average exchange fluxes of the nutrients at the SWI of groups A0, A5, A10, and A20 were 0.91, 2.18, 3.58 and 4.99mmol  $m^{-2}d^{-1}$ , respectively. The average exchange fluxes of nutrients of groups A5, A10 and A20 were 2.39, 3.91 and 5.47 times higher than that of group A0. The changes in the  $NH_4^+$ -N exchange fluxes of groups A10 and A20, which varied greatly, were significantly different from that of group A0  $(P<0.05)$ .

For  $NO_3 + NO_2-N$  (Fig.2b), the average values of exchange flux of the nutrients at the SWI of groups A0, A5, A10 and A20 were 0.42, 0.97, 1.63 and 2.47 mmol m<sup>-2</sup> $d^{-1}$ , respectively. The average values of exchange flux of nutrients of groups A5, A10, and A20 were 2.28, 3.85 and 5.83 times higher than that of the group A0. The changes in  $NO_3^- + NO_2^- - N$  exchange flux were more significant for groups A10 and A20 when compared with that in group A0  $(P<0.05)$ .

The average values of exchange flux of  $PO_4^3$ -P in the SWI of groups A0, A5, A10, and A20 were 0.09, 0.15,

0.27, and 0.37 mmol  $m^{-2}d^{-1}$ , respectively (Fig.2c). The average values of exchange flux of nutrients of groups A5, A10, and A20 were 1.62, 2.86 and 3.92 times higher than that of group A0. In the first ten days of the experiment,  $PO<sub>4</sub><sup>3–</sup>-P$  exchange flux was observed at the SWI for groups A10 and A20, which changed faster as compared with that of group A0. As the experiment proceeding, the average exchange flux in groups A5, A10 and A20 decreased was gradually and tended to be stable.

In the experiment of  $SiO<sub>3</sub><sup>2</sup> - Si (Fig.2d)$ , the average values of exchange flux of the nutrients at the SWI of groups A0, A5, A10 and A20 were 0.42, 0.80, 1.09 and 1.45 mmol  $(m^2 d)^{-1}$ , respectively. The average values of exchange flux of nutrients in groups A5, A10, and A20 were 1.56, 2.14 and 2.87 times higher than that of A0 group. The fluxes of the three groups varied from the sediment to the water. In the first ten days of the experiment, the exchange flux in all the groups was found to fluctuate widely. While in the following days, the average value of exchange fluxes in group A10 and A20 decreased gradually and became stable.

In the culture experiment,  $NH_4^+$ -N was the main component of the DIN. In A0 group, the ratios of  $NH_4^+$ -N,  $NO<sub>3</sub><sup>-</sup>-N$  and  $NO<sub>2</sub><sup>-</sup>-N$  to the DIN were 82%, 13% and 5%, respectively. The DIN constituted 68% and 66% of  $NH_4^+$ -N, followed by  $NO_3^-$ -N (22%, 22%) and  $NO_2^-$ -N (9%, 10%) in groups A5 and A10, respectively. In group A20,  $NH_4^+$ -N accounted for 66% of the DIN, whereas  $NO_3^-$ -N and  $NO<sub>2</sub><sup>-</sup>-N$  contributed 21% and 13%, respectively. The mean exchange fluxes of DIN in groups A5, A10, and A20 were, respectively, quantified as 2.36, 3.89 and 5.58 times higher than that in group A0. Similarly, the changes of DIN exchange flux in groups A5, A10, and A20 were larger than that in the A0 group  $(P<0.05)$ .



Fig.2 Benthic nutrient fluxes during the experimental period, (a)  $NH_4^+$ -N, (b)  $NO_3^-$ + $NO_2^-$ -N, (c)  $PO_4^3$ -P, and (d)  $SiO_3^2$ -Si.

## **3.2 Vertical Distribution Characteristics of Nutrients in the Pore Water**

The average concentration of  $NH_4^+$ -N within the pore water of the sediments in the four groups were 1.34, 1.64, 2.13, and 2.95 times higher than that in the overlying water, respectively (Fig.3). A higher concentration of  $NH_4^+$ -N was found with increasing depth. The vertical distribution of NH4 + -N in the pore water of A0 group was similar to that in group A5, and the  $NH_4^+$ -N of group A10 was similar to that of group A20. However, a more significant change was found in the vertical distribution of group A20.

With respect to  $NO_3^- + NO_2^- - N$ , its average concentra-

tion in the pore water was lower than that in the overlying water for groups A0 and A5, with average concentration 0.43 and 0.75 times higher than that in the overlying water, respectively (Fig.4). The average concentrations in the pore water of groups A10 and A20 were 1.11 and 1.82 times higher than those in the overlying water, respectively. The  $NO_3^- + NO_2^- - N$  concentrations in group A0 showed a minor change, whereas those in groups A5, A10, and A20 were found to vary highly at the depth of 1 to 10 cm, and the concentrations in all the four groups tended to increase initially and decrease subsequently. At the depth of 11 cm of the sediment, the concentrations of  $NO<sub>3</sub><sup>-+</sup>$  $NO<sub>2</sub><sup>-</sup>-N$  in all the four groups decreased slowly and showed a tendency to be stable.



Fig.3 Vertical distribution characteristics of NH<sub>4</sub><sup>+</sup>-N in the pore water ( $\mu$ mol $L^{-1}$ ).



Fig.4 Vertical distribution characteristics of  $NO_3^- + NO_2^- - N$  in pore water ( $\mu$ mol $L^{-1}$ ).

Fig.5 represents that the average concentrations of  $PO<sub>4</sub><sup>3-</sup>P$  in the pore water of the sediments were higher than those in the overlying water for groups A0, A5, A10, and A20, the values of which were 1.22, 1.71, 2.45, and 3.29 times higher than that in the overlying water, respectively. The concentrations of  $PO<sub>4</sub><sup>3-</sup>P$  in groups A10 and A20 tended to increase significantly at the depth of  $1-3$ cm. At the depth of 5–10 cm, the concentrations of  $PO_4^{3-}$ -P of groups A10 and A20 decreased rapidly. When the depth of the sediment reached 11 cm, the concentration of  $PO<sub>4</sub><sup>3-</sup>P$  in all the four groups decreased slowly and became stable.

In the  $SiO<sub>3</sub><sup>2</sup>$ -Si experiment (Fig.6), the average nutrient concentrations in the pore water of sediments were higher than those in the overlying water for groups A0, A5, A10, and A20, the values of which were 1.08, 1.29, 1.56 and 1.98 times higher than that in the overlying water, respectively. For groups A0, A5, and A10, the data showed similar vertical distribution of  $SiO<sub>3</sub><sup>2</sup> - Si$  in the pore water. Stable concentrations of  $SiO<sub>3</sub><sup>2</sup>$ -Si were observed in all of four groups at the depth 10cm of the sediment.







Fig.6 Vertical distribution characteristics of  $SiO<sub>3</sub><sup>2</sup>$ -Si in pore water (µmol $L^{-1}$ ).

NH4 + -N was the main component of DIN in the pore water (Table 2). The mean concentrations of DIN in groups A5, A10, and A20 were 1.37, 1.86 and 2.76 times higher than that in group A0, respectively; there was an

apparent difference in the change of DIN concentration between the groups A10 and A0, and a significant difference could also be found between the groups A20 and A0  $(P<0.05)$ .

Group	The ratio of $NH_4^+$ -N (%)	The ratio of $NO_3$ , $N$ (%)	The ratio of $NO2$ -N $(\% )$
A <sub>0</sub>	72	∸	
A5	64	، ،	
A10	62	∼	
A20	57	30	

Table 2 The ratio of  $NH_4^+$ -N,  $NO_3^-$ -N and  $NO_2^-$ -N to DIN in the pore water

## **3.3 Nutrient Diffusive Fluxes Across the SWI**

The diffusion rate of nutrients in the SWI was calculated based on Fick's diffusion law (Table 3). Compared with group A0 (without bioturbation), the nutrients in groups A10 and A20 were found to migrate from the pore water to the overlying water.  $NO_3^- + NO_2^- - N$  in group A0 were found to migrate from the overlying water to the pore water, while other nutrients were found to be transported in the opposite pathway.

Table 3 Nutrient diffusive flux across the SWI (mmol  $m^{-2} d^{-1}$ )

Group	$PO43–-P$	$SiO32-Si$	$NO3-+NO2--N$	$NH_4$ <sup>+</sup> -N	DIN
A0	$0.007 \pm 0.006$	$0.101 \pm 0.037$	$-0.339\pm0.052$	$0.293\pm0.067$	$-0.046\pm0.041$
A5	$0.009 \pm 0.008$	$0.120 \pm 0.045$	$0.056 \pm 0.059$	$0.276\pm0.098$	$0.332\pm0.053$
A10	$0.018 \pm 0.011$	$0.214\pm 0.071$	$0.068 \pm 0.032$	$0.326\pm0.131$	$0.394\pm0.061$
A20	$0.027\pm0.016$	$0.317\pm 0.174$	$0.127\pm0.045$	$0.398\pm0.183$	$0.525 \pm 0.105$

Note: Positive values denote the movement of nutrients from the sediment to water; Negative values denote the movement of nutrients from water to the sediment.

## **4 Discussion**

#### **4.1 Nutrient Diffusion Across the SWI**

*S. subcrenata* plays an important role in the cycling of nutrients in the sediment ecosystem, and has a significant effect on the exchange of nutrients across the SWI. Generally, dissolved oxygen diffuses a few millimeters above the surface of the sediment.  $NO_3^- + NO_2^- - N$  are dominant in the aerobic layer, so  $NH_4^+$ -N that exists in the anaerobic layer is not released unless the overlying water becomes hypoxic. In this study, the sediment-water interfacial exchange flux of  $NH_4^+$ -N was found to be significantly affected by the bioturbation of *S. subcrenata* (*P*< 0.05), and the bioturbation in anoxic sediments also facilitated the release of  $NH_4^+$ -N from the sediments into the water (Bartlett *et al*., 2008). Many studies have also shown that bioturbation can significantly alter the exchange flux of nutrients at the SWI (Hewitt *et al*., 2006; Nizzoli *et al*., 2007; Norling *et al*., 2007). This study observed a releasing trend of  $NH_4^+$ -N from the sediments to the overlying water due to the bioturbation of *S. subcrenata*. The exchange flux was much higher than the groups without bioturbation. Similar finding has been reported that NH4 + -N was released rapidly when polychaete *Nereis diversicolor* settled at the beginning and the releasing rate decreased with culture proceeding (Hansen and Kristensen, 1997). Due to the bioturbation of *S. subcrenata*, the exchange flux of  $NO_3^- + NO_2^- - N$  increased significantly  $(P<0.05)$ . This could be explained by the fact that more nitrifying bacteria could reach the surface of the sediments and participate in the nitrification reaction, caused by the change of the sediment porosity during the

digging process of *S. subcrenata*. Some part of NH<sub>4</sub><sup>+</sup>-N in an anaerobic layer is directly released by bioturbation because the anaerobic layer faces the overlying water with rich dissolved oxygen. The others are nitrified to  $NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup>-N$ , which are also released from the sediment. Bioturbation not only promoted the diffusion of the dissolved oxygen into the sediments to stimulate the nitrification process in the sediments, it also accelerated the migrating process of NO<sub>3</sub><sup>-</sup> into the SWI (Nizzoli *et al.*, 2007). With or without the effect of *S. subcrenata* bioturbation, no apparent exchange flux was found for  $NO_3^-$ + NO<sub>2</sub><sup>-</sup>-N. Although A20 group showed a decreasing trend within the 6–10 days, other groups presented an increasing trend. The exchange flux of NO<sub>3</sub><sup>−+</sup>NO<sub>2</sub><sup>−</sup>-N increased significantly because of the bioturbation effect. In this study, the exchange flux of  $PO<sub>4</sub><sup>3-</sup>P$  in the SWI increased significantly  $(P < 0.05)$ , which may be caused by the change in the physical properties of the sediment by *S. subcrenata*. The feeding and digging processes of macrobenthos are often accompanied by the modification of the physical structure of the sediments, which could radically alter the physical and chemical characteristics of the sediments (Rowe, 1974; Gilbert *et al*., 2007). In addition, the deposition of organics and dissolved oxygen in the overlying water promotes the release of PO<sub>4</sub><sup>3-</sup>-P (Peña *et al*., 2010). The decomposition of organics by the bacteria at the surface of the sediment as well as the resuspension effect of bioturbation also facilitates the release of  $PO<sub>4</sub><sup>3–</sup>$ -P. The exchange flux of  $PO<sub>4</sub><sup>3–</sup>$ -P was much higher than the group without bioturbation. The exchange flux of SiO<sub>3</sub><sup>2–</sup>-Si increased significantly due to bioturbation (*P* < 0.05). This could be explained by the bioturbation that promoted the microbial activity, as the micro-organisms

could accelerate the dissolution process of the bio-silicon in the sediments (Bidle and Azam, 2001; Bidle *et al*., 2003; Kinoshita *et al*., 2003). Therefore, bioturbation might accelerate the regeneration rate of bio-silicon in the sediments. The diatom was fed by the filter feeding shellfish, and the fine debris was discharged into the sediment after digestion, increasing the contact area between the micro-organisms and diatom, further accelerating the dissolution of the bio-silicon.

### **4.2 Effects of** *S***.** *subcrenata* **on Nutrient Diffusion Across the SWI**

Activities of benthos are capable of altering the permeability, porosity, and spatial heterogeneity of the sediments (Stockdale *et al*., 2009), which promote the diffusion of nutrients into the sediments. In our experiment,  $NH_4^+$ -N in the pore water was the main nitrogen source for the DIN, and the percentage of  $NH_4^+$ -N gradually decreased with more *S. subcrenata*. Some studies have suggested that the concentration of  $NH_4^+$ -N in the pore water is mainly controlled by the redox environment (Canfield *et al*., 1993), and its concentration in the pore water is therefore higher than that in the overlying water. Although culturing *S. subcrenata* decreases the concentration of dissolved oxygen at the surface of the SWI (Shen *et al*., 2008), the bioturbation of *S. subcrenata* accelerates the diffusion of the dissolved oxygen in the water and the surface sea water with more oxygen is transferred to the bottom consequently. From the daily water quality data, it was found that the dissolved oxygen concentration in the *S. subcrenata* group was slightly lower than that in the control group. As denoted in Fig.3, a concentration gradient was formed as the concentration of  $NH_4^+$ -N in the deeper sediments was higher than that in the surface sediments, the result being that  $NH_4^+$ -N in the pore water migrated to the overlying water with a high content of oxygen in the surface sediments and were converted into  $NO<sub>2</sub><sup>-</sup>-N$  and  $NO<sub>3</sub><sup>-</sup>-N$  by the effect of nitrifying bacteria (Dong *et al*., 2009). The respiration and metabolism of *S. subcrenata* may also contribute to the increase in the concentration of  $NO<sub>3</sub><sup>-</sup>-N$ .

The concentration of  $NO_3^- + NO_2^- - N$  in the pore water reached the maximum at the depth of  $2-6$  cm, and then decreased with the increasing depth. When  $NO_3^- + NO_2^- - N$ entered the deeper sediments, the concentration of  $NO<sub>3</sub><sup>-+</sup>$  $NO<sub>2</sub><sup>-</sup>-N$  decreased gradually in the sediments. The oxygen in  $NO_3^- + NO_2^- - N$  is used for the decomposition of anaerobic bacteria, producing  $NH_4^+$ -N in the deeper regions of the sediment. Although the apparently low concentration of  $NO_3^- + NO_2^- - N$  was observed when the depth of the sediment was greater than 2 cm (Couceiro *et al*., 2013),  $NO_3^- + NO_2^- - N$  could move to the depth of 6 cm because of the bioturbation activity of *S. subcrenata.* 

Generally, phosphorus exists in the sediment with a wide variety of chemical forms such as loosely absorbed phosphorus, iron bound phosphorus, and calcium bound phosphorus. The change in salinity affects the release of PO<sub>4</sub><sup>3-</sup>-P from loosely absorbed phosphorus (Froelich, 1988; Suzumura *et al*., 2000). Hypoxic or anoxic condi-

tions enhance the release of  $PO<sub>4</sub><sup>3−</sup>-P$  from the iron bound phosphorus (Bostrom *et al*., 1988; Hose and Denison, 2000; Mayer and Jarrell, 2000). The decrease in pH causes the release of  $PO_4^{3-}$ -P from the calcium bound phosphorus (Lijklema, 1994; Gomez *et al*., 1999). However, the increase in the release rate of  $PO_4^3$ <sup>-</sup>-P was found to be related to the biological process, and not to the chemical process. The average concentration of  $PO<sub>4</sub><sup>3-</sup>-P$ in the pore water was  $1.22 - 3.29$  times higher than that in the overlying water, and a higher average concentration level could be found with a larger density of *S. subcrenata*. Active organic debris could be found on the surface of the sediments. The organic phosphorus in the organic debris layer maintains a high concentration of  $PO_4^3$ <sup>-</sup>-P during conversion and dissolution, resulting in a higher concentration of  $PO<sub>4</sub><sup>3-</sup>P$  in the pore water than that in the overlying water (Huang *et al*., 2007). The concentration of  $PO_4^3$ <sup>-</sup>-P changed significantly at the depth of 1-10cm in the sediment and the bioturbation of benthic organisms at the same depth range increased the discharge of phosphorus from the sediments. It was found that the oligochaete *Limnodrilus hoffmeisteri* accelerated the diffusion rate of phosphorus in the sediment (Wu, 2010). In this study, the biological effect of *S. subcrenata* caused more  $PO<sub>4</sub><sup>3-</sup>P$  to spread to the deeper sediments, which could reach to the depth of 10cm.

The average concentration of  $SiO<sub>3</sub><sup>2</sup> - Si$  in the pore water was  $1.41-1.83$  times higher than that in the overlying water. At the depth of  $1-8$ cm, the average concentration of  $SiO<sub>3</sub><sup>2</sup> - Si$  in the bioturbation group was significantly higher than that in the control group  $(P<0.05)$ . Bidle et al. studied the effects of microorganism on the dissolution rate of bio-silicon in the laboratory and found that they can speed up the dissolution of bio-silicon (Bidle and Azam, 2001; Bidle *et al*., 2003). The microorganisms in the surface of sediment speed up the dissolution of biogenic silica under aerobic condition, leading to an increase of  $SiO<sub>3</sub><sup>2</sup> - Si concentration on the surface sedi$ ments. Influenced by the bioturbation effect, the dissolved  $SiO<sub>3</sub><sup>2</sup>$ -Si diffused into the depth of 8cm in the sediment. The disturbance of *S. subcrenata* lessened as the depth increased, resulting in a decrease in the  $SiO<sub>3</sub><sup>2</sup>$ -Si concentration. Karlson *et al.* (2005) also believed that  $SiO<sub>3</sub><sup>2</sup> - Si$ can mainly migrate through diffusion.

The diffusion rate of nutrient molecules in group A20 was  $1.22-1.87$  times as high as that of group A10, which indicated that the biological effect of culturing *S. subcrenata* and the increase of biological density could improve the diffusion rate of nutrients at the SWI. Some researchers also believe that the fiddler crab *Uca tangeri* or other benthic polychaetes significantly affect the vertical distribution of the sediments and the concentrations of nutrients (Wolfrath, 1992; Honda and Kikuchi, 2002; Palmer, 2010; Musale and Desai, 2011).

## **4.3 Contribution of** *S***.** *subcrenata* **Culture to the Primary Productivity of Haizhou Bay**

The average primary productivity of Haizhou Bay in 2011 was 482.07 mg C(m<sup>2</sup>d)<sup>-1</sup> (Yang, 2015) and the cul-

ture area of *S. subcrenata* in Haizhou Bay was 352.387 hm<sup>2</sup>. According to the proportional data of DIN,  $PO_4^{3-}$ -P and  $SiO<sub>3</sub><sup>2</sup> - Si$ , the nutrients that were extracted from marine water could be estimated by combining with the Redfield ratio (C:N:P:Si = 106:16:1:16) as  $2.99 \times 10^8$  mg  $d^{-1}$ , 4.14 × 10<sup>7</sup> mg  $d^{-1}$ , and 5.98 × 10<sup>8</sup> mg  $d^{-1}$ , respectively. The average density of the bottom sowing was  $50 \pm 10$  ind m<sup>-2</sup>, which is similar to the density of group A10. Referring to the nutrient exchange flux in group A10, the contribution of *S. subcrenata* culture to the primary productivity in Haizhou Bay could be estimated. It could be concluded that the exchange of nutrients in the SWI would provide a part of  $86\%$  DIN,  $71\%$  PO<sub>4</sub><sup>3-</sup>-P and  $18\%$ SiO<sub>3</sub><sup>2−</sup>-Si for the culture area of *S. subcrenata*, meaning that *S. subcrenata* culture has played a key role in nutrient exchange across the SWI in Haizhou Bay at autumn. It can be found that the contribution of *S. subcrenata* culture to Si is much lower than that to N and P, which may be explained by two reasons. On one hand, the filter feeding shellfish could not discharge the soluble Si; on the other hand, studies have found that the concentration of  $SiO<sub>3</sub><sup>2</sup>$ -Si in the sediments at the depth of 4–5 cm is relatively large and the bio-silicon would be buried in the sediments, resulting in inactive Si recycle and decline of the content of Si in the water (Dixit *et al*., 2001).

In conclusion, the bioturbation effect of *S. subcrenata* was found to significantly improve the diffusion rate of the nutrients at the SWI, which could diffuse nutrients to the surface of the sediment within the depth of  $6-10$  cm. Also, the release of nutrients in the sediments can be improved by the bioturbation of *S. subcrenata*. Referring to group A10 (similar to the actual breeding density), the average nutrient fluxes showed a movement from the pore water to the overlying water during the cultivation. The culture of *S. subcrenata* plays a greatly important role in nutrient exchange across the SWI in Haizhou Bay. However, the current study is conducted under the condition without feeding, and the excretion of ammonium by the *S. subcrenata* has not been considered. These potential factors may affect the nutrient exchange at the SWI, which should be considered in the future studies.

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