

## The effects of temperature on oxygen uptake and nutrient flux in sediment inhabited by molluscs

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### ABSTRACT

Benthic invertebrates play an important role in oxygen uptake and nutrient cycling in the benthic boundary layer. The invertebrate activity, as well as the effects of the bioturbation on biogeochemistry, is greatly influenced by temperature. The present study aimed to investigate the influence of temperature on the sediment oxygen uptake and nutrient flux across the sediment-water interface in sediment inhabited by molluscs – animals often dominating the benthic community. Microcosms were constructed using sediment, lake water, and molluscs from lake Taihu (China). The clam *Corbicula fluminea* (Bivalvia: Corbiculidae) and the snail *Bellamya aeruginosa* (Gastropoda: Viviparidae) were selected for the experiment because they dominate the benthic community in the lake. The effect of molluscs on sediment oxygen uptake and nutrient flux was simultaneously examined using 6 treatments representing a combination of temperature (15 and 25°C) and biota (*C. fluminea*, *B. aeruginosa*, and none). *C. fluminea* significantly increased the sediment oxygen uptake and release of soluble reactive phosphorus (SRP) and ammonium from the sediment to the overlying water at both temperatures. The net sediment oxygen uptake, net SRP and ammonium fluxes caused by the clam were significantly higher at 25°C than at 15°C. Moreover, *B. aeruginosa* significantly increased the sediment oxygen uptake at both experimental temperatures, however the net sediment oxygen uptake induced by the snail did not differ significantly between 15 and 25°C. *B. aeruginosa* also produced significantly more net SRP release at 25°C than that at 15°C. In contrast, the influence of *B. aeruginosa* on the net ammonium flux at each temperature was not statistically significant. The result indicates that temperature is an important environmental factor that influences the effect of molluscs on nutrient flux across the sediment-water interface.

**Key words:** *Corbicula fluminea*, *Bellamya aeruginosa*, bioturbation, benthic invertebrate, lake Taihu.

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### INTRODUCTION

Benthic invertebrates live in or on the substrates of freshwater and marine ecosystems. Most of these invertebrates actively rework the sediment, a process that may disturb the natural deposited stratification, facilitate the horizontal movement of particles, and restyle the microtopography of the sediment (Gerino *et al.*, 2003; Gingras *et al.*, 2008; Maire *et al.*, 2008). Indeed, to respire, feed, and excrete metabolites, benthic invertebrates must renew their burrow water through bioirrigation (Kristensen and Kostka, 2005). Burrowing and bioirrigation alter the sediment porosity (Zhang, 2010), sediment pH (Zhu *et al.*, 2006), oxygen level (Wang *et al.*, 2001; Pischedda *et al.*, 2008), and microbial activity (Kogure and Wada, 2005; Kristensen and Kostka, 2005). Therefore, these processes influence the sediment oxygen uptake (Svensson and Leonardson, 1996; Glud, 2008), organic matter mineralisation (Hakenkamp and Palmer, 1999; D'Andrea and DeWitt, 2009), and nutrient cycling (Vanni, 2002; Welsh, 2003), all of which are vital for primary production and water quality.

As bioturbators are important in ecosystem health and

functioning, discovering the potential effects of environmental factors on the behaviour of invertebrates in sediment is crucial (Przeslawski *et al.*, 2009). Temperature is a major environmental factor, and it varies over days, seasons, and years. With the temperature rise, invertebrates rework sediment surface more intensely, bury surface particles to deeper depth, and create higher biodiffusion and biotransport coefficients (Ouellette *et al.*, 2004; Maire *et al.*, 2007; Przeslawski *et al.*, 2009). Changes in burrowing activity alter the oxygen and pH distributions at the sediment-water interface (Przeslawski *et al.*, 2009). An increase in temperature also influences the nutrient flux across the sediment-water interface (Fitch and Crowe, 2011), and warmer environments are associated with higher invertebrate metabolic rates (Hymel and Plante, 2000; Postolache *et al.*, 2006; Brodersen *et al.*, 2008).

*Corbicula fluminea* (Bivalvia: Corbiculidae) and *Bellamya aeruginosa* (Gastropoda: Viviparidae) are two common dominant molluscs in freshwater systems in China (Cai *et al.*, 2010). Many studies on *C. fluminea* concentrate on its ecological effects, as it is considered an invasive clam in North America, South America and Europe

(Karatayev *et al.*, 2007). This clam is able to increase the sediment organic matter content through filter feeding, reduce sediment organic matter by pedal feeding, and alter benthic community through feeding and bioturbation (Hakenkamp and Palmer, 1999; Hakenkamp *et al.*, 2001; Saloom and Duncan, 2005). At the same time its activity in sediment also enhances the sediment oxygen uptake and release of phosphate, ammonium, and nitrate from the sediment to the overlying water (Zhang *et al.*, 2011). *B. aeruginosa* also influences the oxygen uptake of the sediment, sediment organic matter content, and dissolved organic carbon flux (Zheng *et al.*, 2011). However, the effect of temperature on the oxygen uptake and nutrient flux in the sediment inhabited by these two molluscs remains unknown. In the present study, we aim to investigate the influence of temperature on oxygen uptake and nutrient flux in sediments inhabited by *B. aeruginosa* and *C. fluminea*.

## METHODS

### Field sampling

Lake Taihu is located in the Changjiang (Yangtze) river delta and has an area of 2338 km<sup>2</sup> and a mean depth of approximately 1.9 m (Qin, 2008). The water temperature ranges from 6.0 to 30.1°C annually, with the lowest temperatures occurring in January, and the highest temperatures occurring in August (Chen *et al.*, 2009). The estuary of the Dapu river is a eutrophic area situated in the western portion of lake Taihu. As *C. fluminea* is abundant in this area and dominates the benthic community, a previous study on the bioturbation of the clam has been conducted in this area (Zhang *et al.*, 2011). In order to perform a consistent study, all the sediments, water, and organisms used in the laboratory study too were sampled in the same area (31°18'42.7" N, 119°56'52.2" E).

On November 4<sup>th</sup> 2011, sediment cores were collected using plexiglass tubes (11 cm ID, 50 cm long) and an 11×50 cm gravity corer (Rigo Co. Ltd., Saitama, Japan). The overlying water was collected with a water sampler and simultaneously stored in 30 plastic barrels (25L). In addition, a little sediment was collected for the temporary cultivation of *C. fluminea* specimens before the experimentation in the laboratory. The *C. fluminea* and *B. aeruginosa* specimens were collected using a Petersen Grab sampler (length: 36 cm, width: 20 cm, height: 15 cm) on the same day. The sediment cores, mollusc specimens, and lake water were brought to the laboratory and maintained at 15±1°C. Before the experimentation, the molluscs were temporarily cultivated in a tank filled with sediment and water coming from their original site. The temporary cultivation was kept at 12 h intervals of dark and light, under a temperature of 15±1°C, and the water was aerated by a mini air pump to provide the molluscs with enough oxygen.

### Microcosms

The top 12 cm of the cored sediments were sectioned into 4-cm intervals, and the intervals from the same depth were pooled together. To remove macrofauna and large particles, the pooled sediments were sieved through a 0.6 mm mesh. Each pool was then fully homogenised by a dough mixer and placed into 18 plexiglass tubes (11 cm ID, 17-cm long) at the depth from which the pooled sediments had been sampled. Lake water was gently added to the top of each artificial core using intravenous needles. Hence, 18 microcosms having 12 cm of sediment and 5 cm of water were produced. All of the microcosms were placed in a plexiglass tank and submerged in lake water at a temperature of 15±1°C. The overlying water was recycled slowly, and the microcosms were pre-incubated for 16 days at 12 h intervals of dark and light before the introduction of the molluscs.

### Experimental design

After 16 days of pre-incubation, the 18 sediment cores were randomly divided into 6 groups of three replicates, each representing a different combination of temperature (15, 25°C) and biota (*C. fluminea*, *B. aeruginosa*, none). The groups that included *C. fluminea* and *B. aeruginosa* were identified as the C and B treatments, respectively, and the treatments without the addition of molluscs were identified as R (reference). The sediment oxygen uptake rate and the nutrient flux across the sediment-water interface were measured for the 6 groups (the method is described below) and found not to significantly vary among the six groups (one-way ANOVA result, oxygen uptake: F=1.009, P=0.454; SRP flux: F=0.618, P=0.689; ammonium flux: F=1.146, P=0.388). Three groups labelled as 25°C treatment were then moved to another plexiglass tank and also submerged in lake water. The water temperature of the second tank was controlled at 25±1°C by another thermostatic circulator. The other 3 groups labelled as 15°C treatment remained in the first tank at 15±1°C. Molluscs were introduced 5 days later to balance the temperature in and out the sediment cores in the 25°C tank.

For the *C. fluminea* treatment, 10 *C. fluminea* were added to each core, for a total biomass of 29 g wet weight (WW), which was equal to the density and biomass previously found at the field sampling site (1072 ind. m<sup>-2</sup>; 2918 g WW m<sup>-2</sup>) (Zhang *et al.*, 2011). Active *C. fluminea* specimens weighing between 2.46 g and 3.15 g and whose shell length ranged between 1.76 and 2.24 cm were selected. For the *B. aeruginosa* treatment, the density of the snail was based on the published data for this snail (Cai *et al.*, 2010; Liu *et al.*, 2010), and 3 specimens were added per core, for a total biomass of 7.5 g. The *B. aeruginosa* specimens used in the experiment ranged from 1.80 to 2.24 cm in shell length. Thus, the three groups in each tank were treated differently.

The sediment cores with *B. aeruginosa* were covered with nylon net (0.6×0.6 cm hole) to prevent the snails from escaping. The lake water in both tanks was renewed daily with the original lake water, and a small aerator was installed in each tank to maintain the oxygen saturation of the overlying water. Ten days after the molluscs were introduced in the sediment cores, the sediment oxygen uptake and the nutrient flux across the sediment-water interface were examined.

### Oxygen uptake measurement

For the measurement of the sediment oxygen uptake, the lake water in the tank was siphoned to a level lower than that of the top end of the sediment core tubes. The top end of the tubes was then sealed with a lid with a hole in the centre, which in turn was sealed with a rubber stopper. The oxygen uptake measurement was based on the O<sub>2</sub> depletion in the enclosed overlying water. The oxygen concentration in the overlying water was determined using an optical fibre O<sub>2</sub> microoptode, with a tip diameter <0.1 mm (PreSens, Regensburg, Germany), inserted through the central hole. For each measurement, the O<sub>2</sub> microoptode tip was gently moved downwards from the water surface to 2 mm above the sediment-water interface at 1 mm intervals, and at each interval one measurement was obtained. The mean of the profile data indicates the oxygen concentration in the overlying water. The oxygen concentrations were recorded approximately every 0.5 h until they decreased to 80% of the saturation concentration. Oxygen uptake was calculated by linear regression of oxygen concentration with time.

### Nutrient flux measurement

Nutrient flux measurements were conducted on the next day of the oxygen uptake measurement. The principle of nutrient flux was similar to that of the oxygen uptake. So, to get the linear regression result of nutrient concentration on time, we had to sample the overlying water and determine nutrient concentration at different times. Likewise, lake water in the tanks was siphoned exactly as it was done for the oxygen uptake measurement. The water was sampled at the time when the oxygen level reached 80% saturation (t) in each group. Specifically, water samples were collected at 0 (immediately after the sediment core tube was first sealed), 1/4 t, 1/2 t, 3/4 t, and t time, to get a series of data at different times. At each sampling time, 10 mL of the overlying water was sampled, and 10 mL of the siphoned water was then replenished to the core tube. The soluble reactive phosphorus (SRP) and ammonium were analysed using the molybdenum blue method (Murphy and Riley, 1962) and the Nessler's reagent colourimetric method (SEPA, 2002), separately. Nutrient fluxes were estimated by linear regression of nutrient concentration with time, with a correction for the volume of the replacement water. Both oxygen uptake and nutrient fluxes were measured in the dark to eliminate the influence of photosynthesis.

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### Statistical analysis

The impact of temperature and molluscs on the oxygen uptake and nutrient flux were evaluated using a two-way ANOVA, with temperature and treatment as two factors. If the treatment effect was significantly different, a Tukey *post-hoc* test was then used to detect significant differences between the molluscs and the reference treatments in each temperature group. The effects of the different experimental temperatures on the net oxygen uptake and nutrient flux caused by the molluscs were tested using a one-way ANOVA. All of the statistical analyses were conducted using SPSS 13.0 software (SPSS, IBM Corporation, Armonk, NY, USA).

## RESULTS

The introduction of the two molluscs disrupted the surface sediment structure (Fig. 1). *B. aeruginosa* was active and usually glided over the sediment surface, leaving a concave trail 1~2 mm in depth (Fig. 1a). At times, the snails attached to the tube wall or buried themselves in the sediment; nearly all of the snails remained in the sediment at the end of the experiment. In contrast, once it established itself after burrowing, *C. fluminea* seldom moved (Fig. 1b). No difference between the effects of the two temperatures on the activities of the snail or clam was observed. The reworking of the sediment by the two molluscs next to their bodies produced oxic burrow walls which were in the same colour of the surface oxic sediment (Fig. 1c).

The activities of the molluscs significantly altered the sediment oxygen uptake, which was significantly influenced by the temperature as well (Fig. 2a, Tab. 1). Both *C. fluminea* and *B. aeruginosa* produced greater levels of sediment oxygen uptake than those observed for the corresponding reference groups at 15 and 25°C (Fig. 2a). The clam produced higher net oxygen uptake rate at 25°C than that at 15°C, whereas the effect of the temperature on the net oxygen uptake generated by *B. aeruginosa* was not statistically significant (Fig. 3a).

The SRP flux across the sediment-water interface was significantly affected by the two molluscs and temperature (Fig. 2b, Tab. 1). Specifically, at 15°C *C. fluminea* significantly accelerated the SRP flux from the sediment to the overlying water, whereas *B. aeruginosa* did not significantly increase the SRP flux compared with the reference group. Instead, at 25°C each type of mollusc increased the SRP release from the sediment compared with the reference group (Fig. 2b). Both *C. fluminea* and

*B. aeruginosa* enhanced the higher SRP flux from the sediment to the overlying water at the higher temperature (Fig. 3b).

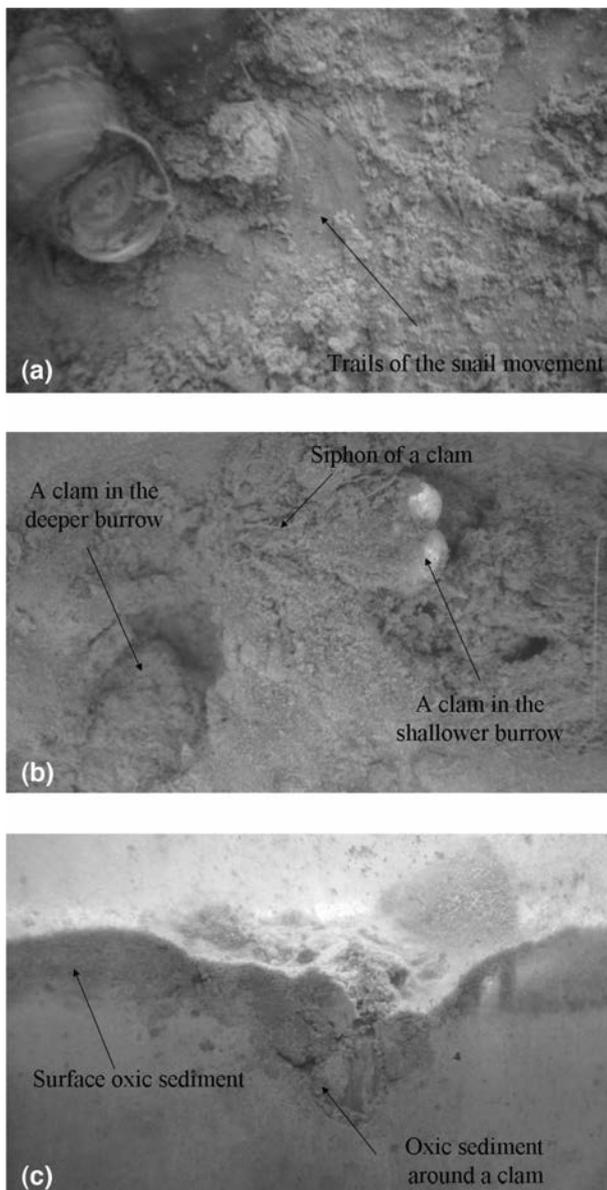
The molluscs and temperature also significantly changed the ammonium flux across the sediment-water interface (Fig. 2c, Tab. 1). *C. fluminea* significantly enhanced the ammonium release from the sediment to the overlying water, whereas *B. aeruginosa* produced no significant variation compared with the corresponding reference group at either temperature (Fig. 2c). As was found for the oxygen uptake, *B. aeruginosa* created no signifi-

cant difference in the ammonium flux between 15 and 25°C, whereas *C. fluminea* caused more ammonium release from the sediment to the overlying water at 25°C than at 15°C (Fig. 3c).

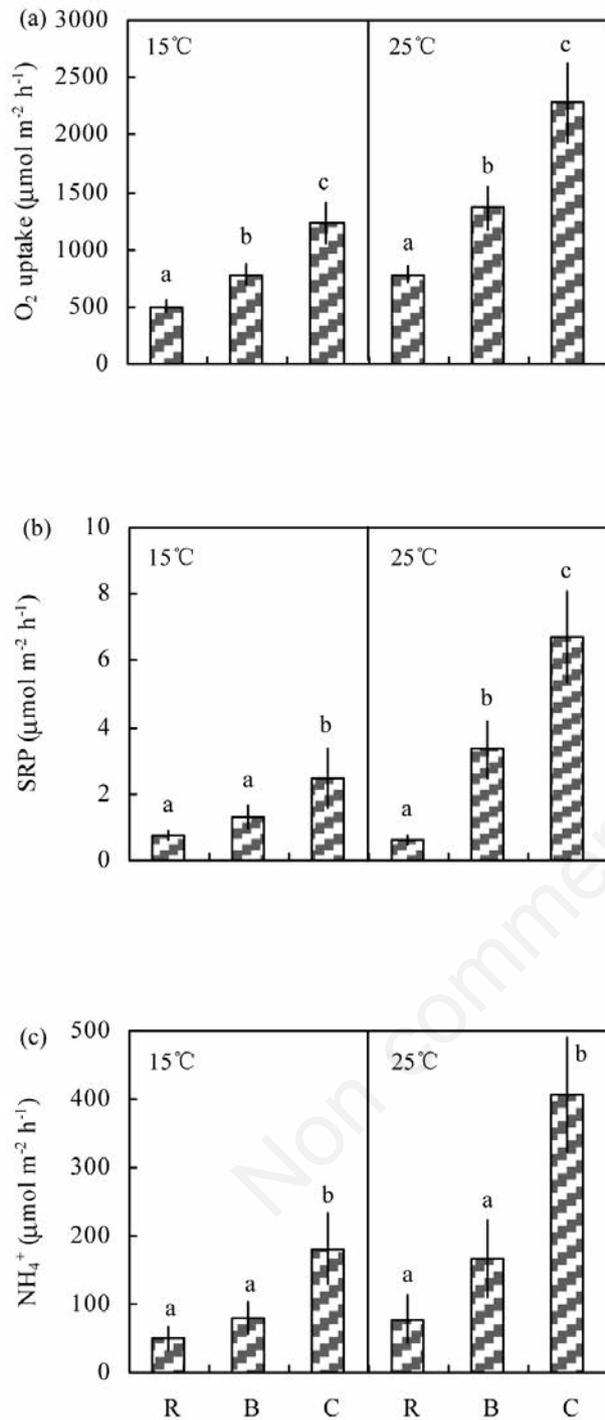
## DISCUSSION

Oxygen is a central molecule for global element cycling, and oxygen uptake by sediment is widely studied to determine benthic metabolism and organic matter mineralisation (Rasmussen and Jørgensen, 1992; Glud, 2008). The enhancement of the sediment oxygen uptake by benthic invertebrates has been attributed to their respiration, to anoxic sediment oxidation, and to microbial activity induced by their activities in the sediment (Glud, 2008). *C. fluminea* has been reported to enhance sediment oxygen uptake, and this enhancement was also explained by the factors cited above (Zhang *et al.*, 2011). The present study further indicates that the effect of the clam on the sediment oxygen uptake increased with an increase in temperature. The respiration of *C. fluminea* increases with increasing temperature over a moderate range (McMahon, 1979; Liu *et al.*, 1999). McMahon (1979) has indicated that clam respiration under oxygen saturation triples when the temperature is increased from 10°C to 20°C; furthermore, that respiration at 30°C is less than that at 20°C but greater than that at 10°C. Liu *et al.* (1999) have shown that a 1.7 g WW clam increases its respiration by approximately 40% as the temperature increases from 20 to 28°C. Such variation in results may be due to the difference in experimental temperatures and the change in clam metabolism from 10 to 30°C. Although respiration is not the sole factor responsible for the enhanced oxygen uptake by the sediment, these results clearly indicate that the clam's respiration is a factor that accelerates the oxygen uptake when the temperature rises.

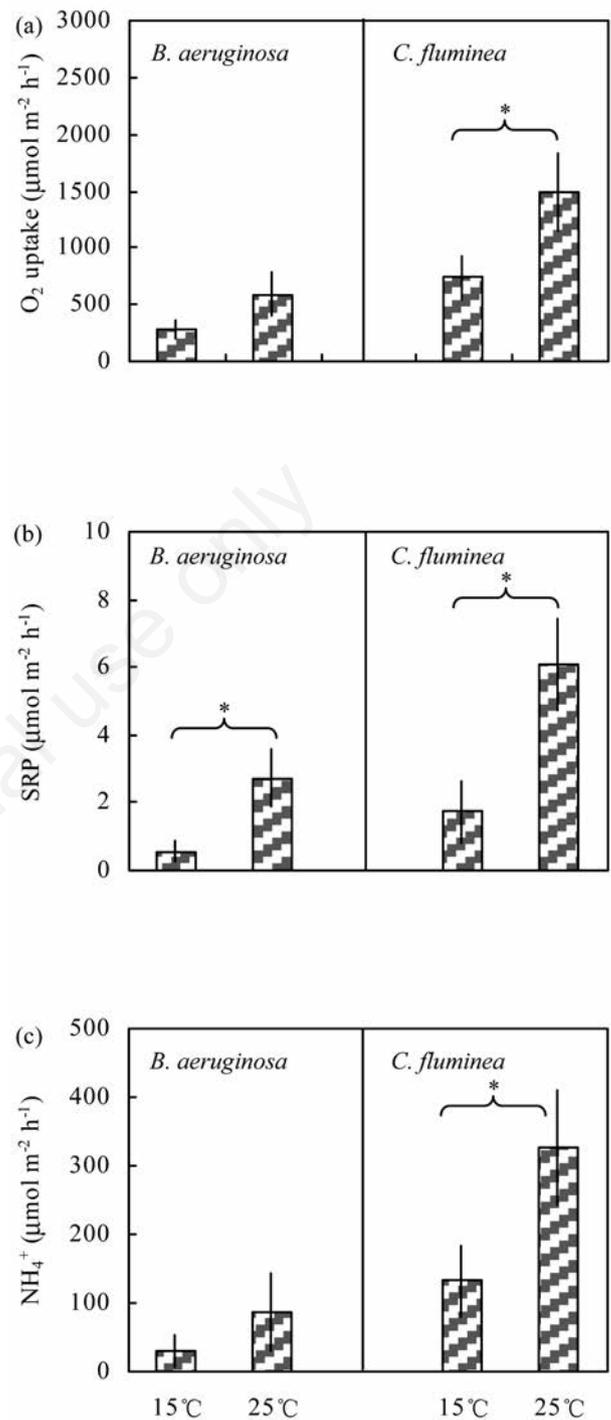
The sediment oxygen uptake became greater in the reference group when the temperature was increased to 25°C (Fig. 2a). This result was explained by an increase in microbial respiration and the oxidation reactions through diffusion accompanying the temperature increase (Kristensen, 2000). It is reasonable, then, that the oxygen uptake through biodiffusion occurring around the clam burrow will also increase (Kristensen, 2000; Welsh, 2003). Besides the increased respiration, this may be another important reason for the enhanced net sediment oxygen uptake with increasing temperature. On the other hand, if temperature rises, the oxygen content of the water will decrease, and this decrease can produce a higher ventilatory flow rate in *C. fluminea* (Tran *et al.*, 2000). Increased filtration through siphon and deposit feeding will increase the oxygen exchange across the sediment-water interface, facilitating oxygen uptake by the sediment and by organisms. It was also reported that a bivalve (*Abra ovata*) increased its reworking of the sediment surface as



**Fig. 1.** Sediment surface in a) *B. aeruginosa* and b) *C. fluminea* treatment, and profile in c) *C. fluminea* treatment.



**Fig. 2.** a) Oxygen uptake rates, b) SRP fluxes, and c) ammonium fluxes across the sediment-water interface in different groups after the introduction of the molluscs at 15 and 25°C. SRP=soluble reactive phosphorus; NH<sub>4</sub><sup>+</sup>=ammonium; R=reference group; B=*B. aeruginosa* group and C=*C. fluminea* group. All the results are expressed as the mean±1 SD (n=3). The positive nutrient fluxes values indicate the release of the nutrient from the sediment to the overlying water. At each temperature, different letters indicate that the differences in the fluxes were significant (P<0.05).



**Fig. 3.** a) Net oxygen uptake, b) SRP fluxes, and c) ammonium fluxes attributable to *B. aeruginosa* and *C. fluminea* after subtracting the corresponding values of the reference group at different temperatures (15 and 25°C). SRP=soluble reactive phosphorus; NH<sub>4</sub><sup>+</sup>=ammonium. All the results are expressed as the mean±1 SD (n=3). The positive nutrient fluxes values indicate the release of the nutrient from the sediment to the overlying water. An asterisk indicates that the mollusc created significantly different (P<0.05) net fluxes at 15°C and 25°C.

the temperature increased (Maire *et al.*, 2007). However, this effect may not apply to the clams in the present study because, unlike *Abra ovata*, the *C. fluminea* individuals usually buried themselves in the sediment without moving after they became established. In our study, this behaviour was observed both at 15 and at 25°C. For this reason, the enhancement of sediment oxygen uptake induced by *C. fluminea* may be due to enhanced oxygen diffusion and respiration.

The presence of *C. fluminea* has been reported to accelerate the release of SRP and ammonium from the sediment to the overlying water. This acceleration has been attributed to excretion by the clam, to nutrient regeneration, and to enhanced diffusion through the burrow wall (Chen *et al.*, 2005; Zhang *et al.*, 2011). Wittmann *et al.* (2008) reported that the clam's rate of excretion of SRP at 19°C was 3.25 times greater than that at 4°C, whereas the rate of ammonium excretion at 19°C was 16 times greater than that at 4°C. Another reason for this outcome may be that an increase in temperature will decrease the oxygen concentration in the overlying water and the depth of oxygen penetration in the sediment, thereby reducing the redox potential and increasing the ammonium and SRP concentrations in the pore water (Zhong *et al.*, 2009a, 2009b). Moreover, *C. fluminea* is able to accumulate organic matter from the overlying water and reduce the organic matter content of the sediment (Hakenkamp and Palmer, 1999; Chen *et al.*, 2005). The decay of organic matter will also release ammonium and SRP into the sediment pore water (Welsh, 2003). Consequently, the diffusion gradients of ammonium and SRP will become steeper. Moreover, the increase of temperature from 15 to 25°C will increase the diffusion coefficients of  $\text{HPO}_4^{2-}$  and ammonium by 27.9% and 25.3%, respectively (Krom and Berner, 1980). Therefore, the diffusion of SRP and ammonium through the sediment-water interface and bioturbated burrow walls can increase. Accordingly, both the increased excretion and diffusion contribute to differences in the net SRP and ammonium fluxes at 15 and 25°C in our study.

Although we know little about *B. aeruginosa*, the snail has been reported to excrete large amounts of ammonium and phosphorus into the water (Lu *et al.*, 2007), and its metabolism also increases with temperature rise over a range

of 5 to 25°C (Yan and Liang, 2003). In addition, the snail also removes *algae* from the overlying water and decreases the amount of organic matter in the sediment (Zheng *et al.*, 2011). As compared with *C. fluminea*, we observed *B. aeruginosa* was active on the sediment, and its gliding movement on the sediment surface was often accompanied by a concave trail 1~2 mm in depth. Wang *et al.* (2009) reported that the depth of oxygen penetration in lake Taihu ranged from 4~6 mm. Accordingly, we maintain the movements of the snail can increase the oxic-anoxic sediment interface but cannot have access to the anoxic sediment – which is important for nutrient exchange and oxygen uptake – under the superficial oxic sediment. Apart from this, in our study the influence of the snail on the sediment oxygen uptake and nutrient release was similar to that observed for *C. fluminea*. Still, in the *B. aeruginosa* treatment, each sediment core was given only 3 *B. aeruginosa* individuals, while the in the *C. fluminea* treatment, as many as 10 specimens of *C. fluminea* were added. These differences in biomass and density may be the reason why the snails did not produce a significant difference in the oxygen uptake and ammonium flux between 15 and 25°C. Thus, we expect that an increase in the biomass of the snails would significantly increase the net oxygen uptake and ammonium flux with temperature rise.

## CONCLUSIONS

The present study examined the effects of temperature on sediment oxygen uptake and nutrient flux in sediment inhabited by two molluscs. The results indicated that *C. fluminea* significantly increased more sediment oxygen uptake, SRP, and ammonium flux at 25°C than that at 15°C. *B. aeruginosa*, however, only accelerated higher SRP release at 25°C, but did not produce more sediment oxygen uptake and ammonium flux at 25°C, when compared with that at 15°C. This difference between two molluscs may be due to the biomass of *B. aeruginosa* which is quite smaller than that of *C. fluminea*, so that a significant increase of sediment oxygen uptake and ammonium flux is expected with an increase of *B. aeruginosa*. Overall, the study demonstrates that temperature and mollusc have a great and interactive effect on the biogeochemistry of oxygen and nutrient in aquatic ecosystems. The inter-

**Tab. 1.** Two-way ANOVA results after the introduction of the molluscs.

Parameter	Temperature effect		Treatment effect		Temperature × Treatment effect	
	F (1,12)	P	F (2,12)	P	F (2,12)	P
O <sub>2</sub> uptake	53.5	<0.001	55.8	<0.001	6.47	<0.05
SRP flux	32.6	<0.001	40.0	<0.001	12.3	<0.01
NH <sub>4</sub> <sup>+</sup> flux	23.5	<0.001	34.8	<0.001	6.16	<0.05

SRP, soluble reactive phosphorus; NH<sub>4</sub><sup>+</sup>, ammonium.

action of infauna with temperature and other environmental factors, and the role of the interaction in aquatic biogeochemistry, though, need to be further researched. More studies focused on the interaction of infauna and environmental factors, especially the infauna community and the multiple factors, will provide more profound understanding of aquatic biogeochemistry.

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