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Citation	Journal of Experimental Marine Biology and Ecology, 383(1): 8-16
Issue Date	2010-01-31
Doc URL	<a href="http://hdl.handle.net/2115/42633">http://hdl.handle.net/2115/42633</a>
Right	
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**Reevaluation of the nutrient mineralization process by infaunal bivalves (*Ruditapes philippinarum*) in a shallow lagoon in Hokkaido, Japan**

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## Abstract

Previous estimations of nutrient mineralization in the water column by infaunal bivalves might have been overestimated because of underestimation of the uptake process by microphytobenthos in the field. We conducted field surveys of environmental conditions and quantitative sampling of *Ruditapes philippinarum* in a shallow lagoon system (Hichirippu Lagoon, eastern Hokkaido, Japan) in August 2006. We recorded the spatial distribution pattern and the molar ratio of dissolved inorganic nutrients to determine the limiting nutrients for microphytobenthos, to evaluate the input and output of nutrients at the entrance of the lagoon station, and to estimate potential nutrient mineralization by *R. philippinarum*. Our aim was to reevaluate the nutrient mineralization process by infaunal bivalve species. In this study, the mean standing stock of microphytobenthos inhabiting surface sediment (5 mm thick) on the tidal flats was 100 times higher than that of phytoplankton (1 m depth). Low N/P and high Si/N ratios (mean = 2.6 and 17.6, respectively) near the entrance of the lagoon compared to those of microphytobenthos (N:P:Si = 10.1:1:18) clearly suggest N deficiency. The flux of  $\text{NH}_4\text{-N}$  coming into the lagoon was  $3.4 \text{ kmolN d}^{-1}$ , and the flux out was  $-3.7 \text{ kmolN d}^{-1}$ . Thus, assuming that there would have been no phytoplankton and microphytobenthos uptake during the day,  $0.3 \text{ kmolN d}^{-1}$  of  $\text{NH}_4\text{-N}$  was produced within the lagoon. However, the  $\text{NH}_4\text{-N}$  mineralization rate of the clams has been estimated to be approximately  $7.7 \pm 6.8 \text{ kmolN d}^{-1}$ . Thus, 96% ( $7.4 \text{ kmolN d}^{-1}$ , i.e.,  $7.7 \text{ kmolN d}^{-1}$  minus  $0.3 \text{ kmolN d}^{-1}$ ) of the  $\text{NH}_4\text{-N}$  mineralized by the clam was consumed by microphytobenthos. In contrast, if all the  $\text{NH}_4\text{-N}$  inflow ( $3.1 \text{ kmolN d}^{-1}$ ) was consumed by the microalgae before outflow, 52% ( $4.0 \text{ kmolN d}^{-1}$ , i.e.,  $7.7 \text{ kmolN d}^{-1}$  minus  $3.7 \text{ kmolN d}^{-1}$ ) of the  $\text{NH}_4\text{-N}$  mineralized by the clams should have been consumed by microphytobenthos. Microphytobenthos on the tidal flats ( $11.3 \pm 11.8 \text{ kmolN}$ ) used all of the surplus nutrients (between  $4.0$  and  $7.4 \text{ kmolN d}^{-1}$ ), and the temporal division rate [= ( $\text{NH}_4\text{-N}$  uptake)/(standing stock of microphytobenthos)] of microphytobenthos would have to be between  $0.35$  and  $0.65 \text{ d}^{-1}$ . Residual  $\text{NH}_4\text{-N}$  ( $0.3\text{--}3.7 \text{ kmolN d}^{-1}$ ) was the water column source and accounted for 12–148% of  $\text{NH}_4\text{-N}$  in the water column near the entrance of the lagoon ( $2.5 \pm 1.4 \text{ kmolN}$ ) per day. This is the first field-based observation with a quantitative evaluation of nutrient mineralization by infaunal bivalves and nutrient uptake by microphytobenthos.

*Key words:* infaunal bivalve species, nutrient mineralization, microphytobenthos, dissolved inorganic nutrients, lagoon, *Ruditapes philippinarum*

## 1. Introduction

One of the important features of the structure of coastal ecosystems is that they are often dominated by bivalve species. In particular, the role of bivalves in nutrient mineralization has been studied for the last two decades. The epifaunal bivalve *Crassostrea virginica* (American oyster) has a significant effect on the nutrient availability for phytoplankton (Dame et al., 1985, 1989). Asmus and Asmus (1991) also suggest that the filtration activity of the mussel *Mytilus edulis* reduces standing stocks of phytoplankton, and that nutrient mineralization sustains primary phytoplankton production. Nutrient mineralization by infaunal bivalve species has been studied in laboratory and mesocosm experiments (Doering et al., 1987), in situ benthic chambers, sediment core incubations (Cockcroft, 1990; Yamamuro and Koike, 1993; Bartoli et al., 2001), and by quantification of the total upward flux of nutrients from sediments and subsequent estimation of the relative contribution of animal excretion (Nakamura et al., 1988; Kiibus and Kautsky, 1996; Magni et al., 2000; Hiwatari et al., 2002; Kohata et al., 2003). These studies have shown that nutrient mineralization is an important source for water-column primary production, and this concept is incorporated in the ecological model for infaunal bivalve fisheries (e.g., Zaldivar et al., 2003; Spillman et al., 2008). However, a recent study showed that nutrient mineralization by infaunal bivalve species (*Ruditapes philippinarum*, *Musclista senhousia*) affects nutrient concentrations in the water column as well as pore water (up to 10 cm depth) (Magni and Montani, 2006).

In recent years, microphytobenthos as primary producers have been recognized as being as important as phytoplankton in the neritic region due to solar radiation reaching the sea floor (MacIntyre et al., 1996; Cahoon, 1999; Underwood and Kromkamp, 1999). Microphytobenthos take up nutrients from both the water column and pore water (Henriksen et al., 1980; Sundbäck et al., 1991; Cahoon and Cooke, 1992; Cahoon, 1999; Sundbäck et al., 2000; Srithongouthai et al., 2003). Even though it is easy to assume that there is an important relationship between microphytobenthos and infaunal bivalve species, this relationship can only be derived from laboratory and in situ measurement studies (e.g., Swanberg, 1991; Sandwell et al., 2009). Previous estimates of nutrient mineralization in the water column by infaunal bivalves might have been overestimated as a result of the underestimation of the uptake process by microphytobenthos in the field.

We conducted field surveys of environmental conditions and quantitative samplings of *R. philippinarum* in a shallow lagoon system (Hichirippu Lagoon in the eastern part of Hokkaido, Japan) in August 2006. We recorded both the spatial distribution pattern and the molar ratio of dissolved inorganic nutrients to determine the limiting nutrients for microphytobenthos, to evaluate input and output of the nutrients at the entrance of the lagoon station based on a continuous survey for 25 h, and to estimate potential nutrient mineralization by *R. philippinarum*. Our aim was to reevaluate the nutrient mineralization process by infaunal bivalve species, and to provide a proof of the concept and broad heuristic insights.

## 2. Materials and Methods

### 2.1 Study Area

Hichirippu Lagoon is ca. 3.56 km<sup>2</sup> in area, shallow (mean water depth ca. 1 m), and brackish. It borders the Pacific Ocean in Hokkaido, Japan (44°03' N, 145°03' E; Fig. 1). At the extreme low water level, tidal flats are ca. 0.19 km<sup>2</sup> in area and cover about 5.3% of the lagoon. Both naturally occurring and artificial tidal flats, accounting for 30% and 70% of the tidal flats, respectively, are present. According to the results of our recent study on clam populations on the tidal flats of this lagoon, the population persisted throughout the year, and was stable at around 8,000 individuals m<sup>-2</sup> in density (Komorita et al., 2009). The shell length of the clam population was ranged between 5 mm and 50 mm (Komorita et al., 2009). Furthermore, 30–92 metric tons of the clam were harvested from the tidal flats between 1998 and 2004 (based on the fishery statistics provided by Chirippu Fishery Cooperative Union). The study was conducted at 15 subtidal stations (Stations 0–12, 14, and 15) and 5 tidal flat stations (Stations A, B, D, F, and G; Fig. 1).

### 2.2 Sampling Procedure

In August 2006, sampling of seawater and sediment was conducted during spring tide at the subtidal and tidal flat stations, respectively. For seawater sampling, we monitored temperature, salinity, nutrients [NH<sub>4</sub>-N, NO<sub>3</sub> + NO<sub>2</sub>-N, PO<sub>4</sub>-P, and Si(OH)<sub>4</sub>-Si], and chlorophyll-*a* (Chl-*a*) of floodwater at the subtidal

stations, and carried out horizontal and continuous surveys. For the horizontal survey, conducted at 14:00–16:00 on August 8, hydrological measurements were performed vertically at 10 cm intervals using a conductivity, temperature, and depth profiler (YSI 556; YSI Hydrodata, Letchworth, UK), and seawater samples (2 L) were collected from the surface and 10 cm above the sea floor (samples at station 0 and the other 14 stations were obtained from the surface and both layers, respectively) using a motor pump (YPM-12; flow rate: 70 L min<sup>-1</sup>, intake diameter: 105 mm; Meiwa, Tokyo, Japan). For the hourly continuous survey conducted at station 0 (cross-sectional area 240 m<sup>2</sup>, 80 m in width and 3 m in depth; Fig. 1), we obtained 26 surface seawater samples and vertical profiles of water temperature and salinity at 20-cm intervals from 14:00 on August 8 to 15:00 the next day. At station T (Fig. 1), the tidal height was monitored using a tide indicator (RMD-5225A; Rigo Co. Ltd, Tokyo, Japan) every 10 min from 13:00 on August 8. Data for the hourly solar radiation were obtained from the Nemuro Meteorological Agency Station, located approximately 50 km northeast of Hichiripu Lagoon.

On August 6, field investigations were carried out during low tide at 5 tidal flat stations. In the present study, we conducted parallel field investigations for Chl-*a* concentration in surface sediment and macrobenthic assemblages. On each sampling occasion, sediment samples for Chl-*a* analysis were randomly collected at 10 locations using an acrylic core tube (3 cm in diameter) within 1 m radius of the station. The sediment was carefully extruded and the surface layer (0–0.5 cm) sliced off. Macrobenthic samples were collected for 5 replicates using a 100-cm<sup>2</sup> stainless steel corer (10 cm depth) at each station; these samples were sieved through a 1-mm mesh screen. Residues of each sample were stored in plastic bags.

### 2.3 Sample treatment

In the laboratory, Chl-*a* was extracted from duplicate subsamples of wet sediment (ca. 0.5 g) using 90% acetone. Seawater samples were filtered through glass fiber filters (GF/F, Whatman, Florham, NJ, USA) and Chl-*a* was extracted from these using 90% acetone. After 24 h darkness at –20°C, samples were sonicated for 5 min. The concentrations of Chl-*a* (µg Chl-*a* L<sup>-1</sup>) in the supernatants, which were obtained before and after acidification with 1 N HCl according to Lorenzen's (1967) method, as described by Parsons et al. (1984), were analyzed fluorophotometrically (Turner 10-AU-5, Turner Designs, Sunnyvale, CA, USA). For sediment samples, the volume of pore water was substituted by the dry weight (DW) of the sediment. Values thus expressed were corrected for porosity as measured by the water content in µg Chl-*a* g<sup>-1</sup> DW. This was obtained after drying a sediment sample (ca. 1 g) at 60°C for 24 h. Measurement of the Chl-*a* content in the surface sediment (0.5 cm depth) was also expressed over area (mg Chl-*a* m<sup>-2</sup>) by accounting for the bulk density of the sediment particles as 2.5 g cm<sup>-3</sup> and taking the spatial and temporal variations of the pore water content into consideration (details see in Montani et al., 2003). Part of each sediment sample was centrifuged at 3,000 rpm and the extracted pore water was immediately filtered through disposable membrane filters (DISMIC-25cs; pore size, 0.45 µm; Advantec, Dublin, CA, USA), attached to a 10-mL sterile syringe, into polystyrene test tubes. Nutrients in the seawater samples were collected after filtration through glass fiber filters (GF/F; Whatman) in a Teflon beaker and transferred into polystyrene test tubes. The filtrates were stored at –20°C in preparation for nutrient analysis using an autoanalyser (QUAATRO; BL TEC, Tokyo, Japan), according to the method described by Strickland and Parsons (1972). At each station, we integrated both Chl-*a* and each nutrient concentration in the seawater with respect to depth (mean depth, 1 m) from surface to bottom to estimate standing stocks of Chl-*a* and each nutrient, respectively.

The clams, *R. philippinarum*, found in sediment samples were cleaned of epibionts and identified. They were weighed for total WW, including the shell. The total biomass of *R. philippinarum* from each sample was then transformed to DW biomass according to the following procedure. A total of 100 individual clams were collected from the stations. In the laboratory, the length of each individual was measured to the nearest 0.05 mm using digital calipers and the total weight (live animal) was determined. All specimens were then depurated in filtered (GF/F; Whatman) seawater for 6–8 h. Soft tissues of each animal were removed from the shell, gently dried on blotting paper, and weighed (WW). The soft tissues were freeze-dried and weighed again to the nearest 1 mg DW soft tissue from all samples.

### 2.4 Data Analysis

The carbon contents of microalgae used were C/Chl = 33 for phytoplankton (Eppley, 1968) and C/Chl = 33.7 for microphytobenthos (Montani et al., 2003). The elemental compositions of these microalgae

used were C:N:P:Si = 106:16:1:15 for phytoplankton (Redfield et al., 1963) and C:N:P:Si = 75.7:10.1:1:17.8 (Montani et al., 2003) for microphytobenthos. The standing stocks of each element in the microalgae were obtained by multiplying these elemental ratios by standing stocks of microalgae. The potential nutrient mineralization rate by *R. philippinarum* in the whole tidal flat area (0.19 km<sup>2</sup>) was estimated based on the experimental data (6.4 μmol NH<sub>4</sub>-N gDW<sup>-1</sup> h<sup>-1</sup>, 1.7 μmolP gDW<sup>-1</sup> h<sup>-1</sup>, and 5.9 μmolSi gDW<sup>-1</sup> h<sup>-1</sup>; Magni and Montani, 2005) under constant water temperature (11.7°C).

The current speed (V; cm s<sup>-1</sup>) was estimated every 10 min at the entrance of the lagoon (Station 0) according to the following equation:

$$V(m) = [TH(m) - TH(m + 10)] \times (A)/S/(10 \text{ min.} \times 60 \text{ s}),$$

where V = current speed (cm s<sup>-1</sup>) at time “m” (every 10 min), TH = tidal height, A = extent of the impact of open ocean (half area of the lagoon 1.78 km<sup>2</sup>; see in section 3.1), and S = cross-sectional area (240 m<sup>2</sup>).

The current speed was shown as a 1-h running mean, and converted from cm s<sup>-1</sup> into m h<sup>-1</sup> by multiplying 100 cm, 60 s and 60 min. Hourly nutrient flux (F; mmol m<sup>-2</sup> h<sup>-1</sup>) was expressed by the following equation:

$$F(h) = C(h) \times V(h),$$

where C = nutrient concentrations (mmol m<sup>-3</sup>), which was converted from μmol L<sup>-1</sup> based on a continuous survey, V(h) = 1-h running mean of current velocity at every hour.

Regression analysis was used to test the significance of the results ( $P < 0.05$ ) using the software StatView (HULINKS, Inc., Tokyo, Japan). Principal component analysis (PCA) was used to explore the relationships among the variables during the continuous survey. PCA was conducted using the software SPSS 11.5 (SPSS Inc., Illinois, USA) with forward selection to test the significance of environmental variables at a level of  $p < 0.1$ .

### 3. Results

#### 3.1 Water Mass Structure

Spatial variation in sea surface temperature, salinity, and nutrients during the flood tide (14:00–16:00) on August 8 is shown in Fig. 2. According to water density, the water column in the lagoon was strongly mixed vertically by the flood tide (data not shown). A clear thermohaline front was observed in the central part of the lagoon (between Stations 10 and 12; Fig. 2a, b). Compared to the temperature near the entrance stations (Stations 0, 1, 2, 11, 12, and 14) where the water mass had low temperature and high salinity [LTHS, 14.6 ± 1.4°C, 32.8 ± 0.1 practical salinity unit (PSU)], the temperature in the inner part of the lagoon (Stations 3–10 and 15) was higher and the salinity was lower (HTLS, 27.9 ± 1.8°C, 31.3 ± 0.6PSU). Based on plots of temperature against salinity for individual samples including the bottom layer (Fig. 3a), temperatures at the entrance (LTHS) were about 10°C lower than that at the central station (HTLS). The open sea (Pacific Ocean) seemed to have an impact on approximately half the area of the lagoon (1.78 km<sup>2</sup>).

The concentration of PO<sub>4</sub>-P and Si(OH)<sub>4</sub>-Si in HTLS (PO<sub>4</sub>-P, 2.7 ± 1.0 μmol L<sup>-1</sup>, Si(OH)<sub>4</sub>-Si, 94.6 ± 18.5 μmol L<sup>-1</sup>) was higher than that in LTHS (PO<sub>4</sub>-P, 1.0 ± 0.4 μmol L<sup>-1</sup>, Si(OH)<sub>4</sub>-Si, 28.0 ± 16.1 μmol L<sup>-1</sup>; Fig. 2e, f and 3d, e). Significant correlations of salinity with both PO<sub>4</sub>-P and Si(OH)<sub>4</sub>-Si [PO<sub>4</sub>-P (μmol L<sup>-1</sup>) = -1.21 × Salinity + 40.7,  $r^2 = 0.80$ ,  $n = 29$ ,  $P < 0.0001$ ; Si(OH)<sub>4</sub>-Si (μmol L<sup>-1</sup>) = -33.31 × Salinity + 1130,  $r^2 = 0.59$ ,  $n = 29$ ,  $P < 0.0001$ ] were observed. In contrast, dissolved inorganic nitrogen (DIN; NH<sub>4</sub>-N and NO<sub>3</sub> + NO<sub>2</sub>-N) in LTHS (NH<sub>4</sub>-N, 1.4 ± 0.8 μmol L<sup>-1</sup>; NO<sub>3</sub> + NO<sub>2</sub>-N, 0.8 ± 0.7 μmol L<sup>-1</sup>) was higher than that in HTLS (NH<sub>4</sub>-N, 0.9 ± 0.5 μmol L<sup>-1</sup>; NO<sub>3</sub> + NO<sub>2</sub>-N, 0.3 ± 0.3 μmol L<sup>-1</sup>; Fig. 2c, d). DIN concentration tended to increase sharply beyond 32.5 PSU salinity (Fig. 3b, c) near the entrance of the lagoon. The molar ratio of N, P, and Si was distinctly divided between LTHS (N/P = 2.6 ± 1.8, Si/N = 17.6 ± 15.6) and HTLS (N/P = 0.5 ± 0.4, Si/N = 127.9 ± 90.4; Fig. 2g, h).

#### 3.2 Continuous survey

Figure 4a shows hourly solar radiation reported for August 8, 2006 at Nemuro Meteorological Agency Station. The solar radiation peaked around noon (10:00 to 14:00) on both days (2.62 to 3.05 MJ m<sup>-2</sup> d<sup>-1</sup>), and the nocturnal period (0 MJ m<sup>-2</sup> d<sup>-1</sup>) was from 20:00 on August 8 to 3:00 on the next day.

During the sampling period, there were two lower and two higher tidal levels (Fig. 4b). The largest difference in the tidal level was 109.3 cm between 2:40 (131.3 cm) and 10:30 (22.0 cm) on August 9. The current speed reached the highest outflow (-44.0 cm s<sup>-1</sup>) at 8:30 and inflow (39.6 cm s<sup>-1</sup>) at 13:10 before

and after the lowest low tide, respectively.

From the beginning of the survey (14:00 on August 9) to the highest high tide (2:40 on August 9), temperature varied between 12.4°C and 13.1°C and salinity varied between 32.8 PSU and 32.9 PSU, respectively, with tidal height (Fig. 4c). Temperature and salinity reached the highest and lowest values at 12:00 (22.7°C) and at 13:00 (32.1 PSU), respectively, after the lowest low tide. Although both factors showed high variation over 24 h, the highest temperature and lowest salinity almost coincided with the thermohaline front in the central part of this lagoon (14.9°C to 26.3°C and 31.8 PSU to 32.8 PSU, respectively) during flood tide (Fig. 2a, b).

Temporal variations of PO<sub>4</sub>-P and Si(OH)<sub>4</sub>-Si varied with the tidal height and peaked at 2.0 μmol L<sup>-1</sup> and 73.1 μmol L<sup>-1</sup>, respectively, at 12:00 on August 9 (Fig. 4d). In contrast, temporal variations of DIN were less obvious than PO<sub>4</sub>-P and Si(OH)<sub>4</sub>-Si (Fig. 4e), and ranged from 1.1 μmol L<sup>-1</sup> to 3.3 μmol L<sup>-1</sup> for NH<sub>4</sub>-N, and from 0.2 μmol L<sup>-1</sup> to 1.8 μmol L<sup>-1</sup> for NO<sub>3</sub> + NO<sub>2</sub>-N, respectively (Fig. 4e). At the lowest low tide (10:30 on August 9), the nutrient concentration also coincided with the thermohaline front in the central part of this lagoon.

In the forward selection procedure of PCA, the rank next to the second PC was excluded on both eigenvalues and degrees of freedom. The variable explained 65.5% of the variance of the first PCA component (Table 1). The factor loadings (r) showed that the significant variables for physical factors were solar radiation (r = 0.796, p < 0.01), current velocity (r = 0.514, p < 0.01), and tidal height (r = -0.914, p < 0.01) because the highest solar radiation and lowest tidal height were reached at almost the same time around noon (Fig. 4a, b). This component seemed to reflect tidal height, which showed the highest factor loadings. The tidal height strongly affected temperature (r = 0.979, p < 0.01), salinity (r = -0.976, p < 0.01), PO<sub>4</sub>-P (r = 0.918, p < 0.01), and Si(OH)<sub>4</sub>-Si (r = 0.963, p < 0.01). In contrast, factor loadings of DIN (NH<sub>4</sub>-N and NO<sub>3</sub> + NO<sub>2</sub>-N) were relatively low and negative (NH<sub>4</sub>-N: r = -0.527, p < 0.01, NO<sub>3</sub> + NO<sub>2</sub>-N: r = -0.675, p < 0.01).

### 3.3 Standing Stocks and Mass Balance of Biophilic Elements

We obtained a linear equation of the plots of total weight versus DW of soft tissues in all specimens of *R. philippinarum* (Fig. 5,  $y = 0.053x - 0.012$ ,  $r^2 = 0.910$ ,  $n = 100$ ). We used this equation to calculate the DW bivalve biomass of animal samples collected during field surveys [5.3% of the total (live) weight].

Table 2 shows the daily flux of seawater and nutrients at station 0, and the daily nutrient mineralization rate by *R. philippinarum* based on biomass ( $265 \pm 234$  gDW m<sup>-2</sup>) on the tidal flat area (0.19 km<sup>2</sup>). The amount of each nutrient [NH<sub>4</sub>-N, NO<sub>3</sub> + NO<sub>2</sub>-N, PO<sub>4</sub>-P, and Si(OH)<sub>4</sub>-Si] and the amount of each element in microalgae are shown in Fig. 6. The amount of each nutrient (i.e., storage) was integrated with respect to the depth from surface to bottom (mean depth: 1 m), and multiplied by the area of LTHS (1.79 km<sup>2</sup>). Multiplying the area of LTHS (1.79 km<sup>2</sup>) and tidal flat (0.19 km<sup>2</sup>) with the standing stocks of each element in phytoplankton and microphytobenthos, respectively, gave the amount of each element in microalgae.

While the daily budget of seawater showed a slight excess of imports over exports (0.03 m<sup>3</sup> d<sup>-1</sup>), the outflow of NH<sub>4</sub>-N and Si(OH)<sub>4</sub>-Si were 0.4 kmolN d<sup>-1</sup> and 12.5 kmolSi d<sup>-1</sup> higher than the inflow (Table 2; Fig. 6a, c), respectively. The mean biomass in unit area of microphytobenthos in surface sediment on the tidal flats ( $149 \pm 155$  mgChl-*a* m<sup>-2</sup>) was about 100 times higher than that of phytoplankton in LTHS ( $1.3 \pm 1.3$  mgChl-*a* m<sup>-2</sup>).

## 4. Discussion

### 4.1 Nutrient Budget

In this study, low N/P and high Si/N (mean = 2.6 and 17.6, respectively; Fig. 2g, h) near the entrance of the lagoon compared to that of microphytobenthos (N:P:Si = 10.1:1:18; Montani et al., 2003) clearly suggest N deficiency in LTHS. While temporal variation of PO<sub>4</sub>-P and Si(OH)<sub>4</sub>-Si depended on tidal height, factor loadings of DIN as the deficient nutrient were lower (Table 1). It was almost certain that temporal variation of DIN reflected the influence of biological activities (particularly nutrient mineralization by *R. philippinarum* and the photosynthetic activity of microalgae).

The flux of NH<sub>4</sub>-N coming into the lagoon was 3.4 kmolN d<sup>-1</sup> and the flux out was 3.7 kmolN d<sup>-1</sup> (Table 2). Thus, assuming that there was absolutely no phytoplankton and microphytobenthos uptake during the day, 0.3 kmolN d<sup>-1</sup> of NH<sub>4</sub>-N was produced within the lagoon. However, the ammonium mineralization rate of clams has been estimated to be approximately 7.7 kmolN d<sup>-1</sup> (Table 2). Thus, 96%

(7.4 kmolN d<sup>-1</sup>, i.e., 7.7 kmolN d<sup>-1</sup> minus 0.3 kmolN d<sup>-1</sup>) of the NH<sub>4</sub>-N mineralized by the clams was consumed by microalgae within the lagoon. In contrast, if all of the NH<sub>4</sub>-N inflow (3.4 kmolN d<sup>-1</sup>) was consumed by microalgae before outflow, 52% (4.0 kmolN d<sup>-1</sup>, i.e., 7.7 kmolN d<sup>-1</sup> as mineralization by the clam minus 3.7 kmolN d<sup>-1</sup> as out flow) of the NH<sub>4</sub>-N mineralized by the clam should have been consumed by the microalgae. In addition, the flux of NO<sub>3</sub> + NO<sub>2</sub>-N coming into the lagoon was 1.7 kmolN d<sup>-1</sup>, and the flux out was 1.4 kmolN d<sup>-1</sup> (Table 2, Fig. 4). Thus, 0.3 kmolN d<sup>-1</sup> of NO<sub>3</sub> + NO<sub>2</sub>-N would be consumed by microalgae.

In the neritic region, the standing stocks of microphytobenthos inhabiting the sediment surface (several mm) often either equaled or surpassed that of phytoplankton inhabiting the water column due to solar radiation reaching the sea floor (Cahoon, 1999). Microphytobenthos take up nutrients not only from the water column but also from pore water (Henriksen et al., 1980; Sundbäck et al., 1991; Cahoon and Cooke, 1992; Cahoon, 1999; Sundbäck et al., 2000; Srithongouthai et al., 2003). In this study, the mean standing stock of microphytobenthos inhabiting surface sediment (5 mm thick) on tidal flats was 100 times higher than that of phytoplankton (1 m depth; see section 3.3).

Assuming that microphytobenthos on the tidal flats ( $11.3 \pm 11.8$  kmolN) used all of the surplus nutrients (between 4.0 and 7.4 kmolN d<sup>-1</sup>), the temporal division rate [(NH<sub>4</sub>-N uptake)/(the standing stock of microphytobenthos)] of microphytobenthos would have to be between 0.35 and 0.65 d<sup>-1</sup>. Microphytobenthos could achieve this because the division rate of microphytobenthos was reported to be between 0.6 and 3.9 d<sup>-1</sup> (Admiraal, 1977a, b; Admiraal and Peletier, 1980; Montani et al., 2003; Ichimi et al., 2008). Conversely, assuming that the phytoplankton in LTHS ( $0.9 \pm 0.9$  kmolN) used all the surplus nutrients, the temporal division rate would have to be between 4.4 and 8.2 d<sup>-1</sup>. Reported rates of phytoplankton division are not nearly that high (e.g., 0.22 to 0.65 d<sup>-1</sup>; Shinada et al., 2000), thus phytoplankton alone are not likely to be responsible for the observed levels of ammonium uptake in the lagoon.

Nizzoli et al. (2006) suggested that the combination of direct interaction of *Tapes philippinarum* (synonym of *Ruditapes philippinarum*) with the sediment and biodeposition maintains a balanced benthic metabolism. In this study, it seemed that the growth rate of microphytobenthos would be somewhat underestimated because we neglected the effect of defecation of *R. philippinarum*.

Previously, in situ measurements using light and dark chambers on tidal flats have shown that the efflux of NH<sub>4</sub>-N from the sediment depends on the photosynthetic activity of microphytobenthos (Sundbäck et al., 1991; Sundbäck et al., 2000; Sandwell et al., 2009). In the continuous survey of this study, the factor loading of NH<sub>4</sub>-N was significantly negative but lower than the other nutrients, it seemed to reflect the effect of both physical variables (i.e., tidal height) and photosynthetic activity (i.e., solar radiation) (Table 1, Fig. 4d). The vague relationships between solar radiation and NH<sub>4</sub>-N concentration during the continuous survey implied that solar radiation almost coincided with the tidal height, which masked the light and dark effect.

Another nitrogen pathway is the denitrification that is carried out by many heterotrophic, generally facultative, anaerobic bacteria. These bacteria demand NO<sub>3</sub> + NO<sub>2</sub>-N as the terminal electron acceptor. The major sources of NO<sub>3</sub>-N for sediment denitrification are NO<sub>3</sub>-N diffusing into the sediment from the water column and NO<sub>3</sub>-N produced in the sediments through nitrification of NH<sub>4</sub>-N released from benthic oxidation of organic matter (Seitzinger, 1988). According to Rysgaard et al. (1995), when the water column concentration of NO<sub>3</sub>-N and NH<sub>4</sub>-N is low (several μmol L<sup>-1</sup>) in the Kertinge Nor estuary (Denmark), there is strong competition between microphytobenthos, nitrifiers, and denitrifiers for inorganic nitrogen. Rysgaard et al. (1995) suggested that benthic primary production would reduce the denitrification rate in such a situation. This study area was shallow (mean depth 1 m) and had a relatively high biomass of microphytobenthos ( $149 \pm 155$  mgChl-*a* m<sup>-2</sup>), and we did not attempt to measure nitrification and denitrification directly. In our calculations, we assumed that nitrification and denitrification made negligible contributions to the nutrient budget.

For PO<sub>4</sub>-P, standing stocks of microphytobenthos on the tidal flats were  $1.0 \pm 1.1$  kmolP. The uptake rate by microphytobenthos was estimated to be between 0.4 and 0.7 kmolP d<sup>-1</sup> based on the division rate of NH<sub>4</sub>-N (0.35–0.65 d<sup>-1</sup>). Inflow and outflow of PO<sub>4</sub>-P was 2.4 and -2.1 kmolP d<sup>-1</sup>, respectively, and the mineralization rate by the clam was  $2.3 \pm 2.0$  kmol d<sup>-1</sup> (Table 2; Fig. 4b). There was considerable discrepancy between budgets through the entrance, uptake, and mineralization rate of PO<sub>4</sub>-P because phosphate is more particle reactive than both nitrogen and silicon (Froelich, 1988; Sundby et al., 1992). In this study area, where photosynthesis of microphytobenthos was active, the sediment surface was maintained under aerobic conditions (e.g., Yamaguchi et al., 2007). Hence, particles such as iron, calcium, carbonates, and organic matter acted as buffer-absorbed phosphate.

According to the molar ratio of nutrients (Fig. 2g, h), Si was the most surplus element for growth of microphytobenthos. Outflow ( $-80.6 \text{ kmolSi d}^{-1}$ ) of  $\text{Si(OH)}_4\text{-Si}$  far exceeded inflow ( $68.1 \text{ kmolSi d}^{-1}$ ) because it was mineralized by the clam ( $7.1 \pm 6.3 \text{ kmolSi d}^{-1}$ ) and stored in LTHS ( $49.8 \pm 28.7 \text{ kmolSi}$ ), and silicate seemed to effuse directly to the open sea.

#### 4.2 Nutrient Sources

Nutrient sources in relatively unpolluted estuarine systems show regeneration from sediments and riverine input (Mann, 2000). In this study, there were significant negative correlations between salinity and both  $\text{PO}_4\text{-P}$  and  $\text{Si(OH)}_4\text{-Si}$  (see section 3.1). We estimated the concentrations of freshwater based on the y intercept (0 PSU) of regression lines; the concentrations were  $40.7 \mu\text{mol L}^{-1}$  ( $\text{PO}_4\text{-P}$ ) and  $1,140 \mu\text{mol L}^{-1}$  ( $\text{Si(OH)}_4\text{-Si}$ ). Mean riverine concentrations of  $\text{PO}_4\text{-P}$  and  $\text{Si(OH)}_4\text{-Si}$  in Japan are  $3.7 \mu\text{mol L}^{-1}$  and  $310 \mu\text{mol L}^{-1}$ , respectively (National Astronomical Observatory of Japan, 2006). Though this lagoon was isolated from highly populated areas, the estimated values were much higher than the mean values in Japan. Thus, it was highly unlikely that the sources of these nutrients were mainly riverine. For  $\text{PO}_4\text{-P}$  and  $\text{Si(OH)}_4\text{-Si}$ , the major source of these nutrients seemed to be nutrient mineralization by the sedimentary bacteria due to indicate the impact on diffusive flux from the sediment on the inner part of the lagoon.

Similarly, there was a slight possibility that sources of DIN were mainly riverine because both  $\text{NH}_4\text{-N}$  and  $\text{NO}_3 + \text{NO}_2\text{-N}$  concentrations tended to increase with increasing proximity to the entrance of the lagoon (Fig. 2c, d and 3b, c). Around the same time (July 15, 2006) as this study,  $\text{NO}_3\text{-N}$  concentration of the surface layer fell below measurable limits at the sampling station ca. 10 km offshore ( $42^\circ 50'\text{N}$ ,  $144^\circ 50'\text{E}$ ; Fisheries Research Agency, 2008). Thus, it was highly unlikely that the high DIN concentration in LTHS in this study (Figs. 2c, d and 3b, c) was supplied from the open sea.

LTHS was formed near the entrance of the lagoon, including the tidal flats (Fig. 1, 2). The clam population on the tidal flats persisted at high biomass ( $265 \pm 234 \text{ gDW m}^{-2}$ ). Bartoli et al. (2001) showed a linear increase in the  $\text{NH}_4\text{-N}$  flux to the water column with increasing biomass of the clam *R. philippinarum*. Magni et al. (2000) reported that the nutrient mineralization rate of infaunal bivalves (*R. philippinarum* and *M. senhousia*) was between 16 and 82 times higher than the diffusive flux from the sediment of tidal flats. Therefore, in this study, mineralization by the clam seemed to be a major source of DIN in the water column in LTHS. In this study, the potential  $\text{NH}_4\text{-N}$  mineralization rate of the clam was estimated to be  $7.7 \pm 6.8 \text{ kmolN d}^{-1}$  (Table 2; Fig. 4a). Uptake by microphytobenthos was estimated to be between 4.0 and 7.4  $\text{kmolN d}^{-1}$  (see section 4.1), and the residual  $\text{NH}_4\text{-N}$  ( $0.3 \sim 3.7 \text{ kmolN d}^{-1}$ ) was the source for the water column. Even the residual mineralized  $\text{NH}_4\text{-N}$  accounted for between 12% and 148% of  $\text{NH}_4\text{-N}$  in the water column in LTHS ( $2.5 \pm 1.4 \text{ kmolN}$ ) per day.

#### 5. Conclusion

Most (52–96%) of the  $\text{NH}_4\text{-N}$  (as a deficient element) mineralized by the infaunal bivalve (*R. philippinarum*) was consumed by microalgae (mainly microphytobenthos) before outflow. In contrast, non-deficient elements, particularly  $\text{Si(OH)}_4\text{-Si}$ , were released into the water column directly. Further study of nutrient mineralization by infaunal bivalve species in shallow water ecosystems is needed to determine the extent of mineralized nutrient contribution to pore water. This is the first field-based observation on a quantitative evaluation between nutrient mineralization by infaunal bivalves and nutrient uptake by microphytobenthos.

#### Acknowledgments

Sampling was conducted with the cooperation of Chirippu Fishery Cooperative Union. We would like to express our thanks to Dr. I. Kudo and anonymous referees for their constructive comments.

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#### Web reference

Fisheries Research Agency, 2008. A-line data home page, <http://hnf.fra.affrc.go.jp/a-line/>

## Captions

**Table 1.** Summary of results of principal component analysis using 9 variables.

**Table 2.** Daily fluxes of water and nutrients at the entrance of the lagoon, and nutrient excretion rate by the clam in the whole tidal flat area.

“–” indicates export from the lagoon.

**Fig. 1.** Study area and location of sampling stations. Letters (Stations A, B, D, F, G) and numbers (Stations 0–12, 14, and 15) indicate tidal flats and subtidal sampling sites, respectively. “0” and “T” represent stations of continuous observation and tidal level monitoring.

**Fig. 2.** Spatial distribution of temperature (a), salinity (b), nutrients (c,  $\text{NH}_4\text{-N}$ ; d,  $\text{NO}_3 + \text{NO}_2\text{-N}$ ; e,  $\text{PO}_4\text{-P}$ ; and f,  $\text{Si(OH)}_4\text{-Si}$ ), and molar ratios of nutrients (g, N/P; and h, Si/N).

**Fig. 3.** Plots of salinity vs. temperature (a), and nutrients (b,  $\text{NH}_4\text{-N}$ ; c,  $\text{NO}_3 + \text{NO}_2\text{-N}$ ; d,  $\text{PO}_4\text{-P}$ ; and e,  $\text{Si(OH)}_4\text{-Si}$ ). Closed and open symbols represent high temperature low salinity (HTLS) and low temperature high salinity (LTHS), respectively. Circles and triangles represent water samples of surface and bottom layers, respectively.

**Fig. 4.** Solar radiation reported at Nemuro Meteorological Agency Station (a), tidal height and velocity (b), temperature and salinity (c), and concentrations of  $\text{PO}_4\text{-P}$  and  $\text{Si(OH)}_4\text{-Si}$  (d), and  $\text{NH}_4\text{-N}$  and  $\text{NO}_3 + \text{NO}_2\text{-N}$  (e) at the entrance of the lagoon during a 25-h period in August 8–9, 2006. Gray and white areas represent flood and ebb tides, respectively.

**Fig. 5.** Relationship between soft tissue expressed dry weight (DW) and total weight (living individual) for *Ruditapes philippinarum*.

**Fig. 6.** Nutrient budget at the entrance of the lagoon, mineralization rate of *Ruditapes philippinarum* on tidal flat, and total amounts of each element in LTHS during summer. The amount (kmol) and budget ( $\text{kmol d}^{-1}$ ) of each element are represented with and without bracketed numbers, respectively. PHY and MPB represent the total amount of each element in phytoplankton in LTHS and microphytobenthos on tidal flats, respectively.

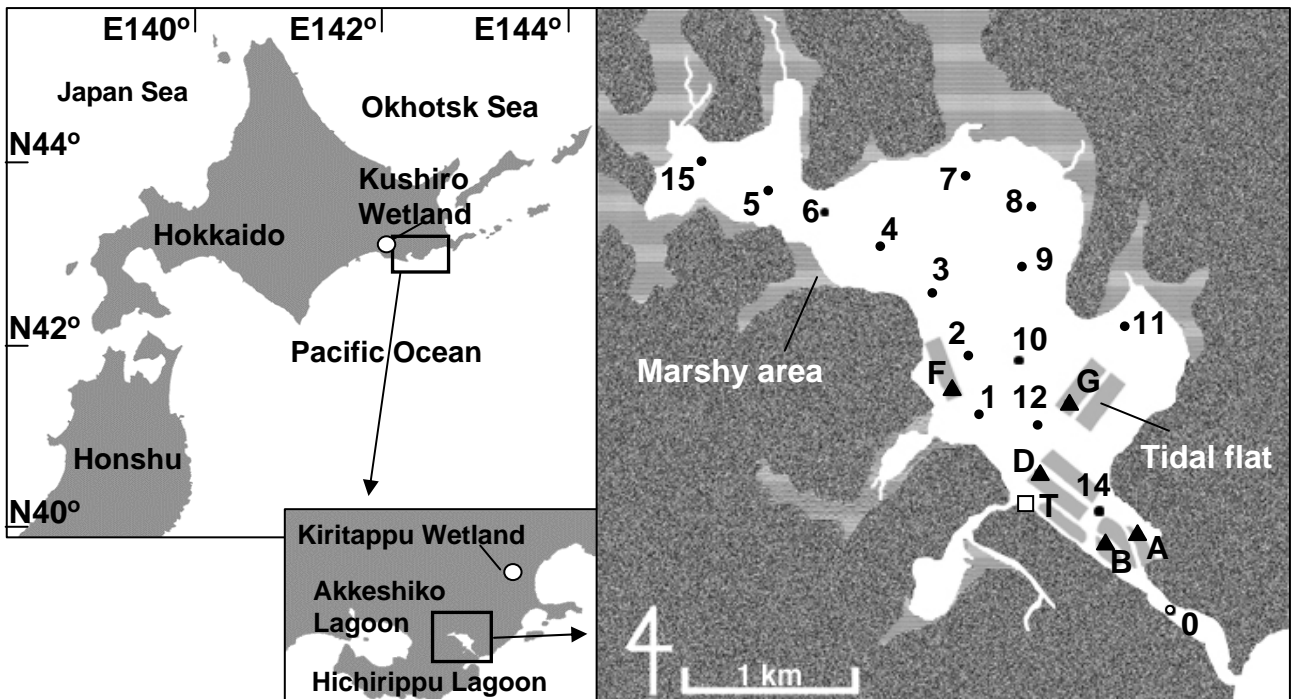
Table 1

	1st PC
Eigen value	8.5
Cummulative % varia	65.5
Factor loading of variables	
Solar radiation	0.796
Velocity	0.514
Tidal height	-0.914
Water temperature	0.979
Salinity	-0.976
NH <sub>4</sub> -N	-0.527
NO <sub>2</sub> +NO <sub>3</sub> -N	-0.675
PO <sub>4</sub> -P	0.918
Si(OH) <sub>4</sub> -Si	0.963

Table2

	Water Flux $\text{m}^3 \text{d}^{-1}$	$\text{NH}_4\text{-N}$ $\text{kmol d}^{-1}$	$\text{NO}_2+\text{NO}_3\text{-N}$ $\text{kmol d}^{-1}$	$\text{PO}_4\text{-P}$ $\text{kmol d}^{-1}$	$\text{Si(OH)}_4\text{-Si}$ $\text{kmol d}^{-1}$
Tide					
Influx	0.24	3.4	1.7	2.4	68.1
Efflux	-0.21	-3.7	-1.4	-2.1	-80.6
Budget	0.03	-0.4	0.3	0.3	-12.5
Mineralization rate		7.7		2.1	7.1
by <i>R.</i>		(6.8)		(1.8)	(6.3)

Fig. 1



**Fig. 2**

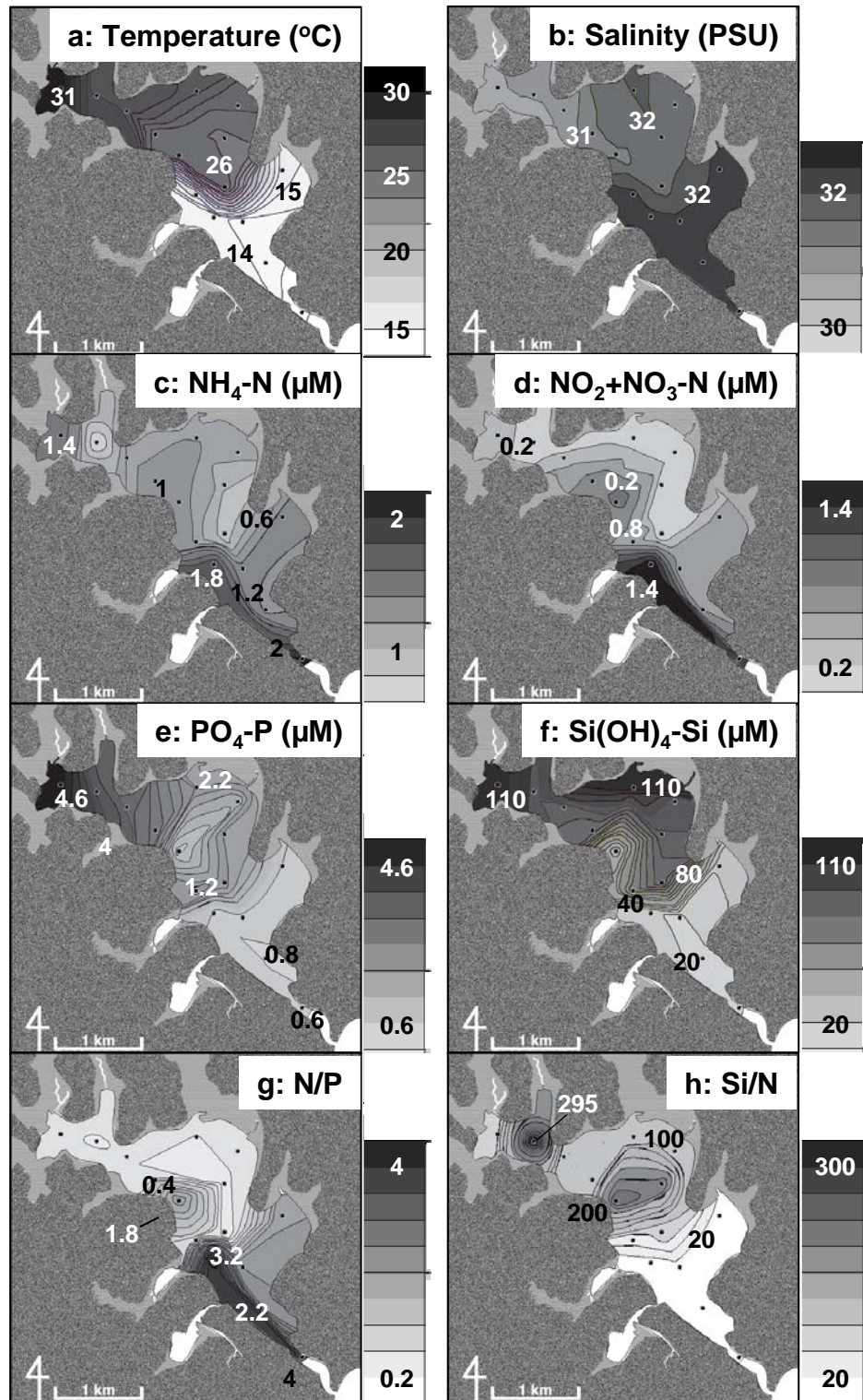


Fig. 3

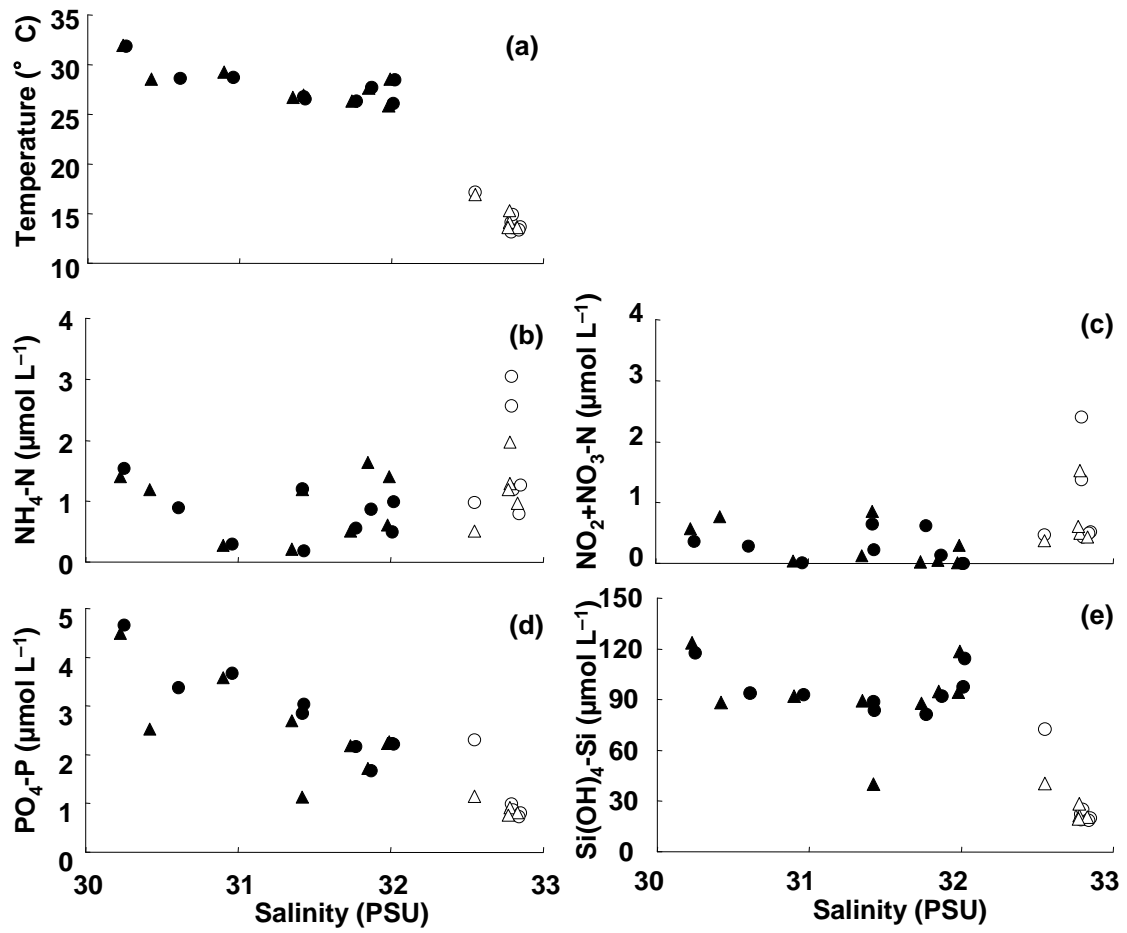
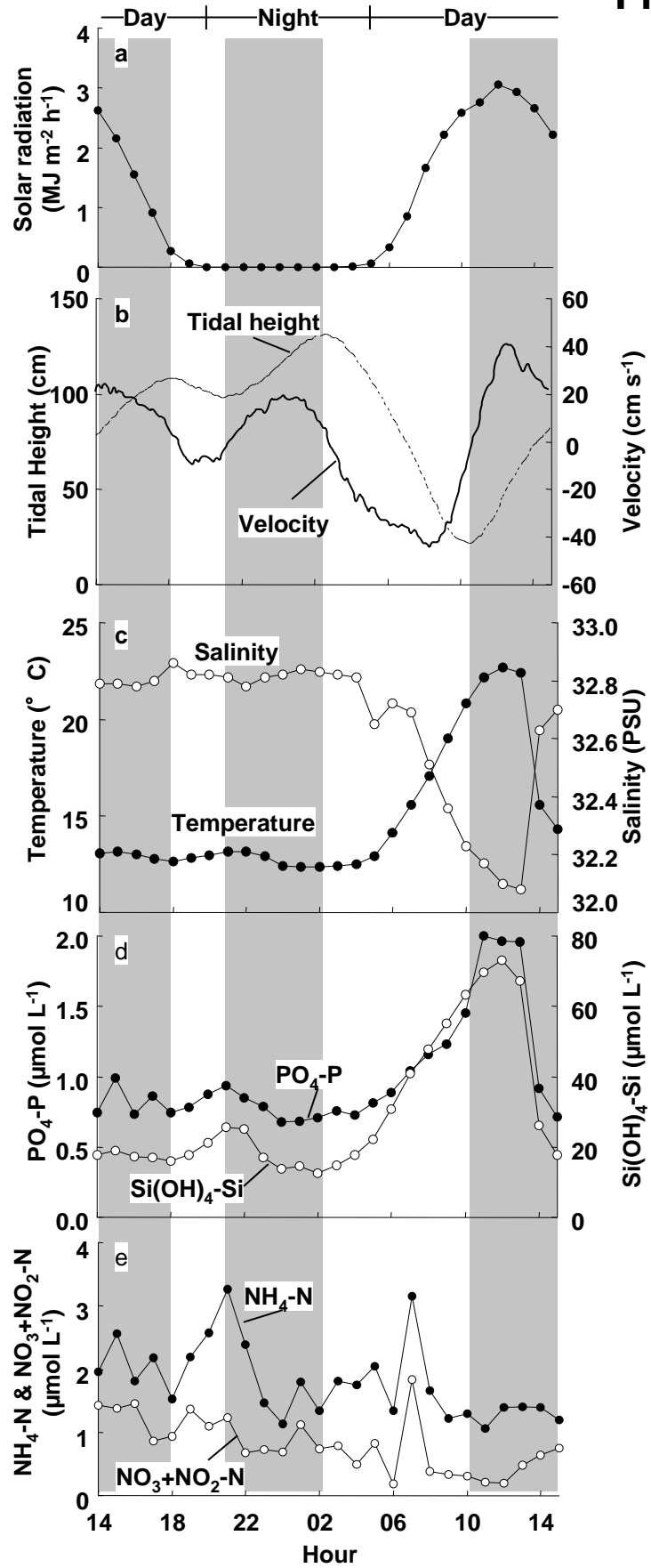


Fig. 4



**Fig. 5**

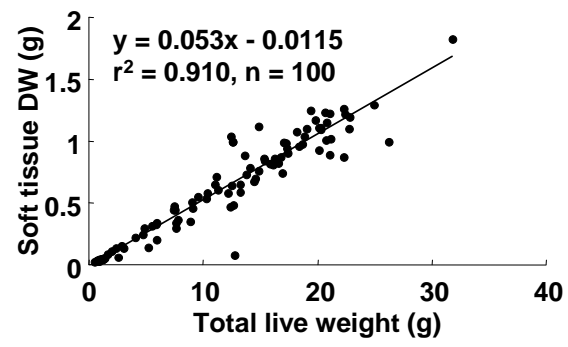


Fig. 6

