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Benthic microalgal biomass in the estuarine tidal flat of the Mae Klong River, Thailand: Relationship with environmental factors at the sediment–water interface

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Abstract: The present study describes and quantifies the short–term variations of microphytobenthic biomass and associated limiting factors in the tidal mudflat of the Mae Klong River estuary. Surficial sediment (0–0.5 cm) was collected during exposure in September (wet monsoon) and November (post monsoon) 2006 at 25 stations, covering the entire tidal flat area in order to determine microphytobenthic biomass (benthic chlorophyll *a*), loss on ignition (LOI) and nutrients in pore water. The average microphytobenthic biomass of September (8.49 $\pm$2.86 mg m^{-2}) was 63% higher than that of November (5.21$\pm$2.58 mg m^{-2}), and statistically different between two–month observations ($P<0.0001$) due to the available irradiance during aerial exposure. The higher microphytobenthic productions in September have also contributed largely to the sedimentary composition in surficial sediments. Furthermore, the longer aerial exposure periods might allow the sediment–water interface to become oxic conditions, resulting in increasing $NO_2^-+NO_3^-$ concentration in pore water via nitrification processes, and contribution of $NO_2^-+NO_3^-$ was calculated as 52.5% to dissolved inorganic nitrogen (DIN; $NO_2^-+NO_3^-$–N+NH_4^+–N). These results suggested that variability of microphytobenthos have been regulated largely by the supplied irradiance during exposure, and may have great role on sedimentary composition in the estuarine tidal flat of the Mae Klong River.

Keywords: *microphytobenthos, chlorophyll a, nutrients, sediment–water interface, estuary, Mae Klong River.*

1. Introduction

The microphytobenthos play a great role in estuaries and shallow water ecosystems, where the available irradiance extends to the seafloor, and much attention has recently been paid to their potentially important production. The microphytobenthos are well documented, frequently referred to as the major primary carbon source for the shallow ecosystem food web, and also serve as an important component in nutrient cycles in tidal estuaries (MACINTYRE *et al.*, 1996 and references therein). According to the previous studies, the microphytobenthos may contribute up to 50% of the entire primary production, depending on environmental factors (FIELDING *et al.*, 1988; de JONGE and COLIJN, 1994; BLACKFORD, 2002). Furthermore, the microphytobenthos can regulate oxygen concentration, which can mediate nutrient transformations and fluxes between the sediment and overlying water via their photo-

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synthesis and also play an important role in sediment stability (SUNDBÄCK *et al.*, 1991; CAHOON and COOKE, 1992; BARRANGUET, 1997; WELKER *et al.*, 2002).

The production and biomass of microphytobenthos are largely influenced by several environmental factors including nutrients, substrate types, tidal rhythm and irradiance, and biological factors such as herbivore grazing (BARRANGUET *et al.*, 1998; LIGHT and BEARDALL, 1998; SMITH and UNDERWOOD, 2000; BLACKFORD, 2002; RIAUX–GOBIN and BOURGOIN, 2002; PERKINS *et al.*, 2004; CARTAXANA *et al.*, 2006; SKINNER *et al.*, 2006; KOH *et al.*, 2007; YAMAGUCHI *et al.*, 2007; DU *et al.*, 2009; JESUS *et al.*, 2009; LOASSACHAN *et al.*, 2009). There have been some reports that microphytobenthos and their primary production influenced sediment stability and nutrient fluxes in an estuarine area (GERBERSDORF *et al.*, 2004; WILSON and BRENNAN, 2004; CIBIC *et al.*, 2007). BARRANGUET (1997) found the production and biomass of microphytobenthos play a great role in regulating oxygen concentration at the sediment–water interface in a mussel cultured area. Moreover, the oxygenation of the organically enriched sediments by microphytobenthos may influence the abundance of the macrobenthic fauna in the western Seto Inland Sea (YAMAGUCHI *et al.*, 2007). A negative correlation among Chl *a* contents in surface sediments and measured silicic acid fluxes using core incubation technique was documented in a coastal shallow ecosystem (Shido Bay, the Seto Inland Sea), suggesting that the microphytobenthos greatly reduced the upward silicic acid flux of sediment–water interface during their nutrient uptake requirement (SRITHONGOUTHAI *et al.*, 2003). LOASSACHAN *et al.* (2009) also found that the microphytobenthos have a great effect on the nutrient availability, especially silicic acid at the sediment–water interface during the large supply of irradiance (winter periods) for their photosynthetic growth in a coastal shallow water, the Seto Inland Sea, Japan.

The present study aims to examine the temporal dynamics of microphytobenthic biomass at the estuarine intertidal flat and its relation to the environmental factors at the sediment–water interface. This study provides considerable information on microphytobenthos, a primary producer, in the Mae Klong River estuarine system, which is a highly productive area and important fishing ground in the upper Gulf of Thailand.

2. Materials and Methods

2.1 Study site

The Mae Klong is one of the most important large rivers, which discharge fresh water into the upper Gulf of Thailand, and is also considered as an important source of nutrients and materials loaded into the western part of the head of the Gulf of Thailand. This river is strongly influenced by the wet southwest monsoon from May to October, and the dry northeast monsoon from November to April.

The Mae Klong River estuary is one of the most important fishing grounds in the upper Gulf of Thailand with its high production of commercial aquatic species, such as razor clams (*Solen* spp.), blood clams (*Anadara granosa*), and green mussels (*Perna viridis*), and is the largest habitat of the razor clam in Thailand. Furthermore, the tidal flat of the Mae Klong River estuary (Don Hoi Lot) has been listed in the Ramsar Convention as an international important natural wetland (www.ramsar.org). The estuarine area consists of a large tidal flat and coastal wetland. The tidal flats are generally characterized as muddy fine sand (>50% of grain size fractions are 125–250 μm) and accumulated from the Mae Klong River, covering an area of 875 km^2 (87,500 ha).

2.2 Sampling strategies

Observations were carried out in September and November 2006 at 25 stations, covering the entire area of the tidal flat (Fig. 1). All the observation and sampling were performed during exposure. Duplicate undisturbed cores were collected carefully at each station using an acrylic pipe of 4 cm in diameter. The surficial sediment (0–0.5 cm) from one core sample was carefully sliced off into a glass vial for analysis of chlorophyll *a* (Chl *a*), and the surficial sediment from another core was also cut–off into a plastic bag for analysis of water content, loss on ignition (LOI) and extraction of pore water. All samples were stored in a cooler box for

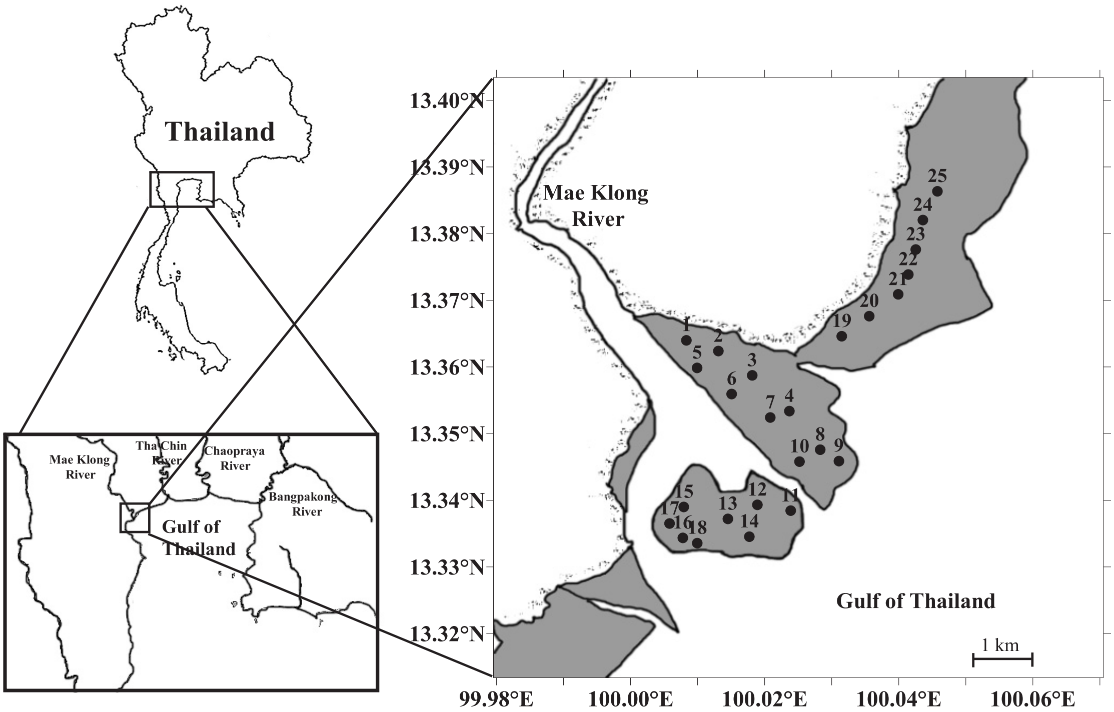

Fig. 1. Sampling stations in the tidal flat (gray zones) of Mae Klong River estuary.

several hours until further analysis in a laboratory.

Water content, LOI and Chl *a* concentrations were immediately determined on the fresh sediment samples. The residual sediments from water content analysis were homogenized and divided into two sub-samples for LOI determination and pore water extraction. Aliquots of the sediment samples were centrifuged to extract the pore water (3,000 rpm, 15 min at 4℃), and the supernatant was then filtered through a Whatman GF/F filter for inorganic nutrient analysis. The filtered pore water samples were kept at −20℃ until the nutrients were analyzed.

2.3 Analysis

The water content of the sediment was determined from the weight loss after drying the wet sediment at 105℃ until a constant weight was obtained (approximately 24 h). For LOI determination, sediment samples were dried to constant weight at 60℃ for 3 days, ignited in a muffle furnace at 550℃ for 3 h, and then LOI was calculated from the loss of weight after combusting the dried sediment samples. For Chl *a* determination, the sediment samples were extracted in 90% acetone and kept at *ca.* 4℃ in the dark for 24 h. The Chl *a* concentrations were analyzed following the spectrophotometric method of LORENZEN (1967) described in PARSONS *et al.* (1984) using a spectrophotometer (Cecil, 1000 series). LOI and Chl *a* contents were expressed as mg m^{-2} DW, which was calculated from the sediment core area. The concentrations of inorganic nutrient in pore water, ammonium (NH_4^+-N), nitrite and nitrate (NO_2^- + NO_3^--N), phosphate (PO_4^{3-}-P) and silicic acid ($Si(OH)_4$-Si) were analyzed using a nutrient auto analyzer (SKALAR, The SAN^{Plus} Segmented Flow Analyzer), and the nutrient concentrations were expressed as μmol l^{-1}. Microphytobenthos species were also roughly observed by a microscope. Moreover, the entire exposure periods in the present study were calculated from the tide table.

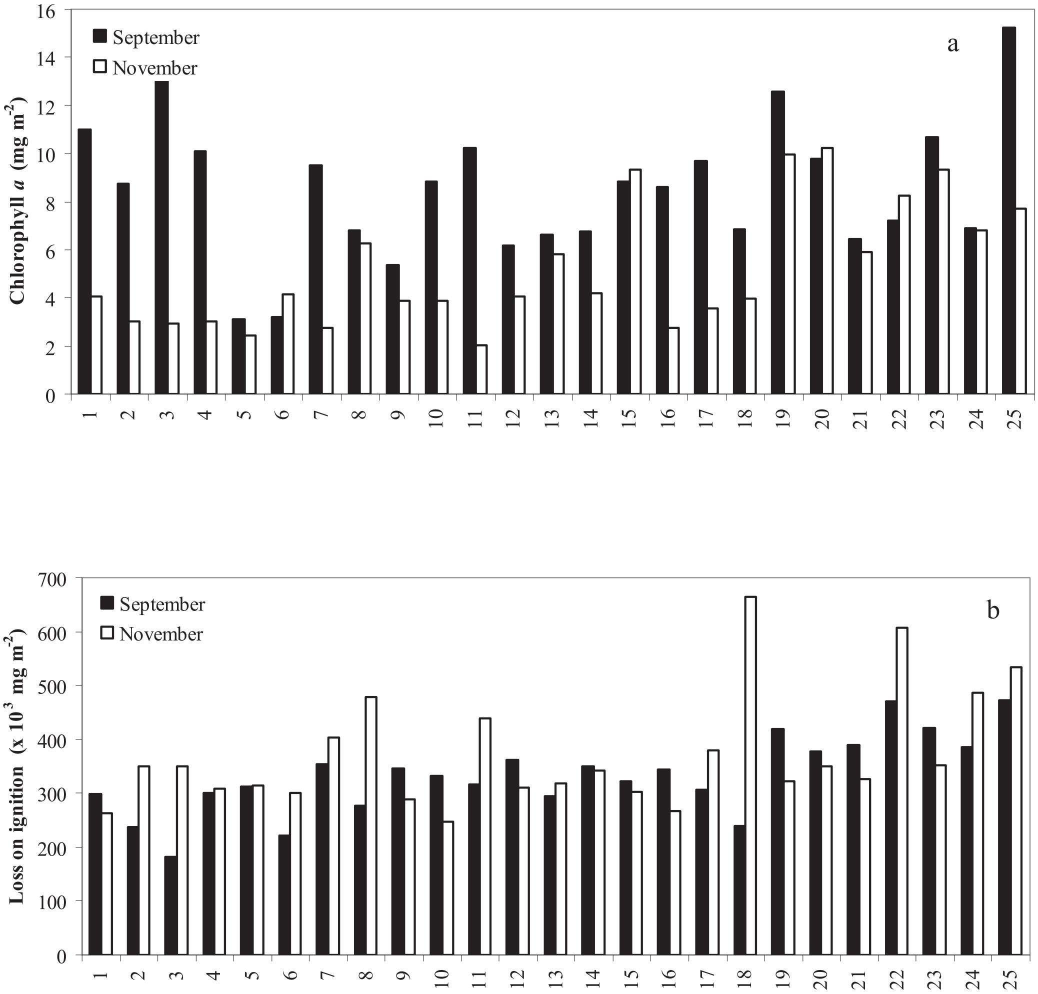

Fig. 2. Variability of (a) benthic Chl *a* content and (b) LOI in surficial sediment layer (0–0.5 cm).

2.4 Statistical methods

Differences in microphytobenthic biomass, LOI, Chl *a*/LOI ratio and nutrient concentrations in pore water during two–month observations were tested through non–parametric tests followed by Mann–Whitney *U* Test. The correlations between microphytobenthic biomass and other parameters were examined by Spearman's rank correlation coefficient. These analyses were performed using SPSS 16.0 for Microsoft Windows.

3. Results

3.1 Chlorophyll a concentration and loss on ignition in the surficial sediments

The variability of benthic Chl *a* concentration within the surficial sediments (0–0.5 cm) is shown in Fig. 2a. In September, the benthic Chl *a* ranged between 3.09 mg m^{-2} (at Stn. 5) and 15.2 mg m^{-2} (at Stn. 25), averaging 8.49 mg m^{-2}. While the benthic Chl *a* of November varied from 2.04 mg m^{-2} (at Stn. 11) to 10.2 mg m^{-2} (at Stn. 20) with an average of 5.21 mg m^{-2}; this was statistically lower than those of Chl *a* contents collected in September (*P*

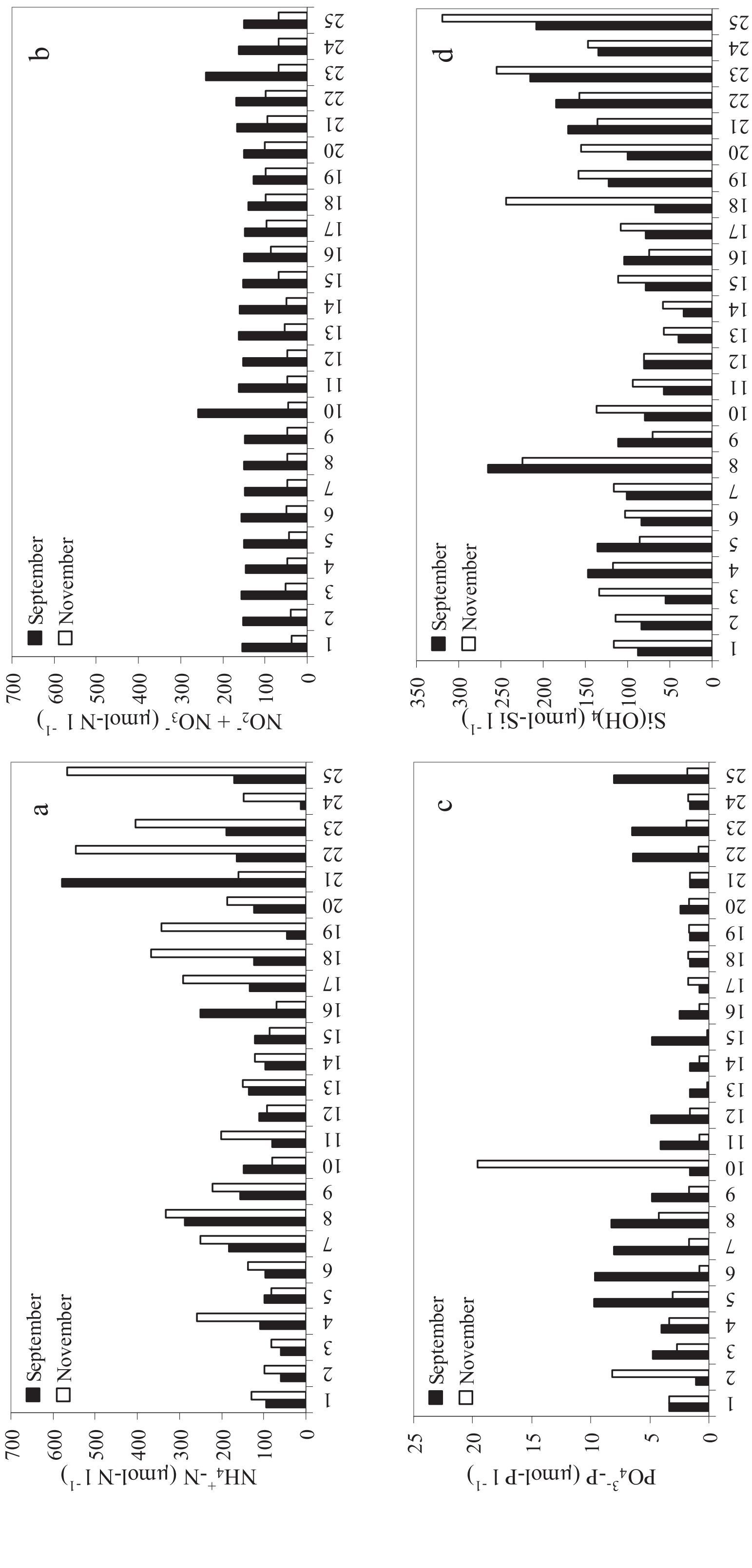

Fig. 3. Variability of (a) NH_4^+–N, (b) $NO_{2^-}+NO_{3^-}$–N, (c) PO_4^{3-}—P and Si (OH) $_4$–Si concentration in pore water.

< 0.0001, $n = 50$). The variability of LOI in surficial sediments is illustrated in Fig. 2b. The LOI of September ranged between 181,000 mg m^{-2} (at Stn. 3) and 472, 000 mg m^{-2} (at Stn. 25), averaging 333,000 mg m^{-2}. In November, the LOI ranged from 247,000 mg m^{-2} (at Stn. 10) to 664,000 mg m^{-2} at (Stn. 18) with an average of 372,000 mg m^{-2}. However, there was no significant difference in the sedimentary LOI in the surficial layer in the estuarine tidal flat between September and November observation.

3.2 *Variability of inorganic nutrients in pore water*

Concentrations of the inorganic nutrient in pore water are presented in Fig. 3. In September, NH_4^+–N concentration varied from 11.3 μmol–N l^{-1} (at Stn. 24) to 579 μmol–N l^{-1} (at Stn. 21), averaging 145 μmol–N l^{-1}. Concentration of $NO_2^- + NO_3^-$–N ranged from 128 μmol–N l^{-1} (at Stn. 19) to 258 μmol–N l^{-1} (at Stn. 10), with an average of 160 μmol–N l^{-1}. PO_4^{3-}–P concentration was between 0.806 μmol–P l^{-1} (at Stn. 17) and 9.75 μmol–P l^{-1} (at Stn. 5), with an average of 4.22 μmol–P l^{-1}. Si $(OH)_4$–Si concentration varied between 33.4 μmol–Si l^{-1} (at Stn. 14) and 265 μmol–Si l^{-1} (at Stn. 8), averaging 113 μmol–Si l^{-1}. In November, NH_4^+–N concentration varied from 68.9 μmol–N l^{-1} (at Stn. 16) to 566 μmol–N l^{-1} (at Stn. 25), with an average of 216 μmol–N l^{-1}. Concentration of $NO_2^- + NO_3^-$–N ranged from 36.1 μmol–N l^{-1} (at Stn. 1) to 100 μmol–N l^{-1} (at Stn. 20), averaging 63.9 μmol–N l^{-1}. PO_4^{3-}–P concentration was between 0.162 μmol–P l^{-1} (at Stn. 13) and 19.6 μmol–P l^{-1} (at Stn. 10), with an average of 2.72 μmol–P l^{-1}. Si $(OH)_4$–Si concentration varied between 57.1 μmol–Si l^{-1} (at Stn. 13) and 319 μmol–Si l^{-1} (at Stn. 25), averaging 135 μmol–Si l^{-1}. However, there was no significant difference in the nutrient concentrations in the pore water in the estuarine tidal flat between September and November, except the concentration of $NO_2^- + NO_3^-$–N. The higher $NO_2^- + NO_3^-$–N concentration was observed throughout September observations (Fig. 3b).

4. Discussion

4.1 *Temporal variation of microphytobenthic biomass (Chl a) in the surficial sediments*

Benthic Chl *a* content is widely used to determine the microphytobenthic biomass in sediments. This biomass in the intertidal mudflat estuary has been regulated by various environmental factors. Although we have no quantitative data on the abundance of microphytobenthos in the current study, we usually microscopically observed various species of pennate diatoms, *e.g. Navicula* spp. and *Nitzschia* spp. contained in surficial sediment samples.

In the present study, the average microphytobenthic biomass of September was 63% higher than that of November. The difference in microphytobenthic biomass between two observations might be explained by considering the available irradiance during exposure. Unfortunately, we have no irradiance data at surface sediment, whereas the exposure periods obtained from the tide table were used for discussion in the present study. Table 1 shows the entire aerial exposure periods and the aerial exposure periods in daytime (from 6 a.m.) at the tidal flat of the Maklong River estuary (Hydrographic Department, Royal Thai Navy, 2006). In August and September, the tidal flat was entirely exposed for 113 and 87 h, with a daily average of 3.65 and 2.90 h day^{-1}, respectively, and the aerial exposure periods in day-

Table 1. Entire aerial exposure periods (to air) and daytime aerial exposure periods (to sunlight from 6 a.m.) at the tidal flat of Maklong River estuary.

	Entire aerial exposure periods (to air)		Daytime aerial exposure periods (to sunlight)	
	Total (h)	Daily Average (h d^{-1})	Total (h)	Daily Average (h d^{-1})
August	113	3.6	93	3.0
September	87	2.9	54	1.8
October	48	1.5	14	0.5
November	29	1.0	0	0.0

* Data were obtained from Tide tables of Thai waters (Hydrographic Department, Royal Thai Navy, 2006).

time accounted for 82.3% and 62.1% of the entire exposure periods, respectively. Otherwise, the aerial exposure periods decreased continually to 1 h day^{-1}, and without aerial exposure in daytime in November. The longer daytime aerial exposure periods in September may well provide sufficient photosynthetically active radiation (PAR), which promotes the increase of the microphybenthos biomass in the tidal flat. On the other hand, the tidal flat was exposed a few hours during the night, and flooded throughout daytime in November; thus, the available PAR on the tidal flat would be less than that of September.

Irradiance is one of the most important factors that can regulate the variability of the microphytobenthos (MACINTYRE *et al.*, 1996; BARRANGUET *et al.*, 1998; YAMAGUCHI *et al.*, 2007; LOASSACHAN *et al.*, 2009). SUNDBÄCK and GRANÉLI (1988) found that microphytobenthic biomass (measured as Chl *a* content) decreased slightly and remained at a constant level for several weeks during exposure to no–light conditions, and increased markedly when exposed to light. KOH *et al.* (2007) also recently reported that the increase of surficial benthic Chl *a* (at 0–0.5 cm) reached 164%, accounting for 52 mg m^{-2} h^{-1} during daytime aerial exposure in the intertidal mudflat Ariake Sea, Japan. Furthermore, ADMIRAAL (1977) previously reported that the minimal daily quantum irradiance for light–saturated growth of estuarine benthic diatom investigated in a culture experiment ranged from 29 to 58 μmol photon m^{-2} s^{-1}. The growth of *Nitzschia* sp. isolated from surface sediment of Kaita Bay in Hiroshima, Japan showed a peak at 50 μmol photon m^{-2} s^{-1}, and its growth was inhibited under higher irradiance that (YAMAMOTO *et al.,* 2004). In contrast, COLIJIN and VAN BUURT (1975) reported the photosynthetic rate of microphytobenthos collected from the eastern part of the Dutch Waddensea was saturated by a light intensity of approximately 185 μmol photon m^{-2} s^{-1} (~10,000 lux), and no photo–inhibition was found at higher irradiance. In addition, MONTANI *et al.* (2003) also demonstrated that the photosynthetic rate of *Navicula* sp. isolated from an estuarine sand flat of the Seto Inland Sea was saturated at a light intensity of 165 μmol photon m^{-2} s^{-1} at 21℃, and no photo–inhibition was found at higher irradiance up to 400 μmol photon m^{-2} s^{-1}. Moreover, PINCKNEY and ZINGMARK (1993) also reported that the daily production of the microphytobenthos was highly variable, primarily due to the daily fluctuations in irradiance.

4.2 Relationship between microphytobenthos and sedimentary parameter

In the present study, however, there was no significant difference in the sedimentary LOI

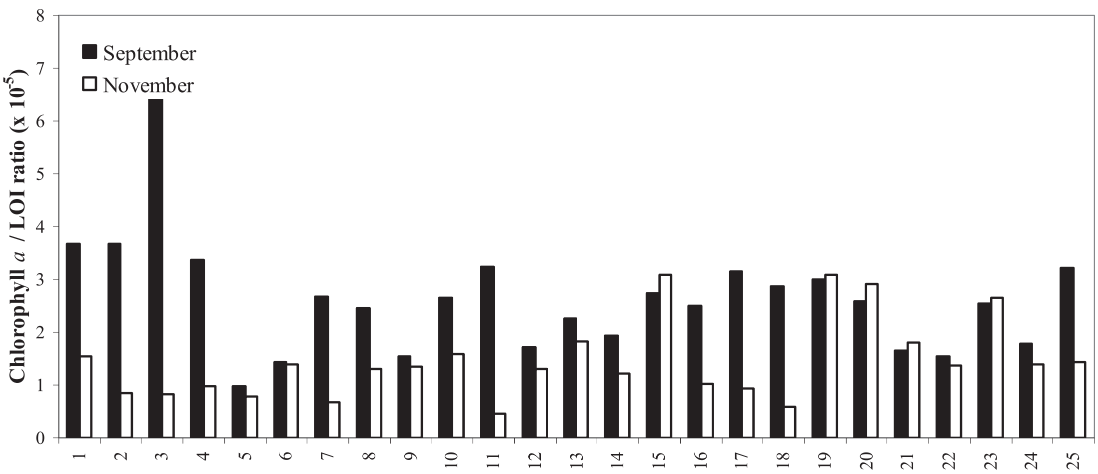

Fig. 4. Variability of benthic Chl *a* content to LOI ratio in surficial sediments.

in the surficial layer in the estuarine tidal flat between September and November. On the other hand, the Chl *a* to LOI ratio (Fig. 4) was statistically different between September and November ($P<0.0001$, $n=50$). The ratio of Chl *a* to LOI in September was significantly higher than that of November. Chl *a* to LOI ratio provides an index of sediment photo–autotrophy (LIGHT and BEARDALL, 1998), where high values correspond to high photo–autotrophic capacity relative to sedimentary organic matter. The result clearly suggested that the presence of higher microphytobenthic biomass observed in September contributed greatly to the sedimentary organic matter rather than that of November. This result corresponded well with the result of microphytobenthic biomass (discussed above). Chl *a* contents seem to be a small fraction of LOI in the sediments, because LOI contents did not change following the increasing Chl *a* contents in sediments. However, the increase in Chl *a* contents in September might result in the change of sedimentary organic matter in the tidal flat.

The inorganic nutrients at the sediment–water interface are one of the most important factors that control the variability of microphytobenthic production. On the other hand, microphytobenthos may also influence the nutrient concentrations at the sediment–water interface, acting as a filter (SUNDBÄCK *et al.*, 1991; WELKER *et al.*, 2002). Unfortunately, the correlations between benthic Chl *a* and NH_4^+–N, PO_4^{3-}–P and Si $(OH)_4$–Si contents in pore water, and also the difference of NH_4^+–N, PO_4^{3-}–P and Si $(OH)_4$–Si concentrations in pore water among two observations were not observed significantly in the present study. However, the significant correlation found between benthic Chl *a* and $NO_2^-+NO_3^-$–N concentration in pore water ($r=0.569$, $P<0.01$, $n=50$) coincided well with a previous study. TANTANASARIT and MEKSUMPUN (2007) reported a good relationship between Chl *a* concentration and $NO_2^-+NO_3^-$–N concentration in the water column at the Mae Klong River estuary. They concluded that $NO_2^-+NO_3^-$–N should be one of the most important factors regulating the growth of phytoplankton in the Mae Klong River estuary. Theses results suggested that $NO_2^-+NO_3^-$–N may also be the one of the important factors controlling photosynthetic growth of phytoplankton and microphytobenthos in the estuary.

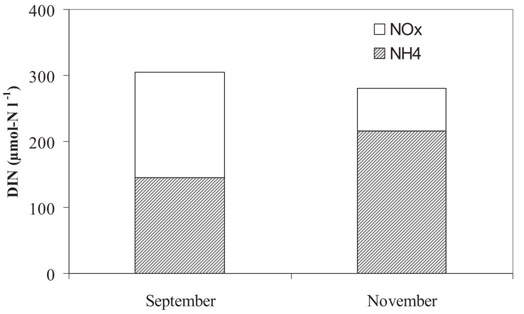

Fig. 5. Average concentrations of DIN ($NO_2^-+NO_3^-$–N$+NH_4^+$–N) in pore water.

In the present study, moreover, the higher $NO_2^-+NO_3^-$–N concentration was observed throughout September observations (Fig. 3b), and also statistically different from those observed in November ($P<0.001$, $n=50$). Otherwise, no significant difference in NH_4^+–N and DIN ($NO_2^-+NO_3^-$–N$+NH_4^+$–N) was observed between the two sampling months (Fig. 3b and Fig. 5). In addition, the average $NO_2^-+NO_3^-$–N concentrations of 160 μmol–N l^{-1} were found in September observation, contributing to the 52.5% to DIN concentration in pore water, and the contribution of $NO_2^-+NO_3^-$–N to DIN reduced to 22.8% (63.9 μmol–N l^{-1}) in November (Fig. 5). These results might be explained by considering that the oxygenation at the sediment–water interface was influenced by a longer aerial exposure period in September (discussed above), which might allow sediments at the sediment–air interface to reach aerobic conditions (THORNTON *et al.*, 1999). Also, the photosynthesis of microphytobenthos during daytime exposure might contribute partially to the oxygenation in the tidal flat, resulting in a higher $NO_2^-+NO_3^-$–N concentration in pore water via nitrification processes in the tidal flat of the Mae Klong River estuary.

Unfortunately, we planed firstly to investigate the spatial distribution of microphytobenthos in the tidal flat, whereas the all sedimentary parameter data between an each zone (3 zones) were not significantly different. Hence, we could not discuss clearly about spatial distribution of all parameters in the present study.

5. Conclusion

This study describes and quantifies the short–term variation of microphytobenthic biomass and associated controlling factors, as well as the influence of the microphytobenthic production on sediment quality in the tidal mudflat of the Mae Klong River estuary. Our results demonstrated that: (1) the difference of microphytobenthic biomass among two observations was due to irradiance available during aerial exposure in daytime, and (2) the higher microphytobenthic biomass might change the sedimentary composition in surficial sediments. Finally, (3) the oxygenation at sediment–water interface influenced by longer aerial exposure periods might allow sediments at the sediment–water interface to become aerobic conditions, resulting in increase of $NO_2^- + NO_3^-$–N concentration in pore water via nitrification processes in the tidal flat of the Mae Klong River estuary.

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