

## Changes of Chlorophyll *a* in an Intertidal Bangtaboon Estuary in Relation to Tidal Driven Salinity and Nutrients

Khanitta Buakaew<sup>1</sup>, Charumas Meksumpun<sup>2,\*</sup>, Shettapong Meksumpun<sup>3</sup>, Chakhrit Ruengsorn<sup>3</sup>,  
Patrawut Thaipichitburapa<sup>4</sup> and Pichasit Sangmek<sup>5</sup>

### ABSTRACT

This study analyzes the impacts of tidal-driven salinity and orthophosphate on chlorophyll *a* in a tropical intertidal Bangtaboon estuary, along the western Gulf of Thailand. Field surveys were carried out at 12 stations during low-, medium-, and high-loading periods in May, July, and November 2015. Results indicated that chlorophyll *a* varied by season. Particularly during high loading periods when orthophosphate increased, chlorophyll *a* levels were higher. In general, the levels of chlorophyll *a* here reflected hypertrophic condition of the ecosystem. The median concentration of chlorophyll *a* can be recognized ca. 2-3 hours after the highest tidal amplitude. In addition, the water masses with 24 psu salinity experienced the greatest chlorophyll *a* production. The role of salinity in chlorophyll *a* production was found to be enhanced by amplitudes and pulses of the tidal patterns. To support environmental conservation and maintain sustainable production in the estuary, orthophosphate concentrations along the highly productive water mass should be carefully monitored and further controlled by suitable management approaches.

**Keywords:** Bangtaboon estuary, chlorophyll *a*, estuarine phytoplankton, orthophosphate, tidal amplitude

### INTRODUCTION

Temporal stability of primary production is one of the crucial factors in determining sustainability of fishery resources of both capture and culture fisheries of the estuarine ecosystem (Pauly and Christensen, 1995; Duarte, 2009; Lallu *et al.*, 2014). However, research attempting to explain estuarine primary production in Thailand is scarce. Additional data is needed to determine the impacts of tidal mixing, environmental functions, aquaculture, and other utilization pressures.

Levels of primary production in the estuarine ecosystem vary greatly (Bucci *et al.*, 2012; Aoki *et al.*, 2016). Such variation implies impacts from different tidal (spring or ebb) phases and variation of salinity, turbidity, and nutrients (Montani *et al.*, 1998; Azhikodan *et al.*, 2016; Menéndez *et al.*, 2016; Mao *et al.*, 2017). In addition to the impact of tidal phases, the topographical structures of particular sites affect the mixing magnitude of freshwater and marine water masses that further influence both quantitative levels of primary producers and their species compositions in the estuarine ecosystem (Bukaveckas *et al.*, 2011; Aoki *et al.*, 2016).

<sup>1</sup> Center for Advanced Studies in Agriculture and Food, Kasetsart University, Bangkok, Thailand

<sup>2</sup> Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

<sup>3</sup> Department of Marine Science, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

<sup>4</sup> Department of Aquatic Science, Faculty of Science, Burapha University, Chonburi, Thailand

<sup>5</sup> Agro-Bioresources Program, Faculty of Natural Resource and Agro- Industry, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand

\* Corresponding author, e-mail address: ffiscmc@ku.ac.th

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It is also crucial to monitor the changes of primary production and the relations to other environmental factors in the estuary. This study attempts to determine the causes of changes in chlorophyll *a* concentrations in a famous intertidal clam-culturing estuary, Bangtaboon Bay, located along the western coast of the inner Gulf of Thailand, in Phetchaburi Province. Environmental impacts were analyzed to depict integrated roles of tidal regimes and land-based freshwater discharge on primary production. Such information is needed to determine suitable water sampling times for obtaining representative samples of chlorophyll *a* and related factors in the estuarine intertidal ecosystem. Moreover, related knowledge will assist in the creation of suitable conservation and management plans for sustainability of aquaculture production in the estuarine areas.

## MATERIALS AND METHODS

The study site, Bangtaboon Bay, is located in Phetchaburi Province, a coastal province in the western part of the upper Gulf of Thailand. This bay is a eutrophic tidal estuary with muddy organic

enriched substrates (Buakaew *et al.*, 2015). The Bangtaboon Bay is also one of the most important blood clam (*Anadara* sp.) culture areas of Thailand. Since 2011, *Anadara* production from this area (more than 15,000 tons per year, or 30% of national production) made Phetchaburi Province the most important clam production site in Thailand (Department of Fisheries, 2014). In the estuary, almost the entire tidal flat of the inner bay is being used for *Anadara* clam culture. The upper tidal flat is separated from the lower flat by a river channel, which runs offshore from the west to the east (Figure 1). Previous study here indicated that salinity in the overall area varies widely, in the range of 8-28 psu. Sedimentary organic content is moderately high, in the range of 5-12%. The southeastern part of the estuary has higher potential for *Anadara* production, due to the highly suitable quality of its fine-grained bottom deposits. The upper and the middle parts of the estuary have comparatively coarser deposits and receive significant influence from the land-based river runoff (Sangmek and Meksumpun, 2014).

For this study, three sampling periods were set, representing the low-, medium-, and high freshwater loading periods of May, July, and

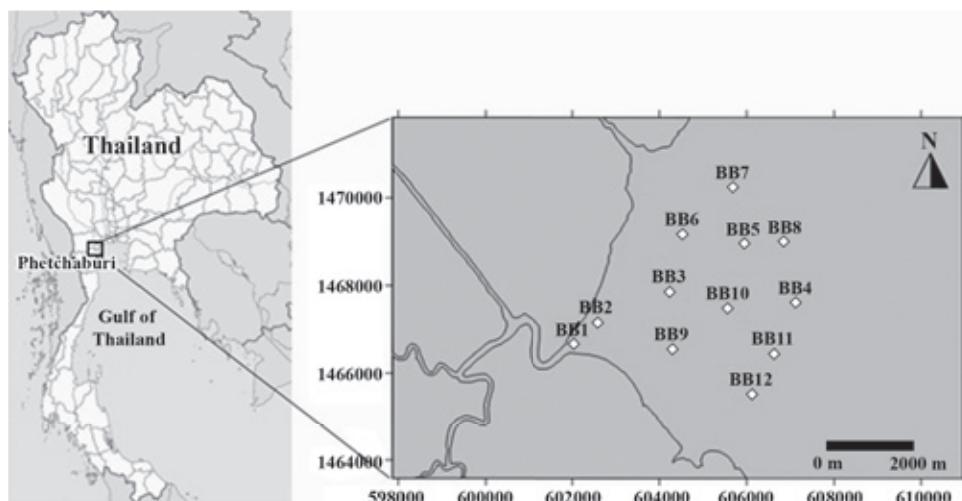


Figure 1. Map of sampling stations located in the Bangtaboon estuary, Phetchaburi Province, in the western coastal zone of Thailand.

November 2015, respectively (Sangmek and Meksumpun, 2015). These periods were influenced by different inflow quantity, corresponding to the SE and NW monsoon seasons of Thailand (TMD, 2014). In each sampling period, field surveys were carried out at 12 stations in the Bangtaboon estuary (Figure 1; Stns BB1-BB12). The sampling stations in the estuary represent three zones: the channel (BB1-4), the northern tidal flat (BB5-8), and the southern tidal flat (BB9-12). At BB9, the water samples were monitored at every 2-h interval for 24 hours. The inflow volumes were determined by measurement of water flow through the cross section of the innermost part of the estuary.

Water temperature, conductivity, salinity, dissolved oxygen, and pH were measured by a multi-parameter probe (YSI Model 660QS). Samples for determination of chlorophyll *a* levels were collected and placed in polyethylene bags that were kept away from direct light before analysis. The samples were filtered through pre-weighed GF/F (Whatman; pore size 0.7  $\mu\text{m}$ ). Chlorophyll *a* was analyzed according to a spectrophotometric method (Parsons *et al.*, 1984). Water samples were also collected and filtered onboard through GF/F. The filtrates were then analyzed for nutrients (phosphate,  $\text{PO}_4^{3-}$ ; and ammonium,  $\text{NH}_4^+$ ) by an auto-nutrients analyzer (SKALAR). Phytoplankton samples were collected by sieving the surface water (0.5 m depth) with a phytoplankton net (20  $\mu\text{m}$ ). The specimens were preserved in 2% formaldehyde solution and later identified and counted in the laboratory. The data were statistically analyzed using the Spearman rank correlation coefficient ( $r_s$ ) for determining the relationships between chlorophyll *a*, salinity, and nutrients in the estuary.

## RESULTS AND DISCUSSION

Results from this study are presented here as follows: land-based freshwater inflows and temporal variations, tidal characteristics of sampling seasons, spatial distribution of chlorophyll *a* and tidal driven salinity, phytoplankton abundance and species composition, and diel variation of chlorophyll *a*. Related water parameters are also presented.

### *Land-based freshwater inflows and their temporal variation*

Inflow volumes of freshwater varied seasonally. During the low-tide periods in the low- and medium-loading seasons of 2015 (May and July, respectively), the inflows were determined to be  $376 \text{ m}^3 \cdot \text{s}^{-1}$  and  $432 \text{ m}^3 \cdot \text{s}^{-1}$ , respectively. In the high-loading season of November, the highest inflow was  $861 \text{ m}^3 \cdot \text{s}^{-1}$ , during the low-tide period. The inflow levels also appeared to be influenced by the tidal phase (high or low tide). Depending on tidal phase, inflows here can vary ca. 2-8 times. The levels along the river mouth in May, which were commonly more than  $300 \text{ m}^3 \cdot \text{s}^{-1}$ , decreased to less than  $50 \text{ m}^3 \cdot \text{s}^{-1}$  during the highest high tide.

### *Tidal characteristics during the sampling seasons*

In the three sampling seasons, the lowest and the highest tidal levels were noted (The Royal Thai Navy, 2016). During the spring tide of the low-loading season (18 May 2015), the largest difference in tidal levels (between the highest high tide and the lowest low tide) was 2.4 m. Such tidal differences varied slightly by season: 2.0 m in the medium-loading season (28 July) and 2.4 m in the high-loading season (18 November). At the lowest low tides of the three seasons, the water levels were reduced to minimum depths of 1.0, 0.7, and 1.2 m, respectively (Figure 2).

### *Spatial distribution of chlorophyll *a*, salinity, and nutrients during the spring tides*

In each sampling season of 2015, there were wide ranges of chlorophyll *a* distributed in the Bangtaboon estuary. The ranges of chlorophyll *a* during the low-, medium-, and high-loading seasons were 18.96-202.93, 13.02-32.46, and 42.36-133.17  $\mu\text{g} \cdot \text{L}^{-1}$ , respectively (Figure 3). Generally high concentrations of chlorophyll *a* (average of 71.85  $\mu\text{g} \cdot \text{L}^{-1}$ ) during the high-loading period in November (Figure 3c) were affected by the spring phase of the tidal cycle (Figure 2), which increased salinity of the water mass during the sampling period. Conversely, long hours of ebb tide (ca. 12 hours) on the sampling day of the medium-loading season provided dilution of phytoplankton density, reducing

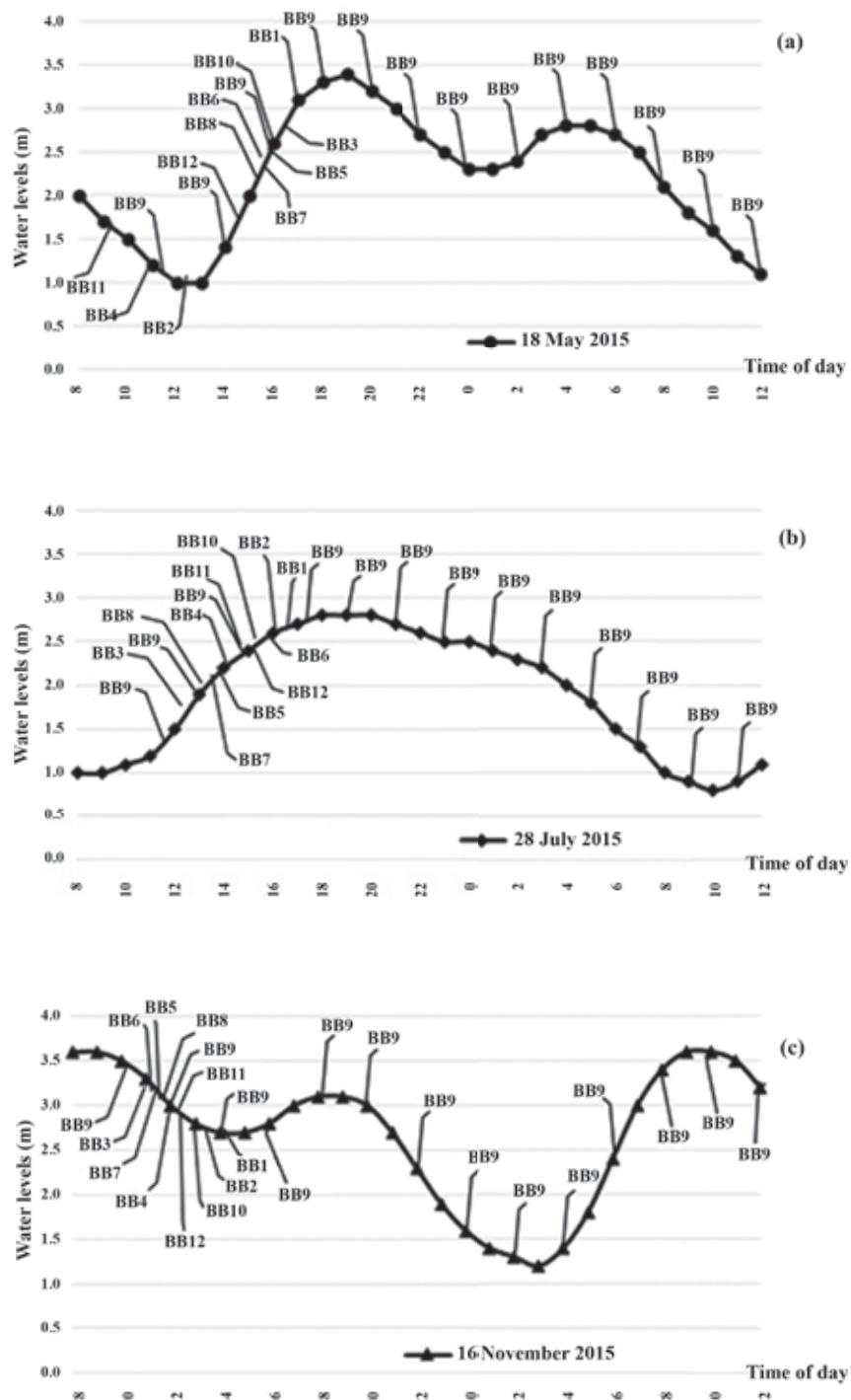


Figure 2. Tidal cycles during the low-loading (a), medium-loading (b), and high-loading (c) seasons at the survey stations (BB1-BB12) of the Bangtaboon estuary, Phetchaburi Province, Thailand (at BB9, water samples were taken at every 2-h interval for 24 hours).

chlorophyll *a* concentrations to an average of  $17.26 \mu\text{g}\cdot\text{L}^{-1}$  in the entire estuarine area (Figure 3b). Median values of salinity in each season were similar: 26.15, 26.92, and 24.32 psu during low-, medium-, and high-loading seasons, respectively

At one particular station, Stn BB8 in the outermost part of the estuary, maximum levels of chlorophyll *a* were frequently recorded. The highest chlorophyll *a* levels at Stn BB8 during the low-, and high-loading seasons occurred while the levels of  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  were in the ranges of 6.0-13.6 and 0.6-1.4  $\mu\text{M}$ , respectively. Those nutrient levels were apparently high, but similar to those of other stations, such as Stn BB9 (3.7-10.9

$\mu\text{M}$  of  $\text{NH}_4^+$  and 0.6-1.3  $\mu\text{M}$  of  $\text{PO}_4^{3-}$ ) located in the inner estuary.

#### Phytoplankton investigation in the shallow clam culture zone

Examination of phytoplankton species was carried out in the shallow clam culture zone of Stn BB9 during the daytime (10-11 am). In that zone, the group of diatoms (Division Bacillariophyceae) was found to be the major phytoplankton group (Table 1). Among this group, the genera *Cylindrotheca* sp., *Skeletonema* sp., and *Thalassiosira* sp. were usually found in comparatively high numbers with maximum densities of 643,960, 207,584 and 22,880

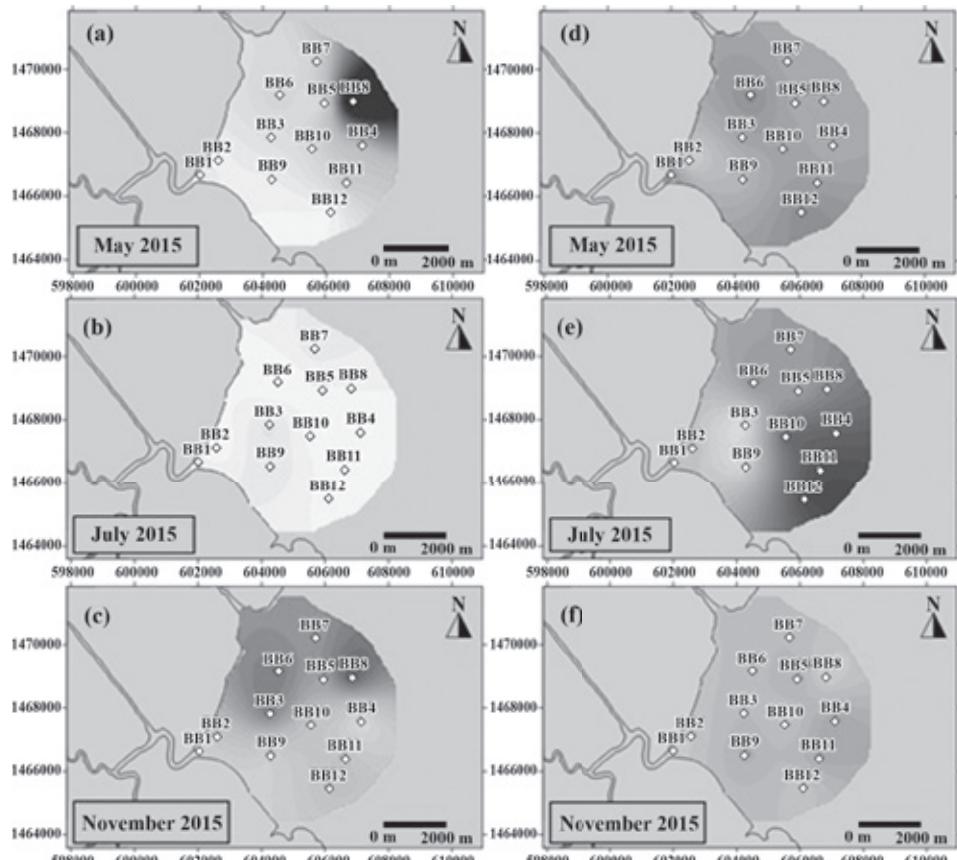


Figure 3. Spatial distributions of chlorophyll *a* (in the left-hand side; a-c) and salinity (in the right-hand side; d-f) during the low-, medium-, and high-loading seasons of the Bangtaboon estuary, Phetchaburi Province, Thailand.

Table 1. Phytoplankton species composition, cell densities, and chlorophyll *a* (Chl *a*) of the samples from the shallow station (Stn BB9) in the Bangtaboon estuary, Thailand, during three study periods.

Phytoplankton species	Cell density (cells•L <sup>-1</sup> )		
	18-May-2015 (Chl <i>a</i> 25 µg•L <sup>-1</sup> )	28-Jul-2015 (Chl <i>a</i> 30 µg•L <sup>-1</sup> )	16-Nov-2015 (Chl <i>a</i> 56 µg•L <sup>-1</sup> )
Cyanophyceae			
<i>Oscillatoria</i> sp.	-	-	832
Bacillariophyceae			
<i>Bacillaria</i> sp.	-	1,913	-
<i>Chaetoceros</i> sp.	248	-	832
<i>Coscinodiscus</i> sp.	558	2,380	416
<i>Cylindrotheca</i> sp.	7,130	643,960	16,640
<i>Diploneis</i> sp.	186	-	-
<i>Entomoneis</i> sp.	62	2,635	-
<i>Haslea</i> sp.	186	1,190	416
<i>Melosira</i> sp.	62	-	-
<i>Meunier</i> sp.	-	-	1,248
<i>Navicula</i> sp.	124	17,255	2,496
<i>Nitzschia</i> sp.	682	10,795	1,664
<i>Odontella</i> sp.	62	-	-
<i>Pleurosigma</i> sp.	62	850	-
<i>Pseudonitzschia</i> sp.	-	680	1,248
<i>Pseudosolenia</i> sp.	-	680	-
<i>Rhizosolenia</i> sp.	372	3,655	2,080
<i>Skeletonema</i> sp.	1,116	3,995	207,584
<i>Thalassionema</i> sp.	-	680	416
<i>Thalassiosira</i> sp.	3,720	18,785	22,880
<i>Trachyneis</i> sp.	62	1,700	832
Dinophyceae			
<i>Protocentrum</i> sp.	-	-	832
<i>Protoperidinium</i> sp.	310	8,330	6,240
<b>Total density (cells•L<sup>-1</sup>)</b>	<b>14,942</b>	<b>719,483</b>	<b>266,656</b>

cells•L<sup>-1</sup>, respectively. The cell densities were higher than those reported by Meksumpun *et al.* (2012). During the medium-loading season, the dominant phytoplankton *Cylindrotheca* sp. had increased more than 90 times over the levels found in the low-loading season. Likewise, total phytoplankton density had increased ca. 50 times. The occurrence of *Cylindrotheca*, a benthic form, implied an impact from re-suspension in the sampling times.

In addition, a planktonic diatom in the water column, genus *Skeletonema*, was normally observed in lower densities. Increases of ca. 4 times were found during the medium-loading season.

Thereafter, during high-loading season, the densities increased more than 50 times (Table 1). Interestingly, the high densities were found during times of comparatively higher water depth (3.7 m). The levels of chlorophyll *a* did not correlate with cell densities because of the selectivity of the plankton net used in this study.

#### *Diel variation of chlorophyll *a* and related water quality parameters*

Diel dynamics of chlorophyll *a* levels measured at Stn BB9 were observed (Figure 4). The ranges of the levels of the low-, medium, and

high-loading seasons were 5.24-45.48, 6.58-35.65 and 3.35-55.96  $\mu\text{g}\cdot\text{L}^{-1}$ , respectively. The median values were 19.17, 11.77 and 16.77  $\mu\text{g}\cdot\text{L}^{-1}$ , respectively. Such levels illustrated a moderately eutrophic water mass. During 24-hour monitoring, the highest variation of chlorophyll *a* levels (+52.61  $\mu\text{g}\cdot\text{L}^{-1}$ ) was observed on the day with the highest tidal amplitude (2.4 m; 16 November 2015), while the lowest variation of chlorophyll *a* levels (+29.07  $\mu\text{g}\cdot\text{L}^{-1}$ ) was noticed on the day with the lowest tidal amplitude (2.0 m; 28 July 2015) which also had comparatively smooth changes of the tidal pattern. Interestingly, the median values of chlorophyll *a* levels in each sampling time can be obtained by

sampling the surface water ca. 2-3 hours after the highest high tidal levels.

Ranges of water temperature during low-, medium-, and high-loading seasons were 31.76-34.92, 30.39-31.06 and 29.99-31.82 °C, respectively. Comparatively higher temperatures were found in the low-loading period (May 2015), corresponding to the summer weather of Thailand. In addition, the DO levels fluctuated greatly by season and during the surveyed days. Such high DO variations are likely due to complex functions of both phytoplankton photosynthetic DO production and the de-oxygenation of re-suspended turbid water enhanced by tidal

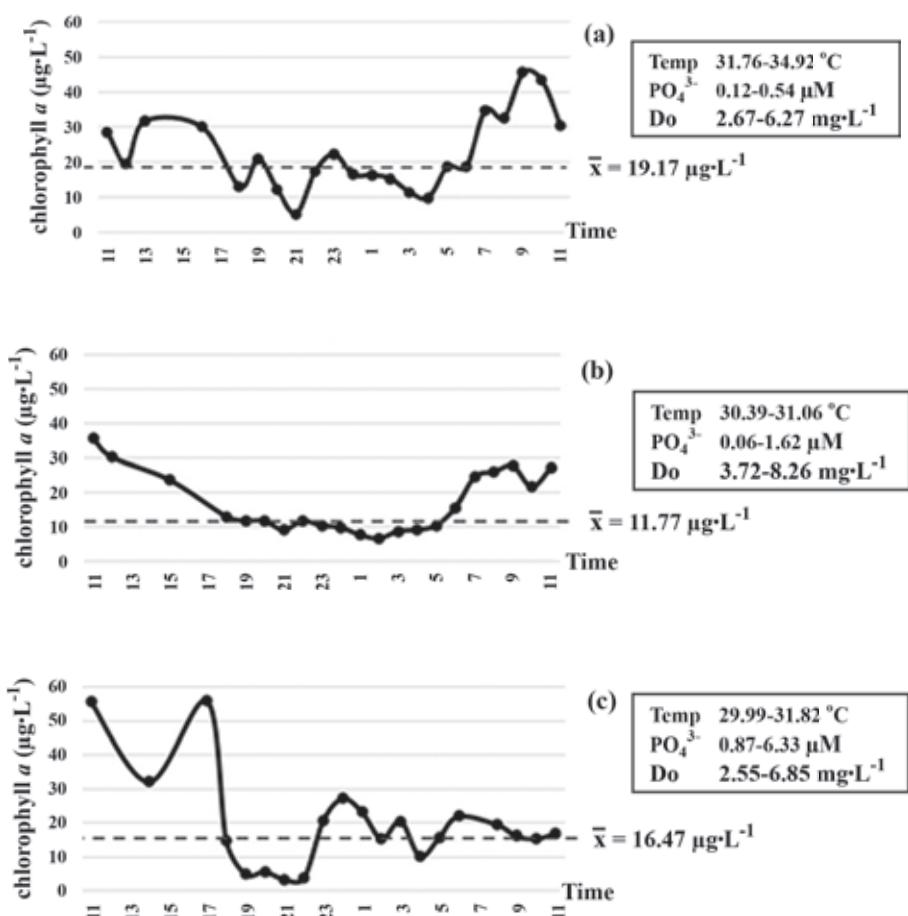


Figure 4. Diel changes of chlorophyll *a* levels during the periods of low- (a), medium- (b), and high- (c) loading seasons.

regimes in the area. Among biologically important nutrients, wide ranges of orthophosphate phosphorus concentrations (0.12-0.54, 0.06-1.62, and 0.87-6.33  $\mu\text{M}$ , respectively, during low-, medium-, and high-loading seasons) were noticed.

*Integrated impacts of environmental factors on chlorophyll a*

The overall changes in chlorophyll *a* and phytoplankton composition of the estuary reflected influences of the tidal regimes in the area. Nevertheless, the levels of DO during the peaks of chlorophyll *a* did not coincide with the chlorophyll *a* levels. This phenomenon was considered to be due to integrated impacts from de-oxygenation and/or re-suspension during the spring or ebb tidal mixing processes which occurs in natural estuarine areas (Montani *et al.*, 1998; Aoki *et al.*, 2016). In accordance with prior studies of tidal and nutrient impacts on primary production (Fisher *et al.*, 1988; Montani *et al.*, 1998; Huang *et al.*, 2004; Azhikodan *et al.*, 2016; Menéndez *et al.*, 2016; Otsuka *et al.*, 2016; Mao *et al.*, 2017), this study found that the patterns of tidal changes and their amplitudes play more important roles in patchiness and distribution of the chlorophyll *a* than the freshwater inflows. In this study, the maximum orthophosphate levels were also increased during the high-loading period. Such phenomena could be due to either the impacts of higher freshwater runoff during that season, or comparatively higher magnitude of the tidal cycle during the period. The influences of tidal driven salinity on nutrients, and their related impacts on chlorophyll *a* were analyzed and discussed as follows.

*Influences of tidal driven salinity on changes in nutrients*

Orthophosphate phosphorus concentrations in all sampling periods decreased with increases of salinity. During the low-loading season in May, orthophosphate levels in the estuary were lowest, less than 0.60  $\mu\text{M}$ , due to comparatively lower freshwater runoff. In the medium-loading season of July, orthophosphate concentrations doubled. During that period, levels of more than 1.2  $\mu\text{M}$  were found in the areas with salinity less than 23.5 psu,

while lower levels of less than 0.6  $\mu\text{M}$  were found in the water mass with salinity more than 28.8 psu.

The highest concentrations of orthophosphate in this estuary were observed during the high-loading season in November, when the estuary receives the highest amounts of freshwater runoff. Orthophosphate levels increased *ca.* 10 times over those found during the low-loading season, but still varied between 0.87-6.33  $\mu\text{M}$ . High nutrient phenomena reflected the nutrient-enriched condition of the estuary. During this high-loading season, almost all of the sampled areas had high orthophosphate levels (more than 2  $\mu\text{M}$ ). The lowest orthophosphate level was found in the water mass with comparatively lower salinity of *ca.* 17 psu.

The pattern of decreased orthophosphate concentrations along the salinity gradients in different inflow seasons seemed to be influenced by time-specific tidal characteristics (Figure 5). Along the periods of low-loading (LL), medium-loading (ML), and high-loading seasons (HL), the orthophosphate concentrations during those periods ( $P_{LL}$ ,  $P_{ML}$ ,  $P_{HL}$ ) were reduced significantly ( $P < 0.01$ ) by the tidal driven salinity ( $S$ ) as shown by Equations 1) - 3) with the correlation coefficients ( $r$ ) of 0.618, 0.705, and 0.939, respectively.

$$P_{LL} = -0.06 S + 1.75 \quad 1)$$

$$P_{ML} = -0.08 S + 2.77 \quad 2)$$

$$P_{HL} = -0.55 S + 15.82 \quad 3)$$

Such strength of decreasing functions of the salinities, which can be seen by the levels of "Slope" or "Decreasing coefficients;  $C_{DE}$ " of the above equations, should be enhanced by mixing potentials of tidal currents in the estuary. In this aspect, "amplitudes" and "frequency pulses" of the tidal characteristics (Figure 2) were analyzed. It was found that combination of the 1<sup>st</sup> Amplitude ( $A_{1st}$ ; m) and the 2<sup>nd</sup> Amplitude ( $A_{2nd}$ ; m) on the sampling day, multiplied by the period of time ( $T$ ; hr) between the two amplitude peaks can reflect the strength of decreasing functions ( $C_{DE}$ ) of the salinity on the quantity of orthophosphate as in Equation 4).

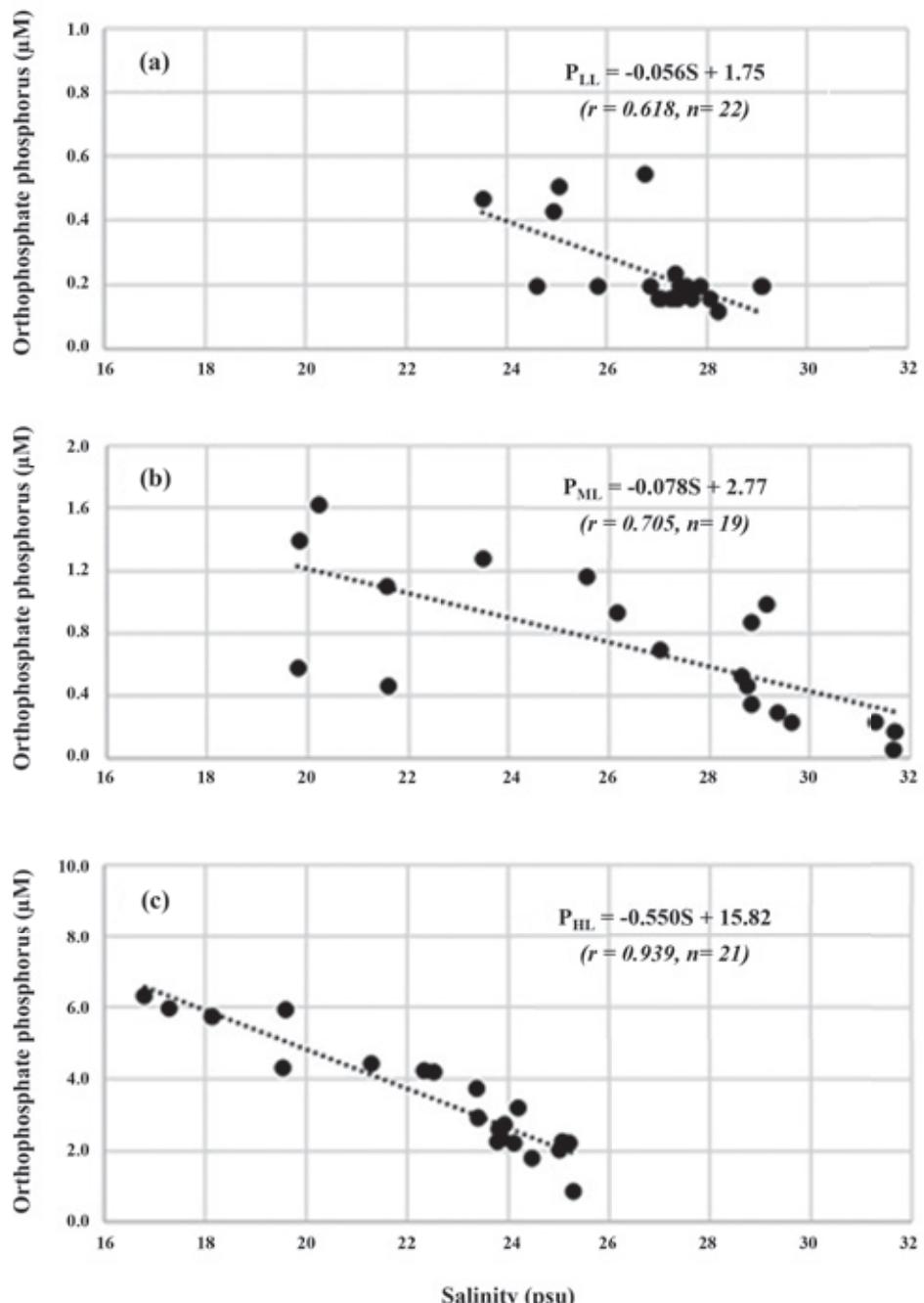


Figure 5. Changes of orthophosphate phosphorus (P<sub>LL</sub>, P<sub>ML</sub>, P<sub>HL</sub>) levels along the salinity (S) gradients during the periods of low- (a), medium- (b), and high- (c) loading seasons, respectively, in the Bangtaboon estuary, Phetchaburi Province, Thailand.

$$C_{DE} = 3.4 \times 10^{-3} (A_{1st} + A_{2nd}) T \quad 4)$$

Such a relationship implied the importance of survey planning in studies of nutrients and further interpretation of related water quality information for the estuarine ecosystem. Careful consideration of the tidal characteristics, in particular the amplitudes and tidal periods, is, thus, necessary because of their significant impacts on the concentrations of available nutrients.

*Influences of tidal mixing of salinity and nutrients on the changes of chlorophyll a*

Tidal mixing and concentrations of nutrients impact phytoplankton chlorophyll in several patterns (Liu *et al.*, 2011; Paches *et al.*, 2014). In this study, during comparatively low levels of orthophosphate (less than 0.5  $\mu$ M), the chlorophyll a concentrations increased from less than 10  $\mu$ g•L<sup>-1</sup> to more than 50  $\mu$ g•L<sup>-1</sup> (Figure 6a). When orthophosphate

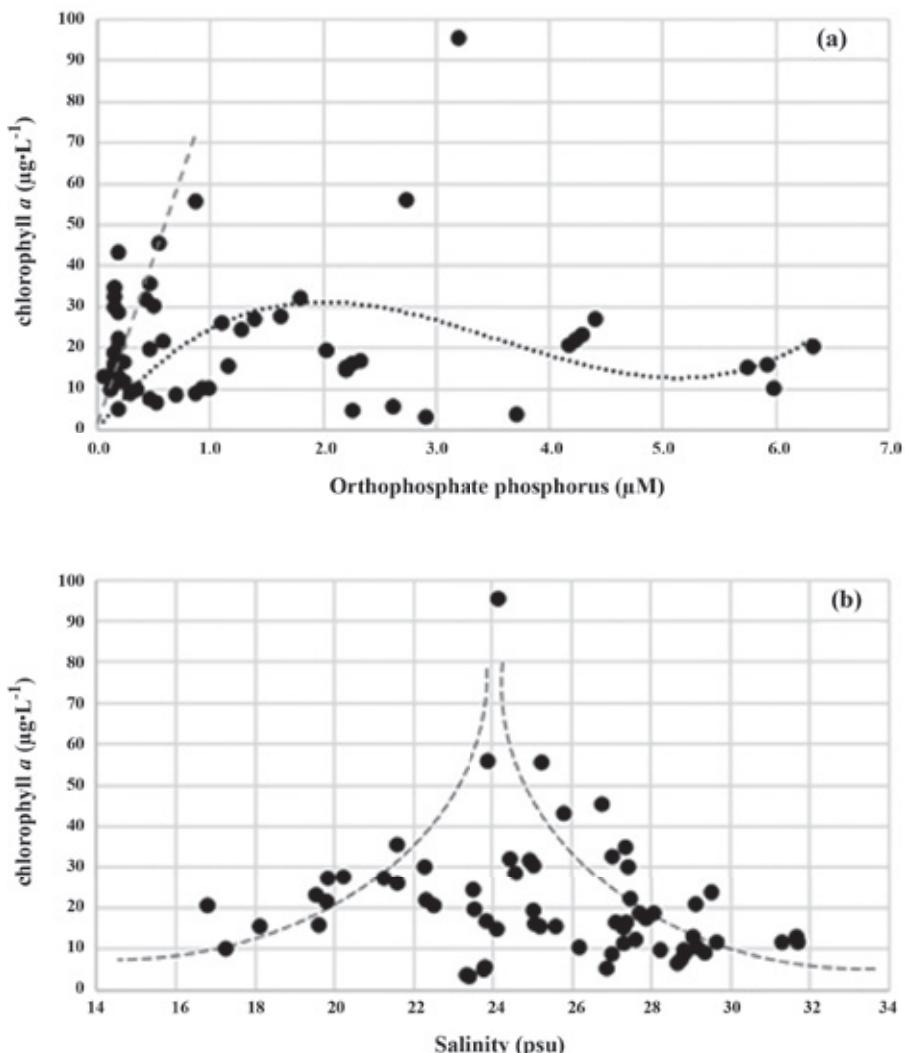


Figure 6. Changes of chlorophyll a concentrations along orthophosphate gradients (a) and salinity gradients (b) in the Bangtaboon estuary, Phetchaburi Province, Thailand.

concentrations became higher, levels of phytoplankton chlorophyll *a* fluctuated greatly. Such occurrences were considered to be due to either the excess levels of orthophosphate concentrations or the impacts of mixing in the estuarine intertidal zones. In general, the nutrients and phytoplankton chlorophyll *a* levels here remarkably reflected eutrophic conditions of the aquatic ecosystem.

Along the gradient of water salinity from 14 to 34 psu, phytoplankton chlorophyll levels were most scattered in the water mass of 24 psu salinity, with the maximum of  $95.39 \mu\text{g L}^{-1}$  (Figure 6b). Such a distribution pattern of chlorophyll could imply that the 24 psu water mass should be the most productive for phytoplankton production of the estuary. The lower or higher salinity sites should be in the zones that received more freshwater or tidal mixing dilution. Accordingly, the phytoplankton chlorophyll *a* in comparatively lower or higher salinity water masses was then diluted. The water mass with 24 psu salinity, thus, should be the most enriched water resource for further extension of related aquaculture (i.e. filter-feeding organisms such as oysters and mussels) in the estuarine area.

## CONCLUSION

This study illustrated patterns of changes in chlorophyll *a* concentrations in a famous clam culture estuary located along the western coast of the Gulf of Thailand. The levels of chlorophyll *a* apparently varied by seasons. Particularly during the high loading period, the levels were comparatively higher due to orthophosphate enhancement. Such chlorophyll *a* levels raised the enrichment status of the estuary to be as high as hypertrophic condition. In general, the estuary received integrated impacts from freshwater runoff and/or orthophosphate concentrations, and from the tidal amplitude and mixing potentials. The effect of tidal-driven salinity on primary production was interestingly found to be enhanced by the amplitudes and the times of pulses of the tidal regimes. Such findings can further contribute to the determination of nutrient levels and related primary production along salinity gradients.

This study also found that the median value of primary production in each sampling day can be obtained *ca.* 2-3 hours after the highest tidal amplitude. This knowledge can be applied for the development of a suitable plan for water-quality monitoring. In addition, the chlorophyll *a* levels were increased significantly along the water mass with 24 psu salinity. As such, the 24-psu water mass was a key source of pelagic primary production of the estuarine area. For ecosystem conservation and sustainable production by aquaculture, it is vital to maintain ecosystem conditions and stability along this productive zone. Accordingly, the nutrient concentrations along the zone should be carefully monitored and further controlled by suitable upstream management approaches.

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