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Physico-Chemical Factors Influencing Blooms of *Chaetoceros* spp. and *Ceratium furca* in the Inner Gulf of Thailand

Nittaya Somsap[1], Nantana Gajaseni[2,*] and Ajcharaporn Piumsomboon[3,*]

ABSTRACT

Physico-chemical factors influencing the blooms of two common phytoplankton—*Chaetoceros* and *Ceratium*—in the Inner Gulf of Thailand (IGoT), were investigated during the northeast (NE) and the southwest (SW) monsoons from a fixed station near Si Chang Island, on the east coast of the IGoT. The results showed that the dominance of each phytoplankton occurred under different ecological conditions. The density (mean ± SD) of *Chaetoceros* spp. was about $0.13 \pm 0.13 \times 10^5$ cells.L-1 in the NE monsoon period but increased to $2.27 \pm 3.11 \times 10^5$ cells.L-1 during the bloom in the early months of the southwest monsoon. This density increase was significantly related to values of the ratio of dissolved inorganic nitrogen to dissolved silicate (DIN:DSi molar ratio), dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP) and temperature. The density of *Ceratium furca* increased from $0.08 \pm 0.04 \times 10^3$ cells.L-1 during the NE monsoon period to $7.14 \pm 3.17 \times 10^5$ cells.L-1 during the red tide at the end of the SW monsoon under conditions of low salinity, high DIN and DIP.
Keywords: *Ceratium*, *Chaetoceros*, DIN, DIN:DSi, DIP

INTRODUCTION

Phytoplankton blooms known as red tide phenomena occur frequently in the coastal area of the Inner Gulf of Thailand (IGoT) which is sometimes called the Upper Gulf of Thailand. During the southwest (SW) monsoon season which occurs from May to September, occurrences of red tide have been reported from the east coast of the IGoT, while later in the year, during the northeast (NE) monsoon from November to February, red tides appear in the northern and western parts of the IGoT due to the counter-clockwise current (Buranapratheprat *et al*., 2006). Many species of *Chaetoceros*—a chain-forming diatom—and two dinoflagellates—*Ceratium* (*C. furca* in particular) and *Noctiluca scintilans*—have been recorded as major bloom-forming phytoplankton in the IGoT (Lirdwitayaprasit *et al*., 2006; Piumsomboon *et al*., 2008).

According to previous studies (Levasseur *et al*., 1984; Lehman and Smith, 1991; Aubry *et al*., 2004; Brogueira *et al*., 2007; Shipe *et al*., 2008; Drake *et al*., 2010; Song, 2010; Abdenadher *et al*., 2012; Huang *et al*., 2012), the phytoplankton composition, bloom and succession are under the influence of ecological factors such as light, temperature, salinity and nutrient concentrations, especially nitrogen (N) and phosphorus (P). River discharge carries nutrients from various

1 Biological Science Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.
2 Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.
3 Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.
* Corresponding author, e-mail: aun.nantana@gmail.org, ajcharaporn.p@chula.ac.th

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sources including agricultural fertilizers, livestock, domestic wastewater and soil erosion toward the sea, where these nutrients enhance mineral components in seawater and finally become available for phytoplankton utilization, while in cases of excessive discharge, this results in eutrophication due to an unbalanced N:P ratio (N:P >> 16:1) and growth-limiting nutrients of phytoplankton (Correll, 1998). Under such circumstances, phytoplankton blooms or red tides normally occur and impact on other aquatic organisms. Nutrients from river discharge are the most important ecological factors promoting long term eutrophication during the SW monsoon in the east coast of the IGoT (Buranapratheprat *et al.*, 2002a).

However, *Chaetoceros curvisetus* is reported as one of causative species of harmful algal blooms in the Yangtze estuary in the East China Sea (Shen *et al.*, 2011). The blooms of *Ceratium* have been recognized in association with anoxic effects (Okaichi, 2003) and as a consequence of the mortality of oysters, shrimp larvae and fish (Vargas-Montero and Freer, 2004). There has also been a report of massive fish mortality in Costa Rica that coincided with the bloom of *Ceratium dens*, *C. furca* and *C. fusus* (Vargas-Montero and Freer, 2004). In addition, Granéli and Hansen (2006) also suggested that *Ceratium* sp. could produce unknown allelopathic substances inhibiting the growth of other microalgae. Thus, the goal of the current study was to focus on the ecological factors affecting the blooms of *Chaetoceros* and *Ceratium* in the IGoT.

MATERIALS AND METHODS

Study site and sampling period

Collections of seawater and phytoplankton samples were conducted at a fixed-station (latitude 13° 9′ 32.6″ N and longitude 100° 49′ 05.2″ E) near the coastal area of Si Chang Island in Chonburi province, on the east coast of the IGoT (Figure 1) to compare the temporal changes in abundance of bloom-forming phytoplankton between two different monsoon seasons: the northeast (NE) monsoon from December 2010 to February 2011 and the southwest (SW) monsoon from May to

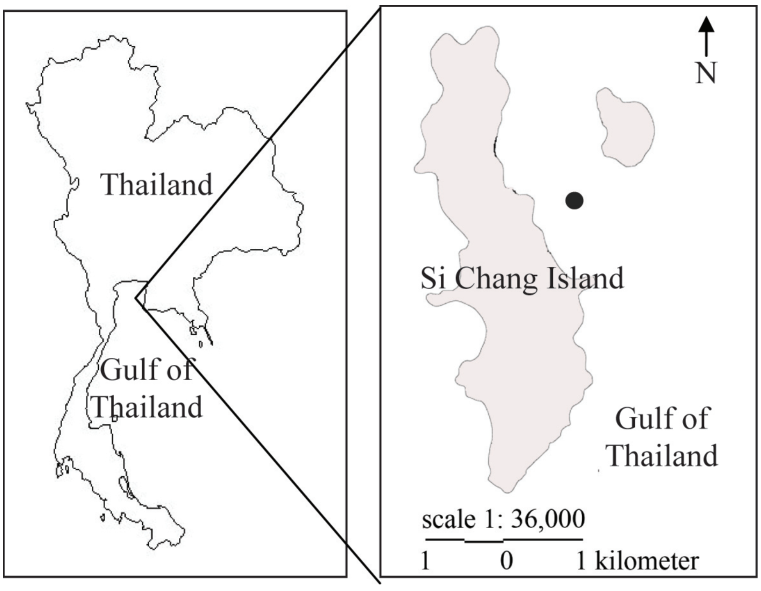

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Figure 1 Sampling site (•) near the coastal area of Si Chang Island, east coast of the Inner Gulf of Thailand.

October 2011. Sampling was conducted on a regular basis once a week except during the red tide phenomena when samples were collected every 1–2 d.

Measurement of physico-chemical parameters

At the sampling site, physico-chemical parameters were measured prior to sampling. These parameters included a Secchi-disc transparency, *in situ* seawater temperature and dissolved oxygen (DO) was measured using a DO meter (YSI-DO 200; YSI Inc., Yellow Springs, OH, USA) and the pH using a pH meter (YSI- pH 100; YSI Inc., Yellow Springs, OH, USA) at three different depths (surface to approximately 0.5 m, mid depth to approximately 2.5–3.0 m and bottom to approximately 5–7 m) depending on the total depth on the day of sampling. Duplicate seawater samples from each concurrent depth were sampled using a Van Dorn bottle. A small amount of seawater from each sample was used for the measurement of salinity using a refractometer. The rest of the seawater samples was kept in pre-cleaned bottles and filtered through a glassfiber filter type C in the laboratory. The filtrate was frozen at -20 °C until analyzed for nitrite-nitrogen, nitrate-nitrogen, phosphate-phosphorus and silicate-silicon (Parsons *et al*., 1984). For analysis of ammonia-nitrogen, seawater samples were collected separately using a pre-cleaned stainless water sampler and kept frozen at -20 °C until analyzed in the laboratory using a spectrophotometric technique (Parsons *et al*., 1984).

Collection and analysis of *Chaetoceros* and *Ceratium* bloom-forming phytoplankton

After the collection of seawater for nutrient analyses, about 10 L of seawater was collected using a Van Dorn water sampler. Seawater samples were filtered onto a 20 µm meshed phytoplankton net. The cells retained on this net were preserved to a final concentration of 1% (volume per volume) neutralized formalin-seawater solution (Sournia, 1978). Sampling was repeated twice at each depth. Cell assemblages were counted using a Sedgewick Rafter slide under a compound microscope, at a magnification of 100× and cell densities were determined as the number of cells per liter according to Lobban *et al*. (1988). The identification of *Chaetoceros* and *Ceratium* was based on Tomas (1997).

Data analysis

The mean values of the physico-chemical parameters (transparency, temperature, salinity, DO, pH, dissolved inorganic nitrogen (DIN; ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen), dissolved inorganic phosphorus (DIP) and dissolved inorganic silicon (DSi; in the form of silicate) and densities of *Chaetoceros* and *Ceratium* were tested for differences among sampling dates and seasons using a one way analysis of variance. Finally, the relationships between physico-chemical factors and phytoplankton were determined using Pearson's correlation coefficient.

RESULTS

Physico-chemical parameters

The variations in the physico-chemical factors are summarized in Table 1 and illustrated in Figures 2–3. During the NE monsoon, seawater was characterized by low values of temperature and pH. While the concentrations of dissolved inorganic nitrogen (NO_3^--N, NO_2^--N and NH_4^+-N) or DIN, were about 5.000 µmol.L^{-1} during the study, exceptionally high concentrations of DIN (above 10.00 µmol.L^{-1}) were noticed twice in the early and toward the end of the SW monsoon period (Figure 3). A high concentration of DIP (above 1.000 µmol.L^{-1}) was recorded in the NE monsoon period (December) and decreased to concentrations lower than 1.000 µmol.L^{-1} for the rest of the study. These caused the high molar ratio of DIN:DIP in the late NE monsoon (February) and in the SW monsoon month of August. The concentration of silicon in the form of silicate

Table 1 Range and mean (± SD) of physico-chemical parameters during the NE and the SW monsoons in the coastal area of Si Chang Island, the Inner Gulf of Thailand.

Physico-chemical parameter	Northeast monsoon		Southwest monsoon	
	Range	Mean ± SD	Range	Mean ± SD
Transparency (m)	3.00 – 4.00	3.15 ± 0.34	1.50 – 4.00	3.15 ± 0.63
Temperature (°C)	27.50 – 28.70	27.80 ± 0.46	29.00 – 31.50	29.80 ± 3.46
Salinity (‰)	30.30 – 34.70	32.50 ± 0.35	15.70 – 32.50	26.70 ± 0.88
DO ($mg.L^{-1}$)	5.48 – 7.69	6.99 ± 0.30	2.69 – 8.95	6.04 ± 0.67
pH	7.77 – 8.78	8.07 ± 0.20	8.07 – 8.90	8.38 ± 0.15
DIN ($\mu mol.L^{-1}$)	0.551 – 3.791	2.026 ± 0.818	0.558 – 37.859	4.359 ± 2.436
DIP ($\mu mol.L^{-1}$)	0.018 – 1.926	1.005 ± 0.606	0.016 – 1.618	0.202 ± 0.091
DIN: DIP	0.60 – 90.30	12.00 ± 18.00	8.16 – 133.00	33.50 ± 16.10
DSi ($\mu mol.L^{-1}$)	1.691 – 12.954	7.585 ± 2.896	12.682 – 106.377	37.066 ± 19.586
DIN: DSi	0.10 – 1.65	0.44 ± 0.42	0.05 – 0.57	0.14 ± 0.06

DO = Dissolved oxygen; DIN = Dissolved inorganic nitrogen; DIP = Dissolved inorganic phosphorus; DSi = Dissolved silicate.

(DSi) showed the same pattern of variation as the DIN, being low in the NE monsoon period and increasing to concentrations higher than 80.00 $\mu mol.L^{-1}$ in late SW monsoon season. The values of salinity, DO and the calculated molar ratio of DIN:DSi in the NE monsoon period were higher than those in the SW monsoon. The variations in these physico-chemical parameters within and between monsoon periods were significant ($P < 0.05$) with the exception of transparency.

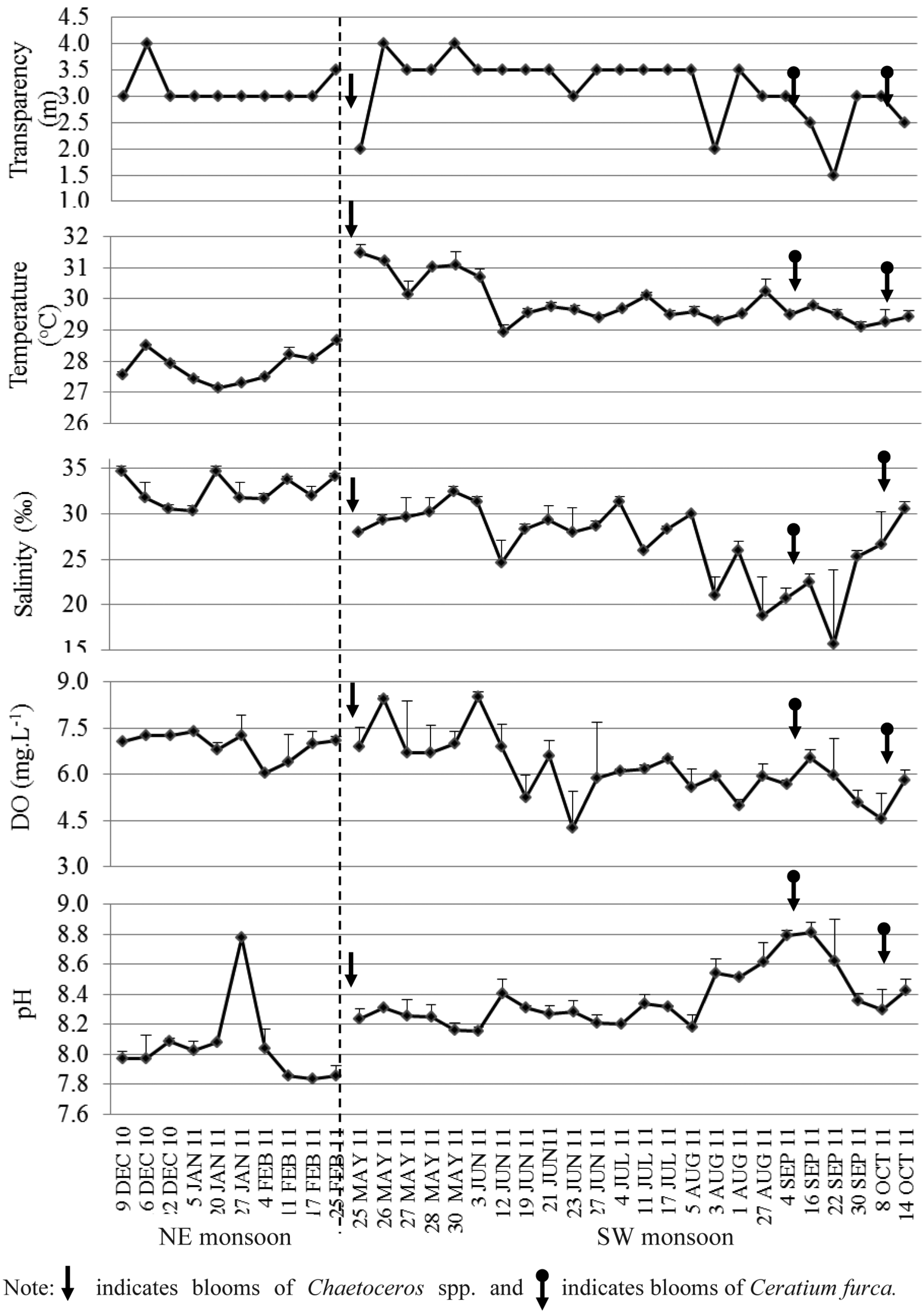
Population dynamic of *Chaetoceros* spp. and *Ceratium furca*

During the NE monsoon, the mean (± SD) cell density of *Chaetoceros* spp. ($0.13 \pm 0.13 \times 10^5$ $cells.L^{-1}$) was the same magnitude (no significant differences, $P > 0.05$) as in the SW monsoon ($0.11 \pm 0.76 \times 10^5$ $cells.L^{-1}$). However, temporal variations in the cell density in the SW monsoon were much larger than in the NE monsoon. A bloom of *Chaetoceros* spp. with *C. curvisetus* as the dominant species ($2.27 \pm 3.11 \times 10^5$ $cells.L^{-1}$; Figure 4a) was recorded on 25 May 2011, in the early SW monsoon (Figure 5). A significant difference ($P < 0.05$) between the densities of *Chaetoceros* spp. on different dates was found during this monsoon.

In contrast, *Ceratium furca* (Figure 4b) showed significant differences ($P < 0.05$) in cell densities between seasons. The density of *C. furca* in the SW monsoon or rainy season was about 1,000 times greater than that in the NE monsoon or dry period with values of $0.66 \pm 1.94 \times 10^5$ $cells.L^{-1}$ and $0.0008 \pm 0.0004 \times 10^5$ $cells.L^{-1}$, respectively. Significant differences ($P < 0.05$) were found within each period and between these two periods. Red tides from this species were observed twice during the late SW monsoon season, on 16 September 2011 ($7.14 \pm 3.27 \times 10^5$ $cells.L^{-1}$) and 14 October 2011 ($3.50 \pm 4.50 \times 10^5$ $cells.L^{-1}$) as shown in Figure 5.

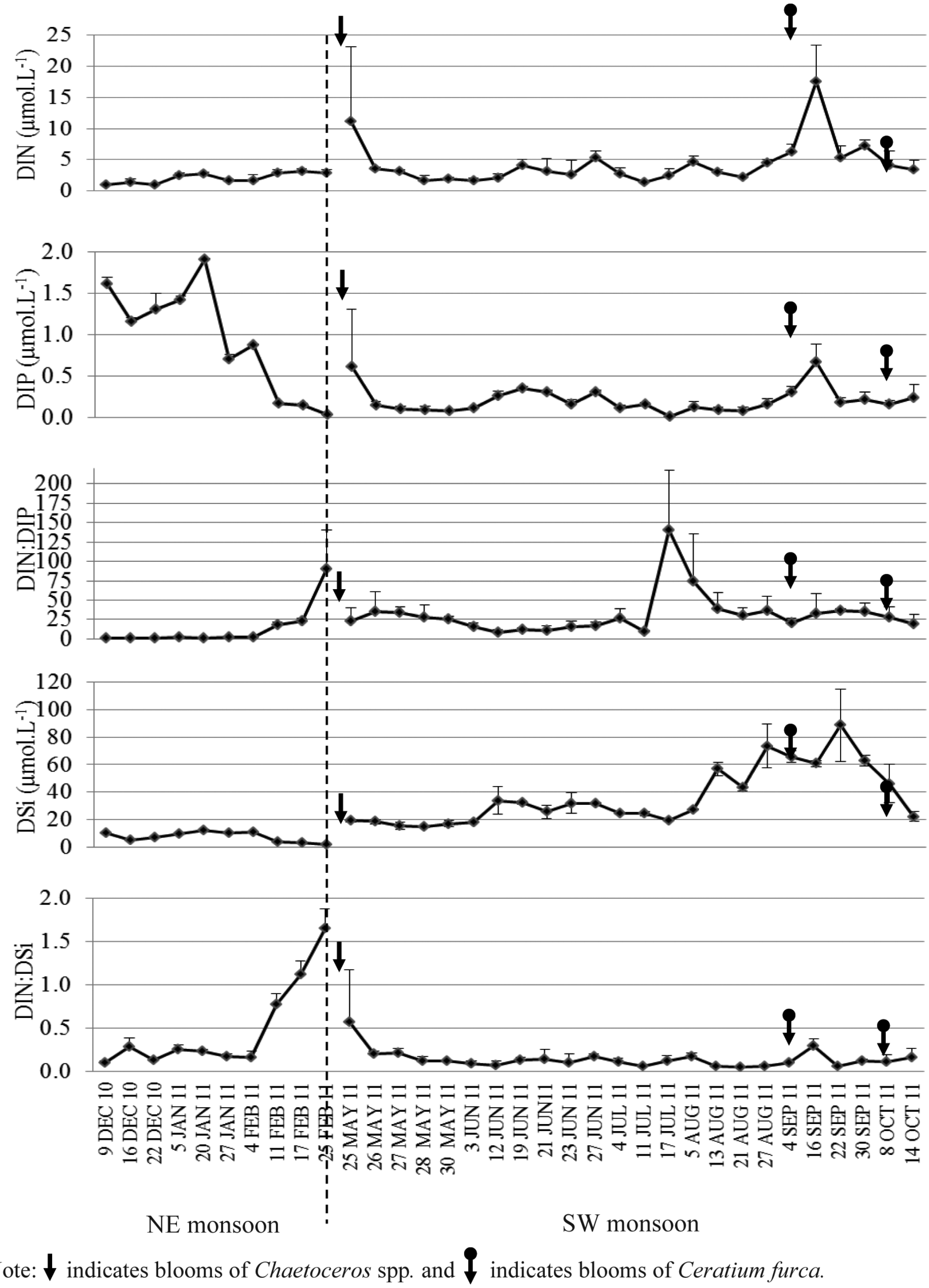
Physico-chemical factors and phytoplankton blooms

The abundance of both *Chaetoceros* spp. and *Ceratium furca* tended to increase with increasing DIN and DIP (Table 2). The moderate relationships between these phytoplanktons with these parameters were highly significant ($P < 0.01$). The appearance of *Chaetoceros* spp. also showed a very strong correlation ($P < 0.01$) with the ratio of DIN:DSi. Furthermore, there was also a



Note: ↓ indicates blooms of *Chaetoceros* spp. and ↧ indicates blooms of *Ceratium furca*.

Figure 2 Physico-chemical parameters from each sampling date in the coastal area of Si Chang Island, the Inner Gulf of Thailand. (DO = Dissolved oxygen, NE = Northeast, SW = Southwest.)



Note: indicates blooms of *Chaetoceros* spp. and indicates blooms of *Ceratium furca.*

Figure 3 Concentrations of inorganic nutrients and molar ratios from each sampling date in the coastal area of Si Chang Island, the Inner Gulf of Thailand. (DIN = Dissolved inorganic nitrogen; DIP = Dissolved inorganic phosphorus; DSi = Dissolved silicate, NE = Northeast, SW = Southwest.)

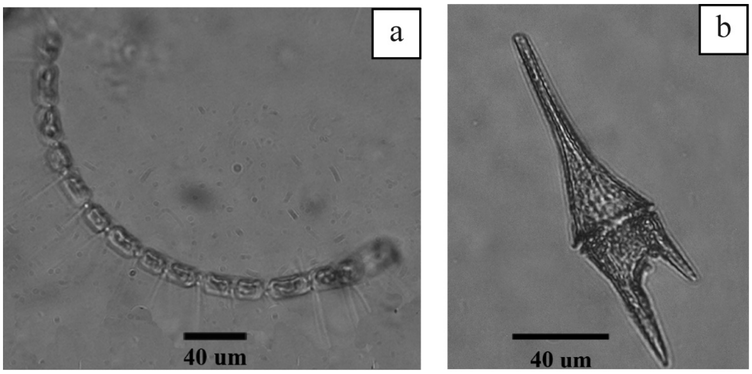
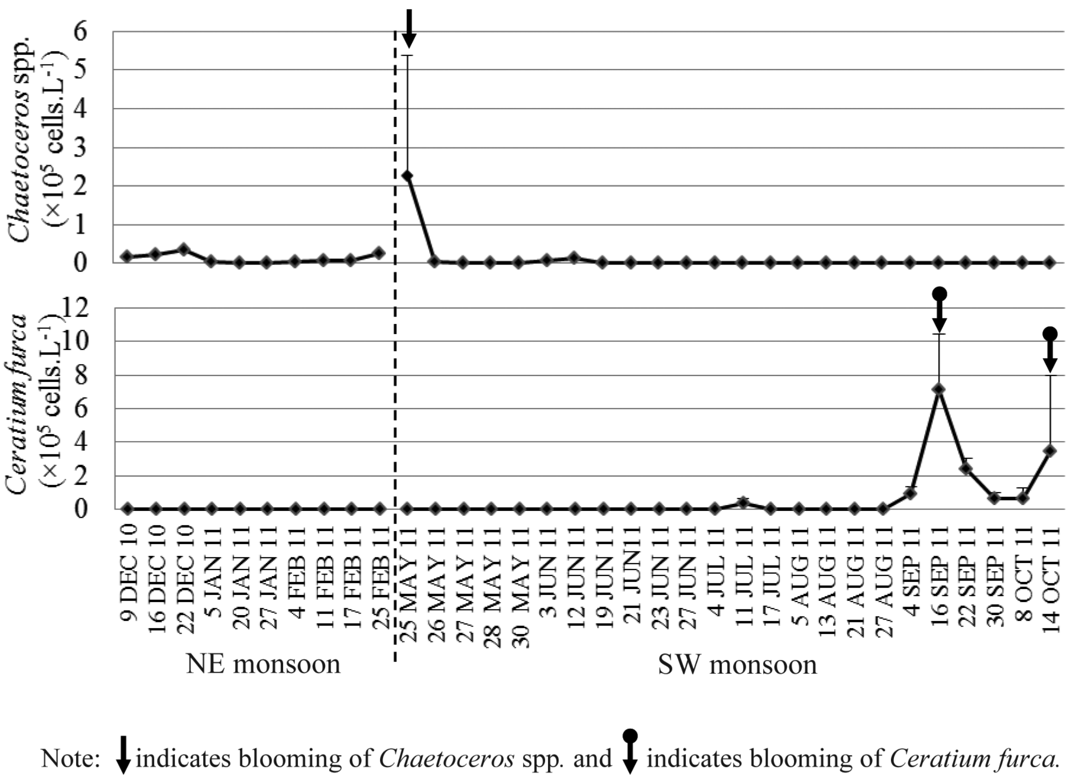


Figure 4 Bloom-forming phytoplankton in the coastal area of Si Chang Island: (a) *Chaetoceros curvisetus;* (b) *Ceratium furca*.



Note: ↓ indicates blooming of *Chaetoceros* spp. and ↓ indicates blooming of *Ceratium furca.*

Figure 5 Variation in cell densities of *Chaetoceros* spp. and *Ceratium furca* in coastal area of Si Chang Island, the Inner Gulf of Thailand. (NE = Northeast, SW = Southwest.)

tendency of increasing abundance of *Chaetoceros* with temperature. On the other hand, the presence of *Ceratium furca*, somehow tended to increase with decreasing transparency and pH (Table 2).

DISCUSSION

Physico-chemical conditions affecting the blooms of *Chaetoceros* spp. and *Ceratium furca*

The temporal variations in the physico-chemical conditions between the two monsoons in 2010 and 2011 were distinguished by high values of seawater temperature, DIN, molar ratio of DIN:DIP and DSi and low salinity during the SW monsoon which indicated the effect of phosphorus as a limiting factor to phytoplankton growth in this study area. These variations were due to the influence of freshwater discharge from the Bang Pakong River to the IGoT as reported by Pianjing (2000), Buranapratheprat *et al.* (2002a, 2002b, 2008), Junchompoo *et al.* (2006), Sriwoon *et al.* (2008), Haii (2012) and Sojisuporn (2012).

Growth of *Chaetoceros* spp. was enhanced under conditions of high temperature (31.50 ± 0.26 °C) during the bloom in comparison to the average temperature of 27.8 ± 0.46 °C during the non-bloom period. This result agreed well with the studies of Sigaud and Aidar (1993) who found that *Chaetoceros* sp. had a maximum growth rate at 31°C. Furthermore, Hemalatha *et al*. (2012) reported that *Chaetoceros simplex* reached its highest cell density at 29 °C.

In the early SW monsoon on 25 May 2011, red tide from *Chaetoceros* spp. occurred together with the high molar ratio of DIN:DSi in seawater which indicated the depleted dissolved silicate during and maybe after the bloom. This caused the high DIN:DSi ratio as seen on 25 May 2011. The density of this diatom also increased simultaneously with the high concentrations of DIN (11.18 ± 11.94 $\mu mol.L^{-1}$) and DIP (0.61 ± 0.70 $\mu mol.L^{-1}$). However, these resulted in a DIN:DIP ratio of 23.50 ± 16.23 which was a little higher than the ratio of N:P≈16:1 reported in Redfield (1934). When the bloom of *Chaetoceros* declined on 26 May 2011, seawater had depleting values of DIN (3.60 ± 0.27 $\mu mol.L^{-1}$), and DIP (0.14 ± 0.05 $\mu mol.L^{-1}$) and resulted in a higher DIN:DIP value (35.00 ± 25.62). This indicated that the rapid growth of *Chaetoceros* spp. in the IGoT was stimulated by both high concentrations of DIN and DIP as reported by Hauss *et al.* (2012) on the enhancement of *Chaetoceros* biomass by increasing the nitrogen supply. The bloom of *Chaetoceros* spp. under a high DIN:DIP ratio was also supported by Huang *et al*. (2012). However, the current results did not show any positive relationship between the density of *Chaetoceros* spp. and DIN:DIP. This may have been due to the actual concentrations of DIN and DIP, since Song (2010) suggested that the proportion of DIN:DIP that could stimulate growth of phytoplankton depended on the concentration of DIN and DIP, with each value not being lower than the optimal concentration limit. Furthermore, the

Table 2 Pearson's correlation matrix between physico-chemical parameters and density of *Chaetoceros* spp. and *Ceratium furca* near the coastal area of Si Chang Island, the Inner Gulf of Thailand during the SW monsoon (May to October 2011).

Species	Trans-parency	Temp	Salinity	DO	pH	DIN	DIP	DIN:DIP	DSi	DIN:DSi
Chaetoceros	-0.13	0.31**	0.04	0.09	-0.10	0.52**	0.69**	-0.03	-0.11	0.84**
C. furca	-0.41**	-0.15	-0.26**	0.01	0.52**	0.40**	0.49**	0.01	0.31**	0.08

DO = Dissolved oxygen; DIN = Dissolved inorganic nitrogen; DIP = Dissolved inorganic phosphorus; DSi = Dissolved silicate
** = Correlation is significant at the 0.01 level (2-tailed); * = Correlation is significant at the 0.05 level (2-tailed).

high DIN:DIP ratios at both times in comparison to the ratio reported in Redfield (1934) may indicate the presence of a phosphorus limitation to phytoplankton growth during this SW monsoon period. Then, the increased concentrations of DIP and DIN stimulated the rapid growth of this diatom as Song (2010) mentioned an increase in the cell density of *Chaetoceros* spp. after an increase in the phosphorous concentration.

The intense bloom of *Ceratium furca* during the late SW monsoon (16 September 2011) occurred under conditions of low salinity (22.50 ± 0.87 %). This result was consistent with Lirdwitayaprasit and Mardnui (2005) who reported that seven red tides incidents involving *C. furca* in the IGoT in 2003–2004 occurred in the SW monsoon from August to October with ranges of salinity between 11.4 and 29.9 parts per trillion (ppt), while Baek *et al.* (2007) found that the rapid growth of *C. fusus* in the coastal area of Sagami Bay, Japan occurred within a salinity range of 24–30 ppt. Fluctuations in the DIN and DIP concentrations were detected during the pre-bloom (4 September 2011), bloom (16 September 2011) and post-bloom (22 September 2011) of *C. furca*. During this period, DIN increased from 6.29 ± 1.23 $\mu mol.L^{-1}$ to 17.59 ± 5.76 $\mu mol.L^{-1}$ and decreased to 5.32 ± 1.92 $\mu mol.L^{-1}$, respectively. The concentrations of DIP, started from 0.31 ± 0.07 $\mu mol.L^{-1}$, increased to 0.67 ± 0.22 $\mu mol.L^{-1}$ and later exhausted to 0.18 ± 0.07 $\mu mol.L^{-1}$. It was clear that the increases in the DIN and DIP concentrations supported the blooming of *C. furca* and the decreases in these nutrients resulted from the uptake of these nutrients by *C. furca* to support its growth. During these different bloom phases, the DIN:DIP ratios changed from 20.79 ± 5.64 to 33.10 ± 25.24 and 36.06 ± 2.82, respectively. This evidence of *C. furca* blooming under high DIN:DIP in this study was also supported by Baek *et al.* (2008) who revealed that *C. furca* in Sagami Bay Japan had rapid growth under a high DIN:DIP ratio > 16.

CONCLUSION

This study indicated that physico-chemical factors, the concentrations and the ratio of inorganic nutrients in particular, clearly affect the presence and the bloom of *Chaetoceros* spp. and *Ceratium furca.* Significant physico-chemical factors that promoted rapid growth of *Chaetoceros* spp. were high values of dissolved silicate (DSi), dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP) and temperature. Blooming of *Ceratium furca* was stimulated by conditions of low salinity, high DIN and DIP. Consequently, these significant differences in the physico-chemical parameters between the NE and the SW monsoons contributed to the differences in cell density and blooms of *Chaetoceros* spp. and *Ceratium furca.*

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