Mar. Res. Indonesia Vol. 39, No. 1, 2014: 39−50

SEASONAL CHANGE IN THE DIEL PATTERN OF THE *PSEUDO-NITZSCHIA* POPULATION IN THE CISADANE RIVER ESTUARY: RESPONSE TO THE CHANGES IN THE WATER'S PHYSICAL-CHEMICAL PARAMETERS

Arief Rachman[*] **and Hikmah Thoha**

Research Center for Oceanography Indonesian Institute of Sciences
Jl. Pasir Putih I, Jakarta Utara 14430, Indonesia
[*]E-mail: red_rackham_dead@yahoo.com

Received: April 2014 Accepted: May 2014

ABSTRACT

Seasonal changes of *Pseudo-nitzschia* diel dynamics in an estuarine ecosystem have been known to be regulated by salinity, water temperature and nutrient dynamics. In this study, we aimed to investigate the effect of these physical-chemical factors on the diel pattern of the *Pseudo-nitzschia* population in the Cisadane River estuary. Plankton samples were collected from a diel station at two different periods, in the dry season (May–June) and the wet season (September). Plankton samplings were done in a 24 hour period with 3 hourly sampling intervals. The result showed a higher abundance of *Pseudo-nitzschia* in the dark-period during the dry season, while during the wet season the abundance was higher in the light-period. Nitrate and phosphate concentrations were suggested as important factors in facilitating *Pseudo-nitzschia* maxima. The low abundance of *Pseudo-nitzschia* in the estuary could be due to the relatively low N:P ratio (<10). The results showed that the Cisadane River estuary was strongly influenced by seawater during the night and by freshwater during day. The GLM analysis suggested that there were changes in the species composition of the *Pseudo-nitzschia* population in the ecosystem. The oligohaline species was found to be abundant in the dry season, the maxima occurred at night, which is probably related to a higher marine influence in the ecosystem. Conversely the polyhaline species was abundant in the wet season, with the maxima occurring during daylight, which might be related to a stronger freshwater influence in the ecosystem.

Keywords: Diel dynamics, *Pseudo-nitzschia*, N:P ratio, estuarine, salinity tolerance.

INTRODUCTION

Pseudo-nitzschia Peragallo is a common and important genus of pennate diatom which is abundant in the estuarine, coastal and oceanic ecosystems in nearly all biogeographic zones, from the tropics to the arctic (Hasle *et al.*, 1996; Hernandez-Becerril, 1998; Casteleyn *et al.*, 2008). *Pseudo-nitzschia* is one of the components of the marine phytoplankton community, and recently attracted many researchers' attention, mainly due to its ability to produce domoic acid, a neurotoxin responsible for amnesic shellfish poisoning (Hernandez-Becerril, 1998; Casteleyn *et al.*, 2008). Both toxic and non-toxic members of *Pseudo-nitzschia* are widespread in the coastal regions with variable salinities, although some species occurred only in low density at low salinity areas of the coastal ecosystem (Thessen *et al.*, 2005). In the past, phytoplankton taxonomists classified *Pseudo-nitzschia* as a member of the genus *Nitzschia Hassall*, but later it was separated into a different genus due to its different morphological and ecological features (Hasle *et al.*, 1996; Hernandez-Becerril, 1998; Skov *et al.*, 1999). In the previous studies in Indonesia, *Pseudo-nitzschia* spp. has commonly been classified as *Nitzschia*, which is a common phytoplankton found in both seasons in Jakarta Bay (Sidabutar, 2010; Thoha, 2010).

DOI : 10.14203/mri.v39i1.83

The Cisadane River estuary is located at the mouth of the Cisadane River that is connected to the Jakarta Bay. As a shallow and macrotidal estuary, this ecosystem shows a highly variable condition due to the strong influence from tides and river flows (Devassy and Goes, 1988; Jouenne *et al.*, 2005; Hadikusumah and Lekalette, 2008). The short-term changes in the parameters of the water in the Cisadane River estuary, especially salinity, turbidity and nutrient concentration, could greatly affect the dynamics of the phytoplankton community (Harding *et al.*, 1981; Devassy and Goes, 1988; Olli, 1998; Brunet and Lizon, 2003; Domingues *et al.*, 2005; McLusky and Eliott, 2006). The periodic variation in freshwater input, tides and wind, is suggested as one of the important factors that regulates the dynamics of organisms in an estuarine ecosystem (De Madariaga *et al.*, 1992; Jouenne *et al.*, 2005). Seasonal changes in rainfall, the volume of surface run off, river input and tidal action were other important factors that might regulate the phytoplankton community in the terms of the species composition and spatial distribution (Harding *et al.*, 1981; Devassy and Goes, 1988).

In this research, we focused on the seasonal changes in diel dynamics of the *Pseudo-nitzschia* population of the Cisadane River estuary to understand the relationship between *Pseudo-nitzschia* spp. diel dynamics and physical-chemical factors affecting the water.

MATERIALS AND METHODS

Study Area

The Cisadane River is one among 13 main rivers that flow into Jakarta Bay (Fig.1). The sampling site was located at 5º29'34.98"S and 106º39'02.77"E. The Cisadane River is known as a major source of nutrients, pollutants and sediments in Jakarta Bay and it is large enough to influence the salinity of the bay (Hadikusumah, 2010).

The estuary was categorized in the hyper-eutrophic class of ecosystems in Jakarta Bay (Sidabutar, 2010). The riparian ecosystem of the Cisadane River has long been heavily stressed by anthropogenic activities and its water quality has severely degraded. The water of the river is turbid, with a pale to dark brown color. Drifting trash is commonly found floating on the surface and sometimes forms a trail of aggregated mass. Decayed organic matter at the bottom of river is often circulated to the surface via bubbles of gases

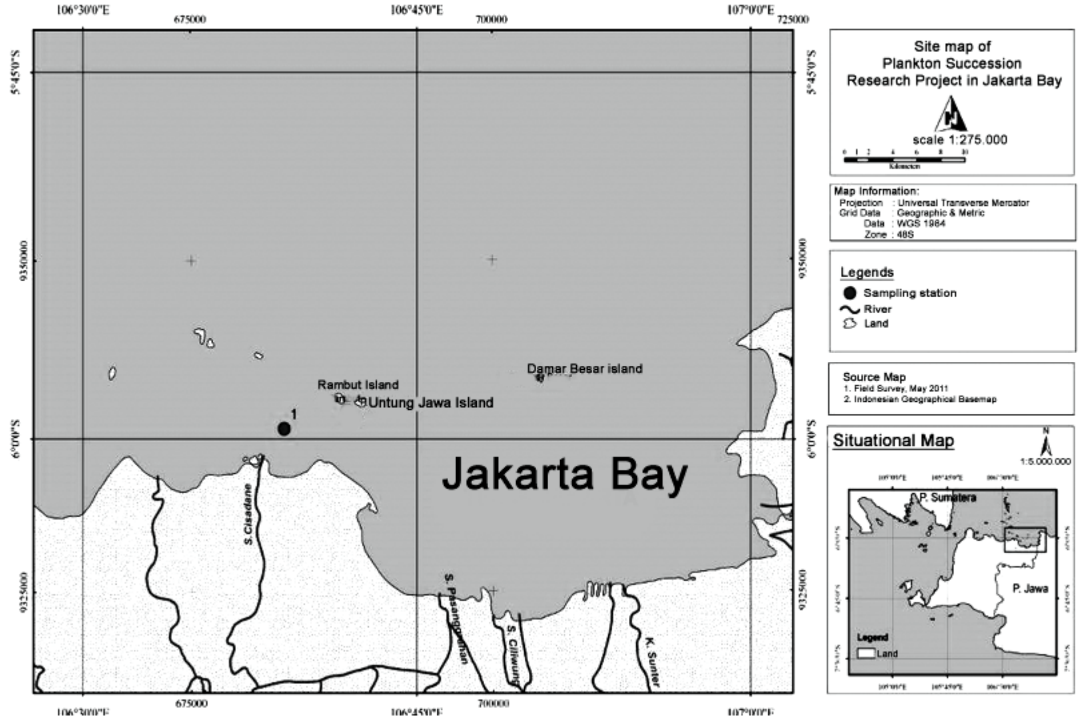

Figure 1. Sampling location of the study.

Table 1. Cell density (cells l^{-1}) of all phytoplankton genera found in the Cisadane River estuarine during the dry season (May–June).

No.	Phytoplankton (cells l^{-1})	Sampling Time in Dry Season (May-June)							
		14:00	17:00	20:00	23:00	02:00	05:00	08:00	11:00
Diatoms									
1	*Amphora*	0	0	0	0	0	0	16	0
2	*Bacteriastrum*	41	2,073	602	915	1,183	0	390	0
3	*Chaetoceros*	2,496	9,919	1,366	6,780	11,073	24,045	10,260	0
4	*Coscinodiscus*	0	0	0	0	0	0	33	0
5	*Ditylum*	0	0	0	0	0	0	0	0
6	*Eucampia*	0	0	0	0	0	0	0	0
7	*Guinardia*	0	0	0	0	37	41	0	65
8	*Hemiaulus*	16	0	0	0	0	0	0	0
9	*Lauderia*	0	163	146	37	0	102	0	341
10	*Navicula*	0	0	0	0	0	0	0	0
11	***Pseudo-nitzschia***	**236**	**1,850**	**732**	**2,646**	**1,476**	**0**	**407**	**4,472**
12	*Odontella*	24	0	0	0	0	0	16	0
13	*Rhizosolenia*	57	163	260	329	0	0	81	49
14	*Skeletonema*	2,325	18,455	3,382	13,451	4,402	18,049	34,390	28,537
15	*Stephanopyxis*	0	0	0	0	0	0	0	0
16	*Streptotheca*	98	0	0	49	0	0	0	0
17	*Surirella*	0	20	0	0	0	0	0	0
18	*Thalassiosira*	390	915	211	146	207	142	537	276
19	*Thalassiothrix*	138	0	0	0	98	102	634	65
Total Diatoms		***5,821***	***33,557***	***6,699***	***24,354***	***18,476***	***42,480***	***46,764***	***33,805***
Dinoflagellates									
20	*Ceratium*	0	0	49	24	24	0	16	0
21	*Dinophysis*	0	0	0	0	0	0	16	0
22	*Gonyaulax*	0	0	0	0	0	20	0	0
23	*Prorocentrum*	0	20	16	0	49	0	16	0
24	*Protoperidinium*	0	0	33	12	0	20	49	16
Total Dinoflagellates		***0***	***20***	***98***	***37***	***73***	***41***	***98***	***16***
Total Phytoplankton		***5,821***	***33,577***	***6,797***	***24,390***	***18,549***	***42,520***	***46,862***	***33,821***

(possibly consisting of sulfuric, ammoniac and carbon dioxide gases).

Plankton sampling

Sampling was carried out on two occasions, first in May–June (early in the dry season) and secondly in September 2011 (early in the wet season). A diel station (mooring station) was established in the bay about 2 km from the mouth of the Cisadane River (Fig. 1). Phytoplankton samplings and water parameter measurements were done in a 24-hour period at 3 hourly intervals.

Phytoplankton samples were collected by vertical hauls at a depth of 10 m at high tide and 5 m at low tide. Samples were taken using a Kitahara plankton net (mesh size 80 μm), because high levels of suspended matter and phytoplankton in the ecosystem easily clog nets with a smaller-sized mesh. The collected samples were placed in 250 ml glass bottles and fixed in 4% formaldehyde (v/v, final concentration). Salinity and temperature were measured during each sampling time with a hand refractometer and mercury thermometer. Water samples were taken at each sampling time with a Nansen bottle at the surface layer (0 m) and at a deeper layer (5 m). The collected samples were placed in 500 ml plastic bottles and used in nutrient analysis. Concentrations of nitrate (NO_3) and phosphate (PO_4) were analyzed with spectrophotometry according to the methods of Strickland and Parsons (1968).

Phytoplankton observation and enumeration

Phytoplankton samples were observed and enumerated at the Plankton and Primary Productivity Laboratory in the Research Center for Oceanography, Jakarta. Phytoplankton enumeration was done by taking a 0.1 ml fraction from the 250 ml sample. Only a small fraction of the volume was necessary because of the extreme density of the phytoplankton cells in the sample. The sample fraction was placed in a Sedgewick Rafter Counting Chamber and observed with a Nikon 104 biological microscope under 200–400× magnification. Identification of phytoplankton taxa was done up to genus level, and grouped into: diatoms, dinoflagellates, and other phytoplankton. Phytoplankton was identified with the help of several references: Shirota (1966), Yamaji (1966), Praseno and Sugestiningsih (2000) and Nontji (2008). In this study, our focus was *Pseudo-nitzschia*, one common phytoplankton genus in Jakarta Bay. The number of *Pseudo-nitzschia* cells counted in the samples was then converted into cells m^{-3} using the equation suggested in Semina (1978) and Arinardi (1997):

$$\mathbf{Vt = R.a.P} \quad \text{........................} \quad (1)$$

Where **Vt** is the volume of filtered water by plankton net (m^3), **R** is the number of flow-meter rotations during the haul, **a** is the area of the plankton-net mouth (m^2), and **P** is the calibration value for the flow-meter used in the net. The density of phytoplankton was then estimated using the following equation:

$$\mathbf{N = (Vs / Vsub) \; x \; (1 / Vt) \; x \; n} \quad \text{..............} \quad (2)$$

Where **N** is the density of phytoplankton for a given volume (m^{-3} or l^{-1}), **n** is the number of phytoplankton cells counted in the sub-sample, **Vsub** is the volume of the sample fraction or volume of sub-sample, **Vs** is the volume of the phytoplankton sample, and **Vt** is the volume of filtered water.

Data analysis

Time series data of *Pseudo-nitzschia* spp. absolute and relative abundance were analyzed to remove noise in the data, and to observe the general trend. The data smoothing was done with Microsoft Excel Data Analysis Add-Ins in 3rd degree (cubic) polynomial smoothing. The data were statistically analyzed by generating correlations (Pearson) between *Pseudo-nitzschia* populations and the measured physical-chemical parameters of the water (Bakus, 2007). A response curve between *Pseudo-nitzschia* population and physical-chemical parameters of the water was constructed by using Generalized Linear Model (GLM) generated with CANOCO for Windows ver. 4.5 (Leps and Smilauer, 2003). The non-linear one-dimensional model was generated in 2nd degree (quadratic) polynomials using a log-link function (Poisson).

RESULTS

Pseudo-nitzschia population in Cisadane River

At least 2 species of *Pseudo-nitzschia* were found in this study (Fig. 2), although identification up to species level was not possible due to the lack of electron microscopy. Based on the light microscopic analysis, all the *Pseudo-nitzschia* species found in the Cisadane River estuary were categorized as being in the delicatissima complex group, with a valve width of 3–4 µm (Hasle *et al.*, 1996; Skov *et al.*, 1999). It was interesting to note that the *Pseudo-nitzschia* cells found were very short (Fig. 2), with a length that varied between 10–20 µm. The reduction of cell length might be related to the late generation from vegetative cell divisions (Cerino *et al.*, 2005) or to some environmental pressure such as salinity stress (Thessen *et al.*, 2005). The number of *Pseudo-nitzschia* cells in the chain was also varied, with the smallest chain consisted of two cells and the longest with more than 10 cells.

The abundance of *Pseudo-nitzschia* in the estuarine ecosystem of the Cisadane River was

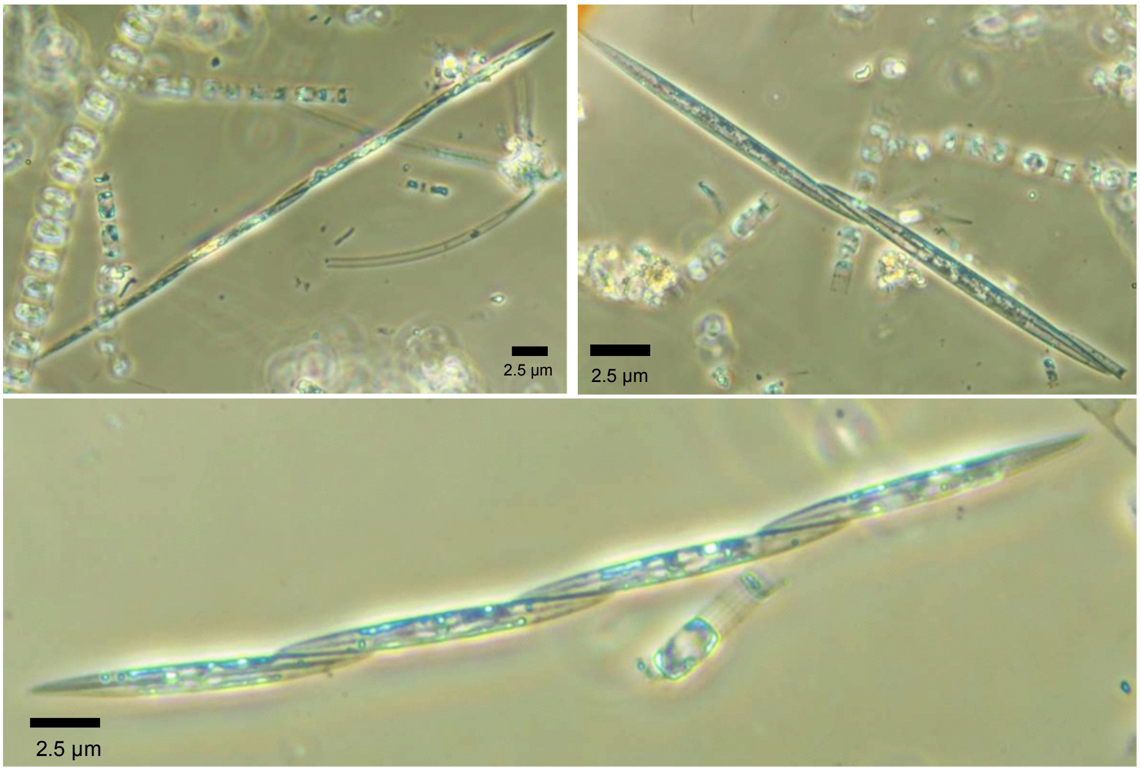

Figure 2. Light micrographs of *Pseudo-nitzschia* cells found in the Cisadane River estuary.

not as high as we assumed. The density of *Pseudo-nitzschia* was very low compared to other common phytoplankton observed, such as *Chaetoceros*, *Skeletonema*, and *Thalassiosira* (Tables 1 and 2). *Skeletonema* was dominant in the Cisadane River ecosystem with a maximum cell density of 80,000 cells l^{-1} during the wet season (Table 2). The range of *Pseudo-nitzschia* abundance in the diel timescale was different in different seasons. In the dry season the abundance of *Pseudo-nitzschia* ranged from 0–5,000 cells l^{-1} (Table 1), while in the wet season the abundance was 40–500 cells l^{-1} (Table 2).

Table 2. Cell density (cell l^{-1}) of all phytoplankton genera found in the Cisadane River estuarine during the wet season (September).

No.	**Phytoplankton (cells l^{-1})**	**Sampling Time in Wet Season (September)**							
		14:00	**17:00**	**20:00**	**23:00**	**02:00**	**05:00**	**08:00**	**11:00**
Diatoms									
1	*Amphora*	0	0	0	20	0	0	0	0
2	*Bacteriastrum*	102	0	0	752	0	81	0	163
3	*Chaetoceros*	8,659	12,012	9,289	6,748	10,996	8,577	6,423	11,890
4	*Coscinodiscus*	0	0	0	0	0	0	0	0
5	*Ditylum*	0	0	0	20	0	0	0	0
6	*Eucampia*	0	0	0	0	20	0	0	0
7	*Guinardia*	0	0	0	0	0	0	61	0
8	*Hemiaulus*	41	61	0	20	41	0	102	0
9	*Lauderia*	81	183	0	0	0	41	0	142
10	*Navicula*	0	0	0	0	0	0	0	0
11	***Pseudo-nitzschia***	**366**	**488**	**122**	**41**	**102**	**447**	**122**	**61**
12	*Odontella*	0	0	0	0	0	0	0	0
13	*Rhizosolenia*	122	20	81	102	203	41	20	0
14	*Skeletonema*	27,093	80,772	79,695	33,923	34,268	29,492	41,423	42,663
15	*Stephanopyxis*	0	0	0	0	0	0	0	0
16	*Streptotheca*	0	0	0	0	0	0	0	0
17	*Surirella*	0	0	0	0	0	0	0	0
18	*Thalassiosira*	183	935	467	366	122	81	0	772
19	*Thalassiothrix*	0	0	0	41	0	0	0	41
Total Diatoms		***36,646***	***94,472***	***89,654***	***42,033***	***45,752***	***38,760***	***48,150***	***55,732***
Dinoflagellates									
20	*Ceratium*	0	81	41	20	20	41	61	0
21	*Dinophysis*	0	41	0	0	0	0	0	0
22	*Gonyaulax*	0	0	0	0	0	0	0	0
23	*Prorocentrum*	0	0	0	0	0	0	0	20
24	*Protoperidinium*	0	61	0	0	0	0	41	20
Total Dinoflagellates		***0***	***183***	***41***	***20***	***20***	***41***	***102***	***41***
Total Phytoplankton		***36,646***	***94,654***	***89,695***	***42,053***	***45,772***	***38,801***	***48,252***	***55,772***

Seasonal difference of diel pattern in *Pseudo-nitzschia* population

The diel dynamics of the *Pseudo-nitzschia* population were different in each season (Fig. 3). In general the abundance of *Pseudo-nitzschia* was higher in the dry season (Fig. 3A) than in the wet season (Fig. 3B). *Pseudo-nitzschia* also contributed more to the total abundance of phytoplankton in the dry season (Fig. 3C) than in the wet season (Fig. 3B). It was noteworthy that *Pseudo-nitzschia* was found to be more abundant at nighttime during the dry season (Fig. 3A), while its abundance was higher in the daytime in the wet season (Fig. 3B).

In the dry season, the maximum cell concentration of *Pseudo-nitzschia* was recorded at night (23:00) and at noon (11:00) (Fig. 3A). During the night, the maximum concentration was at 23:00, cell density of *Pseudo-nitzschia* was 2,646 cells l^{-1} (Table 1), then it declined during the following hours until it disappeared in the morning (05:00) (Fig. 3A). *Pseudo-nitzschia* abundance then gradually increased for the next six hours until it reached a daytime maximum at 11:00, with a density of 4,472 cells l^{-1} (Fig. 3A). On the other hand, peaks of *Pseudo-nitzschia* cell number were observed in the afternoon (17:00) and morning (05:00) in the wet season (Fig. 3B). During the afternoon maximum at 17:00, *Pseudo-nitzschia* abundance was 488 cells l^{-1} (Table 2) and it gradually declined in the next nine hours until it reached the lowest abundance level at 23:00 (Fig. 3B). After that the abundance increased until it reached the morning maximum at 05:00, with a density of 447 cells l^{-1} (Fig. 3B).

Slightly different patterns were observed in the dynamics of the relative abundance of *Pseudo-nitzschia* (Figs 3C and 3D). In the dry season, the maximum value of *Pseudo-nitzschia* relative abundance was first observed at 23:00 and the second at 11:00, with value of 10.85 % and 13.22%, respectively (Fig. 3C). Meanwhile the maxima for relative abundance in the wet season were observed at 14:00 and 05:00 in the wet season, with value of 1% and 1.15% (Fig. 3D).

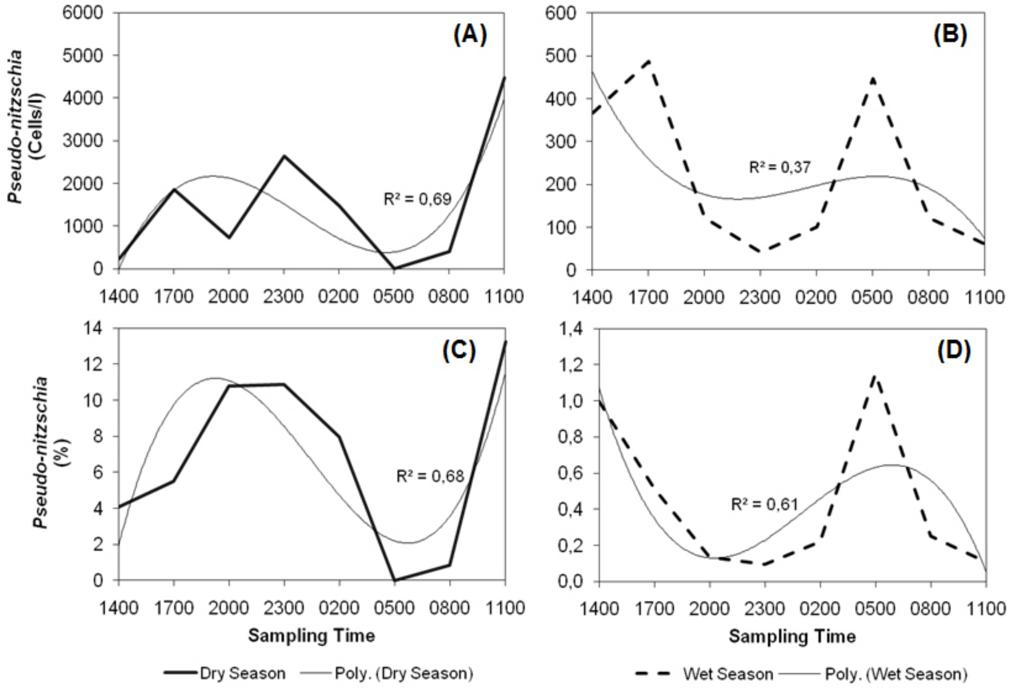

Figure 3. Diel pattern of *Pseudo-nitzschia* population in Cisadane River estuary. Absolute abundance (cells/l) in dry season (May-June) (A) and wet season (September) (B); Relative abundance in total phytoplankton cell numbers (%) in dry season (C) and wet season (D). Thin lines showing the polynomial pattern.

Physical-chemical parameters of Cisadane River estuary

Fluctuation of the water's physical-chemical parameters was observed during the study period, and seasonal variation was also observed for several parameters (Fig. 4). The pH range of the Cisadane River estuary was 6.24–7.70 in the dry season and 5.45–7.71 in the wet season. In this study pH was the only parameter that seems to be stable, with only small fluctuations in the diel cycle (Fig. 4F).

As is characteristic of tropical aquatic ecosystems, there were no extreme temperature changes measured in the water of the Cisadane estuary (Fig. 4D). The temperature range was 28.85–31.45°C in the dry season and 28.25–31.30°C in the wet season. Salinity of the Cisadane River estuary was higher during nighttime (Fig. 4B), and there was no significant difference between the dry and wet seasons. Diel fluctuation in salinity was high, with the highest salinity at 30.50 ppt and the lowest at 8.50 ppt. Low salinity was measured at noon in both seasons (Fig. 4B), which might be related to the tidal dynamics of the Cisadane River estuary. The tidal dynamics of Jakarta Bay probably cause the Cisadane River estuary to receive more influence from the marine system during the night, and more influence from the freshwater system during the day (Fig. 4B). In this study the salinity level in the Cisadane River estuary was similar in both the wet and dry seasons (Fig 4B), thus changes in the volume of rainfall in different seasons did not heavily affect the ecosystem.

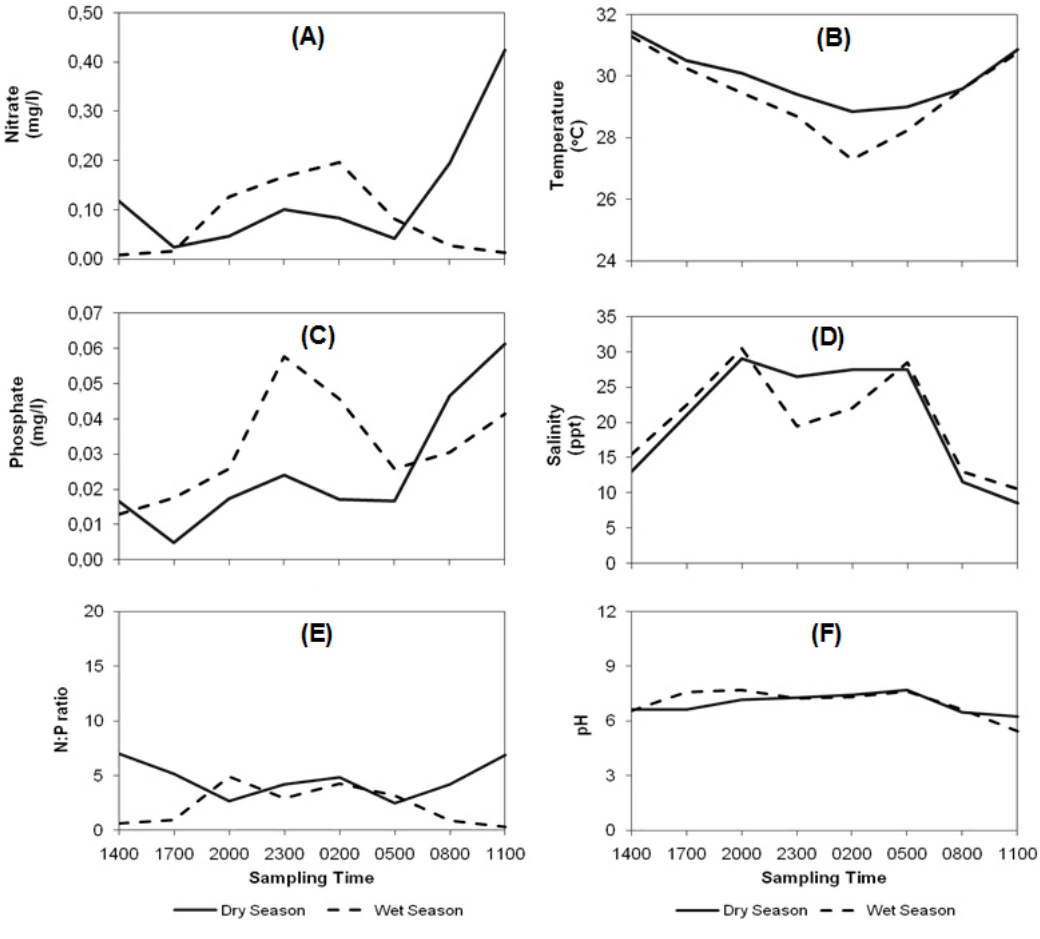

Figure 4. Water physical-chemical parameters of the Cisadane River estuary. (A) Nitrate (NO_3); (B) temperature; (C) phosphate (PO_4); (D) salinity; (E) N:P ratio; (F) pH.

A clear seasonal difference was found in the diel dynamics of nutrients in the Cisadane River estuary (Figs 4A and 4C). Concentration of nitrate was higher during the day in the dry season, but in the wet season it was higher during the night (Fig. 4A). A similar trend was also found in phosphate and N:P ratio dynamics (Figs 4C and 4E). The N:P ratio (mass/mass ratio) in the water of the Cisadane River estuary was higher during the dry season compared to the wet season (Fig 4E), although it was still far from the Redfield Ratio. The N:P ratio in the ecosystem during the dry season was around 2.48–7.02 while in the wet season it was 0.33–4.88.

DISCUSSION

Response of *Pseudo-nitzschia* population dynamics to changes in the physical-chemical parameters of the water

It was interesting to note that maximum population density of *Pseudo-nitzschia* in the dry season occurred during the night. In the wet season, a severe decline in the cell density of *Pseudo-nitzschia* was also observed in the early morning. This pattern was not repeated in the following wet season, when the *Pseudo-nitzschia* maximum density occurred only during the day. Although we lack actual data to support this assumption, we suggested that this seasonally specific pattern might be related to the changes in tidal dynamics in the entire ecosystem of Jakarta Bay. Hadikusumah and Lekalette (2008) suggested that the dominant physical factor in the Cisadane River ecosystem was the tidal dynamics, rather than the inflow or freshwater input by the river itself. During the wet season, the wind-generated currents in Jakarta Bay force the oceanic water closer to the land whereas the wind forces it off the coastline during the dry season.

From the correlation analysis, *Pseudo-nitzschia* has a strong positive correlation with nitrate and N:P ratio ($r_{\text{pseudo-nitrate}}$: 0.591; $r_{\text{pseudo-N:Pratio}}$: 0.528), while a weak positive correlation was found with phosphate ($r_{\text{nitzschia-phospate}}$: 0.19). This relationship suggested that the abundance of *Pseudo-nitzschia* should increase along with the increase of nitrate and N:P ratio. In aquatic ecosystems, the N:P ratio for aquatic plants is generally ranged between 10–45, whereas the generalized Redfield ration is 16. Blooms of phytoplankton occur when the N:P ratio is close to or higher than the Redfield ratio (Cobelo-Garcia *et al.*, 2012). Nitrate (NO_3) is generally the limiting factor in marine ecosystems, while phosphate (PO_4) is the limiting factor in freshwater ecosystems (Domingues *et al.*, 2005). Since the Cisadane River estuary is a combination of marine and freshwater systems, phosphate and nitrate might become very important regulating factors for the growth of the *Pseudo-nitzschia* population. Although low N:P ratio was found in this study, it was also suggested that this system was actually N-limited rather than P-limited (Domingues *et al.*, 2005).

GLM revealed the trends of higher abundance of *Pseudo-nitzschia* when nitrate and phosphate are higher, although the relationship between *Pseudo-nitzschia* and phosphate is almost linear. Higher N:P ratio was also assumed to cause a higher abundance of *Pseudo-nitzschia* in the Cisadane River estuary. Between those parameters, only the N:P ratio was found having significant relationship with *Pseudo-nitzschia* ($p < 0.05$) in the GLM model. Based on this model we hypothesized that when the nitrate concentration is higher than 0.4 mg l^{-1} and the N:P ratio higher than 8 in the environment, the growth of *Pseudo-nitzschia* population will rapidly increase. Although we have to consider the possibility that *Pseudo-nitzschia* might unable to compete with other co-dominant phytoplankton (Tables 1 and 2), a high availability of nutrients in the water is probably still insufficient to support a higher growth rate of *Pseudo-nitzschia* population in the Cisadane River estuary.

In this study, temperature was not regarded as a limiting factor for *Pseudo-nitzschia* growth, but we estimated that the optimum temperature for *Pseudo-nitzschia* was around 30–31°C. Correlation analysis showed that *Pseudo-nitzschia* has a weak positive correlation with temperature ($r_{\text{pseudo-temperature}}$: 0.242). Since the pH range of Cisadane River estuary was normal, pH was not regarded as a limiting factor either, and the optimum pH range for *Pseudo-nitzschia* is estimated around 6–7. Correlation analysis revealed that *Pseudo-nitzschia* prefers a lower pH in the ecosystem, since the relationship was weakly negatively correlated ($r_{\text{pseudo-pH}}$: -0.160).

It was unique that the GLM curve of *Pseudo-nitzschia* and salinity did not show either a normal or logarithmic curve. The peaks in the curve occurred around the extreme zones (left and right), which suggested that *Pseudo-nitzschia* abundance was higher in either low or high salinity waters. This trend suggested that the *Pseudo-nitzschia* population in this research might consist of two species groups with different tolerance ranges for salinity. One group has its optimum range in low salinity (oligohaline), while the other has it in high salinity (polyhaline), if different species or populations indeed exist. Thus, we assumed that salinity was a very important factor that regulates *Pseudo-nitzschia* abundance in the Cisadane River estuary. Salinity stress was reported as an important factor that strongly affects phytoplankton primary production and abundance in estuarine systems (Jouenne *et al.*, 2005; De Madariaga *et al.*, 1992; McLusky and Eliott, 2006). It was suggested that diatoms (*Pseudo-nitzschia*) dominate an estuarine ecosystem as the water became more saline (De Madariaga *et al.*, 1992; Thessen *et al.*, 2005). According to Thessen *et al.* (2005), some species of *Pseudo-nitzschia* could grow at low salinity (<15 ppt). Although the maximum growth rate for most *Pseudo-nitzschia* species was observed at an intermediate salinity level (20–30 ppt), some *Pseudo-nitzschia* species, such as *P. delicatissima*, has a maximum growth rate in very low salinity. On the contrary, other species such as *P. pseudodelicatissima*, have a maximum growth rate at very high salinity (Thessen *et al.*, 2005).

From the results of the *Pseudo-nitzschia* – salinity GLM model (Fig. 5D) combined with *Pseudo-nitzschia* diel dynamics (Fig. 3), we found that there was a change in *Pseudo-nitzschia* species composition along with the changes in the season.

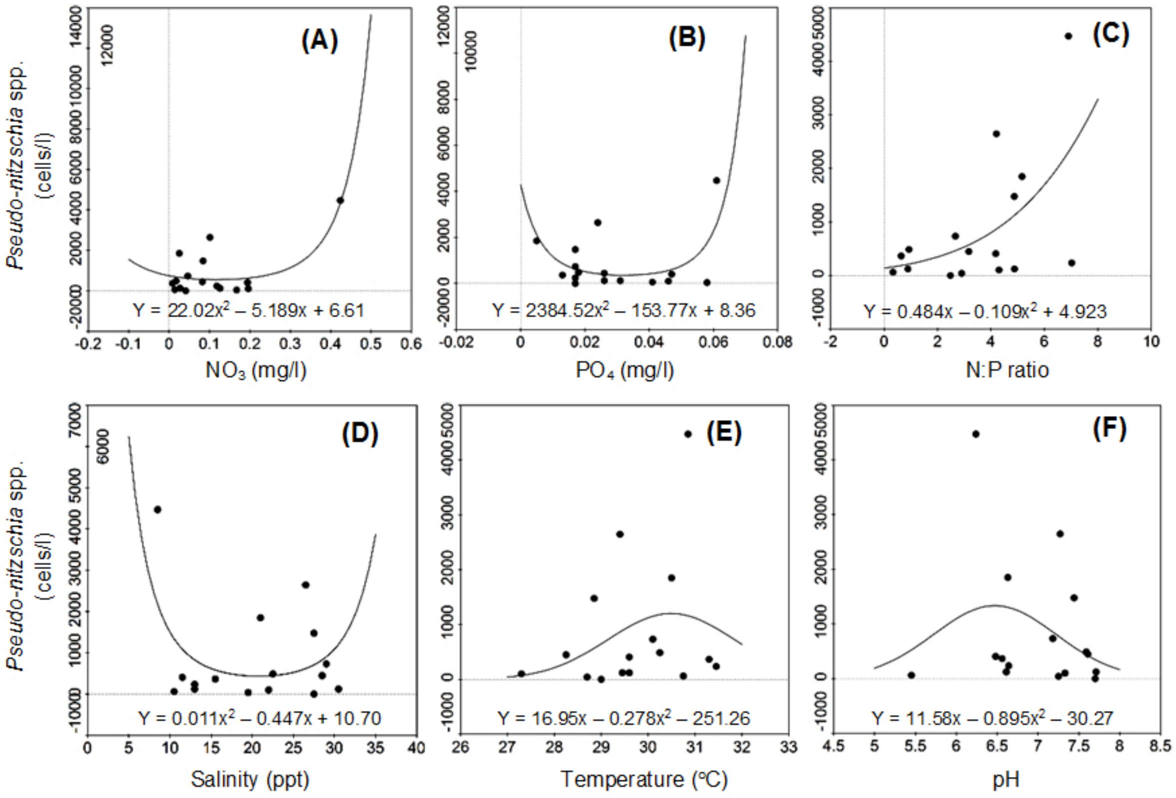

Figure 5. Response of *Pseudo-nitzschia* population to water physical-chemical parameters of Cisadane River estuary. (A) *Pseudo-nitzschia* and nitrate concentration; (B) *Pseudo-nitzschia* and phosphate concentration; (C) *Pseudo-nitzschia* and N:P ratio; (D) *Pseudo-nitzschia* and salinity; (E) *Pseudo-nitzschia* and temperature; and (F) *Pseudo-nitzschia* and pH.

Table 3. Pearson correlation of *Pseudo-nitzschia* and water parameters in the Cisadane River estuary.

	Pseudo-nitzschia	Temp	Salinity	pH	PO4	NO3	N:P ratio
Pseudo-nitzschia	**1.00**						
Temperature	0.242	1.00					
Salinity	-0.160	**-0.534***	1.00				
pH	-0.192	**-0.618***	**0.86****	1.00			
PO4	0.177	-0.259	-0.481	-0.299	1.00		
NO3	**0.591***	-0.103	-0.334	-0.120	**0.749****	1.00	
N:P ratio	**0.528***	0.003	0.002	0.083	0.124	**0.662****	1.00

* **Significant** at 0.05 level (2 tailed)
** **Significant** at 0.01 level (2 tailed)
Bold numbers indicating strong correlation (r > 0.5)

The oligohaline species, or probably freshwater species, might dominate the *Pseudo-nitzschia* population during the wet season. Since the Cisadane River estuary was highly influenced by the freshwater system during light period, oligohaline *Pseudo-nitzschia* species might be abundant during the daytime. Meanwhile the polyhaline species might dominate the *Pseudo-nitzschia* population during the dry season. The Cisadane River estuary was highly influenced by the marine system during darkness, thus the highest abundance of polyhaline *Pseudo-nitzschia* species occurred during the night. The GLM results also suggested that there was no true estuarine *Pseudo-nitzschia* species in the Cisadane River estuary which has a wide range of salinity tolerance (euryhaline).

CONCLUSIONS

We concluded that seasonal change in *Pseudo-nitzschia* diel dynamics in the Cisadane River estuary was most likely related to changes in the salinity and nutrient ratio in the water column. An N:P ratio higher than the Redfield ratio was suggested as the main factor that leads to the high abundance of *Pseudo-nitzschia* in the ecosystem. Meanwhile salinity might limit the abundance of oligohaline or polyhaline *Pseudo-nitzschia* species in the Cisadane River estuary, thus causing the change in its population structure during different seasons.

ACKNOWLEDGEMENTS

This work was conducted under the RISTEK Competitive Grants 2011 and as part of Plankton Succession Research Project. We would like to express our greatest appreciation and dedication for Djoko Hadikunarso, coordinator of this research, who passed away in early 2012. We thank Elly Asnariati, Trimaningsih and Sugestiningsih, our laboratory assistants who helped in many aspects of this research. This study was implemented with partial support from the Asian CORE Program of the Japan Society for the Promotion of Science.

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