BIODIVERSITAS
Volume 17, Number 2, October 2016
Pages: 673-678

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d170241

The abundance of phytoplankton and its relationship to the N/P ratio in Jakarta Bay, Indonesia

TUMPAK SIDABUTAR[1,3,♥], DIETRIECH G. BENGEN[2], SAM WOUTHUYZEN[3], TRI PARTONO[2]
[1]School of Graduates, Institut Pertanian Bogor. IPB Campus at Darmaga, Bogor 16680, West Java, Indonesia
[2]Faculty of Fisheries, Institut Pertanian Bogor. IPB Campus at Darmaga, Bogor 16680, West Java, Indonesia
[3]Research Centre for Oceanography, Indonesian Institute of Sciences. Jl. Pasir Putih I, East Ancol, North Jakarta 14430, Jakarta, Indonesia. Tel.: +62-21-64713850, Fax.: +62-21-64711948, ♥email: tumpaksid@gmail.com

Manuscript received: 4 January 2016. Revision accepted: 22 August 2016.

Abstract. *Sidabutar T, Bengen DG, Wouthuyzen S, Partono T. 2016. The abundance of phytoplankton and its relationship to the N/P ratio in Jakarta Bay, Indonesia. Biodiversitas 17: 673-678.* The occurrence of phytoplankton blooms in Jakarta Bay has increased significantly, and resulted in, the mass mortality of fish and other organisms. Phytoplankton bloom events are indicated by a change in the color of the sea's surface. Generally, phytoplankton growth is influenced by the levels of nutrients in the water, while spatial distribution is influenced by the pattern of the current. In connection with this phenomenon, research was conducted in 2010, 2011 and 2013, to determine the abundance and distribution of phytoplankton and their connection with the N/P ratio. The results showed that the abundance of phytoplankton ranged from $40x10^6$ cells/m^3 up to $1699.1x10^6$ cells/m^3, with the highest recorded data was during the east monsoon in 2010 and the lowest during the first transition period of 2011. The predominant phytoplanktons were frequently diatoms such as *Skeletonema*, *Chaetoceros* and *Thalassiosira*. The distribution of phytoplankton seemingly follows the nutrient concentration ratio where phosphate acted as the limiting factor and nitrogen as the triggering factor. The higher the N/P ratio, the more potentially uncontrolled growth of phytoplankton occurred. When the availability of nutrients increased an increase in total algal biomass occurred, however, the alteration in nutrient composition led to a change in composition of community.

Keywords: Abundance, limiting factor, nutrient ratio, phytoplankton, triggering factor

INTRODUCTION

The abundance of phytoplankton has a close relationship with the availability of nutrients such as nitrates, phosphates and silicates, and these parameters can be used as a benchmark for the productivity of the waters (Weyl 1970; Odum 1971). The continuous discharge of organic material into the waters causes nutrient enrichment, namely eutrophication (Nixon et al. 2008; Nixon 2009). Nutrients such as phosphate, nitrate and silicate play an important role in supporting the life of phytoplankton. However, in excess, they can result in excessive growth of phytoplankton population, or a bloom. The surface water discoloration due to dense phytoplankton cells is known as a red tide or algal bloom, and sometimes, is called an exceptional bloom or noxious bloom and has lately been referred to as a harmful algal bloom (Hallegraeff and Fraga 1998; Reynolds 2006; Heisler et al. 2008).

A phytoplankton bloom may lead to mass mortalities of fish as recently happened in Jakarta Bay. The nature of the relationship between eutrophication and the expansion of algal blooms is still unclear, although in general eutrophication leads to explosions in the phytoplankton population. Increasing phytoplankton blooms coincide with nutrient enrichment, in the South China Sea and Hong Kong (Lam and Ho 1989; Qiu et al. 2010). How eutrophication can stimulate the presence of toxic algal bloom species, is not yet completely understood and is still under debate. Discharge of any organic material to the waters can lead to an overall increase in nutrient availability, and changes in nutrient composition or nutrient ratio. In general, nutrient availability is associated with increased biomass, and can lead to changes in the community. Phytoplankton populations often increase in quantity but decline in quality. The abundance of phytoplankton cells increases significantly, but the number of species does not increase, indicating there is a predominance of certain species.

This observation had been carried out in relation to the increased amount of nutrients, especially nitrate and phosphate, in the waters of Jakarta Bay and is discussed in this paper.

MATERIALS AND METHODS

Study area

This research was conducted in the waters of Jakarta Bay, Indonesia. It is a shallow bay with an average depth of 15 m, its shoreline is about 149 km long and covers an area of approximately 595 km^2. The bay is located in the north of Jakarta, the capital of Indonesia. Thirteen rivers around the bay are known to discharge a large amount of anthropogenic material from land-based sources into it, including industrial effluent, sewage, and agricultural discharges. A map of the bay and sampling stations is shown in Figure 1.

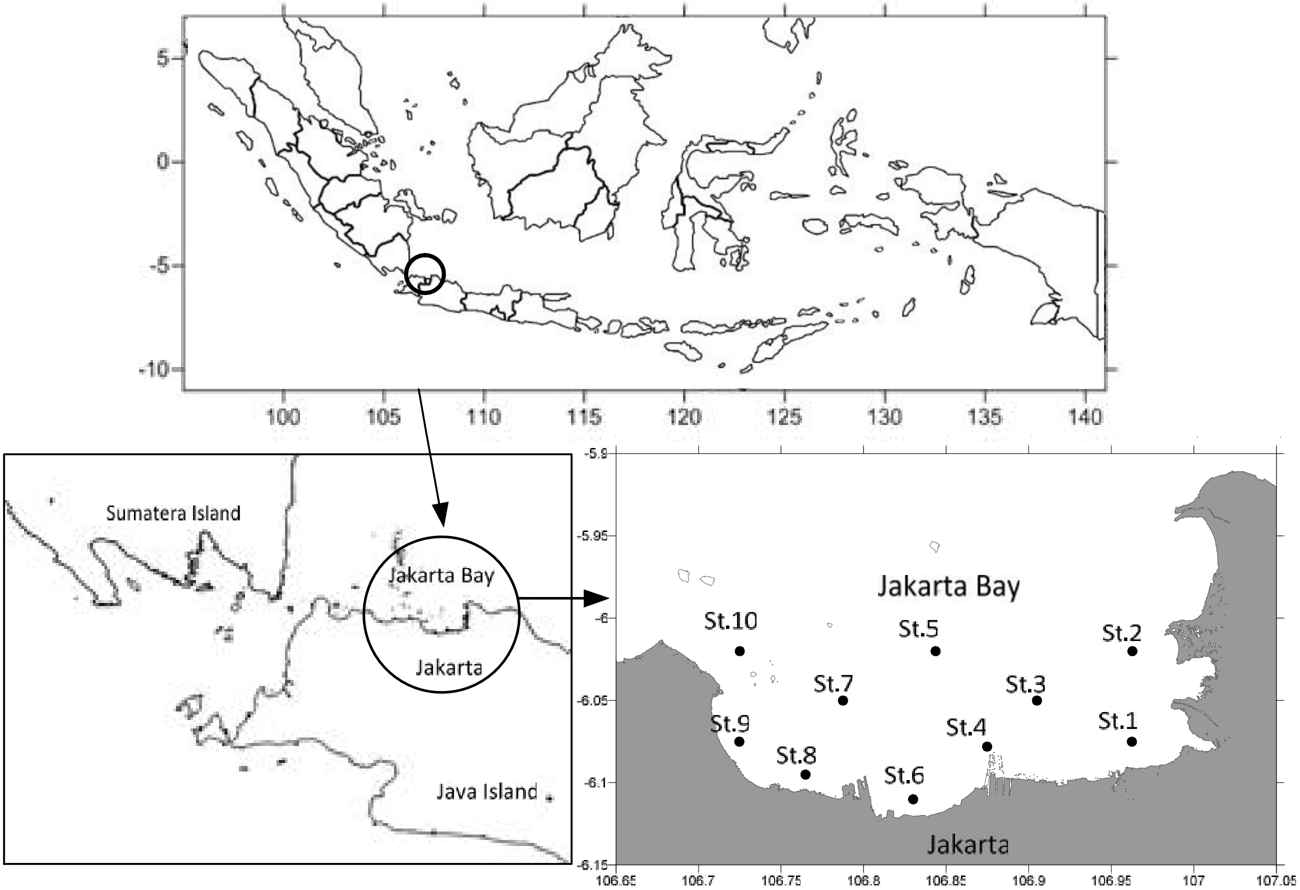

Figure 1. Map of Indonesia and Jakarta Bay with the sampling stations

Sampling period

This study was conducted in 2010, 2011 and 2013 and each period included the first inter-monsoon (MP1) and the east monsoon (MT) when algal blooms mostly appeared. Jakarta Bay is controlled by the annual monsoon pattern where the highest total precipitation falls during the northwest monsoon, and drier conditions occur during the southeast monsoon. The transitional or inter-monsoon generally runs from March to May while the east monsoon is from June to August. Jakarta Bay receives a lot of organic material through the rivers during the rainy seasons and the first inter-monsoon.

Sampling techniques

Sample collection was done using plankton net with mesh size of 20 um, one meter in length and 25 cm diameter of mouth. It was also weighted at the aperture of the net so it could be lowered vertically to a depth of the waters. Samples were then collected in small bottles and put in 2% formaldehide preservative. The volume of filtered water was calculated using formula $v = \pi r^2 \times d$; where v: volume of filtered water; r: radiant of net mouth and d: depth of plankton net lowered into the water. Identification and counting of phytoplankton was performed using Sedgwick-Rafter counting chambers (Sournia 1978) and the results expressed in cells/m^3 (Omori 1991; Michael 1995) through a light microscope at 400x and 1000x magnification. The identification and enumeration of phytoplankton was carried out using references such as Yamaji (1966), Taylor (1976), Newell and Newell (1977), Thomas (1993), Davis (1995), and Hallegraeff (1995). Phytoplankton abundance is calculated by the following formula (Sournia 1978):

$$N = n \times \frac{Vt}{Vs} \times \frac{1}{V}$$

Where,
N : the amount of all phytoplankton
V : volume of filtered water
Vt : the initial sample volume
Vs : sub-sample volume (fraction)
n : number of phytoplankton in sub-sample

Water samples for nutrient analysis were collected from an average depth of 1.0 m using a Kemmerer sampler. Each sample was immediately decanted into an acid-washed bottle and was acidified with 1% v/v HNO3. Nutrients (phosphate, nitrate) were determined based on the colorimetric method (Strickland and Parson 1972; Parson et al. 1984) using a spectrophotometer with wavelengths for phosphate (690 nm) and nitrate (543 nm).

RESULTS AND DISCUSSION

Composition of population

The composition of the phytoplankton populations collected from these waters is presented in Table 1. The amount of phytoplankton recorded was around 35 genera that included approximately 12-25 genera of diatoms and 9-11 genera of dinoflagellates. It seemed that the genera of diatoms and dinoflagellates in these waters were higher in the inter-monsoon (MP1) than in the east monsoon (MT). Generally, the abundance and number of diatom genera in this bay is higher than dinoflagellates (Sidabutar 2006, 2008). During the study, the highest composition of phytoplankton was recorded in the inter-monsoon in the year 2011. About 34 genera were found including 25 diatoms and 9 dinoflagellates. The composition of the species that make up the plankton community can illustrate the diversity of the population or the number of species in a community. This kind of diversity can be increased if the community is more stable or diminish when the environment is unstable or impaired (Krebs 1972; Michael 1995).

Predominant phytoplankton

The predominant phytoplankton in these waters during the study included *Skeletonema*, *Chaetoceros* and *Thalassiosira*. They are almost always found and commonly appear in high numbers in these waters. Therefore, they are classified as a common species. The relative abundance of phytoplankton in these waters during the study is shown in Table 2. The relative abundance of *Skeletonema* ranges between 30-87%. The relative abundance of *Chaetoceros* ranges between 14-57%, being the next most abundant after *Skeletonema*. The *Chaetoceros* was found to be higher in the east monsoon (MT) while *Skeletonema* was found to be higher in inter-monsoon (MP1). The *Skeletonema* predominate in the inter-monsoon (MP1) during high rainfall, while *Chaetoceros* predominate in the east monsoon (MT), when the rainfall is relatively low. However, both of them often found simultaneously as the predominant phytoplankton. It seems that their occurrence is influenced by the season. Therefore those two species seem seasonally dependent, with *Skeletonema* predominant during rainy season and *Chaetoceros* predominant in the dry season.

The predominant phytoplanktons are the members of the population with an abundance of more than 10% and therefore these species are thought to play an important role in the life of the waters. In addition they can usually be used as a biological indicator for the waters (Day et al. 1989). The change in water quality due to climate change and anthropogenic material input into these waters could affect the abundance of planktonic organisms (Hadikusumah 2008)

Other kinds of phytoplankton that sometimes predominant in these waters include *Thalassiosira* with a relative abundance range between 18-26%. The predominance of *Thalassiosira* is seen in the east monsoon (MT) while in the inter-monsoon (MP1) it tends to be lower than 10%. Nevertheless, these three genera of phytoplankton; *Skeletonema*, *Chaetoceros* and *Thalassiosira* play an important role in Jakarta Bay.

Phytoplankton abundance

The results of this study noted that the abundance of phytoplankton in Jakarta Bay during first inter-monsoon (MP1) and east monsoon ranging from 40.90×10^6 up to $1699.10\ \times10^6$ cells/m^3. The highest abundance of phytoplankton was recorded in the east monsoon 2010 (MT_2010) and the lowest was in the first inter-monsoon 2011 (MP1_2011). An abundance graph of the phytoplankton during the study in Jakarta Bay is shown in Figure 2. It appears that the abundance of phytoplankton tends to be higher in the east monsoon compared to the first inter-monsoon. The abundance of phytoplankton in the east monsoon period in 2010 was very high and there was a phenomenon categorized as a bloom that caused greenish-brown discoloration in the surface water. The predominant phytoplanktons during this time were *Skeletonema* (30%), *Chaetoceros* (24%), *Thalassiosira* (18%) and others (28%). The resulting color apparently comes from the combination pigments from these three predominant species.

Generally, algal bloom events in Jakarta Bay appear in the first inter-monsoon (MP1) such as in April and May and also in the second inter-monsoon (MP2) such as in September, October and November (Sediadi 2011), while they rarely occur at other times. According to Wouthuyzen et al. (2007), during the period 2004-2007, there were seven cases of fish mass mortality in Jakarta Bay due to algal bloom events. In 2004, there were two cases (May and December), while in 2005, three cases were recorded (April, June and October) and in 2007, two cases were recorded (April and November). In 2006 and 2008, there were no cases of mass fish mortality although algal blooms occurred.

The relation of N/P ratio to algal bloom.

The most important elements for phytoplankton growth are nitrogen and phosphorus, and, particularly for diatoms, silicate (Egge and Askne 1992). Nutrient enrichment in the waters may cause eutrophication where the nutrient nitrogen serves as the primary trigger for the occurrence of blooming (Anderson et al. 2002). Nitrate concentrations in estuarine or coastal waters are generally higher than the level of phosphate, because a lot of input is from organic material discharged through the rivers and run-off from the mainland. The opposite may occur where phosphate levels are even higher than the levels of nitrates in the waters. The consequences of high nutrient availability will increase the total algal biomass, and the change of nutrient composition or N/P ratio can lead to changes in the community composition (species composition) (Gilbert et al. 2005, 2008). Jakarta Bay is unique as the estuaries of 13 rivers bring fresh water and other input materials there, and also the condition of the bay is much influenced by the open sea. Therefore, it is estimated that the N/P ratio is very volatile and dependent on season, whether it is the rainy or dry season. Comparisons of the nitrate and phosphate concentrations in these waters at the time of the study are presented in Figure 3. In general, the increase of

Table 1. The composition of phytoplankton genera in each sampling period

Genera	Sampling period MP1_ 2010	MT_ 2010	MP1_ 2011	MT_ 2011	MP1_ 2013	MT_ 2013
Diatomae						
Amphora	+	+	+			
Asteromphalus			+			
Asterionella			+	+	+	
Bacteriastrum	+	+	+	+	++	+
Coscinodiscus	+	+	+	+	+	+
Chaetoceros	+	++	++	++	+	++
Climacodium			+			
Dytilum	+		+		+	
Eucampia	+		+	+	+	
Guinardia	+		+	+	+	+
Hemiaulus	+	+	+	+	+	
Lauderia	+	+	+	+	+	+
Leptocylindrus	+	+	+	+	+	+
Nitzschia	+	+	+	+	+	+
Navicula			+	+		+
Odontela	+		+		+	+
Pleurosigma	+		+	+	+	
Rhizosolenia	+	+	+	+	+	+
Surirella			+			
Skeletonema	++	++	++	+	++	++
Streptotheca	+		+	+	+	+
Stephanopyxis			+			
Thalassiosira	+	+	+	++	+	+
Thalassiothrix	+	+	+	+	+	+
Dinoflagellate						
Alexandrium	+	+			+	+
Ceratium	+	+	+	+	+	+
Diplopsalis	+	+			+	
Dinophysis	+	+	+	+	+	+
Dictyocha			+	+		
Gonyaulax	+	+	+	+	+	+
Gymnodinium	+	+	+	+	+	+
Noctiluca	+	+	+	+	+	
Prorocentrum	+	+	+	+	+	+
Protoperidinium	+	+	+	+	+	+
Pyrophacus	+	+		+	+	+
Scriepsiella	+	+	+		+	+
Diatomae	19	12	24	17	18	14
Dinoflagellate	11	11	9	9	11	9
Total genera	30	23	33	26	29	23

Note: ++ predominant; MP: transition period; T: east monsoon; 2010, 2011,2013: years

Table 3. The concentration of phosphate, nitrate and N/P ratio

Period	Ortho fosfat (ugA/L)	Nitrate (ugA/L)	Ratio N/P	Status
MP1_2010	0.056	6.134	110.2	P-limiting
MT_2010	0.056	6.134	110.2	P-limiting
MP1_2011	0.68	0.268	0.4	N-limiting
MT_2011	3.135	5.956	1.9	N-limiting
MP1_2013	0.272	5.034	18.5	P-limiting
MT_2013	1.372	15.893	11.6	P-limiting

Note: MP: inter-monsoon, MT: east monsoon

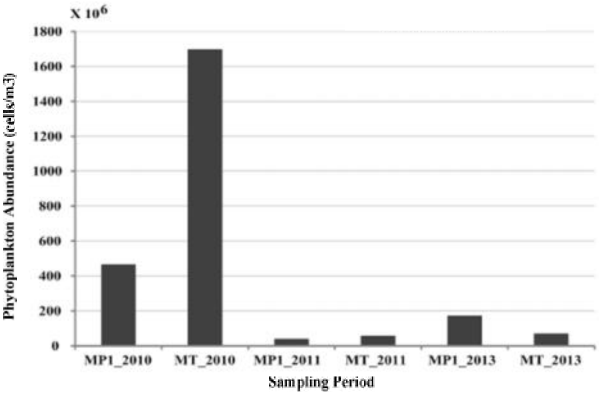

Figure 2. The abundance of phytoplankton in each sampling period

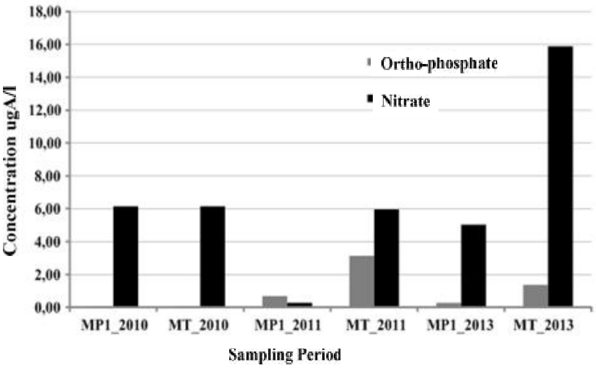

Figure 3. The concentration of nitrate and orthophosphate

Table 2. The relative abundance of predominant genera

Genera	Relative abundance (%)					
	MP1_2010	MT_2010	MP1_2011	MT_2011	MP1_2013	MT_2013
Skeletonema	87	30	43	0	79	67
Chaetoceros	0	24	39	57	14	14
Thalassiosira	0	18	0	26	0	0
Others	13	28	18	17	7	19
Total (10^6 cells/m^3)	465.90	1699.10	40.90	57.90	172.70	70.00

Note: 0: percentage <10 %, MP1_2011: first inter-monsoon 2011, MT_2010: east monsoon 2010.

phytoplankton abundance is in accordance with the increase of nutrient composition or N/P ratio. The ratio of nitrate to phosphate during this study was relatively high only in 2010 and 2013, while in 2011 it was relatively low. When the N/P ratio is relatively high, it indicates that nitrate is as a triggering factor and phosphate is as a limiting factor, but in 2011, while the N/P ratio was relatively low, it indicates that phosphate is as a triggering factor and nitrate is as a limiting factor (Table 3).

There is a link between high levels of nitrate or N/P ratio with the abundance of phytoplankton. An N/P ratio above 10, as seen in MP1-2010, MT-2010, MP1-2013 and MT-2013, indicates phosphate is the limiting factor. Phosphate and nitrate are nutritional components that play important role in supporting the life of aquatic organisms. However, if the amount is excessive, it will deviate from providing the normal benefits as a nutrient and an explosion of phytoplankton species may occur. When the ratio of N/P is greater than 10, this indicates phosphate as a factor limiting phytoplankton growth, which means that the shortage of phosphates in the water prevents the further growth of microalgae. Similarly, a ratio of less than 10 indicates nitrogen is the growth limiter. Ortho-phosphates can be a limiting factor for the process of photosynthesis. According to Qiu et al. (2010) eutrophication is one of the main factors that led to the deterioration of the aquatic environment in the estuary. Therefore, understanding the role of nutrients, especially nitrogen and phosphorus as a limiting factor of phytoplankton, is an important aspect for reducing and regulating eutrophication (Paerl 2009). Sometimes, the atomic ratio of dissolved nutrients in the water is very different from those required for the growth of phytoplankton. In normal sea waters, the N/P ratio is 15: 1. The N/P ratio increase will potentially cause an algal bloom where there is uncontrolled growth of phytoplankton.

The spatial distribution of phytoplankton abundance tends to follow the spatial distribution pattern of nutrient ratios. The pattern of algal bloom distribution can be predicted by the pattern of nutrient distribution in a particular location. Nutrient and phytoplankton distribution is determined by the currents, but the growth of phytoplankton cells is highly dependent on the ratio of N/P and solar energy. The results showed that an N/P ratio more than 10 indicates the availability of nitrate to trigger the growth of phytoplankton, rather than the availability of phosphate as limiting factor. The ratio of N/P in estuaries or coastal waters can indicate the patterns of phytoplankton growth as follows: when the N/P ratio ≤ 5, it means that N is limiting factor while if the N/P ratio is between 5-10, it is referred to as intermediate, and when the N/P ratio ≥ 10, phosphate is the limiting factor for growth of phytoplankton (Duarte 2009). At a time when the N/P ratio is high (more than 10), the abundance of phytoplankton tends to be high, whereas when the ratio of N/P is low (less than 5) the abundance of phytoplankton tends to be low.

Phytoplankton, such as *Skeletonem*a, *Chaetoceros* and *Thalassiosira*, plays an important role in Jakarta Bay. They are not only known as common species but also as seasonally dependent species. The explosion of phytoplankton populations in the Jakarta Bay is closely related to the ratio of nitrogen and phosphate in the waters. A bloom will occur at least when the N/P ratio is at higher than 10, indicating that nitrate is acted as a trigger and phosphate is as a limiting factor. Besides that, current patterns might play a role in the distribution and accumulation of phytoplankton cells that lead to the appearance of discoloration in surface waters. In future, it is interesting to study the potentially harmful species and toxigenic phytoplankton in this bay, especially which was designated as the main cause of blooms.

ACKNOWLEDGEMENTS

This paper is as part of my dissertation collaborating with project program of Research Center for Oceanography of Indonesian Institute of Sciences (LIPI) in 2010, 2011 and 2013. The authors would like to thanks to the Research Centre for Oceanography, Indonesian Institute of Sciences and also researchers and technicians who assisted in the implementation of this research.

REFERENCES

Anderson DM, Glibert PM, Burkholder JM. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25: 704-726.

Egge JK, Aksne DL. 1992. Silicate as regulating nutrient in phytoplankton competition. Mar Ecol Prog Ser 83: 281-289.

Davis CC. 1995. The Marine and Fresh Water Plankton. Michigan State Univ. Press, East Lansing, MI.

Day JWD, Hall CAS, Kemp WM, Arancibia AY. 1989. Estuarine Ecology. John Wiley and Sons, New York.

Duarte CM. 2009. Coastal eutrophication research: a new awareness . Hydrobiologia 629: 263-269.

Krebs CJ.1972. Ecology. The Experimental Analysis of Distribution and Abundance. Harper & Row Publisher, New York.

Gilbert PM, Seitzinger S, Heil CA, Burkholder HM, Parrow MW, Codispoti LA, Kelly V. 2005. The role of eutrophication in the global proliferation of Harmful Algal Blooms. Oceanography 18 (2): 198-209.

Gilbert PM, Burkholder JM, Graneli E, Andersen DM. 2008. Advance and insights in the complex relationships between eutrophication ans HABs: Preface to special issue. Harmful Algae 8: 1-2.

Hadikusumah. 2008. The Changes of water masses in relation to Global Climate Change in Jakarta Bay. In: Aziz A, Ruyitno, Syahailatua A, Muchtar M, Pramudji, Sulistijo, Susana T (eds.). Study of Ecological Change of Jakarta Bay waters. P2O-LIPI, Jakarta.

Heisler J, Glibert P, Burkholder J, Anderson D, Cochlan W, Dennison W, Gobler C, Dortch Q, Heil C, Humphries E, Lewitus A, Magnien R, Marshall H, Sellner K, Stockwell D, Stoecker D, Suddleson M. 2008. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8: 3-13.

Hallegraeff GM. 1995. Harmful algal blooms: A global overiew. In: Halegraeff GM. Anderson DM, Cembella AD (eds.). Manual on Harmful Marine Microalgae. UNESCO, Paris.

Hallegraeff GM, Fraga S. 1998. Bloom dynamics of the toxic dinoflagellate *Gymnodinium catenatum*, with emphasis on Tasmanian and Spanish coastal waters. In Anderson DM, Cembella AD, Hallegraeff GM (eds.). Physiological Ecology of Harmful Algal Blooms. Springer, Berlin.

Lam CWY, Ho KC. 1989. Red tides in Tolo Harbour, Hong Kong. In: Okaichi T, Anderson DM, Nemoto T (eds.). Red Tides: Biology, Environmental Science and Toxicology. Elsevier, Amsterdam.

Michael P. 1995. Ecological Methods for Field and Laboratory Investigations. McGraw-Hill Publishing Co. Ltd., New York.

Odum EP. 1971. Fundamental of Ecology. 3rd ed. W.B. Sounders Company. Philadelphia.

Newell GE, Newell RC. 1977. Marine Plankton. A Practical Guide. Hutchinson, London.

Nixon SW, Buckley B, Granger S, Harris LA, Oczkowski AJ, Fulweiler RW, Cole LW. 2008. Nutrient (N and P) inputs to Narragansett Bay: Past, present, and future. In: Desbonnet A, Costa-Pierce BA (eds.). Ecosystem based Management: A Case Study of Narragansett Bay. Springer Series in Environmental. Springer, New York.

Nixon SW. 2009. Eutrophication and macros scope. Hydrobiologia 629: 5-19.

Omori M. 1991. Methods in Marine Zooplankton Ecology. Krieger Publishing Company, Malabar, FL.

Paerl HW. 2009. Controlling eutrophication along the freshwater-marine continuum: dual nutrient (N and P) reduction are essential. Estuar Coast 32: 593-601.

Parsons TR, Takahashi M, Hargrave B. 1984. Biological Oceanographyc Processes. 3rd ed. Pergamon Press, New York.

Reynolds CS. 2006. The Ecology of Phytoplankton. Cambridge University Press, Cambridge.

Qiu D, Huang L, Zhang J, Lin S. 2010. Phytoplankton dynamics in and near the highly eutrophic Pear River Estary, South China Sea. Continent Shelf Res 30: 177-186.

Sidabutar T. 2006. Red tide phenomenon which can result in losses to the fisheries sector. Proceedings of the annual national seminar II results research of fisheries and marine resources. Department of Fisheries and Marine Resources, Faculty of Agriculture, University of Gajah Mada, Yogyakarta.

Sidabutar T. 2008. Plankton condition of Jakarta Bay: an assessment of ecosystem changes of Jakarta Bay. In: The study of ecological changes of Jakarta Bay. Research Center for Oceanology, Jakarta.

Strickland JDH, Parsons TR. 1972. A Practical Handbook of Sea Water Analysis. Fisheries Research Board of Canada, Ottawa.

Sediadi A. 2011. The study of spatial and temporal quality of the waters of Jakarta Bay. [Ph.D. Dissertation]. Universitas Indonesia, Jakarta.

Sournia A. 1978. Phytoplankton Manual. Monographs on Oceanographic Methodology. UNESCO, Paris.

Taylor FJR. 1976. Bibloitheca Botanica. Dinoflagellate. International Indian Ocean Expedition. Stuttgart.

Thomas CR. 1993. Marine Phytoplankton. Academic Press, San Diego, CA.

Weyl PK. 1970. Oceanography: An Introduction to the Marine Environment. John Wiley and Sons, New York.

Wouthuyzen S, Tarigan S, Sugarin, Suryani R, Raharusun I, Lekalete J. 2007. Early detection of algal bloom events (Harmful Algal Blooms / HAB) in the waters of Jakarta Bay. Competitive project. LIPI, Jakarta.

Yamaji IE. 1966. Illustration of the Marine Plankton of Japan. Houkusho. Osaka, Japan.