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Autoecology of *Ceratium furca* and *Chaetoceros didymus* as potential harmful algal blooms in tourism and aquaculture sites at Teluk Pandan Bay, Lampung, Indonesia

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Abstract. *Hasani Q, Yusup MW, Caesario R, Julian D, Muhtadi A. 2022. Autoecology of* Ceratium furca *and* Chaetoceros didymus *as potential harmful algal blooms in tourism and aquaculture sites at Teluk Pandan Bay, Lampung, Indonesia. Biodiversitas 23: 5670-5680.* Harmful algal blooms (HABs) phenomenon has been observed around tourism and aquaculture sites in Teluk Pandan Waters, Lampung Bay. The dominant potential HABs found in Teluk Pandan waters were *Ceratium furca* and *Chaetoceros didymus*. This study aimed to analyze the relationship between the environmental physicochemical factors and the abundance of *C. furca* and *C. didymus* as potential HABs. Phytoplankton samples were collected from four stations (Hurun Bay, Sidodadi Coastal Waters, Sari Ringgung Beach and Cikunyinyi Bay) during May-August 2022. Physical parameters (temperature, depth, brightness, current velocity and salinity), as well as chemical parameters (dissolved oxygen, pH, phosphate, nitrite, nitrate and ammonia) were all measured in conjunction with phytoplankton sampling. Canonical correlation analysis and multiple regression were used to predict the effect of environmental parameters on phytoplankton density. Blooms of *C. furca* were found with the highest density at 5.417×10^6 cells/L in Cikunyiyi Bay, while *C. didymus* predominated in Hurun Bay (2.890×10^4 cells/L), Sidodadi Coastal Waters (3.923×10^4 cells/L) and Sari Ringgung Beach (3.531×10^4 cells/L). The main factors influencing the increase in *C. furca* population at Cikunyinyi Bay were pH, phosphate and nitrate. The increase in *C. didymus* population at Sidodadi Coastal Waters was affected by phosphate, nitrate and nitrite, while phosphate and nitrate influenced the increase in *C. didymus* population at Sari Ringgung Beach. Potentially HABs density was found to be higher near intensive shrimp farming sites. The use of fertilizer and artificial feed in high quantities is thought to be responsible for increased water eutrophication. Periodic monitoring and consistent determination of environmental carrying capacity for intensive shrimp farming activities are required to control HABs and ensure the sustainability of aquaculture and tourism activities in Teluk Pandan Waters.

Keywords: Fertilizer, nutrient, sustainable aquaculture, sustainable tourism

INTRODUCTION

Phytoplankton are the basis of the aquatic food chain (Zhou et al. 2016; Moruff et al. 2016), and the origin of life for organisms in the oceans (Sunardi et al. 2017; Kostryukova et al. 2018). Phytoplankton contains chlorophyll which is capable of carrying out photosynthesis (Dimowo 2013; Moruff et al. 2016), which produces oxygen and organic compounds such as carbohydrates (Islam et al. 2020) and can be used as an indicator of waters environment quality (Pirzan and Pong-Masak 2008). Phytoplankton are harmless and beneficial to aquatic organisms, but if there is abundant growth in waters, due to high nutrients in water bodies, it can cause Harmful algal blooms (HABs) or red tides (Sidabutar and Srimariana 2020). These HABs have caused occasional massive losses for the aquaculture industry and have chronically affected socioeconomic interests in some countries due to fish and shellfish mortality (Karlson et al., 2021). HABs are often causing fish mortality in the fish farming floating nets in Indonesia (Eong and Sulit 2017; Sidabutar et al. 2021).

Waters of Teluk Pandan District, which are part of Lampung Bay, are a popular tourist destination as well as an important aquaculture activities center in Pesawaran Regency, Lampung Province, Indonesia (Hasani et al. 2012). Various beach attractions around Teluk Pandan District are growing rapidly, so Teluk Pandan District is proposed to become a Tourism Special Economic Zone (KEK) in Lampung Province (Purnomo et al. 2019). Apart from being a popular destination for coastal tourism, Teluk Pandan waters are also an important aquaculture center in Lampung Province (Hasani et al. 2012; Sholihin et al. 2014; Irawan et al. 2015). Aquaculture sites in Teluk Pandan District include Hurun Bay (fish farming with cage culture (KJA) and pearl farming); Sidodadi Coastal waters (intensive vannamei shrimp farming); Sari Ringgung Beach (grouper farming with KJA); and Cikunyinyi Bay (intensive vannamei shrimp farming) (Hasani et al. 2012). In addition, these waters are also close to residential areas. Teluk Pandan Bay is multi-functional, providing numerous livelihood opportunities to most people surrounding the bay (Sidabutar et al. 2021).

Various tourism, household and aquaculture activities in Teluk Pandan waters have the potential to release organic materials into the seas, resulting in enrichment and changes in the trophic status of the waters. The enrichment of organic matter in the water can then rapidly trigger the growth of phytoplankton, resulting in harmful algal blooms (HABs). Indications of the emergence of various phytoplankton causing HABs in Teluk Pandan Bay have been discussed by researchers (Sidabutar and Srimariana 2020; Sidabutar et al. 2021). The are five types of HABs toxins: Amnesic Shelfish Poisoning (ASP), Diarrhetic Shelfish Poisoning (DSP), Neurotoxic Shelfish Poisoning (NSP), Paralytic Shelfish Poisoning (PSP) and Ciguatera Fish Poisoning (CFP) (Kazmi et al. 2022). Phytoplankton that does not emit poisonous substances might nonetheless have a negative impact due to its high density, such as a decrease in dissolved oxygen level caused by the decay process (O_2 depletion/anoxius), gill blockage, etc. (Hasani et al. 2012; Kazmi et al. 2022). A large phytoplankton bloom, even though not toxic, can cause hypoxia (low dissolved oxygen) (Patten et al. 2012).

Phytoplankton that has the potential to cause HABs in Teluk Pandan waters is *Ceratium furca* (Hasani et al. 2012) and *Chaetoceros didymus* (Hasani et al. 2012; Sholihin et al. 2015; Irawan et al. 2015). This study aimed to analyze the relationship between the environmental physicochemical factors and the abundance of phytoplankton *C. furca* and *C. didymus* as potential HABs. Adequate information about the relationship of a species of HABs with environmental and nutrient factors is important for handling and mitigating the impacts (Sidabutar and Srimariana 2020; Chen et al. 2021). Autoecology is a study of ecology to investigate the relationship between certain species of organisms with their habitats and environmental conditions (Master et al. 2020). Autoecological analysis is required to answer various problems caused by a species (Walter and Hengeveld 2014), particularly phytoplankton, that creates HABs in Teluk Pandan waters. The autoecological study will be the basic data for determining the invasion control strategy in a particular location (Sutomo and Fardila 2015; Master et al. 2020). In this case, it is a strategy to control phytoplankton that causes HABs in Teluk Pandan waters.

MATERIALS AND METHODS

Research site

Several points of tourism and aquaculture sites around Teluk Pandan waters, Lampung, Indonesia were selected as research stations for this study. The determination of research stations was based on various types of aquaculture and tourism activities. The study does not include assessment of impacts originating from upland or land-based activities near than study location such as plantations, agriculture and others. Four research stations were specified at each location which are: Hurun Bay, Sidodadi Coastal Waters, Sari Ringgung Beach and Cikunyinyi Bay. Two sample points were established for each station (Figure 1).

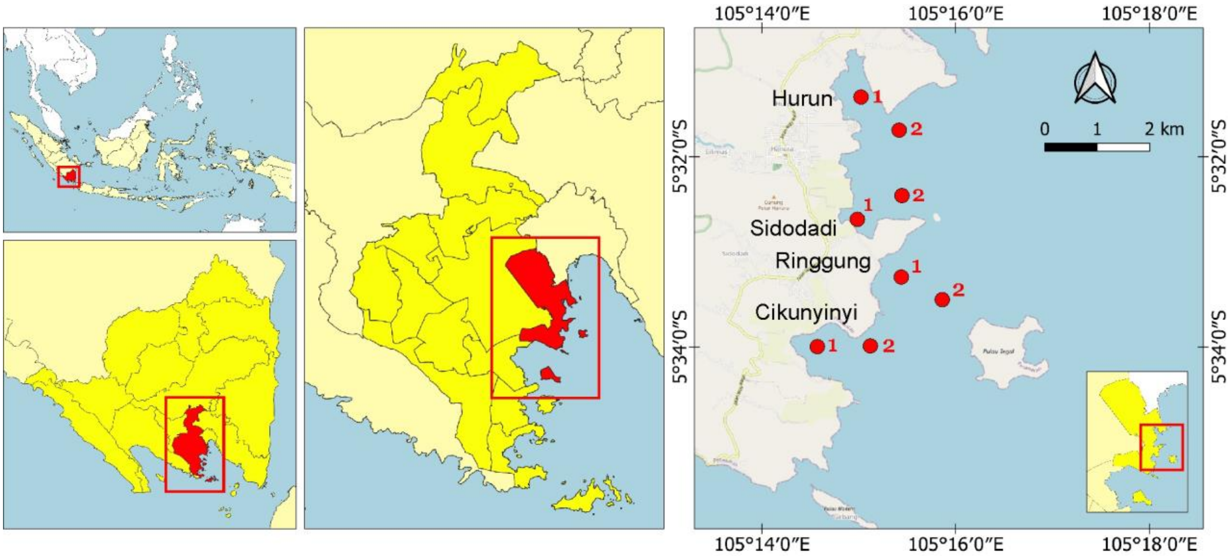

Figure 1. Map of research stations in Teluk Pandan waters, Lampung Province, Indonesia. Note:

1. Hurun Bay, close to settlements, there are KJA activities, private and community-owned intensive shrimp farming and marine tourism at Bensor Resort Beach. Station 1 is located at 105°15'08" E and 05°31'61" S. The coordinate of station 2 is 105°16'36" E and 05°31'76" S.
2. Sidodadi Coastal Waters. constitutes more open waters, there are intensive shrimp farming systems, close to the tourist area of Lahu Island. The coordinate of station 1 is 105°14'95" E and 05°32'26" S while station 2 is 105°15'23" E and 05°32'45" S.
3. Sari Ringgung Beach. It is a tourist location for Sari Ringgung Beach and Tegal Mas Island. There are activities of grouper farming in KJA that are managed by the community. Station 1 at coordinates 105°15'31" E and 05°33'40" S. Station 2 at coordinates 105°14'51" E and 05°33'34" S.
4. Cikunyinyi Bay which has intensive shrimp farming activities and mangrove tourism Cikunyinyi. Coordinate for station 1 is 105°14'71" E and 05°34'02" S while for station 2 is 105°15'06" E dan 05°33'97" S.

Water quality parameter measurement

Environmental parameters (water quality) examined in this study include physical parameters (temperature, depth, brightness, current velocity and salinity) and chemical parameters (dissolved oxygen, pH, phosphate, nitrite, nitrate and ammonia). The temperature was monitored using a thermometer, brightness with a Secchi disk, depth with a Hondex PS-7 portable depth sounder and current velocity with a current meter. Salinity was determined using a hand refractometer; pH is measured with a pH meter; phosphate (PO_4-P), Nitrite (NO_2-N); Nitrate (NO_3-N) and ammonia (NH_3-N) were analyzed in the laboratory according to APHA (2017).

Sampling and identification of phytoplankton

Phytoplankton samples were collected at each location six times every two weeks. Phytoplankton sampling was carried out actively using a modified Kitahara plankton net with a mouth diameter of 0.30 m, a length of 1.0 m and a mesh size of 53 µm. Plankton net withdrawals were taken vertically from the bottom of the sea to the surface to represent varied depths in the plankton sample (Muhtadi et al. 2020; Hasani et al. 2021). Sampling was done at each station in a consistent sequence for each sampling activity, beginning at Station 1 and finishing at Station 4. The samples were preserved in glass bottles with Lugol's solution (APHA 2017; Dissanayake et al. 2021; Hasani et al. 2021). The collected samples were frozen and stored at -20°C for laboratory nutrient analysis (Chen et al. 2021).

Observation and identification of phytoplankton were performed with an Olympus compound microscope model CX23 with an optical infinity system, binocular with observation at 40-400 times magnification. Phytoplankton identification was carried out down to the species level and grouped by genus using the identification books by Al-Kandari et al. (2009); Patten et al. (2012); Al-Yamani and Saburova (2019); Middleton et al. (2021). Monitoring and enumeration of phytoplankton were performed with a Sedgwick-rafter counting chamber of 50 × 20 × 1 mm^3 (APHA 2017; Dissanayake et al. 2021). The calculation of the abundance of phytoplankton (cells/L) was carried out with a strip technique using the formulation APHA (2017); Muhtadi et al. (2020); Hasani et al. (2021) as follows:

$$\frac{\mathrm{No}}{\mathrm{mL}} = \frac{\mathrm{C} \times 1000\ \mathrm{mm}^2}{\mathrm{L} \times \mathrm{D} \times \mathrm{W} \times \mathrm{S}} \quad \text{and} \quad \frac{\mathrm{Sel}}{\mathrm{L}} = \frac{\mathrm{No}}{\mathrm{mL}} \times \frac{\mathrm{Vt}}{\mathrm{Vd}} \times \mathrm{Fp}$$

Where:

C: Number of organisms based on observation; L: strip length (mm); D: strip depth (mm); W: strip width (mm); and S: number of chopped strips. Whereas No/mL: the abundance of phytoplankton in the chamber; Vt: Volume of filtered water or volume of the sample in the container (ml); Vd: Volume of water filtered into the plankton net during sampling (l) and Fp: dilution factor.

Two approaches were applied in quantitative analysis, which were: (i) Multiple regression analysis between environmental parameters and abundance of phytoplankton HABs at the research station. (ii) Canonical correlation analysis (CCA) to describe the relationship of the environmental parameters on the occurrence of HABs. Analyses were performed with Microsoft Excel 2010 and Canoco for Windows 5.12.

RESULTS AND DISCUSSION

Potential of *Chaetoceros didymus* and *Ceratium furca* as HABs

A total of four Ceratium species were found in this study, namely *C. extensum, C. furca, C. tenue* and *C. tripos.* All species were found in low density at all stations. *C. furca* species were also found in low density at all stations, but specifically at Cikunyinyi Station 1, the density increased sharply and reached a peak at 3rd sampling with an abundance of 5.417×10^6 cells/L. Then it decreased at the 4th sampling (1.541×10^6 cells/L) and continued to decrease to only 473 cells/L at the end of observation (6th sampling). The highest density of *C. furca* in this study was 5.417×10^6 cells/L at Cikunyinyi Bay station 1 (Table 1; Figure 2).

Table 1. Range of harmful phytoplankton abundance over the study period

Species	**Phytoplankton density range (cells/L)**							
	Hurun 1	**Hurun 2**	**Sidodadi 1**	**Sidodadi 2**	**Ringgung1**	**Ringgung 2**	**Cikunyinyi 1**	**Cikunyinyi 2**
Ceratiaceae								
Ceratium extensum	0-484	14-106	57-434	55-166	41-227	0-653	26-398	57-203
Ceratium furca	95-986	53-1,142	98-3,726	162-1,489	146-2,570	78-2,951	473-5,417,51	120-19,212
Ceratium tenue	38-379	0-199	36 -199	0-123	0-98	0-118	0-174	0-203
Ceratium tripos	0-242	0-331	142-781	0-47	0-70	12-118	0-227	0-118
Chaetocerotaceae								
Chaetoceros didymus	1,872-28,904	2,090-10,930	1,231-39,325	805-15,245	563-18,463	2,264- 35,316	408-20,593	1572-8,353
Chaetoceros peruvianis	129-1,612	243-2,130	308-3,011	127-2,216	82-2,100	216-2,435	75-2,051	136-337
Chaetoceros pseudocrinitus	142-1,160	53-795	186-2,358	85-2,183	81-497	220-1,405	275-1,420	437-1,555
Chaetoceros vorticella	468-3,503	346-3,202	71-1,250	184-1,184	69-2,946	321-2,112	0-170	265-856

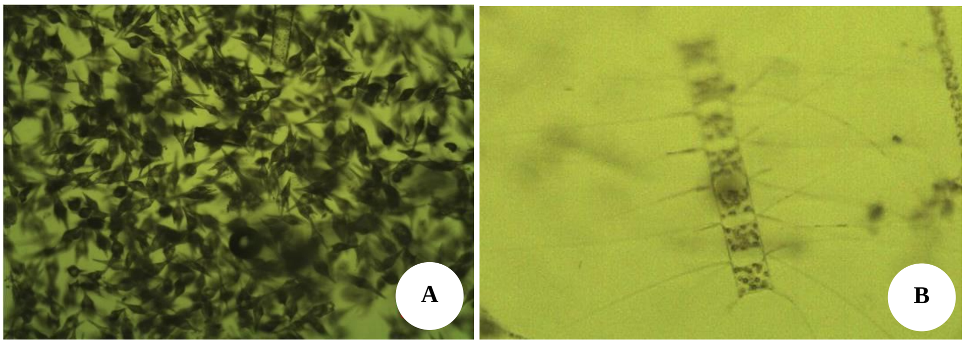

Figure 2. Hight abundance of *Ceratium furca* (A); *Chaetoceros didymus* (B). The potential HABs found at the Teluk Pandan waters, Lampung, Indonesia

The density of *C. didymus* is the highest compared to other species of the Chaetocerotaceae genus, namely: *C. peruvianis*; *C. pseudocrinitus* and *C. vorticella*. Species of *C. didymus* are HABs found abundant at all research sites with the lowest abundance of 408 cells/L at Cikunyinyi Bay Station 1 and the highest with an abundance of 35,316 cells/L at Ringgung Station 2 (Table 1). Several species of phytoplankton were identified in relatively small numbers at several stations, although their populations increased dramatically at particular times, namely *C. vorticela*, *Protoperidinium* sp, *Pyrodinium bahamense*, *Noctiluca scintillans*. Species of *C. furca* and *C. didymus* are non-toxic and harmless to aquatic organisms. However, because of its extremely high density, it can cause negative and destructive impacts for the aquatic organisms (Patten et al. 2012; Dissanayake et al. 2021). The very high density of phytoplankton species can trigger depletion of dissolved oxygen in the waters due to the decomposition process (O_2 depletion), eventually causing the waters to become anoxius (Patten et al. 2012; Kazmi et al. 2022). Phytoplankton density that is too high can also cause clogging of gills by phytoplankton cells in fish and can emit aerosol gases that are lethal to aquatic organisms (Hasani et al. 2012; Kazmi et al. 2022).

The results of this study are in line with previous research regarding HABs in Teluk Pandan waters. Sholihin et al. (2015) found that the highest density of *Cochlodinium* sp, *Trichodesmium erythraeum* and *C. furca* are several HABs that commonly emerge around fish farming sites at Sari Ringgung Beach. *Ceratium* sp. is one of the species of phytoplankton that is a potential cause of HABs in Lampung Bay (Hasani et al. 2012). The occurrence of phytoplankton that causes HABs is influenced by water nutrients (Hasani et al. 2012; Sholihin et al. 2015) and can cause the death of farmed fish (Hasani et al. 2012; Irawan et al. 2015). These types of phytoplankton were also found in this study, although at varying densities and stations. There are nine species bloom makers in Lampung Bay are namely *Pyrodinium* sp., *Noctiluca* sp., *Phaeocystis* sp., *Dinophysis* sp., *Trichodesmium* sp., *Ceratium* sp., *Prorocentrum* sp., *Pseudonitzhia* sp., and *Cochlodinium* sp. (Sidabutar et al. 2021). The most frequent causative species, such as green *Noctiluca* and *Trichodesmium*, co-occurring during blooms and causing fish mortalities in the fish farming floating nets. This study adds that *C. furca* and *C. dydimus* are potential HAbs to be wary of.

Water physicochemical parameters

The physical parameters of the water at the research sites, were relatively uniform. Water depths range from 5.4-23.4 m. The lowest depth is at Sidodadi 1 and the highest is at Ringgung Station 2. Brightness range from 2.5-13.8 m. Brightness at some stations is relatively low, especially at stations that are on the small bay and close to the coast, such as in Hurun 1 (3.0-4.9 m) and Cikunyinyi 1 (2.5-5.7 m). The low rates of brightness at these two stations are due to the input of suspended particles from the mainland through a small river located at both locations. Velocity at each research site is low, ranging from 0.02-0.17 m/s. Cikunyinyi Bay 1 and Ringgung Beach 1 have the lowest current velocity, whereas the highest velocity is at Hurun 1, Sidodadi 1, Sidodadi 2 and Ringgung 2. In general, the current velocity of station 2 at each site is higher than station 1. Meanwhile, the temperature at the research site is relatively high, ranging from 28.4-31.4°C (Table 2).

Table 2. Range of physic-chemical parameters value of water at the research site

Parameters	Station							
	Hurun		Sidodadi		Ringgung		Cikunyinyi	
	I	II	I	II	I	II	I	II
Depth (m)	8.2-10.9	17.1-20.1	5.4-8.2	8.4-10.0	13.9-15.9	21.8-23.4	6.8-8.5	7.5-11.1
Brightness (m)	3.0-4.9	5.1-8.9	4.1-6.4	4.6-7.8	6.8-12.4	7.0-13.8	2.5-5.7	4.5-11.1
Velocity (m/s)	0.05-0.18	0.05-0.14	0.03-0.17	0.03-0.17	0.02-0.16	0.05-0.17	0.02-0.15	0.03-0.13
Temperature (°C)	28.9-31.4	28.8-31.2	28.5-30.6	28.5-30.4	28.4-29.9	28.4-30.0	28.5-29.5	28.4-29.9
Salinity (‰)	32-33	33	33	33	33	33	32-33	33
pH	8.04-8.17	8.03-8.18	7.94-8.17	8.04-8.19	7.99-8.13	8.02-8.19	7.97-8.12	8.00-8.18
DO (mg/L)	5.26-5.75	5.94-6.19	5.35-5.94	5.64-6.01	5.57-6.01	6.00-6.04	5.42-6.03	5.81-6.01

Table 3. Range of nutrient levels of N and P water at the research site

Site	Station							
	Hurun		**Sidodadi**		**Ringgung**		**Cikunyinyi**	
PAR	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
NO_2^-N (mg/L)	0.003-0.035	0.002-0.093	0.002-0.022	0.001-0.012	0.003-0.006	0.001-0.005	0.002-0.072	0.001-0.247
NO_3^-N (mg/L)	0.022-0.234	0.002-0.237	0.023-0.240	0.002-0.257	0.006-0.173	0.035-0.196	0.003-0.074	0.019-0.312
NH_4^-N (mg/L)	0.001-0.871	0.001-0.061	0.001-0.079	0.001-0.225	0.001-0.072	0.001-0.097	0.001-0.053	0.001-0.081
PO_4^-P (mg/L)	0.008-0.045	0.013-0.031	0.005-0.210	0.003-0.056	0.009-0.081	0.001-0.074	0.035-0.146	0.013-0.023

The salinity range at all stations was roughly similar, usually 33 ‰, except for Hurun 1 and Cikunyinyi 1 which had salinity ranges of 32-33‰. The pH range observed during the study indicates that the water was alkaline (7.9-8.18). Such conditions are still favorable for the growth of phytoplankton and marine aquaculture (Zhang et al. 2020). This pH value is also suitable for the growth of marine fish juvenile (Shuangyao et al. 2018). Research by Hansen (2002) shows that the growth rate was highest at pH 7.5 to 8.0 for *Ceratium* sp., *Heterocapsa* sp., and *Prorocentrum* sp. Species of cyanobacteria, diatoms and planktonic green algae preferably near neutral to alkaline pH. The growth was comparatively lower at pH 6.3. The high pH levels adversely affect the growth and significantly reduced at pH 10.6 (Rai and Rajashekhar 2014), while in the laboratory, the optimal pH for phytoplankton growth is between 6.3 and 10 (Takarina et al. 2021).

Nutrients N and P are important and determinant factors for phytoplankton growth (Malerba et al. 2012; Piranti et al. 2021). During the research, observation on nutrient N was represented by nitrite-nitrogen (NO_2^-N), nitrate-nitrogen (NO_3^-N) and ammonium-nitrogen (NH_4^-N). Meanwhile, nutrient P was represented by orthophosphate (PO_4^-P). The level of nitrite-nitrogen in the water ranged from 0.001 mg/L to 0.247 mg/L. The highest concentration was found in Cikunyinyi Bay 2 (Table 3).

The levels of ammonium-nitrogen (NH_4^-N) showed a range of 0.001 mg/L-0.225 mg/L. This value is equivalent to 0.001 mg/L-0.250 mg/L NH_3^-N. Research by Aryawati et al. (2016), showed that the concentration of Ammonium-nitrate 0.05 mg/L could trigger an increase in four potential HABs dinoflagellates (*Ceratium, Dinophysis, Gymnodinium* and *Pyrodinium*) in Banyuasin Coastal Water, South Sumatera, Indonesia.. During the study, the amounts of nitrate-nitrogen (NO_3^-N) in the waters ranged from 0.002 mg/L-0.312 mg/L. Hurun 2 and Sidodadi 2 had the lowest values, whereas Cikunyinyi Bay 2 had the highest range. Nitrate levels at the research sites were often higher than those found in natural waters because generally natural waters only have nitrate-nitrogen levels of 0.1 mg/L, meanwhile, nitrate-nitrogen levels exceeding 0.2 mg/L can result in eutrophication and then trigger rapid growth or blooms of algal (Hasani et al. 2012). The levels of orthophosphate (PO_4^-P) indicated a range between 0.001 mg/L-0.2 mg/L (Table 3). The nitrogen (NO_3) and phosphorus (PO_4) are most influential nutrients on the growth and development of Phytoplankton in estuarine and ocean, including total nitrogen and silicates (Barrera-Alba and Moser 2016; Muhtadi et al. 2020).

Autoecology of *Ceratium furca* and *Chaetoceros didymus*

Studies on autoecology were conducted on numerous species of phytoplankton that were abundant at certain times and stations. At Cikunyinyi Station 1, *C. furca* reached a peak at 3rd sampling with an abundance of 5.417×10^6 cells/L and then went decreased to 1.451×10^6 cells/Liter at 4th sampling. *C. didymus* was also the dominant phytoplankton which reached the highest abundance of 2.831×10^4 cells/L at Hurun 1, 3.392×10^4 cells/L at Sidodadi Station 1 and 3.531×10^4 cells/L at Ringgung Station 2 (Table 1).

The relationship between *C. didymus* and *C. furca* as potential HABs at research sites with nutrients and the tendency to be found at certain stations are illustrated through triplot canoco diagram which displays the interaction between nutrients, HABs and research station. The Triplot Canoco Diagram shows that *C. furca* tends to be at Cikunyinyi 1, while *C. didymus* tends to be at Hurun 1, Sidodadi 1, Ringgung 1, Ringggung 2 and Cikunyinyi 2 (Figure 3). These results are consistent with the fact on the field that blooms of *C. furca* abundance were found at Cikunyinyi 1, while *C. didymus* has the highest abundance at Hurun 1, Sidodadi 1 and Ringgung 2. The analysis results showed that the cumulative value of variance percentage in the correlation between environmental variables and phytoplankton is 100.0 on the axis.

In addition to canoco triplot diagrams, the autoecological study in this research also used multiple regression analysis to examine the effect of environmental variables on the abundance of *C. furca* and *C. didymus* at different stations. The result showed that blooms of *Ceratium furca* population at Cikunyinyi 1 were mostly affected by pH, PO_4^-P and NO_2^-N, each of which showed $p<0.1$, with a correlation coefficient of 0.869 and showed a significant F value. The abundance of *C. didymus* at Hurun 1 was affected by PO_4^-P, NH_4^-N and NO_3^-N, by PO_4^-P, NO_2^-N and NO_3^-N at Sidodadi 1 (NO_2^-N and NO_3-N are significant at $p<0.05$), and by NH_4-N, NO_2-N and NO_3-N at Ringgung 2 (Table 4).

Single regression analysis was done to determine the effect of each nutrient form on the abundance of dominant HABs. A study of single regression is carried out by considering various forms of regression (linear, logarithmic, polynomial, or exponential) and then selecting the regression form that gives the best coefficient of determination.

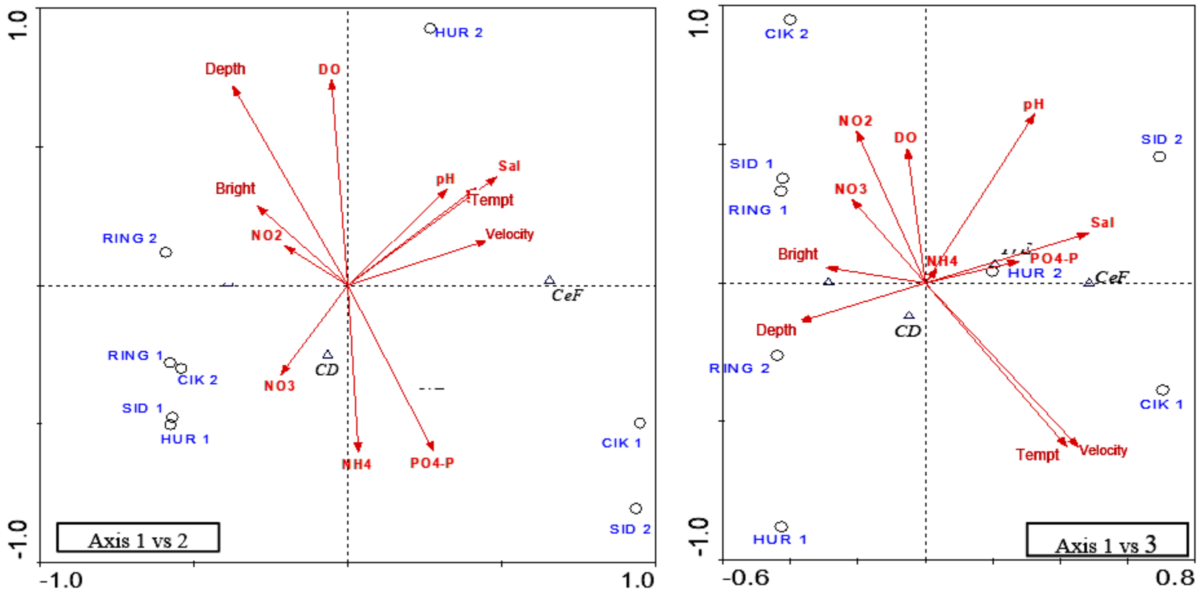

Figure 3. Triplot canoco diagram illustrating the relationship between *Ceratium furca* and *Chaetoceros didymus*, nutrients and research station. Note: CD: *Chaetoceros didymus*, CeF: *Ceratium furca*

Table 4. Relationship pattern of *Ceratium furca* and *Chaetoceros didymus* with nutrients at the research station

Station	Intercept (βo)	Coefficient (β)						R^2	F	F tab
		Current	pH	$PO_4^{-}P$	$NH_4^{-}N$	$NO_2^{-}N$	$NO_3^{-}N$			
Ceratium furca										
Cik 1	487,399,531**		-60,271,547**	27,800,319		-131,508,446**		0.869	4.422	0.190
Chaetoceros didymus										
Hur 1	-20,299			604,833**	261,415		109,137	0.868	4.390	0.191
Sid 1	-11,348*			82,758**		1,317,391*	115,021*	0.996	152.269	0.007
Ringg 2	-13,976	109,998			-27,933	1,557,173	146,329	0.975	9.770	0.235

Note: *) significant at p<0,05; **) significant at p<0,1

The relationship of nutrients to the abundance of *Ceratium furca*

Ceratium furca is phytoplankton found to have the highest abundance in this research, reaching 5.417×10^6 cells/L in the third sampling and 1.451×10^6 cells/L in the fourth sampling before declining to 473 cells/L at the end of the study. Blooms of *C. furca* phenomenon was only found at Cikunyinyi 1, whereas at the other stations, *C. furca* abundance was relatively low, namely in the range of 95-986 cells/L (Hurun 1), 53-1.142 cells/L (Hurun 2), 18-3.726 cells/L (Sidodadi 1), 162-1.489 cells/L (Sidodadi 2), 146-2.570 cells/L (Ringgung 1), 78-2.951 cells/L (Ringgung 2) and 120-19.212 cells/L (Cikunyinyi 2).

Based on water nutrient level data, the increase in *C. furca* population at Cikunyinyi 1 is thought to be triggered by an increase in orthophosphate and nitrite levels in Cikunyinyi Bay on 3th sampling. The density of *C. furca* at Sidodadi 1 showed a correlation between nitrate and the abundance of *C. furca*. This is demonstrated by the coefficient of determination (R^2), which has a value of 0.348 at level α 0.05 and takes the form of a polynomial equation, $y = -2E+09x^2 + 2E+08x-221691$. Meanwhile, the relationship between nitrite and the abundance of *C. furca* shows a coefficient of determination, $R^2 = 0.329$, with a regressional relationship in the form of the equation as $y = 9E+08x^2-1E+08x + 3E+06$. Orthophosphate and ammonium showed a low relationship with the abundance of *C. furca*, with R^2 values of 0.259 and 0.0167 respectively (Figure 4). The result of this simple linear analysis proved that the increased phosphate at Cikunyinyi 1 has triggered an increased abundance of *C. furca*. Ceratium species did not correlate significantly with such environmental factors as irradiation, salinity and N-nutrients. In contrast, they were significantly correlated with phosphate levels (Baek et al. 2011).

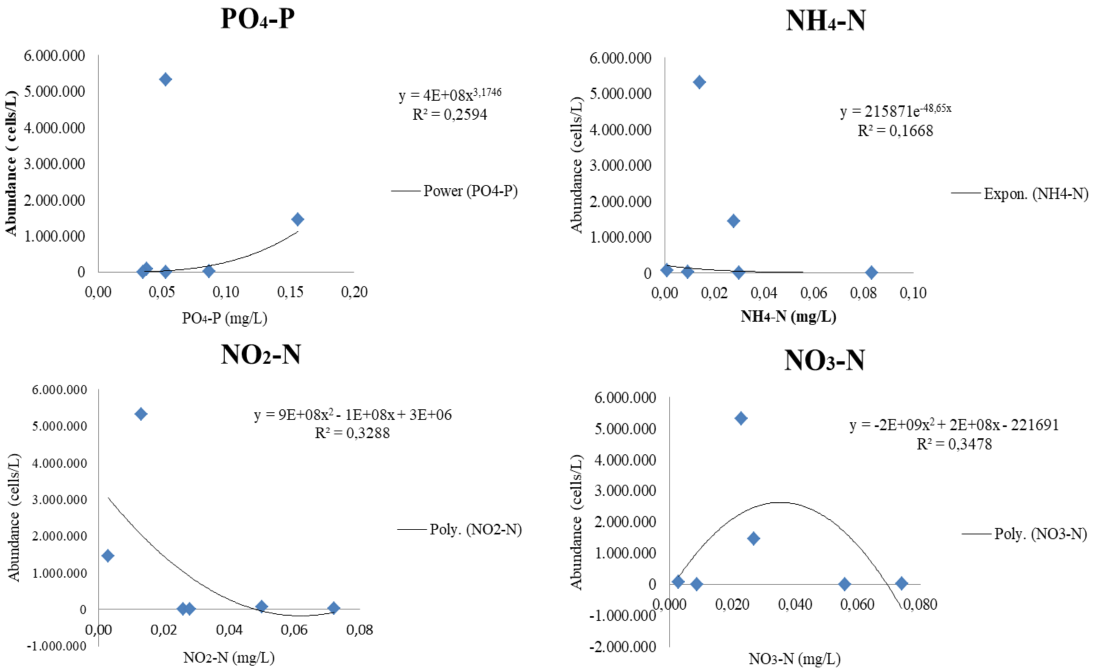

Figure 4. Relationship pattern of *Ceratium furca* abundance with each nutrient at Cikunyinyi 1

The increased abundance of *C. furca* at Cikunyinyi 1 was also shown through multiple regression analysis. The result of multiple regression analysis with the best coefficient of determination was shown by the environmental variables pH, orthophosphate and nitrite, with the equation $Y = -87{,}399{,}531 - 60{,}271{,}547\text{pH} + 27{,}800{,}319\text{PO}_4\text{-P} - 131{,}508{,}446\ \text{NO}_2\text{-N}$, which showed R^2 value of 0,869 at value $p<0{,}1$ for pH dan nitrite (Table 4). Thus it can be concluded that the increase in *C. furca* abundance at Cikunyinyi 1 is influenced by pH, orthophosphate and nitrite. Casuistically (in Sagami Bay) an increase in *C. furca* density is highly connected with phosphate, Chlorophyll-a levels and salinity (Baek et al. 2008). In different places and conditions (Jinhae Bay), it can also be affected by nitrate and nitrite nutrients (Baek et al. 2011). Ceratium furca has a competitive advantage by adapting to low nutrient conditions through the physiological characteristics of nutrient uptake (Baek et al. 2008).

The relationship of nutrients to the abundance of *Chaetoceros didymus*

Chaetoceros didymus is a phytoplankton found in high abundance at all research stations. In this study, *C. didymus* reached the highest abundance of 2.890×10^4 cells/L at Hurun 1, then 3.932×10^4 cells/L at Sidodadi 1 and 3.531×10^4 cells/L at Ringgung 2. A single regression study of various forms of N and P nutrients on the abundance of *C. didymus* at Hurun 1, Sidodadi 1 and Ringgung 2 gave the best results as follows Figure 5, Figure 6 and Figure 7.

The abundance of *C. didymus* at Hurun 1 was mainly influenced by PO_4^-P with a polynomial relationship pattern. Relationship pattern of PO_4^-P with *C. didymus* at Hurun 1 generates the equation $y = 6E{+}07x^2 - 3E{+}06x + 41{,}035$ ($R^2 = 0.766$). Ammonium and Nitrate also show a fairly good effect with a coefficient of determination of 0.308 for Ammonium and 0.317 for nitrate (Figure 5). The result of this regression is similar to the result of a multiple regression between environmental variables and *C. didymus* abundance at Hurun 1 (Table 4), which forms the equation $Y = -20{,}299 + 604{,}833\ \text{PO}_4^-\text{P} + 261{,}415\ \text{NH}_4^-\text{N} + 109{,}137\ \text{NO}_3^-\text{N}$ ($R^2 = 0.868$). In this equation, PO_4^-P shows a significant value ($p<0.1$).

The relationship pattern of nutrients to the abundance of *C. didymus* at Sidodadi 1 shows that NO_3^-N is the nutrient that has a strong relationship to the abundance of *C. didymus* with the equation $Y = 1E{+}06x^2 + 32582x + 4595.9$ (polynomial) with a value of R^2 is 0.714. Phospate and nitrite also shows good coefficient of determination respectively 0.594 and 0.423 with the equation $Y = 3E{+}06x^2 - 65{,}8305x + 35{,}275$ for PO_4^-P and $Y = -2E{+}08x^2 + 6E{+}06x - 9{,}774$ for $\text{NO}_2\text{-N}$ (Figure 6). As shown in Table 4, multiple regression analysis shows that the abundance of *C. didymus* was influenced by PO_4^-P, NO_2^-N and NO_3^-N with the equation $Y = -11.348 + 82.758\text{PO}_4^-\text{P} + 1.317.391\ \text{NO}_2^-\text{N} + 115.021\ \text{NO}_3^-\text{N}$ ($R^2=0.996$). In this equation, NO_2^-N and NO_3^-N show a significant value at $p<0.05$, while $\text{PO4}^-\text{P}$ showed a value ($p<0.1$).

Based on single regression analysis, the abundance of *C. didymus* at Ringgung 2 resulted in a regression pattern similar to the conditions at Ringgung 1, that the abundance of *C. didymus* was mainly influenced by nitrate, phospate and ammonium which formed a polynomial relationship with equations as $Y = 4E{+}06x2 - 50{,}0281x + 18{,}286$ for nitrate; $Y=1E{+}07x^2 - 1E{+}06x + 25{,}955$ for phosphate; and $Y = -7E{+}06x^2 + 725906x + 2313{,}3$ for ammonium, with a coefficient of determination (R^2) of 0.720 ($\text{NO}_3\text{-N}$), 0.433 ($\text{PO}_4\text{-P}$) and 0.311 for $\text{NH}_4\text{-N}$ respectively. While nitrite shows a weak effect (Figure 7).

The multiple regression analysis between the environmental variables and the abundance of *C. furca* at Ringgung 2 can be presented through the equation as Y=-13,976-27,933 NH_4^-N + 1,557,173 NO_2^-N + 146,329 NO_3-N, ($R^2 = 0.975$) (Table 4). It also shows that the abundance of *C. furca* at Ringgung 2 is affected by the current, NH_4^-N, NO_2^-N, and NO_3^-N. There is no environmental factor that has a significant effect on this equation ($p<0.05$). This result confirms the previous study that the increase in *C. furca* and *C. didymus* at the aquaculture site in Hurun Bay and Cikunyinyi Bay was affected by phosphate, nitrite, and nitrate (Hasani et al. 2012; Sholihin 2015).

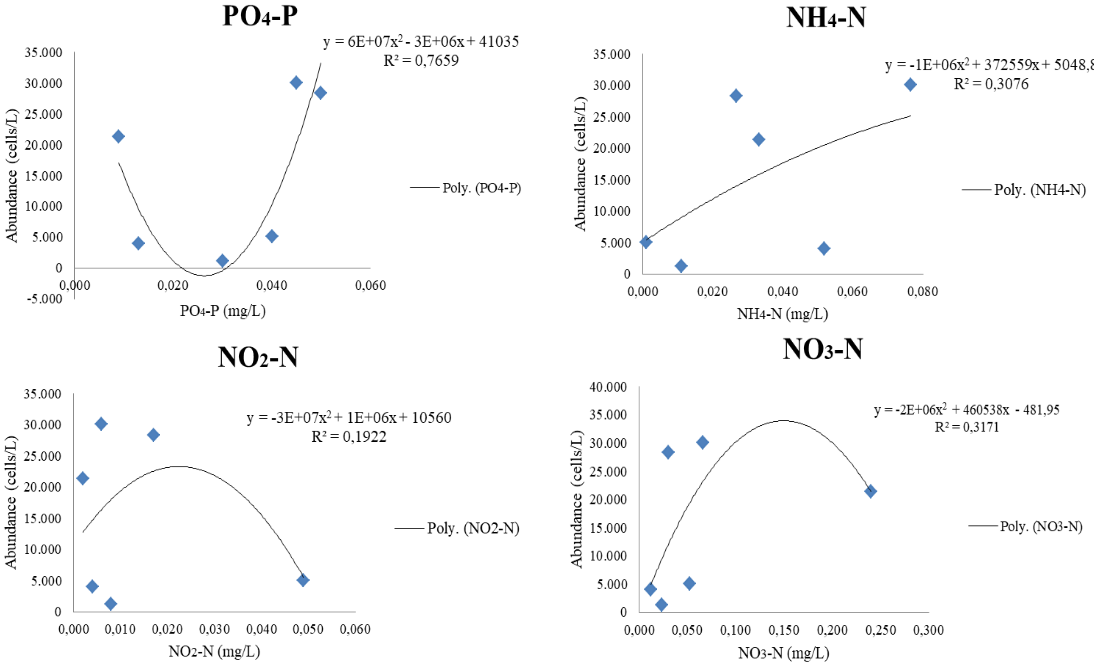

Figure 5. Relationship pattern of *Chaetoceros didymus* abundance with each nutrient at Hurun 1

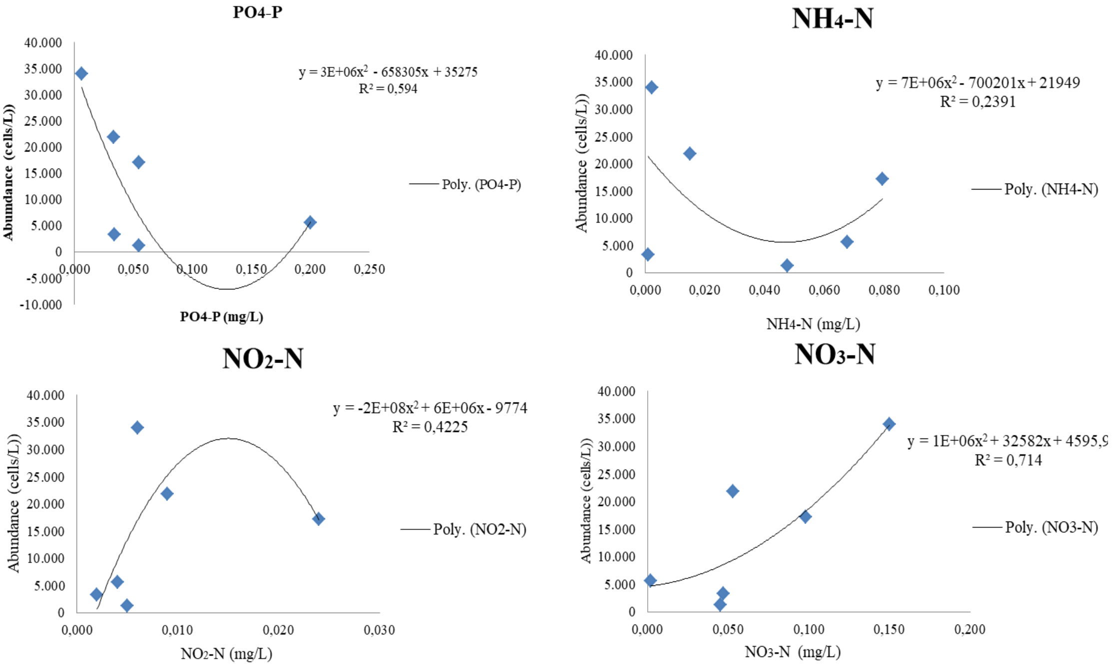

Figure 6. Relationship pattern of *Chaetoceros didymus* abundance with each nutrient at Sidodadi 1

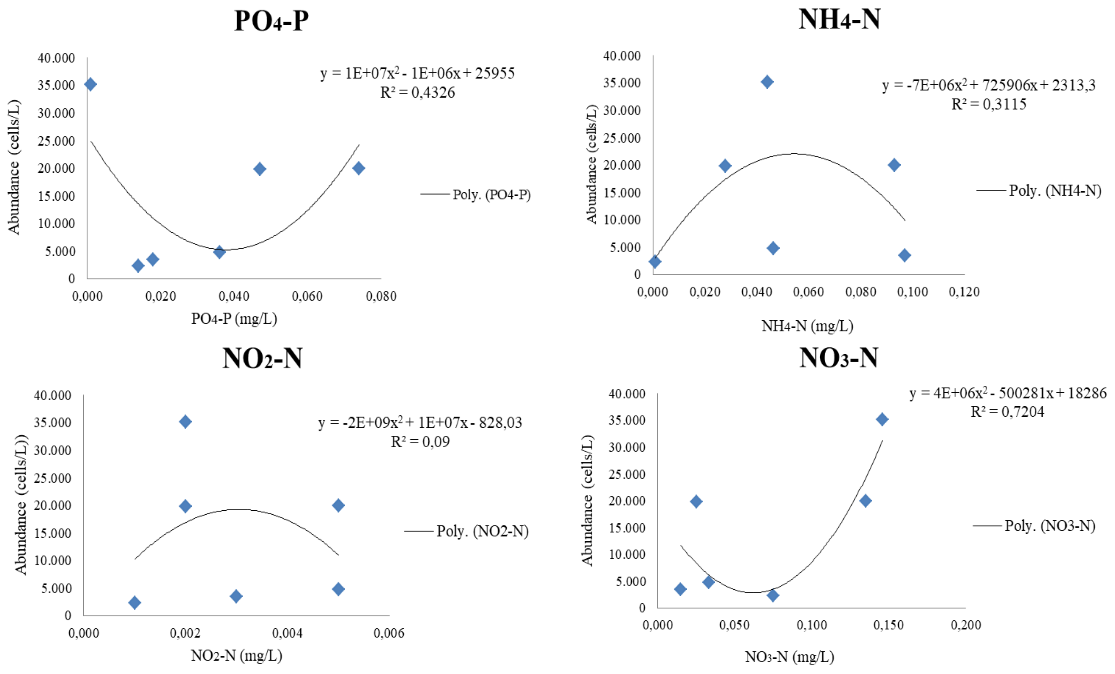

Figure 7. Relationship pattern of *Chaetoceros didymus* abundance with each nutrient at Ringgung 2

Chaetoceros didymus was found in high density at the stations in Hurun Bay (KJA farming), Sidodadi Beach (intensive vannamei shrimp farming) and Ringgung Beach (KJA farming and tourist site). Phosphate concentration tends to be higher near shrimp ponds (Sidodadi Beach and Cikunyinyi Bay) than in KJA farming and tourist sites. *C. furca* was dominantly found in Cikunyinyi Bay which is the location of intensive shrimp farming. Such conditions are thought to be caused by fertilizer and feed waste in shrimp farming activities (Muaddama et al. 2018). Phosphorus fertilizers that are generally applied to shrimp farming are super phosphate and triple super phosphate (Supono 2018). According to Supito (2017), semi-intensive shrimp farming pond uses approximately 100 kg/ha of triple super phosphate (TSP) fertilizer. The growth of diatoms in shrimp ponds requires approximately 75-150 kg/ha of urea and 25-50 kg/ha of TSP (Hendrajat et al. 2007). Meanwhile, 75 kg of urea and TSP are necessary for the growth of diatome (Ratnawati 2008). According to Supono (2018), urea can be stocked up to 200 kg/ha to accelerate the decomposition of pond soil. Furthermore, shrimp farming likewise uses artificial feed that contains P elements, but only 20-40% are converted into fish or shrimp meat, while the rest is passed into the water as waste. In contrast to shrimp farming in ponds, fish farming activities in KJA do not use fertilizer, resulting in less phosphate waste, as well as in tourist areas.

Nitrate concentration in waters close to ponds also tends to be higher than in KJA farming and tourist sites. The nitrate content is thought to come from nitrogen fertilizers that are applied in intensive shrimp farming activities. These nitrogen fertilizers are applied together with phosphate fertilizer to grow phytoplankton in shrimp ponds (Supito 2017). Nitrogen fertilizers containing nitrate (Calcium nitrate-$Ca(NO_3)_2$) are preferred over fertilizers containing ammonium (e.g. Ammonium sulfate/ ZA-$(NH_4)_2SO4$) because they are more water-soluble.

In conclusion, *C. furca* and *C. didymus* are two phytoplankton species as potential HABs that are found dominantly in the waters around fish farming and tourist sites in Teluk Pandan, Lampung Bay. In general, these two species occur in higher density in intensive shrimp farming sites. In tourist areas, those species are identified at a lower density. The increase in the abundance of potential HABs phytoplankton is correlated significantly with the concentration of phosphate and nitrate-nitrite in the waters. The use of a high quantity of artificial feed and fertilizer to increase water fertility in shrimp ponds is thought to be responsible for increasing the fertility of the waters around the study site. At this time, the waters around tourist sites do not seem to have experienced a significant increase in the emergence of HABs. However, due to its location close to the aquaculture center, rising alertness of increased water fertility and the possibility of the appearance of HABs in tourist areas in the future is needed. The increase in the density of HABs may pose a threat to the continuity of aquaculture and tourism activities. Therefore, periodic monitoring and consistent determination of the environmental carrying capacity for intensive shrimp farming activities are required to maintain the sustainability of aquaculture and tourism activities around the waters of Teluk Pandan.

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