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Identification of potentially harmful microalgal species and eutrophication status update in Benoa Bay, Bali, Indonesia

Yulianto Suteja[a], I Gusti Ngurah Putra Dirgayusa[a], Afdal[b], Muhammad Reza Cordova[b], Arief Rachman[b], Wingking Era Rintaka[c], Noverita Dian Takarina[d], Wike Ayu Eka Putri[e], Isnaini[e], Anna Ida Sunaryo Purwiyanto[e,*]

[a] Marine Science Department, Faculty of Marine and Fisheries, Udayana University Indonesia. Jl. Raya Kampus Universitas Udayana, Bukit Jimbaran, Bali, Indonesia
[b] Research Center for Oceanography, Indonesian Institute of Sciences, Jl. Pasir Putih 1, Ancol Timur, Jakarta, Indonesia
[c] Institute for Marine Research and Observation, Ministry of Marine Affairs and Fisheries Republic of Indonesia Jl. Baru Perancak, Jembrana, Bali, Indonesia
[d] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, 16424, Indonesia
[e] Marine Science Department, Faculty of Mathematics and Natural Science, Sriwijaya University, Palembang, Indonesia

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ABSTRACT

Harmful algal blooms (HABs) often occurred in a eutrophicated coastal areas, which causes ecological, economic, and health problems to the ecosystem and coastal communities. Benoa Bay is a coastal ecosystem in Indonesia which threatened by eutrophication and vulnerable to the occurrence of HABs in the future. This study focuses on identifying the potential harmful microalgal species and updating the trophic status in Benoa Bay. Sampling was done during the dry season in 2017 from 30 stations. Tewnty-nine species of phytoplankton were identified in Benoa Bay. *Coscinodiscus* spp., *Pseudo-nitzschia* spp., *Skeletonema* spp., *Chaetoceros* spp., *Rhizosolenia* spp., and *Ceratium* spp., were categorized as potentially harmful microalgal. Among those, *Coscinodiscus* spp. was the most abundant and widely distributed species in Benoa Bay. Phytoplankton cell density was highest in the area between Suwung Landfill and Floating Net Cage (16,584 cells L^{-1}), while the lowest (20 cells L^{-1}) was found in the Sama River estuary. The tb-RDA analysis showed salinity and dissolved oxygen percentage saturation (D%O) were two most significant factor that drives the density and distribution of phytoplankton species in Benoa Bay. In general, Benoa Bay was in the oligotrophic state based on phytoplankton abundance and chlorophyll-a concentration, while in mesotrophic states based on TRIX. The occurrence of some potentially harmful microalgal species, along with relatively high trophic levels in Benoa Bay, requires serious attention to prevent damages from any HABs event in the future.

1. Introduction

Harmful algal blooms (HABs) is a rapid growth of the microalgal (phytoplankton) population that cause many negative ecological impacts to the ecosystems via the production of harmful biotoxins, by damaging or clogging the gills of aquatic organisms, or by inducing anoxia or hypoxia conditions in the water column that could lead to a mass mortality event in the aquatic ecosystem (GEOHAB, 2006; Watson et al., 2015). Bloom caused by toxin producer phytoplankton species is not only harmful or deadly to aquatic organisms (Wardiatno et al., 2004; Wells et al., 2020) but also could cause severe poisoning cases to any humans who consume fish or shellfish that has been contaminated by the toxins (Berdalet et al., 2016). The biotoxins or phytotoxins in the organism generally will accumulate in the tissue or gastrointestinal tracts of the contaminated aquatic organisms (Anderson et al., 2008; Wardiatno et al., 2004), which later can cause several potentially fatal diseases in humans, such as Ciguatera Fish Poisoning (CFP) (Armstrong et al., 2016; Chan, 2015), Amnesic Shellfish Poisoning (ASP) (James et al., 2005), Neurotoxic Shellfish Poisoning (NSP) (Watkins, 2008), and Paralytic Shellfish Poisoning (PSP) (Cetinkaya and Mus, 2012). Casualties from consuming shellfish or fish containing biotoxins were reported in Canada (Todd, 1993), The United States of America (Lipp and Rose, 1997), Mexico (Altamirano and Sierra-Beltrán, 2008), Korea (Park et al., 2013), and Chile (Lagos, 1998). The potential for the occurrence of HABs phenomena is generally higher in eutrophicated areas.

Eutrophication is one of the rising environmental problem faced by

* Corresponding author.
E-mail addresses: yuliantosuteja@unud.ac.id (Y. Suteja), anna_is_purwiyanto@unsri.ac.id (A.I.S. Purwiyanto).

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many developing countries (Yang et al., 2008) and has been reported in various rivers, lakes, and coastal areas in the world (Le Moal et al., 2019; Ménesguen and Lacroix, 2018; Zhang et al., 2020). It was predicted that the intensity, duration, and frequency of eutrophication would increase in this century due to the change of rainfall pattern combined with growing anthropogenic activities on the land and coastal area (Sinha et al., 2017). Eutrophication itself is the nutrients enrichment process in aquatic ecosystems, commonly caused and accelerated by human activities that induce the overgrowth of algae, disruption of ecosystem balance, and water quality degradation (Nixon, 1995). Anthropogenic sources (agriculture, intensive aquaculture, waste disposal, residential, and industrial) contribute more than 80% of the nutrients load to the aquatic ecosystem (Zhou et al., 2020). Besides high nutrient load, other factors that further enhance the effect of eutrophication in aquatic ecosystems are weak currents, calm wave, sediment resuspension, and environmental factors (water depth, sunlight, salinity, temperature, carbon dioxide, etc.) (Qin et al., 2006; Roy et al., 2013). One major concern of eutrophication impact in an aquatic ecosystem is HABs, commonly known as the red tide (Anderson, 2009; Guy, 2014).

HABs events that occurred in the eutrophic and hypertrophic coastal or estuaries in Indonesia have been reported in several studies, such as in the Lampung Bay, Jakarta Bay, Ambon Bay, and Pieh Island-West Sumatera (Irawan et al., 2017; Likumahua, 2015; Praseno et al., 1999; Thoha et al., 2010). Some of those HABs events have damaged the aquatic ecosystem and the coastal communities' health by causing mass fish or shrimp mortalities or severe poisoning cases in humans (Praseno et al., 1999). Besides, poisoning cases caused by consumption of phytotoxin contaminated shellfish or fish were also reported from several areas in Indonesia, such as Ambon Bay, Makassar waters, Sebatik Island in East Kalimantan, the Lewotobi and Lewouran regions of East Nusa Tenggara, Lampung Bay, and Cirebon (Aditya et al., 2015; Nurlina and Liambo, 2018; Wardiatno et al., 2004). In this research, we focused our observation on Benoa Bay, Bali, one of many important coastal ecosystems in Indonesia.

Benoa Bay is an estuary of six rivers (Badung, Mati, Sama, Loloan, Bualu, and Buaji rivers) and is the largest bay in the southern part of Bali Island. The research conducted by Suteja and Purwiyanto (2018) concludes that nutrients, mainly nitrate and phosphate in the six rivers, were high and often found at the eutrophic level. The nutrient level in the waters of Benoa Bay was beyond the maximum limitation for biota that were set by local (The Bali Governor Regulation Number 16 of 2016) and central government (The Decree of Environment Minister number 51 of 2004) regulations (Rahayu et al., 2017). Furthermore, Suteja and Dirgayusa (2017) reported that the Benoa Bay generally were hypertrophic (based on Total Phosphorus/TP) and oligotrophic-mesotrophic (based on Total Nitrogen/TN) condition. However, determining the trophic state based only on the nutrients' concentration was insufficient and missing several key information. Therefore, we used a combination of chlorophyll-a (Ignatiades, 2005), phytoplankton abundance (Karydis, 2009), and Trophic Index (TRIX) (Primpas and Karydis, 2011; Vollenweider et al., 1998) to determine, as well as to update, the trophic states information of Benoa Bay waters. This study focuses on identifying the potential harmful microalgal species and updating the trophic status in Benoa Bay.

2. Materials and methods

2.1. Study site description

Benoa Bay is a semi-enclosed bay used by the local communities for cultivation (fish and crabs), fishing zones (fish and shellfish), and tourism activities. Benoa Bay is surrounded by 1002.2 ha of mangrove ecosystem (Pratama et al., 2019) and a part of Ngurah Rai Forest Park. The coastal land of Benoa Bay is heavily impacted by anthropogenic activities, mainly from the Bali International Airport on the western side, the landfill on the northern side, and the International Harbor for passengers, goods, and fish on the eastern side. The concentration of suspended material and sedimentation process in Benoa Bay is high, especially in the river mouth area (Maharta et al., 2018; Risuana et al., 2017). The surface water of Benoa Bay was reported to contain high microplastics and organic matter, especially in the area around the landfill (Suteja et al., 2021; Yuspita et al., 2017). Furthermore, Benoa Bay mangrove, sediment, crab, and plankton are contaminated by Chromium and Lead from the screen printing and textile industry (Dirgayusa et al., 2017; Sudarmawan et al., 2020; Suteja et al., 2020b; Suteja and Dirgayusa, 2018). Benoa Bay is generally characterized by shallow waters and becomes tidal flat at low tide when the water column condition is also strongly influenced by the tidal processes (Madyawan et al., 2020; Raharja et al., 2018) that propagate from the Indian Ocean (Suteja et al., 2020a). In general, the bottom substrate is dominated by sand in the middle of the bay and mud around the river mouth surrounding the Benoa Bay.

2.2. Field sampling

The field sampling was conducted during the southeast monsoon (dry season) in 2017. There were 30 stations in this research, including 24 stations located in Benoa Bay and 6 stations in the rivers that empty into the bay (Fig. 1). Sampling in the rivers was done at the lowest water level on spring tide to get a freshwater sample. In contrast, the sampling process in Benoa Bay was carried out at the highest water level on spring tide to avoid shipwrecking in the shallow area. The environmental parameters (salinity, temperature, transparency, dissolved oxygen (DO) percent saturation (D%O), and turbidity) were conducted on-site. SonTek CastAway®-CTD was used to measure the salinity (accuracy +0.1 and resolution 0.01) and temperature (accuracy +0.05 and resolution 0.01) in all stations. Horiba DO-meter 110-K series (resolution 0.1%) and Lutron TU-2016 turbidity meter (accuracy 0.01 NTU) were used for measuring D%O and turbidity, respectively. Furthermore, we also conduct chlorophyll and plankton sampling at all stations. Water sampling procedures for chlorophyll-a were adapted from previous studies (Likumahua, 2015). Portable Nansen Water Sampler was used to take 1.5 L of surface water (±0.5m below the surface). The water sample was then transferred to pre-sterilized HDPE bottles. Plankton sampling procedures followed previous studies (Liang et al., 2020). Plankton sampling was done using a plankton net (0.5 m in diameter, 20 μm of mesh size, 1.5 m of net length) mounted with Hydro-Bios Flowmeter (series 438–115) in the opening area. During the sampling process, the plankton net is attached to the ship portside to reduce vessel-based contamination. Plankton net was horizontally drawn by boat on a circular pattern around the research station for ±10 min at ± 3 knots of speed. Specifically, for plankton sampling in rivers, the plankton net is placed in a fixed position and utilizes current for the filtering process. After the trawling process was completed, the plankton net was flushed from the outside to make all stick plankton drops to the bottom layer (cod-end). Plankton in cod-end then transferred to sterile HDPE bottles and preserved using formaldehyde 4% and Lugol 1.5% (Syakti et al., 2019). All samples (water and plankton) were then stored in insulation ice-boxes and taken to Analytical Laboratory at Udayana University for further analysis.

2.3. Laboratory process

2.3.1. Chlorophyll-a

The chlorophyll-a extraction procedure was adapted from previous studies (Likumahua et al., 2019; Siedlewicz et al., 2020). 750 ml of water sample filtered using a two-stage vacuum pump (DSZH 1 HP, model WK-8BM) through pre-weighed sterile cellulose nitrate filter paper (Whatman 7184–004, pore size 0.45 μm, diameter 47 mm). The filter paper was then transferred to a glass tube and added 10 ml of 90% acetone. The sample was then stored in a dark condition in the refrigerator at 4 °C for 2 h. The pigment extraction using 90% acetone in

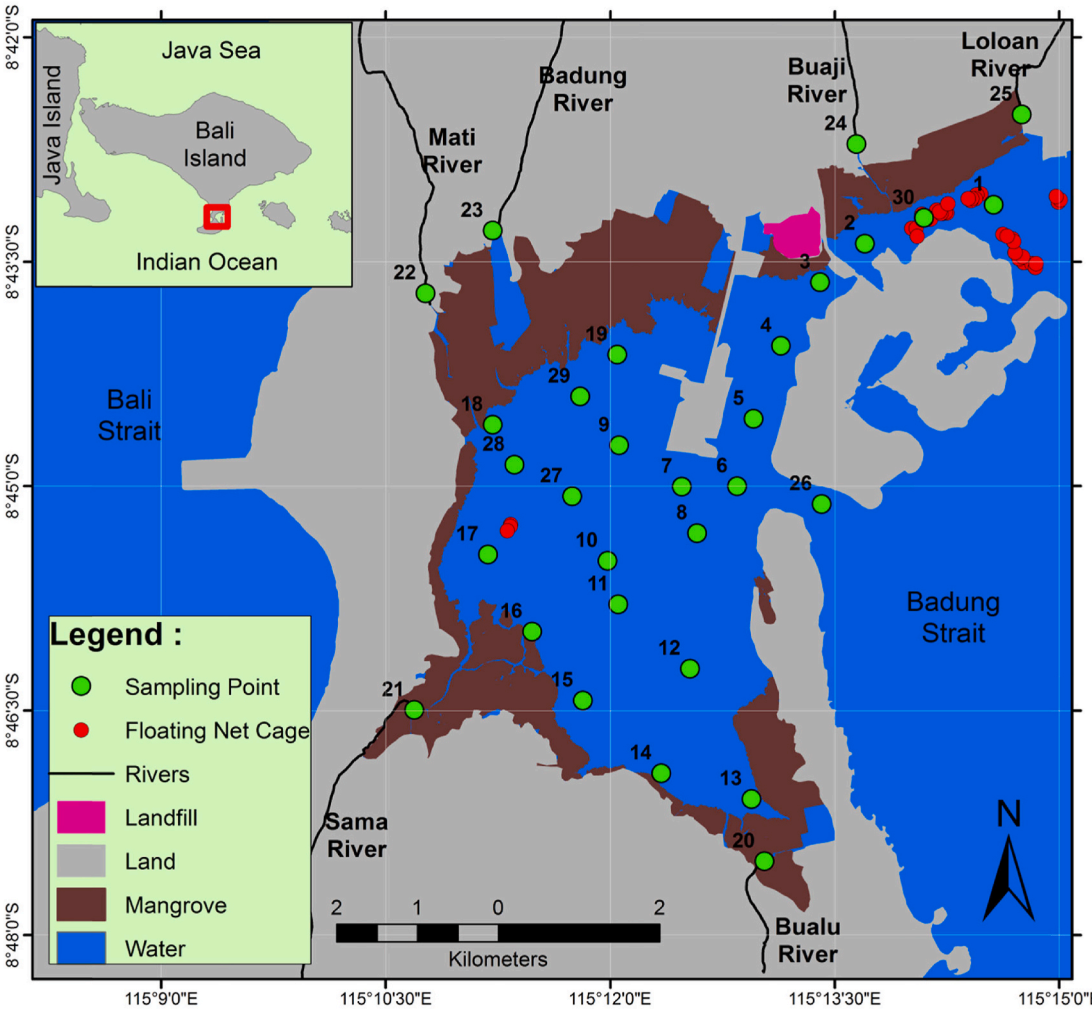

Fig. 1. Sampling location in Benoa Bay.

phytoplankton ranged from 2 to 24 h (Arar and Collins, 1997). For optimizing the extraction process, the samples were ground using a glass spatula. The samples were centrifuged for 20 min at 6000 rpm, and the extracts chlorophyll-a (supernatant) was recorded using a spectrophotometer (Shimadzu series UV-1700) at 630, 645, 665, and 750 nm wavelengths. The equation described by Strickland and Parsons (1972) was used to calculate the concentration of chlorophyll-a. The chlorophyll-a concentrations were expressed in μg L^{-1}.

2.3.2. Phytoplankton

The identification of phytoplankton followed previous research procedures (El Gammal et al., 2017). Before the identification process, plankton sample was stirred in the bottle to make it homogeneous. 1 ml sample was taken and transferred to the gridded Sedgwick-Rafter counting chamber. The Sedgwick-Rafter was then closed slowly to prevent air bubbles. Phytoplankton identification and counting processes were carried out under a binocular Nikon (model E200) microscope (magnification x100 and x400) equipped with an Optilab camera (12.6 MP resolution, Advance plus series). Phytoplankton taxonomy identification was based on cell morphological descriptions in books (Hasle and Syvertsen, 1997; Throndsen, 1997), journals (Garcia-Baptista, 1993), and online data (http://www.marinespecies.org/and https://www.algaebase.org/). Specifically, the potentially harmful microalga species category was based on the previous description (Furuya et al., 2018; Hallegraeff, 2004; Manivasagan and Kim, 2015).

2.4. Data analysis

The phytoplankton abundance in Benoa Bay was calculated based on (APHA, 1995) and expressed in cells L^{-1}. On the other hand, TRIX at each station was calculated based on the equation (Fiori et al., 2016; Paula Filho et al., 2020; Vollenweider et al., 1998):

$$TRIX = \frac{log10\,(chlorophyll - a\ x\ DIN\ x\ DIP\ x\ aD\%O) - (k)}{m}$$

The data of DIN and DIP for TRIX were obtained from Suteja and Dirgayusa (2017). Before being included in TRIX calculations, the unit of DIN and DIP were converted to μg L^{-1}. The $aD\%O$ is an absolute percentage deviation of DO saturation ($Abs|100 - D\%O| = aD\%O$). The k (3.84) is the term of scalar value for the minimum logarithmic sum from each variable ($\sum log10\,(\mathrm{min}value)$), while m (0.63) is a term of scalar factor obtained from the sum of the maximum logarithmic for each variable minus k, and multiplied by 0.1 ($((\sum log10(\mathrm{max}value)) - k)x\,0.1$).

The trophic states based on the abundance of phytoplankton, chlorophyll-a and TRIX were determined based on predefined categories (Table 1) (Håkanson and Blenckner, 2008; Karydis, 2009; Vollenweider et al., 1998). All of the data was mapped spatially for determining the distribution pattern. The variations between stations were performed using one-way ANOVA statistical tests (significant level $p < 0.05$). The relationship between phytoplankton abundance and chlorophyll-a concentration was investigated using the Pearson correlation. It was also

Table 1
Classification of trophic state based on phytoplankton abundance (Karydis, 2009), chlorophyll-a (Håkanson and Blenckner, 2008), and TRIX (Vollenweider et al., 1998) in Benoa Bay, Indonesia.

Trophic states	Phytoplankton abundance (cells L^{-1})	Chlorophyll-a (μg L^{-1})	TRIX
Oligotrophic	$\leq 6 \times 10^3$	<2	0–4
Mesotrophic	6×10^3–150×10^3	2–6	4–5
Eutrophic	$\geq 150 \times 10^3$	6–20	5–6
Hypertrophic	–	>20	6–10

used to determine the relationship between the abundance of phytoplankton (potentially harmful and non-harmful) to TRIX. Transformation-based redundancy analysis (tb-RDA) was used to determine the association between studied environmental parameters and phytoplankton species in Benoa waters. The RDA was also used to investigate environmental parameters' response to the trophic state (especially TRIX). The analysis was done by transforming the species data using Hellinger transformation and standardizing all the explanatory variables (environmental parameters). The RDA Full Model was then subjected to posthoc analysis using variance inflation factor (VIF) to detect multicollinearity within explanatory variables and Monte-Carlo Permutations. Those analyses were performed to detect the significance of RDA axis and marginal effects of explanatory variables. Explanatory variables with VIF value > 10 showed collinearity with other variables and were removed in the Reduced RDA Model. The Reduced RDA Model was also subjected to the same posthoc analysis. It was done to find the explanatory variables with the most significant effect on the phytoplankton communities in Benoa Bay. Forward RDA Model selection was performed using the result of RDA analysis. The RDA and its posthoc analysis were performed using the "vegan" package in R (Borcard et al., 2011; Zuur et al., 2007).

3. Result and discussion

3.1. Environmental parameters

Environmental parameters in Benoa Bay were different and significantly different ($p < 0.05$) between stations (Table 2, Table S1). The average salinity in Benoa Bay was 27.01 ± 11.39 PSU, with the lowest salinity (0 PSU) recorded in some river stations (stations 22, 23, 24, and 25) and the highest (33.58 PSU) at station 6 (Table S1). The salinity in the Bualu and Sama Rivers was 26.50 and 10.50 PSU, respectively. Both rivers are affected by ocean tides and categorized as seasonal rivers (Suteja and Purwiyanto, 2018). The transparency of Benoa Bay waters is low (the average < 1.5 m), which is associated with high levels of turbidity and suspended materials at the study site. The lowest DO was recorded at rivers stations (54.58–59.02%) and the FNC and Suwung landfill (Table S1). This condition might be due to the high organic matter at that location (Yuspita et al., 2017), which required a high amount of oxygen for the decomposition process. The low DO in the aquatic environment could be followed by increased ammonia concentration due to the decomposition of organic matter by bacteria under a hypoxic condition (Suteja and Dirgayusa, 2017). The average concentration of DIN and DIP in Benoa Bay is very high and exceeds the government quality standards. It is a major concern considering that these nutrients are needed by phytoplankton to grow in aquatic ecosystems.

Table 2
Environmental parameters in surface water of Benoa Bay, Indonesia. Note: *indicating that the values were obtained from a previous study (Suteja and Dirgayusa, 2017).

Environmental Parameters	Minimum	Maximum	Average	Standard Deviation
Temperature (°C)	25.20	32.50	27.96	1.40
Salinity (PSU)	0.00	33.58	27.26	11.18
Transparency (m)	0.16	4.00	1.32	1.02
Turbidity (NTU)	0.00	42.82	7.19	11.34
DO (%)	54.58	87.12	69.63	10.00
DIN (mg L^{-1})*	0.09	16.25	1.67	3.16
DIP (mg L^{-1})*	0.03	1.83	0.58	0.43

3.2. Phytoplankton

3.2.1. Potentially harmful phytoplankton

From this research, 29 species of phytoplankton were identified in the Benoa Bay (Table 3), which consists of 2 species of green algae (Chlorophyta), 1 species of blue-green algae (Cyanophyta), 23 species of diatoms (Bacillariophyta), and 1 species of dinoflagellates (Dinophyta). Among those, 6 species were categorized as potentially harmful, noxious, or bloom-forming (Table 3). Among those potentially harmful species (Fig. 2), *Pseudo-nitzschia* spp., is known with at least 26 of its cogeners that is a toxin producer (Bates et al., 2018). Some Pseudo-nitzschia species (*Pseudo-nitzschia pungens* and *Pseudo-nitzschia multiseries*) produce domoic acid (DA) toxins that cause ASP syndrome in humans (Kudela et al., 2020; Likumahua et al., 2019). *Pseudo-nitzschia* spp. is also a common marine diatom genus that has been found in various places in Indonesian waters, such as Ambon Bay (Likumahua et al., 2019), Lampung Bay (Barokah et al., 2017), and Jakarta Bay (Sidabutar et al., 2016; Thoha et al., 2010). Aside from Indonesian waters, *Pseudo-nitzschia* species were also found and causing harmful blooms in the waters of Australia (Ajani et al., 2020), California coast (Zhu et al., 2017), Gulf of Maine-North America (Clark et al., 2019), Adriatic Sea (Turk Dermastia et al., 2020), and English Chanel (Husson et al., 2016). On the other hand, others genera (*Coscinodiscus* spp., *Skeletonema* spp., *Chaetoceros* spp., and *Rhizosolenia* spp.) are commonly abundant diatoms in coastal waters. However, according to previous research, those species are considered as harmful red-tide maker or bloom-forming and has known to blooms (Al-Ghelani et al., 2005; Furuya et al., 2018; GEOHAB, 2010; Karthik et al., 2020) and cause damages to the ecosystem or fisheries (Al Gheilani et al., 2011; Al Shehhi et al., 2014; Parry et al., 1989; Thoha et al., 2010). Therefore, those diatoms are considered as potentially harmful genera in this study.

Among the potentially harmful phytoplankton species, *Coscinodiscus* spp. was the most abundant species found in almost all sampling stations (26 stations) (Table 3), followed by *Pseudo-nitzschia* spp., *Skeletonema* spp., *Chaetoceros* spp., *Rhizosolenia* spp., and the lowest was *Ceratium* spp. The diatoms *Coscinodiscus* spp. often found bloom-forming in the shallow and rich nutrients estuary and coastal waters (Carstensen et al., 2015). The dominance of *Coscinodiscus* spp. in Bali's coastal waters has been previously reported in Gilimanuk waters, Bali Western National Park, where it contributes to >90% of total cell phytoplankton density in that ecosystem (Thoha, 2010). Although blooms of *Coscinodiscus* spp. usually did not cause harmful effects, some of its species (*Coscinodiscus wailesii*) produce harmful blooms to the ecosystem. These species caused depleting the DO (Manabe and Ishio, 1991), bleaching of Nori (*Porphyra thalli*) (Nishikawa and Yamaguchi, 2008; Ono et al., 2006), and producing a high amount of sticky mucilage (Armbrecht et al., 2014; Boalch and Harbour, 1977). The greyish sticky mucilage often contains large amounts of plankton remains and some solid materials, damaging the fishing net (Boalch and Harbour, 1977) or cause gills clogging in fishes. Hence, the blooming of *Coscinodiscus* spp. could cause mass mortality of fish (Al Gheilani et al., 2011). Harmful *Coscinodiscus* spp. blooms have been reported in several regions, such as the Arabian Sea (Al Gheilani et al., 2011; Al Shehhi et al., 2014), Seto Inland Sea - Japan (Manabe and Ishio, 1991), India West Coast (Karthik et al., 2020), and Southern San Juan Channel - United States of America (USA) (Zamon, 2002).

The bloom-forming ability of *Coscinodiscus* spp. is due to its ability to adapt and grow well in a wide range of salinity, temperature, nutrients, and light (Nagai and Imai, 1999; Nishikawa et al., 2000; Nishikawa and Hori, 2004; Nishikawa and Yamaguchi, 2008). The *Coscinodiscus* spp. as

Table 3
Checklist and present-absent codes of phytoplankton species in Benoa Bay, Indonesia. Potentially harmful species marked with pink shade. Note: (++++) = cell density >10^4 cells L^{-1}; (+++) = cell density between 10^3 and 10^4 cells L^{-1}; (++) = cell density between 10^2 and 10^3 cells L^{-1}; (+) = cell density between 1 and 10^2 cells L^{-1}; (−) = not present.

Species	Sampling Stations																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Chlorophyta																														
Pterosferma spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Scenedesmus spp.	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyanophyta																														
Lyngbya spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	++	+	-	-	-	-
Bacillariophyta																														
Achnanthes spp.	+	++	+	+	-	+	-	+	-	+	+	+	++	+	+	+	+	++	++	-	-	-	-	-	-	-	+	-	-	++
Amphora spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
Bacillaria spp.	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Bidulphia spp.	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chaetoceros spp.	+	+	-	+	-	+	+	+	++	+	+	+	-	+	+	+	+	+	-	-	+	-	-	-	-	-	-	+	-	+
Coscinodiscus spp.	+++	++++	+++	+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+	+	-	-	-	-	++	+++	++	+++	+++
Detonula spp.	-	-	++	-	-	-	+	+	+	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	+	-
Diploneis spp.	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gyrosigma spp.	-	-	-	-	-	-	-	+	+	++	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-
Leptocylindrus spp.	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Licmophora spp.	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Melosira spp.	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
Pleurosigma spp.	-	-	-	+	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-
Porosira spp.	++	+++	++	+	+	+	+	+	+	+	++	+	++	++	+	+	+	++	++	-	-	-	-	-	-	-	-	+	++	++
Pseudo-nitzschia spp.	+	++	+	+	+	+	+	+	++	++	++	++	++	+	+	+++	++	++	++	-	-	-	-	-	-	+	+	++	++	-
Rhabdonema spp.	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizosolenia spp.	-	-	-	+	+	-	-	+	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-
Skeletonema spp.	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+
Tabellaria spp.	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Thalassiothrix spp.	-	++	-	-	-	-	+	+	-	-	+	-	-	-	-	-	+	+	++	-	+	-	+	-	-	+	-	-	+	-
Thalassoinema spp.	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-
Unknown sp2.	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unknown sp.	-	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	+	++
Dinophyta																														
Ceratium spp.	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-

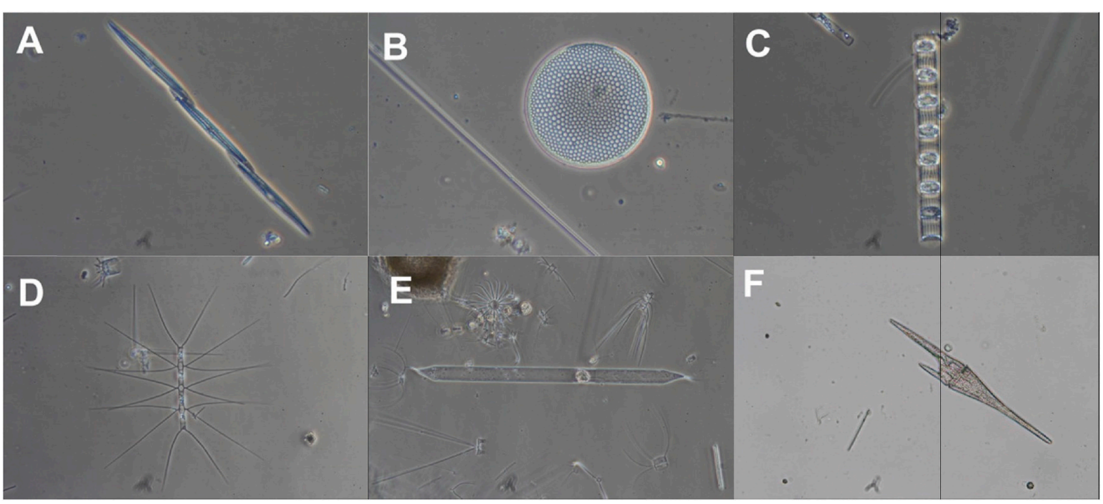

Fig. 2. Potentially harmful species in surface water of Benoa Bay, Indonesia. *Pseudo-nitzschia* spp. (A), *Coscinodiscus* spp. (B), *Skeletonema* spp. (C), *Chaetoceros* spp. (D), *Rhizosolenia* spp. (E), and *Ceratium* spp. (F).

dominant phytoplankton usually found in the estuary with a low light condition (Ramakrishnan et al., 2018), high freshwater and nutrient input (Gómez and Souissi, 2010). Benoa Bay is a semi-enclosed bay in Bali, consisting of six rivers estuaries, a nutrient-enriched water column (Suteja and Purwiyanto, 2018) with a high suspended material (Risuana et al., 2017), and seasonal freshwater discharge. These conditions are ideal for supporting the rapid growth of many bloom-forming diatoms, including *Coscinodiscus* spp. However, *Coscinodiscus* spp. was not observed in Benoa Bay's sediment (Ananingtyas et al., 2017), even this species can live in the surface sediment.

On the other hand, other potentially harmful phytoplankton, mainly *Skeletonema* spp. and *Chaetoceros* spp. are commonly dominant diatoms in eutrophic waters, such as in Jakarta Bay, Lampung Bay, or Ambon Bay (Adnan, 1992; Damar et al., 2012; Pello et al., 2016; Sidabutar et al., 2016). Their dominance is often shifting between seasons as their abundance was highly linked with changes in rainfall and freshwater loads (Adnan, 1992; Sidabutar et al., 2016). In Jakarta Bay, *Skeletonema* spp. was found abundant during the rainy season, while *Chaetoceros* spp. during the dry season (Sidabutar et al., 2016). The *Skeletonema* spp. blooms in Jakarta Bay have been linked to mass fish mortality events in 2004 (Thoha et al., 2010).

Generally, *Rhizosolenia* spp. is considered one of the essential diatoms genera. Its blooms were considered crucial in highly productive marine ecosystems (Gárate-Lizárraga et al., 2003). For example, *Rhizosolenia chunii* in Port Phillip Bay - Southeastern Australia could damage the mussels (*Mytilus edulis* planulatus) mariculture by causing pungent and bitter taste in the mussels' tissue. It causes 500 tonnes of mussels unmarketable and an economic loss of approximately USD 1 million (Parry et al., 1989). Blooms of *Rhizosolenia delicatula* in Bay of Brest - France cause growth anomalies in king scallops (*Pecten maximus*) (Lorrain et al., 2000), while *Rhizosolenia setigera* in Ariake Sea - Japan have caused nutrient depletion in the waters and damaged cultures of seaweed laver (*Porphyra tenera*) (Nagasaki et al., 2004). *Rhizosolenia* spp. is common phytoplankton in Indonesian water. For example, its species' high density was reported during the rainy season in Ambon Bay's inner side (Serihollo et al., 2015).

The low abundance of non-toxic dinoflagellate in Benoa Bay was *Ceratium* spp. However, two of its cogeners (*Ceratium furca* and *Ceratium fusus*) were frequently blooms in marine ecosystems, such as in the Northern Sea, North Atlantic Ocean, the Indian Ocean, and South-Eastern Asian ocean (Baek et al., 2008). In Indonesian waters, *Ceratium* spp. could be found in some eutrophic coastal waters. In Jakarta Bay, this species found in higher abundance one month after a *Pseudo-nitzschia* (*Nitzschia*) blooms (Thoha and Rachman, 2015). Blooms of *Ceratium furca* were also reported several times from the Cikunyinyi area in Lampung Bay, Indonesia (Hasani et al., 2013).

3.2.2. Abundance

The cell density of phytoplankton in Benoa Bay was varied and significantly different ($p < 0.05$) between stations (Fig. 3), with an average cell density of 2432 cells L^{-1}. The highest density of phytoplankton (16,584 cells L^{-1}) was found at station 2, which was located between the Suwung Landfill and the Floating Net Cage (FNC) (Fig. 3). This high density of phytoplankton might occur due to the availability of high nutrients from landfills and FNC. Previous studies in Benoa Bay concluded that the concentration of nitrate and phosphate in sediments and waters around Suwung landfill and FNC was high and exceeded the government's quality standards (Dewi et al., 2017; Rahayu et al., 2017; Suteja and Dirgayusa, 2017). Those conditions occurred due to the decomposition process of waste in landfills that produced macronutrients and micronutrient-rich leachate (Podder et al., 2020), promoting rapid microalga growth (Dogaris et al., 2019). Previous studies in Shanghai, China, have shown that leachate contains more than 10 mg L^{-1} of total nitrogen (TN) and 70 mg L^{-1} of total phosphorus (TP) (Liu et al, 2011, 2015). Thus, without proper management, landfills become a significant contributor to environmental pollution (Guerrero et al., 2013; Ngoc and Schnitzer, 2009). On the other hand, nutrients from FNC come from food residue, fish excretion, and other wastes related to the fish farming process (Price et al., 2014). It was estimated that the production of 1 ton of fish from the FNC would release 243.9 kg of TN and 54.1 kg of TP into surrounding waters (Mansur et al., 2013). The excessive supply of nutrients in the waters will stimulate ecosystem imbalances, particularly the algal blooms (Afraei Bandpei et al., 2016). Therefore, more attention to the waste generated from intensive aquaculture is needed to reduce environmental damage and improve natural resources' sustainable management (FAO, 2018; Tovar et al., 2000).

In general, the lowest abundance of phytoplankton was found in 6 rivers that flow into Benoa Bay with a range from 20 cells L^{-1} (station 21/Sama River) to 97 cells L^{-1} (station 24/Buaji River). Low phytoplankton abundant was most likely due to high turbidity and low transparency water column, limiting the primary productivity of phytoplankton in the ecosystem. All river mouth stations (stations 20–25) in this research were found with water column transparency <1m and turbidity between 11.4 and 42.8 NTU (Table S1). Additionally, high concentrations of ammonia were also measured in river mouth stations, which could inhibit many phytoplankton growths in the ecosystem. It was known

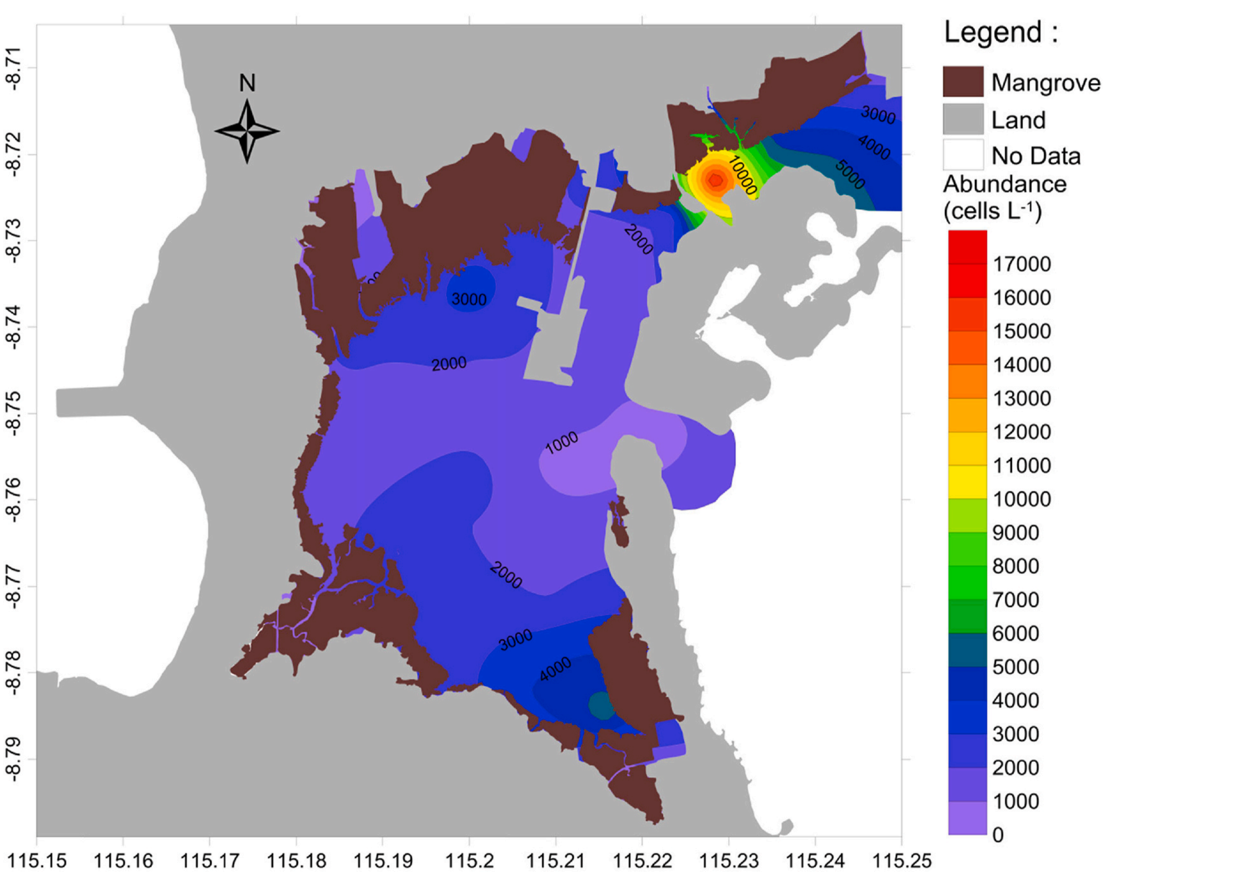

Fig. 3. Phytoplankton abundance in surface water of Benoa Bay, Indonesia.

that high ammonia concentration has proven toxic to phytoplankton (Källqvist and Svenson, 2003), while suspended solid material inhibits the penetration of sunlight that is needed by phytoplankton in the photosynthesis process (Bilotta and Brazier, 2008). Moreover, low salinity in river mouth stations, limiting the distribution and growth of most marine diatoms species found in this research.

Spatially, the phytoplankton abundance in Benoa Bay was higher in areas closer to the estuaries and mainland due to high nutrient concentrations in those areas (Rahayu et al., 2017; Suteja and Dirgayusa, 2017). A similar phytoplankton distribution pattern was found in Penerusan Bay, Bali (Damayanti et al., 2017), and East Belitung Waters (Simanjuntak, 2009).

The abundance of phytoplankton in Benoa Bay of this study was different from previous studies. The average of phytoplankton abundance in Benoa bay (2432 cells L^{-1}) was lower than reported in Jakarta Bay-Indonesia (40,000–1,699,100 cells L^{-1}) (Sidabutar et al., 2016), and Morodemak waters-Indonesia (17,060–28,090 cells L^{-1}) (Yusuf, 2019), but was higher than the phytoplankton density in surface water of Bintan Island (ranged from 0.1 to 2050 cells L^{-1}) (Syakti et al., 2019), Downstream of Perancak Estuary (42 cells L^{-1}) (Hastuti et al., 2018), and Estuary of Delta Mahakam (140–2200 cells L^{-1}) (Effendi et al., 2016). Those differences in the phytoplankton abundance were driven by different environmental parameters such as residence time of water, nutrient (nitrate, phosphate, silicate) input, suspended solid material, light penetration, rainfall, season, and flood (Bharathi et al., 2018; Bharathi and Sarma, 2019; Syakti et al., 2019; Vineetha et al., 2020; Wang and Zhang, 2020; Wardiatno et al., 2004).

Based on the phytoplankton species composition, in general, it was found that phytoplankton in Benoa Bay was dominated by potentially harmful microalgal species (Fig. 4). The combined average cell density of potentially harmful species in all stations (*Coscindoscus* spp., *Pseudo-nitzschia* spp., *Skeletonema* spp. *Chaetoceros* spp., *Rhizosolenia* spp., and *Ceratium* spp.) (Table 3), contributed 85.2% of phytoplankton's total cell density in Benoa Bay. The potentially harmful species were found in the southern rivers (Bualu and Sama rivers) and absent in other rivers (Mati, Badung, Loloan, and Buaji rivers) (Fig. 4). It is presumably due to the differences in salinity that limit phytoplankton distribution, especially the diatom species that dominated in the Benoa Bay. Moreover, the Bualu and Sama rivers were seasonal rivers with minimal water flow during the dry season and caused high salinity. During sampling, we obtained that the salinity in the Bualu and Sama rivers were 26.5 and 19.5 PSU, respectively. Meanwhile, the Mati, Badung, Loloan, and Buaji rivers were permanent rivers with flowing water throughout the year. It causes freshwater salinity (0 PSU) was recorded during sampling. A study in the Pearl River estuary, China, found that planktonic diatoms' distribution strongly correlates with salinity (Zong et al., 2010). The statistical analysis for investigating the effect of environmental parameters on the phytoplankton species composition can be seen in section 3.4 Trophic State. The high abundance and dominance of potentially harmful species in Benoa Bay must be used as an early warning, especially for the local government and community. Appropriate mitigation efforts must be taken so that the adverse effects of algae blooming can be

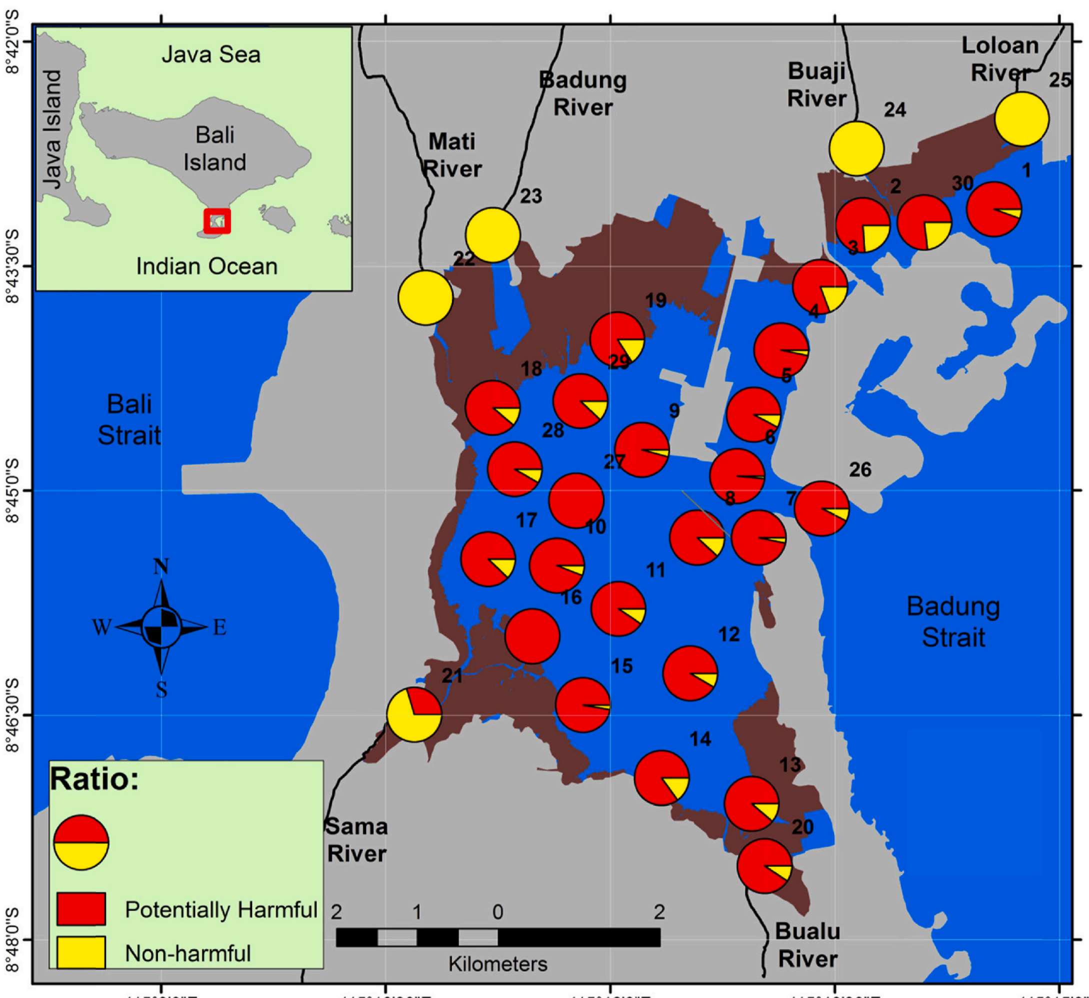

Fig. 4. The ratio of potentially harmful and non-harmful species in surface water of Benoa.

prevented and avoided.

3.3. Chlorophyll-a

The concentration of surface water chlorophyll-a in Benoa Bay was varied significantly ($p < 0.05$) between stations (Fig. 5). The lowest chlorophyll-a concentration (0.23 μg L^{-1}) was observed at station 7 and the highest (10.58 μg L^{-1}) at station 22, while the average concentration was 1.81 ± 2.21 μg L^{-1}. The average chlorophyll-a concentrations in Benoa Bay were much lower than those obtained in Jakarta Bay during the wet season (>5.2 μg L^{-1}) and dry season (>17.0 μg L^{-1}) (Damar et al., 2019). Due to the high nutrient input in Jakarta Bay (Koropitan et al., 2009), and induce hypertrophic conditions in the area close to the mainland and rivers estuary (Damar et al., 2019).

Spatially, the chlorophyll-a concentration in surface water of Benoa Bay was high in areas closer to the river estuaries or mainland and then gradually decreases to the middle of the bay (Fig. 5). The chlorophyll-a concentration pattern was in line with the nutrients distribution pattern in the Benoa Bay (Rahayu et al., 2017; Suteja and Dirgayusa, 2017). The result of this study was similar to some previous research, in which chlorophyll-a concentration is strongly influenced by phosphate, nitrite, nitrate, and ammonium (Baliarsingh et al., 2015; Bharathi and Sarma, 2019; Damar et al., 2019; Longphuirt et al., 2019; Niu et al., 2020; Paczkowska et al., 2019; Van De Poll et al., 2013; Wang and Zhang, 2020) but not significantly affected by silicates in tropical estuaries (Damar et al., 2019; Saifullah et al., 2019). The spatial distribution pattern of chlorophyll-a concentration in Benoa Bay surface water was in line with prior studies in several locations in Indonesia such as Jakarta Bay (Damar et al., 2019; Dharmaputra et al., 2013), Toli Bay-Sulawesi (Wirasatriya, 2011), and Ambon Bay (Likumahua et al., 2019).

The correlation between chlorophyll-a concentration and phytoplankton abundance in Benoa Bay based on Pearson statistical analysis was negatively weak ($r = -0.26$). It indicated that the abundance of phytoplankton was not significantly affected the chlorophyll-a concentration in Benoa Bay. That results were similar to previous research in Bintan Island-Indonesia, which found that the abundance of phytoplankton was not related to chlorophyll-a concentration (Syakti et al., 2019). The other studies state that the chlorophyll-a concentration is not only influenced by the phytoplankton abundance but also influenced by the average cell volume (Jiménez et al., 1987; Vörös and Padisák, 1991). The higher the average cell volume, the lower chlorophyll-a concentration in the marine environment (Vörös and Padisák, 1991). The observations using a microscope showed that phytoplankton in Benoa Bay is dominated by various sizes of giant diatoms (*Coscinodiscus* spp.). Unfortunately, when the research was conducted in Benoa Bay, the size was ignored and not recorded. However, the result of this study was contradicted with the results in other studies that obtained a strong positive correlation ($r > 0.6$) between the abundance of phytoplankton and chlorophyll-a concentration (Bharathi and Sarma, 2019; Gameiro et al., 2004; Ridho et al., 2020; Saifullah et al., 2019). It is mainly caused by differences in dominant phytoplankton species and water conditions that affect chlorophyll-a concentration. Gameiro et al. (2004) noted that the chlorophyll-a concentration in the estuary is influenced by season and water turbidity affecting the sunlight penetration. In Indonesian tropical estuary, the influence of seasons on chlorophyll-a concentration is still being debated. For example, the highest concentration of chlorophyll-a was found during the dry season in Jakarta Bay (Damar et al., 2019), but in the Ambon Bay was reported in the rainy season (Likumahua et al., 2019). The chlorophyll-a concentration in the aquatic environment is also influenced by tides (Gianie et al., 2019). Unfortunately, the effect of seasons and tides on chlorophyll-a concentration in Benoa Bay is still unknown. Thus further research is needed to understand the ecosystem dynamics in Benoa Bay.

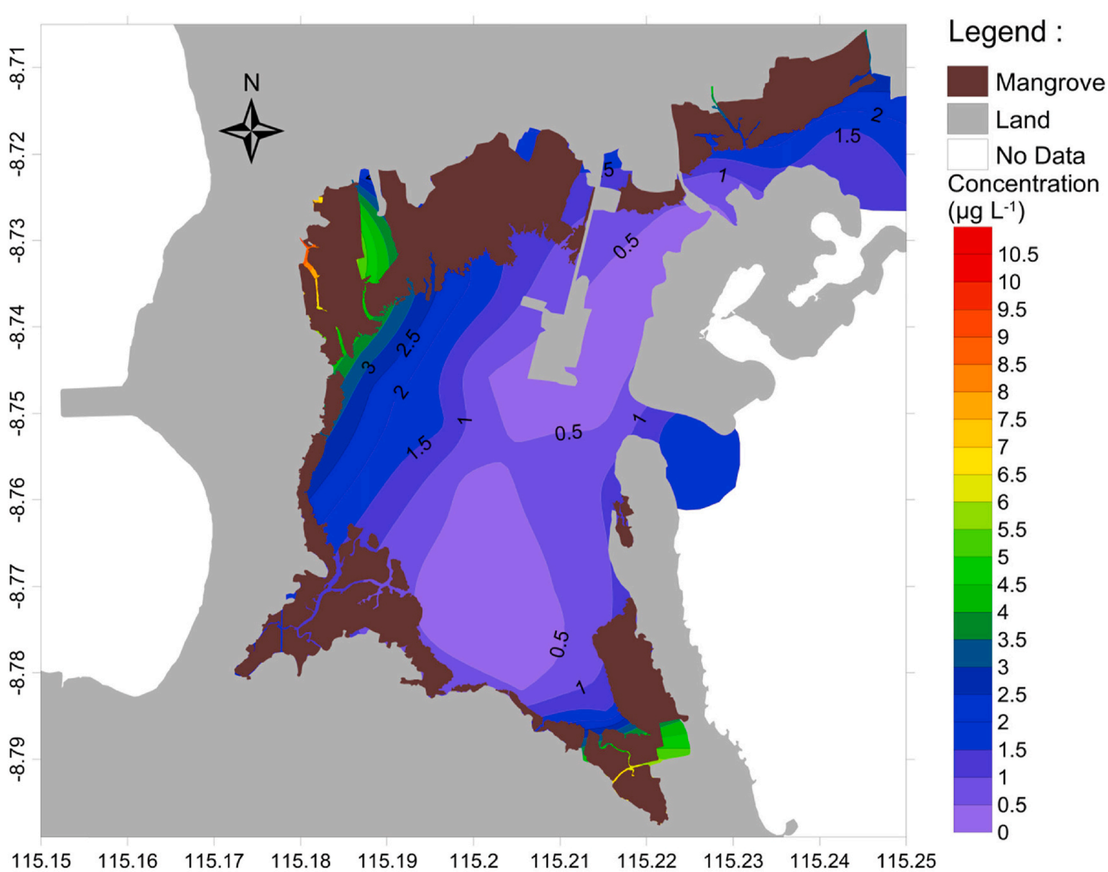

Fig. 5. Chlorophyll-a concentration in surface water of Benoa Bay, Indonesia.

3.4. Trophic state

The tropical state in the surface water of Benoa Bay was varied based on phytoplankton abundance, chlorophyll-a, and TRIX (Fig. 6). Trophic state calculation using the TRIX index is based on chlorophyll-a, DIN, DIP, and D%O data. This factor causes very significant differences in the trophic state based on the TRIX index compared to the abundance of phytoplankton or chlorophyll-a separately. Based on phytoplankton abundance (Fig. 6 top panels) and chlorophyll-a (Fig. 6 middle panels), Benoa Bay surface waters were generally in the oligotrophic (high water quality) category. The mesotrophic (good water quality) category based on phytoplankton abundance was only found in the FNC and the Suwung Landfill. On the other hand, based on chlorophyll-a, the hypertrophic category was found in Bualu (station 20) and Mati (station 22) rivers. The oligotrophic category in Benoa Bay was similar to earlier research based on TN concentration in the water (Suteja and Dirgayusa, 2017). Based on TRIX, the trophic state in Benoa Bay ranged from 2.70 (oligotrophic) to 9.20 (hypertrophic) with an average of 4.89 + 1.61 (mesotrophic). The mesotrophic state was around the mainland, while the oligotrophic state was in the middle of the bay (Fig. 6 bottom panels). Trophic state based on TRIX was contrary to trophic state based on TP which dominated by hypertrophic state (Suteja and Dirgayusa, 2017). Oligotrophic waters are characterized by low water productivity, high water transparency, lack of DO in the bottom zone, and lack of water color anomalies. Meanwhile, the mesotrophic state is indicated by moderate water productivity, occasional anomalies in water colors, changes in water turbidity, and hypoxia occurrence at the bottom layer (Fiori et al., 2016).

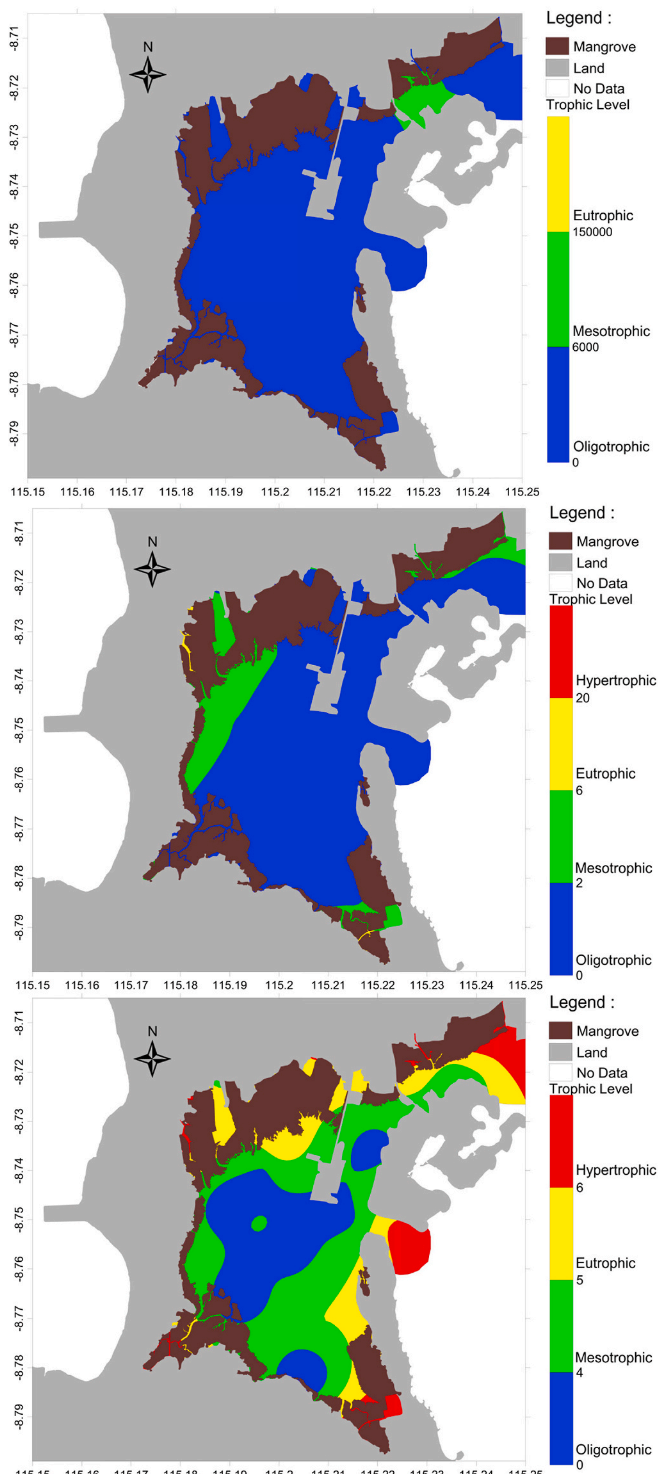

Fig. 6. Trophic state based on phytoplankton abundance (top), chlorophyll-a (middle), and TRIX (bottom) in surface water of Benoa Bay, Indonesia.

In this research, three RDA models (Full Model, Reduced Model, and Simplified Model) were created by combining all environmental and species data into one primary dataset. The Full RDA Model explains the most variance among the three models but has a low significance (p-value <0.1) (Fig. S1), which could be the result of multicollinearity problems among the explanatory variables. The second model, the Reduced RDA Model (Fig. 7 top panels), was constructed by eliminating the potentially collinear explanatory variables based on its VIF values. Even so, the Monte-Carlo permutation test showing that the majority of environmental parameters in the Reduced RDA Model was not significantly affecting the density of phytoplankton species in this study (Table S2). In that case, the Simplified RDA Model was created by Forward Model selection using the Reduced RDA Model as the 'full model', in which only the highest significant variables will remain at the end (Fig. 7 bottom panels) (Table S2). Despite its lowest percentage in explaining the data variables (lowest R^2 or Adjusted R^2), the Simplified RDA Model was the most significant. The eigenvalues and partitioning of correlations for each RDA model are shown in Table S3.

The Reduce RDA Model showed that the trophic level based on TRIX is more influenced by chlorophyll-a and DIN rather than DIP and D%O (Fig. 7 top panels). A slight change in the chlorophyll-a and DIN concentrations would significantly affect the TRIX calculation results compared to DIP and D%O. The RDA results obtained in this study are different from Krivokapic et al. (2016), who obtained TP as the dominant factor affecting TRIX on the Montenegrin coast. However, the effect of chlorophyll-a on TRIX was also found in the Zmiinyi Island Area (Black Sea) (Kovalova and Medinets, 2012). We can conclude that different factors influenced the trophic states based on TRIX in each area.

Based on the Reduced RDA Model, potentially harmful species (Pseudo-nitzschia spp., Skeletonema spp., and Ceratium spp.) were associated with highly oxygenated waters. Their density should increase along with an increase in D%O (Fig. 7 top panels). On the other hand, the most abundant potentially harmful species (Coscinodiscus spp.) was mainly found in higher salinity areas. The other potentially harmful species (Chaetoceros spp.) seem to be abundant in the lower salinity area but have higher temperature, turbidity, DIN, and chlorophyll-a. Interestingly, areas with a higher TRIX value would also associate with a higher Chaetoceros spp. density. Rhizosolenia spp. does not seem highly associated with most environmental parameters measured in this study. However, it would be expected that the density would be higher in lower DIP concentration and more transparent water (Fig. 7 top panels). The Simplified RDA Model clearly showed that the main forces that drive the variation in the phytoplankton abundance were the salinity level and D %O.

The importance of salinity as the main driver for phytoplankton density in Benoa Bay was clearly shown by the stark contrast between

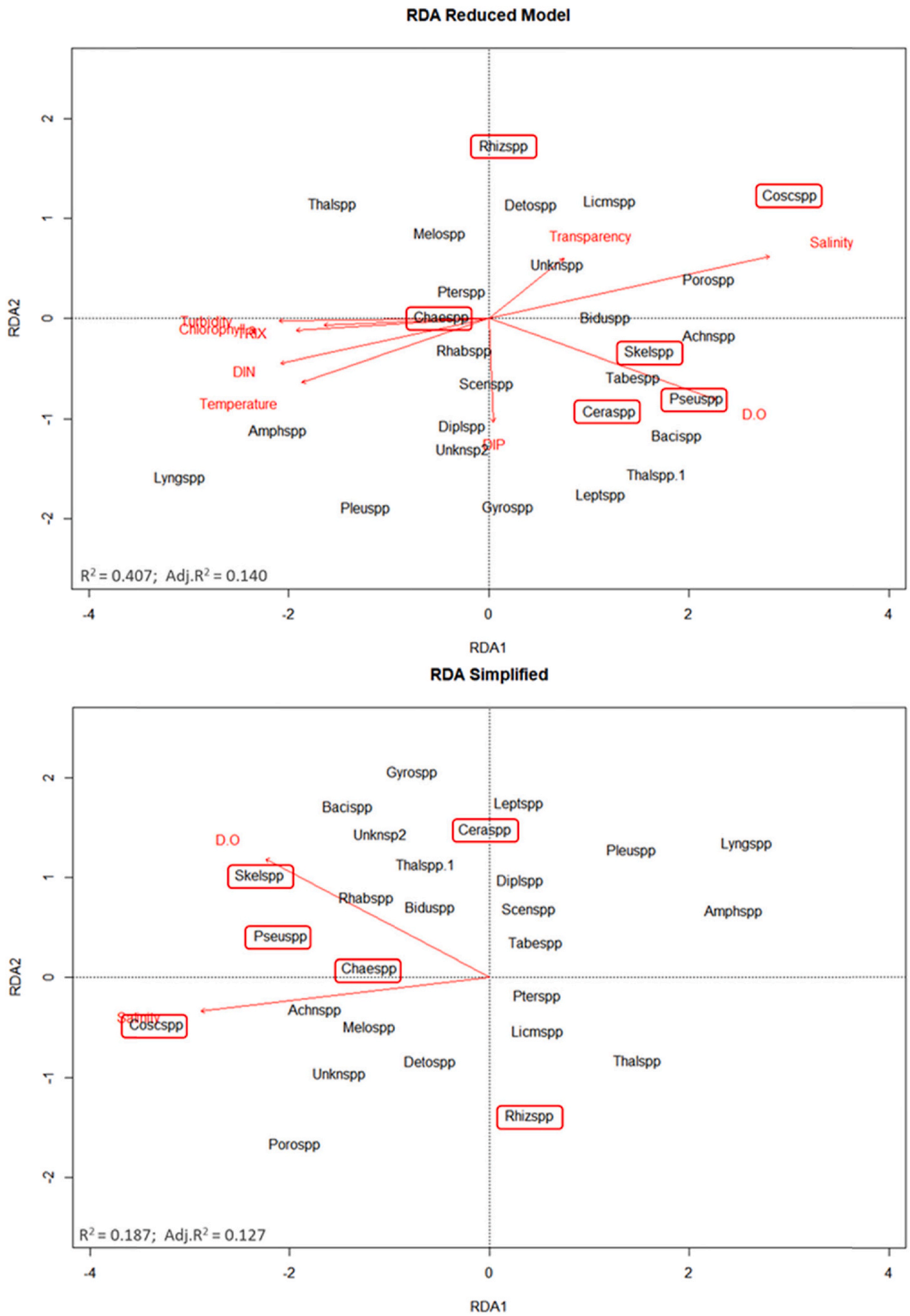

Fig. 7. Biplot of RDA analysis on phytoplankton communities in Benoa Bay using only explanatory variables with VIF value < 10 or the Reduced Model (top) and only the most significant explanatory variables chosen by forward model selection (bottom). The Reduced Model was significant at confidence level of 95% (p-value <0.05), while the Simplified Model was significant at confidence level of 99% (p-value <0.001). Potentially harmful species marked with a red box. Notes: Achnspp = *Achnantes* spp., Amphspp = *Amphora* spp., Biduspp = *Bidulphia* spp., Chaespp = *Chaetoceros* spp., Coscspp = *Coscinodiscus* spp., Detospp = *Detonula* spp., Diplspp = *Diploneis* spp., Gyrospp = *Gyrosigma* spp., Leptospp = *Leptocylindrus* spp., Licmspp = *Licmophora* spp., Melospp = *Melosira* spp., Pleuspp = *Pleurosigma* spp., Porospp = *Porosira* spp., Pseuspp = *Pseudo-nitzschia* spp., Rhabspp = *Rhabdonema* spp., Rhizspp = *Rhizosolenia* spp., Skelspp = *Skeletonema* spp., Tabespp = *Tabellaria* spp., Thalspp = *Thalassiosira* spp., Thalspp.1 = *Thalassionema* spp., Unknsp2 = *Unknown* sp2., Unknspp = *Unknown* spp., Pterspp = *Pterosferma* spp., Scenspp = *Scenedesmus* spp., Lyngspp = *Lyngbya* spp., Ceraspp = *Ceratium* spp. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the phytoplankton communities in the bay and the river estuaries. Most diatoms and dinoflagellates found in this study were abundant in the high salinities area, while the other groups (chlorophytes and cyanobacteria) were abundant in lower salinity (riverine area). Salinity acts as a barrier to species dispersal. It directly affects cell metabolism by affecting osmoregulation and limiting their distribution in the ecosystem. In diatom-dominated phytoplankton communities (like in Benoa Bay), salinity often acts as the main driver that formed the community structure. High salinity limits other groups' growth and primary productivity as the cyanobacteria (Gasinaite et al., 2005). Generally, the optimum salinity for diatoms and cyanobacteria were 35 and 25 PSU, respectively (Khatoon et al., 2010). The potentially toxic and harmful Pseudo-nitzschia spp. has known to have a wide tolerance range salinity (1–35 PSU), such as in Lousiana and Texas coast (Thessen et al., 2005). Salinity also could be the most important factor that regulates the diameter of Coscinodiscus spp. frustules. The higher salinity would reduce the average diameter of the cell's frustules (Mukherjee et al., 2013).

It was interesting to find that D%O seems to be an essential factor regulating the variation shown in phytoplankton density data (Fig. 7 bottom panels) (Table S2). However, a study done by Burris (1981) found that higher oxygen concentration in the water leads to a lower photosynthetic quotient. A high photorespiration rate causes it in the cells due to the presence of high oxygen concentration. Unfortunately, it did not answer why some of the most common diatoms in Benoa Bay were associated with highly oxygenated waters (Fig. 7 top and bottom panels). It is might that oxygen concentration in the water affecting the macro and micronutrient (nitrogen (N) and iron (Fe)) availability as it will determine the biogeochemical pathway (Thackeray, 2010). For example, the decomposition of organic materials in hypoxic or anoxic eutrophic waters will produce toxic compounds (ammonia, hydrogen sulphide, or methane), which could be lethal for most aquatic organisms, including phytoplankton (Thackeray, 2010). Phytoplankton blooms could also cause hypoxia or anoxia in the water caused by excess biomass decomposition during or after the blooms.

The tropic level correlation based on TRIX was positive low with both potentially harmful ($r = 0.22$) and non-harmful ($r = 0.10$) phytoplankton species. The low level of correlation occurs because the basis in TRIX did not involve the type of phytoplankton. Based on Anderson et al. (2002) and Glibert et al. (2008), there is a relationship between HAB and trophic levels, especially if there is eutrophication. However, Anderson et al. (2008) stated a low correlation between HAB and trophic levels; a similar result was found in this study. The conceptual understanding of HABs in eutrophic systems was based on the simplicity of the notion that higher nutrients will produce higher algal biomass. Nevertheless, a high trophic level (eutrophic category) did not always lead to microalgal blooms since it was also known to occur in a low trophic level (oligotrophic category). For example, some dinoflagellate species (toxin-producer *Alexandrium* spp.) were found abundant in relatively pristine waters with minimal anthropogenic impacts in Alaska, northern Japan, and north-eastern Canada (Anderson et al., 2002). On the other hand, *Gymnodinium breve* was well known to form a wide area of blooms in the Gulf of Mexico's oligotrophic waters (Smayda and Reynolds, 2001). Others dinoflagellate species (*Amphisolenia* spp., *Dinophysis* spp., *Histioneis* spp., and *Ornithocercus* spp.) were also commonly abundant in oligotrophic waters. Those species could tolerate a long period of nutrient depletion and low irradiance levels in the water column (Smayda and Reynolds, 2001). Dinoflagellate has also been known to dominate the microalgal community under nutrient-limited conditions, such as phosphorus limited, due to their ability to utilize various phosphorus forms and their unique mixotrophic survival strategy (Ou et al., 2008).

This research was done once during the tidal flood and dry season. Therefore, the trophic state information during the tidal ebb and another season (wet and transition seasons) was still missing, in which a more in-depth study is needed to describe a complete trophic state in Benoa Bay. Unlike this research, Canu et al. (2003) build a model which calculated the average TRIX value for one year, including all seasons and tidal condition in Venice lagoon, Italy. Canu et al. (2003) model's showed the TRIX index value along the coast (closed to the sewage treatment and rivers) would be higher than the open sea. Under different conditions, TRIX's supporting factors (chlorophyll-a, DIN, DIP, and D%O) will have different levels, both in the estuary and in the open sea. It was proven by Krivokapic et al. (2016), where the TRIX results on Boka Bay and the open sea fluctuated every month. In certain months (April, July, August, and October), the TRIX on Boka Kotorska Bay will be higher than the open sea. However, in other months (May, June, September, and November), the opposite occurs. It proves that changes in the supporting factors of TRIX will provide different TRIX values and conclusions. The same thing will happen in Benoa Bay. TRIX analysis would be more comprehensive if the data were gathered from time-series data (i.e., more than 2 years). Thus, suggested further works are needed to do long-term monitoring. It reinforces that monitoring of the trophic state in Benoa Bay in other conditions is essential. Moreover, Benoa Bay has a vital role in economic and ecological function in southern Bali Island. From this research, we could conclude that the local government should improve Benoa Bay marine spatial planning zonation without decreasing the environmental quality than inducing the HABs event. Furthermore, the local government needs continuous monitoring for physical, chemical, and biological water quality to ensure Benoa Bay's ecosystem's sustainability.

4. Conclusion

The research has successfully investigated the latest conditions in Benoa Bay, Bali, Indonesia. Based on the phytoplankton identification, six potentially harmful microalgal species in Benoa Bay were found (*Pseudo-nitzschia* spp., *Coscinodiscus* spp., *Skeletonema* spp., *Chaetoceros* spp., *Rhizosolenia* spp. and *Ceratium* spp.). Almost in all observation stations, we found *Coscinodiscus* sp. (the giant diatom). This species could be adapted and grow well in a wide range of salinity, temperature, nutrients, and light intensity. The highest phytoplankton abundance (16,584 cells L^{-1}) was observed at the station between the Suwung Landfill and the FNC due to high nutrient availability. Meanwhile, the low phytoplankton abundance in the river mouth stations might occur due to the combination of high turbidity, low transparency, low salinity, and high ammonia concentration in the water column. The important note in this study was the potentially harmful species contribute 82.5% of phytoplankton total cell density in Benoa Bay. The phytoplankton abundance and chlorophyll-a were significantly different between stations and increased in areas close to the estuaries and mainland. On the other hand, the statistical analysis shows that the correlation between those two factors was negatively weak. The trophic state in Benoa Bay was different at each station. In general, Benoa Bay was in the oligotrophic state (high water quality) based on phytoplankton abundance and chlorophyll-a concentration, while in mesotrophic states (good water quality) based on TRIX. Based on the RDA analysis, the TRIX calculation in Benoa Bay was more influenced by chlorophyll-a and DIN than DIP and D%O.

Credit author contribution statement

Yulianto Suteja: Conceptualization, Supervision, Formal analysis, Investigation, Writing – original draft preparation, Methodology, Validation, Visualization, Resources, Funding acquisition. I Gusti Ngurah Putra Dirgayusa: Writing-Review and Editing. Afdal: Validation, Writing – review & editing. Muhammad Reza Cordova: Validation, Writing – review & editing, Formal analysis. Arief Rachman: Validation, Writing – review & editing, Formal analysis, Methodology, Investigation. Wingking Era Rintaka Siwi: Writing-Review and Editing. Noverita Dian Takarina: Writing-Review and Editing. Wike Ayu Eka Putri: Writing-Review and Editing. Isnaini: Writing-Review and Editing. Anna Ida Sunaryo Purwiyanto: Validation, Methodology, Data curation, Formal analysis, Investigation, Writing-Review and Editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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