A count model approach on the occurrences of harmful algal blooms (HABs) in Ambon Bay

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ABSTRACT

Climate change and increased anthropogenic activities have resulted in an imbalance in the aquatic ecosystem and have triggered the appearance of harmful algae in Ambon Bay, Indonesia. This study aims to identify the phytoplankton community structure, measure physicochemical water quality (temperature, salinity, pH, DO, nitrate and phosphate) in Ambon Bay, and create a prediction model to estimate the occurrence of harmful algal bloom based on these water quality measures. The results of the statistical count model (Poisson regression) showed that three phytoplankton divisions were observed: Bacillariophyta, Dinophyta and Cyanophyta. Of these, Bacillariophyceae were the most abundant. The only species of the Cyanophyta division identified was *Trichodesmium*, a type of harmful algae that can produce high biomass that may clog fish gills and generate low oxygen. Our Poisson regression model suggested that all water quality factors measured affected the abundance of *Trichodesmium* in Ambon Bay and that, moreover, rising levels of nitrate and salinity will cause a surge in *Trichodesmium*.

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Introduction

Algae are common organisms found in water which are situated at the base of the food chain in the aquatic ecosystem and play an important role as a primary producer. However, algae can be harmful if they accumulate in massive quantities or if the community is dominated by toxin-containing species which can damage the food web and, and further put human lives in peril. Harmful algal blooms (HABs) occur when the growth of colonies of harmful algal causes ecosystem imbalances in marine, brackish and freshwater environments (Townhill et al., 2018; Watson et al., 2015). A great number of HABs are caused by increases in microscopic algae (phytoplankton), specifically Cyanophyta (also known as blue-green algae), though macroalgae are also relevant (Anderson et al., 2012). There is different effect as the result of high and small abundance of HABs (Gobler, 2020). Small abundance HABs usually produce toxins that threatened other organisms. Meanwhile, massive

bloom can cover the coastal and marine area which reduce light transparency and adversely affect primary producers (Lind et al., 2018).

Many researchers have suggested that HABs are the result of climate change and rapid human population growth (Berdalet et al., 2015; Hallegraeff, 2010; Moore et al., 2008). Anthropogenic activities almost inevitably produce pollutants, which are then discharged into water. Waste from agriculture, aquaculture, and domestic tasks is composed of elements which affects the biochemical compounds in water (Widyastuti et al., 2015). Land use changes for agricultural purposes increase the utilisation of fertilizer consisting mainly of nitrogen and phosphorus, which causes the nutrients nitrate and phosphate in water to climb (Xu et al., 2015). Nutrients are essential for phytoplankton growth, and the high concentration of nutrients in aquatic ecosystems is thus associated with excessive abundance of phytoplankton. Moreover, global warming – indicated by rising average temperatures worldwide, among other attributes on Earth's surface – contributes to the community structure shift in certain ecosystems. In addition, many eutrophic ecosystem which host recurring HABs were showing low dissolved oxygen and low pH (Griffith & Gobler, 2020). Another study also demonstrated that subtle changes of salinity can completely alter the community of algae in a system

(Chakraborty et al., 2011). All these factors are considered to be determinants of HABs, even though the probability of the occurrence may vary on a given location (Cha et al., 2014). Some previous studies also suggest that ammonium as the factor affecting HABs, but it has been experimented that there was no significant difference between the growth of algae that cultivated in ammonium and nitrate (Abadie et al., 2015).

HABs are mostly reported in marine and coastal regions (Anderson et al., 2012). These activities create untreated waste comprised of organic matter which is discharged into the water, leading to nutrient enrichment or eutrophication (Suryanto, 2011). Previous studies of Ambon Bay showed that organisms in the Cyanophyceae class had appeared in the phytoplankton community structure and that the bay's temperatures were quite warm, ranging from 29 °C to 31 °C (Sellano, 2011). The United States Environmental Protection Agency included Cyanophyta on its Contaminant Candidate List due to Cyanophyta's massive bloom, which produces toxins under hostile environmental conditions (Kimambo et al., 2019).

Analyzing the occurrence of HABs is very important in developing strategies for managing HABs, whether to minimise their frequency and acuteness or to take action to diminish their impact (Carpenter et al., 1998; Freeman, 2011). The analyzed model can be built based on the availability of factors influencing algal bloom. While the explanatory factors of this phenomenon are generally well recognised, they may differ from site to site (Glibert et al., 2010). HAB occurrences and phytoplankton abundance are both considered count data (Cha et al., 2014). The relationship between phytoplankton abundance and its predictors are statistically analysed using linear regression (Gayoso, 1998; Yan et al., 2004) in which the method treats response variables as continuous rather than count data. The model used for this kind of data is Poisson regression (Agresti, 2002). The objective of this study was to create a count data model for HAB occurrence in Ambon Bay utilising Poisson regression. The resulting model enables the estimation of the presence or absence of Cyanophyceae and its quantity as the function of specific environmental conditions, which can be used to mitigate the occurrence of HABs.

Material and methods

Study area

This study was conducted in Ambon Bay, Indonesia, between January and April 2015. There were six sampling sites, which were determined purposively. The sampling was done once in every week. The study area is displayed in Fig. 1. Ambon Bay is a coastal area in the eastern part of Indonesia which fulfils a variety of important roles; the area contains aquaculture and capture fisheries, harbours, fishing ports, conservation areas, sea transportation lines, recreation areas, and hot water waste disposal from the state electricity company (Pello, Adiwilaga et al., 2014; Sellano, 2011)

Materials

Several water quality measures (temperature, pH, DO, transparency, NO_3 and PO_4) were used in this research as the predictors for HABs and count in Ambon Bay. The water samples were obtained using a Van Dorn water sampler. The instrument CTD SBE 19 was used to measure temperature, pH and DO, while NO_3 and PO_4 were evaluated ex situ using a GENESYS 10S UV–Vis spectrophotometer.

Water samples were taken to identify the harmful algal and were concentrated using plankton nets with pore size 30 μm at euphotic depth. The water samples were preserved by adding formalin and were then analysed in the university laboratory.

Identification of the phytoplankton was carried out using the Olympus CX21LED microscope at 400x magnification, and morphological characteristics were determined in accordance to the World Register of Marine Species (http://www.marinespecies.org). The algae density was calculated by applying the census method to Sedgwick Rafter Counting Cells (APHA, 1989).

Data analysis

The water quality parameters of Ambon Bay were analysed descriptively. Meanwhile, HAB occurrence was predicted using a generalised Poisson regression model (Cha et al., 2014; Consul & Famoye, 1989) as a function of the water quality parameter. This model is the derivative of Poisson probability distribution, assigning its parameter μ as a function of one or more predictors (Cameron & Trivedi, 1998).

The properties of Poisson distribution is $E(Y) = Var(Y) = \mu$. In other words, variance and mean of Poisson distribution have identic value or called as equidispersion. This is the underlying condition that should be fulfilled. Statistical test to check whether the variance and mean of certain data satisfy the equidispersion, then Wald test need to be performed (Cameron & Trivedi, 1998).

Poisson regression model applied to cross section data that consists of n independently observations, with the i-th observation denoted by $(y_i, \mathbf{x}_i)$, where y_i is the number of event of interest that observed, and $\mathbf{x}_i$ is vector of the predictors. Regression model based on the conditional distribution of predictors $\mathbf{x}_i^t = [x_{1i}, ..., x_{ki}]$ dan parameter $\boldsymbol{\beta}$ can be written as follows (Cameron & Trivedi, 1998)

$$f(y_i|\mathbf{x}_i) = \frac{e^{-\mu_i}\mu_i^{y_i}}{y_i!} \tag{1}$$

So that

$$E(y_i|\mathbf{x}_i) = \boldsymbol{\mu}(\mathbf{x}_i, \boldsymbol{\beta}) = \exp(\mathbf{x}_i^t\boldsymbol{\beta}) \tag{2}$$

or

$$\ln(\mu_i) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + ... + \beta_k x_{ki} \tag{3}$$

Eqs. (1)–(3) altogether defined Poisson regression model. Parameter estimation for this model obtained by using Maximum Likelihood Estimation (MLE) method with likelihood function (4)

$$l(\beta_0, ..., \beta_k) = \sum_{i=1}^{n} \{y_i(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + ... + \beta_k x_{ki})$$
$$- \exp(\beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + ... + \beta_k x_{ki})\} \tag{4}$$

If the equidispersion were violated, then Poisson regression model will useless. Thus, the alternative model is Generalized Poisson Regression (GPR). Probability function of GPR showed in Eq. (5) (Consul & Famoye, 1989)

$$f(y|\mu, \alpha) = \left[\frac{\mu}{1+\alpha\mu}\right]\frac{(1+\alpha y)^{y-1}}{y!}\exp\left(-\frac{\mu(1+\alpha y)}{1+\alpha\mu}\right) \tag{5}$$

Properties of the function are

$$E(y) = \mu$$

$$Var(y) = \mu(1+\alpha\mu)^2$$

Generalized Poisson distribution has two parameters, namely μ and α which called as overdispersion parameter. Eqs. (6) and (7) are the regression model that fitted to this probability distribution

$$\boldsymbol{\mu}(\mathbf{x}_i, \boldsymbol{\beta}) = \exp(\mathbf{x}_i^t\boldsymbol{\beta}) \tag{6}$$

$$\ln(\mu_i) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + ... + \beta_k x_{ki} \tag{7}$$
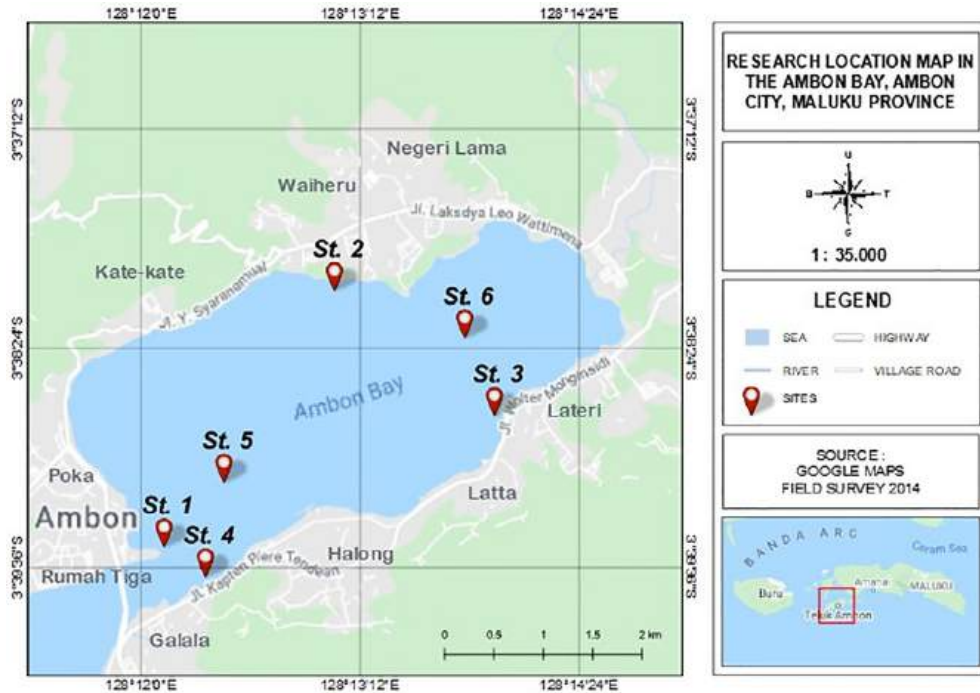
Fig. 1. Sampling sites in Ambon Bay.

Similar to Poisson regression, GPR parameter estimation is also carried out by MLE method (Consul & Famoye, 1989). Data analysis was performed using R version 3.6.1.

Results and discussion

Water quality factors in Ambon Bay

The water temperature of Ambon Bay was measured to be 28 °C, an optimal temperature for algae growth (Renaud et al., 2002) (see Table 1). Specifically, for HABs species algae such as *Trichodesmium* sp, the optimum temperature is 26–27 °C (Boatman et al., 2017). The fluctuation of salinity in marine waters also plays a significant role in the composition of phytoplankton communities (Kirst & Karsten, 1989). Increases in salinity result in the depletion of phytoplankton growth, due to the obstruction of the osmoregulation mechanism (Chakraborty et al., 2011; Redden & Rukminasari, 2008). Here, the salinity varied between 32.11 and 32.64 PSU. Ambon Bay has relatively low salinity compared to the preferred salinity for open water (34–37 PSU); as a result, its conditions are favourable for algae development (Nurul Salma et al., 2013).

The pH values recorded in Ambon Bay exceeded 7 and can thus be classified as alkaline. However, the growth rate of some marine phytoplankton is uncorrelated with pH and CO_2 (Berge et al.,

2010). In addition, DO concentration was observed to be 5.70–6.25 mg/L. This parameter is strongly affected by temperature; rising temperatures result in DO reduction and diminish phytoplankton diversity and biomass (Takarina, 2017).

The other important parameters for phytoplankton growth are the concentrations of nitrate and phosphate. These nutrients are known contributors to algae growth, and their unavailability is a limiting factor in phytoplankton production (Vrede et al., 2009). A remarkable finding of this study is that nitrate concentration in all sites are under the water quality standard specified by the Indonesian Government and WHO (Ministry of Environment, 2001; WHO, 2004) as much as 10 mg/L. The phosphate concentration was likewise lower than the standard set by the Indonesian Ministry of Environment in 2001 (0.2 mg/L); however, the phosphate magnitude was not as great as the nitrate magnitude. Even though both nutrient concentrations are not exceeding the standard value, nutrient enrichment must be controlled because they can trigger algal bloom and eutrophication (Lusiana et al., 2019)

Composition and phytoplankton abundance in Ambon Bay

The total phytoplankton abundance in Ambon Bay was relatively uniform across the six sampling sites, ranging from approximately 18,796,1000 cells/L to 23,150,000 cells/L (Fig. 2) which show harmful bloom in low risk (de la Cruz et al., 2017). The high-

Table 1
Physicochemical water quality measurement results.

| Parameter	Site						Min.	Max
	I	II	III	IV	V	VI		
Temperature (°C)	28.00 ± 1.414	28.00 ± 1.414	28.50 ± 0.707	28.50 ± 0.707	28.50 ± 0.707	28.50 ± 0.707	27.00	29.00
Salinity (PSU)	32.64 ± 0.658	32.61 ± 0.700	32.11 ± 0.000	32.23 ± 0.071	32.10 ± 0.000	32.10 ± 0.000	32.10	33.10
pH	7.21 ± 0.679	7.22 ± 0.679	7.36 ± 0.912	7.26 ± 0.771	7.25 ± 0.742	7.20 ± 0.679	6.71	8.00
DO (mg/L)	6.05 ± 0.354	5.80 ± 0.141	5.95 ± 0.354	6.25 ± 0.636	5.90 ± 0.283	5.80 ± 0.141	5.70	6.70
Nitrate (mg/L)	2.41 ± 1.435	2.10 ± 1.068	2.48 ± 1.478	2.78 ± 0.622	2.04 ± 1.315	2.16 ± 1.153	1.11	3.52
Phosphate (mg/L)	0.03 ± 0.032	0.03 ± 0.033	0.03 ± 0.035	0.03 ± 0.036	0.03 ± 0.032	0.03 ± 0.033	0.0041	0.0550
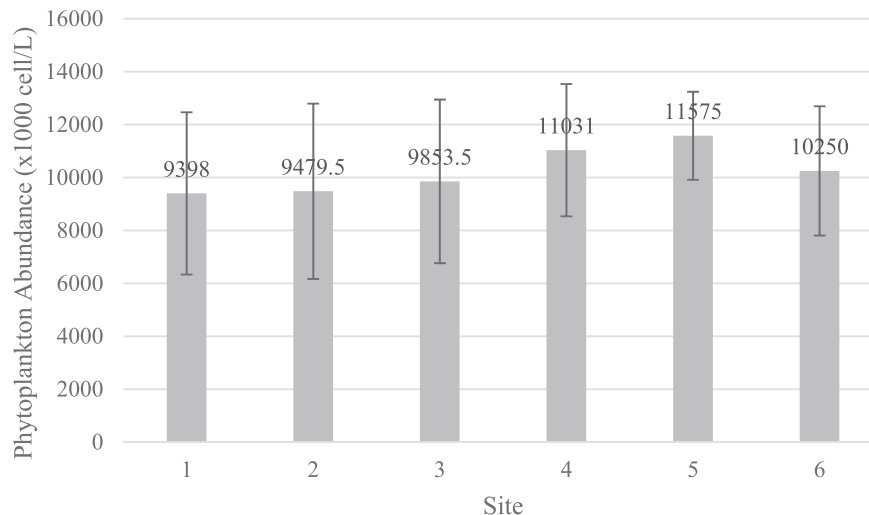
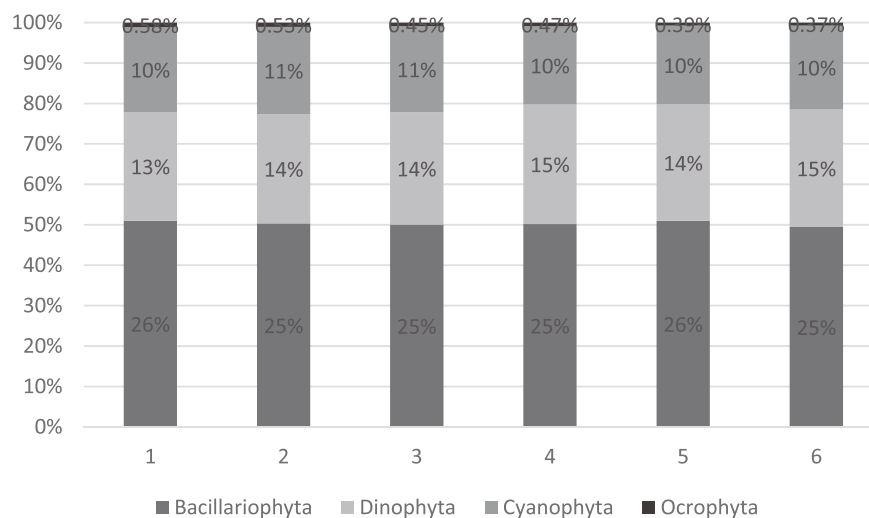Fig. 2. Phytoplankton abundance in sampling sites.

Fig. 3. Phytoplankton community structure in Ambon Bay.

est biomass was found in Site 5 and the lowest in Site 2 (Fig. 2). The collected phytoplankton (Fig. 3) can be divided into three divisions and 17 species (genera). The detailed abundance is presented in Fig. 3. Bacillariophyta were the most dominant division across all sites, accounting for more than 50% of total algae with a total of 12 species (*Rhizosolenia*, *Chaetoceros*, *Skeletonema*, *Thalassionema*, *Nitzschia*, *Bacteriastrum*, *Thalassiothrix*, *Coscinodiscus*, *Noctiluca* sp., *Thalassiosira* sp., *Biddulphia* sp.). The proportion of Dinophyta was slightly below 30% and consisted of four species (*Ceratium* sp., *Pyrodinium*, *Dinophysis*, and *Alexandrium*). Only one species (*Trichodesmium*) of the Cyanophyta division and *Distephanus,* sp. from Ocrophyta were observed, amounting to 20% and <1% of the overall algae abundance, respectively.

Some species of the observed phytoplankton might be harmful if they bloom like *Ceratium* sp. The species can be considered a harmful algae (Syakti et al., 2019), if it jeopardises nutrient availability under rapid growth biomass conditions (Baek et al., 2008). However, in this study, *Ceratium* sp. appeared only in small amounts so did any others species; so, they are not considered harmful in these conditions. However, *Trichodesmium* is a filamentous marine cyanophyte which is a significant contributor to nitro-

gen fixation (Rynearson & Palenik, 2011) and some strains can produce a neurotoxin called saxitoxin but only in small amount (Sacilotto Detoni et al., 2016). This neurotoxin can cause paralytic shellfish poisoning, flaccid paralysis, respiratory failure and death (Yunes, 2019). Extensive bloom of *Trichodesmium* can choke or clog the fish gills and resulting mortality (D'Silva et al., 2012). The bloom of *Trichodesmium* have been reported worldwide and specifically in Indonesia (the Java Sea and Lampung Bay), where they have caused red tides and fish mortality (Puspasari et al., 2018)

Table 2
Parameter estimation of Poisson model for predicting HAB abundance (dependent) based on water quality measures (independent).

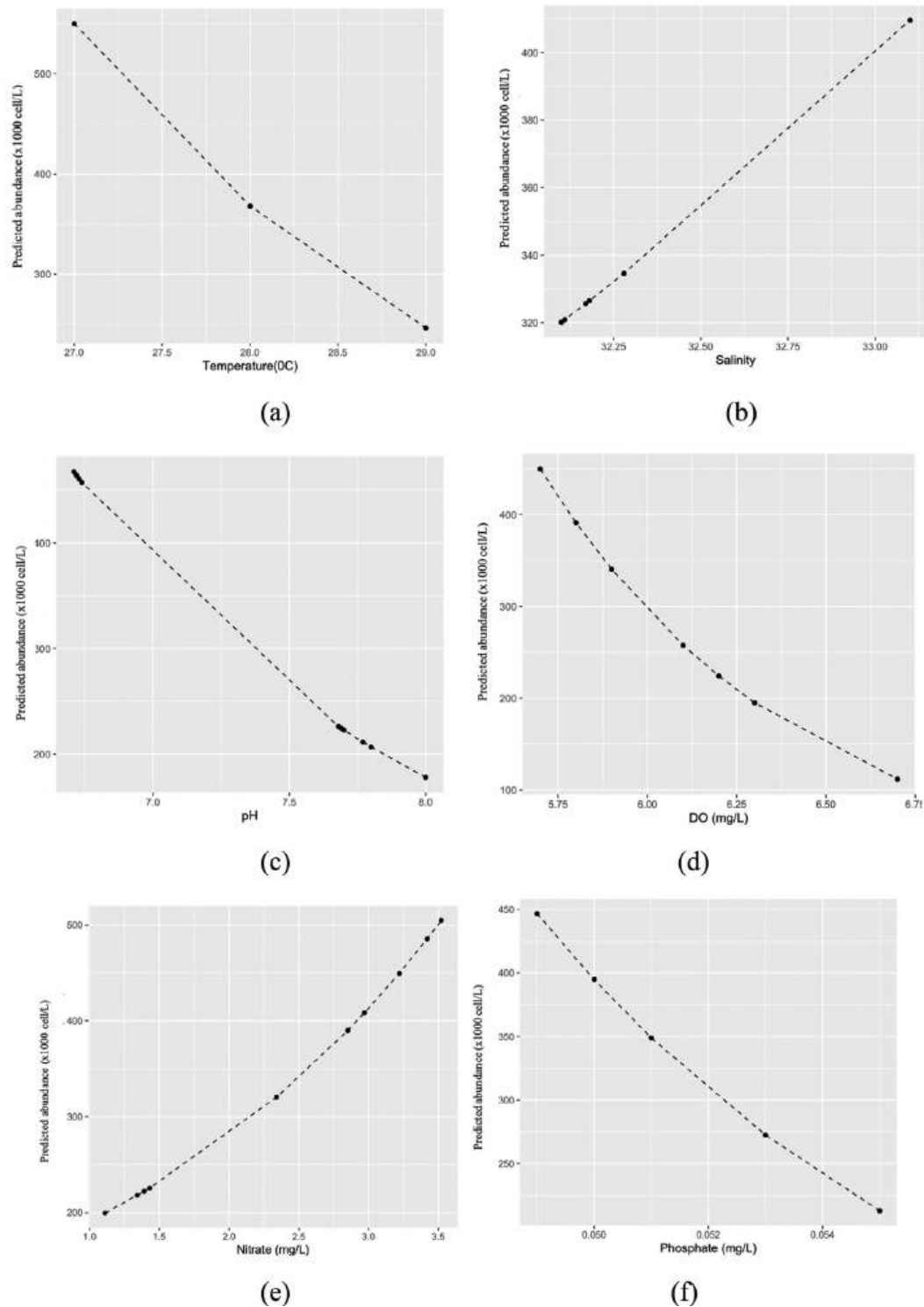| Variable	Parameter estimate	Standard error	p-value
Temperature	−0.40235	0.5574	<2e−16
Salinity	0.24662	0.04027	9.14e−10
DO	−1.39210	0.06947	<2e−16
pH	−0.74938	0.03224	<2e−16
Nitrate	0.38517	0.01873	<2e−16
Phosphate	−123.5102	8.6571	<2e−16

Fig. 4. Line graph of HAB abundance (*Trichodesmium* sp count) as the function of (a) temperature; (b) salinity; (c) pH; (d) DO; (e) nitrate concentration; and (f) phosphate concentration.

Poisson model for HAB occurrence in Ambon Bay

All the observed water quality parameters had different effects (parameter estimate) on HAB abundance in Ambon Bay (Table 2). However, all parameters significantly affected algae density because their p-values were less than the specified significance level α (0.05). The abundance of *Trichodesmium* is highly associated with nutrient availability, temperature and salinity (Jiang et al.,

2017). Specifically, previous study suggested that the assemblage of phytoplankton in Ambon Bay was affected by ammonium, salinity, and water temperature (Wagey, 2002). Our findings show that there are more extensive environmental factors which are temperature, salinity, DO, pH, nitrate and phosphate that significantly affect the abundance of *Trichodesmium* in marine water.

Trichodesmium biomass in current study area comprised of around 10% or 400,000 cells/L. Previous reports on *Trichodesmium*

bloom showed that its contribution to phytoplankton structure between 12.5% and 75% which were equivalent to 136,000–4,140,000 saxitoxin cells/L (Mohanty et al., 2010). Moreover, Indonesians Institute of Sciences data also noted that Ambon Bay have been suffered from numerous HABs (9 events) since 1990, which mostly caused by *Trichodesmium* bloom (Thoha, 2016). Hence, it is very likely and predicted that this genus will create HABs in Ambon Bay.

Interestingly, our research found that temperature and DO had a negative effect on *Trichodesmium* abundance (Fig. 4a and d). This finding contradicts several previous studies claiming that global warming has increased *Trichodesmium* growth rates (Capone et al., 2005; Jiang et al., 2017). However, this may be due to the fact that other water quality factors were not controlled. A study of the physiological constraints of *Trichodesmium* showed a positive effect of temperature on its growth rates through the control of other biochemical determinants (Breitbarth et al., 2007).

On the other hand, salinity was demonstrated to have a positive relationship with HABs. As illustrated in Fig. 4b, *Trichodesmium* abundance is predicted to rise continuously even after 33 PSU. *Trichodesmium* can actively grow in a wide range of salinities (22–43 PSU) and is thus classified as euryhaline (Blondeau-Patissier et al., 2018). The optimum growth of *Trichodesmium* occurs in the range 33–37 PSU (Fu & Bell, 2003). However, an increase of pH caused a decrease in algae biomass (Fig. 4c). Hence, acidification contributes to the decrease of growth and dinitrogen fixation (Hallegraeff, 2010; Hong et al., 2017; Moore et al., 2008).

The influence of nutrients (nitrate and phosphate) is depicted in Fig. 4c and d. The surge of nitrate concentration leads to the growth of *Trichodesmium* biomass. *Trichodesmium* requires nitrogen through the uptake of nitrate to perform dinitrogen fixation (Capone et al., 2005). While nitrogen is highly abundant on Earth, this molecule is highly unreactive and cannot be assimilated by most organisms and used in their nutrition (Breitbarth et al., 2007). Therefore, nitrogen needs to be fixed in order to be utilised by an organism; Cyanophyta are the organisms capable of doing this (Holl & Montoya, 2005). In contrast, due to the scarcity of phosphate in Ambon Bay (Table 2), *Trichodesmium*'s phosphate uptake might not be sufficient, thus making phosphate the limiting factor of algae growth (Lomas et al., 2014; Shetye et al., 2013).

Conclusion

Many activities in Ambon Bay have resulted in untreated waste being discharged into water, creating nutrient enrichments that can shift the community structure of phytoplankton. The present study revealed the occurrence of harmful algal genera of the Cyanophyta division, namely *Trichodesmium*. Analysis of algae biomass using a count model (Poisson regression) during this study period showed that all physicochemical factors measured (temperature, salinity, pH, DO, nitrate and phosphate) in the study area had a significant effect on *Trichodesmium* abundance. In particular, a significant amount of *Trichodesmium* are expected to occur in accordance with the rise in salinity and nitrate. This study did not present the effect of temporal variability where it might be a major driver on HABs occurrences especially *Trichodesmium* sp (Jiang et al., 2017). Thus, future study is advised to include temporal variability to get a better prediction on HABs.

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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