BIODIVERSITAS
Volume 21, Number 7, July 2020
Pages: 3101-3107

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210730

Environmental factors and seasonal effect on the potential harmful algae presence at Ambon Bay, Indonesia

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Manuscript received: 4 May 2020. Revision accepted: 16 June 2020.

Abstract. *Mahmudi M, Lusiana ED, Herawati EY, Serihollo LG. 2020. Environmental factors and seasonal effects on the potential harmful algae presence at Ambon Bay, Indonesia. Biodiversitas 21: 3101-3107.* Marine and coastal areas are susceptive to harmful algae presence which can lead to Harmful Algae Blooms (HABs). Major drivers for this event are climate change, domestic and industrial activities. These alter the environmental condition in marine ecosystems which caused the shift of phytoplankton community. This study aims to analyze the environmental factors affecting the harmful algae occurrence in Ambon Bay as well as the seasonal change during west monsoon and first transition in regards to this issue. There were six environmental variables accounted in this research which measured in situ and ex-situ. The results showed that there were three algae divisions that formed the phytoplankton structure in Ambon Bay, namely Bacillariophyceae, Dinophyceae, and Cyanophyceae. These divisions have been reported as the cause of previous HABs in the area. Furthermore, algae density during first transition season was higher than during west monsoon season, but the composition of phytoplankton community was stable in which dominated by harmful algae such as *Chaetoceros, Skeletonema, Nitzschia, Ceratium, Pyrodinium, Dinophysis, Alexandrium,* and *Trichodesmium.* Meanwhile, based on Canonical Correspondence Analysis (CCA), temperature and nutrients were the main factors that highly associated with the presence and abundance of harmful.

Keywords: Canonical correspondence analysis, climate change, HABs, monsoon season

INTRODUCTION

Harmful Algae Blooms (HABs)occurrence in marine and coastal areas is associated with transforming environmental factors as the consequence of both climate change and anthropogenic activities (Davidson et al. 2014; Watson et al. 2015). Climate change has induced progressive acidification, warming, and deoxygenation of marine ecosystem (Gobler 2020). Many eutrophic habitats where HABs occurred already experience low dissolved oxygen (DO), thermal extremes, and low pH, which creating these locations as potential places for conditions that will become more regular in larger-scale systems as climate change proliferates. Coastal areas are also commonly susceptible to anthropogenic runoffs such as municipal, industrial, and agricultural waste (Kirby and Beaugrand 2009; Yuan et al. 2011). The discharges are usually untreated and carry pathogens, organic and inorganic nutrient, heavy metals, and detergents that are considered to harm marine and coastal ecosystems (Nixon 1995; Halpern et al. 2008). These substances can transform food-web dynamics in coastal areas. For instance, waste outflow increase nutrients that can promote bottom-up effects (Davis et al. 2010) and alter the community structure in marine ecosystems to configure massive blooms harmful algal species (Berdalet et al. 2015).

Most harmful algae species come from phytoplankton division. Nevertheless, there are different effects as the result of high and small abundance of HABs. High abundance of HABs will result in oxygen depletion in bottom waters since the bloom sinks and bacteria decomposed it, then it commonly is known as non-toxic HABs (Gobler 2020). Cultured and wild fish may also experience death resulting from the choking of gills due to mucus production of phytoplankton. In contrast, low density of HABs can produce biotoxins, and then they are being concentrated by filter feeder and other organisms that may subsequently be ingested by humans (Davidson et al. 2014). Dinophyceae and Cyanophyceae are two phytoplankton divisions that have been widely reported to cause HABs both in freshwater and marine ecosystems.

In Indonesia, HABs have been reported since 1990 which initially happened in Java Sea due to *Trichodesmium erythraeum* bloom. After that, any other algae blooms have regularly occurred in different locations (Thoha 2016; Syakti et al. 2019). Interestingly, according to Indonesians Institute of Sciences data, there were 23 HABs reported between 1990 and 2015, in which 9 of them took place in Ambon Bay caused by Cyanobacteria, dinoflagellates, and diatoms (Thoha 2016). Ambon Bay has numerous significant roles as conservation area, aquaculture and capture fisheries, recreation area, and waste disposal site from state electricity company (Sellano 2011). Therefore, the occurrence of HABs at high potential to threaten its biodiversity. It is also may damage the sustainability of activities that bring wealth of its surrounding communities.

It is important to further analyze the factors associated with the occurrence of HABs species in Ambon Bay. However, previous studies only revealed the community structure of phytoplankton in this area (Padang 2010; Serihollo et al. 2015). There was no study that investigates the relationship between algae community especially potential HABs and its environmental factors under different periods of time. This is significant to prevent the occurrence and minimize the impact of future HABs. Hence, the objectives of this study were to analyze the environmental factors affecting the presence of harmful algae in Ambon Bay and its patterns during west monsoon and first transition season.

MATERIALS AND METHODS

Study area

This study was performed in Ambon Bay, Indonesia in 2015. Samplings were conducted in January for west monsoon April for first transition season (four replications per season) at six sampling sites (Figure 1). The determination of sampling sites was using purposive method where the site determined based on its specific characteristics as follows: (i) Site 1: area of state electricity company, near to residential, (ii) Site 2: near to residential and military facilities, (iii) Site 3: floating net cages area, (iv) Site 4: ship harbor, (v) Site 5 and 6: middle part of the Ambon Bay.

Materials and sampling procedure

The main material of this research was phytoplankton which taken from Ambon Bay water samples. They were grasped using Van Dorn water sampler at 0-20 m in depth, then stored in a 650 ml volume bottle. After that, they concentrated in plankton net (pore size 30 μm) and preserved with formalin (4%) addition. The phytoplankton identification was conducted by utilizing microscope type Olympus CX21LED at 400x magnification and its classification made conforming to the World Register of Marine Species (http://www.marinespecies.org). The phytoplankton abundance was estimated by using Sedgwick Rafter method (APHA 1989). The environmental factors used in this research were temperature (°C), pH, DO ($mg.L^{-1}$), salinity (PSU), nitrate ($mg.L^{-1}$), and phosphate ($mg.L^{-1}$) which were also taken at 0-20 m in depth. The first four factors were measured using CTD SBE 19, while nitrate and phosphate were preserved in cooler before being measured using a GENESYS 10S UV-Vis spectrophotometer.

Data analysis

The association of algae growth and its environmental factors was analyzed using Pearson correlation. Meanwhile, t-test was used to check equality of algae density during west monsoon dan first transition season. On the other hand, the relationship between abundance of certain species and its environmental variables can be analyzed by using Canonical Correspondence Analysis (CCA). It is a constrained ordination method in which the variation of a set response variables (environmental variables) explained by a set predictor variable (species abundance) (Greenacre 2010). As an exploratory constrained method, CCA highlights graphical representations of the results (González et al. 2008). The data analysis in this research performed in PAST version 4.0.

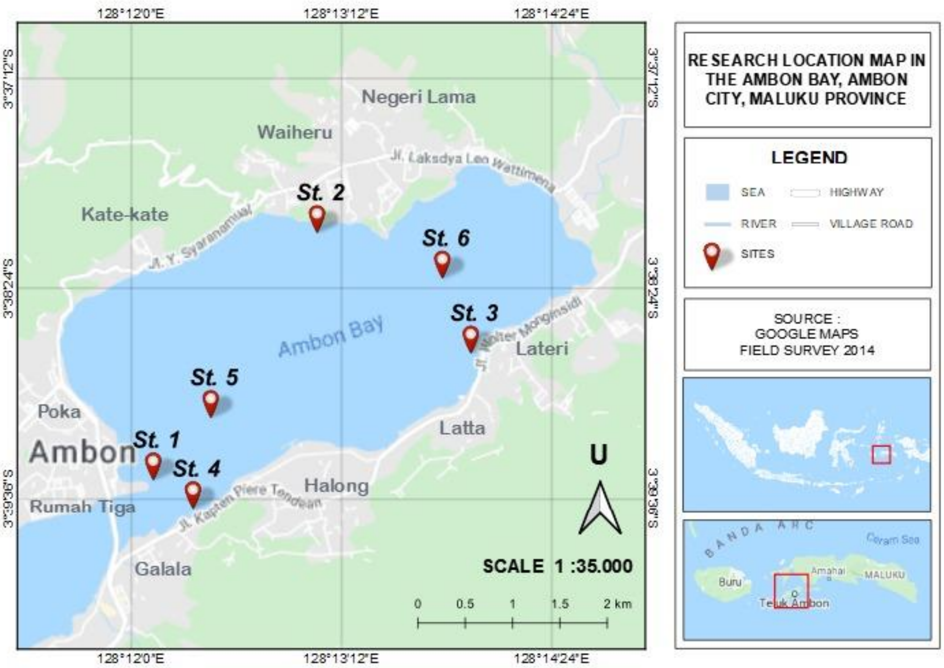

Figure 1. Research location in Ambon Bay, Indonesia where the samples were collected during the west monsoon and first transition period (2015)

RESULTS AND DISCUSSION

Environmental factors measurement results

The result of environmental measurements during this study is presented in Table 1.

It can be seen in Table 1 that the measurement range of water temperature was between 27 and 29^{0}C. These fall in the ideal temperature for phytoplankton growth (Boyd et al. 2013). The water temperature during first transition season (April) was constant at 29 ^{0}C, while during west monsoon season (January) were recorded 27-28^{0}C. On the other hand, salinity also determines the community structure of phytoplankton in marine open waters (Redden dan Rukminasari 2008). It was stated that a rise in salinity will inhibit the growth rate of phytoplankton because of osmoregulation disruption (Redden and Rukminasari 2008; Chakraborty et al. 2011). In this study, the salinity was shown between 32.10 and 33.10 practical salinity unit (PSU) where can be considered as low salinity compared to most open water which lies from 33 to 37 PSU (Srokosz and Banks 2019).

In January, the pH was reported around 6.7 classified as acidic while pH in April was measured more than 7.60 or alkaline. This is an ideal pH for marine phytoplankton growth which suggested between 6.3 and 10 (Hinga 2002). There was significant correlation between pH and phytoplankton density in this study (r=0.68, p=0.012). Similarly, DO concentrations in first transition season were higher than those in west season. It was recorded between 5.70 and 6.70 mg.L^{-1}. This factor has strong negative association with temperature (Koralay et al. 2018), whereas the decrease of DO will result in obstruction of algae diversity and biomass (Haas et al. 2014). However, this study suggested positive correlation from both factors instead. It might be caused by the influence of another environmental factor to DO such as nitrate (Akaahan dan Azua 2016).

Nitrate and phosphate (nutrient) are major determinants for algae growth. Inadequacy of these nutrients is limiting factors in algae production (Wisha et al. 2018). Generally, nitrate concentrations during first transition season were lower than those during west monsoon season. These measurement results in both seasons and sites were beneath the water quality standard by the Indonesian Government that set 10 mg.L^{-1}. Furthermore, phosphate concentrations were also ranged under the official standard set by Ministry of Environment in Indonesia (0.2 mg.L^{-1}) (Ministry of Environment 2001). However, phosphate measurements in first transition season were higher compared to west monsoon season. This study showed that there was significant correlation of nitrate and algae growth (r=0.54, p=0.068) as well as phosphate and algae growth (r=0.71, p=0.009). Although both nutrients were not surpassing the standard value, uncontrolled nutrient enrichment will result to eutrophication and harmful algae bloom (Lusiana et al. 2019).

Phytoplankton community in Ambon Bay

The distribution of phytoplankton community in Ambon Bay is showed in Table 2, while the community structure depicted in Figures 2 and 3.

From Table 2, the community structure of phytoplankton during west monsoon and first transition season was quite similar. Only *Noctiluca* sp and *Thalassiosira* sp from Bacillariophyceae division that was absent during first transition period. In total, the number of algae biomass in Ambon Bay amidst west monsoon and first transition season was estimated at 7.585 x 10^6 cell.L^{-1} and 9.409 x 10^6 cell.L^{-1}, respectively. Figure 2 and Figure 3 show the algae were comprised of three divisions that were Bacillariophyceae, Dinophyceae, and Cyanophyceae. Bacillariophyceae was the most dominant division which represented around 60% of the overall biomass (5.458 x 10^6 cell/L in first transition season and 5.059 x 10^6 cell.L^{-1}during west monsoon season). This division consisted of 11 genera (*Rhizosolenia, Chaetoceros, Skeletonema, Thalassionema, Nitzschia, Bacteriastrum, Thalassiothrix, Noctiluca* sp, *Thalassiosira* sp, *Biddulphia* sp) in west monsoon season. *Chaetoceros* has mechanics effect to respiratory organs of the fish (Li et al. 2017), while *Skeletonema* will cause hypoxia and anoxia to the water (Shumway et al. 2018). On the other hand, *Nitzschia* contains biotoxin that threatens human health namely ASP or Amnesic Shellfish Poisoning (Shumway et al. 2018).

Dinophyceae division comprised 27% of overall phytoplankton biomass in west monsoon season and 40% amidst first transition season. There were 4 genera from this group (*Ceratium, Pyrodinium, Dinophysis, Alexandrium*). Algae from genus *Ceratium* create blooms as the result of its versatility and hostility to sedimentation and enhanced utilization of light and nutrients (Donagh et al. 2005). *Ceratium* bloom can create anoxic situation to aquatic environment, and threaten local population of aquatic organisms such as lobsters (Pitcher and Probyn 2011).

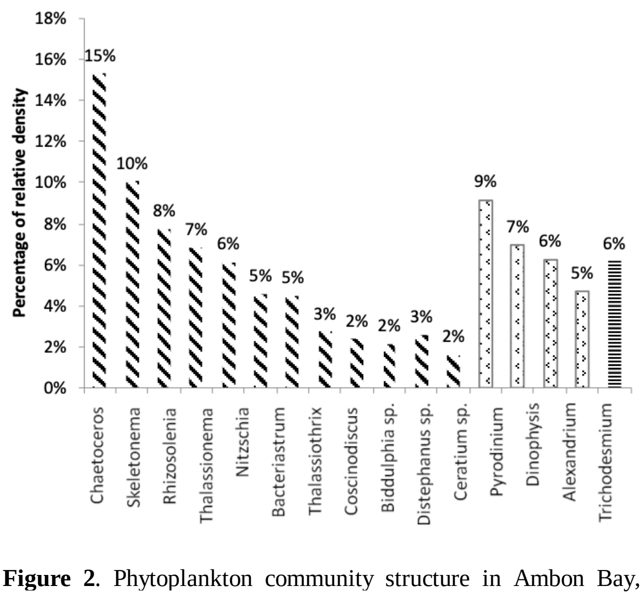

Figure 2. Phytoplankton community structure in Ambon Bay, Indonesia during west monsoon season (2015)

Table 1. Water parameters in Ambon Bay, Indonesia during the west monsoon and first transition period (2015)

Variables	**West monsoon season**						**First transition season**					
	St. 1	**St. 2**	**St. 3**	**St. 4**	**St. 5**	**St. 6**	**St. 1**	**St. 2**	**St. 3**	**St. 4**	**St. 5**	**St. 6**
Temperature (°C)	27.00 ± 0.82	27.00 ± 0.96	28.00 ± 0.96	28.00 ± 0.96	28.00 ± 1.50	28.00 ± 1.41	29.00 ± 1.50	29.00 ± 1.41	29.00 ± 1.00	29.00 ± 1.00	29.00 ± 1.26	29.00 ± 1.29
Salinity (PSU)	33.10 ± 0.14	33.10 ± 0.22	32.11 ± 0.59	32.28 ± 0.17	32.10 ± 0.62	32.10 ± 0.62	32.17 ± 0.57	32.11 ± 0.64	32.11 ± 0.60	32.18 ± 0.58	32.10 ± 0.68	32.10 ± 0.72
pH	6.73 ± 0.44	6.74 ± 0.34	6.71 ± 0.43	6.71 ± 0.40	6.72 ± 0.48	6.72 ± 0.48	7.69 ± 0.07	7.70 ± 0.05	8.00 ± 0.05	7.80 ± 0.13	7.77 ± 0.16	7.68 ± 0.20
DO ($mg.L^{-1}$)	5.80 ± 0.41	5.70 ± 0.19	5.70 ± 0.41	5.80 ± 0.28	5.70 ± 0.36	5.70 ± 0.37	6.30 ± 0.54	5.90 ± 0.26	6.20 ± 0.44	6.70 ± 0.65	6.10 ± 0.32	5.90 ± 0.39
Nitrate ($mg.L^{-1}$)	3.42 ± 0.09	2.85 ± 0.01	3.52 ± 0.04	3.22 ± 0.22	2.97 ± 0.03	2.97 ± 0.08	1.39 ± 0.04	1.34 ± 0.01	1.43 ± 0.02	2.34 ± 0.13	1.11 ± 0.10	1.34 ± 0.01
Phosphate ($mg.L^{-1}$)	0.0042 ± 0.002	0.0042 ± 0.002	0.0041 ± 0.002	0.0045 ± 0.002	0.0041 ± 0.002	0.0041 ± 0.002	0.050 ± 0.014	0.051 ± 0.014	0.053 ± 0.013	0.055 ± 0.013	0.049 ± 0.014	0.051 ± 0.012

Table 2. Phytoplankton found in Ambon Bay, Indonesia during west monsoon and first transition period (2015)

Phytoplankton	West monsoon season	First transition season
Bacillariophyceae		
Rhizosolenia	+	+
Chaetoceros	+	+
Skeletonema	+	+
Thalassionema	+	+
Nitzschia	+	+
Bacteriastrum	+	+
Thalassiothrix	+	+
Coscinodiscus	+	+
Noctiluca sp	+	-
Thalassiosira sp	+	-
Biddulphia sp	+	+
Distephanus sp	+	+
Dinophyceae		
Ceratium	+	+
Pyrodinium	+	+
Dinophysis	+	+
Alexandrium	+	+
Cyanophyceae		
Trichodesmium	+	+

Meanwhile, particular *Pyrodinium* species produce biotoxin like ciguatera, Diarrhetic shellfish poisoning (DSP), Neurotoxic shellfish poisoning (NSP), and Paralytic shellfish poisoning (PSP) (Wang 2008). Moreover, some Dinophysis species create diarrhoetic toxins and pectenotoxins, These are causing and cause gastrointestinal infection, even at little densities (Shumway et al. 2018). Genus *Alexandrium* has widely known as one of the most important HABs species with regard to the severity and distribution of the impact. Organism from this genus capable to produce three different toxins (saxitoxin, spirolides, and goniodomins) (Jedlicki et al. 2012).

Trichodesmium was the only genera found from Cyanophyceae division that comprised only 6% and 2% of the overall phytoplankton biomass. It contains saxitoxin but has not been announced to create threaten human health yet. However, *Trichodesmium* can easily live in disadvantageous environment. Hence, it usually presences in massive bloom which covers marine area and familiarly called red tide (Jiang et al. 2017). Based on the t-test, the algae density during west monsoon dan first transition season was significantly different (t=2.074, Sig.=0.000).

Relationship analysis of environmental factors and potential HABs

This following Figure 4 presents triplot from the CCA which used data combination taken from west monsoon and first transition season. The analysis required water parameters or the environmental variables (temperature, salinity, pH, DO, nitrate and phosphate) which considered independent variables, while the algae abundance (potential HABs) that classified into its division became the group of dependent variables.

Figure 4 shows that Bacillariophyceae species were likely to present in high salinity, mid to low nutrient concentration and DO, colder temperature, and acidic waters. The shortest projection of Bacillariophyceae was on to temperature, pH, and phosphate concentration. These variables measurement during first transition season were greater than those during west monsoon season. Hence, Bacillariophyceae density amidst this season was totaled to 5.458 x 10^6 cell.L^{-1} compared to 5.059 x 10^6 cell.L^{-1} during west monsoon season. Specifically, algae from *Chaetoceros* genus can reach its maximum growth rate under high temperature (Spilling et al. 2015) and proportional to nutrient concentration (Hemalatha et al. 2012). Therefore, *Chaetoceros* density experienced remarkable increase from west monsoon season to first transition season (Figures 2 and 3).

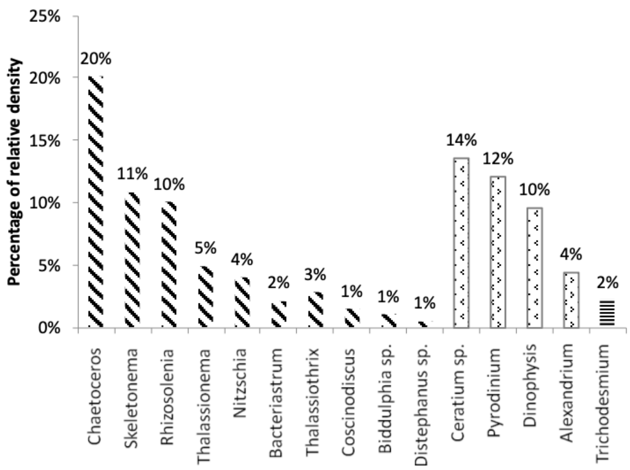

Figure 3. Phytoplankton community structure in Ambon Bay, Indonesia during first transition season (2015)

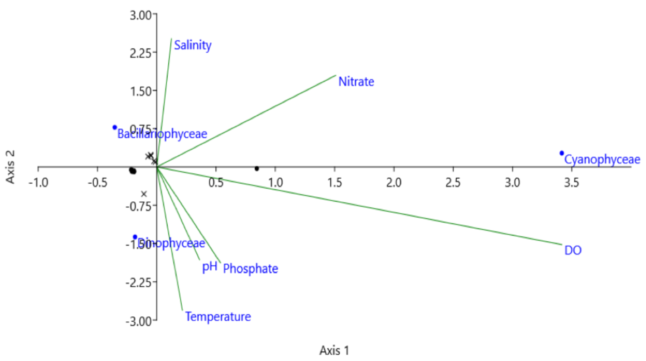

Figure 4. Triplot of CCA Result

The appearance of Dinophyceae was corresponding with high temperature and phosphate concentration, low DO and nitrate concentration, high values of pH and salinity. It has nearest projection on temperature and pH. Identically to Bacillariophyceae, this genus also rises considerably from January (2058 cell/L) to April (3748 cell/L). In particular, *Ceratium* showed notably increasing among other genera in Dinophyceae division. The density of *Ceratium* follows seasonal patterns whereas it reached maximum in warmer season (Pereira et al. 2016). Furthermore, the highest growth of this genus observed at pH 7.5-8.0 (Hansen 2002) which was exactly reported in this study during first transition season (Table 1).

Lastly, Cyanophyceae, which only represented by *Trichodesmium*, realized on high measurement of all environmental factors. This object has close distance to nitrate. As a result, Cyanophyceae had greater abundance during west monsoon season which contains high nitrate concentration. *Trichodesmium* uptake nitrogen from nitrate to conduct dinitrogen fixation (Eichner et al. 2017). Nitrogen cannot be assimilated by most organisms and used in their nutrition (Breitbarth et al. 2007). As a consequence, nitrogen requires to be fixed so that it can be used by organisms, whereas one of the organisms that can do this is Cyanobacteria (Holl and Montoya 2005).

The result of this study reflects that even in environmental conditions during west monsoon and first transition season, which consider to retain algae bloom, yet the phytoplankton community was dominated by genera that classified as HABs, such as *Chaetoceros, Skeletonema, Nitzschia, Ceratium, Pyrodinium, Dinophysis, Alexandrium,* and *Trichodesmium.* They comprised around 90% of the biomass. As a consequence, HABs occurrence in Ambon Bay has been reported frequently (Thoha 2016). Environmental factors that highly associated with the presence of the HABs organism were temperature and nutrient. Warmer temperature that might be induced by climate change and global warming is likely to trigger HABs occurrence (Watson et al. 2015; Redzuan and Milow 2019; Gobler 2020). Nutrient enrichment also needs to be taken into account to prevent further HABs presence and even eutrophication by reducing waste discharge into waters (Lusiana et al. 2019).

From this study, it can be concluded that Ambon Bay has frequently experienced HABs during the past decade. The blooms caused by algae from Bacillariophyceae, Dinophyceae, and Cyanophyceae division. This research demonstrated that many observed algae genera were dominated by harmful algae species during west monsoon and first transition season. In general, the phytoplankton biomass amidst first transition season was higher than that in west monsoon season, mostly caused by warmer temperature. Moreover, nutrient availability and pH also highly determine the presence of harmful algae in Ambon Bay. Future research is suggested to observe the harmful algae presence during east monsoon and second transition season too. This will enhance the temporal analysis related to the issue.

ACKNOWLEDGEMENTS

We wish to show appreciation to our colleagues in AquaRES research group for their comments and advice during this manuscript preparation. This research was not a part of any grant from funding agencies either public or private institutions.

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