

Detection of marine microalgae (phytoplankton) quality to support seafood health: A case study on the west coast of South Sulawesi, Indonesia

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Abstract. *Tambaru R, Burhanuddin AI, Massinai A, Amran MA. 2021. Detection of marine microalgae (phytoplankton) quality to support seafood health: a case study on the west coast of South Sulawesi, Indonesia. Biodiversitas 22: 5179- 5186.* The research aimed to detect marine microalgae quality to support seafood health was carried out from January to November 2020 along the west coast of South Sulawesi, Indonesia. Samples were collected from the coastal waters of Pangkep District, Maros District, and the northern part of Makassar City. Phytoplankton cell counts were obtained using the deposition method developed by Uthermol. Phytoplankton cell abundances were calculated through sweeping (census) using a Sedgwick Rafter Cell (SRC). Two-way analysis of variance (ANOVA) was used to compare the distribution of marine microalgae community abundance between observation stations and periods. Based on the types and relative abundance of phytoplankton present, i.e., harmful algal bloom (HAB) forming or not (non-HAB), the results showed the quality of marine microalgae, specifically, phytoplankton was relatively good. Many more non-HAB (94-98%) than HAB (2-6%) marine microalgae were detected. Thus, the phytoplankton flourishing in these waters is mostly suitable as food for other organisms, including fish and shellfish. This also means that if fishers harvest these fish and shellfish, they should be fit and safe for human consumption.

Keywords: HABs, health, marine, microalgae, phytoplankton, seafood

INTRODUCTION

Microalgae are the most common and abundant unicellular organisms found in all water bodies, including coastal and marine waters (George et al. 2012; Desrosiers et al. 2013). These organisms are a group of microscopic plants, some of which are single-celled while others are colonial, which live in all seas and freshwater bodies. These microalgae are often referred to as phytoplankton (Sunda 2012; Shadrin et al. 2017).

Periodic increases in microalgae populations are natural phenomena (Visciano et al. 2016). They do not always cause detrimental effects so long as algal growth remains within normal limits and does not cause a significant disturbance to the surrounding ecosystems (Gao et al. 2020). However, if there is a dramatic increase in the population of unwanted or harmful organisms, the situation can require an immediate response (Zohdi and Abbaspour 2019). These population explosions can trigger problems that impact the lives of other organisms; examples include mass fish kills (Work et al. 2017), the contamination of seafood leading to public health problems (food poisoning) (Kudela et al. 2018), and changes to aquatic ecosystem community structure (Lu et al. 2018; Todd et al. 2019).

Rapid or excessive increases in the microalgae (phytoplankton) population, termed algal blooms, can occur when environmental conditions are conducive to algal growth and reproduction (Glibert 2017; Paerl et al. 2018; Meesters and Tapilatu 2020). When phytoplankton

population explosions include toxic microalgae, they can be extremely dangerous (Tian et al. 2018). These phenomena involving toxic microalgae are generally termed Harmful Algae Blooms, more often referred to by the acronym HABs, and have adverse effects on local coral reef, fishery resources, including mariculture and capture fisheries commodities such as oysters, shellfish, and fish (Anderson et al. 2015; Berdalet et al. 2016; Glibert 2017; Brown et al. 2019; Meesters and Tapilatu 2020).

Over time, environmentalists have become increasingly concerned about the incidence of HABs (Sha et al. 2021). Frequently discussed topics include the emergence of new types of HAB, the rising frequency of occurrence, the expansion of the geographic area affected, and the prolonged duration of their occurrence (Fu et al. 2012; Anderson et al. 2015; Wells et al. 2015; Berdalet et al. 2016; D'Costa et al. 2017; Paerl et al. 2018; Xiao et al. 2019; Trainer et al. 2020). Recent studies have also found novel toxins in HABs (Anderson et al. 2015; Lassus et al. 2016). These factors all contribute to increases in HAB-associated mortality of marine organisms (Fukuyo et al. 2011).

HABs are phenomena that commonly occur in water bodies, especially marine and coastal waters. HABs occur when certain groups of phytoplankton (HABs forming species) experience population explosions and have negative impacts on other aquatic organisms as well as on human health (Berdalet et al. 2016, 2018). According to Xiao et al. (2019), the factors that can trigger a

phytoplankton population explosion that can be qualified as a HABs include nutrient enrichment (eutrophication), the occurrence of heavy rains increasing the flow of nutrient-loaded river water into the sea (Hughes et al. 2011), and upwellings (Loureiro et al. 2011).

Some of the acute diseases caused by toxins from microalgae in the HABs group are paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) (Fukuyo et al. 2011; Krock et al. 2018), and ciguatera fish poisoning (CFP) (Skinner et al. 2011). These toxins are hazardous to human health because they attack the nervous system and interfere with respiration and digestion. These diseases are related to the human consumption of fish and shellfish. Many types of toxic phytoplankton can be found in Indonesian coastal waters, including several Dinoflagellate species from the genera *Noctiluca*, *Gymnodinium*, *Cochlodinium*, *Ceratium*, *Peridinium*, *Gonyaulax*, *Ostreopsis*, *Prorocentrum*, and *Gambierdiscus* (Adnan 1984; Adnan 1989; Skinner et al. 2011; Hasani et al. 2013; Aditya et al. 2015). One HAB event in 1993 took place in Jakarta Bay, where a mass fish-kill was caused by the excessive abundance of phytoplankton that can cause HABs. Similar HAB incidents have occurred in the waters of Lewotobi and Lewouran (East Nusa Tenggara), Sebatik Island (East Kalimantan), and Ambon Bay (Mahmudi et al. 2020).

Other events of interest have been observed in the northern and southern coastal waters of South Sulawesi. In November 2014, a mass fish-kill incident took place in estuarine coastal waters of Pangkep District, attracting public attention and suddenly creating awareness in an area where such an incident had never happened before. Environmental experts put forward various hypotheses to explain the mass fish-kill; causal factors proposed included pollution by organic matter, reduced oxygen content, the blooming of certain types of phytoplankton, and a decrease in the quality of river sediment (Rukminasari and Tahir 2020). Furthermore, in August 2016, around 63 local residents were poisoned due to eating shellfish, specifically clams or cockles of the genus *Anadara*. The clams were collected in the coastal waters of Mallasoro Village (on the south coast of South Sulawesi in Bangkala District, Jeneponto District. It is suspected that the clams were contaminated with toxins from potentially dangerous (toxin-producing) phytoplankton. Thus, more research is required to test the hypotheses and provide definitive answers regarding the incidents mentioned earlier.

Microalgal blooms can occur, are known to contaminate aquatic organisms such as fish and shellfish, and may have been a factor in both cases. In the Pangkep region, aquaculture and mariculture activities are common in both riverine and coastal waters (Lestari et al. 2021). In some cases, use various drugs and fertilizers to increase production; this is also the case in the coastal waters of Mallasoro Village, Jeneponto District, South Sulawesi. To date, there has not been any definitive answer regarding the factors which caused the two aforementioned possible HAB events. However, the potential role of the ongoing nutrient enrichment of riverine and coastal waters is one strong reason to justify analyzing the phytoplankton

community and monitoring change, specifically regarding the types of microalgae that can form HABs. Increases and or changes in nutrient concentration can occur due to seasonal changes between the east and west monsoons (Vajravelu et al. 2018; Rastina et al. 2020). These fluctuations will impact the microalgal communities, including the presence of HAB forming species whose presence can be seasonal (Narale and Anil 2017; Vajravelu et al. 2018; Trainer et al. 2020), and can therefore become a factor triggering the emergence and rapid growth of HABs.

There is a lack of comprehensive research on the presence and development of HABs in the coastal waters of South Sulawesi. The information available regarding the HAB-forming microalgae which may occur in the region is limited and partial. However, HABs species have been reported incidentally or as a small part of some studies in the waters of South Sulawesi with limited spatial or temporal coverage (Mujib et al. 2015; Rukminasari and Tahir 2020; Samawi et al. 2020; Lestari et al. 2021). Meanwhile, information on HAB forming phytoplankton is crucial for anticipating and early detection of HABs, and a basis for evaluating the quality of coastal waters concerning the potential impacts on the life of organisms at higher tropical levels (Glibert 2017).

To evaluate the prevalence of HAB forming phytoplankton, this study aimed to detect the quality of marine microalgae to support seafood health in a case study on the west coast of South Sulawesi, Indonesia. This research relates to the lives and livelihoods of the local communities, especially fishermen who depend on the coast and the sea for their lives. It will be of benefit through providing up-to-date data and information on the quality of marine microalgae along the west coast of South Sulawesi, in order to inform sustainable management of coastal resources, as an early detection mechanism for HABs-associated risks, and as a tool for evaluating water quality in the region.

MATERIALS AND METHODS

Research site and timeframe

The research was conducted from June to November 2020. Data were collected in the coastal waters off the west coast of South Sulawesi Province, Indonesia, during three periods (Table 1). The four research sites (Figure 1) were in Pangkep District (PK), Maros District (Kuri: KR, and Maros: MR), and the northern waters of Makassar City (Tallo: TL).

Table 1. Timing of sample collection and in situ measurement of parameters

Period	Time of day	Season
Jun-Jul 2020	10:00-16:00	Dry season
Aug-Sep 2020	10:00-16:00	Dry to the rainy season
Oct-Nov 2020	10:00-16:00	Beginning of the rainy season

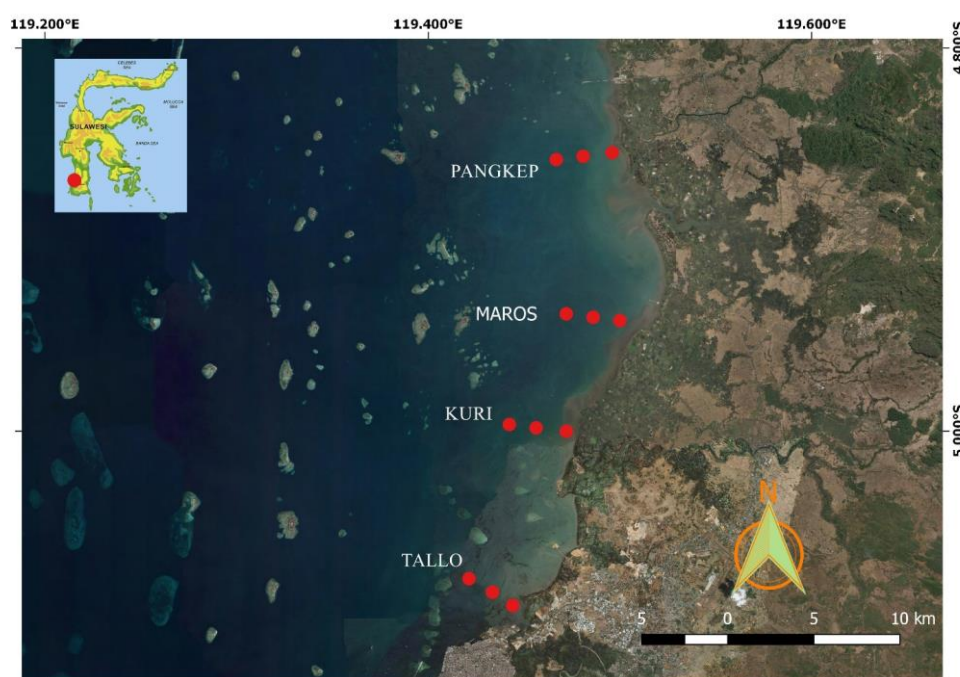


Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi, Indonesia

Materials and research design

The primary materials used in this research were seawater samples collected from three zones along an onshore-offshore gradient at each of the four stations during each period. Several oceanographic parameters were measured in the field (in situ), while others were measured in the laboratory. This study was non-experimental. The variables were observed without any manipulation or intervention by the researchers. The variables analyzed were the abundance and composition of the phytoplankton community, which included both HAB and non-HAB forming species.

Phytoplankton analysis in the laboratory

Seawater samples were collected using a 2 L Kemmerer Water Sampler. At each site, 1 L of water was taken from each station (zone) to count and identify phytoplankton in the laboratory. Phytoplankton cells were precipitated out of the samples using the method developed by Utermöhl (Vadrucci et al. 2018). A 100 mL sub-sample was placed in a measuring beaker (volume 100 mL) and preserved in Lugol solution for one week. Once precipitation had occurred, the precipitated material (10 mL) was separated from the supernatant by siphoning the supernatant out of the beaker. This precipitate was then placed in a bottle to which more Lugol solution was added. The abundance of phytoplankton cells was calculated using a sweeping (census) method (Rocha et al. 2015) using a 50 mm x 20 mm x 1 mm Sedgwick Rafter Cell (SRC). A 1 mL aliquot of the precipitate was placed in the SRC using a graduated pipette. The SRC was observed under a binocular microscope (Olympus CX21) at 10x10 magnification. Phytoplankton cells were identified using several standard

reference works (Tomas 1997; Castellani and Edwards 2017).

Statistical analysis

The data were analyzed descriptively through tabulation and graphical approaches. These included the use of graphs and maps to represent the taxonomic composition and the spatial and temporal distribution of the microalgae identified. A two-way analysis of variance (ANOVA) was used to evaluate the spatial and temporal differences in microalgal community abundance, in particular that of HAB-forming phytoplankton. Post-hoc Tukey tests were carried out if the ANOVA indicated significant differences at the 95% confidence level (α : 0.05). Prior to testing, all parameters were first tested for normality of the data distribution using Kolmogorov-Smirnov and Levene's Test of Equality. These analyses were implemented in SPSS 17 and Excel Stat 2017 (Brahem et al. 2017).

RESULTS AND DISCUSSION

Detection of marine microalgae (phytoplankton)

During this study, 22 phytoplankton genera belonging to 3 classes were identified. These comprised 16 genera of the Class Bacillariophyceae, 1 genus of the Class Cyanophyceae, and 5 genera of the Class Dinophyceae. The genus-level composition varied spatially (between sites/stations) and temporally (between study periods); however, for almost all sampling periods and sites, the dominant phytoplankton genera were *Chaetoceros*, *Coscinodiscus*, *Nitzschia*, *Odontella*, and *Rhizosolenia*, all of which belong to the Class Bacillariophyceae.

The phytoplankton density in the samples (Figure 2) indicates relative abundance. Over the study period, mean phytoplankton abundance was higher at the Tallo and Kuri sites than at the Maros and Pangkep sites. The analysis of variance indicated a significant difference in phytoplankton abundance between the sites/stations ($p < 0.01$). The observed phytoplankton abundance also varied between the periods. However, the analysis of variance indicated that the differences in the abundance of phytoplankton over time were not statistically significant throughout observation ($p > 0.05$).

Types of marine microalgae (phytoplankton) considered dangerous because they can form HABs were present at almost all sites/stations (Figure 3). The taxa identified included 5 potentially HAB-forming phytoplankton genera belonging to the Class Dinophyceae: *Ceratium*, *Dinophysis*, *Gymnodinium*, *Prorocentrum*, and *Protoperdinium* (Figure 4). These genera were present in most sites/stations and observation periods.

The presence and abundance of the potentially HAB forming taxa varied between sites/stations and periods of observation (Figure 3). The analysis of variance revealed highly significant differences ($p < 0.01$) in the abundance of HAB forming taxa between sites/stations.

Quality of marine microalgae (phytoplankton)

The phytoplankton identification and counts revealed that non-HAB phytoplankton were both more taxonomically diverse and far more abundant than the HAB phytoplankton. The non-HAB phytoplankton comprised 94-98% of the microalgae cells, counted while HAB forming phytoplankton accounted for 2-6% (Figure 5).

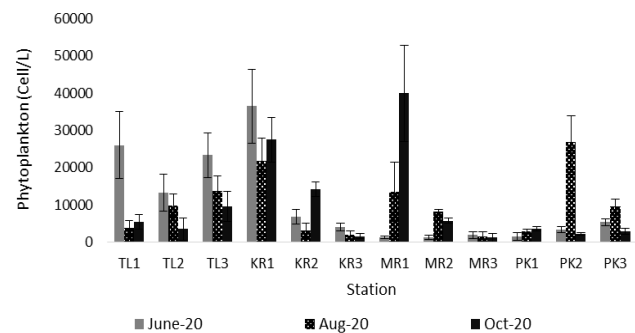


Figure 2. Phytoplankton density at the sampled sites in South Sulawesi over the research period (mean \pm SD)

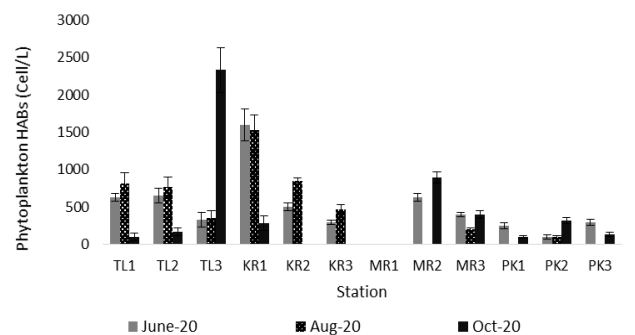


Figure 3. HAB forming phytoplankton density in samples from South Sulawesi over the study period (mean \pm SD)

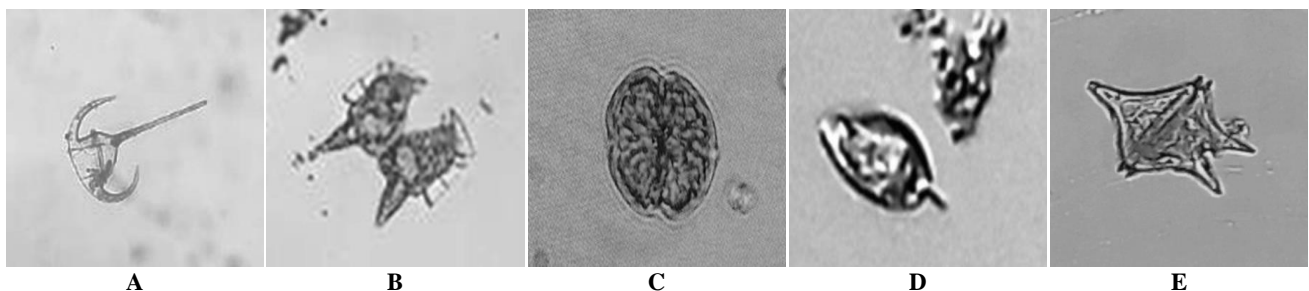


Figure 4. Examples of phytoplankton from class Dinophyceae. A. *Ceratium*, B. *Dinophysis*, C. *Gymnodinium*, D. *Prorocentrum*, E. *Protoperdinium*

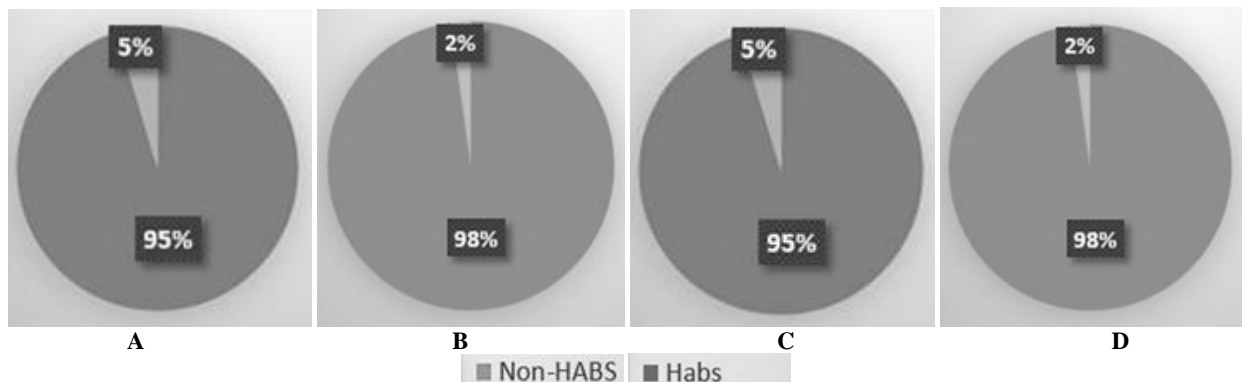


Figure 5. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period. A. Tallo, B. Kuri, C. Maros, D. Pangkep

Discussion

The dynamics of marine microalgal communities vary from one site to another and from one time to another. Changes in community composition frequently occur (Marinov et al. 2010; Fujiwara et al. 2018). The survival and replication of phytoplankton are greatly influenced by various environmental parameters (Baek et al. 2020). In response to environmental changes, these microalgal communities will comprise different types of phytoplankton with shifts in the relative abundance of each type. At certain times some groups are found to be abundant, at other times other groups will dominate the community (Thovyan et al. 2020).

Changes in various environmental parameters have an impact on the population dynamics of the phytoplankton that can cause HABs (Cui et al. 2018). If these changes favor the growth and replication of HAB-forming taxa, the equilibrium of the ecosystem can be affected, reducing the quality of marine microalgae with knock-on effects on other organisms. Ecologically, phytoplankton forms the basis of most food chains; thus, their abundance and composition affect the existence of almost all aquatic biota (Brett et al. 2009; Cavicchioli et al. 2019). Therefore, information on the characteristics of the phytoplankton present in particular waters can indicate their ability to support aquatic life. During the study, five genera were dominant at almost all sites/stations and observation periods: *Chaetoceros*, *Coscinodiscus*, *Nitzschia*, *Odontella*, and *Rhizosolenia*. All of these species belong to the Class Bacillariophyceae. *Chaetoceros* were the most abundant genus and classes for all sites, zones, and periods of observation. These taxa are often present at high densities in marine waters worldwide (Sunesen et al. 2008; Angara et al. 2013), including Indonesia (Takarina et al. 2019). Usually, *Chaetoceros* species are abundant in areas where nitrogenous nutrients, especially nitrates, are below the optimal concentration range for the growth of phytoplankton. Indeed, Ferreira et al. (2020) stated that *Chaetoceros* can still exhibit comparatively fast growth when low N-type nutrient concentrations.

The Tallo site/stations had the highest phytoplankton abundance, differing significantly from Maros and Pangkep ($p < 0.01$); however, the abundance at the Kuri site was not significantly different from that at Tallo. Different results occurred in observing the abundance of phytoplankton based on the observation period. Based on the analysis of variance, the abundance of phytoplankton was not significantly different based on the period of observation ($p > 0.05$). The analysis results are supported by *in-situ* water quality parameters, as several parameters that can affect phytoplankton growth had almost the same value during the three periods.

Types of marine microalgae which are considered dangerous as HABs forming phytoplankton were present at almost all sites/stations (Figure 3). These observations are an important finding as, under certain conditions, these microorganisms can negatively affect water quality if they multiply excessively (blooming), endangering the lives of other aquatic organisms, even humans when they become dominant. Through the food chain, the accumulation of

toxins in the body of organisms that consume HABs forming microalgae can cause health problems and even death in humans (Pettersson and Pozdnyakov 2013).

The taxa identified included five potentially HABs forming phytoplankton genera belonging to the Class Dinophyceae: *Ceratium*, *Dinophysis*, *Gymnodinium*, *Prorocentrum*, and *Protoberidinium*. These genera were present in most sites/stations and observation periods. The classification of these taxa as presenting a risk of HABs can be justified because the Dinophyceae contain the most toxic genera (Tillmann et al. 2010). These five HABs genera produce toxic metabolites, so they belong to the toxin producer group (Kudela et al. 2018). If these metabolites accumulate in the bodies of marine organisms such as shellfish and fish, they can cause poisoning in humans who consume this seafood (Farabegoli et al. 2018).

The abundance of HABs in surface waters can be influenced by aquatic sediment dynamics. HABs can remain for long periods in aquatic sediments in the form of cysts, such as *Gymnodinium* cysts from the Class Dinophyceae (Dzhembekova et al. 2018). According to Fukuyo et al. (2011), phytoplankton in this class play an important role in recurring HAB events and contribute to the geographical expansion of HAB occurrences. These repeat HABs can occur when many cysts of potentially HAB-forming Dinophyceae are deposited in marine sediments (Narale and Anil 2017; Trainer et al. 2020). Turbulence can disturb the sediment and raise these cysts to the surface layer of the water. Such events can trigger an explosion of HABs if prevailing environmental conditions support their growth.

Ceratium does not belong to the toxic phytoplankton group. However, this genus can cause various problems in the waters if it blooms (Praseno and Sugestiniingsih 2000). In addition, this organism is considered to be a threat to the aquatic environment because it can cause oxygen deprivation through decreasing oxygen concentration in the water. *Ceratium* can result in mass mortality of marine life due to decreased oxygen levels if the population becomes too abundant (Thoha and Rachman 2012). *Dinophysis* belongs to the HAB-forming group because it contains toxins that can cause diarrhetic shellfish poisoning (DSP) if humans consume contaminated shellfish (Taylor et al. 2013). According to (Dietrich et al. 2019), the symptoms of poisoning caused by *Dinophysis* (DSP) include diarrhea induced by okadaic acid. *Gymnodinium* is a type of HAB-forming phytoplankton that causes red tides and oxygen depletion (Pitcher and Probyn 2016). *Gymnodinium* can cause paralytic shellfish poisoning (PSP) so that consuming contaminated shellfish can cause paralysis in humans (Rodrigues et al. 2012). *Protoberidinium* species contain toxins called azaspiracids; the symptoms in humans who ingest this poison are somewhat similar to those of DSP, and poisoning can cause nausea in the victim within 3-5 days (Trainer et al. 2013).

The presence and abundance of the HAB forming taxa varied significantly (ANOVA, $p < 0.01$) between sites/stations and periods of observation (Figure 3). While visually Tallo and Kuri appear to differ from the Maros and Pangkep sites, the Tukey test showed that the Pangkep site

differed significantly from the other three sites (Tallo, Kuri, and Maros). Although the abundance of HAB taxa differed between the periods, the lack of statistical significance (ANOVA, $p > 0.05$) indicates that the presence and abundance of taxa capable of forming HABs can be considered similar throughout the study period.

Overall, the quality of marine microalgae is determined by the types and relative abundance of phytoplankton that can form HABs (HAB) and those which do not form HABs (non-HAB) phytoplankton in the water. HAB-forming phytoplankton can multiply rapidly when changes occur in ambient environmental conditions. The Dinophyceae can tolerate environmental changes such as nutrient enrichment (eutrophication) (Tester et al. 2020). If eutrophic conditions occur, phytoplankton from the Dinophyceae will tend to multiply more rapidly and may form HABs (Glibert 2017). Once the HABs have begun to form, non-HAB phytoplankton may experience a decline in abundance and or diversity (Glibert 2020; Lestari et al. 2021). One reason for this decline is that when the HABs phytoplankton is multiplying, they release toxins (Glibert 2017, 2020); these may impede the growth and reproduction of the non-HAB phytoplankton as well as affect other organisms (Dorham 2014; Glibert 2017). Conversely, a greater variety of non-HAB phytoplankton indicates a thriving phytoplankton community that can support the life of other organisms.

The results indicate that changes in water quality may occur at the study sites, but these changes can still be tolerated by non-HAB phytoplankton. In addition to the taxonomic composition, the abundance of each taxon also plays a role in determining the quality of the microalgal community present. Good quality means, *inter alia*, that non-HAB phytoplankton abundance substantially exceeds that of HAB phytoplankton (Glibert 2017). As this was the case at all study sites and observation periods, there is a reasonable likelihood that the waters are still in good condition and have not significantly changed, as shown by the relative abundance of HAB phytoplankton and non-HAB phytoplankton (Figure 5). The types of phytoplankton present can mostly be considered of good quality for supporting the life of other organisms, including fish and shellfish. Their presence also indicates that fisheries produced from the study sites should be fit and safe for consumption, as contamination from HAB taxa is very unlikely at the low levels detected. This information is certainly encouraging for coastal communities, as their seafood is still suitable for consumption concerning HABs.

In conclusion, the observed composition of the marine microalgal communities present in the coastal waters along the west coast of South Sulawesi indicates that the phytoplankton quality can be considered reasonable. This evaluation is based on the taxonomic composition and abundance of non-harmful (non-HAB) phytoplankton, which was far more abundant than the taxa, which can cause HABs. This shows that seafood is still suitable for consumption because HAB taxa can be considered not yet present at contaminating levels. Even though the proportions of HAB forming taxa present were very low, their presence is an early warning that vigilance is needed. These results highlight the importance of maintaining water

quality and the necessity of regular water quality monitoring, as suggested by Anderson et al. (2015), to ensure that HABs do not develop undetected.

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