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(NIP. 196901251993031002, Universitas Hasanuddin)

Judul: 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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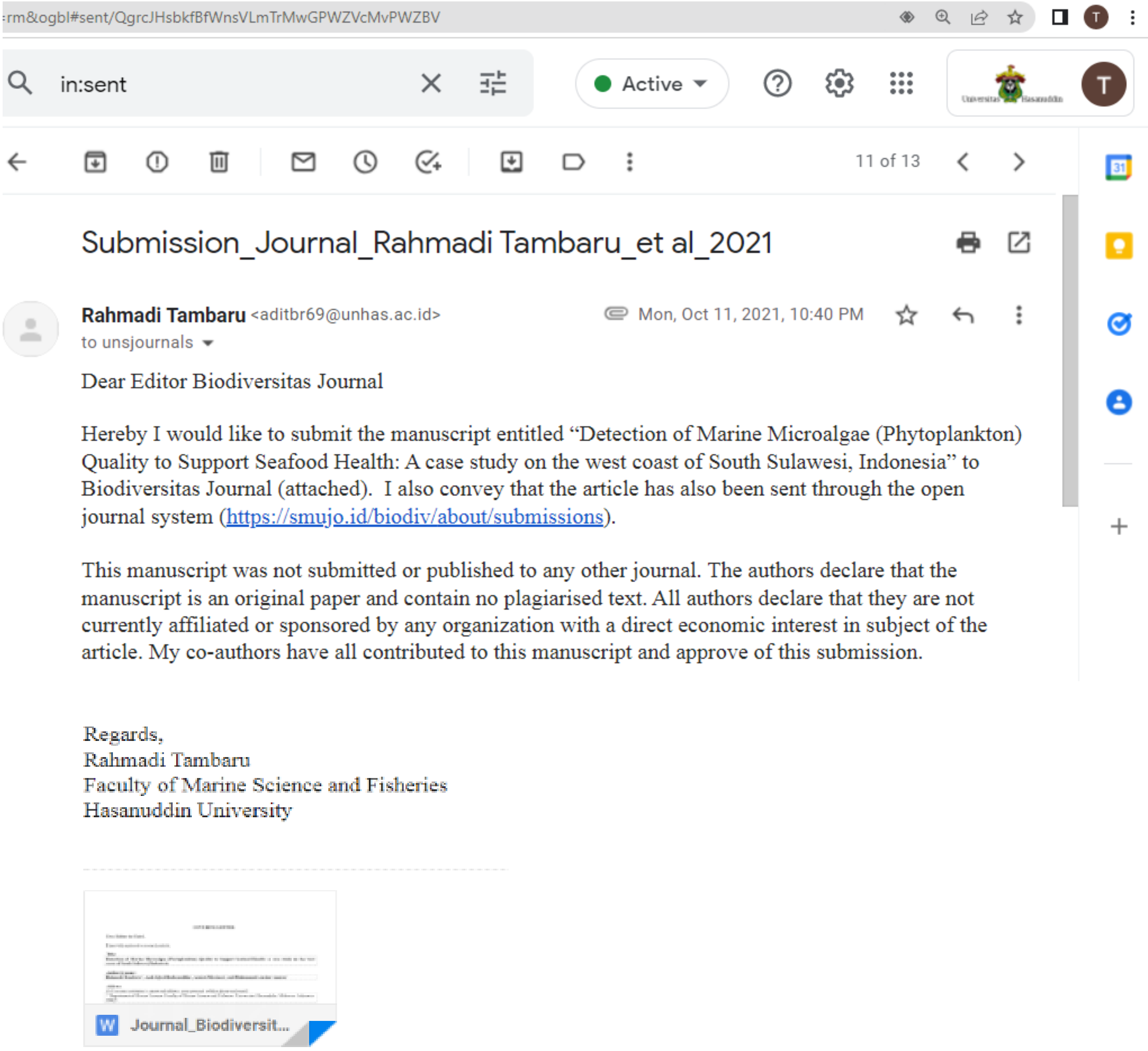

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Hereby I would like to submit the manuscript entitled "Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sulawesi, Indonesia" to Biodiversitas Journal (attached). I also convey that the article has also been sent through the open journal system (https://smujo.id/biodiv/about/submissions).

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A2. COMMENTS FOR EDITOR: NOVELTY STATEMENTS

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sulawesi, Indonesia

Author(s) name:

Rahmadi Tambaru[1*], Andi Iqbal Burhanuddin[1], Arniati Massinai[1], and Muhammad Anshar Amran[1]

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(Fill in your institution's name and address, your personal cellular phone and email)

[1*] Department of Marine Science, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia, 90425

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Answering the hypothesis regarding mass fish deaths caused by organic matter pollution, and the blooming of certain types of phytoplankton in coastal waters, especially in Pangkep waters.

This study provides the latest data and information on the quality of marine microalgae in supporting food health in the Coastal area, for sustainable management of coastal waters, as early detection, and as evaluation material in observing water quality, especially in the coastal waters of South Sulawesi.

In particular, research on Marine Microalgae (Phytoplankton) especially HABs (Harmful Algae Blooms) for the coastal waters of South Sulawesi has never been carried out comprehensively. The available information regarding the types that fall into the category of HABs, is only a small part of the conclusions of a study, and incidentally reported HABs in a study. Thus, the results of this study are important as part of information and evaluation to examine the quality of coastal waters in relation to the life of organisms at higher trophic levels.

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Place and date:

Makassar, October 8, 2021

Sincerely yours,

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Dr Ir Rahmadi Tambaru, M.Sc

A3. PRELIMINARY DRAFT (STATUS SUBMITTED)

Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sulawesi, Indonesia

RAHMADI TAMBARU[1♥], ANDI I. BURHANUDDIN[1], ARNIATI MASSINAI[1], MUHAMMAD A. AMRAN[1]

[1]Department of Marine Science, Department of Marine Science, Faculty of Marine Science and Fisheries, Universitas Hasanuddin. Jl. Perintis Kemerdekaan KM 10 Tamalanrea, Makassar, South Sulawesi, Indonesia, 90425 Tel./Fax. +62-411-586025, ♥email: aditbr69@unhas.ac.id

Manuscript received: DD MM 2021 (Date of abstract/manuscript submission). Revision accepted: .... 2021.

Abstract. The research aims to detect the quality of marine microalgae to support seafood health was carried out from January to November 2020 in the west coast of South Sulawesi, Indonesia. The marine microalgae in question are phytoplankton. Samples were collected from the coastal waters of Pangkep Regency, Maros Regency, and the northern part of Makassar City. Phytoplankton cell counts were determined through the deposition method developed by Uthermol. The abundance of phytoplankton cells was 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 time periods. The results showed that the quality of marine microalgae, specifically phytoplankton, was relatively good, based on the type of phytoplankton present, i.e. harmful algal bloom (HABs) forming or not (non-HABs), and their relative abundance. There were many more non-HAB (94-98%) than HAB (2-6%) marine microalgae detected. This means that the quality of the types of phytoplankton flourishing in these waters makes them suitable as food for other organisms, including fish and shellfish. It also means that if these fish and shellfish are caught by fishermen they should be fit and safe for human consumption.

Key words: Marine, microalgae, phytoplankton, HABs, seafood, health

Abbreviations : Harmful algal bloom (HABs)

Running title: Phytoplankton Quality to Support Seafood Health

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 (Shadrin et al. 2017; Sunda 2012).

Periodic increases in microalgae populations are natural phenomena (Visciano et al. 2016) and 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 et al. 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) (Grattan et al. 2016), and changes to aquatic ecosystem community structure (Todd et al. 2019; Lu et al. 2018).

Rapid or excessive increases in the population of microalgae (phytoplankton), termed algal blooms, can occur when environmental conditions are conducive to algal growth and reproduction. 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.

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. 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), upwellings (Loureiro et al. 2011), and the occurrence of heavy rains increasing the flow of nutrient-loaded river water into the sea (Hughes 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), and diarrhetic shellfish poisoning (DSP) (Krock et al. 2018). These toxins are very dangerous because they attack the human nervous system, respiration, and digestion. These diseases are related to the human consumption of fish and shellfish. In fact, all types of toxic phytoplankton can be found in Indonesian coastal waters, including several Dinoflagellate species from the genera *Ostreopsis*, *Prorocentrum,* and *Gambierdiscus* (Hasani et al. 2013). One HABs 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. Several similar HABs 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 Regency, attracting public attention and suddenly creating awareness in an area where such an incident had never happened before. Environmental experts put forward a variety of different 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 an incident occurred with around 63 local residents being 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 Regency) and were suspected of having been contaminated with potentially dangerous (toxin-producing) phytoplankton.

Clearly, research is needed to test these hypotheses and to provide definitive answers regarding the above-mentioned incidents. Microalgae blooms can occur and are known to have contaminated aquatic organisms such as fish and shellfish. This may have been a factor in both cases. In the Pangkep region aquaculture activities are common in both riverine and coastal waters, using a lot of drugs and fertilizers to increase their production, as is also the case in the coastal waters of Mallasoro Village, Jeneponto Regency, South Sulawesi. However, to date, there has not been any definitive answer regarding the factors which caused these two events.

The potential role of the ongoing nutrient enrichment of riverine and coastal waters is a strong enough reason to justify analyzing developments in the phytoplankton community, specifically the types of microalgae that can form HABs. Increases and or changes in the concentration of these nutrients can occur due to seasonal changes such as between the east and west monsoons. These fluctuations will have an impact on the microalgal communities, including the presence of HABs forming species whose presence can be seasonal, and become a factor triggering the emergence and rapid growth of HABs.

There has not been any comprehensive research on the presence and development of HABs in the coastal waters of South Sulawesi. The information available regarding the HABs-forming microalgae which may occur in the region is limited and partial, with HABs species being reported incidentally as a small part of some previous studies. Meanwhile, information on HABs forming phytoplankton is very important for anticipating and early detection of HABs, as well as a basis for evaluating the quality of coastal waters concerning the life of organisms at a higher tropical level.

To evaluate the prevalence of HABs 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 will be of benefit through providing up-to-date data and information on the quality of marine microalgae in the west coast of South Sulawesi 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. In the end, it relates to the life of the community, especially fishermen who have been depending on the coast and the sea for their lives.

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 time periods (Table 1). The four research sites (Figure 1) were in Pangkep Regency (PK), Maros Regency (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

Number	Time period	Time of day	Season
1	June-July 2020	10:00-16:00	Dry season
2	August-September 2020	10:00-16:00	Dry to the rainy season
3	October-November 2020	10:00-16:00	Beginning of the rainy season

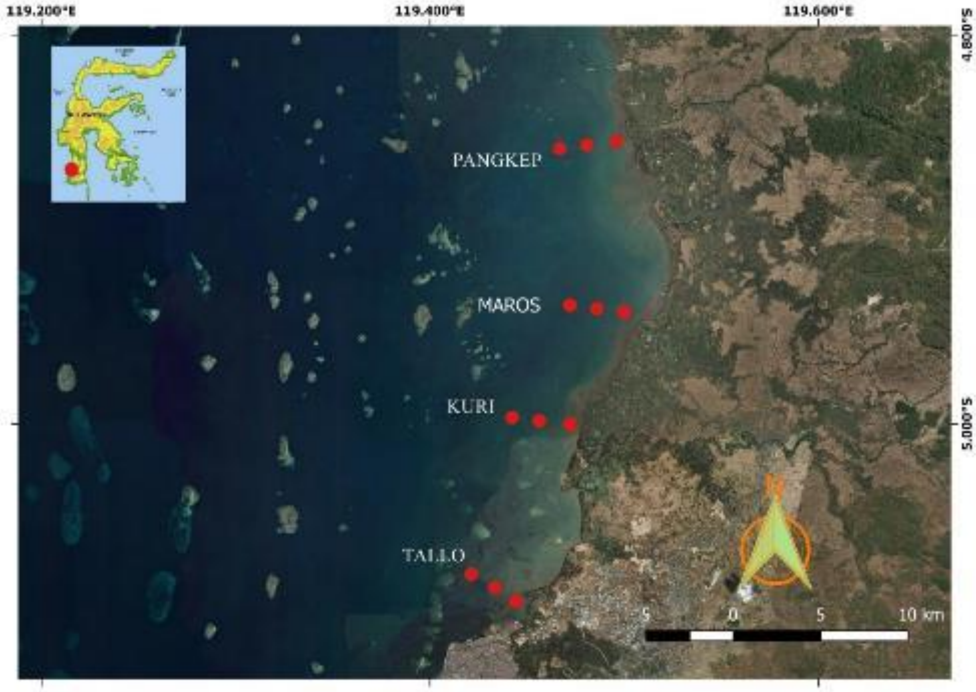

Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi

Materials and research design

The primary materials used in this research were seawater samples collected from the three zones at each of the four stations during each time 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 HABs and non-HABs forming species.

Phytoplankton analysis in the laboratory

Seawater samples were collected using a 2 liter Kemmerer Water Sampler. At each site, 1 liter of water was taken from each station (zone) for the counting and identification of 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's solution for 1 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's solution was added. A 1 mL aliquot of the precipitate was then placed in a *Sedgwick Rafter Cell* (SRC) using a graduated pipette. The SRC measured phytoplankton cell abundance using a sweeping (census) method (Rocha et al. 2015). Phytoplankton was identified using several standard reference works such as (Castellani and Edwards 2017; Tomas 1997).

Statistical analysis

The data were analyzed descriptively through tabulation and graphical approaches including graphs and maps to represent the taxonomic composition as well as the spatial and temporal distribution of the microalgae identified. A two-way Anova was used to evaluate the spatial and temporal differences in microalgal community abundance, in particular, HABs forming phytoplankton. These analyses were implemented in SPSS 17 and Excel Stat 2017 (Brahem et al. 2017).

RESULTS AND DISCUSSION

Detection of marine microalgae (phytoplankton)

The dynamics of marine microalgal communities vary from one location to another and from one time to another. Changes in community composition occur frequently (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 the community will be dominated by other groups. 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 HABs 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 all aquatic biota. Information on the characteristics of the phytoplankton present in particular waters can indicate their ability to support aquatic life.

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 locations/stations) and temporally (between study periods); however, for almost all sampling periods and sites the dominant phytoplankton genera were *Chaetoceros*, *Coscinodiscus*, *Nitzschia*, *Odontela*, and *Rhizosolenia*, all of which belong to the Class Bacillariophyceae.

The genus *Chaetoceros* and Class Bacillariophyceae 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 around the world (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 N-type nutrient concentrations are low.

The phytoplankton density in the samples (Figure 2) indicates relative abundance. Over the study period as a whole, mean phytoplankton abundance was higher at the Tallo and Kuri sites than at the Maros and Pangkep stations.

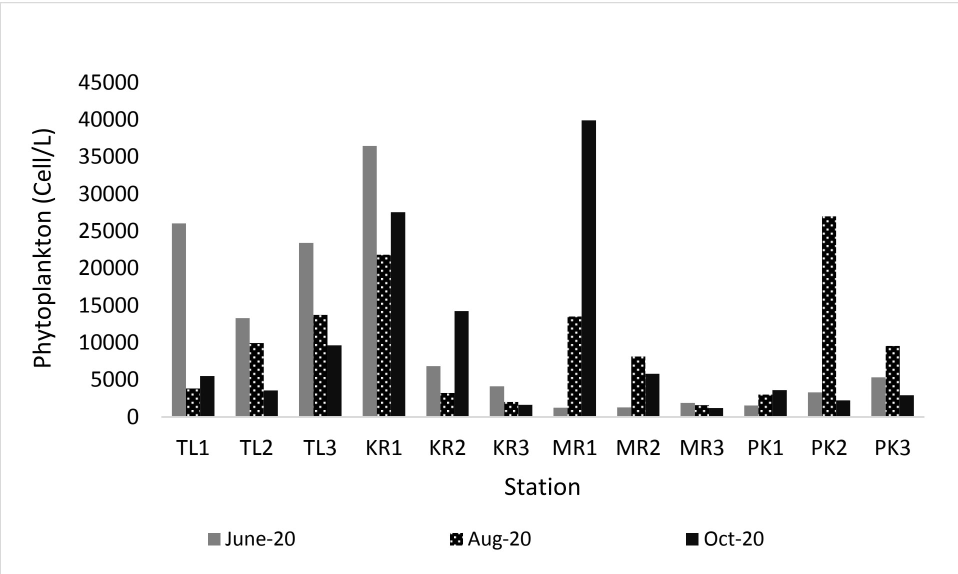

Figure 2. Mean sample phytoplankton density over the research period

The analysis of variance indicates a significant difference in phytoplankton abundance between the locations/stations ($p < 0.01$). The Tallo location/stations had the highest phytoplankton abundance, differing significantly from Maros and Pangkep; however, the abundance at the Kuri location was not significantly different from that at Tallo.

The observed phytoplankton abundance also varied between the time periods. However, the analysis of variance indicates that the differences in the abundance of phytoplankton over time were not significantly different based on the period of observation ($p > 0.05$). This is consonant with the results on the in-situ water quality parameter measurements, as several parameters which can affect phytoplankton growth did not vary significantly between the three-time periods. This means that phytoplankton experienced similar conditions for growth throughout the study.

Types of marine microalgae which are considered dangerous as HABs forming phytoplankton were present at almost all locations/stations (Figure 3). This is an important observation 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).

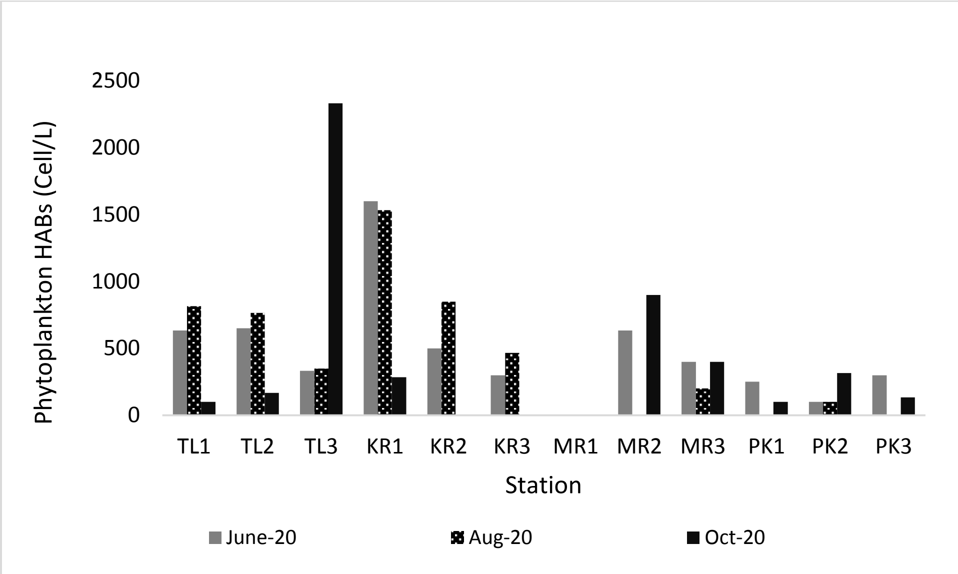

Figure 3. Mean density of HABs forming phytoplankton in the samples collected during the study period

The taxa identified included 5 potentially HABs forming phytoplankton genera belonging to the Class Dinophycea: *Ceratium*, *Dinophysis*, *Gymnodinium*, *Prorocentrum*, and *Protoperidinium*. These genera were present in most locations/stations and observation time periods. The classification of these taxa as presenting a risk of HABs can be justified because the Dinophyceae are known to contain the most toxic genera (Tillmann et al. 2010). These five HABs genera produce toxic metabolites, so they are classified as belonging 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).

Ceratium can be identified from visual characters including horns and two flagella. This genus 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 (Boesch and Rabalais 1991). *Dinophysis* is considered to belong to the HABs 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 HABs 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). *Protoperidinium* 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 HABs forming taxa varied between locations/stations and periods of observation (Figure 3). The analysis of variance (Appendix 2) revealed highly significant differences ($p < 0.01$) in the abundance of HABs taxa between locations/stations. 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 HABs taxa differed between the time periods, the Anova results indicate that these differences were not significant ($p > 0.05$). This means that the presence and abundance of taxa capable of forming HABs can be considered similar throughout the study period.

Quality of marine microalgae (phytoplankton)

The quality of marine microalgae is determined by the types and relative abundance of phytoplankton that can form HABs (HABs) and those which do not form HABs (Non-HABs) phytoplankton in the water. A greater variety of Non-HABs phytoplankton is an indication of a thriving phytoplankton community that can support the life of other organisms. Changes in water quality may occur, but these changes can still be tolerated by non-HABs 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 that non-HABs phytoplankton abundance exceeds that of HABs phytoplankton. If this is the case, there is a reasonable likelihood that the waters are still in good condition and haven't changed greatly.

HABs phytoplankton are phytoplankton that 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). In general, if eutrophic conditions occur, phytoplankton from the Dinophyceae will multiply more rapidly and may form HABs. Once the HABs have begun to form, non-HABs phytoplankton experience a decline. This happens because when the HABs phytoplankton are multiplying they release toxins which impede the growth and reproduction of the non-HABs phytoplankton.

The results of the phytoplankton identification and counts revealed that non-HABs phytoplankton were not only more taxonomically diverse but also far more abundant than the HABs phytoplankton. The non-HABs phytoplankton comprised 94-98% of the microalgae cells counted while HABs forming phytoplankton accounted for 2-6% (Figure 4). This means that the quality of the microalgae present in the waters where this research was conducted was still in a good condition category. This information is certainly encouraging for coastal communities. Seafood is still suitable for consumption because HABs can be considered as not yet contaminating.

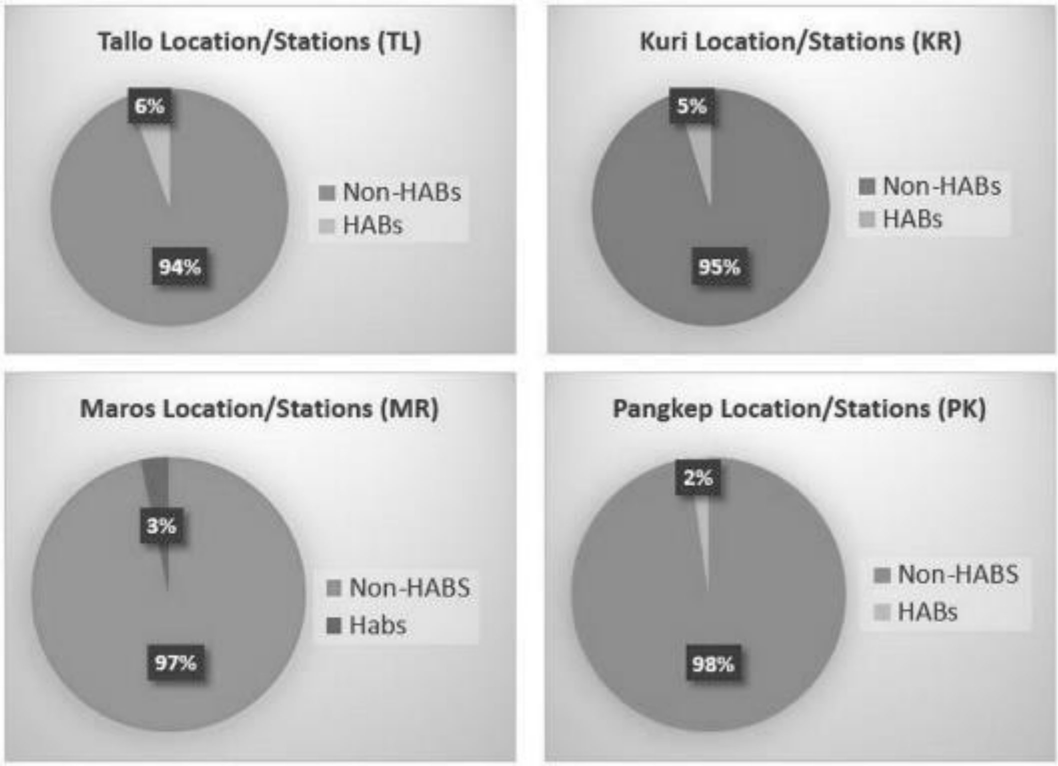

Figure 4. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period

Even though the proportions of HABs 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 to ensure that HABs do not develop undetected.

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 good. This evaluation is based on the taxonomic composition and abundance of non-harmful (non-HABs) phytoplankton which were far more abundant than the taxa which can cause HABs. This shows that seafood is still suitable for consumption because HABs can be considered as not yet contaminating.

ACKNOWLEDGEMENTS

The authors wish to thank the Rector of Universitas Hasanuddin for a research grant provided through the Universitas Hasanuddin Research and Community Service Institute (LP2M) under the 2020 Basic Research scheme.

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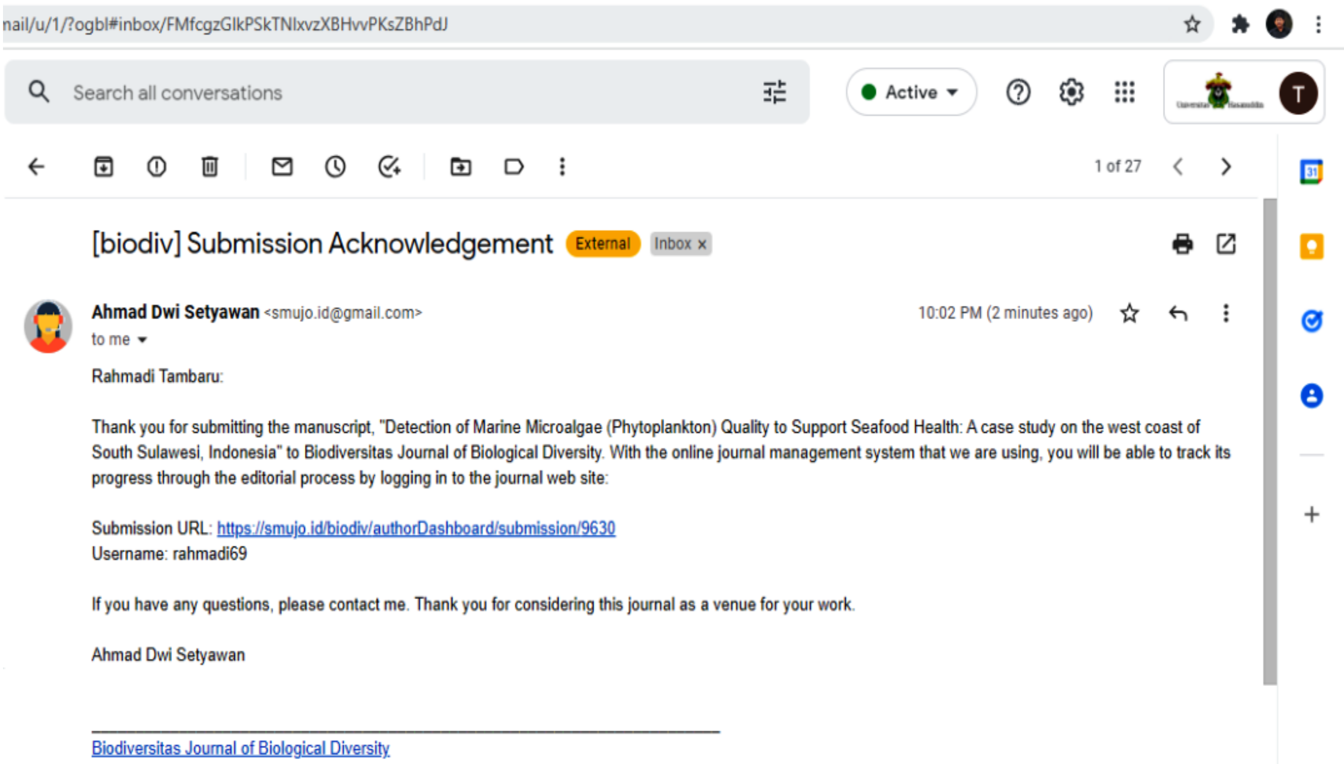

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We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Detection of marine microalgae (phytoplankton) quality to support seafood health: A case study on the west coast of South Sulawesi, Indonesia".

Our decision is to: Accept Submission

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Biodiversitas Journal of Biological Diversity

B2. RESPONSE UNCORRECTED PROOF TO EDITOR

Uncorrected proof

Participants Edit

Smujo Editors (editors)

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Messages

Note	From
Dear Editors, We have read our manuscript carefully. We haven't done any revisions. Manuscript we sent back (attached). Regards, Rahmadi Tambaru Faculty of Marine Sciences and Fisheries Hasanuddin University rahmadi69, 9630-Article Text-51827-1-18-20211103_Rev.doc	rahmadi69 2021-11-04 02:21 PM

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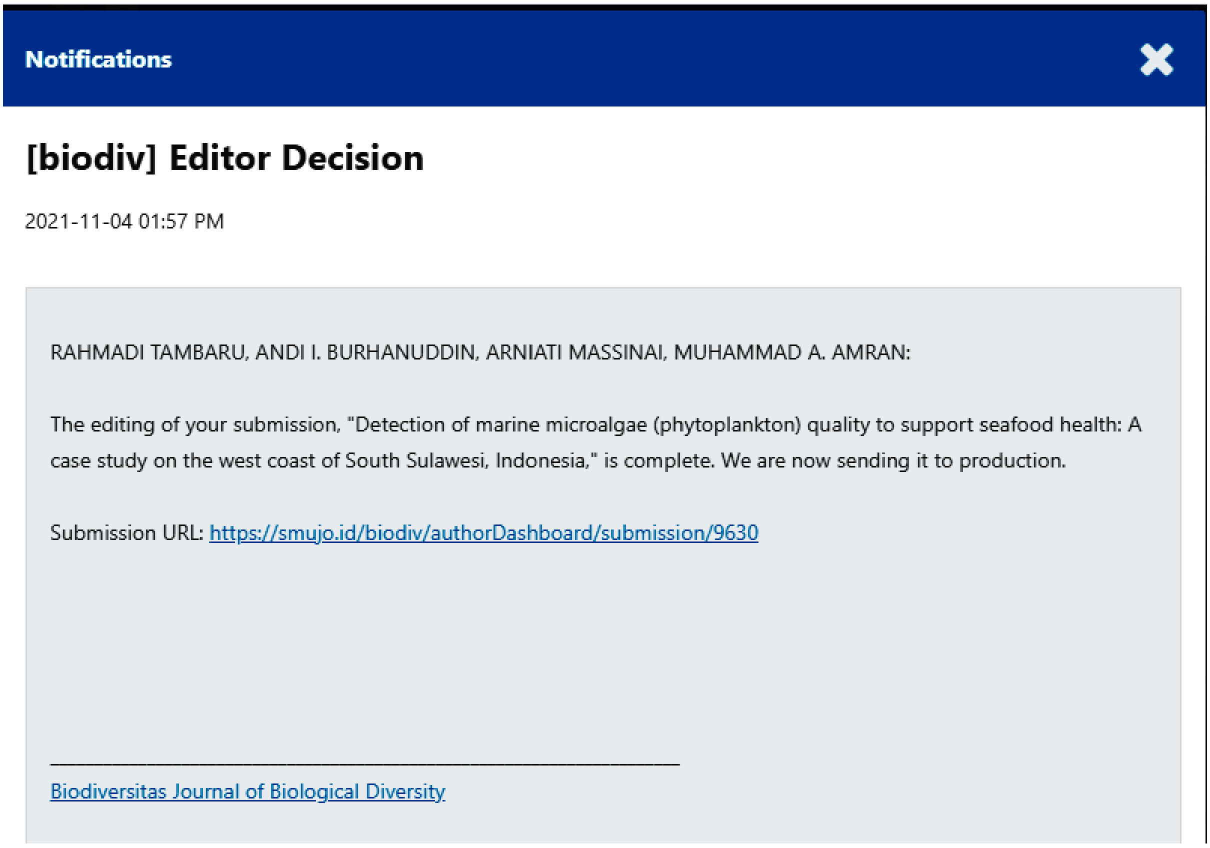
B3. IN EDITING SUBMISSION



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The editing of your submission, "Detection of marine microalgae (phytoplankton) quality to support seafood health: A case study on the west coast of South Sulawesi, Indonesia," is complete. We are now sending it to production.

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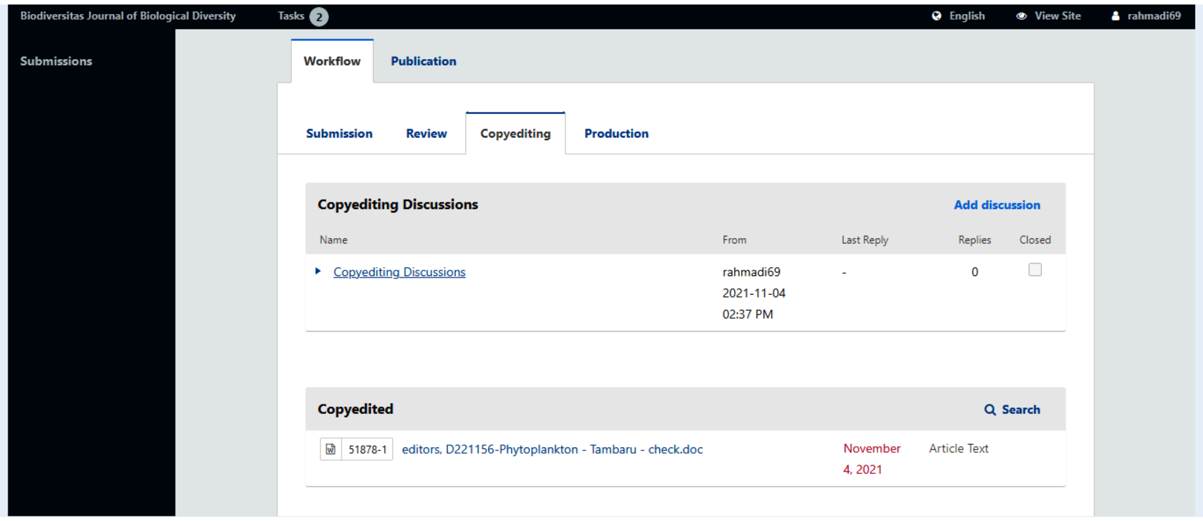
B4. MANUSCRIPT COPYEDITING



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B5. MANUSCRIPT FINAL PROOF

BIODIVERSITAS
Volume 22, Number 11, November 2021
Pages: 5179- 5186

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d221156

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

RAHMADI TAMBARU♥, ANDI I. BURHANUDDIN, ARNIATI MASSINAI, MUHAMMAD A. AMRAN
Department of Marine Science, Faculty of Marine Science and Fisheries, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km 10, Tamalanrea, Makassar 90425, South Sulawesi, Indonesia. Tel./fax.: +62-411-586025, ♥email: aditbr69@unhas.ac.id

Manuscript received: 11 October 2021. Revision accepted: 29 October 2021.

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
Octr-Nov 2020	10:00-16:00	Beginning of the rainy season

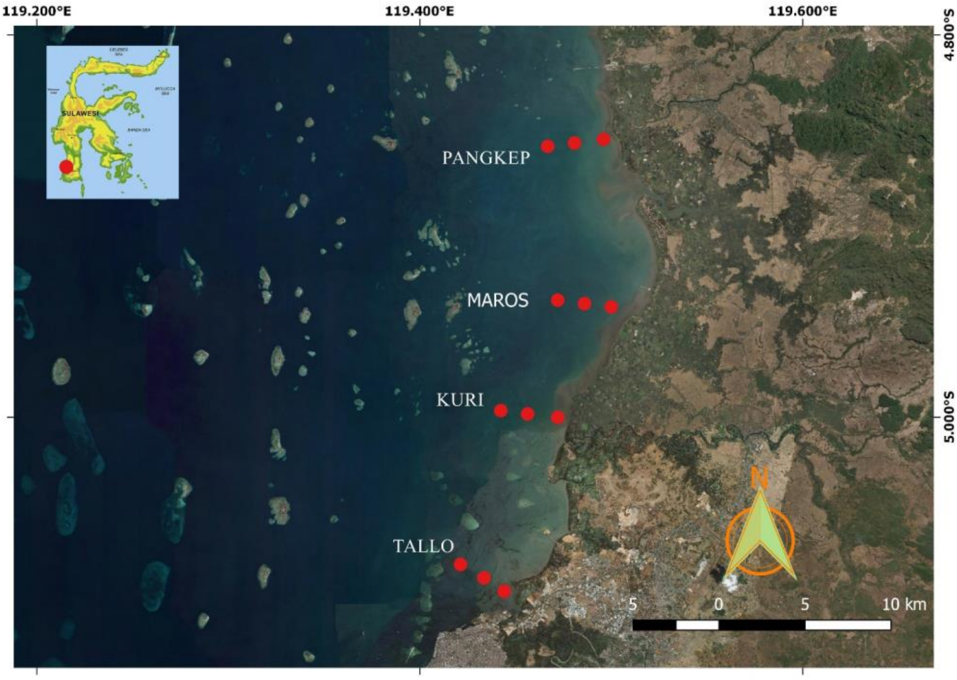

Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi, Indonesia

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 Sedgwig 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 *Protoperidinium* (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).

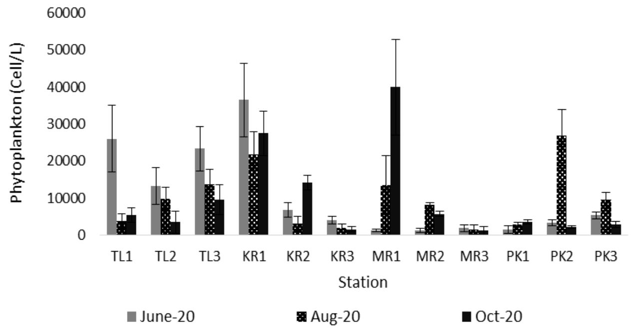

Figure 2. Phytoplankton density at the sampled sites in South Sulawesi over the research period (mean ± SD)

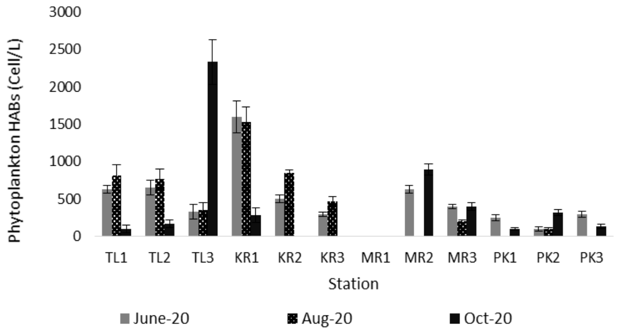

Figure 3. HAB forming phytoplankton density in samples from South Sulawesi over the study period (mean ± SD)

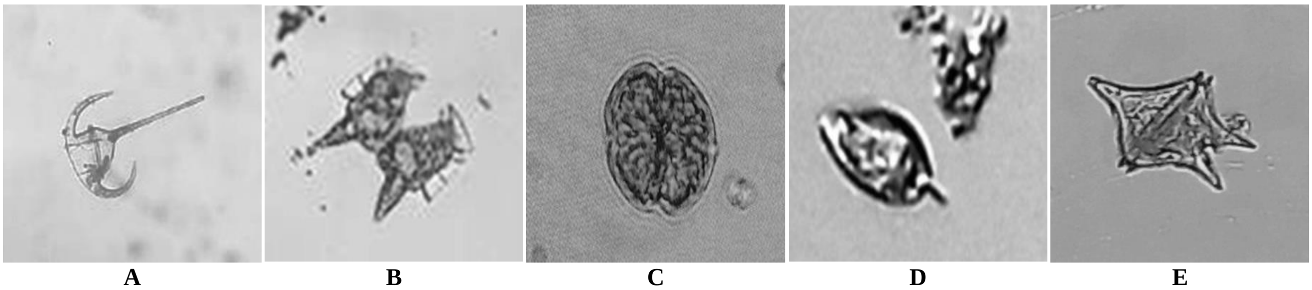

Figure 4. Examples of phytoplankton from class Dinophyceae. A. *Ceratium*, B. *Dinophysis*, C. *Gymnodinium*, D. *Prorocentrum*, E. *Protoperidinium*

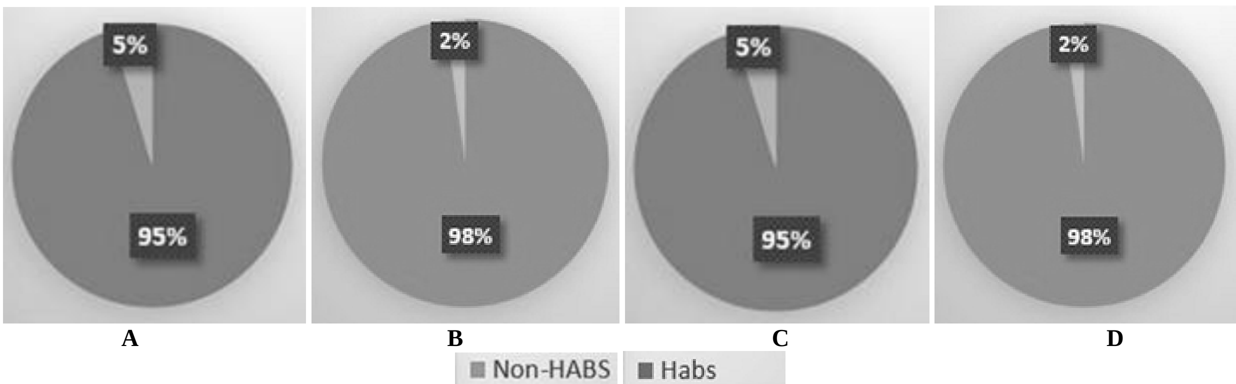

Figure 5. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period. A. Tallo, B. Kuri, C. Maros, D. Pangkep

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 *Protoperidinium*. 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 Sugestiningsih 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). *Protoperidinium* 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 (ANOA, $p>0.05$) 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 (Dorgham 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.

ACKNOWLEDGEMENTS

The authors wish to thank the Rector of Universitas Hasanuddin for a research grant provided through the Universitas Hasanuddin Research and Community Service Institute (LP2M) under the 2020 Basic Research scheme.

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3. FILE PERTANYAAN/PERINTAH PERBAIKAN DARI PARA REVIEWER JURNAL

A. FIRST REVISION FROM REVIEWER (OCTOBER 24, 2021)

A1. STATUS IN REVIEW (EARLIER DRAFT) and COMMENTS FROM REVIEWERS/EDITORS (FIRST ROUND)

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Search all convers

19 of 35

[biodiv] Editor Decision External Inbox ×

Smujo Editors <smujo.id@gm... Sun, Oct 24, 2021, 1:13 PM

to me

Rahmadi Tambaru:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sulawesi, Indonesia".

Our decision is: Revisions Required

Reviewer T:

It is great to see phytoplankton monitoring for HABs coming from Indonesian waters. While no major HAB problems were detected, promising safe seafood is highly dangerous, without routime plankton and toxin monitoring. This work ignores a major body of work on HABs from Indonesian waters. An important lit review by Fukuyo et al. 2011. Ecology and oceanography of HABs. is provided as attachment. Indonesia has major PSP problems from the dinoflagellate Pyrodinium bahamense (427 human poisonings, 17 mortalities), fish kills in Jakarta Bay from Noctiluca and Trichodesmium; Gambierdiscus (ciguatera) is known from Bali (Skinner 2011, Mar.Res.Indonesia 36, 11-23). Nitzschia navis-varingica (ASP) is known from Sulawesi. How can we be certain that these authors did not overlook highly toxic organisms at low concentrations, that require electron microscopy or molecular sequencing for detection.

Note that some HABs are not plankton but benthic (Gambierdiscus, Ostreopsis,Prorocentrum). Protoperidinium is not a HAB (but can be toxic after feeding on Azadinium). Only 1 Gymnodinium (G.catenatum) can produce PSP, this species is known from Ambon Bay.

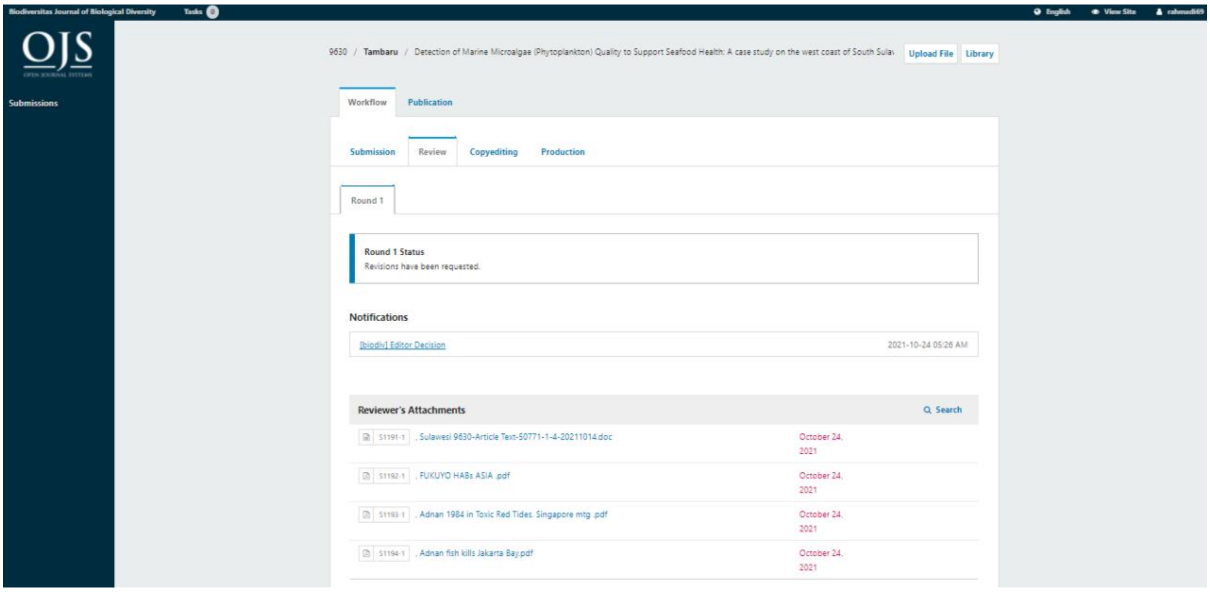
Please separate Results vs Discussion. If possible provide some photographs of key organisms, eg. Dinophysis, Prorocentrum. The conclusion that there were more non-HABs than HABs is globally applicable , 5000-10000 microalgal taxa but only 200+HABs, this is not a justification that water quality is good or seafood safe.

I like to encourage these authors to pursue this publication, but it is not suitable in this format and needs to be phrased much more carefully and taking into account the full HAB literature from Indonesia. I heavily annotated manuscript is attached.

Recommendation: Resubmit for Review

Biodiversitas Journal of Biological Diversity

4 Attachments



Notifications

[biodiv] Editor Decision

2021-10-24 05:26 AM

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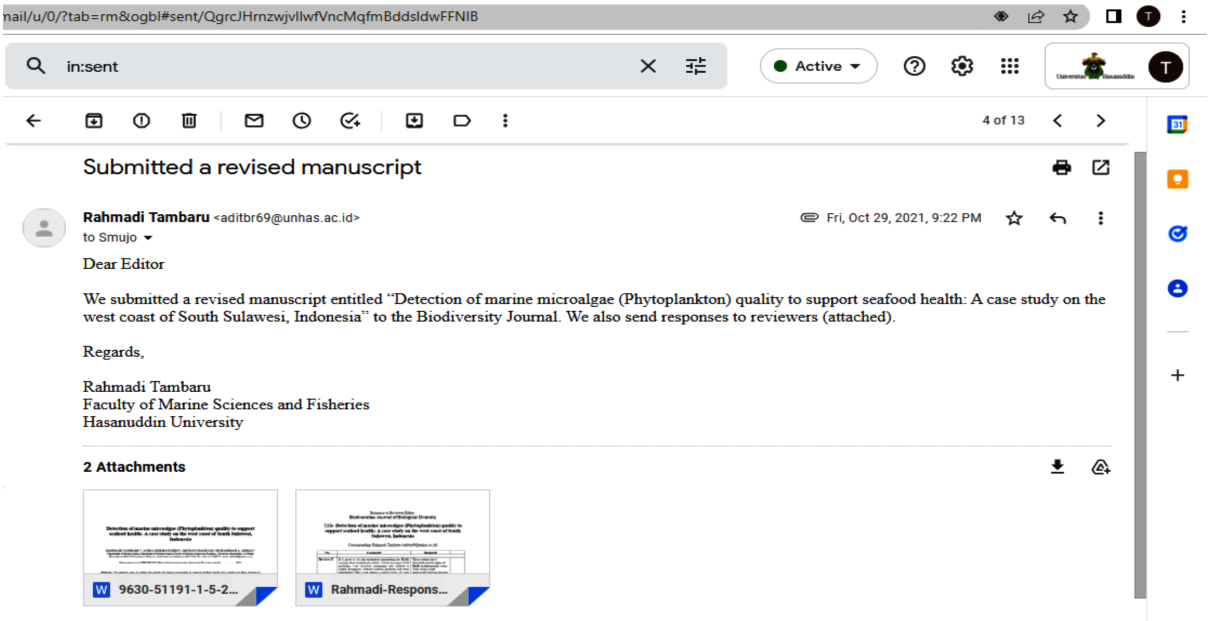
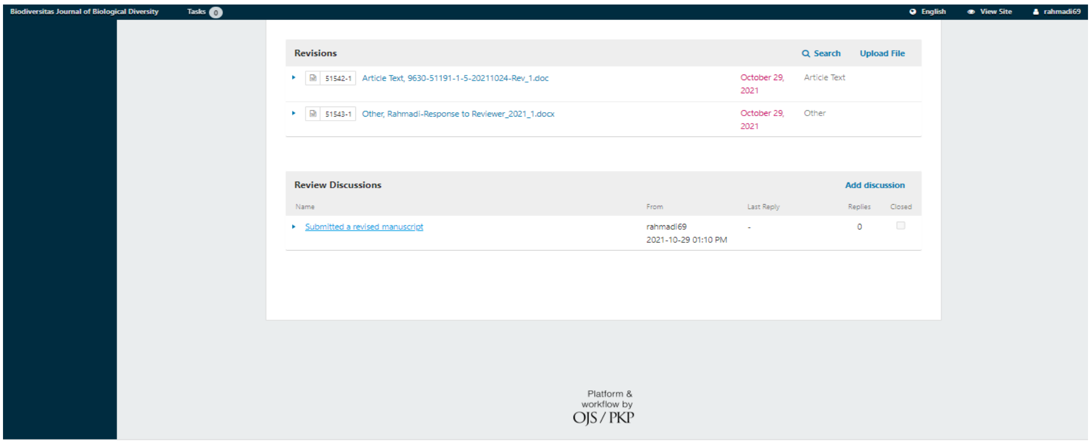
Recommendation: Resubmit for Review

A2. RESPONSE TO REVIEWER/EDITOR (FIRST ROUND) (OCTOBER 29, 2021)



Revisions-Review Discussions



Submitted a revised manuscript

Participants Edit

Smujo Editors (editors)

Rahmadi Tambaru (rahmadi69)

Messages

Note	From
Dear Editor We submitted a revised manuscript entitled "Detection of marine microalgae (Phytoplankton) quality to support seafood health: A case study on the west coast of South Sulawesi, Indonesia" to the Biodiversity Journal. We also send responses to reviewers (attached). Regards, Rahmadi Tambaru Faculty of Marine Sciences and Fisheries Hasanuddin University rahmadi69, Rahmadi-Response to Reviewer_2021_1.docx rahmadi69, 9630-51191-1-5-20211024-Rev_1.doc	rahmadi69 2021-10-29 01:10 PM

Response to Reviewer/Editor (FIRST ROUND) (OCTOBER 29, 2021)
Biodiversitas Journal of Biological Diversity

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

Corresponding: Rahmadi Tambaru (aditbr69@unhas.ac.id)

Nu.	Comments	Responds	
Reviwer T	It is great to see phytoplankton monitoring for HABs coming from Indonesian waters. While no major HAB problems were detected, promising safe seafood is highly dangerous, without routime plankton and toxin monitoring. This work ignores a major body of work on HABs from Indonesian waters. An important lit review by Fukuyo et al. 2011. Ecology and oceanography of HABs. is provided as attachment. Indonesia has major PSP problems from the dinoflagellate Pyrodinium bahamense (427 human poisonings, 17 mortalities), fish kills in Jakarta Bay from Noctiluca and Trichodesmium; Gambierdiscus (ciguatera) is known from Bali (Skinner 2011, Mar.Res.Indonesia 36, 11-23). Nitzschia navis-varingica (ASP) is known from Sulawesi. How can we be certain that these authors did not overlook highly toxic organisms at low concentrations, that require electron microscopy or molecular sequencing for detection.	These authors have described several types of HABs in Indonesian waters. Even using a light microscope (not an electron microscope), they were able to find several types of HABs. Their research results are trusted and have been cited by many other researchers. Light microscopes have been used in general by many researchers. Several expert statements to provide reinforcement regarding the monitoring of phytoplankton HABs have been added on page 1 (paragraphs 3 and 4) Some of the results of research by experts on the types of HABs have been added on page 2 (paragraph 2).	
	Note that some HABs are not plankton but benthic (Gambierdiscus, Ostreopsis, Prorocentrum). Protoperidinium is not a HAB (but can be toxic after feeding on Azadinium). Only 1 Gymnodinium (G.catenatum) can produce PSP, this species is known from Ambon Bay.	Yes, right. Some literature explaining this has been added on page 6 (paragraph 5).	

	Please separate Results vs Discussion. If possible provide some photographs of key organisms, eg. Dinophysis, Prorocentrum. The conclusion that there were more non-HABs than HABs is globally applicable , 5000-10000 microalgal taxa but only 200+HABs, this is not a justification that water quality is good or seafood safe.	The manuscript has been revised by separating the results vs discussion. Some photos of HABs have been added on page 5. Yes, I agree that there are more non-HABs than HABs going on globally. Although only in a low percentage, the presence of HABs must be taken seriously. If environmental conditions are favorable, then HABs bloom can take place. If that happens, the water quality will be low because HABs can poison the waters. In this regard, the conclusion has been revised on page 7 (paragraph 2).	
	I like to encourage these authors to pursue this publication, but it is not suitable in this format and needs to be phrased much more carefully and taking into account the full HAB literature from Indonesia. I heavily annotated manuscript is attached.	The manuscript has been adapted to the Biodiversity Journal format. Several literatures on HABs in Indonesia have been cited including three recommended journals (available on pages 2 and 7).	
The other notes		We proof some words/sentences so that more understandable and common style in the introduction. We also revised some of the sentences, this is meant to be easier to understand (page 2 paragraphs 1, 2, 3, 4).	
		We also added a sentence in the introduction to reinforce the monitoring of phytoplankton HABs (page 1 paragraph 4).	
		We also add an explanation of the main equipment used in this study (sub title : Phytoplankton analysis in the laboratory : page 3)	

A3. REVISED VERSION (FIRTS ROUND) (OCTOBER 29, 2021)

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

RAHMADI TAMBARU[1♥], ANDI I. BURHANUDDIN[1], ARNIATI MASSINAI[1], MUHAMMAD A. AMRAN[1]
[1]Department of Marine Science, Department of Marine Science, Faculty of Marine Science and Fisheries, Universitas Hasanuddin. Jl. Perintis Kemerdekaan KM 10 Tamalanrea, Makassar, South Sulawesi, Indonesia, 90425 Tel./Fax. +62-411-586025, ♥email: aditbr69@unhas.ac.id

Manuscript received: DD MM 2021 (Date of abstract/manuscript submission). Revision accepted: .... 2021.

Abstract. The research aims to detect the quality of marine microalgae to support seafood health was carried out from January to November 2020 along in the west coast of South Sulawesi, Indonesia. Samples were collected from the coastal waters of Pangkep Regency, Maros Regency, and the northern part of Makassar City. Phytoplankton cell counts were obtined 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 time periods. Based on the types and relative abundance of phytoplankton present, i.e. harmful algal bloom (HABs) forming or not (non-HABs) the results showed that the quality of marine microalgae, specifically phytoplankton, was relatively good. There were many more non-HAB (94-98%) than HAB (2-6%) marine microalgae detected. This means that the phytoplankton flourishing in these waters are mostly suitable as food for other organisms, including fish and shellfish. It also means that if these fish and shellfish are harvested by fishermen they should be fit and safe for human consumption.

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

Abbreviations: Harmful algal bloom (HABs)

Running title: Phytoplankton Quality to Support Seafood Health

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 (Shadrin et al. 2017; Sunda 2012).

Periodic increases in microalgae populations are natural phenomena (Visciano et al. 2016) and 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 et al. 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. 2016), and changes to aquatic ecosystem community structure (Todd et al. 2019; Lu et al. 2018).

Rapid or excessive increases in the population of microalgae (phytoplankton), termed algal blooms, can occur when environmental conditions are conducive to algal growth and reproduction. 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 fishery resources, including mariculture and capture fisheries commodities such as oysters, shellfish, and fish (Berdalet et al. 2016; Brown et al. 2019; Glibert 2017).

Over time, the incidence of HABs is increasingly being noticed by environmentalists. The emergence of new types of HAB, including the increasing frequency of occurrence, as well as the expansion of the geographic area affected, and the prolonged duration of their occurrence, are events that are increasingly being discussed. From recent studies, several researchers have found different types of toxins from HBs toxins that have been there before. Of course, this has an impact on the increasing mortality of marine organisms (Fukoyo 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. 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 very dangerous to human health because they attack the nervous system and interfere with respiration and digestion. These diseases are related to human consumption of fish and shellfish. In fact, 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 Regency, attracting public attention and suddenly creating awareness in an area where such an incident had never happened before. Environmental experts put forward a variety of different 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 an incident occurred with around 63 local residents being 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 Regency. It is suspected that the clams were contaminated with toxins from potentially dangerous (toxin-producing) phytoplankton. Clearly, more research is needed to test the hypotheses put forward and to provide definitive answers regarding the above-mentioned incidents.

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 activities are common in both riverine and coastal waters (Lestari et al. 2021;), and in some cases use various drugs and fertilizers to increase production; this is also the case in the coastal waters of Mallasoro Village, Jeneponto Regency, 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 with respect to the types of microalgae that can form HABs. Increases and or changes in nutrient concentration can occur due to seasonal changes such as between the east and west monsoons (Rastina et al. 2020; Vajravelu et al. 2018). These fluctuations will have an impact on the microalgal communities, including the presence of HAB forming species whose presence can be seasonal (Narale and Anil 2017; Trainer et al. 2020; Vajravelu et al. 2018), 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 (Lestari et al. 2021; Mujib et al. 2015; Rukminasari and Tahir 2020; Samawi et al. 2020). Meanwhile, information on HAB forming phytoplankton is very important for anticipating and early detection of HABs, as well as a basis for evaluating the quality of coastal waters with respect to 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 have been depending 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 time periods (Table 1). The four research sites (Figure 1) were in Pangkep Regency (PK), Maros Regency (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

Number	Time period	Time of day	Season
1	June-July 2020	10:00-16:00	Dry season
2	August-September 2020	10:00-16:00	Dry to the rainy season
3	October-November 2020	10:00-16:00	Beginning of the rainy season

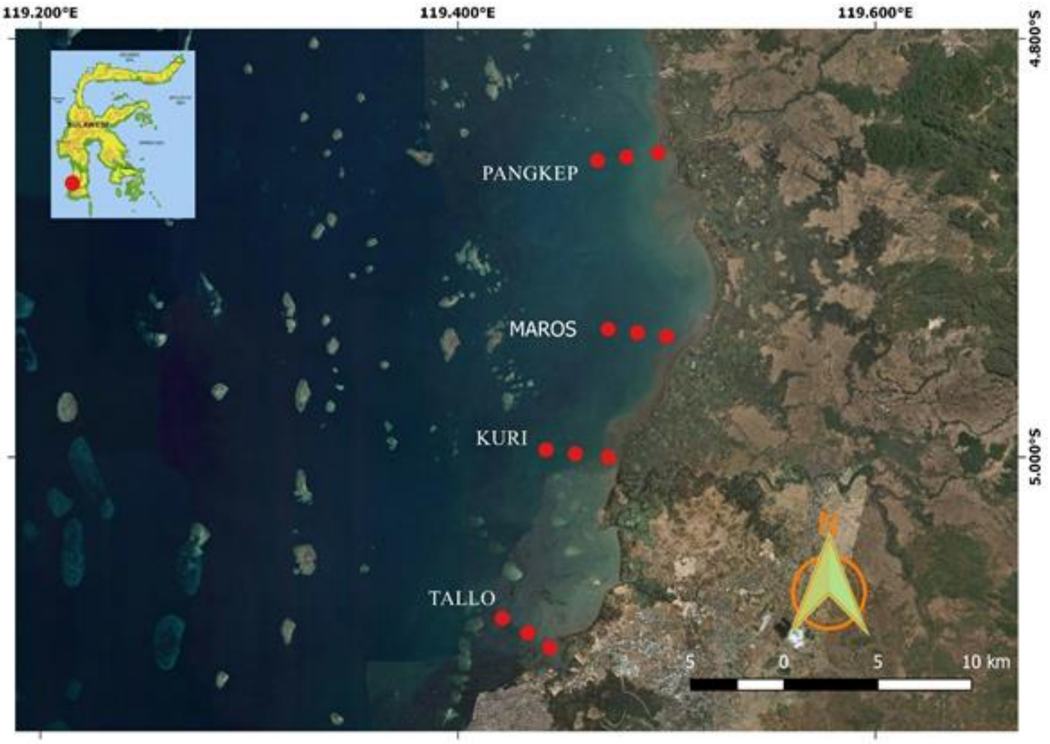


Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi

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 time 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 liter Kemmerer Water Sampler. At each site, 1 liter of water was taken from each station (zone) for the counting and identification of 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's solution for 1 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 Sedgwig 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 such as (Castellani and Edwards 2017; Tomas 1997).

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. 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 locations/stations) and temporally (between study periods); however, for almost all sampling periods and sites the dominant phytoplankton genera were *Chaetoceros*, *Coscinodiscus*, *Nitzschia*, *Odontela*, 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 as a whole, mean phytoplankton abundance was higher at the Tallo and Kuri sites than at the Maros and Pangkep stations. The analysis of variance indicated a significant difference in phytoplankton abundance between the locations/stations ($p < 0.01$). The observed phytoplankton abundance also varied between the time periods. However, the analysis of variance indicated that the differences in the abundance of phytoplankton over time were not statistically significant over the period of observation ($p > 0.05$).

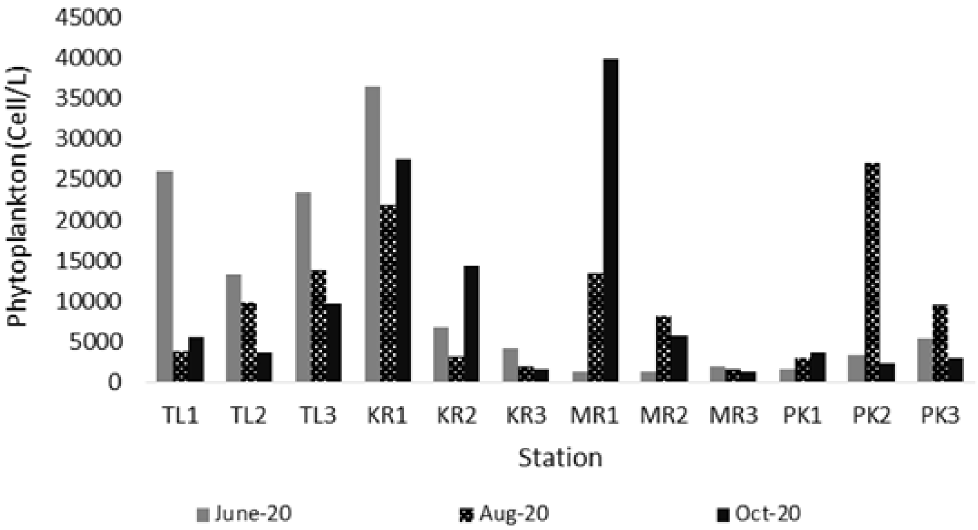


Figure 2. Mean sample phytoplankton density over the research period

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

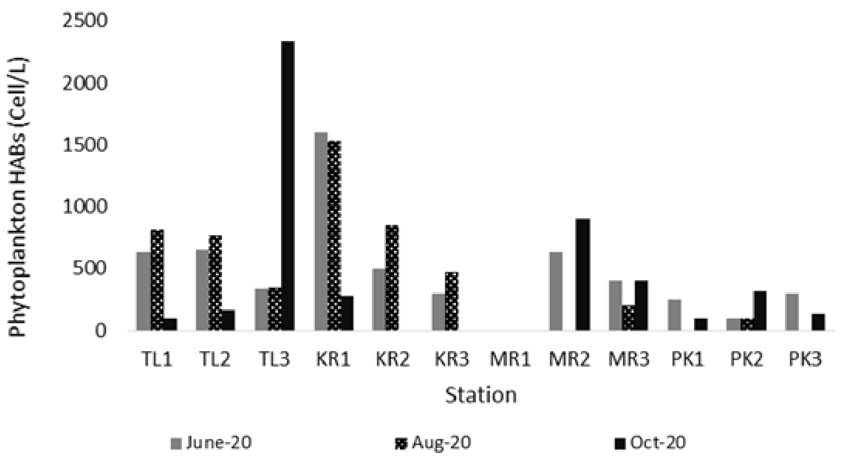
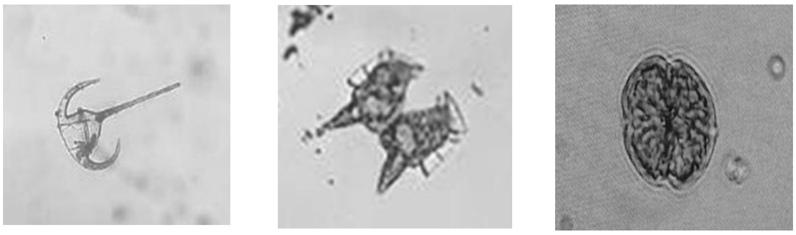
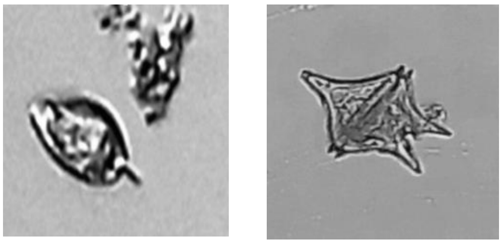


Figure 3. Mean density of HABs forming phytoplankton in the samples collected during the study period

The presence and abundance of the potentially HAB forming taxa varied between locations/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 locations/stations.



(a) *Ceratium* (b) *Dinophysis* (c) *Gymnodinium*



(d) *Prorocentrum* (e) *Protoperidinium*

Figure 4. Examples of phytoplankton from class Dinophyceae

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).

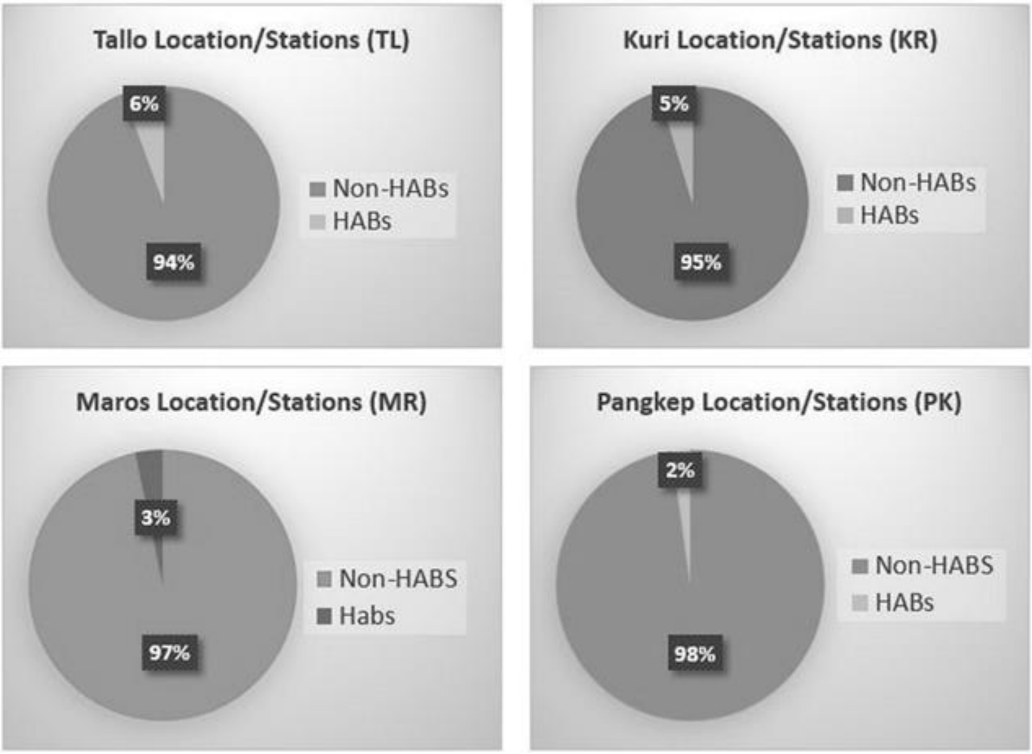


Figure 5. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period

Discussion

The dynamics of marine microalgal communities vary from one location to another and from one time to another. Changes in community composition occur frequently (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 the community will be dominated by other groups.

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 form 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 locations/stations and observation time periods: *Chaetoceros, Coscinodiscus, Nitzschia, Odontela, 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 around the world (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 N-type nutrient concentrations are low.

The Tallo location/stations had the highest phytoplankton abundance, differing significantly from Maros and Pangkep ($p<0.01$); however, the abundance at the Kuri location 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 results of the analysis of variance, the abundance of phytoplankton was not significantly different based on the period of observation ($p>0.05$). This is consonant with the results on the in-situ water quality parameter measurements, as several parameters which can affect phytoplankton growth did not vary significantly between the three-time periods. This means that phytoplankton experienced similar conditions for growth throughout the study.

Types of marine microalgae which are considered dangerous as HABs forming phytoplankton were present at almost all locations/stations (Figure 3). This is an important observation 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 5 potentially HABs forming phytoplankton genera belonging to the Class Dinophycea: *Ceratium*, *Dinophysis*, *Gymnodinium*, *Prorocentrum*, and *Protoperidinium*. These genera were present in most locations/stations and observation time periods. The classification of these taxa as presenting a risk of HABs can be justified because the Dinophyceae are known to contain the most toxic genera (Tillmann et al. 2010). These five HABs genera produce toxic metabolites, so they are classified as belonging 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 addition of the number and abundance of HABs in surface waters can come from aquatic sediments. HAB in aquatic sediments is in the form of cysts, such as *Gymnodinium* cysts from the Dinophyceae class (Dzhembekova et al. 2018). According to Fukoyo et al (2011), phytoplankton in this class play an important role in recurring HAB events as well as contributing to the geographical expansion of HAB occurrences. These repeat HABs can occur when a large number of 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 Sugestiningsih, 2000), and 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* is considered to belong 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). *Protoperidinium* 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 locations/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 time periods, the lack of statistical significance (ANOA, $p>0.05$) indicate 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 are multiplying they release toxins (Glibert 2017, 2020); these may impede the growth and reproduction of the non-HAB phytoplankton as well as affecting other organisms (Dorgham 2014; Glibert 2017). Conversely, a greater variety of non-HAB phytoplankton is an indication of 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 (Gilbert 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 haven't changed greatly, 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, and also indicate that fisheries produce from the study locations 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 with respect to 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 good. This evaluation is based on the taxonomic composition and abundance of non-harmful (non-HAB) phytoplankton which were 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 as 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 advocated by Anderson et al. (2015), to ensure that HABs do not develop undetected.

ACKNOWLEDGEMENTS

The authors wish to thank the Rector of Universitas Hasanuddin for a research grant provided through the Universitas Hasanuddin Research and Community Service Institute (LP2M) under the 2020 Basic Research scheme.

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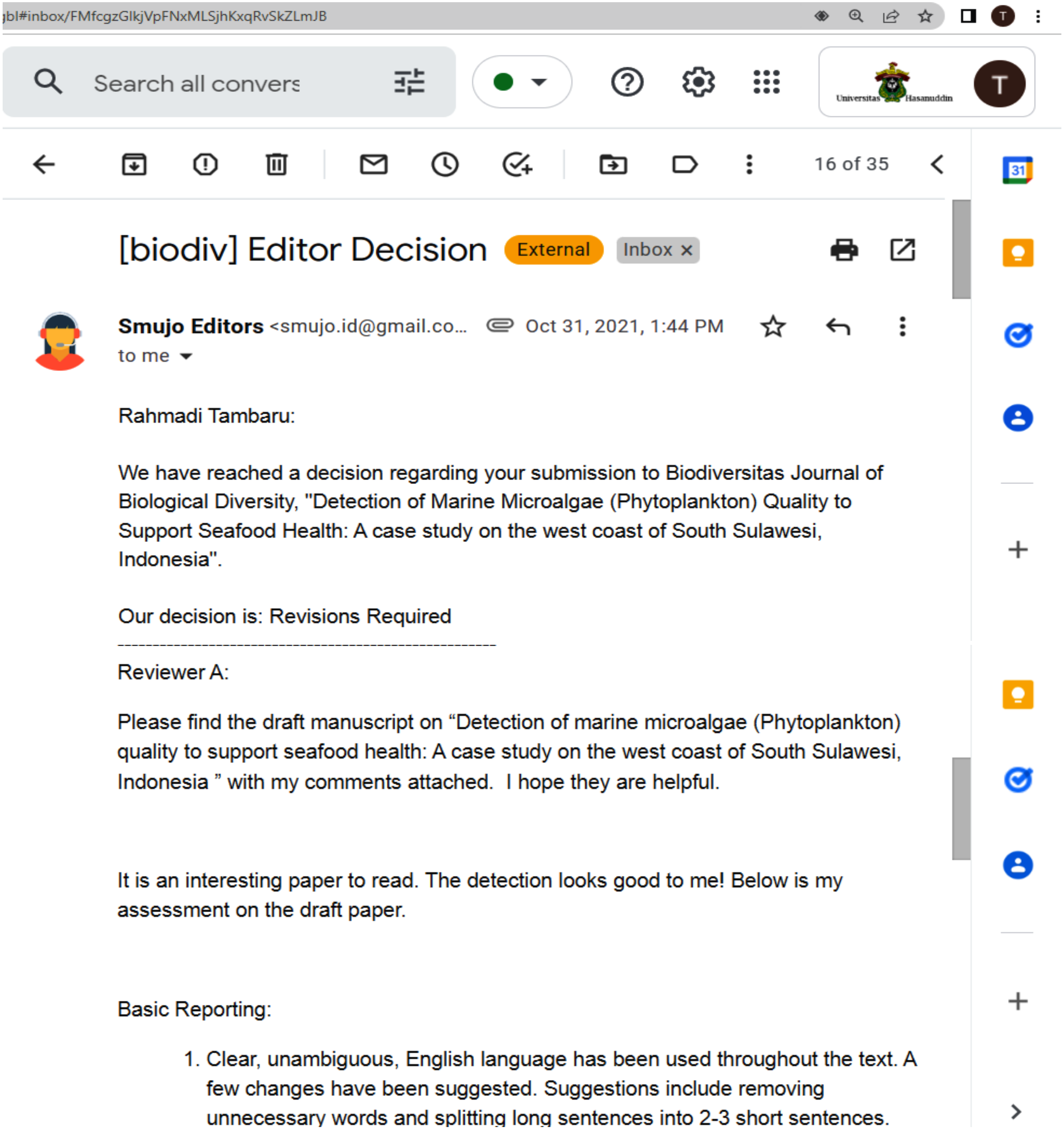
B. SECOND REVISION FROM REVIEWER (OCTOBER 31, 2021)

B1. COMMENTS-DECISIONS FROM REVIEWERS (SECOND ROUND)



[biodiv] Editor Decision External Inbox ×

Smujo Editors <smujo.id@gmail.co... Oct 31, 2021, 1:44 PM

to me

Rahmadi Tambaru:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sulawesi, Indonesia".

Our decision is: Revisions Required

Reviewer A:

Please find the draft manuscript on “Detection of marine microalgae (Phytoplankton) quality to support seafood health: A case study on the west coast of South Sulawesi, Indonesia ” with my comments attached. I hope they are helpful.

It is an interesting paper to read. The detection looks good to me! Below is my assessment on the draft paper.

Basic Reporting:

1. Clear, unambiguous, English language has been used throughout the text. A few changes have been suggested. Suggestions include removing unnecessary words and splitting long sentences into 2-3 short sentences.

2. Intro & background show context. Concepts are well explained and sufficient literature/ background information are provided.
3. Statistical analysis to be completed with tests for normality and equal variances. Please use SD/SE to be combined with mean. Mean would not be stand alone without variations (SD/SE).
4. Literature is well referenced & relevant but its completeness could be improved (e.g. see below).
   Meesters E, Tapilatu RF. 2020. First dive Raja Ampat, Sorido. https://weblog.wur.eu/coastsea/first-dive-raja-ampat-sorido/Thovyan, A.I., Tapilatu, R.F., Sabariah, V. and Venables, S.K., 2020. Plankton abundance and community structure in reef manta ray (Mobula alfredi) feeding habitat in the Dampier Strait, Raja Ampat, West Papua, Indonesia. *AACL Bioflux*, *13*(5), pp.2956-2969.

Recommendation: Revisions Required

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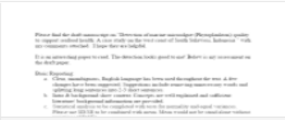
Biodiversitas Journal of Biological Diversity

2 Attachments


A-9630-Article Tex...


A-Summary Comm...

Summary Comments from Reviewer

Please find the draft manuscript on "Detection of marine microalgae (Phytoplankton) quality to support seafood health: A case study on the west coast of South Sulawesi, Indonesia " with my comments attached. I hope they are helpful.

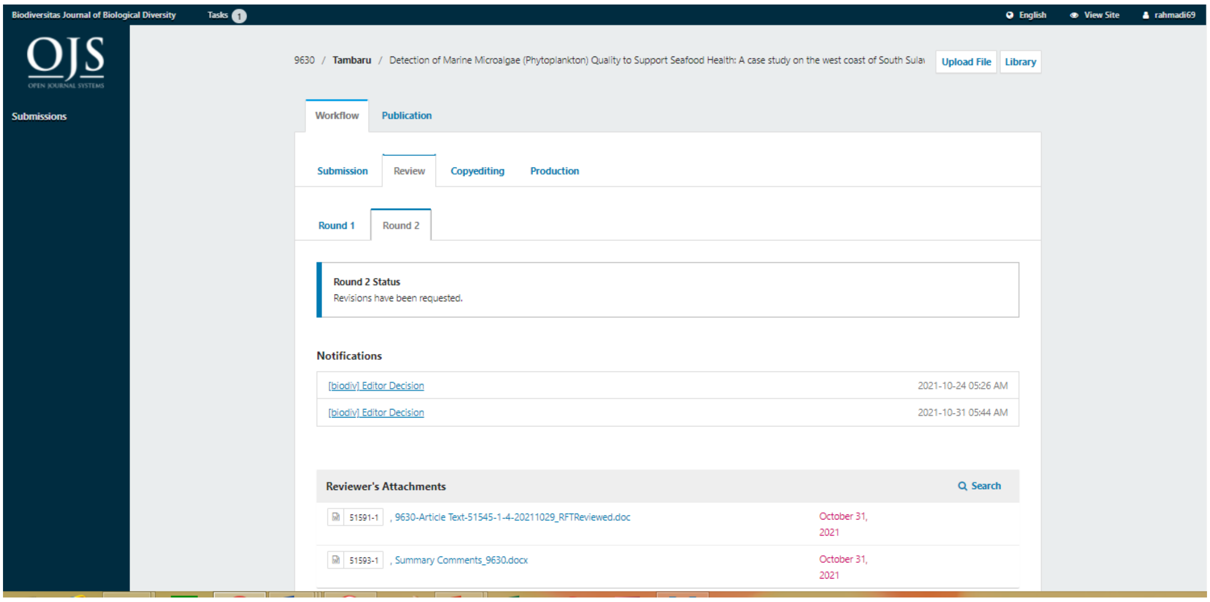
It is an interesting paper to read. The detection looks good to me! Below is my assessment on the draft paper.

Basic Reporting:

a. Clear, unambiguous, English language has been used throughout the text. A few changes have been suggested. Suggestions include removing unnecessary words and splitting long sentences into 2-3 short sentences.
b. Intro & background show context. Concepts are well explained and sufficient literature/ background information are provided.
c. Statistical analysis to be completed with tests for normality and equal variances. Please use SD/SE to be combined with mean. Mean would not be stand alone without variations (SD/SE).

d. Literature is well referenced & relevant but its completeness could be improved (e.g. see below).

1. Meesters E, Tapilatu RF. 2020. First dive Raja Ampat, Sorido. https://weblog.wur.eu/coastsea/first-dive-raja-ampat-sorido/
2. Thovyan, A.I., Tapilatu, R.F., Sabariah, V. and Venables, S.K., 2020. Plankton abundance and community structure in reef manta ray (Mobula alfredi) feeding habitat in the Dampier Strait, Raja Ampat, West Papua, Indonesia. *AACL Bioflux*, *13*(5), pp.2956-2969.



Notifications

[biodiv] Editor Decision

2021-10-31 05:44 AM

Rahmadi Tambaru:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sulawesi, Indonesia".

Our decision is: Revisions Required

Reviewer A:

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Recommendation: Revisions Required

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Biodiversitas Journal of Biological Diversity

B2. MANUSCRIPT PROGRESS: ADVANCED REVIEW OF MANUSCRIPT DRAFT FROM REVIEWERS (SECOND ROUND)

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

Abstract. The research aimed~~s~~ to detect ~~the quality of~~ marine microalgae's quality to support seafood health was carried out from January to November 2020 along ~~in~~ the west coast of South Sulawesi, Indonesia. Samples were collected from the coastal waters of Pangkep Regency, Maros Regency, 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 ~~time~~ periods. Based on the types and relative abundance of phytoplankton present, i.e. harmful algal bloom (HABs) forming or not (non-HABs), the results showed ~~that~~ the quality of marine microalgae, specifically phytoplankton, was relatively good. ~~There were many~~Many more non-HAB (94-98%) than HAB (2-6%) marine microalgae were detected. This means that the phytoplankton flourishing in these waters ~~are~~ is most~~ly~~ suitable as food for other organisms, including fish and shellfish. It also means that if ~~these fish and shellfish are harvested by~~ fishers~~rmen~~ harvest this fish and shellfish, they should be fit and safe for human consumption.

Commented [MOU1]: Unclear Antecedent, please clarify

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

Abbreviations: Harmful algal bloom (HABs)

Running title: Phytoplankton Quality to Support Seafood Health

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 (Shadrin et al. 2017; Sunda 2012).

Periodic increases in microalgae populations are natural phenomena (Visciano et al. 2016). They ~~and~~ 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 et al. 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. 2016), and changes to aquatic ecosystem community structure (Todd et al. 2019; Lu et al. 2018).

Rapid or excessive increases in ~~the population of~~ microalgae (phytoplankton) population, termed algal blooms, can occur when environmental conditions are conducive to algal growth (Meesters and Tapilatu, 2020) and reproduction. 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 (Berdalet et al. 2016; Brown et al. 2019; Glibert 2017, Meesters and Tapilatu, 2020).

Over time, the incidence of HABs is increasingly being noticed by environmentalists. The emergence of new types of HAB, including the increasing frequency of occurrence, ~~as well as~~ the expansion of the geographic area affected, and the prolonged duration of their occurrence, ~~are events that~~ are increasingly being discussed. From recent studies, several researchers have found different types of toxins from HBs toxins that have been there before. Of course, this ~~has an~~

impacts on the increasing mortality of marine organisms (Fukoyo 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. 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 ~~very dangerous~~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. ~~In fact, many~~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 Regency, attracting public attention and suddenly creating awareness in an area where such an incident had never happened before. Environmental experts put forward ~~a variety of different~~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, ~~an incident occurred with~~ around 63 local residents ~~being~~ were poisoned due to eating shellfish, specifically clams or cockles of the genus *Anadara* (REF?). The clams were collected in the coastal waters of Mallasoro Village (on the south coast of South Sulawesi in Bangkala District, Jeneponto Regency. It is suspected that the clams were contaminated with toxins from potentially dangerous (toxin-producing) phytoplankton. ~~Clearly~~Thus, more research is requir~~need~~ed to test the hypotheses ~~put forward and~~ and~~to~~ provide definitive answers regarding ~~the above-mentioned~~ incidents as 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; Any additional REFS?).~~, and~~ I~~i~~n some cases use various drugs and fertilizers to increase production; this is also the case in the coastal waters of Mallasoro Village, Jeneponto Regency, 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 ~~with respect to~~regarding the types of microalgae that can form HABs. Increases and or changes in nutrient concentration can occur due to seasonal changes ~~such as~~ between the east and west monsoons (Rastina et al. 2020; Vajravelu et al. 2018). These fluctuations will ~~have an~~ impact ~~on~~ the microalgal communities, including the presence of HAB forming species whose presence can be seasonal (Narale and Anil 2017; Trainer et al. 2020; Vajravelu et al. 2018), 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 (Lestari et al. 2021; Mujib et al. 2015; Rukminasari and Tahir 2020; Samawi et al. 2020). Meanwhile, information on HAB forming phytoplankton is very important for anticipating and early detection of HABs, ~~as well as~~and a basis for evaluating the quality of coastal waters with respect to 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 ~~have been~~ depend~~ing~~ 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 ~~time~~ periods (Table 1). The four research sites (Figure 1) were in Pangkep Regency (PK), Maros Regency (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

Number	P~~Time p~~eriod	Time of day	Season
1	June-July 2020	10:00-16:00	Dry season
2	August-September 2020	10:00-16:00	Dry to the rainy season
3	October-November 2020	10:00-16:00	Beginning of the rainy season

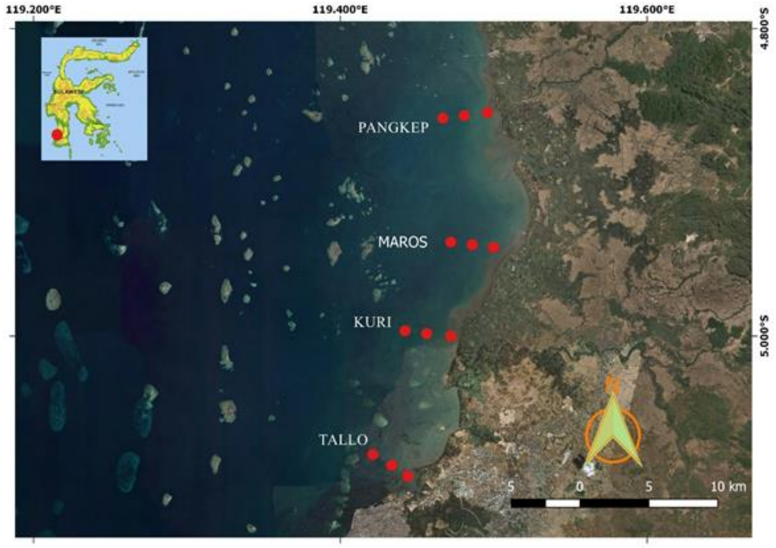

Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi

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 ~~time~~ 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 liter Kemmerer Water Sampler. At each site, 1 liter of water was taken from each station (zone) ~~for the counting and identification of~~ 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's solution for one~~1~~ 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 Sedgwig 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 ~~such as~~ (Castellani and Edwards 2017; Tomas 1997).

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. These analyses were implemented in SPSS 17 and Excel Stat 2017 (Brahem et al. 2017).

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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 locations/stations) and temporally (between study periods); however, for almost all sampling periods and sites, the dominant phytoplankton genera were *Chaetoceros*, *Coscinodiscus*, *Nitzschia*, *Odontela*, 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, ~~as a whole,~~ mean phytoplankton abundance was higher at the Tallo and Kuri sites than at the Maros and Pangkep stations. The analysis of variance indicated a significant difference in phytoplankton abundance between the locations/stations (*p* <0.01). The observed phytoplankton abundance also varied between the~~ time~~ periods. However, the analysis of variance indicated that the differences in the abundance of phytoplankton over time were not statistically significant ~~over the period of~~throughout observation (*p*> 0.05).

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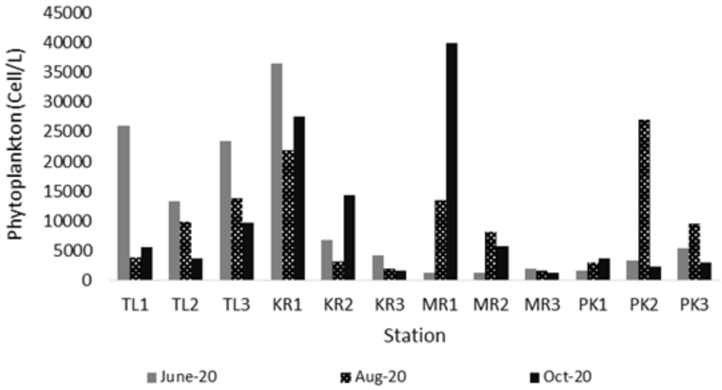

Figure 2. Mean±SD/SE sample phytoplankton density over the research period

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

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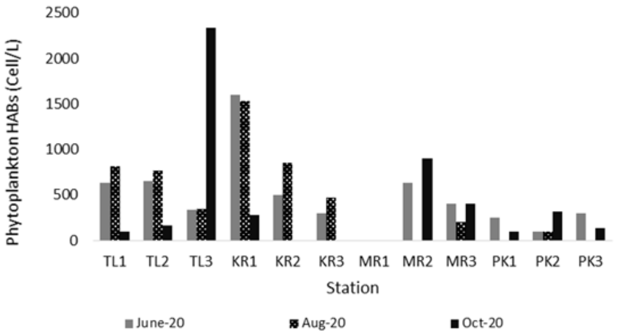
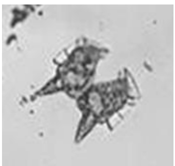


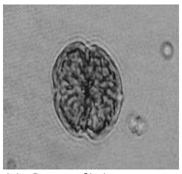
Figure 3. Mean density of HABs forming phytoplankton in the samples collected during the study period

The presence and abundance of the potentially HAB forming taxa varied between locations/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 locations/stations.

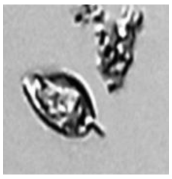
(a) *Ceratium*



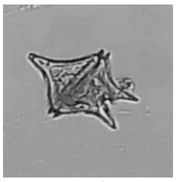
(b) *Dinophysis*



(c) *Gymnodinium*



(d) *Prorocentrum*



(e) *Protoperidinium*

Figure 4. Examples of phytoplankton from class Dinophyceae

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).

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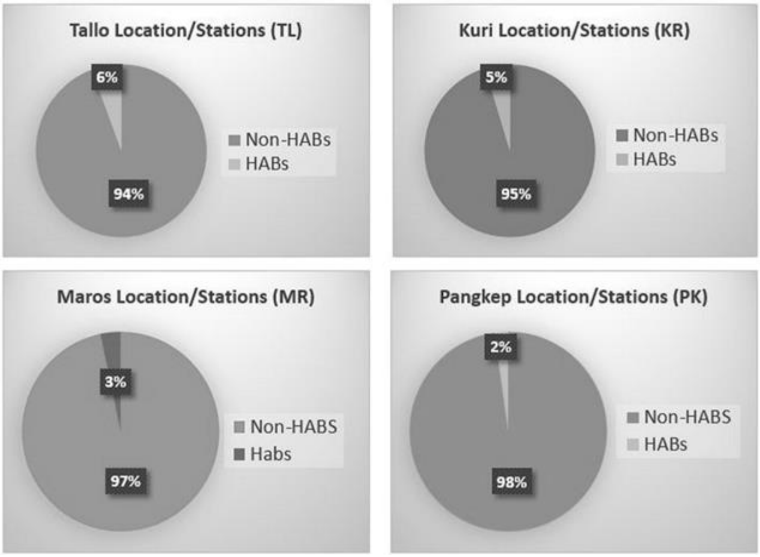

Figure 5. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period

Discussion

The dynamics of marine microalgal communities vary from one location to another and from one time to another. Changes in community composition occur frequently (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)~~the community will be dominated by other groups~~.

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 locations/stations and observation ~~time~~ periods: *Chaetoceros, Coscinodiscus, Nitzschia, Odontela, 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 ~~around the~~ 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 ~~N-type nutrient concentrations are~~ low N-type nutrient concentrations.

The Tallo location/stations had the highest phytoplankton abundance, differing significantly from Maros and Pangkep ($p<0.01$); however, the abundance at the Kuri location 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 results of~~ the analysis of variance, the abundance of phytoplankton was not significantly different based on the period of observation ($p>0.05$). This is consonant with the results on the in-situ water quality parameter measurements, as several parameters which can affect phytoplankton growth did not vary significantly between the three~~-time~~ periods. This means that phytoplankton experienced similar conditions for growth throughout the study.

Types of marine microalgae which are considered dangerous as HABs forming phytoplankton were present at almost all locations/stations (Figure 3). This is an ~~important~~ essential observation 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

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The taxa identified included ~~5~~ five potentially HABs forming phytoplankton genera belonging to the Class Dinophycea: *Ceratium*, *Dinophysis*, *Gymnodinium*, *Prorocentrum*, and *Protoperidinium*. These genera were present in most locations/stations and observation ~~time~~ periods. The classification of these taxa as presenting a risk of HABs can be justified because the Dinophyceae ~~are known to~~ contain the most toxic genera (Tillmann et al. 2010). These five HABs genera produce toxic metabolites, so they ~~are classified as~~ belong~~ing~~ 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 addition of the number and abundance of HABs in surface waters can come from aquatic sediments. HAB in aquatic sediments is in the form of cysts, such as *Gymnodinium* cysts from the Dinophyceae class (Dzhembekova et al. 2018). According to Fukoyo et al (2011), phytoplankton in this class play an important role in recurring HAB events ~~as well~~ and ~~as~~ contribute~~ing~~ to the geographical expansion of HAB occurrences. These repeat HABs can occur when ~~a large number of~~many cysts of potentially HAB-~~f~~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 Sugestiningsih, 2000), and 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* ~~is considered to~~ 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). *Protoperidinium* 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 locations/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 ~~time~~ periods, the lack of statistical significance (ANOA, $p>0.05$) 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 are multiplying, they release toxins (Glibert 2017, 2020); these may impede the growth and reproduction of the non-HAB phytoplankton as well as affect~~ing~~ other organisms (Dorgham 2014; Glibert 2017). Conversely, a greater variety of non-HAB phytoplankton ~~is an indication of~~ 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 (Gilbert 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 ~~greatly~~, 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, and also indicate that fisheries produce from the study locations 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 ~~with respect to~~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 ~~good~~reasonable. This evaluation is based on the taxonomic composition and abundance of non-harmful (non-HAB) phytoplankton, which were 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 ~~as~~ 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

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ACKNOWLEDGEMENTS

The authors wish to thank the Rector of Universitas Hasanuddin for a research grant provided through the Universitas Hasanuddin Research and Community Service Institute (LP2M) under the 2020 Basic Research scheme.

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B3. FURTHER RESPONSE TO REVIEWERS (SECOND ROUND)

Second Round Status on OJS

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9630 / **Tambaru** / Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study or   Upload File   Library

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Submission   Review   Copyediting   Production

Round 1   Round 2

Round 2 Status
Revisions have been submitted.

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Revisions   Search   Upload File

51768-1	Article Text, A-9630-Article Text-51545-1-4-20211029_RFTReviewed_Revisi_A1.doc	November 2, 2021	Article Text
51769-1	Other, Rahmadi-Response to Reviewer_2021_2.docx	November 2, 2021	Other

Review Discussions   Add discussion

Name	From	Last Reply	Replies	Closed
Submitted a revised manuscript	rahmadi69 2021-10-29 01:10 PM	-	0	
2nd Response to Reviewers/Editors	rahmadi69 2021-11-02 03:28 PM	-	0	

2nd Response to Reviewers/Editors

Participants Edit
Smujo Editors (editors)
Rahmadi Tambaru (rahmadi69)

Messages

Note	From
Dear Editors We again submitted a revised manuscript entitled "Quality detection of marine microalgae (Phytoplankton) to support seafood health: A case study on the west coast of South Sulawesi, Indonesia" to the Biodiversity Journal. We also send the 2nd Response to the Reviewer/Editor (attached). Regards, Rahmadi Tambaru Faculty of Marine Sciences and Fisheries Hasanuddin University rahmadi69, Rahmadi-Response to Reviewer_2021_2.docx rahmadi69, A-9630-Article Text-51545-1-4-20211029_RFTReviewed_Revisi_A1.doc	rahmadi69 2021-11-02 03:28 PM

2nd Response Status to Reviewers/Editors
Biodiversitas Journal of Biological Diversity

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

Corresponding: Rahmadi Tambaru (aditbr69@unhas.ac.id)

Date : November 2, 2021

Nu.	Comments	Responds	
Reviwer A	Clear, unambiguous, English language has been used throughout the text. A few changes have been suggested. Suggestions include removing unnecessary words and splitting long sentences into 2-3 short sentences.	Thank you. We have splitting long sentences into short sentences (lines 209-211 page 6, and lines 242-245 page 7)	
	Intro & background show context. Concepts are well explained and sufficient literature/ background information are provided.	Thank you.	
	Statistical analysis to be completed with tests for normality and equal variances. Please use SD/SE to be combined with mean. Mean would not be stand alone without variations (SD/SE).	We have already described the tests performed before and after the ANOVA Test (lines 127-129 pages 4-5)	
	Literature is well referenced & relevant but its completeness could be improved (e.g. see below). 1. Meesters E, Tapilatu RF. 2020. First dive Raja Ampat, Sorido. https://weblog.wur.eu/coastsea/first-dive-raja-ampat-sorido/ 2. Thovyan, A.I., Tapilatu, R.F., Sabariah, V. and Venables, S.K., 2020. Plankton abundance and community structure in reef manta ray (Mobula alfredi) feeding habitat in the Dampier Strait, Raja Ampat, West Papua, Indonesia. *AACL Bioflux*, *13*(5), pp.2956-2969	We have cited the suggested literature (Meesters : lines 34 and 39, page 1; Thovyan ; line 169)	
The other notes		We replace the word "location" with the word "site" in its entirety (example on lines 139-140 page 4)	
		We revised the sentence on lines 186-188 page 6	
		The word "this" has been revised and clarified (line 15 page 1; line 186 and line 190 page 6)	
		Figure 2 and 3 have been completed with Standard Deviation (page 4)	

		Sentence revision and addition of some supporting literature in paragraphs 3 and 4 on page 1. The literature has been included in the reference list	
		Sentence revision on line 145 page 4	
		The word “REF” (line 68) and the word “Any additional REFS?” (line 74) removed	

B4. SECOND REVISED (NOVEMBER 2, 2021)

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

Abstract. 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 Regency, Maros Regency, 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 are 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: Marine, microalgae, phytoplankton, HABs, seafood, health

Abbreviations: Harmful algal bloom (HABs)

Running title: Phytoplankton Quality to Support Seafood Health

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 (Shadrin et al. 2017; Sunda 2012).

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 (Todd et al. 2019; Lu et al. 2018).

Rapid or excessive increases in microalgae (phytoplankton) population, termed algal blooms, can occur when environmental conditions are conducive to algal growth and reproduction (Glibert 2017; Meesters and Tapilatu 2020; Paerl et al. 2018). 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; Brown et al. 2019; Glibert 2017; 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 (Anderson et al. 2015; Berdalet et al. 2016; D'Costa et al. 2017; Fu et al. 2012; Paerl et al. 2018; Trainer et al. 2020; Wells et al. 2015; Xiao et al. 2019). 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 Regency, 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 Regency. 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 Regency, 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 (Rastina et al. 2020; Vajravelu et al. 2018). These fluctuations will impact the microalgal communities, including the presence of HAB forming species whose presence can be seasonal (Narale and Anil 2017; Trainer et al. 2020; Vajravelu et al. 2018), 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 (Lestari et al. 2021; Mujib et al. 2015; Rukminasari and Tahir 2020; Samawi et al. 2020). Meanwhile, information on HAB forming phytoplankton is very important for anticipating and early detection of HABs, and a basis for evaluating the quality of coastal waters with respect to 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 Regency (PK), Maros Regency (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

Number	Period	Time of day	Season
1	June-July 2020	10:00-16:00	Dry season
2	August-September 2020	10:00-16:00	Dry to the rainy season
3	October-November 2020	10:00-16:00	Beginning of the rainy season

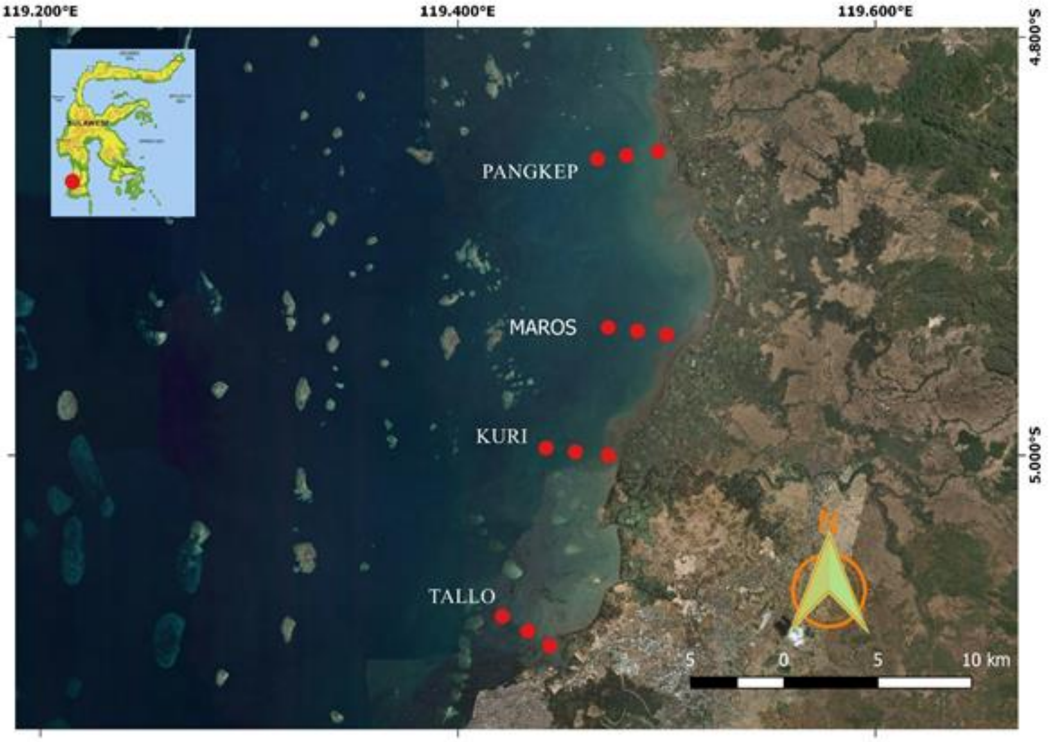


Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi

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 Sedgwig 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 (Castellani and Edwards 2017; Tomas 1997).

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 ($\alpha = 0.05$). 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*, *Odontela*, 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).

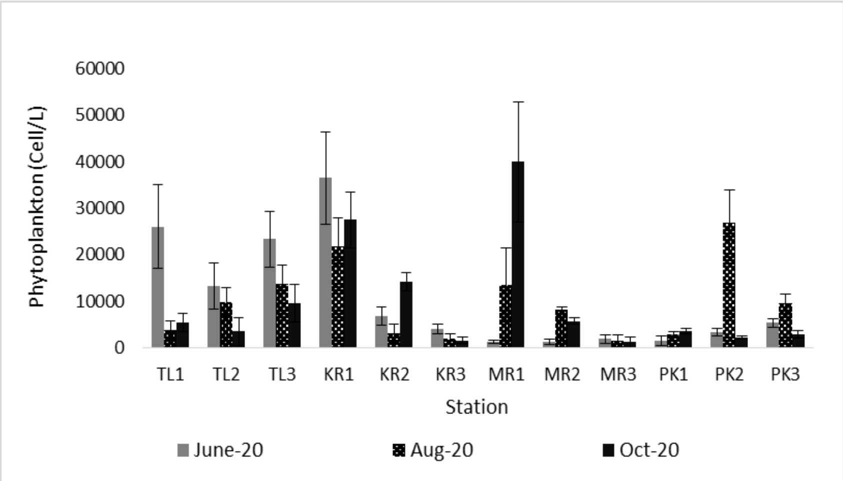


Figure 2. Phytoplankton density at the sampled sites in South Sulawesi over the research period (mean ± SD)

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 *Protoperidinium* (Figure 4). These genera were present in most sites/stations and observation periods.

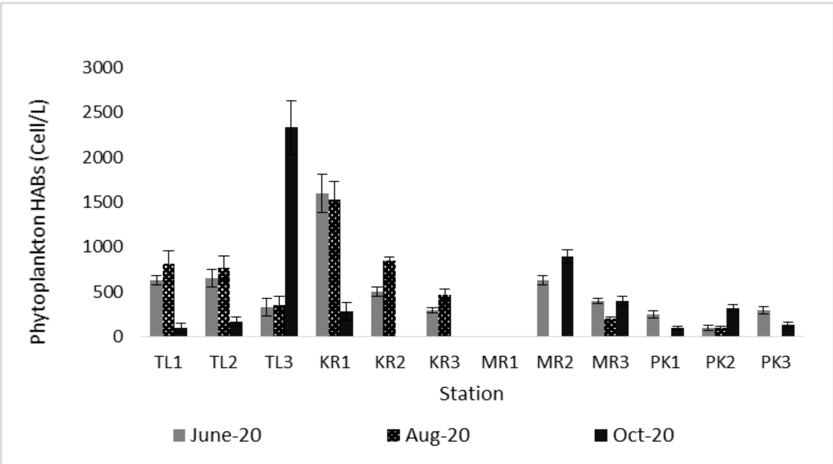
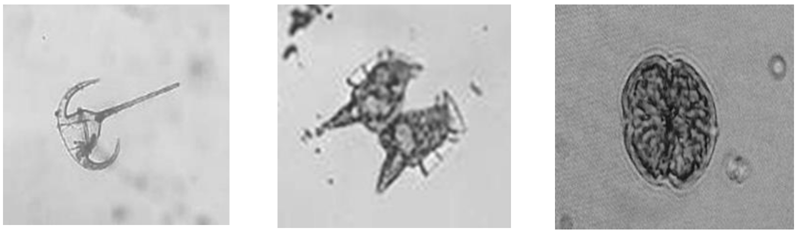
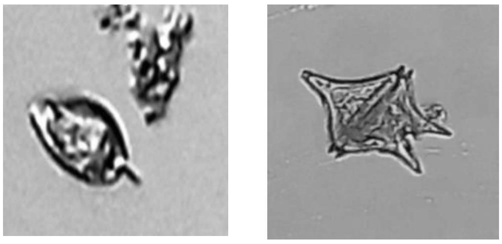


Figure 3. HAB forming phytoplankton density in samples from South Sulawesi over the study period (mean ± SD)

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.



(a) *Ceratium* (b) *Dinophysis* (c) *Gymnodinium*



(d) *Prorocentrum* (e) *Protoperidinium*

Figure 4. Examples of phytoplankton from class Dinophyceae

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).

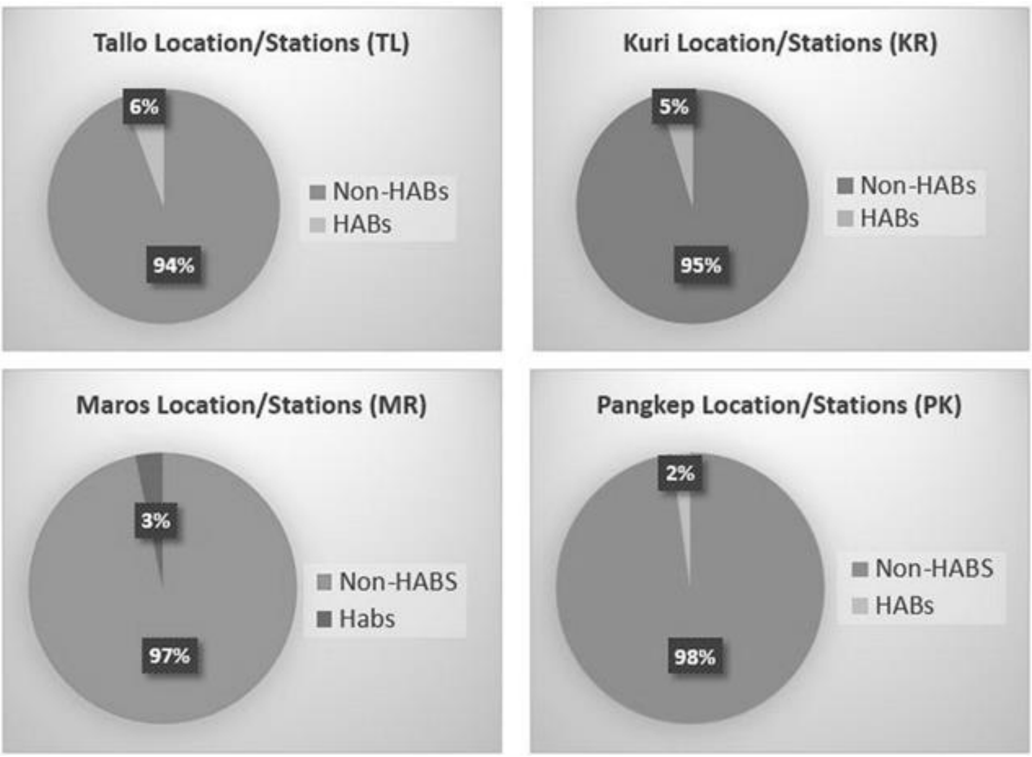


Figure 5. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period

Discussion

The dynamics of marine microalgal communities vary from one site to another and from one time to another. Changes in community composition occur frequently (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, Odontela, 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 results of the analysis are supported by *in-situ* water quality parameters, as several parameters which 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 *Protoperidinium*. 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 Sugestiningsih, 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). *Protoperidinium* 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 (ANOA, $p>0.05$) 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 are 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 (Dorgham 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 (Gilbert 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 produce 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 were 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.

ACKNOWLEDGEMENTS

The authors wish to thank the Rector of Universitas Hasanuddin for a research grant provided through the Universitas Hasanuddin Research and Community Service Institute (LP2M) under the 2020 Basic Research scheme.

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BIODIVERSITAS
Volume 22, Number 11, November 2021
Pages: xxxx

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d2211xx

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

RAHMADI TAMBARU♥, ANDI I. BURHANUDDIN, ARNIATI MASSINAI[1], MUHAMMAD A. AMRAN[1]
Department of Marine Science, Faculty of Marine Science and Fisheries, Universitas Hasanuddin. Jl. Perintis Kemerdekaan KM 10 Tamalanrea, Makassar, South Sulawesi, Indonesia, 90425 Tel./Fax. +62-411-586025, ♥email: aditbr69@unhas.ac.id

Manuscript received: 11 October 2021. Revision accepted: xxx October 2021.

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: xxxx.* 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 Regency, Maros Regency, 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 are 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: Marine, microalgae, phytoplankton, HABs, seafood, health

Abbreviations: Harmful algal bloom (HABs)

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 (Shadrin et al. 2017; Sunda 2012).

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 (Todd et al. 2019; Lu et al. 2018).

Rapid or excessive increases in microalgae (phytoplankton) population, termed algal blooms, can occur when environmental conditions are conducive to algal growth and reproduction (Glibert 2017; Meesters and Tapilatu 2020; Paerl et al. 2018). 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; Brown et al. 2019; Glibert 2017; 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 (Anderson et al. 2015; Berdalet et al. 2016; D'Costa et al. 2017; Fu et al. 2012; Paerl et al. 2018; Trainer et al. 2020; Wells et al. 2015; Xiao et al. 2019). 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 Regency, 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 Regency. 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 Regency, 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 (Rastina et al. 2020; Vajravelu et al. 2018). These fluctuations will impact the microalgal communities, including the presence of HAB forming species whose presence can be seasonal (Narale and Anil 2017; Trainer et al. 2020; Vajravelu et al. 2018), 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 (Lestari et al. 2021; Mujib et al. 2015; Rukminasari and Tahir 2020; Samawi et al. 2020). Meanwhile, information on HAB forming phytoplankton is very important for anticipating and early detection of HABs, and a basis for evaluating the quality of coastal waters with respect to 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 Regency (PK), Maros Regency (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

Number	Period	Time of day	Season
1	June-July 2020	10:00-16:00	Dry season
2	August-September 2020	10:00-16:00	Dry to the rainy season
3	October-November 2020	10:00-16:00	Beginning of the rainy season

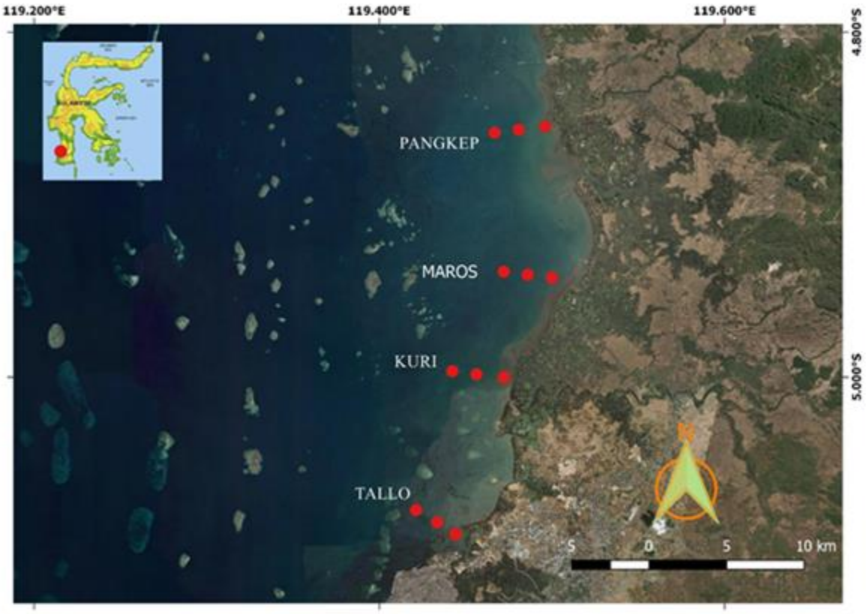


Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi

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 Sedgwig 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 (Castellani and Edwards 2017; Tomas 1997).

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 ($\alpha = 0.05$). 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, Odontela*, 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 *Protoperidinium* (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).

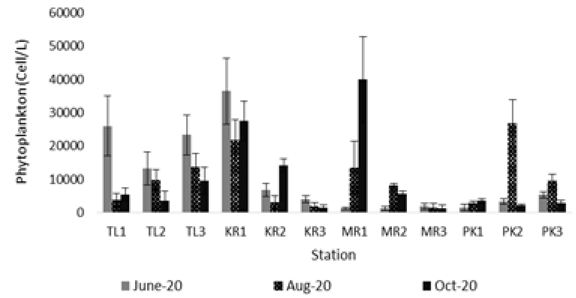


Figure 2. Phytoplankton density at the sampled sites in South Sulawesi over the research period (mean ± SD)

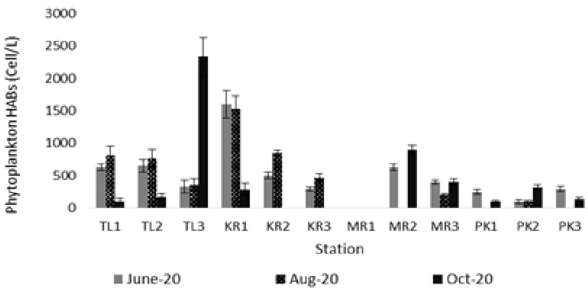
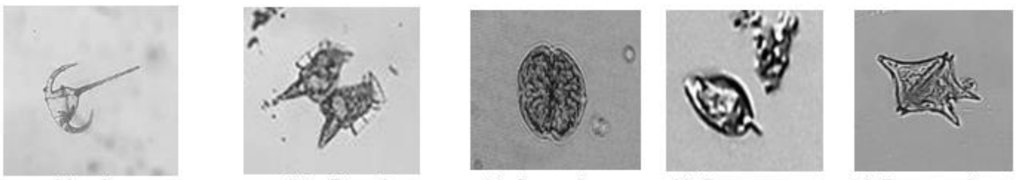


Figure 3. HAB forming phytoplankton density in samples from South Sulawesi over the study period (mean ± SD)



(a) *Ceratium* (b) *Dinophysis* (c) *Gymnodinium* (d) *Prorocentrum* (e) *Protoperidinium*

Figure 4. Examples of phytoplankton from class Dinophyceae

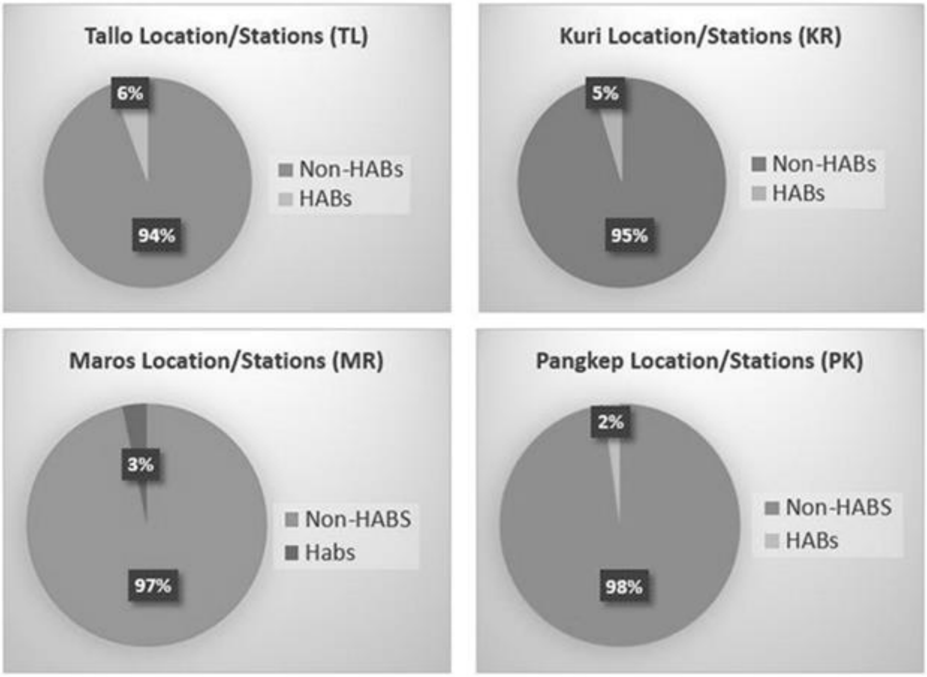


Figure 5. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period

Discussion

The dynamics of marine microalgal communities vary from one site to another and from one time to another. Changes in community composition occur frequently (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, Odontela, 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 results of the analysis are supported by *in-situ* water quality parameters, as several parameters which 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 *Protoperidinium*. 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 Sugestiningsih, 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). *Protoperidinium* 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 (ANOA, $p>0.05$) 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 are 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 (Dorgham 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 (Gilbert 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 produce 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 were 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.

ACKNOWLEDGEMENTS

The authors wish to thank the Rector of Universitas Hasanuddin for a research grant provided through the Universitas Hasanuddin Research and Community Service Institute (LP2M) under the 2020 Basic Research scheme.

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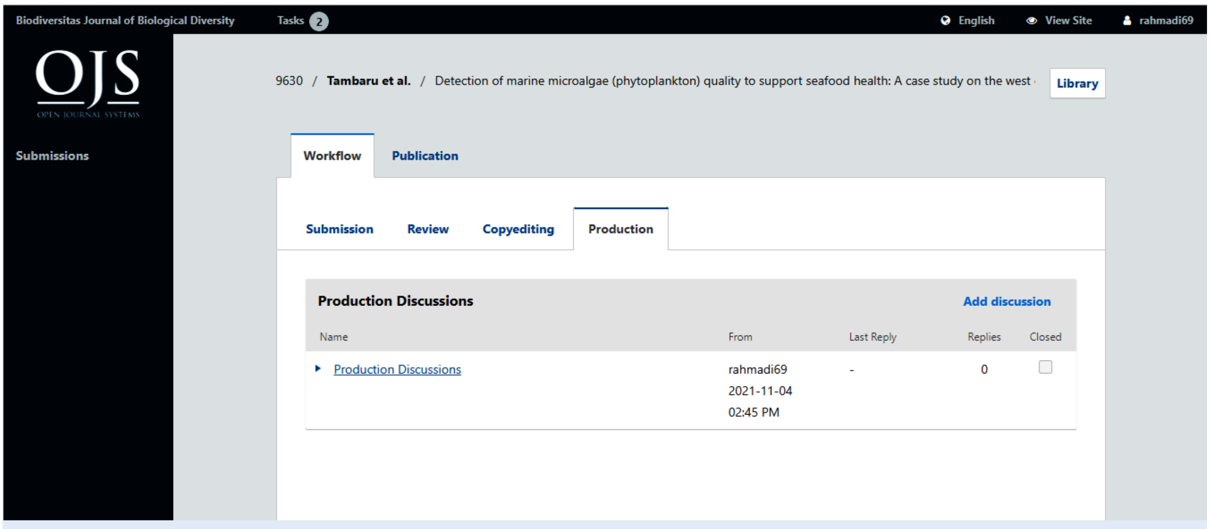

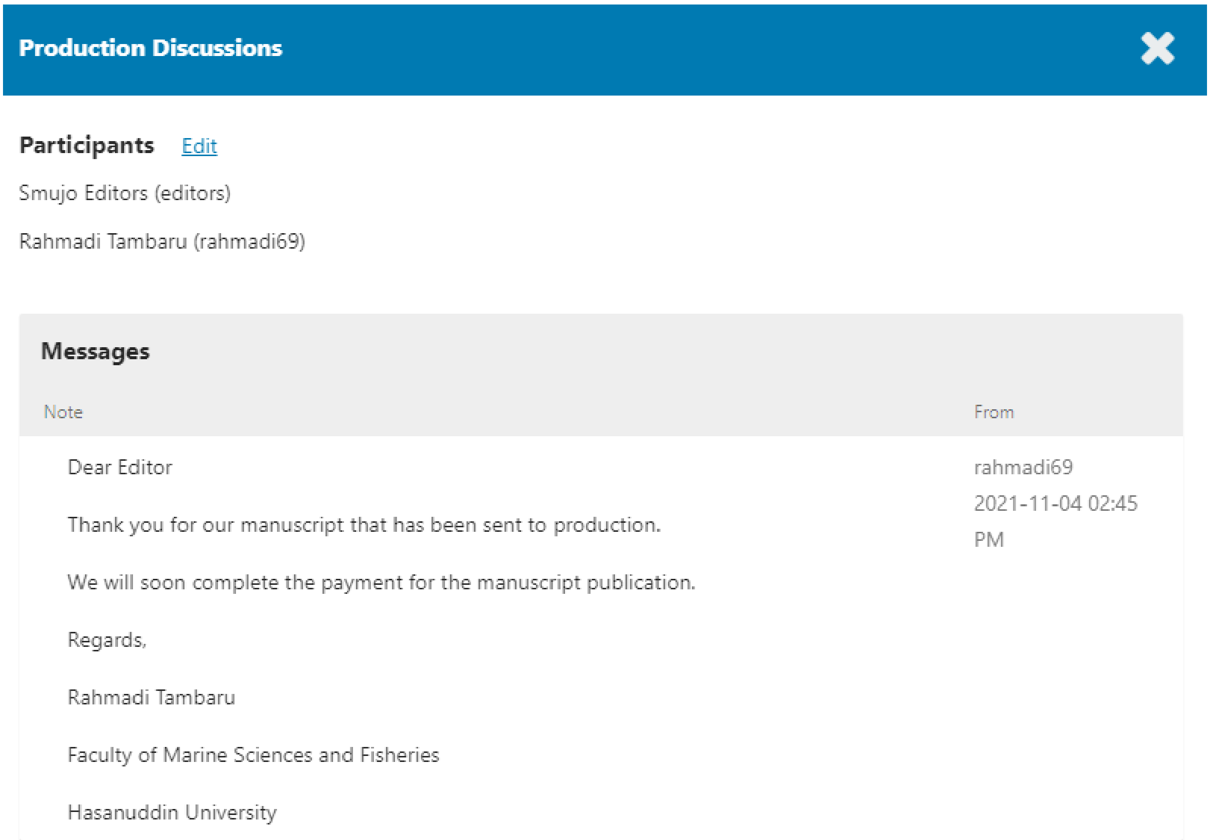

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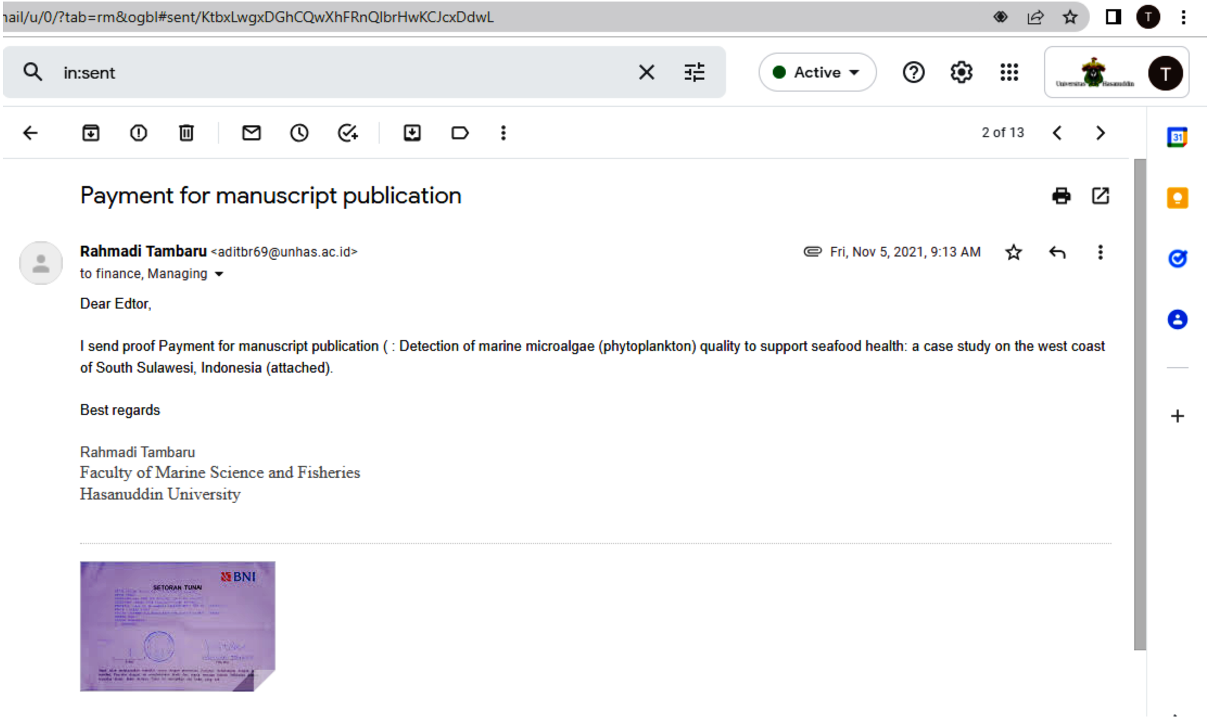

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