

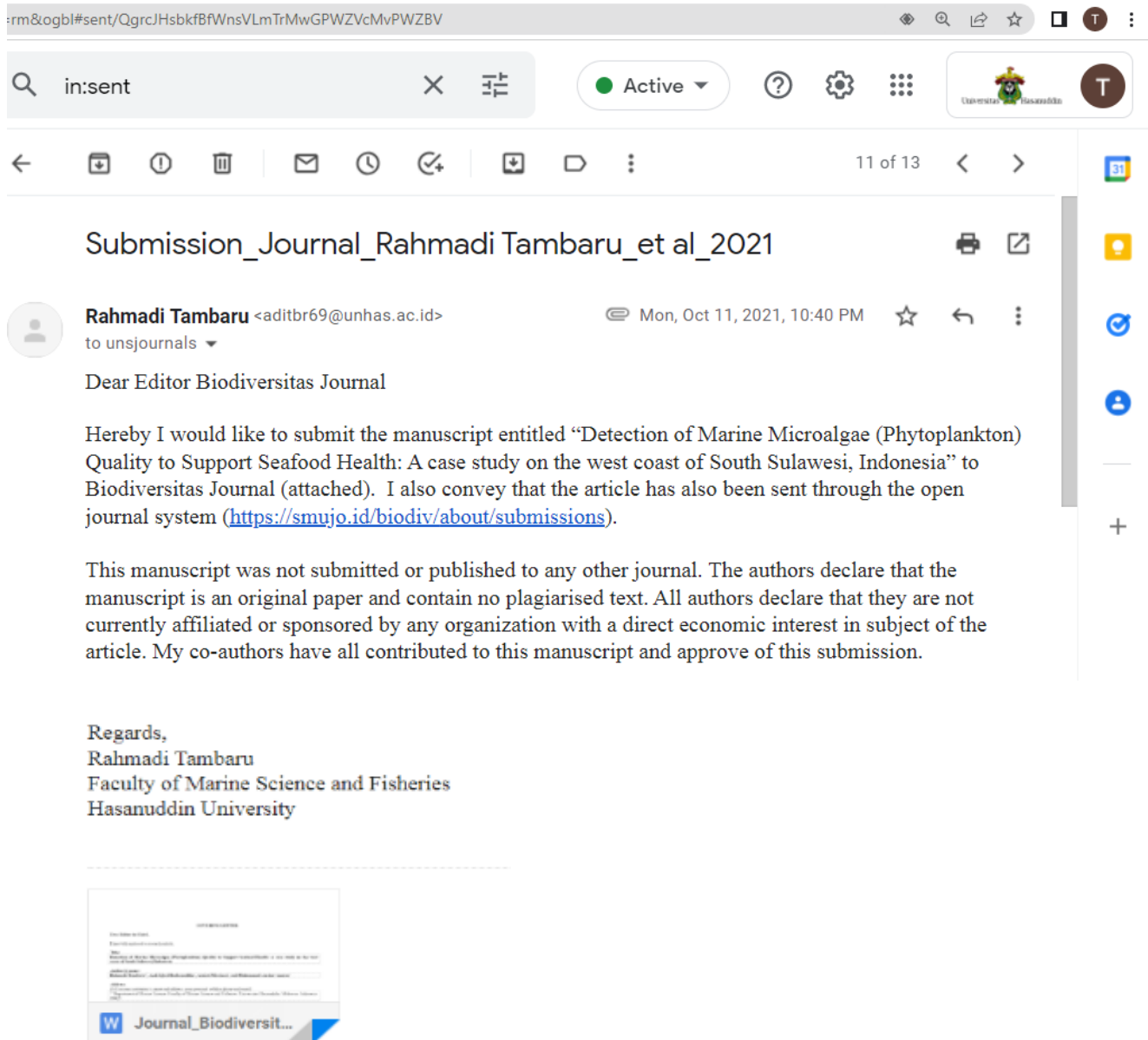
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Judul: Detection of marine microalgae (phytoplankton) quality to support seafood health: A case study on the west coast of South Sulawesi, Indonesia

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Regards,
Rahmadi Tambaru
Faculty of Marine Science and Fisheries
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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 Burhanuddin¹, Arniati Massinai¹, and Muhammad Anshar Amran¹

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^{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,

(fill in your name, no need scanned autograph)

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. BURHANUDDIN¹, ARNIATI MASSINAI¹, MUHAMMAD A. AMRAN¹

¹Department 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

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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

43 into the sea (Hughes et al. 2011).

44 Some of the acute diseases caused by toxins from microalgae in the HABs group are paralytic shellfish poisoning (PSP),
45 amnesic shellfish poisoning (ASP), and diarrhetic shellfish poisoning (DSP) (Krock et al. 2018). These toxins are very
46 dangerous because they attack the human nervous system, respiration, and digestion. These diseases are related to the human
47 consumption of fish and shellfish. In fact, all types of toxic phytoplankton can be found in Indonesian coastal waters, in-
48 cluding several Dinoflagellate species from the genera *Ostreopsis*, *Prorocentrum*, and *Gambierdiscus* (Hasani et al. 2013).
49 One HABs event in 1993 took place in Jakarta Bay where a mass fish-kill was caused by the excessive abundance of phyto-
50 plankton that can cause HABs. Several similar HABs incidents have occurred in the waters of Lewotobi and Lewouran (East
51 Nusa Tenggara), Sebatik Island (East Kalimantan), and Ambon Bay (Mahmudi et al. 2020).

52 Other events of interest have been observed in the northern and southern coastal waters of South Sulawesi. In November
53 2014, a mass fish-kill incident took place in estuarine coastal waters of Pangkep Regency, attracting public attention and
54 suddenly creating awareness in an area where such an incident had never happened before. Environmental experts put for-
55 ward a variety of different hypotheses to explain the mass fish-kill; causal factors proposed included pollution by organic
56 matter, reduced oxygen content, the blooming of certain types of phytoplankton, and a decrease in the quality of river sedi-
57 ment (Rukminasari and Tahir 2020). Furthermore, in August 2016 an incident occurred with around 63 local residents being
58 poisoned due to eating shellfish, specifically clams or cockles of the genus *Anadara*. The clams were collected in the coastal
59 waters of Mallasoro Village (on the south coast of South Sulawesi in Bangkala District, Jeneponto Regency) and were sus-
60 pected of having been contaminated with potentially dangerous (toxin-producing) phytoplankton.

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

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

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

77 To evaluate the prevalence of HABs forming phytoplankton, this study aimed to detect the quality of marine microalgae
78 to support seafood health in a case study on the west coast of South Sulawesi, Indonesia. This research will be of benefit
79 through providing up-to-date data and information on the quality of marine microalgae in the west coast of South Sulawesi
80 to inform sustainable management of coastal resources, as an early detection mechanism for HABs-associated risks, and as
81 a tool for evaluating water quality in the region. In the end, it relates to the life of the community, especially fishermen who
82 have been depending on the coast and the sea for their lives.

83 MATERIALS AND METHODS

84 Research site and timeframe

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

89 **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



90
91 **Figure 1.** Map of sampling sites in the waters off the west coast of South Sulawesi

92 **Materials and research design**

93 The primary materials used in this research were seawater samples collected from the three zones at each of the four
94 stations during each time period. Several oceanographic parameters were measured in the field (*in situ*) while others were
95 measured in the laboratory. This study was non-experimental. The variables were observed without any manipulation or
96 intervention by the researchers. The variables analyzed were the abundance and composition of the phytoplankton commu-
97 nity, which included both HABs and non-HABs forming species.

98 **Phytoplankton analysis in the laboratory**

99 Seawater samples were collected using a 2 liter Kemmerer Water Sampler. At each site, 1 liter of water was taken from
100 each station (zone) for the counting and identification of phytoplankton in the laboratory. Phytoplankton cells were precipi-
101 tated out of the samples using the method developed by Utermöhl (Vadrucci et al. 2018). A 100 mL sub-sample was placed
102 in a measuring beaker (volume 100 mL) and preserved in Lugol's solution for 1 week. Once precipitation had occurred, the
103 precipitated material (10 mL) was separated from the supernatant by siphoning the supernatant out of the beaker. This pre-
104 cipitate was then placed in a bottle to which more Lugol's solution was added. A 1 mL aliquot of the precipitate was then
105 placed in a *Sedgwick Rafter Cell* (SRC) using a graduated pipette. The SRC measured phytoplankton cell abundance using
106 a sweeping (census) method (Rocha et al. 2015). Phytoplankton was identified using several standard reference works such
107 as (Castellani and Edwards 2017; Tomas 1997).

108 **Statistical analysis**

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

113 **RESULTS AND DISCUSSION**

114 **Detection of marine microalgae (phytoplankton)**

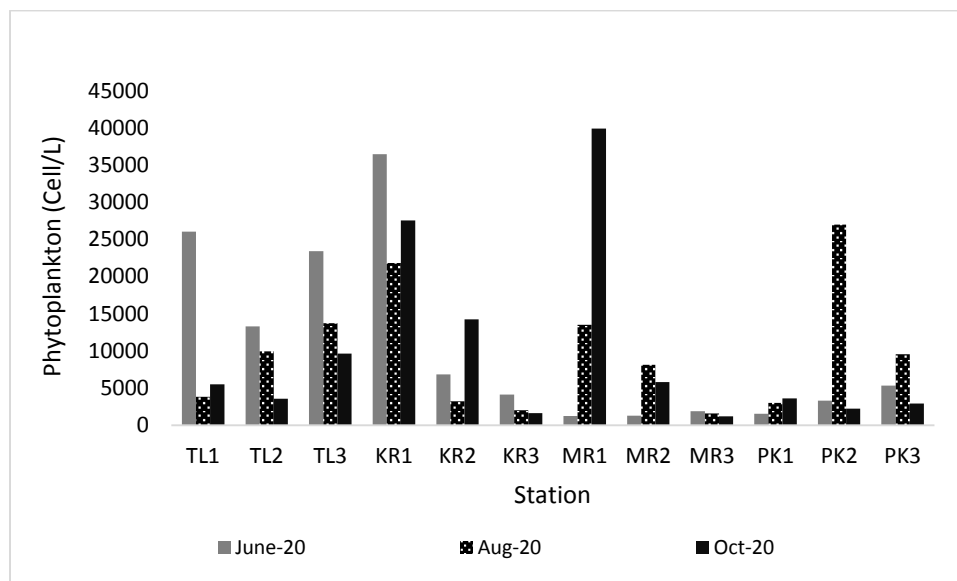
115 The dynamics of marine microalgal communities vary from one location to another and from one time to another.
116 Changes in community composition occur frequently (Marinov et al. 2010; Fujiwara et al. 2018). The survival and replica-
117 tion of phytoplankton are greatly influenced by various environmental parameters (Baek et al. 2020). In response to envi-
118 ronmental changes, these microalgal communities will comprise different types of phytoplankton with shifts in the relative

119 abundance of each type. At certain times some groups are found to be abundant, at other times the community will be
120 dominated by other groups. Changes in various environmental parameters have an impact on the population dynamics of the
121 phytoplankton that can cause HABs (Cui et al. 2018). If these changes favor the growth and replication of HABs forming
122 taxa, the equilibrium of the ecosystem can be affected, reducing the quality of marine microalgae with knock-on effects on
123 other organisms. Ecologically, phytoplankton forms the basis of most food chains, thus their abundance and composition
124 affect the existence of all aquatic biota. Information on the characteristics of the phytoplankton present in particular waters
125 can indicate their ability to support aquatic life.

126 During this study 22 phytoplankton genera belonging to 3 classes were identified. These comprised 16 genera of the
127 Class Bacillariophyceae, 1 genus of the Class Cyanophyceae, and 5 genera of the Class Dinophyceae. The genus-level com-
128 position varied spatially (between locations/stations) and temporally (between study periods); however, for almost all sam-
129 pling periods and sites the dominant phytoplankton genera were *Chaetoceros*, *Coscinodiscus*, *Nitzschia*, *Odontela*, and *Rhi-*
130 *zosolenia*, all of which belong to the Class Bacillariophyceae.

131 The genus *Chaetoceros* and Class Bacillariophyceae were the most abundant genus and classes for all sites, zones, and
132 periods of observation. These taxa are often present at high densities in marine waters around the world (Sunesen et al. 2008;
133 Angara et al. 2013) including Indonesia (Takarina et al. 2019). Usually, *Chaetoceros* species are abundant in areas where
134 nitrogenous nutrients, especially nitrates, are below the optimal concentration range for the growth of phytoplankton. Indeed,
135 Ferreira et al. (2020) stated that *Chaetoceros* can still exhibit comparatively fast growth when N-type nutrient concentrations
136 are low.

137 The phytoplankton density in the samples (Figure 2) indicates relative abundance. Over the study period as a whole,
138 mean phytoplankton abundance was higher at the Tallo and Kuri sites than at the Maros and Pangkep stations.
139

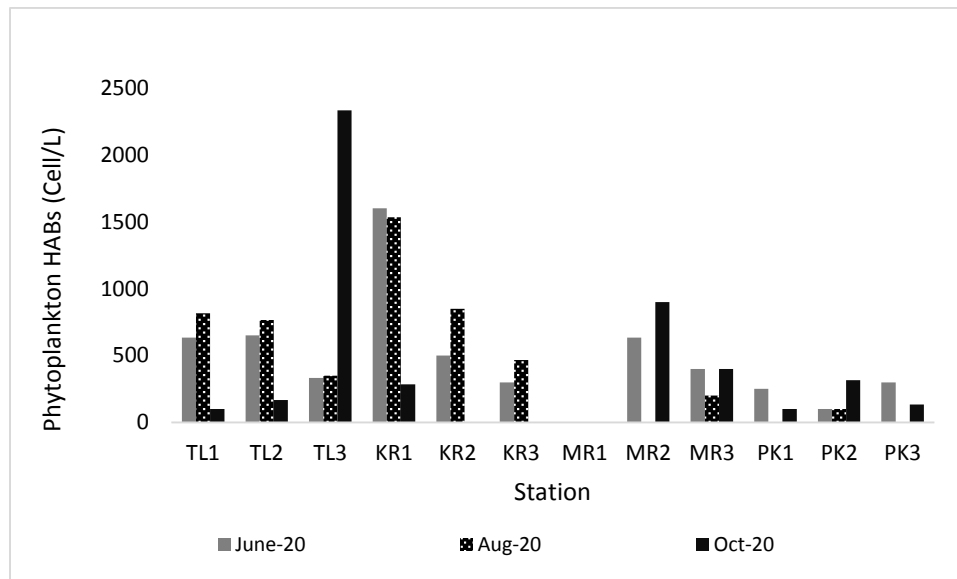


140
141 **Figure 2.** Mean sample phytoplankton density over the research period

142 The analysis of variance indicates a significant difference in phytoplankton abundance between the locations/stations (p
143 <0.01). The Tallo location/stations had the highest phytoplankton abundance, differing significantly from Maros and
144 Pangkep; however, the abundance at the Kuri location was not significantly different from that at Tallo.

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

150 Types of marine microalgae which are considered dangerous as HABs forming phytoplankton were present at almost all
151 locations/stations (Figure 3). This is an important observation as, under certain conditions, these microorganisms can nega-
152 tively affect water quality if they multiply excessively (blooming), endangering the lives of other aquatic organisms, even
153 humans when they become dominant. Through the food chain, the accumulation of toxins in the body of organisms that
154 consume HABs forming microalgae can cause health problems and even death in humans (Pettersson and Pozdnyakov 2013).
155



156

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

158 The taxa identified included 5 potentially HABs forming phytoplankton genera belonging to the Class Dinophyceae:
 159 *Ceratium*, *Dinophysis*, *Gymnodinium*, *Prorocentrum*, and *Protoperidinium*. These genera were present in most locations/sta-
 160 tions and observation time periods. The classification of these taxa as presenting a risk of HABs can be justified because the
 161 Dinophyceae are known to contain the most toxic genera (Tillmann et al. 2010). These five HABs genera produce toxic
 162 metabolites, so they are classified as belonging to the toxin producer group (Kudela et al. 2018). If these metabolites accu-
 163 mulate in the bodies of marine organisms such as shellfish and fish, they can cause poisoning in humans who consume this
 164 seafood (Farabegoli et al. 2018).

165 *Ceratium* can be identified from visual characters including horns and two flagella. This genus is considered to be a
 166 threat to the aquatic environment because it can cause oxygen deprivation through decreasing oxygen concentration in the
 167 water. *Ceratium* can result in mass mortality of marine life due to decreased oxygen levels if the population becomes too
 168 abundant (Boesch and Rabalais 1991). *Dinophysis* is considered to belong to the HABs group because it contains toxins
 169 that can cause diarrhetic shellfish poisoning (DSP) if humans consume contaminated shellfish (Taylor et al. 2013). Accord-
 170 ing to (Dietrich et al. 2019), the symptoms of poisoning caused by *Dinophysis* (DSP) include diarrhea induced by okadaic
 171 acid. *Gymnodinium* is a type of HABs forming phytoplankton that causes red tides and oxygen depletion (Pitcher and Probyn
 172 2016). *Gymnodinium* can cause paralytic shellfish poisoning (PSP) so that consuming contaminated shellfish can cause
 173 paralysis in humans (Rodrigues et al. 2012). *Protoperidinium* species contain toxins called azaspiracids. The symptoms in
 174 humans who ingest this poison are somewhat similar to those of DSP and poisoning can cause nausea in the victim within
 175 3-5 days (Trainer et al. 2013).

176 The presence and abundance of the HABs forming taxa varied between locations/stations and periods of observation
 177 (Figure 3). The analysis of variance (Appendix 2) revealed highly significant differences ($p < 0.01$) in the abundance of
 178 HABs taxa between locations/stations. While visually Tallo and Kuri appear to differ from the Maros and Pangkep sites, the
 179 Tukey test showed that the Pangkep site differed significantly from the other three sites (Tallo, Kuri, and Maros). Although
 180 the abundance of HABs taxa differed between the time periods, the Anova results indicate that these differences were not
 181 significant ($p > 0.05$). This means that the presence and abundance of taxa capable of forming HABs can be considered
 182 similar throughout the study period.

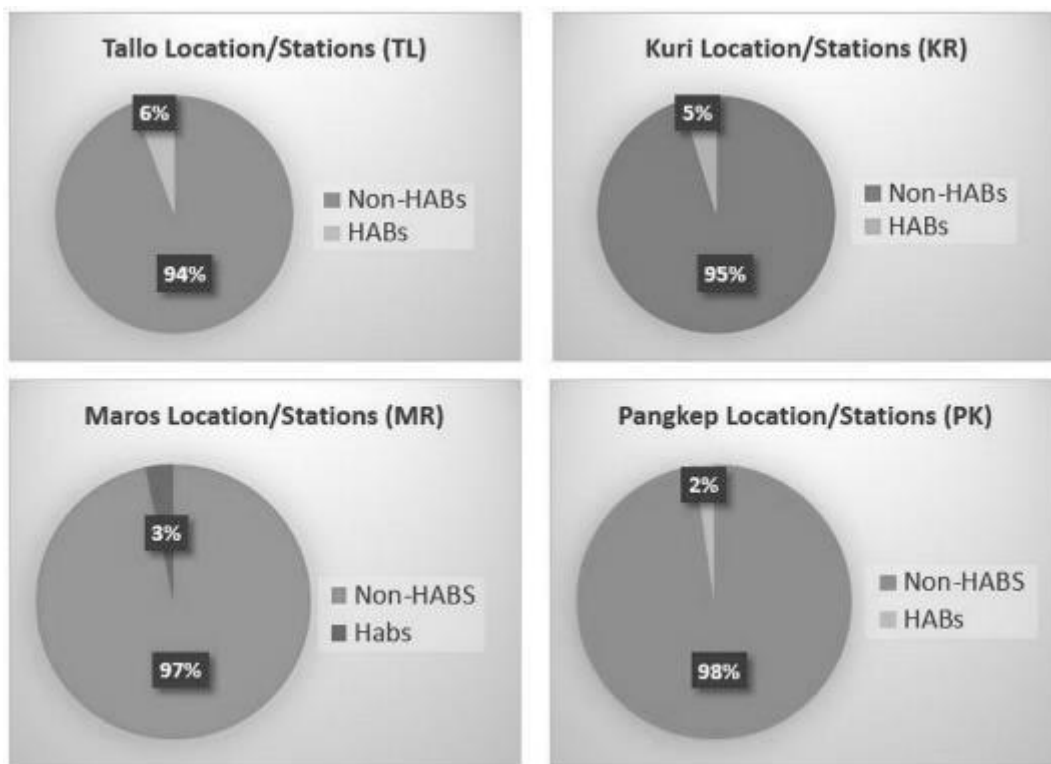
183 **Quality of marine microalgae (phytoplankton)**

184 The quality of marine microalgae is determined by the types and relative abundance of phytoplankton that can form
 185 HABs (HABs) and those which do not form HABs (Non-HABs) phytoplankton in the water. A greater variety of Non-HABs
 186 phytoplankton is an indication of a thriving phytoplankton community that can support the life of other organisms. Changes
 187 in water quality may occur, but these changes can still be tolerated by non-HABs phytoplankton. In addition to the taxonomic
 188 composition, the abundance of each taxon also plays a role in determining the quality of the microalgal community present.
 189 Good quality means that non-HABs phytoplankton abundance exceeds that of HABs phytoplankton. If this is the case, there
 190 is a reasonable likelihood that the waters are still in good condition and haven't changed greatly.

191 HABs phytoplankton are phytoplankton that can multiply rapidly when changes occur in ambient environmental condi-
192 tions. The Dinophyceae can tolerate environmental changes such as nutrient enrichment (eutrophication) (Tester et al. 2020).
193 In general, if eutrophic conditions occur, phytoplankton from the Dinophyceae will multiply more rapidly and may form
194 HABs. Once the HABs have begun to form, non-HABs phytoplankton experience a decline. This happens because when the
195 HABs phytoplankton are multiplying they release toxins which impede the growth and reproduction of the non-HABs phy-
196 toplankton.

197 The results of the phytoplankton identification and counts revealed that non-HABs phytoplankton were not only more
198 taxonomically diverse but also far more abundant than the HABs phytoplankton. The non-HABs phytoplankton comprised
199 94-98% of the microalgae cells counted while HABs forming phytoplankton accounted for 2-6% (Figure 4). This means that
200 the quality of the microalgae present in the waters where this research was conducted was still in a good condition category.
201 This information is certainly encouraging for coastal communities. Seafood is still suitable for consumption because HABs
202 can be considered as not yet contaminating.

203



204

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

206 Even though the proportions of HABs forming taxa present were very low, their presence is an early warning
207 that vigilance is needed. These results highlight the importance of maintaining water quality and the necessity of
208 regular water quality monitoring to ensure that HABs do not develop undetected.

209 In conclusion, the observed composition of the marine microalgal communities present in the coastal waters
210 along the west coast of South Sulawesi indicates that the phytoplankton quality can be considered good. This
211 evaluation is based on the taxonomic composition and abundance of non-harmful (non-HABs) phytoplankton
212 which were far more abundant than the taxa which can cause HABs. This shows that seafood is still suitable for
213 consumption because HABs can be considered as not yet contaminating.

214

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Our decision is to: Accept Submission

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B2. RESPONSE UNCORRECTED PROOF TO EDITOR


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B5. MANUSCRIPT FINAL PROOF

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

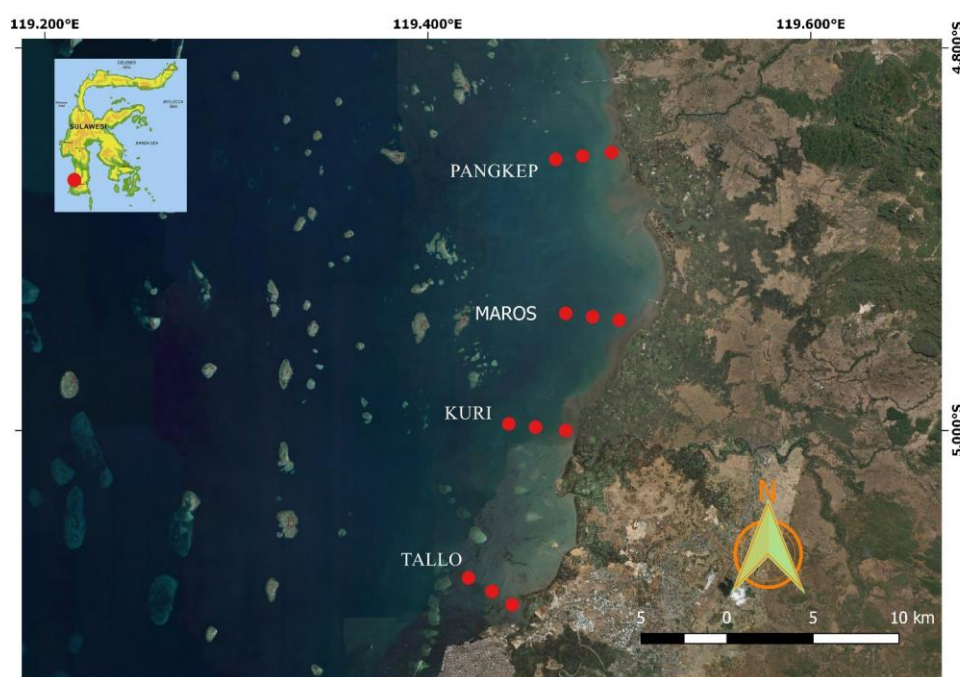


Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi, Indonesia

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 Sedgwick Rafter Cell (SRC). A 1 mL aliquot of the precipitate was placed in the SRC using a graduated pipette. The SRC was observed under a binocular microscope (Olympus CX21) at 10x10 magnification. Phytoplankton cells were identified using several standard

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

Statistical analysis

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

RESULTS AND DISCUSSION

Detection of marine microalgae (phytoplankton)

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

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

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

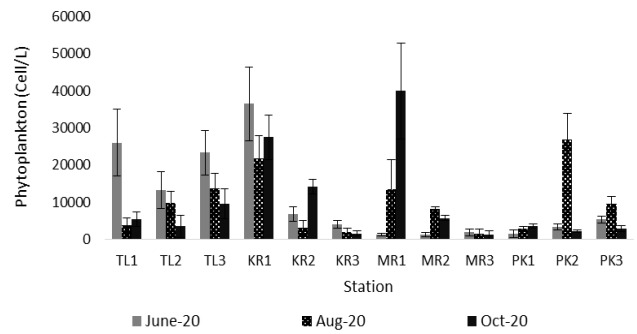


Figure 2. Phytoplankton density at the sampled sites in South Sulawesi over the research period (mean ± SD)

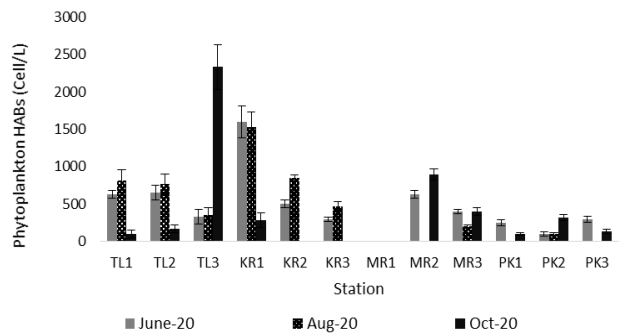


Figure 3. HAB forming phytoplankton density in samples from South Sulawesi over the study period (mean ± SD)

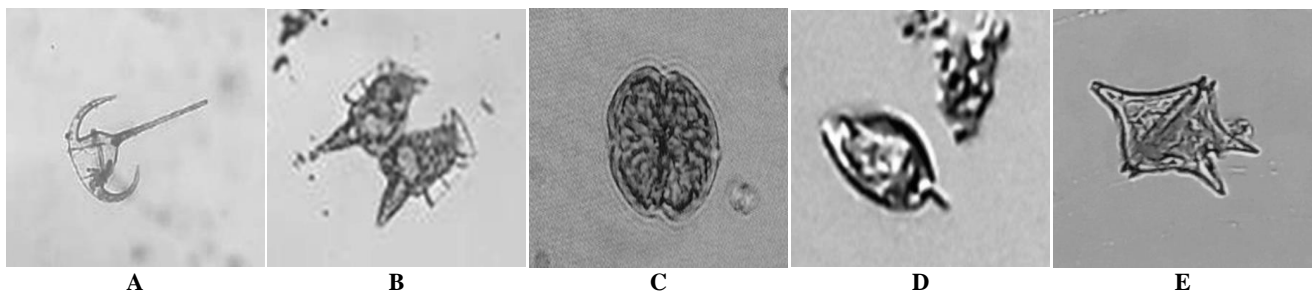


Figure 4. Examples of phytoplankton from class Dinophyceae. A. *Ceratium*, B. *Dinophysis*, C. *Gymnodinium*, D. *Prorocentrum*, E. *Protoperidinium*

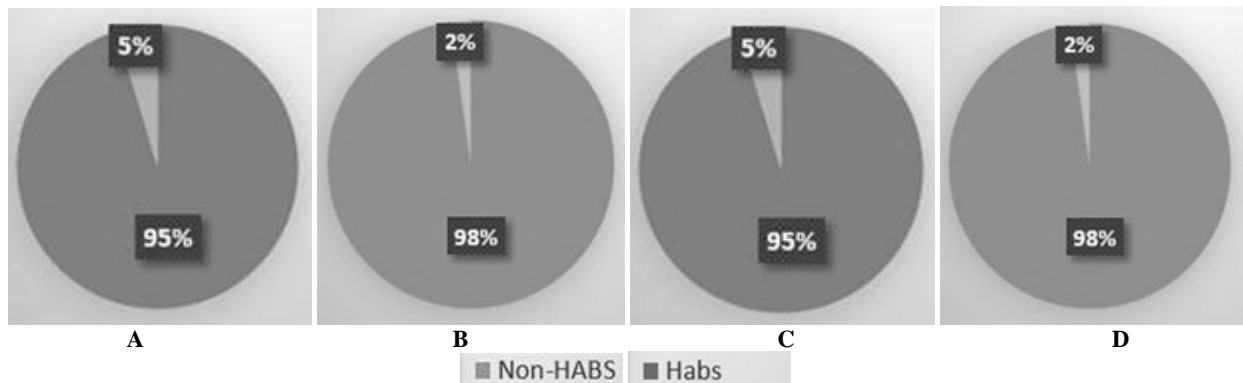


Figure 5. Relative abundance (proportion) of HABS and non-HABS phytoplankton observed during the research period. A. Tallo, B. Kuri, C. Maros, D. Pangkep

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

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

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

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

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

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

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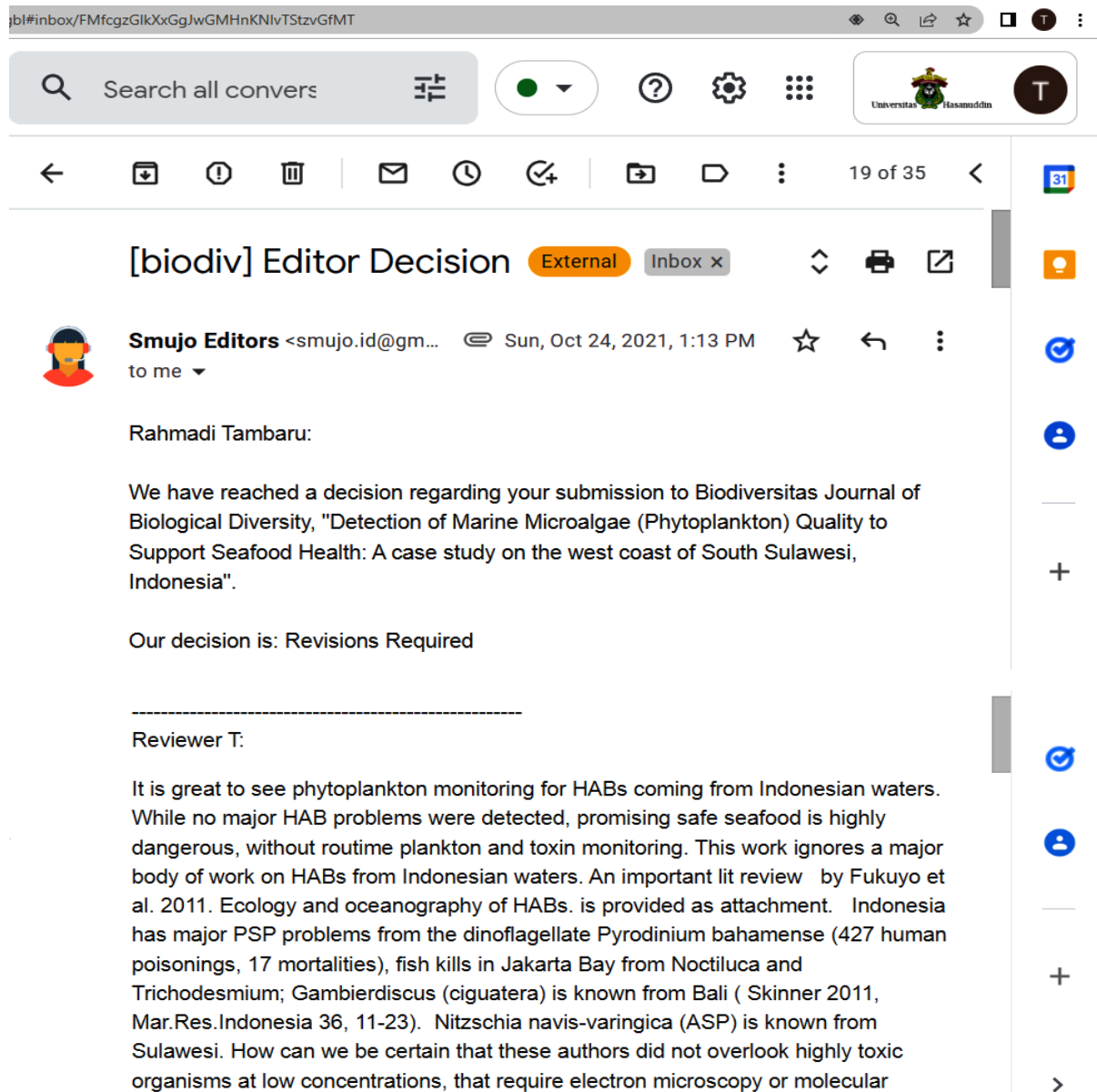
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**3. FILE PERTANYAAN/PERINTAH PERBAIKAN DARI
PARA REVIEWER JURNAL**

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

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



The screenshot shows an email interface with the following details:

- Subject:** [biodiv] Editor Decision
- Sender:** Smujo Editors <smujo.id@gm...>
- Date:** Sun, Oct 24, 2021, 1:13 PM
- Recipient:** to me
- Content:**

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 routine 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, Prochlorocentrum). 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, Prochlorocentrum. 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



The screenshot shows the submission system interface for Biodiversitas Journal of Biological Diversity. The page title is '9630 / Tamburu / Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sula'. The interface includes a 'Workflow' section with 'Submission', 'Review', 'Copyediting', and 'Production' tabs. The 'Review' tab is active, showing 'Round 1' with a status of 'Revisions have been requested'. A notification from the editor is dated 2021-10-24 05:28 AM. The 'Reviewer's Attachments' section lists four files with their IDs and dates: 1. 'Sulawesi 9630-Article Text-50771-1-4-20211014.doc' (ID: 51191-1, Date: October 24, 2021). 2. 'FUKUYO HABs ASIA .pdf' (ID: 51192-1, Date: October 24, 2021). 3. 'Adnan 1984 in Toxic Red Tides: Singapore mng .pdf' (ID: 51193-1, Date: October 24, 2021). 4. 'Adnan fish kills Jakarta Bay.pdf' (ID: 51194-1, Date: October 24, 2021).



[biodiv] Editor Decision

2021-10-24 05:26 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 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 routine 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*). *Prorocentrum* 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

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

Submitted a revised manuscript

Rahmadi Tambaru <aditbr69@unhas.ac.id> to Smujo
Fri, Oct 29, 2021, 9:22 PM

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

2 Attachments

- 9630-51191-1-5-2...
- Rahmadi-Respons...

Revisions-Review Discussions

Biodiversity Journal of Biological Diversity

Revisions

ID	File Name	Date	Type
51542-1	Article Text, 9630-51191-1-5-20211024-Rev_1.docx	October 29, 2021	Article Text
51543-1	Other, Rahmadi-Response to Reviewer_2021_1.docx	October 29, 2021	Other

Review Discussions

Name	From	Last Reply	Replies	Closed
Submitted a revised manuscript	rahmadi69	2021-10-29 01:10 PM	0	<input type="checkbox"/>

Platform & workflow by OJS / PKP



Participants [Edit](#)

Smujo Editors (editors)

Rahmadi Tambaru (rahmadi69)

Messages

Note	From
<p>Dear Editor</p> <p>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).</p> <p>Regards,</p> <p>Rahmadi Tambaru</p> <p>Faculty of Marine Sciences and Fisheries</p> <p>Hasanuddin University</p> <p>rahmadi69, Rahmadi-Response to Reviewer_2021_1.docx</p> <p>rahmadi69, 9630-51191-1-5-20211024-Rev_1.doc</p>	<p>rahmadi69 2021-10-29 01:10 PM</p>

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	<p>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 routine 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.</p>	<p>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.</p> <p>Light microscopes have been used in general by many researchers.</p> <p>Several expert statements to provide reinforcement regarding the monitoring of phytoplankton HABs have been added on page 1 (paragraphs 3 and 4)</p> <p>Some of the results of research by experts on the types of HABs have been added on page 2 (paragraph 2).</p>	
	<p>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.</p>	<p>Yes, right. Some literature explaining this has been added on page 6 (paragraph 5).</p>	

	<p>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 sea-food safe.</p>	<p>The manuscript has been revised by separating the results vs discussion.</p> <p>Some photos of HABs have been added on page 5.</p> <p>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).</p>	
	<p>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.</p>	<p>The manuscript has been adapted to the Biodiversity Journal format.</p> <p>Several literatures on HABs in Indonesia have been cited including three recommended journals (available on pages 2 and 7).</p>	
The other notes		<p>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).</p>	
		<p>We also added a sentence in the introduction to reinforce the monitoring of phytoplankton HABs (page 1 paragraph 4).</p>	
		<p>We also add an explanation of the main equipment used in this study (sub title : Phytoplankton analysis in the laboratory : page 3)</p>	

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 TAMBARU¹✉, ANDI I. BURHANUDDIN¹, ARNIATI MASSINAI¹, MUHAMMAD A. AMRAN¹

¹Department 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 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 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 Algal 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

45 certain groups of phytoplankton (HABs forming species) experience population explosions and have negative impacts on
46 other aquatic organisms as well as on human health (Berdalet et al. 2018). According to Xiao et al. (2019), the factors that
47 can trigger a phytoplankton population explosion that can be qualified as a HABs include nutrient enrichment
48 (eutrophication), the occurrence of heavy rains increasing the flow of nutrient-loaded river water into the sea (Hughes et al.
49 2011), and upwellings (Loureiro et al. 2011).

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

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

71 Microalgal blooms can occur, are known to contaminate aquatic organisms such as fish and shellfish, and may have
72 been a factor in both cases. In the Pangkep region aquaculture activities are common in both riverine and coastal waters
73 (Lestari et al. 2021;), and in some cases use various drugs and fertilizers to increase production; this is also the case in the
74 coastal waters of Mallasoro Village, Jeneponto Regency, South Sulawesi. To date, there has not been any definitive answer
75 regarding the factors which caused the two aforementioned possible HAB events. However, the potential role of the
76 ongoing nutrient enrichment of riverine and coastal waters is one strong reason to justify analyzing the phytoplankton
77 community and monitoring change, specifically with respect to the types of microalgae that can form HABs. Increases and
78 or changes in nutrient concentration can occur due to seasonal changes such as between the east and west monsoons
79 (Rastina et al. 2020; Vajravelu et al. 2018). These fluctuations will have an impact on the microalgal communities,
80 including the presence of HAB forming species whose presence can be seasonal (Narale and Anil 2017; Trainer et al.
81 2020; Vajravelu et al. 2018), and can therefore become a factor triggering the emergence and rapid growth of HABs.

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

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

95

MATERIALS AND METHODS

96 Research site and timeframe

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

101

102 **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



103 **Figure 1.** Map of sampling sites in the waters off the west coast of South Sulawesi
104

105 **Materials and research design**

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

111 **Phytoplankton analysis in the laboratory**

112 Seawater samples were collected using a 2 liter Kemmerer Water Sampler. At each site, 1 liter of water was taken from
113 each station (zone) for the counting and identification of phytoplankton in the laboratory. Phytoplankton cells were
114 precipitated out of the samples using the method developed by Utermöhl (Vadrucci et al. 2018). A 100 mL sub-sample
115 was placed in a measuring beaker (volume 100 mL) and preserved in Lugol's solution for 1 week. Once precipitation had
116 occurred, the precipitated material (10 mL) was separated from the supernatant by siphoning the supernatant out of the
117 beaker. This precipitate was then placed in a bottle to which more Lugol solution was added. **The abundance of**
118 **phytoplankton cells was calculated using a sweeping (census) method (Rocha et al. 2015) using a 50 mm x 20 mm x 1**
119 **mm Sedgwig Rafter Cell (SRC).** A 1 mL aliquot of the precipitate was placed in the SRC using a graduated pipette. The
120 SRC **was observed under a binocular microscope (Olympus CX21) at 10x10 magnification.** Phytoplankton cells were
121 identified using several standard reference works such as (Castellani and Edwards 2017; Tomas 1997).

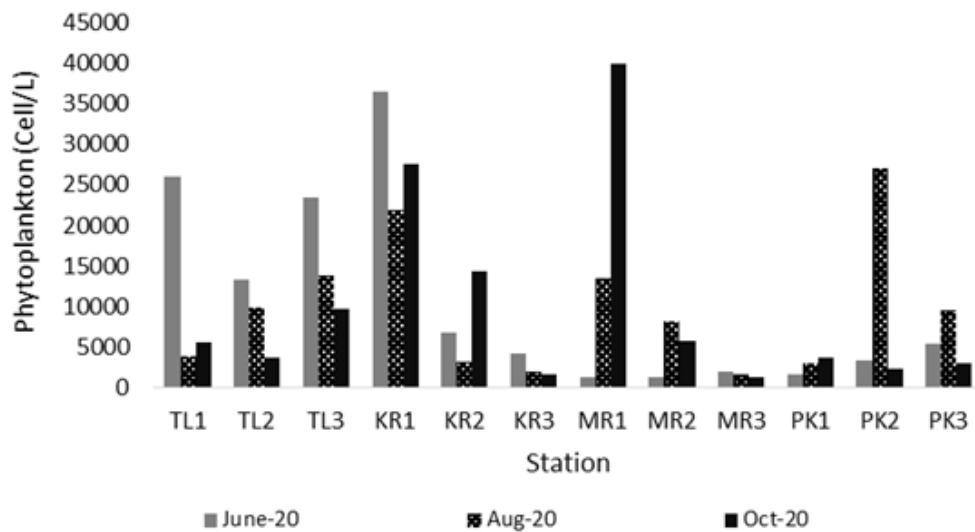
122 **Statistical analysis**

123 The data were analyzed descriptively through tabulation and graphical approaches. **These** included the use of graphs
124 and maps to represent the taxonomic composition and the spatial and temporal distribution of the microalgae identified. A
125 two-way analysis of variance (ANOVA) was used to evaluate the spatial and temporal differences in microalgal
126 community abundance, in particular that of HAB forming phytoplankton. These analyses were implemented in SPSS 17
127 and Excel Stat 2017 (Brahem et al. 2017).

129 **Detection of marine microalgae (phytoplankton)**

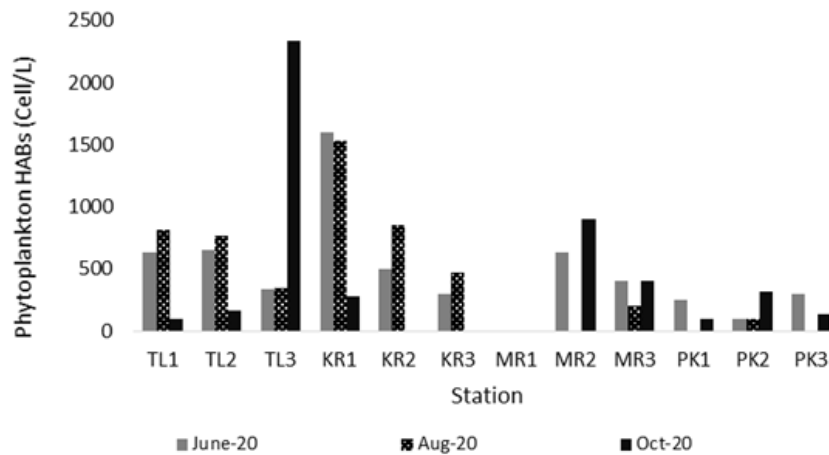
130 During this study 22 phytoplankton genera belonging to 3 classes were identified. These comprised 16 genera of the
 131 Class Bacillariophyceae, 1 genus of the Class Cyanophyceae, and 5 genera of the Class Dinophyceae. The genus-level
 132 composition varied spatially (between locations/stations)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

135 The phytoplankton density in the samples (Figure 2) indicates relative abundance. Over the study period as a whole,
 136 mean phytoplankton abundance was higher at the Tallo and Kuri sites than at the Maros and Pangkep stations. The
 137 analysis of variance indicated a significant difference in phytoplankton abundance between the locations/stations (p
 138 <0.01). The observed phytoplankton abundance also varied between the time periods. However, the analysis of variance
 139 indicated that the differences in the abundance of phytoplankton over time were not statistically significant over the period
 140 of observation ($p > 0.05$).



141
 142 **Figure 2.** Mean sample phytoplankton density over the research period

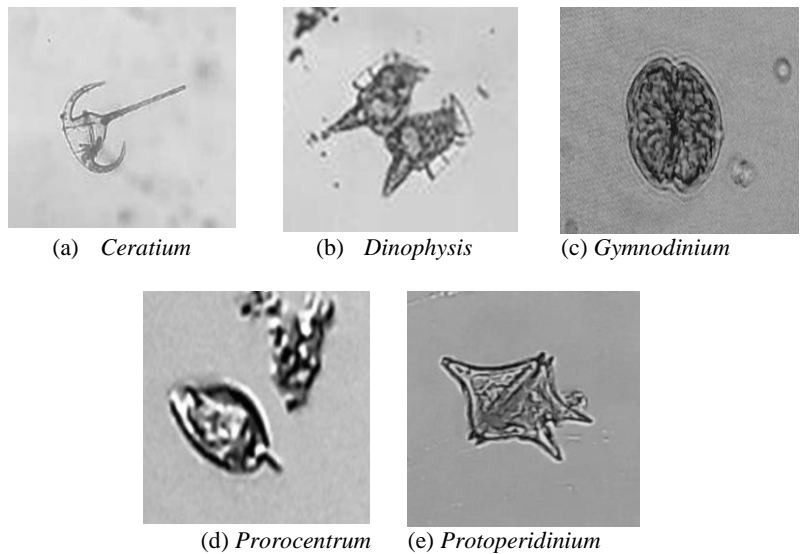
143 Types of marine microalgae (phytoplankton) which are considered dangerous because they can form HABs were
 144 present at almost all locations/stations (Figure 3). The taxa identified included 5 potentially HAB forming phytoplankton
 145 genera belonging to the Class Dinophyceae: *Ceratium*, *Dinophysis*,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



147
 148 **Figure 3.** Mean density of HABs forming phytoplankton in the samples collected during the study period

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

152

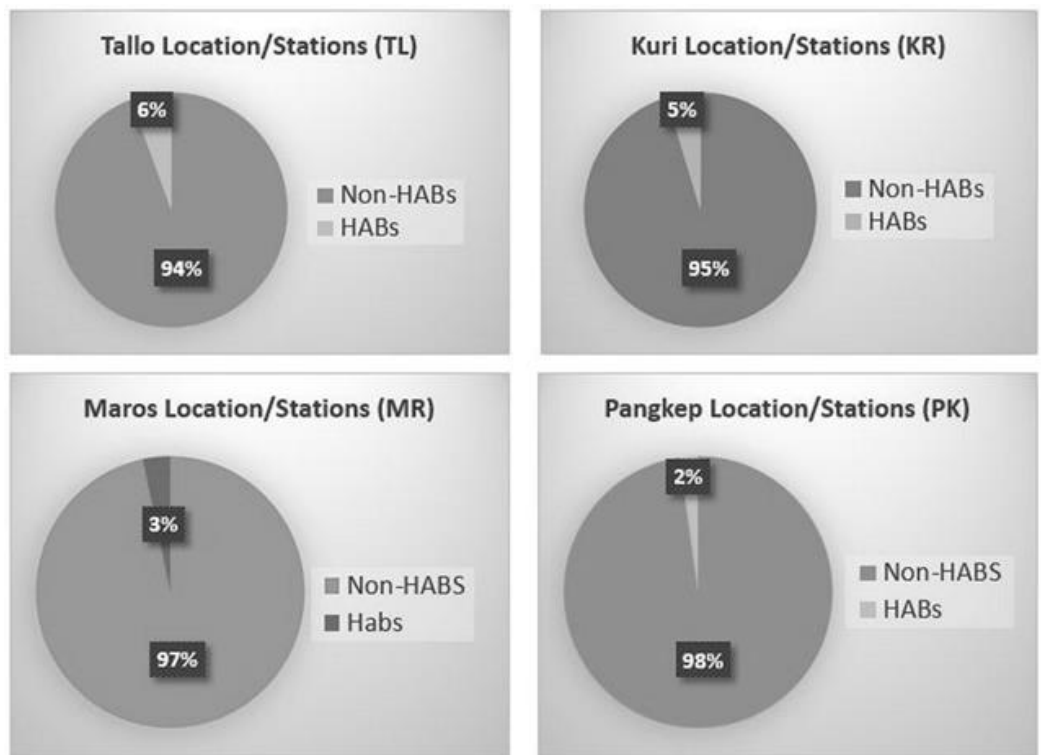


153

154 **Figure 4.** Examples of phytoplankton from class Dinophyceae

155 **Quality of marine microalgae (phytoplankton)**

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



159

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

161 **Discussion**

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

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

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

188 Types of marine microalgae which are considered dangerous as HABs forming phytoplankton were present at almost
189 all locations/stations (Figure 3). This is an important observation as, under certain conditions, these microorganisms can
190 negatively affect water quality if they multiply excessively (blooming), endangering the lives of other aquatic organisms,
191 even humans when they become dominant. Through the food chain, the accumulation of toxins in the body of organisms
192 that consume HABs forming microalgae can cause health problems and even death in humans (Pettersson and Pozdnyakov
193 2013).

194 The taxa identified included 5 potentially HABs forming phytoplankton genera belonging to the Class Dinophyceae:
195 *Ceratium*, *Dinophysis*, *Gymnodinium*, *Prorocentrum*, and *Protoperidinium*. These genera were present in most
196 locations/stations and observation time periods. The classification of these taxa as presenting a risk of HABs can be
197 justified because the Dinophyceae are known to contain the most toxic genera (Tillmann et al. 2010). These five HABs
198 genera produce toxic metabolites, so they are classified as belonging to the toxin producer group (Kudela et al. 2018). If
199 these metabolites accumulate in the bodies of marine organisms such as shellfish and fish, they can cause poisoning in
200 humans who consume this seafood (Farabegoli et al. 2018).

201 **The addition of the number and abundance of HABs in surface waters can come from aquatic sediments. HAB in**
202 **aquatic sediments is in the form of cysts, such as *Gymnodinium* cysts from the Dinophyceae class (Dzhembekova et al.**
203 **2018). According to Fukoyo et al (2011), phytoplankton in this class play an important role in recurring HAB events as**
204 **well as contributing to the geographical expansion of HAB occurrences. These repeat HABs can occur when a large**
205 **number of cysts of potentially HAB forming Dinophyceae are deposited in marine sediments (Narale and Anil 2017;**
206 **Trainer et al. 2020). Turbulence can disturb the sediment and raise these cysts to the surface layer of the water. Such**
207 **events can trigger an explosion of HABs, if prevailing environmental conditions support their growth.**

208 ***Ceratium* does not belong to the toxic phytoplankton group; however, this genus can cause various problems in the**
209 **waters if it blooms (Praseno and Sugestiningih, 2000), and is considered to be a threat to the aquatic environment because**
210 **it can cause oxygen deprivation through decreasing oxygen concentration in the water. *Ceratium* can result in mass**
211 **mortality of marine life due to decreased oxygen levels if the population becomes too abundant (Thoha and Rachman**
212 **2012). *Dinophysis* is considered to belong to the HAB forming group because it contains toxins that can cause diarrhetic**
213 **shellfish poisoning (DSP) if humans consume contaminated shellfish (Taylor et al. 2013). According to (Dietrich et al.**
214 **2019), the symptoms of poisoning caused by *Dinophysis* (DSP) include diarrhea induced by okadaic acid. *Gymnodinium* is**
215 **a type of HAB forming phytoplankton that causes red tides and oxygen depletion (Pitcher and Probyn 2016).**
216 ***Gymnodinium* can cause paralytic shellfish poisoning (PSP) so that consuming contaminated shellfish can cause paralysis**
217 **in humans (Rodrigues et al. 2012). *Protoperidinium* species contain toxins called azaspiracids; the symptoms in humans**
218 **who ingest this poison are somewhat similar to those of DSP and poisoning can cause nausea in the victim within 3-5 days**
219 **(Trainer et al. 2013).**

220 **The presence and abundance of the HAB forming taxa varied significantly (ANOVA, $p < 0.01$) between**
221 **locations/stations and periods of observation (Figure 3). While visually Tallo and Kuri appear to differ from the Maros and**
222 **Pangkep sites, the Tukey test showed that the Pangkep site differed significantly from the other three sites (Tallo, Kuri,**
223 **and Maros). Although the abundance of HAB taxa differed between the time periods, the lack of statistical significance**
224 **(ANOVA, $p > 0.05$) indicate that the presence and abundance of taxa capable of forming HABs can be considered similar**
225 **throughout the study period.**

226 **Overall,** the quality of marine microalgae is determined by the types and relative abundance of phytoplankton that can
227 form HABs (HAB) and those which do not form HABs (non-HAB) phytoplankton in the water. **HAB forming**

228 phytoplankton can multiply rapidly when changes occur in ambient environmental conditions. The Dinophyceae can
229 tolerate environmental changes such as nutrient enrichment (eutrophication) (Tester et al. 2020). If eutrophic conditions
230 occur, phytoplankton from the Dinophyceae will tend to multiply more rapidly and may form HABs (Glibert, 2017). Once
231 the HABs have begun to form, non-HAB phytoplankton may experience a decline in abundance and/or diversity (Glibert
232 2020; Lestari et al. 2021). One reason for this decline is that when the HABs phytoplankton are multiplying they release
233 toxins (Glibert 2017, 2020); these may impede the growth and reproduction of the non-HAB phytoplankton as well as
234 affecting other organisms (Dorgham 2014; Glibert 2017). Conversely, a greater variety of non-HAB phytoplankton is an
235 indication of a thriving phytoplankton community that can support the life of other organisms.

236 The results indicate that changes in water quality may occur at the study sites, but these changes can still be tolerated
237 by non-HAB phytoplankton. In addition to the taxonomic composition, the abundance of each taxon also plays a role in
238 determining the quality of the microalgal community present. Good quality means, *inter alia*, that non-HAB phytoplankton
239 abundance substantially exceeds that of HAB phytoplankton (Gilbert 2017). As this was the case at all study sites and
240 observation periods, there is a reasonable likelihood that the waters are still in good condition and haven't changed greatly,
241 as shown by the relative abundance of HAB phytoplankton and non-HAB phytoplankton (Figure 5). The types of
242 phytoplankton present can mostly be considered of good quality for supporting the life of other organisms including fish
243 and shellfish, and also indicate that fisheries produce from the study locations should be fit and safe for consumption, as
244 contamination from HAB taxa is very unlikely at the low levels detected. This information is certainly encouraging for
245 coastal communities, as their seafood is still suitable for consumption with respect to HABs.

246 In conclusion, the observed composition of the marine microalgal communities present in the coastal waters along the
247 west coast of South Sulawesi indicates that the phytoplankton quality can be considered good. This evaluation is based on
248 the taxonomic composition and abundance of non-harmful (non-HAB) phytoplankton which were far more abundant than
249 the taxa which can cause HABs. This shows that seafood is still suitable for consumption because HAB taxa can be
250 considered as not yet present at contaminating levels. Even though the proportions of HAB forming taxa present were very
251 low, their presence is an early warning that vigilance is needed. These results highlight the importance of maintaining
252 water quality and the necessity of regular water quality monitoring, as advocated by Anderson et al. (2015), to ensure that
253 HABs do not develop undetected.

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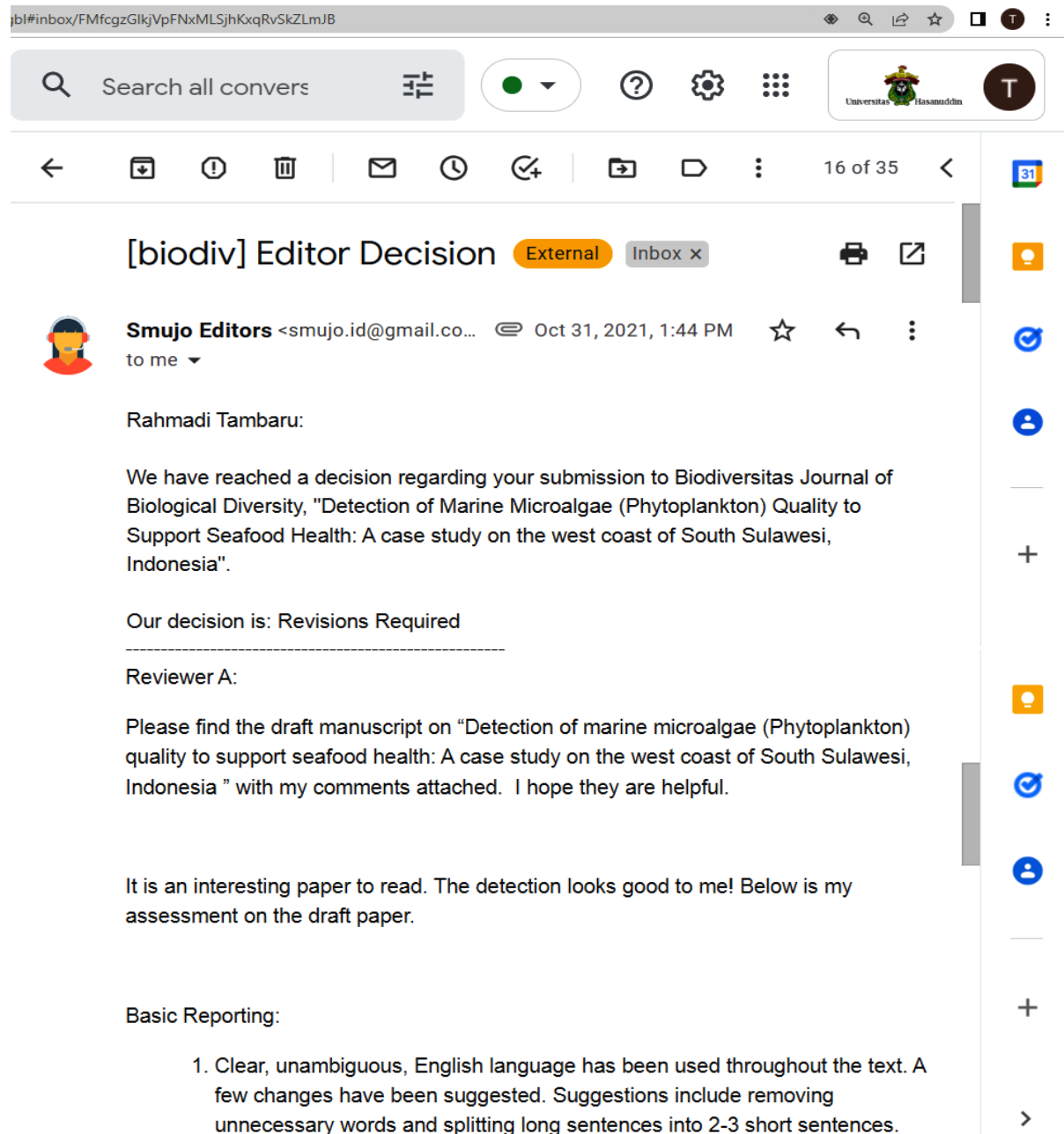
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B. SECOND REVISION FROM REVIEWER (OCTOBER 31, 2021)

B1. COMMENTS-DECISIONS FROM REVIEWERS (SECOND ROUND)



The screenshot shows an email interface with a search bar at the top, navigation icons, and a list of actions. The email subject is "[biodiv] Editor Decision" and it is marked as "External" and "Inbox x". The sender is "Smujo Editors" with the email address "smujo.id@gmail.co...". The email is dated "Oct 31, 2021, 1:44 PM" and is addressed "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

[Biodiversitas Journal of Biological Diversity](#)

2 Attachments



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.

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- 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. *AAFL Bioflux*, 13(5), pp.2956-2969.

The screenshot shows the OJS submission interface. The top navigation bar includes the OJS logo, the journal title 'Biodiversitas Journal of Biological Diversity', and user information 'English View Site ralmad69'. The main content area displays the manuscript title '9630 / Tamburu / Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sulawesi' with 'Upload File' and 'Library' buttons. Below this, there are tabs for 'Workflow' and 'Publication', and sub-tabs for 'Submission', 'Review', 'Copyediting', and 'Production'. The 'Round 1' and 'Round 2' tabs are visible, with 'Round 2' selected. A 'Round 2 Status' box indicates 'Revisions have been requested.' Below this, a 'Notifications' section shows two entries: '[pdoi]v1 Editor Decision' dated 2021-10-24 05:26 AM and another dated 2021-10-31 05:44 AM. The 'Reviewer's Attachments' section includes a search bar and two entries: '51591-1_9630-Article Text-51545-1-4-20211029_RFTReviewed.doc' dated October 31, 2021, and '51593-1_Summary Comments_9630.docx' dated October 31, 2021.



[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".

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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. *AAAL Bioflux*, 13(5), pp.2956-2969.

Recommendation: Revisions Required

**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 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 mostly~~ suitable as food for other organisms, including fish and shellfish. It also means that if ~~these fish and shellfish are harvested by fishermen harvest this 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 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~~

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45 impacts on the increasing mortality of marine organisms (Fukuyo et al. 2011).

46 HABs are phenomena that commonly occur in water bodies, especially marine and coastal waters. HABs occur when
47 certain groups of phytoplankton (HABs forming species) experience population explosions and have negative impacts on
48 other aquatic organisms as well as on human health (Berdalet et al. 2018). According to Xiao et al. (2019), the factors that
49 can trigger a phytoplankton population explosion that can be qualified as a HABs include nutrient enrichment
50 (eutrophication), the occurrence of heavy rains increasing the flow of nutrient-loaded river water into the sea (Hughes et al.
51 2011), and upwellings (Loureiro et al. 2011).

52 Some of the acute diseases caused by toxins from microalgae in the HABs group are paralytic shellfish poisoning
53 (PSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) (Fukuyo et al. 2011; Krock et al. 2018),
54 and ciguatera fish poisoning (CFP) (Skinner et al. 2011). These toxins are ~~very dangerous~~ ~~hazardous~~ to human health
55 because they attack the nervous system and interfere with respiration and digestion. These diseases are related to ~~the~~
56 human consumption of fish and shellfish. ~~In fact, many~~ ~~Many~~ types of toxic phytoplankton can be found in Indonesian
57 coastal waters, including several Dinoflagellate species from the genera *Noctiluca*, *Gymnodinium*, *Cochlodinium*,
58 *Ceratium*, *Peridinium*, *Gonyaulax*, *Ostreopsis*, *Prorocentrum*, and *Gambierdiscus* (Adnan 1984; Adnan 1989; Skinner et al
59 2011; Hasani et al. 2013; Aditya et al. 2015). One HAB event in 1993 took place in Jakarta Bay, where a mass fish-kill
60 was caused by the excessive abundance of phytoplankton that can cause HABs. Similar HAB incidents have occurred in
61 the waters of Lewotobi and Lewouran (East Nusa Tenggara), Sebatik Island (East Kalimantan), and Ambon Bay
62 (Mahmudi et al. 2020).

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

74 Microalgal blooms can occur, are known to contaminate aquatic organisms such as fish and shellfish, and may have
75 been a factor in both cases. In the Pangkep region aquaculture ~~and~~ ~~mariculture~~ activities are common in both riverine and
76 coastal waters (Lestari et al. 2021; ~~Any additional REFS?~~), ~~and~~ ~~in~~ some cases use various drugs and fertilizers to increase
77 production; this is also the case in the coastal waters of Mallasoro Village, Jeneponto Regency, South Sulawesi. To date,
78 there has not been any definitive answer regarding the factors which caused the two aforementioned possible HAB events.
79 However, the potential role of the ongoing nutrient enrichment of riverine and coastal waters is one strong reason to justify
80 analyzing the phytoplankton community and monitoring change, specifically ~~with respect to~~ ~~regarding~~ the types of
81 microalgae that can form HABs. Increases and or changes in nutrient concentration can occur due to seasonal changes
82 ~~such as~~ between the east and west monsoons (Rastina et al. 2020; Vajravelu et al. 2018). These fluctuations will ~~have an~~
83 impact ~~on~~ the microalgal communities, including the presence of HAB forming species whose presence can be seasonal
84 (Narale and Anil 2017; Trainer et al. 2020; Vajravelu et al. 2018), and can therefore become a factor triggering the
85 emergence and rapid growth of HABs.

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

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

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

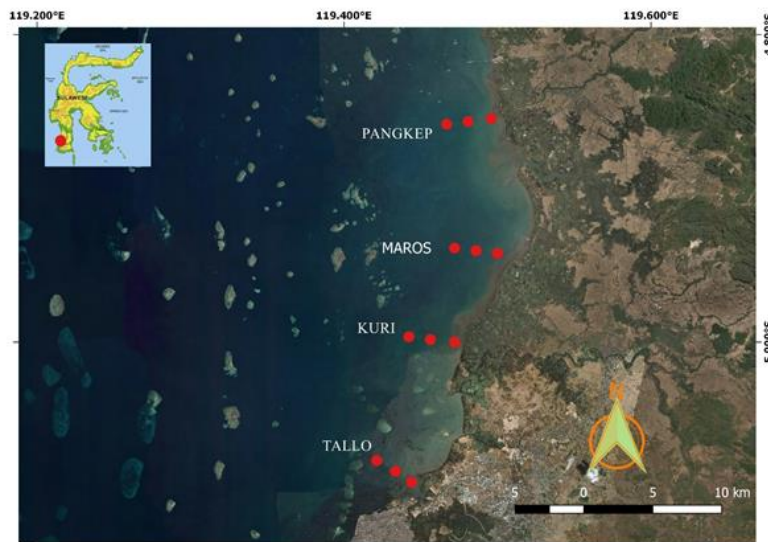


Figure 1. Map of sampling sites in the waters off the west coast of South Sulawesi

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 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 week. Once precipitation had occurred, the precipitated material (10 mL) was separated from the supernatant by siphoning the supernatant out of the beaker. This precipitate was then placed in a bottle to which more Lugol solution was added. The abundance of phytoplankton cells was calculated using a sweeping (census) method (Rocha et al. 2015) using a 50 mm x 20 mm x 1 mm Sedgwick Rafter Cell (SRC). A 1 mL aliquot of the precipitate was placed in the SRC using a graduated pipette. The SRC was observed under a binocular microscope (Olympus CX21) at 10x10 magnification. Phytoplankton cells were identified using several standard reference works, such as (Castellani and Edwards 2017; Tomas 1997).

127 **Statistical analysis**

128 The data were analyzed descriptively through tabulation and graphical approaches. These included the use of graphs
129 and maps to represent the taxonomic composition and the spatial and temporal distribution of the microalgae identified. A
130 two-way analysis of variance (ANOVA) was used to evaluate the spatial and temporal differences in microalgal
131 community abundance, in particular that of HAB forming phytoplankton. These analyses were implemented in SPSS 17
132 and Excel Stat 2017 (Brahem et al. 2017).

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133 **RESULTS AND DISCUSSION**

134 **Detection of marine microalgae (phytoplankton)**

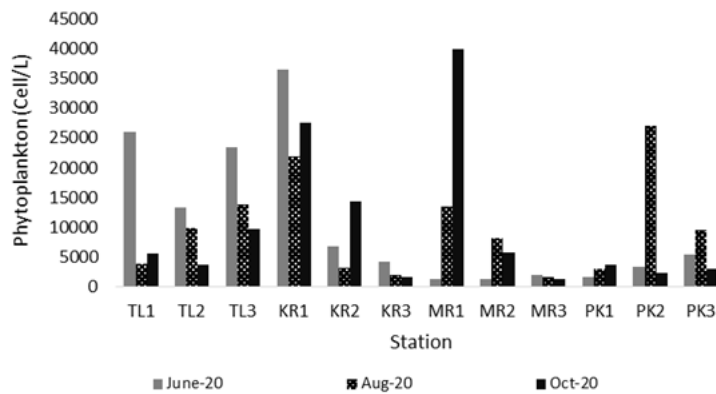
135 During this study, 22 phytoplankton genera belonging to 3 classes were identified. These comprised 16 genera of the
136 Class Bacillariophyceae, 1 genus of the Class Cyanophyceae, and 5 genera of the Class Dinophyceae. The genus-level
137 composition varied spatially (between locations/stations)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

140 The phytoplankton density in the samples (Figure 2) indicates relative abundance. Over the study period, as a whole,
141 mean phytoplankton abundance was higher at the Tallo and Kuri sites than at the Maros and Pangkep stations. The
142 analysis of variance indicated a significant difference in phytoplankton abundance between the locations/stations (p
143 <0.01). The observed phytoplankton abundance also varied between the time periods. However, the analysis of variance
144 indicated that the differences in the abundance of phytoplankton over time were not statistically significant over the period
145 throughout observation ($p > 0.05$).

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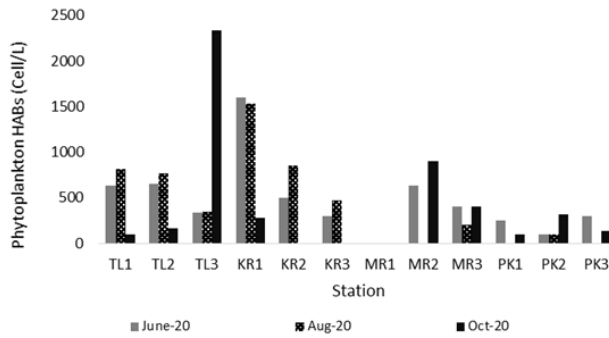
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146 **Figure 2.** Mean±SD/SE sample phytoplankton density over the research period

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

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152

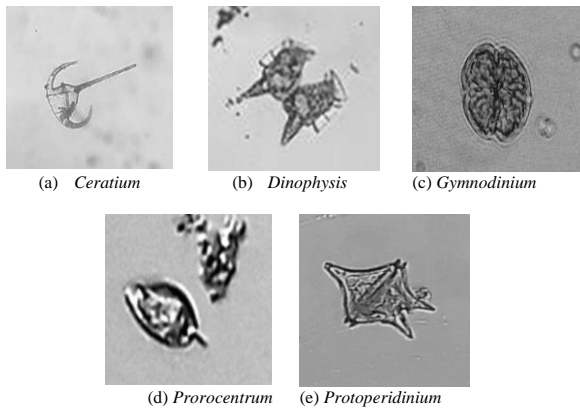
153 **Figure 3.** Mean density of HABs forming phytoplankton in the samples collected during the study period

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

157

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158

159 **Figure 4.** Examples of phytoplankton from class Dinophyceae

160 **Quality of marine microalgae (phytoplankton)**

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

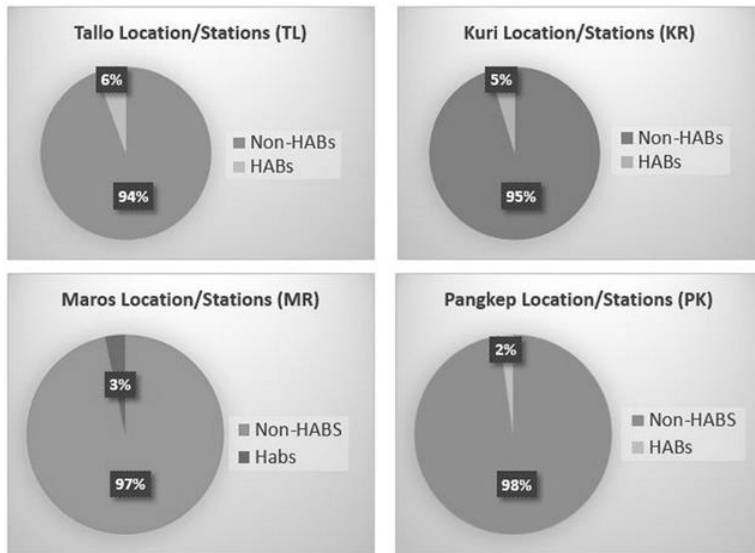


Figure 5. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period

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- *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*, *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 ~~around the world wide~~ (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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198 body of organisms that consume HABs forming microalgae can cause health problems and even death in humans
199 (Pettersson and Pozdnyakov 2013).

200 The taxa identified included ~~5-five~~ potentially HABs forming phytoplankton genera belonging to the Class
201 Dinophyceae: *Ceratium*, *Dinophysis*, *Gymnodinium*, *Prorocentrum*, and *Protoperidinium*. These genera were present in
202 most locations/stations and observation ~~time~~ periods. The classification of these taxa as presenting a risk of HABs can be
203 justified because the Dinophyceae ~~are known to~~ contain the most toxic genera (Tillmann et al. 2010). These five HABs
204 genera produce toxic metabolites, so they ~~are classified as belonging~~ to the toxin producer group (Kudela et al. 2018). If
205 these metabolites accumulate in the bodies of marine organisms such as shellfish and fish, they can cause poisoning in
206 humans who consume this seafood (Farabegoli et al. 2018).

207 The addition of the number and abundance of HABs in surface waters can come from aquatic sediments. HAB in
208 aquatic sediments is in the form of cysts, such as *Gymnodinium* cysts from the Dinophyceae class (Dzhembekova et al.
209 2018). According to Fukuyo et al (2011), phytoplankton in this class play an important role in recurring HAB events ~~as~~
210 ~~well and as~~ contributing to the geographical expansion of HAB occurrences. These repeat HABs can occur when a ~~large~~
211 ~~number of many~~ cysts of potentially HAB-~~f~~forming Dinophyceae are deposited in marine sediments (Narale and Anil
212 2017; Trainer et al. 2020). Turbulence can disturb the sediment and raise these cysts to the surface layer of the water. Such
213 events can trigger an explosion of HABs, if prevailing environmental conditions support their growth.

214 *Ceratium* does not belong to the toxic phytoplankton group; however, this genus can cause various problems in the
215 waters if it blooms (Praseno and Sugestingsih, 2000), and is considered to be a threat to the aquatic environment because
216 it can cause oxygen deprivation through decreasing oxygen concentration in the water. *Ceratium* can result in mass
217 mortality of marine life due to decreased oxygen levels if the population becomes too abundant (Thoha and Rachman
218 2012). *Dinophysis* ~~is considered to belong~~ to the HAB-forming group because it contains toxins that can cause diarrhetic
219 shellfish poisoning (DSP) if humans consume contaminated shellfish (Taylor et al. 2013). According to (Dietrich et al.
220 2019), the symptoms of poisoning caused by *Dinophysis* (DSP) include diarrhea induced by okadaic acid. *Gymnodinium* is
221 a type of HAB forming phytoplankton that causes red tides and oxygen depletion (Pitcher and Probyn 2016).
222 *Gymnodinium* can cause paralytic shellfish poisoning (PSP), so that consuming contaminated shellfish can cause paralysis
223 in humans (Rodrigues et al. 2012). *Protoperidinium* species contain toxins called azaspiracids; the symptoms in humans
224 who ingest this poison are somewhat similar to those of DSP, and poisoning can cause nausea in the victim within 3-5
225 days (Trainer et al. 2013).

226 The presence and abundance of the HAB forming taxa varied significantly (ANOVA, $p < 0.01$) between
227 locations/stations and periods of observation (Figure 3). While visually Tallo and Kuri appear to differ from the Maros and
228 Pangkep sites, the Tukey test showed that the Pangkep site differed significantly from the other three sites (Tallo, Kuri,
229 and Maros). Although the abundance of HAB taxa differed between the ~~time~~ periods, the lack of statistical significance
230 (ANOVA, $p > 0.05$) indicates that the presence and abundance of taxa capable of forming HABs can be considered similar
231 throughout the study period.

232 Overall, the quality of marine microalgae is determined by the types and relative abundance of phytoplankton that can
233 form HABs (HAB) and those which do not form HABs (non-HAB) phytoplankton in the water. HAB-forming
234 phytoplankton can multiply rapidly when changes occur in ambient environmental conditions. The Dinophyceae can
235 tolerate environmental changes such as nutrient enrichment (eutrophication) (Tester et al. 2020). If eutrophic conditions
236 occur, phytoplankton from the Dinophyceae will tend to multiply more rapidly and may form HABs (Glibert, 2017). Once
237 the HABs have begun to form, non-HAB phytoplankton may experience a decline in abundance and/or diversity (Glibert
238 2020; Lestari et al. 2021). One reason for this decline is that when the HABs phytoplankton are multiplying, they release
239 toxins (Glibert 2017, 2020); these may impede the growth and reproduction of the non-HAB phytoplankton as well as
240 affecting other organisms (Dorgham 2014; Glibert 2017). Conversely, a greater variety of non-HAB phytoplankton ~~is an~~
241 ~~indication of indicates~~ a thriving phytoplankton community that can support the life of other organisms.

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

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

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

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B3. FURTHER RESPONSE TO REVIEWERS (SECOND ROUND)

Second Round Status on OJS

The screenshot shows the OJS submission status page for article 9630. The page is titled "Biodiversitas Journal of Biological Diversity" and includes a navigation bar with "English" and "View Site" options. The article title is "Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study of". The submission status is "Publication", and the current round is "Round 2". The "Round 2 Status" section indicates that "Revisions have been submitted."

The screenshot displays the "Revisions" and "Review Discussions" sections of the OJS submission page. The "Revisions" section lists two revisions: "Article Text, A-9630-Article Text-51545-1-4-20211029_RFTReviewed_Revisi_A1.doc" and "Other, Rahmadi-Response to Reviewer_2021_2.docx", both dated November 2, 2021. The "Review Discussions" section shows two discussions: "Submitted a revised manuscript" and "2nd Response to Reviewers/Editors", both dated 2021-10-29 and 2021-11-02 respectively, with 0 replies each.

The screenshot shows the "2nd Response to Reviewers/Editors" message. The participants listed are Smujo Editors (editors) and Rahmadi Tambaru (rahmadi69). The message content is as follows:

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)

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Nu.	Comments	Responds	
Reviewer 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://web-log.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 (<i>Mobula alfredi</i>) feeding habitat in the Dampier Strait, Raja Ampat, West Papua, Indonesia. <i>AAFL Bioflux</i> , 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

45 contribute to increases in HAB-associated mortality of marine organisms (Fukuyo et al. 2011).

46 HABs are phenomena that commonly occur in water bodies, especially marine and coastal waters. HABs occur when
47 certain groups of phytoplankton (HABs forming species) experience population explosions and have negative impacts on
48 other aquatic organisms as well as on human health (Berdalet et al. 2016, 2018). According to Xiao et al. (2019), the
49 factors that can trigger a phytoplankton population explosion that can be qualified as a HABs include nutrient enrichment
50 (eutrophication), the occurrence of heavy rains increasing the flow of nutrient-loaded river water into the sea (Hughes et al.
51 2011), and upwellings (Loureiro et al. 2011).

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

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

72 Microalgal blooms can occur, are known to contaminate aquatic organisms such as fish and shellfish, and may have
73 been a factor in both cases. In the Pangkep region aquaculture and mariculture activities are common in both riverine and
74 coastal waters (Lestari et al. 2021). In some cases use various drugs and fertilizers to increase production; this is also the
75 case in the coastal waters of Mallasoro Village, Jeneponto Regency, South Sulawesi. To date, there has not been any
76 definitive answer regarding the factors which caused the two aforementioned possible HAB events. However, the potential
77 role of the ongoing nutrient enrichment of riverine and coastal waters is one strong reason to justify analyzing the
78 phytoplankton community and monitoring change, specifically regarding the types of microalgae that can form HABs.
79 Increases and or changes in nutrient concentration can occur due to seasonal changes between the east and west monsoons
80 (Rastina et al. 2020; Vajravelu et al. 2018). These fluctuations will impact the microalgal communities, including the
81 presence of HAB forming species whose presence can be seasonal (Narale and Anil 2017; Trainer et al. 2020; Vajravelu et
82 al. 2018), and can therefore become a factor triggering the emergence and rapid growth of HABs.

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

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

96 MATERIALS AND METHODS

97 Research site and timeframe

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

102

103 **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



104 **Figure 1.** Map of sampling sites in the waters off the west coast of South Sulawesi
105

106 **Materials and research design**

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

112 **Phytoplankton analysis in the laboratory**

113 Seawater samples were collected using a 2 L Kemmerer Water Sampler. At each site, 1 L of water was taken from each
114 station (zone) to count and identify phytoplankton in the laboratory. Phytoplankton cells were precipitated out of the
115 samples using the method developed by Utermöhl (Vadrucci et al. 2018). A 100 mL sub-sample was placed in a measuring
116 beaker (volume 100 mL) and preserved in Lugol solution for one week. Once precipitation had occurred, the precipitated
117 material (10 mL) was separated from the supernatant by siphoning the supernatant out of the beaker. This precipitate was
118 then placed in a bottle to which more Lugol solution was added. The abundance of phytoplankton cells was calculated
119 using a sweeping (census) method (Rocha et al. 2015) using a 50 mm x 20 mm x 1 mm Sedgwick Rafter Cell (SRC). A 1
120 mL aliquot of the precipitate was placed in the SRC using a graduated pipette. The SRC was observed under a binocular
121 microscope (Olympus CX21) at 10x10 magnification. Phytoplankton cells were identified using several standard reference
122 works (Castellani and Edwards 2017; Tomas 1997).

123 **Statistical analysis**

124 The data were analyzed descriptively through tabulation and graphical approaches. These included the use of graphs
125 and maps to represent the taxonomic composition and the spatial and temporal distribution of the microalgae identified. A
126 two-way analysis of variance (ANOVA) was used to evaluate the spatial and temporal differences in microalgal
127 community abundance, in particular that of HAB forming phytoplankton. **Post-hoc Tukey tests were carried out if the
128 ANOVA indicated significant differences at the 95% confidence level ($\alpha = 0.05$). Prior to testing, all parameters were first**

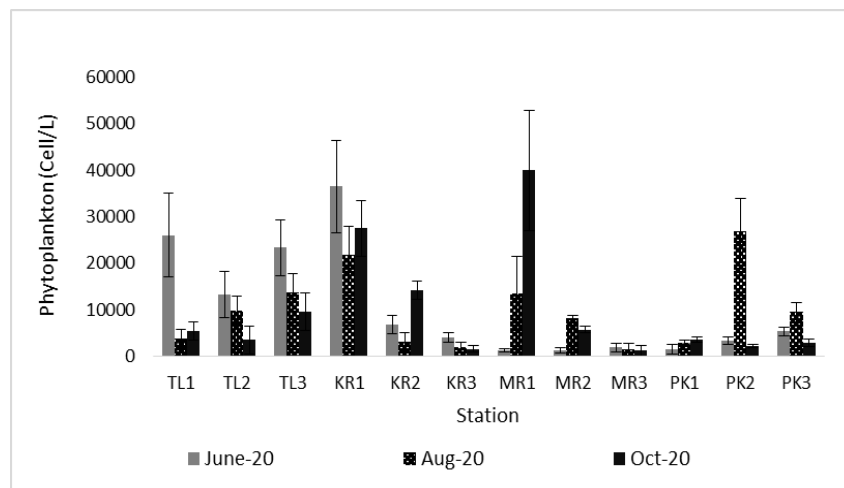
129 tested for normality of the data distribution using Kolmogorov-Smirnov and Levene's Test of Equality. These analyses
130 were implemented in SPSS 17 and Excel Stat 2017 (Brahem et al. 2017).

131 RESULTS AND DISCUSSION

132 Detection of marine microalgae (phytoplankton)

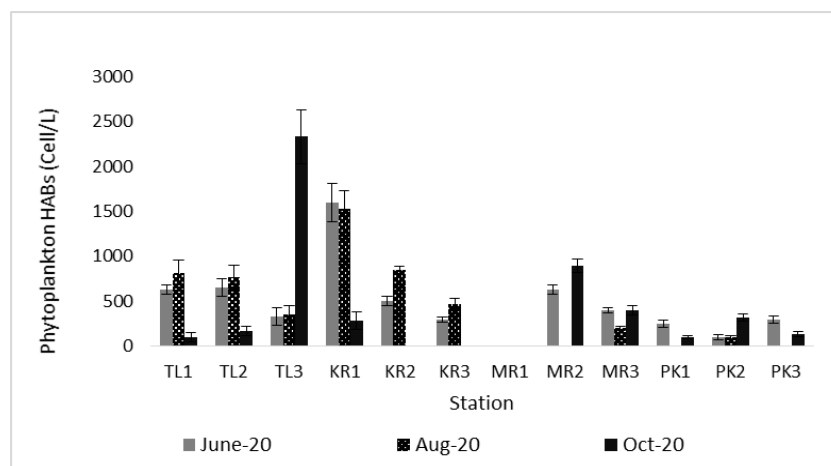
133 During this study, 22 phytoplankton genera belonging to 3 classes were identified. These comprised 16 genera of the
134 Class Bacillariophyceae, 1 genus of the Class Cyanophyceae, and 5 genera of the Class Dinophyceae. The genus-level
135 composition varied spatially (between sites/stations) and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

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



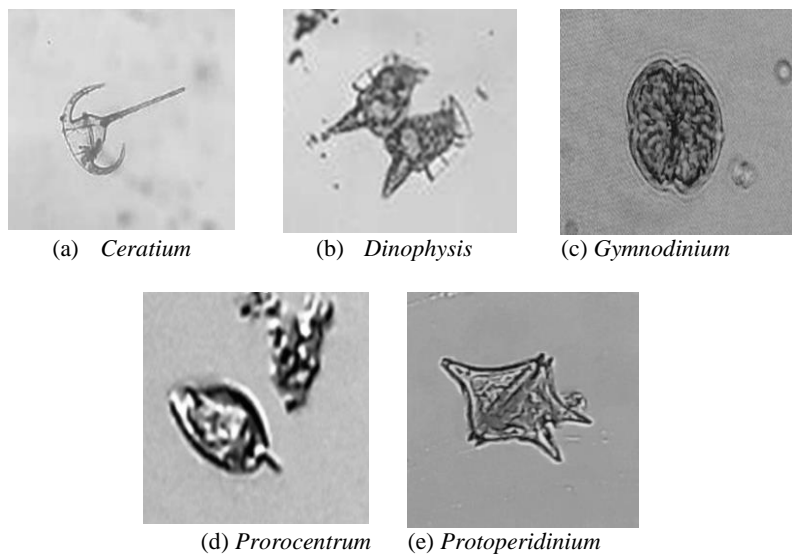
143
144 **Figure 2.** Phytoplankton density at the sampled sites in South Sulawesi over the research period (mean \pm SD)

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



149
150 **Figure 3.** HAB forming phytoplankton density in samples from South Sulawesi over the study period (mean \pm SD)

151 The presence and abundance of the potentially HAB forming taxa varied between sites/stations and periods of
 152 observation (Figure 3). The analysis of variance revealed highly significant differences ($p < 0.01$) in the abundance of HAB
 153 forming taxa between sites/stations.
 154

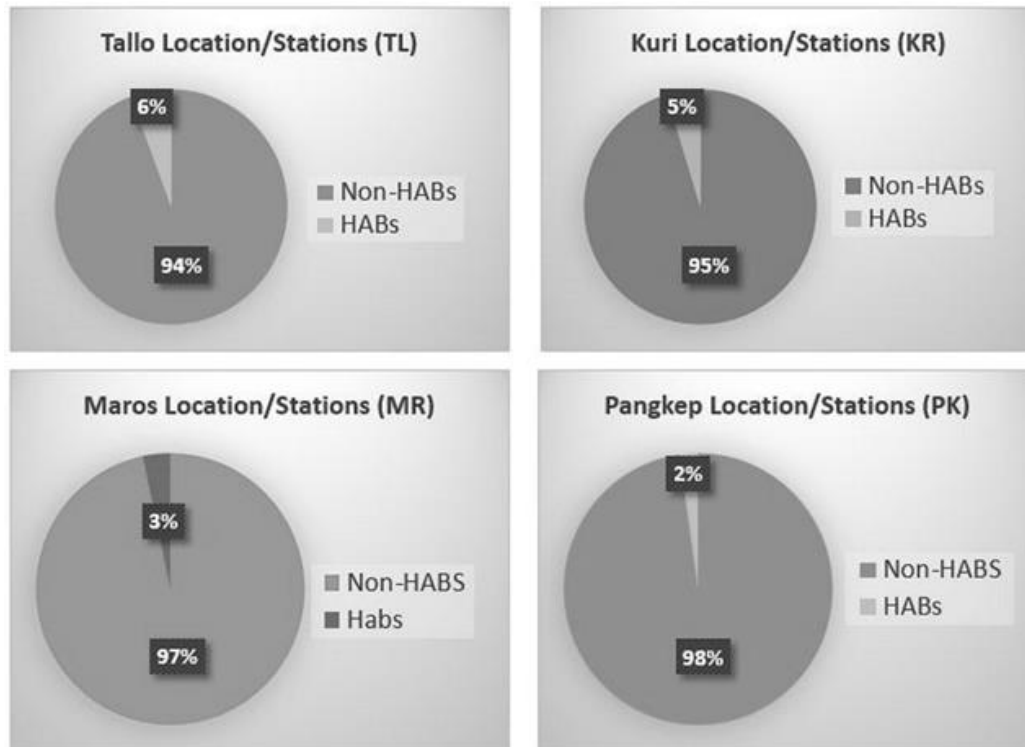


155

156 **Figure 4.** Examples of phytoplankton from class Dinophyceae

157 **Quality of marine microalgae (phytoplankton)**

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



161

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

163 **Discussion**

164 The dynamics of marine microalgal communities vary from one site to another and from one time to another. Changes
 165 in community composition occur frequently (Marinov et al. 2010; Fujiwara et al. 2018). The survival and replication of
 166 phytoplankton are greatly influenced by various environmental parameters (Baek et al. 2020). In response to

167 environmental changes, these microalgal communities will comprise different types of phytoplankton with shifts in the
168 relative abundance of each type. At certain times some groups are found to be abundant, at other times other groups will
169 dominate the community (Thovyan et al. 2020).

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

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

189 Types of marine microalgae which are considered dangerous as HABs forming phytoplankton were present at almost
190 all sites/stations (Figure 3). **These observations are an important finding as**, under certain conditions, these microorganisms
191 can negatively affect water quality if they multiply excessively (blooming), endangering the lives of other aquatic
192 organisms, even humans, when they become dominant. Through the food chain, the accumulation of toxins in the body of
193 organisms that consume HABs forming microalgae can cause health problems and even death in humans (Pettersson and
194 Pozdnyakov 2013).

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

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

209 ***Ceratium* does not belong to the toxic phytoplankton group. However, this genus can cause various problems in the
210 waters if it blooms (Praseno and Sugestiningih, 2000). In addition, this organism is considered to be a threat to the aquatic
211 environment because it can cause oxygen deprivation through decreasing oxygen concentration in the water.** *Ceratium* can
212 result in mass mortality of marine life due to decreased oxygen levels if the population becomes too abundant (Thoha and
213 Rachman 2012). *Dinophysis* belongs to the HAB-forming group because it contains toxins that can cause diarrhetic
214 shellfish poisoning (DSP) if humans consume contaminated shellfish (Taylor et al. 2013). According to (Dietrich et al.
215 2019), the symptoms of poisoning caused by *Dinophysis* (DSP) include diarrhea induced by okadaic acid. *Gymnodinium* is
216 a type of HAB forming phytoplankton that causes red tides and oxygen depletion (Pitcher and Probyn 2016).
217 *Gymnodinium* can cause paralytic shellfish poisoning (PSP), so that consuming contaminated shellfish can cause paralysis
218 in humans (Rodrigues et al. 2012). *Protoperidinium* species contain toxins called azaspiracids; the symptoms in humans
219 who ingest this poison are somewhat similar to those of DSP, and poisoning can cause nausea in the victim within 3-5
220 days (Trainer et al. 2013).

221 The presence and abundance of the HAB forming taxa varied significantly (ANOVA, $p < 0.01$) between sites/stations
222 and periods of observation (Figure 3). While visually Tallo and Kuri appear to differ from the Maros and Pangkep sites,
223 the Tukey test showed that the Pangkep site differed significantly from the other three sites (Tallo, Kuri, and Maros).
224 Although the abundance of HAB taxa differed between the periods, the lack of statistical significance (ANOVA, $p > 0.05$)
225 indicates that the presence and abundance of taxa capable of forming HABs can be considered similar throughout the study
226 period.

227 Overall, the quality of marine microalgae is determined by the types and relative abundance of phytoplankton that can
228 form HABs (HAB) and those which do not form HABs (non-HAB) phytoplankton in the water. HAB-forming
229 phytoplankton can multiply rapidly when changes occur in ambient environmental conditions. The Dinophyceae can
230 tolerate environmental changes such as nutrient enrichment (eutrophication) (Tester et al. 2020). If eutrophic conditions
231 occur, phytoplankton from the Dinophyceae will tend to multiply more rapidly and may form HABs (Glibert, 2017). Once
232 the HABs have begun to form, non-HAB phytoplankton may experience a decline in abundance and or diversity (Glibert
233 2020; Lestari et al. 2021). One reason for this decline is that when the HABs phytoplankton are multiplying, they release
234 toxins (Glibert 2017, 2020); these may impede the growth and reproduction of the non-HAB phytoplankton as well as
235 affect other organisms (Dorgham 2014; Glibert 2017). Conversely, a greater variety of non-HAB phytoplankton indicates
236 a thriving phytoplankton community that can support the life of other organisms.

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

247 In conclusion, the observed composition of the marine microalgal communities present in the coastal waters along the
248 west coast of South Sulawesi indicates that the phytoplankton quality can be considered reasonable. This evaluation is
249 based on the taxonomic composition and abundance of non-harmful (non-HAB) phytoplankton, which were far more
250 abundant than the taxa, which can cause HABs. This shows that seafood is still suitable for consumption because HAB
251 taxa can be considered not yet present at contaminating levels. Even though the proportions of HAB forming taxa present
252 were very low, their presence is an early warning that vigilance is needed. These results highlight the importance of
253 maintaining water quality and the necessity of regular water quality monitoring, as suggested by Anderson et al. (2015), to
254 ensure that HABs do not develop undetected.

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C. UNCORRECTED PROOF FROM REVIEWERS/EDITORS

C1. NEW NOTIFICATION FROM EDITOR

The screenshot shows an email interface. At the top, there is a search bar and navigation icons. The email subject is "[biodiv] New notification from Biodiversitas Journal of Biological Diversity" with "External" and "Inbox" tags. The sender is "Smujo Editors <smujo.id@gmail.com>" and the recipient is "to me". The date and time are "Wed, Nov 3, 2021, 10:22 PM". The email body contains the following text:

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Ahmad Dwi Setyawan

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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 MASSINAI¹, MUHAMMAD A. AMRAN¹

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

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Abstract. *Tambaru R, Burhanuddin AI, Massinai A, Amran MA. 2021. Detection of marine microalgae (phytoplankton) quality to support seafood health: a case study on the west coast of South Sulawesi, Indonesia. Biodiversitas 22: xxx.* 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, Jenepono 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, Jenepono 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.

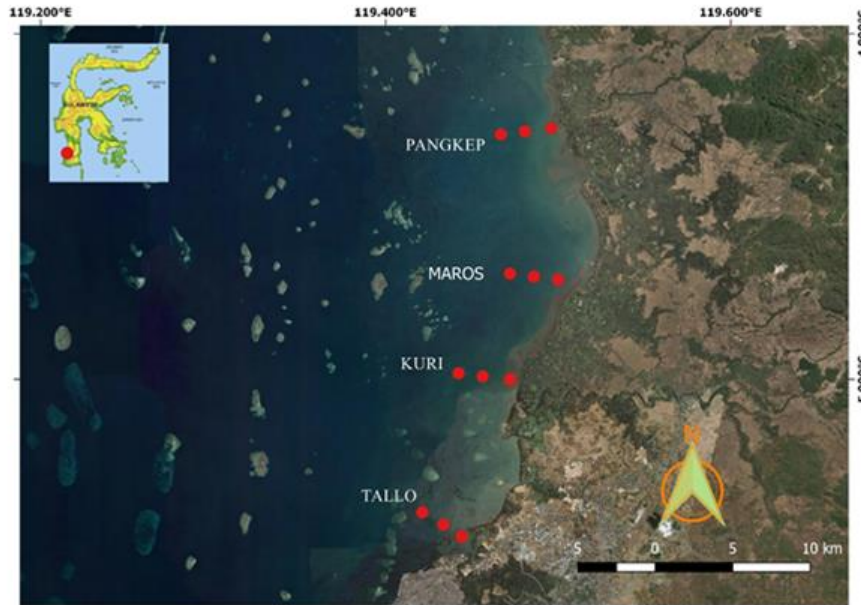
MATERIALS AND METHODS

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 (Vadrucchi et al. 2018). A 100 mL sub-sample was placed in a measuring beaker (volume 100 mL) and preserved in Lugol solution for one week. Once precipitation had occurred, the precipitated material (10 mL) was separated from the supernatant by siphoning the supernatant out of the beaker. This precipitate was then placed in a bottle to which more Lugol solution was added. The abundance of

phytoplankton cells was calculated using a sweeping (census) method (Rocha et al. 2015) using a 50 mm x 20 mm x 1 mm Sedgwick Rafter Cell (SRC). A 1 mL aliquot of the precipitate was placed in the SRC using a graduated pipette. The SRC was observed under a binocular microscope (Olympus CX21) at 10x10 magnification. Phytoplankton cells were identified using several standard reference works (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*, *Odontella*, and *Rhizosolenia*, all of which belong to the Class Bacillariophyceae.

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

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

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

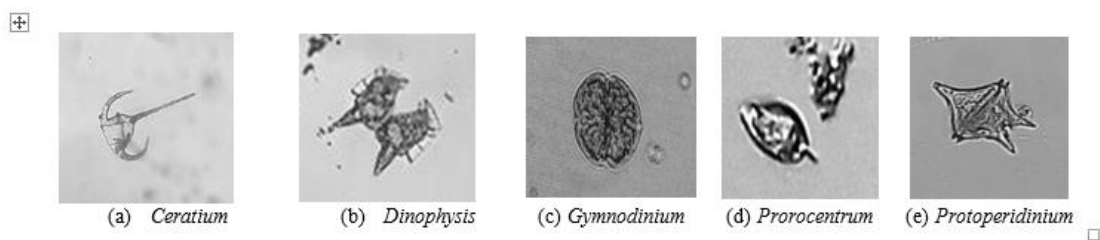


Figure 4. Examples of phytoplankton from class Dinophyceae

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).

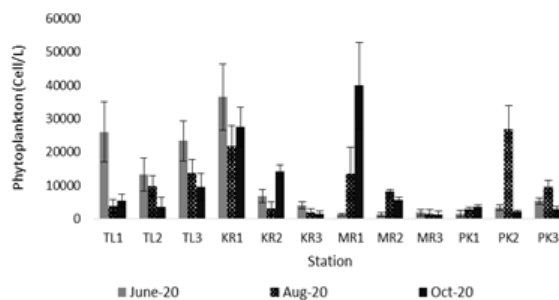


Figure 2. Phytoplankton density at the sampled sites in South Sulawesi over the research period (mean \pm SD)

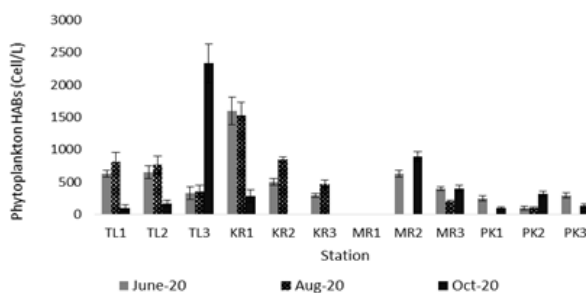


Figure 3. HAB forming phytoplankton density in samples from South Sulawesi over the study period (mean \pm SD)

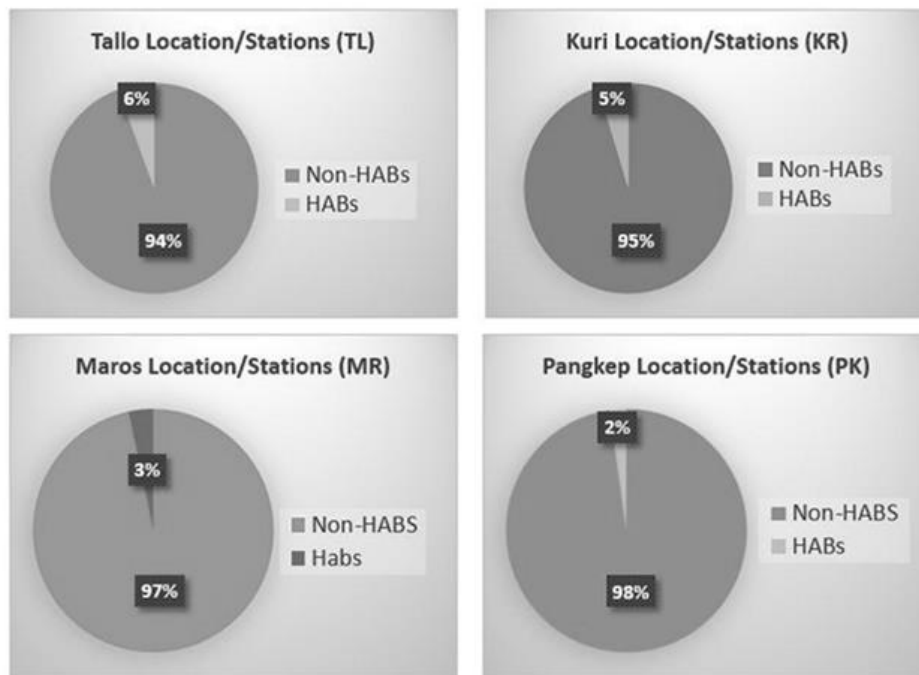


Figure 5. Relative abundance (proportion) of HABs and non-HABs phytoplankton observed during the research period

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*, *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 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 Sugestiniingsih, 2000). In addition, this organism is considered to be a threat to the aquatic environment because it can cause oxygen deprivation through decreasing oxygen concentration in the water. *Ceratium* can result in mass mortality of marine life due to decreased oxygen levels if the population becomes too abundant (Thoah 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

(ANOVA, $p > 0.05$) indicates that the presence and abundance of taxa capable of forming HABs can be considered similar throughout the study period.

Overall, the quality of marine microalgae is determined by the types and relative abundance of phytoplankton that can form HABs (HAB) and those which do not form HABs (non-HAB) phytoplankton in the water. HAB-forming phytoplankton can multiply rapidly when changes occur in ambient environmental conditions. The Dinophyceae can tolerate environmental changes such as nutrient enrichment (eutrophication) (Tester et al. 2020). If eutrophic conditions occur, phytoplankton from the Dinophyceae will tend to multiply more rapidly and may form HABs (Glibert, 2017). Once the HABs have begun to form, non-HAB phytoplankton may experience a decline in abundance and or diversity (Glibert 2020; Lestari et al. 2021). One reason for this decline is that when the HABs phytoplankton 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 (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 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.

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D. BILLING/INVOICE/PAY TO PUBLISH

The screenshot shows an email client interface. At the top, there is a search bar and navigation icons. The main content area displays a notification from Biodiversitas Journal of Biological Diversity, dated Nov 4, 2021, 8:46 AM. The notification text reads: "You have a new notification from Biodiversitas Journal of Biological Diversity: You have been added to a discussion titled 'BILLING' regarding the submission 'Detection of Marine Microalgae (Phytoplankton) Quality to Support Seafood Health: A case study on the west coast of South Sulawesi, Indonesia'." Below the notification are buttons for "Reply" and "Forward".

The screenshot shows a blue notification window titled "BILLING". It lists participants: DEWI NUR PRATIWI (dewinurpratiwi) and Rahmadi Tambaru (rahmadi69). Below the participants is a "Messages" section with a table of messages:

Note	From
Dear Author(s),	dewinurpratiwi
Kindly find attached an invoice for the publication of your manuscript.	2021-11-04 12:45 AM
dewinurpratiwi, 3577.RAHMADI TAMBARU.pdf	

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E. PRODUCTION

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9630 / Tambaru et al. / Detection of marine microalgae (phytoplankton) quality to support seafood health: A case study on the west [Library](#)

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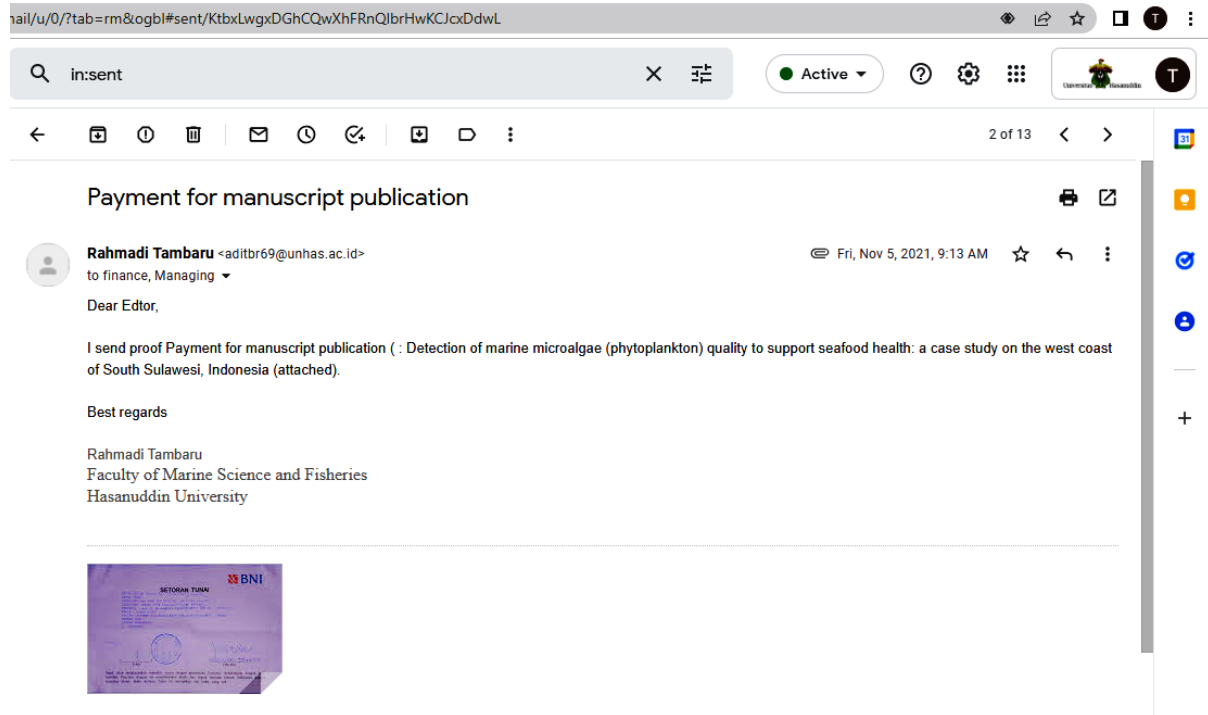
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Rahmadi Tambaru (rahmadi69)

Messages

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Dear Editor	rahmadi69
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Faculty of Marine Sciences and Fisheries	
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F. PAYMENT FOR MANUSKRIP PUBLICATION



G. PUBLICATION (PAPER PUBLISHED: ARCHIVE)

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Submissions

Workflow Publication

Status: Published

This version has been published and can not be edited.

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Title

Detection of marine microalgae (phytoplankton) quality to support seafood health: A case study on the

Abstract

B *I* \times^2 \times_2

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.

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1/1 2 5

1/1	Assigned reviews completed
2	Revisions submitted
5	Open discussions

Last activity recorded on Thursday, November 11, 2021.

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