# **Proses Revisi Pertama**

**Sulastri Arsad,S.Pi,M.Sc,M.Si** <sulastriarsad@ub.ac.id> 19 février 2019 à 09:14 À : NEPT Journal <contact@neptjournal.com>

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Kind regards,

Sulastri

ARSAD

Lecturer and Researcher Faculty of Fisheries & Marine Science Universitas Brawijaya Malang, Indonesia 65145

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Horizontal distribution of Chlorophyll-a in the Gorontalo bay rev.docx 1100K

1	Horizontal distribution of chlorophyll-a in the Gorontalo Bay
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#### 33 Abstract

The concentration of chlorophyll- $\alpha$  in the Gorontalo Bay is necessary to be observed since it could describe the condition of water richness. The semi-enclosed Gorontalo Bay morphology causes the status of water fertility to be largely determined by the input of inorganic or organic materials originating from the mainland. This study aimed to figure out the concentration and horizontal distribution pattern of the chlorophyll-  $\alpha$ then further to decide the relationship between the concentration of chlorophyll- $\alpha$  and the nutrient in the Gorontalo Bay. There were fifteen sub sampling sites selected based on coastal and ecological characteristics. Results showed that the distribution pattern of chlorophyll- a in the Gorontalo Bay in June and July 2017 was dissimilar and its concentration ranged from 0.984 to 3.744 mg m<sup>-3</sup>. In addition, there was a positive and substantial relationship between chlorophyll- $\alpha$  and phosphate (p<0.01). Nonetheless, there was no significant correlation between chlorophyll- $\alpha$  and nitrate (p>0.01) and ammonia (p>0.01). Keywords: *ammonia*, *bay*, *chlorophyll*-α *distribution*, *nitrate*, *phosphate* 

#### 65 Introduction

Bay ecosystems are very dynamic due to the influence from the mainland and human impacts (Madin et al. 2016; Österblom et al. 2017). Physical, chemical, and biological conditions depending on inputs which derived from land activities. Ecosystem balance and sustainability of aquatic biota of bay become an essential aspect that must be maintained. Water fertility is one point that contributes greatly in ensuring the balance of the marine ecosystem. The distribution of chlorophyll- $\alpha$  on the water surface is an indicator which can be used to estimate water fertility.

Geographically Gorontalo Bay is a part of Tomini Bay, morphologically 73 Gorontalo Bay is semi-enclosed waters then likely that the profile of surface 74 chlorophyll- $\alpha$  distribution in these waters is local in type and different from the 75 distribution of chlorophyll- $\alpha$  in Tomini Bay in general. The two main points that 76 underlie the importance of studying the distribution of chlorophyll-a in the Gorontalo 77 Bay are ecological and economic roles. Ecologically, the Gulf of Gorontalo is still semi-78 79 encircled waters to enable inputs from terrestrial activities that carry organic or inorganic materials are very decisive for water fertility. Human activities in the 80 81 mainland around the waters of Gorontalo Bay relatively varied. Output and waste from human activities containing organic or inorganic materials flow into the waters through 82 83 rivers and streams during rain (Cloern et al. 2016).

84 Gorontalo Bay has an economical purpose as this bay is a strategic zone for fishing by local fishermen. Local people whose main livelihoods are fishermen depend 85 severely on catches. The availability of the abundant and varied fish of the bay is 86 meanderingly influenced by the availability of diet. Phytoplankton, the autotrophic part 87 of the plankton community, is a key element in oceanic ecosystems and in 88 biogeochemical cycling (Beltran-Heredia et al. 2017). The presence of it as the food of 89 fish is determined by the water fertility aspect that can be reflected from the distribution 90 of chlorophyll-a. 91

92 The purpose of this study was to determine the concentration and horizontal
93 distribution pattern of chlorophyll-α and to figure out the correlation between
94 chlorophyll-α and nutrient concentrations in the Gorontalo Bay.

95

#### 96 Materials and Methods

Water samples were carried out twice in June and July 2017. Water samples 97 were collected on five stations with dissimilar in land use. In the main land near from 98 site 1, there are rare resident settlements; near, from site 2 there are no human activities 99 yet because of a steep cliff presence. Moreover, land use near from the site 3 is Bone 100 River estuary. In the site 4 there is an oil port and in the site 5 there are densely 101 populated settlements. Sampling at each station was performed out on 3 sub-stations by 102 103 dragging the line-transect to the offshore as presented on the sampling map on the 104 Figure1.

The samples for analysis of chlorophyll- $\alpha$  and nutrient concentration were 105 collected from fifteen different points then compiled into five sample bottles and taken 106 at Hydrobiology Laboratory, Environment and Waters Biotechnology division, 107 Universitas Brawijaya referring to APHA standard procedure. The surface water 108 109 samples collected with phytoplankton net by performing a horizontal towing of the plankton net (Tuney & Maroulakis 2014). The net was held by the side of the boat 110 (while the boat was moving slowly) for approximately 1.5 minutes. A slight turn 111 performed with the boat so that the net was towed from the inner side of the turn to 112 113 avoid drifting the net under the boat.

114 The spectrophotometric method was used for the determination of chlorophyll- $\alpha$ 115 concentration (Rice et al. 2014). The absorbance 664 used to determine the chlorophyll-116  $\alpha$  concentration in the extract through the calculation by inserting the corrected optical 117 densities in the following equations:

118

119 Chlorophyll –  $\alpha$  = 11.85 (absorbance 664) – 1.54 (absorbance 674) – 0.08 (absorbance 630)

120 where:

absorbance 664, 647, and 630 = corrected optical densities (with a 1-cm light path) at
the wavelength.

123 After determining the concentration of pigment the extract, the amount of 124 pigment per unit volume was calculated as follows:

Chlorophyll –  $\alpha$ ,  $\frac{\text{mg}}{\text{m}^3} = \frac{\text{C}_a \text{x extract volume, L}}{\text{Volume of sample, m}^3}$ 

- 125
- 126

#### 128 Data Analysis

Data were analyzed by statistical software SPSS and Microsoft office Excel 2010. Data were subjected by correlate and bi-variety test to find significant relationship between chlorophyll- $\alpha$  and nutrient concentration, while t-test to find significant distinction between nutrients concentration during sampling (p<0.05) considered being significantly different. In addition, the distribution of chlorophyll- $\alpha$  concentrations were visualized by *ArcGIS* version.10 software with spatial interpolation technique.

135

#### 136 **Results and Discussions**

#### 137 Concentration and distribution of chlorophyll-a in the Gorontalo Bay

The concentration of chlorophyll- $\alpha$  among the five stations was varied ranged from 0.984 to 3.740 mg m<sup>-3</sup> during two sampling times, June and July (Fig. 2). Averagely, the concentration of Chlorophyll- $\alpha$  recorded during sampling was higher in July than that in June. Furthermore, the value of chlorophyll- $\alpha$  in the station 4 in July was the highest with the number of 3.744 mg m<sup>-3</sup>, on the other hand the lowest concentration was in the station 5 in June with the number of 0.9844 mg m<sup>-3</sup>.

144 The chlorophyll- $\alpha$  surface concentration in the Gorontalo Bay in June was 145 higher than that of chlorophyll- $\alpha$  in July. This is because a day before the sampling in 146 July there was rain, therefore the inorganic materials that are the source of food for 147 phytoplankton come in the bay waters through run-off. It is generally accepted that 148 phytoplankton biomass can be reflected by concentration of chlorophyll- $\alpha$  (Larsson et 149 al. 2017).

Being the predominant pigment in phytoplankton cells, chlorophyll-  $\alpha$  has been long used as a proxy for estimating the standing stocks of phytoplankton biomass in the water column. The latter is of major importance, as phytoplankton plays a central role in the structure and functioning of aquatic ecosystems, while its abundance reflects the tropic status of a water body (Mandalakis et al. 2017). Moreover, Figure 3 depicts that chlorophyll- $\alpha$  in June 2017 was different from that of July 2017.

Based on the visualization results of horizontal distribution of chlorophyll- $\alpha$ (Fig. 3), it showed that the chlorophyll- $\alpha$  distributed highly in the western area of the bay in June 2017 and in the eastward revealed low concentration. Furthermore, different trend was shown in July 2017 as there was a change in the horizontal distribution 160 pattern of chlorophyll- $\alpha$ . In July the eastern part of the waters has a high concentration 161 of chlorophyll- $\alpha$ , while in the west it has low chlorophyll-a value. This fact indirectly 162 indicates that the tropic status on the west and east part of Gorontalo Bay in these two 163 months is altered.

The main factor suspected to cause this spreading pattern distribution is a 164 seasonal reversing wind season. Local fishermen informed that in June 2017 it was the 165 166 end of west season where the local wind moves from eastward to westward. Otherwise, in July it was the east wind season where the wind blows from westward to eastward. 167 168 Allahdadi et al. (2017) identified that coastal current and its spatial distribution were significantly affected by open boundary conditions. Wind controls the movement of 169 water in semi-enclosed bay of large shallow Lake Taihu, China (Li et al. 2017). The 170 movement of wind causes a water mass transfer in the semi-enclosed Gorontalo bay 171 172 which has chlorophyll- $\alpha$ .

173

## 174 Relationship between chlorophyll-a and nutrient concentrations in the Gorontalo175 Bay

176 The concentrations of ammonia, nitrate and phosphate in the waters of Gorontao Bay in June and July of 2017 are relatively various. In the Gulf of Gorontalo in June and 177 July, the ammonia concentration was  $0.116 \pm 0.213$  mg m<sup>-3</sup> with the least value of 0.035 178 mg m<sup>-3</sup> and the greatest value of 0.336 mg m<sup>-3</sup>. Nitrate concentration was 179  $0.091 \pm$ 0.074 mg m<sup>-3</sup> with the lowest number of 0.042 mg m<sup>-3</sup> and the highest number of 0.146 180 mg/m<sup>3</sup>. The concentration of phosphate was  $0.047 \pm 0.095$  mg m<sup>-3</sup> with the smallest 181 concentration of 0.003 mg m<sup>-3</sup> and the highest value of 0.137 mg m<sup>-3</sup>. Phosphate 182 concentrations in waters are lower than nitrogen (ammonia and nitrate). This is because 183 184 the phosphorus has been considered a key limiting nutrient in marine systems (Redfield 1958). 185

186 The relationship between chlorophyll- $\alpha$  concentration with nutrient, ammonia, 187 nitrate, and phosphate is presented in Figure 4. It can be seen from that figures that there 188 is a negative correlation between chlorophyll- $\alpha$  and ammonia and nitrate. Although 189 based on t-test results in the data showed no significant correlation of chlorophyll- $\alpha$ 190 concentrations to ammonia and nitrate. The determination coefficient between 191 chlorophyll- $\alpha$  and ammonia and nitrate was 40% and 21% respectively. Balali et al. 192 (2013) stated that there is a significant negative correlation between chlorophyll- $\alpha$  and 193 nitrate (R<sup>2</sup> = 26.1%) and ammonia (R<sup>2</sup> = 11%) as the amount of chlorophyll- $\alpha$  was high 194 the amounts of nitrate and ammonia were in the lowest.

195 Otherwise, a positive correlation is indicated by the relationship between 196 chlorophyll- $\alpha$  concentration and phosphate concentration in the waters of the Gorontalo 197 Bay with a coefficient of determination of 94%. Furthermore, there is a positive 198 correlation between chlorophyll- $\alpha$  and phosphate in the waters (Magumba at al. 2013; 199 Hakanson & Eklund 2010; Davis & Cornwell 1991).

200

#### 201 Conclusions

There was a positive and significant relationship between chlorophyll- $\alpha$  and phosphate (p<0.01) but there was no significant correlation between chlorophyll- $\alpha$  and nitrate (p>0.01) and ammonia (p>0.01) in the Gorontalo Bay in June and July 2017. In addition, the chlorophyll- $\alpha$  concentration in the Gorontalo Bay in June and July 2017 ranged from 0.984 to 3.744 mg L<sup>-1</sup>.

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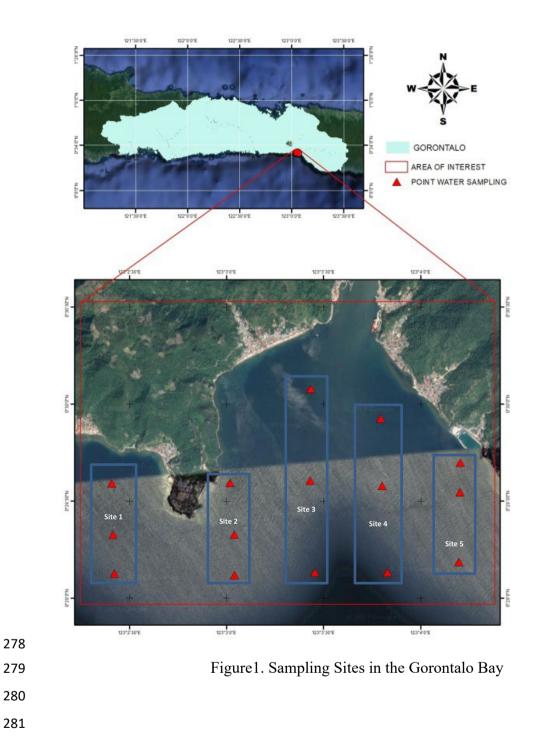
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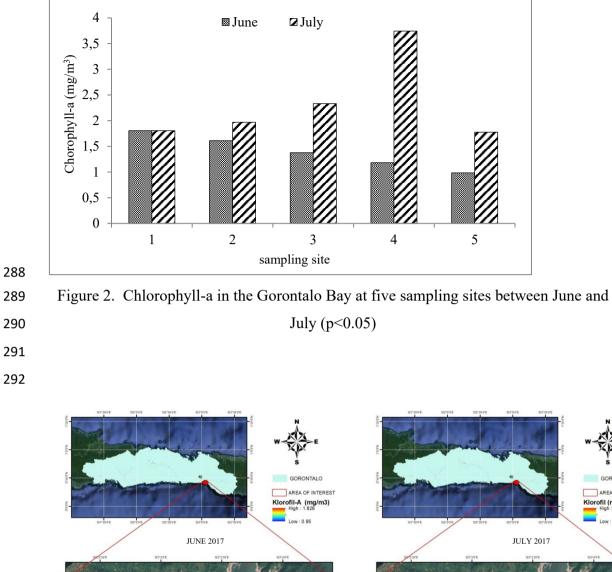
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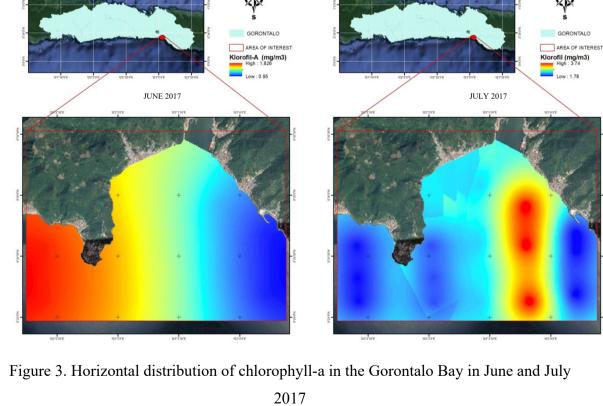
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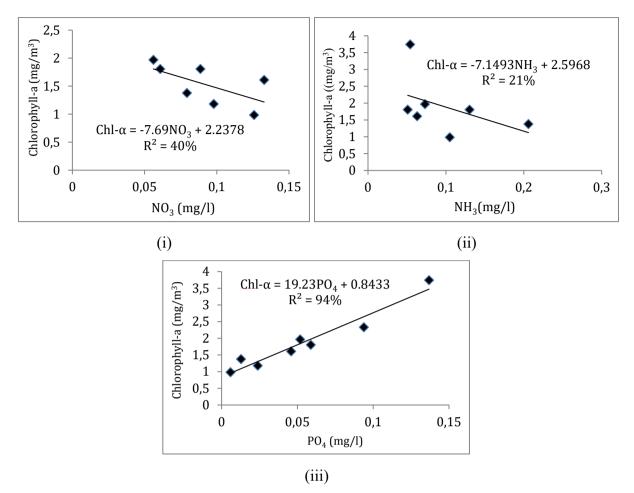


Figure 4. Simple correlation between chlorophyll-a and (i) NO<sub>3</sub> (ii) NH<sub>3</sub> and PO<sub>4</sub> (iii) concentration in the Gorontalo Bay

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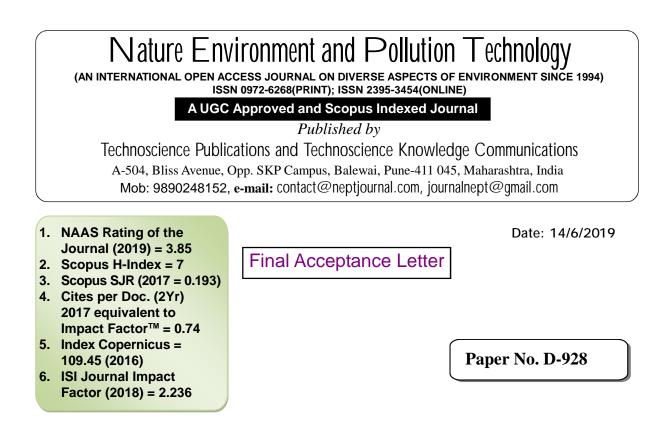
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Miftahul Khair Kadim, NuralimPasisingi and Sulastri Arsad Department of Aquatic Resources Management, Fisheries and Marine Science Faculty, Universitas NegeriGorontalo,Gorontalo 96128, Indonesia

Dear Sir/Madam

We are happy to inform you that your paper entitled **"Horizontal distribution of chlorophyll-a in the Gorontalo Bay"** has been accepted for publication in the scientific journal *Nature Environment and Pollution Technology* [ISSN 0972-6268 (Print), ISSN 2395-3454 (Online)]. The paper is likely to come in Vol. 18, No. 4 (December), Year 2019.

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Sulastri ARSAD Lecturer and Researcher Faculty of Fisheries & Marine Science Universitas Brawijaya Malang, Indonesia 65145

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p-ISSN: 0972-6268 e-ISSN: 2395-3454

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### Horizontal Distribution of Chlorophyll- $\!\alpha$ in the Gorontalo Bay

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\*Department of Aquatic Resources Management, Fisheries and Marine Science Faculty, Universitas Negeri Gorontalo, Gorontalo 96128, Indonesia

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Received: 19-02-2019 Accepted:

Key Words: Ammonia Gorontalo Bay Chlorophyll-α distribution Nitrate Phosphate

#### ABSTRACT

The concentration of chlorophyll- $\alpha$  in the Gorontalo Bay is necessary to be observed since it could describe the condition of water richness. The semi-enclosed Gorontalo Bay morphology causes the status of water fertility to be largely determined by the input of inorganic or organic materials originating from the mainland. This study aimed to figure out the concentration and horizontal distribution pattern of the chlorophyll- $\alpha$  then further to decide the relationship between the concentration of chlorophyll- $\alpha$  and the nutrients in the Gorontalo Bay. There were fifteen sub-sampling sites selected based on coastal and ecological characteristics. Results showed that the distribution pattern of chlorophyll- $\alpha$  in the Gorontalo Bay in June and July 2017 was dissimilar and its concentration ranged from 0.984 to 3.744 mg.m<sup>-3</sup>. In addition, there was a positive and substantial relationship between chlorophyll- $\alpha$  and nitrate (p>0.01) and ammonia (p>0.01).

#### INTRODUCTION

Bay ecosystem is very dynamic because of the influence of the mainland and human impacts (Madin et al. 2016, Österblom et al. 2017). Physical, chemical, and biological conditions depend on inputs which are derived from land activities. Ecosystem balance and sustainability of aquatic biota of bay become an essential aspect that must be maintained. Water fertility is one point that contributes greatly in ensuring the balance of the marine ecosystem. The distribution of chlorophyll- $\alpha$  on the water surface is an indicator which can be used to estimate water fertility.

Geographically Gorontalo Bay is a part of Tomini Bay, morphologically Gorontalo Bay is semi-enclosed waters then likely that the profile of surface chlorophyll- $\alpha$  distribution in these waters is local in type and different from the distribution of chlorophyll- $\alpha$  in Tomini Bay. The two main points that underlie the importance of studying the distribution of chlorophyll- $\alpha$  in the Gorontalo Bay are ecological and economic roles. Ecologically, the Gulf of Gorontalo is still semi-encircled waters to enable inputs from terrestrial activities that carry organic or inorganic materials which are very decisive for water fertility. Human activities in the mainland around the waters of Gorontalo Bay are relatively varied. Output and waste from human activities containing organic or inorganic materials flow into the waters through rivers and streams during rain (Cloern et al. 2016). Gorontalo Bay has an economical purpose as this bay is a strategic zone for fishing by local fishermen. Local people whose main livelihoods are fishermen depend mainly on catches. The availability of the abundant and varied fish of the bay is meanderingly influenced by the availability of diet. Phytoplankton, the autotrophic part of the plankton community, is a key element in oceanic ecosystems and in biogeochemical cycling (Beltran-Heredia et al.2017). The presence of it as the food of fish is determined by the water fertility aspect that can be reflected from the distribution of chlorophyll- $\alpha$ .

The purpose of this study was to determine the concentration and horizontal distribution pattern of chlorophyll- $\alpha$  and to figure out the correlation between chlorophyll- $\alpha$  and nutrient concentrations in the Gorontalo Bay.

#### MATERIALS AND METHODS

Water samples were carried out twice in June and July 2017. Water samples were collected from five stations dissimilar in land use. In the mainland near the site 1, there are rare resident settlements; near, from site 2 there are no human activities yet because of a steep cliff presence. Moreover, land use near the site 3 is Bone River estuary. In the site 4 there is an oil port and in the site 5 there are densely populated settlements. Sampling at each station was performed out on 3 sub-stations by dragging the line-transect to the offshore as presented on the sampling map on the Fig. 1.

The samples for analysis of chlorophyll- $\alpha$  and nutrient concentration were collected from fifteen different points then compiled into five sample bottles and taken at Hydrobiology Laboratory, Environment and Waters Biotechnology division, Universitas Brawijaya. Analysis of water samples was made referring to APHA standard procedures (APHA 1989). The water samples were collected in the column water horizontally by using plankton net (Tuney & Maroulakis 2014). The net was held by the side of the boat (while the boat was moving slowly) for approximately 1.5 minutes. A slight turn performed with the boat so that the net was towed from the inner side of the turn to avoid drifting the net under the boat.

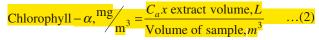
The pigment form (chlorophyll- $\alpha$ ) concentration was estimated by using spectrophotometer (Rice et al. 2014). The absorbance at 664 nm was used to determine the chlorophyll- $\alpha$  concentration in the extract through the calculation by inserting the corrected optical densities in the following equations:

Chlorophyll –  $\alpha = 11.85$  (absorbance664 nm) –1.54 (absorbance 674 nm) – 0.08 (absorbance 630 nm) ...(1)

Where, absorbance 664, 647 and 630 nm = corrected

optical densities (with a 1cm light path) at the wavelength.

After determining the concentration of the pigment extract, the amount of pigment per unit volume was calculated as follows:



#### **DATA ANALYSIS**

Data were analysed by statistical software SPSS and Microsoft Office Excel 2010. Data were subjected to correlate and bi-variety test to find significant relationship between chlorophyll- $\alpha$  and nutrient concentration, while *t*-test to find significant distinction between nutrients concentration during sampling (p<0.05) considered being significantly different. In addition, the distribution of chlorophyll- $\alpha$  concentrations were visualized by *ArcGIS* version.10 software with spatial interpolation technique.

#### **RESULTS AND DISCUSSION**

## Concentration and Distribution of Chlorophyll- $\alpha$ in the Gorontalo Bay

The concentration of chlorophyll- $\alpha$  among the five stations was varied from 0.984 to 3.740 mg.m<sup>-3</sup> during the two sampling times, June and July (Fig. 2). Averagely, the concentration of chlorophyll- $\alpha$  recorded during sampling was

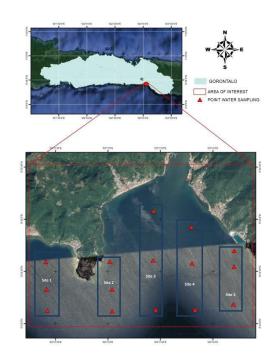


Fig.1: Sampling sites in the Gorontalo Bay.

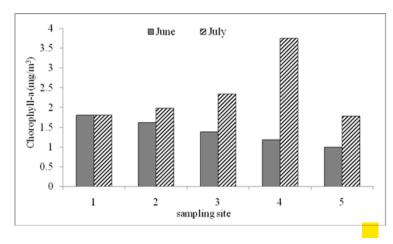


Fig. 2: Chlorophyll- $\alpha$  in the Gorontalo Bay at five sampling sites between June and July (p<0.05).

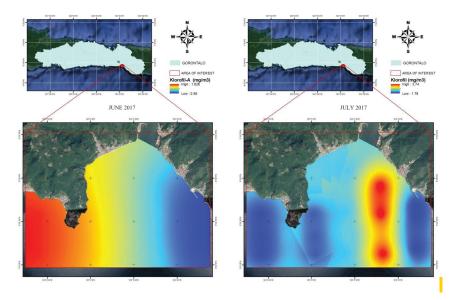


Fig. 3: Horizontal distribution of chlorophyll- $\alpha$  in the Gorontalo Bay in June and July 2017.

higher in July than that in June. Furthermore, the value of chlorophyll- $\alpha$  in the station 4 in July was the highest with the number of 3.744 mg.m<sup>-3</sup>, on the other hand the lowest concentration was in the station 5 in June with the chlorophyll of 0.9844 mg.m<sup>-3</sup>.

The chlorophyll- $\alpha$  surface concentration in the Gorontalo Bay in June was higher than that of chlorophyll- $\alpha$  in July. This is because a day before the sampling in July there was rain, therefore the inorganic materials that are the source of food for phytoplankton came in the bay waters through run-off. It is generally accepted that phytoplankton biomass can be reflected by concentration of chlorophyll- $\alpha$  (Larsson et al. 2017).

Being the predominant pigment in phytoplankton cells, chlorophyll- $\alpha$  has been long used as a proxy for estimating the standing stocks of phytoplankton biomass in the water column. The latter is of major importance, as phytoplankton plays a central role in the structure and functioning of aquatic ecosystems, while its abundance reflects the trophic state of a water body (Mandalakis et al. 2017). Moreover, Fig. 3 depicts that chlorophyll- $\alpha$  in June 2017 was different from that of July 2017.

Based on the visualization results of horizontal distribution of chlorophyll- $\alpha$  (Fig. 3), it showed that the chlo-

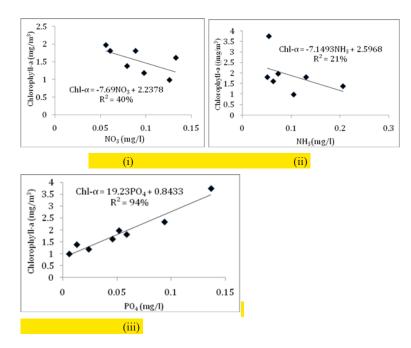


Fig. 4: Simple correlation between chlorophyll-a and (i) NO<sub>3</sub> (ii) NH<sub>3</sub> and PO<sub>4</sub> (iii) concentration in the Gorontalo Bay.

rophyll- $\alpha$  distributed highly in the western area of the bay in June 2017 and in the eastward revealed low concentration. Furthermore, different trend was shown in July 2017 as there was a change in the horizontal distribution pattern of chlorophyll- $\alpha$ . In July the eastern part of the waters has a high concentration of chlorophyll- $\alpha$ , while in the west it has low chlorophyll- $\alpha$  value. This fact indirectly indicates that the trophic status on the west and east parts of Gorontalo Bay in these two months is altered.

The main factor suspected to cause this spreading pattern distribution is a seasonal reversing wind season. Local fishermen informed that in June 2017, it was the end of wet season where the local wind moves from eastward to westward. Otherwise, in July it was the east wind season where the wind blows from westward to eastward. Allahdadi et al. (2017) identified that coastal current and its spatial distribution were significantly affected by open boundary conditions. Wind controls the movement of water in semi-enclosed bay of large shallow Lake Taihu, China (Li et al. 2017). The movement of wind causes a water mass transfer in the semi-enclosed Gorontalo bay which has chlorophyll- $\alpha$ .

## Relationship Between Chlorophyll- $\alpha$ and Nutrient Concentrations in the Gorontalo Bay

The concentrations of ammonia, nitrate and phosphate in the waters of Gorontao Bay in June and July of 2017 were relatively different. In the Gulf of Gorontalo in June and July, the ammonia concentration was  $0.116 \pm 0.213$  mg.m<sup>-3</sup> with the least value of 0.035 mg.m<sup>-3</sup> and the greatest value of 0.336 mg.m<sup>-3</sup>. Nitrate concentration was  $0.091\pm0.074$  mg.m<sup>-3</sup> with the lowest value of 0.042 mg.m<sup>-3</sup> and the highest of 0.146 mg/m<sup>3</sup>. The concentration of phosphate was  $0.047\pm0.095$  mg.m<sup>-3</sup> with the lowest concentration of 0.003 mg.m<sup>-3</sup> and the highest of 0.137 mg.m<sup>-3</sup>. Phosphate concentrations in waters are lower than nitrogen (ammonia and nitrate). This is because the phosphorus has been considered a key limiting nutrient in marine systems (Redfield 1958).

The relationship between chlorophyll- $\alpha$  concentration with nutrients, ammonia, nitrate, and phosphate is presented in Fig. 4. It can be seen from the figure that there is a negative correlation between chlorophyll- $\alpha$  and ammonia and nitrate. Although based on *t*-test results the data showed no significant correlation of chlorophyll- $\alpha$  concentrations to ammonia and nitrate. The determination coefficient between chlorophyll- $\alpha$  and ammonia and nitrate was 40% and 21% respectively. Balali et al. (2013) stated that there is a significant negative correlation between chlorophyll- $\alpha$ and nitrate (R<sup>2</sup> = 26.1%) and ammonia (R<sup>2</sup> = 11%) as the amount of chlorophyll- $\alpha$  was high when the amounts of nitrate and ammonia were the lowest.

Otherwise, a positive correlation is indicated by the relationship between chlorophyll- $\alpha$  concentration and phosphate concentration in the waters of the Gorontalo Bay with a coefficient of determination of 94%. Furthermore, there is a positive correlation between chlorophyll- $\alpha$  and phosphate in the waters (Magumba et al. 2013, Hakanson & Eklund 2010, Davis & Cornwell 1991).

#### CONCLUSIONS

There was a positive and significant relationship between chlorophyll- $\alpha$  and phosphate (p<0.01) but no significant correlation between chlorophyll- $\alpha$  and nitrate (p>0.01) and ammonia (p>0.01) in the Gorontalo Bay in June and July 2017. In addition, the chlorophyll- $\alpha$  concentration in the Gorontalo Bay in June and July 2017 ranged from 0.984 to 3.744 mg.L<sup>-1</sup>.

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#### Equation 1:

Chlorophyll –  $\alpha$  (mg. L<sup>-1</sup>) = 11.85 (absorbance 664) – 1.54 (absorbance 674) – 0.08 (absorbance 630)

Equation 2:

Chlorophyll – 
$$\alpha$$
 (mg. m<sup>-3</sup>) =  $\frac{\text{Chlorophyll} - \alpha (\text{mg. L}^{-1}) \times \text{extract volume (L)}}{\text{Volume of sample (m}^3)}$ 

