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Spatio-temporal distribution of microalgae producing chlorophyll and carotenoid pigments in Bali Strait, Indonesia

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Abstract. *Fauziah A, Bengen DG, Kawaroe M, Effendi H, Krisanti M. 2019. Spatio-temporal distribution of microalgae producing chlorophyll and carotenoid pigments in Bali Strait, Indonesia. Biodiversitas 20: 61-67.* The Bali Strait waters become a place for various development activities that connect Java Island and Bali Island so that it is thought to have an impact in the life of microalgae that play an important role in the wealth of their water resources. This study aims to explore the spatiotemporal distribution of marine microalgae, which has the potential to produce chlorophyll and carotenoid pigments, as well as their relationship with the environmental characteristics of the Bali Strait waters. The research was conducted at the 5 stations in the morning, midday and afternoon. The data obtained were analyzed using Principal component analysis (PCA) and Correspondence analysis (CA). The results showed that chlorophyll-a, chlorophyll-b and carotenoid were mostly contained by the species *Chaetoceros gracilis, Tripos lunula* at station 5 (Pang-pang Bay) in the morning, midday and afternoon, and contained by the species *Fragilariopsis cylindrus, Thalassiothrix fravenfeldii* at station 3 (Ketapang-Gilimanuk ferriage) in the midday and afternoon. Spatially, salinity, ammonia, nitrate, and chlorophyll-a have significantly different values between stations, while temporally the content of chlorophyll-b and carotenoid are influenced by time (in the morning, midday and afternoon).

Keywords: Bali Strait, carotenoid, chlorophyll, microalgae, spatiotemporal

INTRODUCTION

Microalgae are unicellular plants that produce food through photosynthesis (Qasmi et al. 2012). Microalgae have photosynthetic pigments which are able to capture and utilize solar energy and CO2. As an autotrophic organism, microalgae use simple inorganic compounds to build complex compounds and bind sunlight energy, absorb CO2 to increase productivity, and need nutrients to support its growth (Heydarizadeh et al. 2013). Hence, they are mostly known as the producer in the marine ecosystem food chain (Buditama et al. 2017).

The existence of microalgae fluctuates in the waters depending on the environmental conditions, the intensity of light entering the waters, nutrition, competition, and predation (Avecedo-Trejos et al. 2015). Juneja et al (2013) express that understanding synergistic interactions between multiple environmental variables and nutritional factors is required to develop sustainable high productivity bio algae systems. The increasing light intensity led to the increase in the growth, biomass, chlorophyll-a, and carotenoid content of microalgae (Fakhri et al. 2017). Moreover, the increase of nutrient content in the waters will increase the growth of microalgae. The heterogeneity of the aquatic environment is very influential on the presence of marine microalgae, which includes biogeographic patterns, abundance, composition, nature, and the overall distribution in the aquatic environment (Barton et al. 2013).

There are three different groups of photosynthetic pigments in the absorption of light and photoprotective pigments in the ocean, namely chlorophyll, carotenoids and *phycobiliproteins* (Jeffrey et al. 1995). Chlorophyll and carotenoids are generally used as quantitative biomarkers to determine the composition and biomass of marine phytoplankton (Wright and Jeffrey 2005) and (Buditama et al. 2017). Moreover, monitoring of chlorophyll-a phytoplankton pigment is mostly used as an indicator of eutrophication in coastal waters (Jiang et al. 2017). The maximum water pigment value coincides with the part of the water column where nutrients increase in concentration (Lorenzen 1967). The pigment of microalgae as a special characteristic of each species can be used as an indirect assessment to measure cell growth of microalgae species. In addition, it is used as well as a parameter to check trophic levels of waters (Henriques et al. 2007).

This study aims to explore the types of marine microalgae that have the potential ability to produce chlorophyll and carotenoid pigments and examine their relationship with environmental characteristics in the Bali Strait waters.

MATERIALS AND METHODS

Time and location

The study was conducted in the Bali Strait, Indonesia (Figure 1). Samples were taken at 5 different stations. Station 1 was at Menjangan Island, Station 2 at Tabuhan Island, Station 3 at the Ketapang-Gilimanuk ferriage, Station 4 at Muncar Port, and Station 5 at Pang-pang Bay. Stations 1 and 2 are small islands in the northern part of the Bali Strait and there are no river mouths flowing in the area. Stations 3 and 4 are areas occupied by human activities, both vessel crossings area and fishing industries. While Station 5 is in bays with river mouths influenced by the sea and freshwater. The influence of the sea and freshwater fluctuates depends on tides and the inputs of freshwater so that nutrients from the land come together in the bay. Sampling was conducted at each location (station) at three times.

Specifically, the study was conducted in the east season, in August 2017. Microalgae sampling was conducted in the morning at 06.00-08.00, midday at 11.00-13.00, and in the afternoon at 16.00-18.00. The sampling time was chosen based on the availability of sunlight needed by microalgae for photosynthesis.

Water quality measurement

Water quality wes measured in situ with various parameters including the salinity, temperature and dissolved oxygen (DO). The salinity was measured using a refractometer, while the temperature and DO were measured using the WTW Microprocessor Conductivity Meter. The 500 ml of water sample was carried out to measure the physical-chemical parameters of the water by ex-situ measurement. The sample was kept in a cool condition, with temperatures about 4ºC. For Phosphate, ammonia and nitrate tests were conducted at the BBAP-Situbondo Testing Laboratory by using colorimetric specification method was measured using UV-Vis Spectrometry.

Sampling and calculating abundance of microalgae

Sampling was carried out by filtering 50 L of surface water using plankton nets with specification Wildco 8” 10 um Nitex® mesh (Effendi et al. 2016) to find out the abundance of microalgae. The filtered water inside the plankton net was collected in a 250 ml sample bottle, 10% Lugol solution was added, up to the solution looked like tea color and deposited in four days. Then, the sediment of filtered seawater was taken as a sample. It was identified and classified then using a binocular microscope and identification handbook (Davis 1955; Yamaji 1979). The samples were analyzed at the Natural Feed Laboratory-BBAP-Situbondo Testing Laboratory.

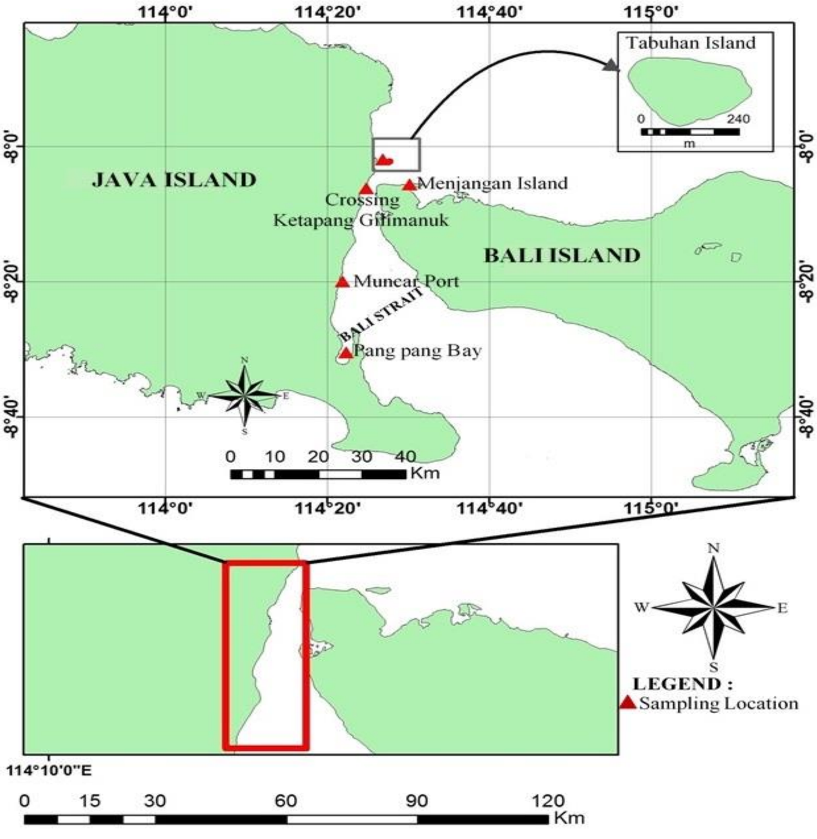

Figure 1. Research station in the Bali Strait waters, Indonesia

Measurement of photosynthetic pigments

The 50 liters of seawater were filtered using plankton nets (Effendi et al. 2016) to measure chlorophyll and carotenoid pigments. The filtered water was stored in a sample bottle and in the cold condition at a temperature of about 4ºC. Furthermore, it was filtered on Whatman paper grade 42, circle diam. 42.5 mm with a vacuum pump.

The sample of pigments was dissolved in acetone extraction (Wasmund et al. 2006). Here in after, the sample was stored in a refrigerator for 12 hours and precipitated by rotating a centrifuge of 4,000 rpms for 15 minutes at 4ºC (Sumanta et al. 2014). The sample of pigments was measured by UV-Vis Spectrometry at the BBAP-Situbondo Testing Laboratory. All the test was measured at the wavelengths of 470 nm, 663 nm, and 645 nm. The content of chlorophyll-a was calculated using the method of Ritchie (2006), and total carotenoids were calculated following the method of Kim et al. (2014).

Chlorophyll-a (μg/mL) $=-1.93 \times A_{645} + 11.93\times A_{663}$
Chlorophyll-b (μg/mL) $= 20.36 \times A_{645}-5.50\times A_{663}$
Total carotene (μg/mL) $= 4 \times A470$

Data analysis

Data analysis was referred to Bengen (2000) using XLSTAT software. Principal Component Analysis (PCA) was used to determine the spatial and temporal distribution in the waters). Moreover, the relationship among water quality parameters (temperature, salinity, DO, phosphate, ammonia, nitrate, chlorophyll-a, chlorophyll-b, and carotenoid) and the spasio-temporal distribution of microalgae was using Correspondence Analysis (CA) method.

RESULTS AND DISCUSSION

Spatio-temporal variation of environmental characteristics of the Bali Strait waters

The characteristics of the water environment from the 5 research stations are presented in Table 1. The results of the Principal component analysis show that the spatial distribution of physical-chemical parameters of water focuses on the three main axes (F1, F2, and F3) with a cumulative character of 73.36% (Figure 2). In the three main axes, four groups of physical-chemical parameters of the waters were linked to the station and research time. Two groups were identified on the F1 axis, while one group was identified on the F2 and F3 axes.

The first group on the F1 axis gives an overview that water environment at station 5 (in the morning, midday and afternoon) and at station 3 (in the midday) are temporally characterized by physical-chemical parameters. The F1 group is characterized correlation by ammonia (0.599), chlorophyll-a (0.678), chlorophyll-b (0.864) and carotenoids (0.848) which is higher than the other four stations. This group contributes to the formation of a positive F1 axis. A high concentration of ammonia in the morning is due to a very calm water conditions of Pang-pang Bay waters compared to the other four stations, so that the nutrition of ammonia concentration entered the waters is well stored and can be used as sources of nutrition that support the formation of chlorophyll-a, chlorophyll-b and carotenoid for microalgae during photosynthesis. Setiapermana (2006) found that the denitrification process optimally occurs in relatively calm (stagnant) waters.

In the bay with horizontal distribution patterns, ammonia concentration is high near a river mouth and it tends to be lower towards the open sea (Hamuna et al. 2018).

Change in the composition of microalgae in producing photosynthetic pigments are influenced by a change in light absorption by phytoplankton pigments in the waters (Marlian et al. 2015). Microalgae is able to produce pigments such as chlorophyll-a, chlorophyll-b, β-carotene, astaxanthin, phycocyanin, lutein, which are identified by derivative analyzes that give color reflection in dominating waters (Minu et al. 2014).

Table 1. The physical-chemical parameters of water at 5 research stations in the Bali Strait, Indonesia

Station/ time	Tem. (^{0}C)	Salinity (Psu)	DO (mg/L)	Phosphate (mg/L)	Ammonia (mg/L)	Nitrate (mg/L)	Chl.a (μg/mL)	Chl.b (μg/mL)	Carotene (μg/mL)
St1.morning	27.6	37.0	6.88	0.153	0.006	1.97	0,04	0,04	0,09
St1.midday	28.1	32.7	7.88	0.020	0.004	2.13	0,00	0,14	0,06
St1.afternoon	28.2	34.3	7.35	0.170	0.003	2.03	0,02	0,13	0,08
St2.morning	27.8	36.0	7.26	0.020	0.003	2.00	0,02	0,01	0,03
St2.midday	28.3	33.0	7.52	0.210	0.002	1.23	0,05	0,03	0,15
St2.afternoon	29.0	34.3	8.76	0.057	0.001	2.20	0,01	0,09	0,02
St3.morning	27.3	36.0	7.32	0.020	0.004	2.10	0,07	0,03	0,43
St3.midday	27.8	24.0	7.33	0.030	0.003	2.13	1,51	0,42	0,17
St3.afternoon	27.6	23.0	8.25	0.027	0.002	2.13	0,70	0,27	0,74
St4.morning	26.7	34.0	7.86	0.040	0.011	2.03	0,42	0,01	0,72
St4.midday	27.9	30.0	7.84	0.180	0.004	1.87	0,59	0,01	0,32
St4.afternoon	27.8	280	8.66	0.317	0.005	1.90	0,38	0,30	0,92
St5.morning	27.3	30.0	8.06	0.047	0.035	1.47	0,37	0,01	0,81
St5.midday	27.5	26.3	7.58	0.033	0.026	1.70	0,87	0,13	0,54
St5.afternoon	28.3	30.0	8.24	0.037	0.021	1.67	0,24	0,63	0,82

Marlian et al. (2015) found that waters close to land (rivers, estuaries, and bay edges) have high nutrient concentration followed by the high horizontal distribution of chlorophyll-a, whereas the waters far from land (the middle of the bay and outermost of the bay) have low nutrient concentration followed by the low horizontal distribution of chlorophyll-a. These nutrients come from the land or runoff, thus contributing very important to aquatic fertility, especially to phytoplankton biomass (chlorophyll-a) in the bay waters.

The high number of chlorophyll-a at station 5 (Pang-pang Bay) is thought to be related to the location located in the mangrove area, aquaculture area, and the bay. Mangrove vegetation areas provide the greatest nutrients to the waters. Mangrove leaf litter that falls into the water experiences decomposition so that it can provide additional nutrients for phytoplankton growth. Mangrove leaf litter is a source of carbon and nitrogen, particularly for the forest itself and surrounding waters generally (Hidayah et al. 2016). Dissolved nutrients in mangrove areas are utilized by plankton. This plankton community, especially phytoplankton, plays an important role in the mangrove ecosystem. Moreover, the productivity of phytoplankton in mangrove waters is four times higher than in the open ocean (Haryadi and Hadiyanto 2012).

The second group gives a temporal description of the morning, midday and afternoon at the first station in Menjangan Island and midday at the second station in Tabuhan Island, located in the Bali Strait characterized by physical-chemical parameters in the form of salinity (-0.832). This group contributes to the formation of a negative F1 axis. The salinity at station 1 and 2 are high because these areas are sandy islands and have no river flow. In the east season or dry season, there is absolutely no freshwater input, which causes high salinity (Young et al. 1994; Falkland 1999). High salinity causes the distribution and abundance of chlorophyll-a becoming smaller than usual (Marlian et al. 2015).

The third group described the afternoon conditions of the second station (Tabuhan Island) and the fourth station (Muncar) in the Bali Strait with a relatively high correlation of temperatures (0.764), DO (0.658) and Phosphate (0.637). This group contributes to the formation of a positive F2 axis.

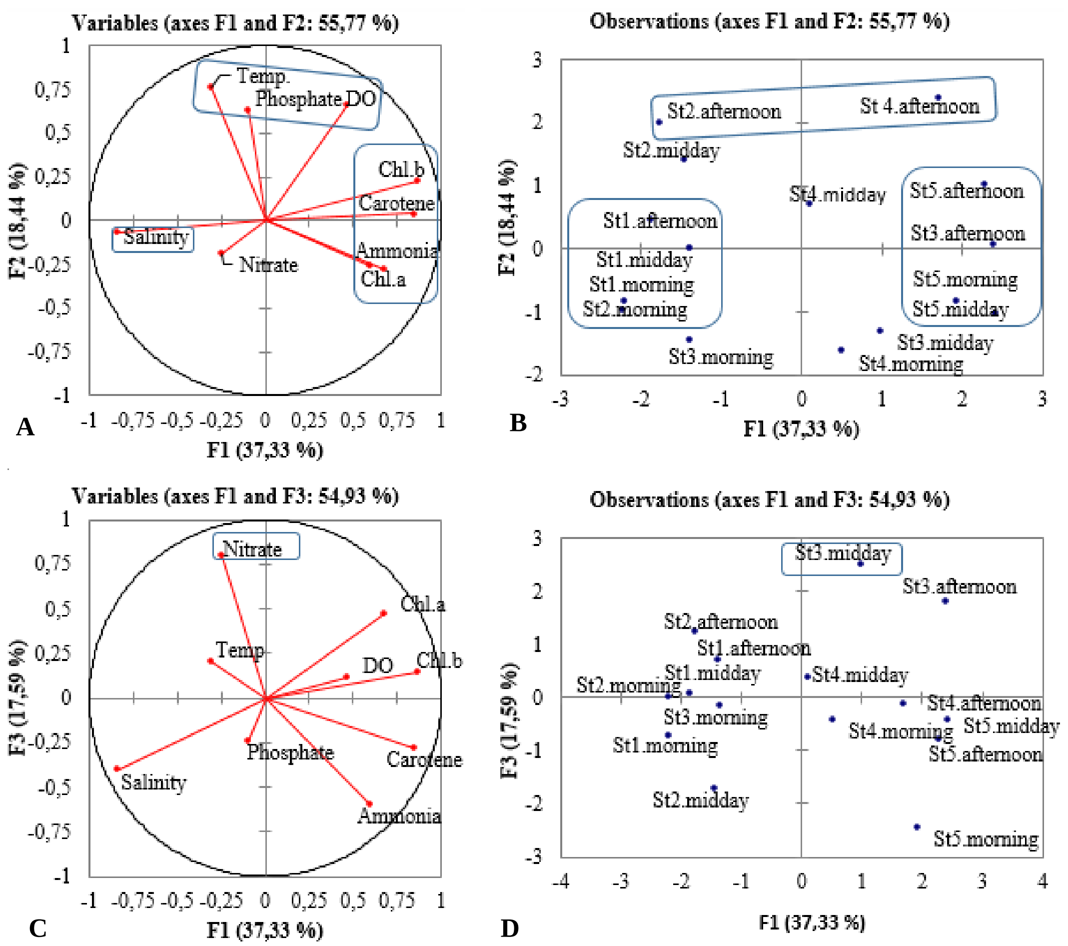

Figure 2. The principal component analysis of the environmental characteristic distribution of water area in the study location on axis 1 (F1), axis 2 (F2) and axis 3 (F3)

The fourth group describes the midday conditions of the third station (Ketapang-Gilimanuk ferriage) located in the Bali Strait with a higher form of Nitrate when compared to the other four stations. This group contributes to the formation of a positive F3 axis. At station 3, the high concentration of nitrates (0.785) in the midday occurs because there is a Ketapang river mouth in the water area carrying the waste load coming from the human activities nearby. Nitrates play a role in distinguishing the abundance of phytoplankton. The differences in nitrate concentration in waters may cause differences in phytoplankton abundance. In addition, increase and growth of phytoplankton population in waters are related to nutrient availability and light (Meiriyani et al. 2011).

Spatial distribution of microalgae in the Bali Strait

The composition of species and abundance of microalgae at the study location is presented in Table 2. Table 2 shows that the Bacillariophyceae group has the highest number of species (8 species), consisting of Bacteriastrum elongatum, Trieres chinensis, Chaetoceros gracilis, Fragilariopsis cylindrus, Leptocylindrus danicus, Navicula distans, Rhizosolenia hebetate, Thalassiothrix fravenfeldii; whereas, Dynophyceae only has 2 species, namely *Tripos* longissimus and Tripos lunula. The composition based on microalgae classes found at each station in the Bali Strait is dominated by Bacillariophyceae Classes (Table 2). This condition is common in marine waters as stated by Nybakken and Bertness (2004) that the composition of phytoplankton in the sea is dominated by the Bacillariophyceae group. According to Effendi et al. (2016), Bacillariophyceae is a sensitive type of algae. It has a very wide degree of environmental tolerance, and ability to adapt to various types or conditions of the environment, both changes in the physical, chemical and biological conditions of the aquatic environment.

In Figure 3, the spatial-temporal distribution of microalgae in the Bali Strait is centralized on 3 (three) major axes with a total value of 71.17%. In the three main axes, there are six groups of microalgae showed a linkage on the distribution of microalgae to the station and research time. The two groups identified are on the axes F1, F2, and F3, respectively.

Based on the results of the Main Component Analysis (PCA) and Correspondence Analysis (CA), there is a relationship between microalgae distribution and the characteristics of the aquatic environment as an indicator of microalgae habitat. Result study found that in the first group on the positive F1 axis, *Fragilariopsis cylindrus, Thalassiothrix fravenfeldii* are found in station 3 (Ketapang-Gilimanuk ferriage). These microalgae are sampled in the midday with high nitrate as its characters and in the afternoon characterized by high ammonia concentration, high chlorophyll-a, chlorophyll-b, and carotenoids rate. The second group on the negative F1 axis, *Trieres sinensis, Navicula distans, Rhizosolenia hebetate* are found in station 1 (Menjangan Island) and station 2 (Tabuhan Island) both in the morning, midday and afternoon and station 4 (Muncar) in the midday, with high temperature, salinity and DO as the characteristic of the waters.

Table 2. Composition and abundance of the Bali Strait microalgae, Indonesia

Station	Diversity of microalgae									
	Bacteriastrum elongatum	***Trieres chinensis***	***Chaetoceros gracilis***	***Fragilariopsis cylindrus***	***Leptocylindrus danicus***	***Navicula distans***	***Rhizosolenia hebetata***	***Thalassiothrix fravenfeldii***	***Tripos longissimus***	***Tripos lunula***
	Bacillariophyceae								**Dinophyceae**	
St1.morning	12.3	0	0	0	0	2.33	22.7	0	2.67	0
St1.midday	7	0	0	0	0	1.33	14	0	0	0
St1.afternoon	2.67	0	0	0	0	2.33	14	0	0.33	0
St2.morning	8	0	0	0	0	4	32	0	0	3
St2.midday	5	0	0	0	0	1	16	0	0	2.67
St2.afternoon	0	0	0	0	0	2.67	8.67	0	0	0
St3.morning	7.33	0.33	0	0	6	0	5.33	0	0	0.33
St3.midday	7.67	1	0	58	1	0.67	13.3	4	0	0
St3.afternoon	1.33	1.33	0	27.7	0	1.33	10.7	5	0	7.67
St4.morning	2.67	1.33	0	0	0	3.33	16	5.67	4.67	0
St4.midday	3.33	1.33	0	0	0	0	5.67	0	11	0
St4.afternoon	2.33	2.33	0	0	0	3.33	9.67	1.33	2	0
St5.morning	0	3	2.67	0	5.33	10	6.33	1.33	0	2.67
St5.midday	0	0.67	0	0	0	3.67	3.67	0	0	5.33
St5.afternoon	1	0	13.7	0	0	0	11	0	0	8.33

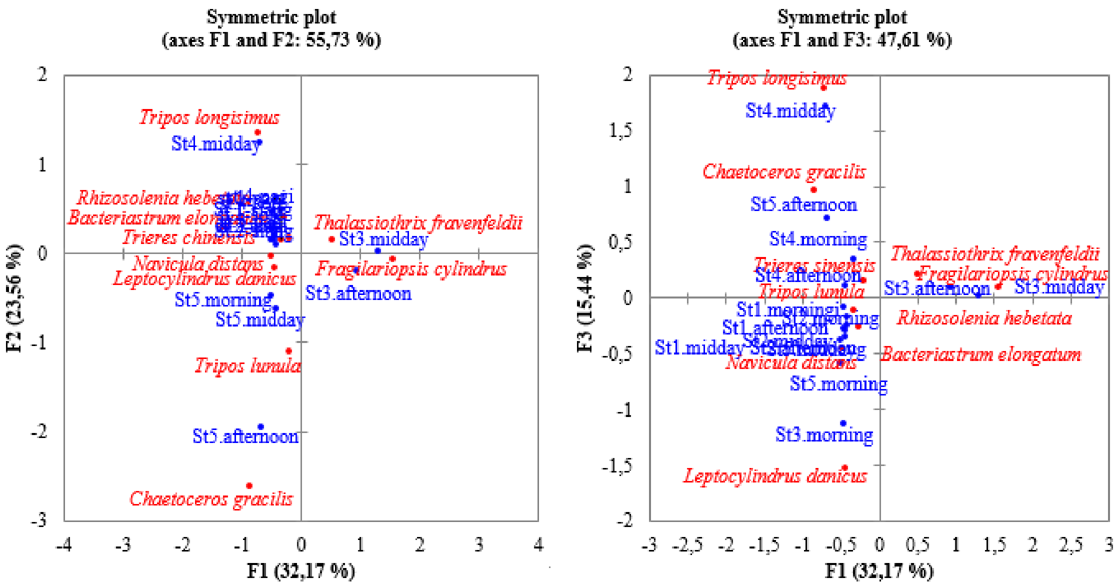

Figure 3. Correspondence analysis (CA) on spatial temporal distribution of microalgae in the Bali Strait on axis 1 (F1), axis 2 (F2) and axis 3 (F3)

The third group on the F2 positive axis, *Bacteriastrum elongatum* is evenly distributed at station 4 (Muncar) in the morning, while the fourth group in the negative F2 axis commonly consists of *Chaetoceros gracilis*, *Tripos lunula*. These microalgae are temporarily found in station 5 (Teluk pang-pang) in the period of morning, midday and afternoon characterized by high ammonia concentration, chlorophyll-a, chlorophyll-b, and carotenoids rate.

The fifth group is on the F3 positive axis, *Tripos longisimus* is found mostly at station 4 (Muncar) during the day, which is characterized by high temperature. The sixth group is on the F3 negative axis, *Leptocylindrus danicus* is evenly distributed at station 3, particularly in the morning.

In conclusion, water environment of the Bali Strait has different physical-chemical characteristics represented by five research stations. The presence of chlorophyll-a, chlorophyll-b and carotenoids are showed by *Chaetoceros gracilis* and *Ceratium lunula* located at station 5 (Pang-pang Bay) sampled in the morning, midday and afternoon. In addition, *Fragilaria cylindrus* and *Thalassiothrix fravenfeldii* located at station 3 (Ketapang-Gilimanuk ferriage) and sampled in the midday showed the presence of chlorophyll-a, chlorophyll-b and carotenoids as well, which are characterized by high nitrate concentration during the day.

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