The influence of nitrate and phosphate concentration on the abundance of plankton at the estuary of Bengawan Solo, Gresik, East Java

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Abstract. The estuary of Bengawan Solo river is influenced by materials brought from the upstream, community activities, and ponds around the estuary. The influx of organic material (nitrate and phosphate) to the estuary may interfere with water quality, which in turn affects the presence of plankton (phytoplankton and zooplankton) in the waters. This study aimed to analyze the influence of nitrate and phosphate on the abundance and diversity of plankton in the downstream of Bengawan Solo River, Ujungpangkah, Gresik. The water sampling was conducted at nine stations at low and high tide. The plankton in the water was filtered and collected horizontally using plankton net, and then analyzed using Sedgewick Rafter Counter Cell (SRCC). Using UV-Vis spectrophotometry, the water samples were analyzed for the nitrate (NO_3) and phosphate (PO_4^{3-}). Data analysis included the density of plankton, plankton diversity index, and regression curve to determine the relationship of plankton with nitrate and phosphate. The abundance of phytoplankton and zooplankton ranges respectively, 1100-2108 cells m^{-3} and 92,150-118,095 cells m^{-3}. The waters at the estuary of Bengawan Solo, Gresik were categorized as oligotrophic. The composition of diatom plankton found was (20 genera), dinoflagellates (3 genera), cyanobacteria (1 genus), hexanauplia (6 genera), branchiopods (1 genus), and Oligotrichea (1 genus). The diversity index (H') of phytoplankton was 1.50-1.81, and of the zooplankton was 1.66-1.79, and both were in the medium category. The relationship between the abundance of plankton with nitrate and phosphate showed that the variables are closely correlated to each other.
Key Words: abundance, organic material, phytoplakton, Ujungpangkah, zooplankton.

Introduction. Ujungpangkah is one of the sub-districts in Gresik, approximately 35 km north to the Gresik Regency, East Java Province. Bengawan Solo Estuary in Ujungpangkah is one of the estuaries of the Bengawan Solo River. As an area of interfusion between freshwater and seawater (Syafarina et al 2018), the estuary has gained influence from water of Bengawan Solo river. Since the river has crossed Yogyakarta to East Java for about 600 km in length, the water has been enriched by nutrients from human activities, such as agriculture, industry, household, aquaculture, etc. The input of nutrients (i.e. nitrate and phosphate) to the estuary can affect the water quality, which further affects the presence of plankton (phytoplankton and zooplankton) in the water.

The availability of phosphate and nitrate are essential for plankton. Plankton uses phosphate and nitrate for its growth process (Aini et al 2018). Nitrates are the most common forms of nitrogen found in water and the main nutrient for phytoplankton growth. Nitrates are a source of nitrogen for plants that can be converted into proteins (Effendi 2003). An orthophosphate is a form of phosphorus that can be used directly by phytoplankton (Effendi 2003). Phosphate is an essential nutrient for the growth of an organism (Widyastuti 2015 in Suryadi et al 2017).

Modeling of nutrients distribution in Bengawan Solo estuary showed relatively high contents of phosphate and nutrients, 0.01-0.23 mg L^{-1} of nitrate and 0.01-0.55 mg L^{-1} of phosphate because of farming and agriculture activities around the research site (Mustain et al 2018). The nutrients can affect the abundance of phytoplankton, and on the other hand, the density of phytoplankton can reduce nutrient content in the water. The changes in phytoplankton composition can affect the composition of zooplankton and plankton communities as a whole in an aquatic ecosystem (Prescott 1970 in Rumanti et al 2014). Therefore, this study aims to analyze the abundance and diversity of plankton, and the influence of nitrate and phosphate on the abundance of plankton in the estuary of the Bengawan Solo River, Ujungpangkah, Gresik.

Material and Method

Research methods. The study was conducted from September 1, 2019 to December 1, 2019 in the Bengawan Solo Estuary, Ujungpangkah, Gresik. Water and plankton sampling was carried out at 9 stations at low tide to high tide. Station 1 was on the main river. Stations 2 and 3 were on the river branches. Stations 4, 5, and 6 were at river estuary, and stations 7, 8, and 9 were at the sea. Figure 1 shows the location of the study.

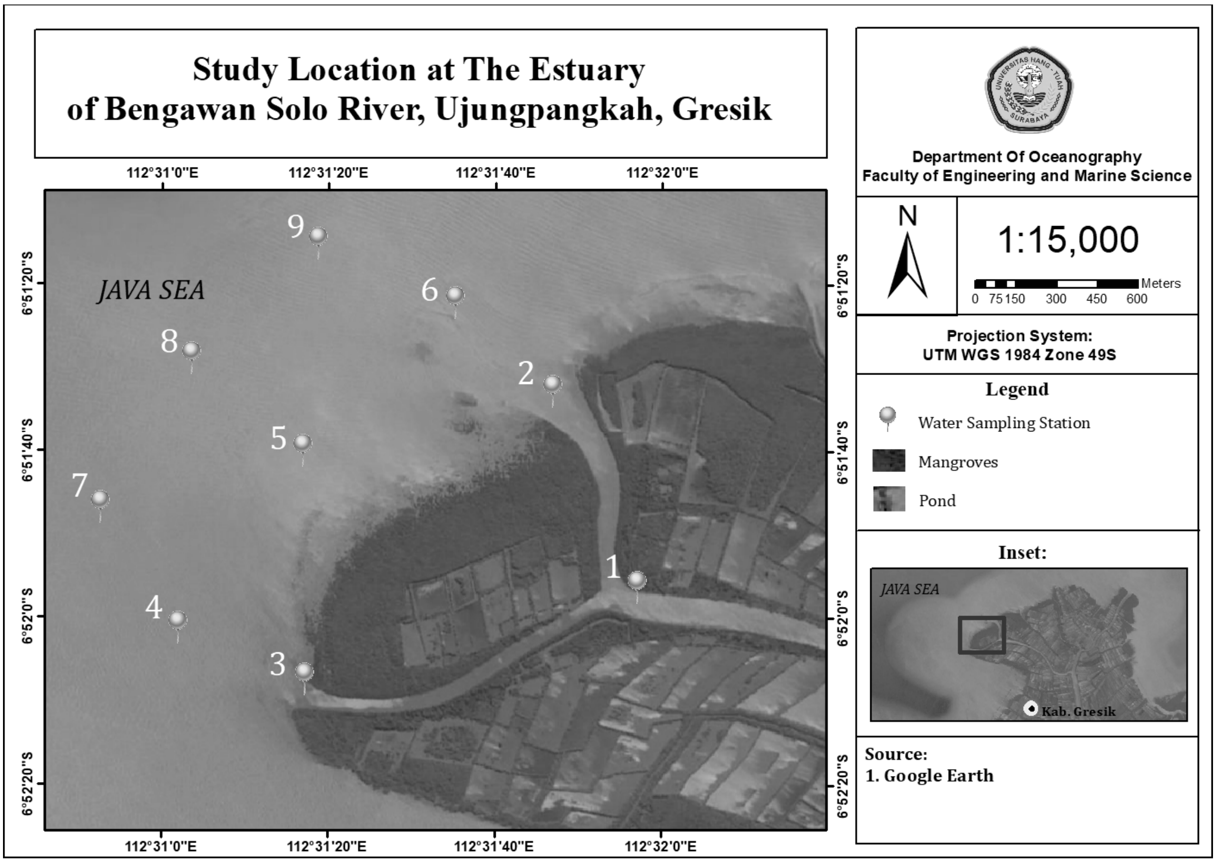


Figure 1. The location of the study at Ujungpangkah, Gresik, Indonesia.

In this study, we observed the abundance and diversity of phytoplankton and zooplankton, the concentration of nitrates (NO^{3-}) and phosphate (PO_4^{3-}), also water physical parameters (i.e. water temperature, transparency, current, and depth) and chemical parameters (i.e. dissolved oxygen (DO), pH, salinity, total suspended solid (TSS)). The tools used in this study were plankton-net, UV-Vis spectrophotometer, oven and scale, DO meter, hand-refractometer, Secchi disk, pH meter, current meter, and water sample bottles.

The setup of plankton sampling. Sampling was conducted in the waters of the estuary of the Bengawan Solo River. Plankton samples were collected by filtering water horizontally using the plankton net size 25 µm. Netting was pulled using a ship on the surface of the water at a speed of 2 knots for 5 minutes. Plankton sampling in shallow waters (< 10 m) was generally done by way of net withdrawal of plankton for approximately 5 minutes horizontally (Khouw 2016). The collected plankton was transferred into a 50 mL bottle, preserved with 4% formalin solution (Setiawan et al 2018), and counted the abundance of plankton using Sedgwick Rafter Counter Cell (SRCC). The type of plankton was identified using Yamaji (1979) plankton identification books.

The plankton density was calculated by the formula, as shown below (Rachman et al 2018):

$$DA = \frac{1}{V1} \times \frac{V2}{V3} \left(\text{Number of cell Genus } i \times \frac{1000}{\text{Number of grids observed}}\right) \quad \ldots\ldots\ldots\ldots \quad (1)$$

Without flowmeter:

$V1 = \pi r^2 s$

where: DA = absolute density of plankton cell genus *i* (cell m^{-3});
V1 = volume of filtered water (m^3);
V2 = volume of plankton sample (mL);
V3 = volume of sample fraction (mL);
π = 3.14 or 22/7;
r = radius of net mouth (m);
s = distance (m).

Shanon-Wiener Diversity Index (Odum 1993) can be formulated as follows:

$$H' = -\sum Pi \ln Pi, \text{ where } Pi = \frac{ni}{N} \quad \ldots\ldots\ldots\ldots \quad (2)$$

where: H' = Diversity Index;
Ni = number of species i cell;
N = total of cell.

According to Krebs (1998) in Dewanti et al (2018), diversity value can be classified as follows:
H' < 1 = low diversity;
1 ≤ H' ≤ 3 = medium diversity;
H' > 3 = high diversity.

Statistical analysis. The nitrate and phosphate concentrations, and plankton identification data were analyzed using double linear regression and Pearson correlation. The way to figure out the relationship between the X-free variables (nitrates and phosphate) with the Y-bound variable (the abundance of phytoplankton) is by multiplying linear regression analysis using Microsoft Excel 2016 (Rizqina et al 2017). Pearson correlation was used to determine how close the relationship between nitrate and phosphate concentrations with the abundance of phytoplankton is. A Pearson correlation coefficient (R) is categorized as follows (Nasution et al 2019):
0 = no correlation between 2 variables;
0-0.20 = weak correlation;
0.20-0.40 = weak but definite correlation;
0.40-0.70 = quite strong correlation;
0.70-0.90 = high or strong correlation;
0.90-1 = very strong correlation;
1 = perfect correlation.

Results. Bengawan Solo river is the longest river in the Java Island that flows for about 600 km. It starts from the Sewu Mountains in the south-west of Surakarta and ends at the estuary in Ujungpangkah, Gresik. It is a water source for many activities in Ujungpangkah, such as ponds, fish auction places, shell farm in tidal lands, and mangrove conservation areas.

The composition of the plankton. Based on the results of research in the estuary waters of the Bengawan Solo river, Ujungpangkah, Gresik, from 9 observation stations in the surface layer of the waters, 24 genera of phytoplankton was found. It was Bacillariophyceae/Diatomeae (20 genera), Dinophyceae or Dinoflagellata (3 genera), and Cyanobacteria class (1 genus). The composition for the type of zooplankton was 3 classes. It was Hexanauplia (6 genera), Branchiopoda (1 genus), and Oligotrichea (1 genus) (Figure 2). These Hexanauplia belong to the crustacean subphylum that can adapt well to environmental changes. Crustaceae is the most easily recognizable type of plankton compared to other types of plankton in terms of both the larva and the mature forms (Tyas et al 2017).

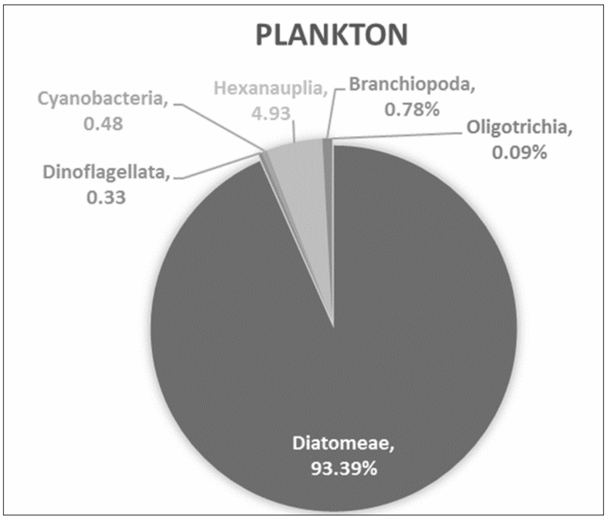
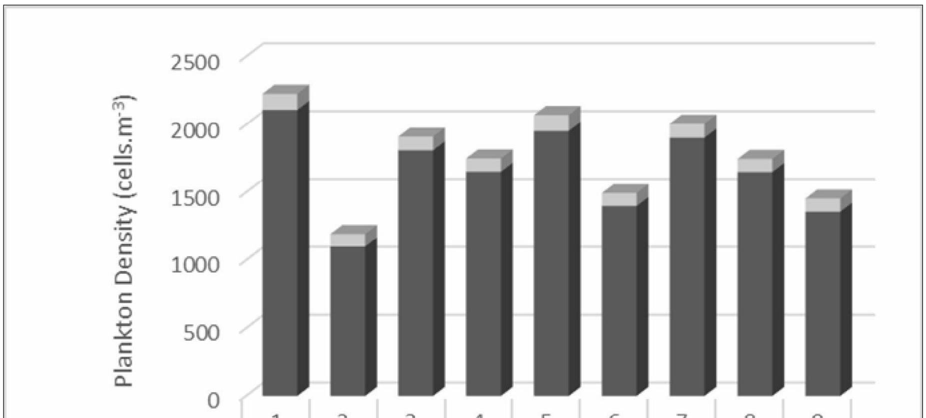


Figure 2. The composition of the plankton at Bengawan Solo Estuary.

The abundance of the plankton. The observation of the plankton in Bengawan Solo Estuary found that the abundance of phytoplankton was different from the abundance of zooplankton. The abundance value of phytoplankton ranges from 1100 to 2108 cells m^{-3} while the zooplankton ranges from 92 to 118 cells m^{-3}. The highest abundance on phytoplankton and zooplankton was both found at station 1, as 2108 cells m^{-3} and 118 cells m^{-3}, respectively. At the same time, the lowest abundance of phytoplankton and zooplankton was at station 2 with 1100 and 92 cells m^{-3}, respectively. Figure 3 shows the density of phytoplankton and zooplankton.



	1	2	3	4	5	6	7	8	9
zooplankton	118	92	101	98	116	96	103	97	98
Phytoplankton	2108	1100	1811	1652	1954	1399	1904	1651	1357

Figure 3. Plankton density in Bengawan Solo Estuary, Ujungpangkah.

Plankton diversity index. The value of diversity index (H') can depict the stability of a community of aquatic organisms. The value of the diversity index of phytoplankton in study location ranged from 1.50 to 1.81 (Table 1). The diversity index of phytoplankton on all observation stations belonged to the medium category with a value of 1 < H' < 3 (Krebs 1998 in Dewanti et al 2018). The value of the diversity of zooplankton index ranged from 1.66 to 1.79. The highest diversity index was at station 3 with a value of

1.79 whereas the lowest diversity index was at station 1 with a value of 1.66. Diversity index of zooplankton in all observation stations was categorized to a medium category, based on Krebs (1988) in Dewanti et al (2018).

Table 1

Diversity index of plankton in Bengawan Solo Estuary, Ujungpangkah

	Stations								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Phytoplankton	1.65	1.81	1.73	1.79	1.69	1.63	1.66	1.68	1.50
Zooplankton	1.66	1.76	1.79	1.78	1.74	1.78	1.76	1.77	1.78

The concentration of nitrate and phosphate in waters. The result of nitrate measurements in the Bengawan Solo Estuary, Ujungpangkah, was obtained with concentration of 0.03503-0.03518 mg L^{-1} (Figure 4). The highest concentration of nitrate was at station 1, a location in the river, within a value of 0.03518 mg L^{-1}. The nitrate concentration in this water exceeded the standard quality for marine life, which is 0.008 mg L^{-1} (KLH 2004). The high concentration of nitrate in station 1 was followed by the abundance of phytoplankton at station 1, which was 2108 cells m^{-3}.

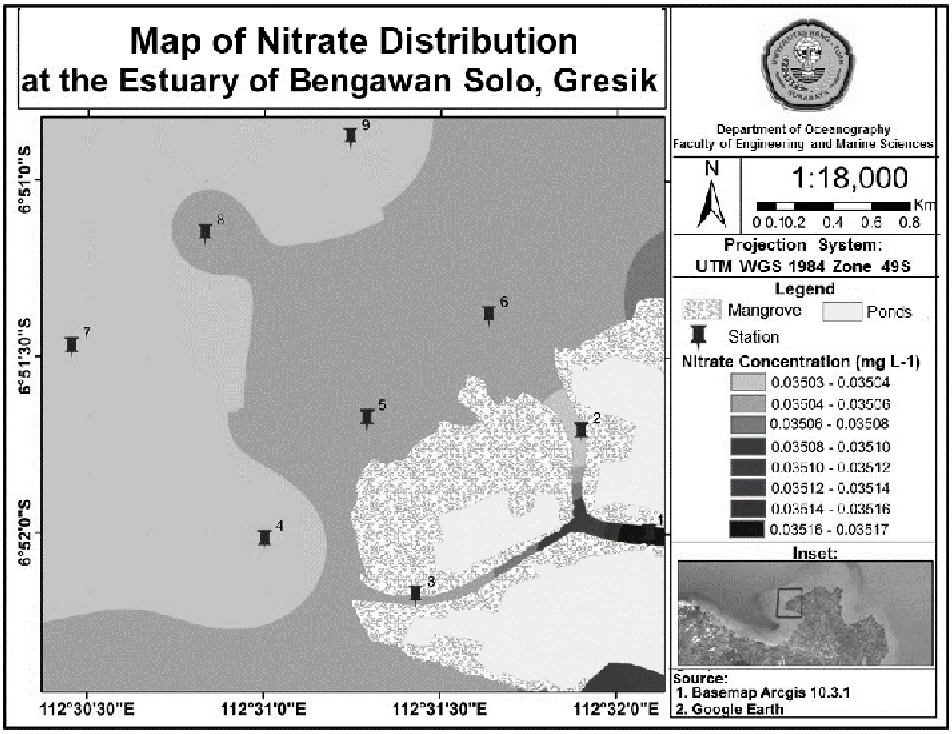


Figure 4. Nitrate concentration at the estuary of Bengawan Solo, Ujung Pangkah.

The results of phosphate analysis showed a range of 0.004 to 2.978 mg L^{-1} (Figure 5). These value exceeded the default threshold value of phosphate for marine life that was 0.015 mg L^{-1} (KLH 2004). The highest phosphate value was at station 7, which reached 2.978 mg L^{-1}. The high phosphate value was presumed because the waters have a shallow depth. According to Permatasari et al (2016), the high concentration of phosphate was caused by a shallower area and it was an area of direct mass mixing water. Based on the statement of Simanjuntak et al (2006) in Permatasari et al (2016), the high level of phosphate was caused by currents and stirring of water masses resulting in the lifting of high phosphate content from the base to the surface layer.

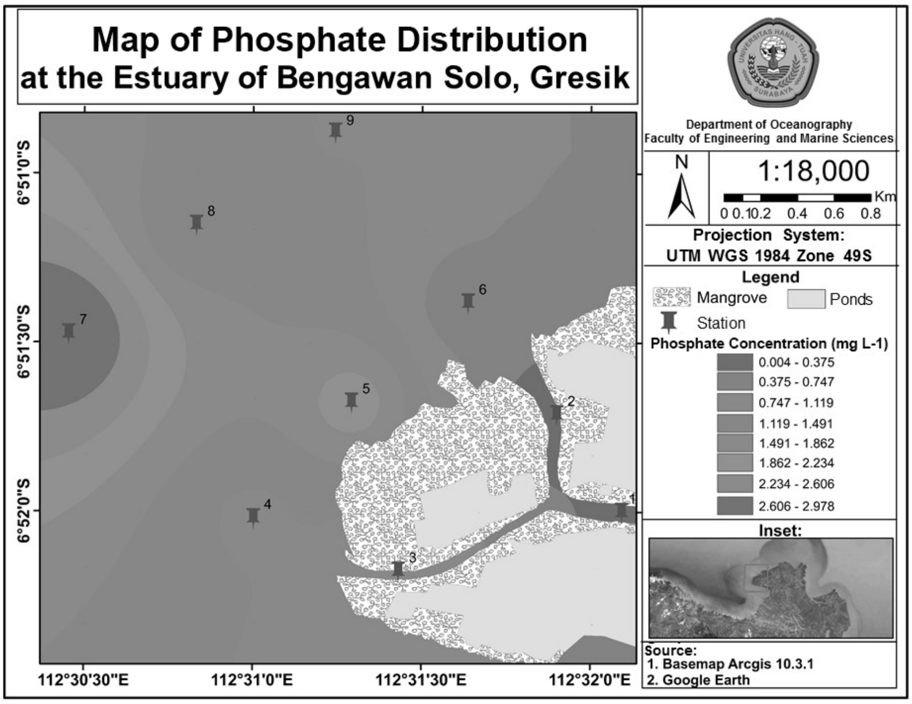
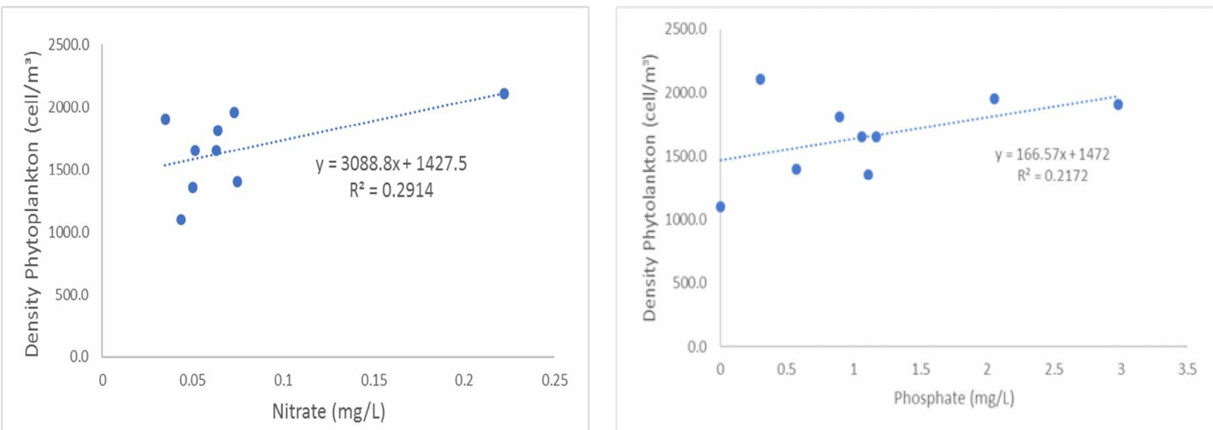


Figure 5. Phosphate concentration at the Bengawan Solo Estuary, Ujung Pangkah.

The relationship between the abundance of phytoplankton with nitrate and phosphate concentrations. The relationship between the abundance of phytoplankton with nitrate-water concentration formed equations of y = 3088.8+1427.5. The correlation coefficient (r) = 0.54 meaning that inter variables have a quite close correlation. Correlation of positive value means there is a positive relationship between nitrate concentration and the abundance of phytoplankton. Nasution et al (2019) stated that the value of the correlation coefficient (r) = 0.40-0.70 belongs to the category of quite strong correlation. The value of determination coefficient (R^2) = 0.2914 indicates that the abundance of phytoplankton is 29.1% influenced by the value of nitrate, and 70.9% influenced by other parameters (Figure 6a).

The relationship between the abundance of phytoplankton and phosphate concentration formed the equation of y = 166.57+1472. The correlation coefficient (r) = 0.47 means that inter variables have a quite close correlation. Correlation of positive value means there is a positive relationship between phosphate concentration and abundance of phytoplankton. The value of determination coefficient (R^2) = 0.2172 indicates that the abundance of phytoplankton is 21.1% influenced by phosphate concentration value, and 79.9% influenced by other parameters (Figure 6b).



a. Nitrate b. Phosphate

Figure 6. The relationship between the abundance of phytoplankton with nitrate and phosphate.

Principal Component Analysis (PCA). The results of the correlation matrix analysis showed that important information describing the correlation between parameters was depicted on two main axes (F1 and F2), with information quality of 32.83% and 26.25% respectively. Therefore, the two main axes amounted to 59.08% of the total variance can explain the various correlations of physical and chemical parameters of the environment with plankton abundance. Other parameters on the F3 axis and so on are not discussed here, because they are considered to have low influence. The most influential environmental parameter on plankton abundance is nitrate concentration, where nitrate has a positive effect on plankton abundance (Figure 7).

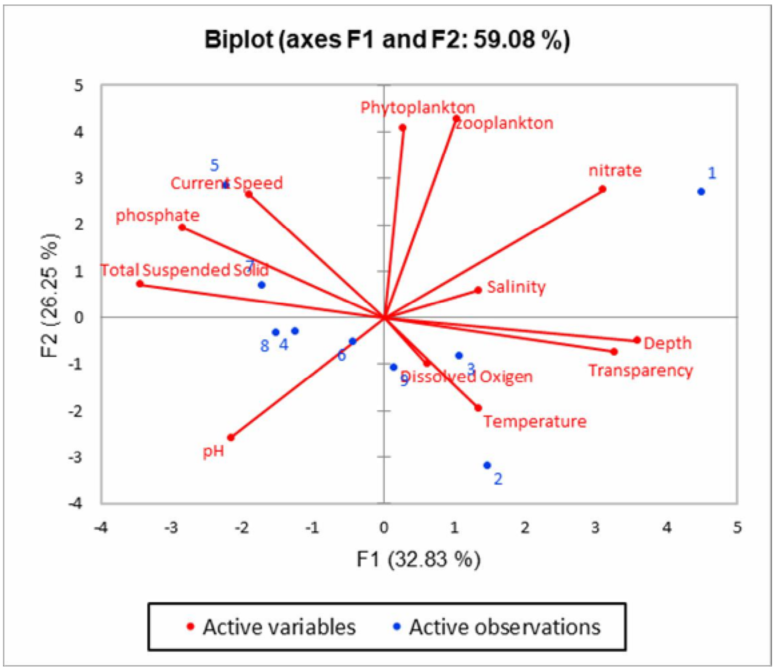


Figure 7. Graph of linkages the biophysical parameters with plankton abundance

Measurement of water quality in the location of study. Data in Table 2 indicated that measurement of water quality has relatively different results at each station. Measurement of water temperature obtained was in a range of 30 to 34.8^{o}C. Depth measurement showed that the range of depth was 0.3-1.7 m. On the other hand, the measurement of transparency had a range of 0.07-0.55 m. Salinity measurement obtained was 34-36 ppm and it was optimal for plankton growth. The optimal temperature for phytoplankton is between 25-30^{o}C while the optimal temperature for zooplankton is 15-35^{o}C (Dewanti et al 2018).

Table 2

Physical and chemical condition of Bengawan Solo Estuary

No	*Parameter*	*Station*								
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1	Temperature (^{o}C)	32	32	34.8	32.1	30	33.1	32.1	31.8	31.7
2	Depth (m)	1.7	1.1	0.9	0.6	0.3	0.8	0.5	0.4	1.5
3	Transparency (m)	0.55	0.47	0.19	0.07	0.09	0.07	0.07	0.07	0.53
4	Current (m s^{-1})	0.3	0.2	0.3	0.33	0.35	0.35	0.33	0.3	0.34
5	Current direction	S	SW	SW	S	SW	SW	S	SW	SW
6	Salinity (ppm)	36	35	34	35	34	35	36	35	35
7	pH	7	7.5	7.2	7.5	7.2	7.2	7.6	7.5	7.6
8	DO (mg L^{-1})	6.8	6.8	8.7	7.1	6.5	7.3	7.2	6.6	6.6
9	TSS (mg L^{-1})	0.2	0.4	0.3	0.8	1.1	0.87	0.6	0.9	0.8

S = South; SW = Southwest.

Sunlight in the waters is essential in the photosynthesis process of the phytoplankton. It can improve the content of DO (Tyas et al 2017). The sunlight does not arrive at the bottom of the waters in all stations. The penetrating power of sunlight into the waters is determined by the color of the waters, the content of organic and inorganic materials suspended in the waters, the density of plankton, and the microorganisms and detritus (Iswanto et al 2015).

Salinity measurement obtained was 34-36 ppm and it was optimal for plankton growth. According to Nontji (1993) in Widyarini et al (2017), a good range of salinity for plankton life was 11-40 ppm. Measurement of acidity degree (pH) obtained a range of 7-7.6. The pH value in this water was still within the optimal pH limit of aquatic, that the normal water pH value is 6-8. DO measurement obtained was 6.5-8.7 mg L^{-1}. DO concentrations at each station vary, this is due to (1) the number of phytoplankton supplying oxygen, (2) aquatic organisms that consume dissolved oxygen and (3) water temperature.

Measurements of TSS were obtained with a range from 0.2 to 1.1 mg L^{-1}. The values showed that it was below the default threshold at 80 mg L^{-1} for marine biota (KLH 2004). Suspended substances in the water can inhibit the penetration of light to the surface of the water, thereby lowering the light intensity used by phytoplankton (Anggraini et al 2016). Surface current velocity obtained results ranging from 0.2 to 0.35 m s^{-1}. The current speed at all of the observation stations was considered at a medium level. Current velocity affected the spread or distribution of the plankton, as plankton moves to hover over the current (Nontji 1993).

Discussion. The analysis showed that the abundance of phytoplankton at all stations was higher than zooplankton because the reproductive cycle of zooplankton is slower than phytoplankton (Rakhesh 2008 in Dewanti et al 2018). Increased abundance of phytoplankton is followed by an increase in the abundance of zooplankton. As well as if abundance of phytoplankton decreases, it will be followed by a decrease in the abundance of zooplankton (Dewanti et al 2018). The composition and number of phytoplankton populations always fluctuate due to differences in water quality (mainly nutrients), grazing by zooplankton and herbivorous fish, and the accumulation of toxic metabolic remnants (Makmur et al 2013). Phytoplankton abundance ranges from 1100,435 to 2107,826 cells m^{-3} while the abundance of zooplankton ranges from 92,150 to 118,095 cells m^{-3}, that means these waters have low fertility rates (oligotrophic). Plankton abundance of 0-2000 ind L^{-1} belongs to the category of oligotrophic waters, plankton abundance of 2000-15000 ind L^{-1} belongs to the mesotropic waters, and plankton abundance of more than 15000 ind L^{-1} is classified as eutrophic waters (Landner 1978 in Sidaningrat et al 2018).

The greater the value of H' indicates the more variety of life in these waters, this condition makes a water a better place to live in (Dewanti et al 2018). The high value of phytoplankton's diversity is due to the chemical and physical factors that support the growth of phytoplankton (Fauzia et al 2016).

The number of plankton is fluctuating and caused by differences in current speed and nutrients at each observation station. This is reinforced by the statement of Sulistiowati et al (2016), that the speed of the flow affects the distribution of nutrients and also plankton, where currents carry nutrients to various surrounding waters. Phytoplankton requires nitrate concentration in the range of 0.9-3.5 mg L^{-1} (Suryadi et al 2017). Phytoplankton, especially diatoms, can absorb nitrates as a minimum of 0.001-0.007 mg L^{-1} (Permatasari et al 2016). Phytoplankton distribution patterns show a relationship with the distribution of nitrates. Phytoplankton abundance is getting higher, in line with the increase on nitrate concentration (Permatasari et al 2016). Likewise, PCA results showed that the most important parameter affecting the abundance of phytoplankton and zooplankton is nitrate.

Based on the results of the linear regression test, the abundance of phytoplankton in the waters of the Bengawan Solo River estuary is influenced by nitrates rather than phosphate. Based on the study of Permatasari et al (2016) in the estuary of Demak River the abundance of phytoplankton in waters is influenced by nutrient nitrate compared to phosphate. Nitrate is the determining factor of the abundance of phytoplankton. It is also supported by Darmawan et al (2018) in the Banjir Kanal Semarang Barat that nitrate is often the main barrier factor in the primary productivity of phytoplankton in the estuary.

Conclusions. The abundance value of phytoplankton ranges from 1100 to 2108 cells m^{-3} while the abundance of zooplankton ranges from 92 to 118 cells m^{-3}. That means these waters have low fertility rates (oligotrophic). The values of the diversity index (H') of phytoplankton and zooplankton are included in the medium category with a value of $1 < H' < 3$. The relationship between plankton's abundance with nitrate indicates the value of the correlation coefficient (r) = 0.54 which means variables between each other have a fairly close correlation and the value of determination coefficient (R^2) = 0.2914 indicates that 29.1% of phytoplankton abundance is influenced by the value of nitrate. While the relationship between plankton's abundance and phosphate shows the value of the correlation coefficient (r) = 0.47 which means variables between each other have a fairly close correlation and the value of determination coefficient (R^2) = 0.2172 indicates that 21.7% of phytoplankton abundance is influenced by phosphate concentration value.

Conflict of interest. The authors declare that there is no conflict of interest.

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Received: 16 February 2021. Accepted: 23 August 2021. Published online: 13 January 2022.
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How to cite this article:
Wijaya N. I., Elfiansyah M., 2022 The influence of nitrate and phosphate concentration on the abundance of plankton at the estuary of Bengawan Solo, Gresik, East Java. AACL Bioflux 15(1):83-95.

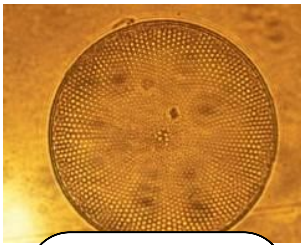
Appendix 1. Figure of the plankton

A. Class Bacillariaphyceae/Diatom

Actinoptychus

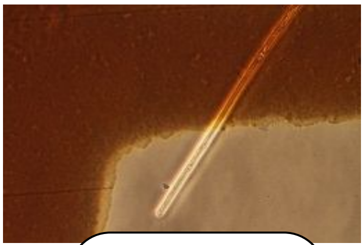
Bacillaria

Chaetoceros



Coscinodiscus

Dactyliosolen



Detonula

Ditylum

Fragilaria

Guinardia

Hemiaulus

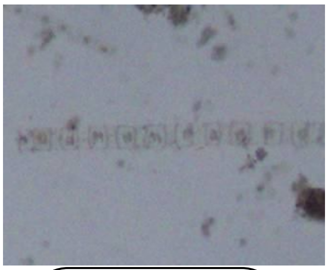
Lauderia

Leptocylindrus

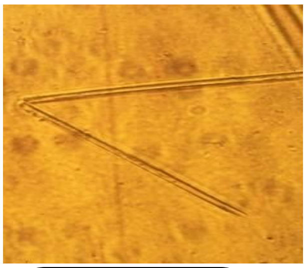
Pleurosigma

Pseudo-Nitzschia

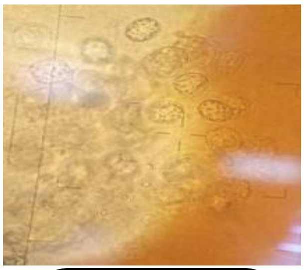
Rhizosolenia



Skeletonema



Thalassionema



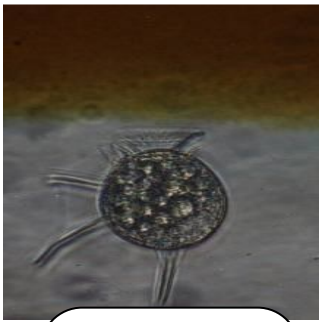
Thalassiosira

Thalassiothrix

Triceratium

B. Class Dinoflagellata

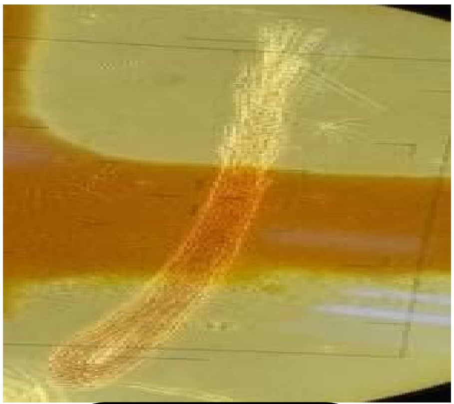
Ceratium



Dinophysis

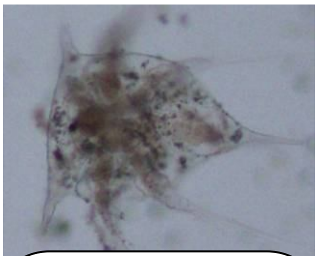
Pyrocystis

C. Class Cyanobacteria



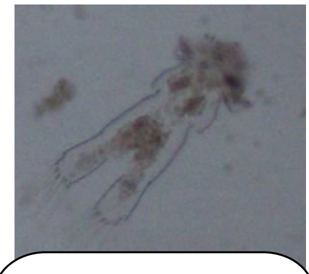
Trichodesmium

D. Class Hexanauplia



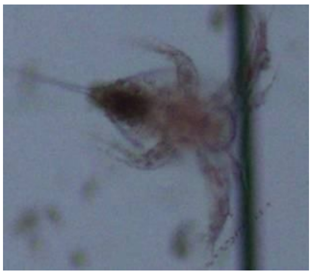
Balanus

Calanus



Calocalanus

Euchaeta



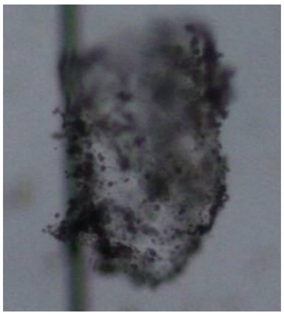
Eurytemora

Pseudodiaptomus

E. Class Branchipoda

Evadne

F. Class Oligotrichea



Leprotintinnus