The dinoflagellate causing ciguatera fish poisoning, *Prorocentrum lima*, in Karimunjawa island waters - Central Java

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**Abstract.** *Prorocentrum lima* is one of the microalgae species in Dinoflagellates group, which could cause Ciguatera Fish Poisoning (CFP) syndrome. *P. lima* commonly found it attached to various kind of macroalgae species and could cause human poisoning through biomagnification process in the food chain. Research on the abundance of *P. lima* and the environmental factors influences had been conducted in Karimunjawa Island, on 24 August 2018, and the objective of the research is to analyze the *P. lima* distributions in Karimunjawa waters, based on the cells abundance and environmental factors. The research was carried out by collecting macroalgae *Padina* and *Sargassum* at four stations, in the reef flat of Karimunjawa Island. Macroalgae were put inside plastic bottles and vigorously shaken by vortex (12500 rpm, 1 minute). Water samples separated from the macroalgae, filtered through a series of sieves, and then observed under a light microscope. The environmental factors are analyzed by Principal Component Analysis (PCA). Results showed a high abundance of *P. lima* found at Station 1 (Tanjung Gelam beach), which are 266 cells/ml on *Padina* and 210 cells/ml on *Sargassum*. Based on PCA analysis, Station 1 was characterized by water current, light intensity, phosphate, and depth. Those environmental factors should support the higher abundance of *P. lima* cells at the station.

**1. Introduction**

*Prorocentrum lima* is one of the benthic Dinoflagellate species, and commonly found attached on various kind of macroalgae. *P. lima* could produce ciguatoxin and causing Ciguatera Fish Poisoning (CFP), the human poisoning syndrome which occurred after the consumption of various tropical fishes associated with coral reefs. *P. lima* has been found in several Indonesian waters such as Belitung Island, Seribu Islands, Lampung Bay, Lombok Island, and Weh Island - Banda Aceh [1,2,3,4,5].

Karimunjawa National Park is geographically positioned at 5°40'39" - 5°55'00" S and 110°05'57" - 110°31'15" E, and located in Karimunjawa District, Jepara Resident, Central Java Province [6]. The majority of Karimunjawa residents are working as fisherman [6]. Biomass potency of reef fish in Karimunjawa National Park waters is higher at large and inhabitant islands, which reach up into 4224.06 kg/ha [7]. Coral reef in Karimunjawa waters is mostly at damage condition, with coverage percentage ranging between 25– 49.9 % which categorized as a medium [8]. The decreasing of coral reef condition in Karimunjawa waters should have an impact on the sustainability of fisheries in the area. Damage on coral reef ecosystem should promote the growth of toxic benthic Dinoflagellates, including *P. lima*, that would make fishes from the area become toxic.
The research was aimed to analyze the *P. lima* distributions in Karimunjawa waters, based on the cells abundance and environmental factors. The research is strongly necessary to further anticipates negative impacts affecting human health and economic activities in the area. Moreover, research on the distribution of *P. lima* is still rarely conducted in Indonesian waters. This makes the research on *P. lima* in Karimunjawa waters is also important to conducts, in order to obtained more database of this species distribution in Indonesian waters.

2. Methods
Samples collected in the reef flat area of Karimunjawa Island waters, on August 24th 2018 (figure 1). Sampling location was divided into four stations, which were Tanjung Gelam Beach (Station 1) and Sunset Beach (Station 2), Nirwana resort area (Station 3), and ship port area (Station 4).

![Figure 1](image1.png)

**Figure 1.** Sampling location in Karimunjawa Island, Central Java Province.

Macroalgae of *Padina* and *Sargassum* were collected at each location and put inside plastic bottles containing seawater. The bottles were shaken in order to release the benthic Dinoflagellate from macroalgae [9], using vortex machine (12500 rpm, 1 minute). The macroalgae as substrate were then separated from the water samples and the volume was measured. Samples were filtered through a series of sieves (125µm dan 20µm) and preserved with ethanol 96%. After that, 1 ml samples were dropped into Sedgewick rafter cells and observed under a microscope with 10x10 magnification. Dinoflagellate cells abundance was expressed as cells/ liter of macroalgae.

Environmental parameters were also measured during sample collection, which was salinity using a refractometer, temperature and dissolved oxygen using DO-meter, waters acidity degree using pH universal indicator, water current, and direction using floating drogue and compass, light intensity using lux-meter, and nutrients (nitrate and phosphate). The relation between sampling stations and environmental parameters were analyzed by Principal Component Analysis.

3. Results and Discussion
*Prorocentrum lima* (figure 2) cells were found at four research stations in sampling location, ranging from 73 – 266 cells/ml macroalgae, both on *Padina* and *Sargassum*. Results showed that *P. lima* cells were mostly found in higher number on *Padina* to *Sargassum*, especially at Station 1, 2 and 4 (figure 3). Research on benthic Dinoflagellates at Pramuka Island, Seribu Islands - North Jakarta, also showed that *P. lima* was most abundantly found on *Padina* [10]. Habitat or substrate preferences occurred in benthic Dinoflagellate community [11]. The morphology of the macroalgae as a host could increasing habitat complexity and might be related to the density of the community [12][13][14]. *Padina* is a group of brown algae or Phaeophyceae which obsess bushy foliose structure, where benthic
Dinoflagellates were commonly found associated with [13]. The complex structure of *Padina* provides *P. lima* cells a shelter, those needed from desiccation due to excessive light and also from water current [15][16].

![Figure 2](image2.jpg)

**Figure 2.** *Prorocentrum lima* cells (Photomicrograph by Widiarti 2018).

![Figure 3](image3.jpg)

**Figure 3.** The abundance of *P. lima* (cells/ml macroalgae) in four stations.

PCA graph on axis 1-2 and axis 1-3 showed that there were the grouping of stations and environmental factors variables (figure 4). There were groups in axis 1+, represented by stations 1 and environmental factors namely current velocity, light intensity, water depth, and phosphate; in axis 2+, represented by station 2 and environmental factors of nitrate and salinity; and axis 1-, represented by station 4 and environmental factors of temperature, pH, and dissolved oxygen. PCA graph on axis 1-3 were also showed similar grouping, which was axis 1+ represented by stations 1 and environmental factors namely current velocity, light intensity, water depth, and phosphate; in axis 2+, represented by station 2 and environmental factor of nitrate; and axis 1-, represented by station 4 and environmental factors of temperature, pH, dissolved oxygen, and salinity. Based on axis 1-2 and 1-3, it could be concluded that station 1 characterized by current velocity, light intensity, water depth, and phosphate;
station 2 characterized by nitrate; and station 4 characterized by temperature, pH, and dissolved oxygen.

![PCA graphs on environmental parameters at each station (axis F1 x F2 and axis F1 x F3).](image)

**Figure 4.** PCA graphs on environmental parameters at each station (axis F1 x F2 and axis F1 x F3).

Results also showed a high abundance of *Prorocentrum lima* found at Station 1 (Tanjung Gelam beach), which were 266 cells/ml on *Padina* and 210 cells/ml on *Sargassum* (figure 3). Based on PCA analysis, Station 1 was characterized by water current, light intensity, phosphate and depth (figure 3), with values higher than other stations (0.196 m/s, 444.5 lux, 0.003 ppm, and 0.5 m, respectively) (Table 1). *Prorocentrum* spp. were known as benthic species which prefer the higher speed of water current, in order to wash their attachment mucus [17,18]. According to [11] observed maximum densities of *P. lima* cells near channel sites. Additional water motion in the channel sites contributes to removing mucosa which *P. lima* produces so that the exposure to the nutrient supply would increase. *P. lima* obsessed mucocyst pores at the anterior end of the cell, which could produce an initial attachment to a substrate [11].

**Table 1.** Environmental parameter values at four stations.

| No. | Parameter         | Station 1 | Station 2 | Station 3 | Station 4 |
|-----|-------------------|-----------|-----------|-----------|-----------|
| 1   | Salinity (‰)      | 35.0      | 35.0      | 34.0      | 35.5      |
| 2   | Temperature (°C)  | 30.7      | 31.3      | 30.4      | 31.9      |
| 3   | DO (mg/l)         | 7.40      | 13.8      | 13.9      | 15.2      |
| 4   | Light Intensity (lux) | 44450 | 30700     | 17000     | 3455      |
| 5   | pH                | 7.05      | 7.25      | 7.25      | 7.40      |
| 6   | Water Current (m/s)| 0.196   | 0.099     | 0.051     | 0.031     |
| 7   | Nitrate (mg/L)    | 0.102     | 0.113     | 0.088     | 0.106     |
| 8   | Phosphate (mg/L)  | 0.003     | 0.002     | 0.003     | <0.002    |
| 9   | Depth (m)         | 0.5       | 0.5       | 0.4       | 0.1       |
The higher phosphate value at Tanjung Gelam beach showed that the area had higher input of nutrient from land, probably because of tourist activities. Besides providing eutrophication in one area, tourist activities were also lead to adverse changes in the reef environment mainly caused by reef disturbances including ship anchoring, dumping, and coral stepping [19,20]. These should increase the new surface for macroalgae to grow, on which benthic Dinoflagellate are more prefer to attach [19,21]. These showed the necessity of routine monitoring in the area, so that the negative impact of the potentially toxic benthic Dinoflagellates that already found by this research, could be minimized.

4. Conclusion
The high abundance of *Prorocentrum lima* in Karimunjawa Island waters was found at Tanjung Gelam beach, where the waters were characterized by high values of water current, light intensity, phosphate, and depth. The higher phosphate value at Tanjung Gelam beach showed that the area had higher input of nutrient from land, probably because of tourist activities. These showed the necessity of routine monitoring in the area.

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