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Composition Analysis in Type of Dinoflagellata as Source of Paralytic Shellfish Poisoning (PSP) Toxins in Pearl Oyster "Pinctada Maxima" in Lombok Waters –West Nusa Tenggara (NTB)

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Abstract : Dinoflagellata has important meaning for fisheries since it was a natural food for fishes with economic value. Objectives of this study were to discovered phytoplankton community structure in type of dinoflagellata which consist of abundance, composition and its diversity in Lombok waters, the cause of *Paralytic Shellfish Poisoning* (PSP) toxin and to analyze composition and identify gastric content of pearl oyster and to found out toxin level of *Paralytic Shellfish Poisoning* (PSP) in pearl oyster "Pinctada maxima" also to discover the custom and feeding habit of pearl oyster. Study was conducted in July 2016 in 2 locations which are Teluk Sekotong (West Lombok) and Teluk Kodek (North Lombok) whereas each location contain 3 sites point for sampling. Result of this study showed that phytoplankton abundance which was found in Teluk Sekotong (West Lombok) or Teluk Kodek (North Lombok) consist of 8 species of Dinoflagellata and 28 types of diatoms. Phytoplankton abundance in Teluk Sekotong was 12601 cell/L and for dinoflagellata was 2107 cell/L higher than phytoplankton abundance in Teluk Kodek with 9044 cell/L and dinoflagellata abundance about 1327 cell/L. PSP content test results toward flesh of pearl oyster were 14.145 µg (Teluk Sekotong) and 13.211 µg (Teluk Kodek), with toxin concentration of pearl oyster still lower than the predetermined tolerance level which is 80 µg STXeq per 100 gram, which was arrange in SNI 3460.1:2009 concerning flesh.
Keywords : Dinoflagellata, *Pinctada maxima*, Toxin, ELISA.

Introduction

Dinoflagellata has important meaning for fisheries since it was a natural food for fishes with economic value. However, Dinoflagellata could also cause negative effect such as HAB (*Harmful Algal Blooming*) phenomenon, which was the increase of toxic and non-toxic algae population occurs in sea or waters and could create disadvantages by contaminating marine biota with toxins[1,2].

The entrance of plankton with high toxic level into the body of pearl oyster would cause the death of oyster. According to Tomaru et. al.[3], infection from toxic substances produced by toxic micro algae would weakened and blocking nutrition absorption process.

Prosperous waters would support the diversity of available biota. Prosperous waters could be indicated by its available abundance of phytoplankton. From this problem, author would like to review by conducting composition analysis in type of dinoflagellate as source of *Paralytic Shellfish Poisoning* (PSP) toxins in pearl

oyster 'Pinctada maxima' in Lombok waters that was assumed as one of the factor which affects mass mortality of pearl oyster.

Other than causing death in pearl oysters, other fatal event could occur due to consuming shellfish that was suspected contain accumulated *Paralytic Shellfish Poisoning* (PSP) toxins, which was caused by type of dangerous dinoflagellata in the water, which accumulated in oysters's body through food chain. If those shellfish was consumed by human, it would cause several chronic symptom such as food poisoning and eventually death.

PSP illness was initially detected in 1700s in North America whereas there were 12 species of Dinoflagellata that was producing toxic which causing PSP and were part of genus Alexandrium, Pyrodinium, Gonyaulax and Gymnodinium. PSP illness was not only caused by shellfish consumption but it might also caused by consuming small crab, gastropods, mackerel and plankton-eater fish.

When *Pyrodinium* blooming occurs, this species would produce toxin known as *Paralytic Shellfish Poisoning* (PSP) with higher concentration. Through food chain process, this toxin would transfer and accumulated into zooplankton and shellfish that consuming it. Zooplankton would also eaten by fish thus it might cause fish mortality. Similar thing occurs when shellfish was eaten by other animals or human, so that animal and human would get poisoning and eventually causing death.

Methodology

This study was conducted in Lombok Island, West Nusa Tenggara Province in July 2016 at 2 stations of Lombok waters whereas both held oysters cultivation which are St. 1 Teluk Sekotong (West Lombok) located between 115°46' – 116°28'BT and 8°25' – 8°55'LS and St. 2 Teluk Kodek (North Lombok) located between 8°21'.42'' LS – 116°21'.54''BT. There were 3 sampling sites. Study sites could be seen in (Figure 1).

Figure 1. Study Sites Location

Several parameters measured in this study were physics parameters such as temperature, brightness and current. Chemical parameters such as salinity, dissolved oxygen level (DO), phosphate level, nitrate level, in the water. For biology parameters, it consist of abundance and type of plankton in the water, also gastric content of pearl oyster. *Paralytic Shellfish Poisoning* (PSP) toxins was also measured.

Plankton Sampling

Sampling for phytoplankton was done in 2 (two) stations, whereas each station contain 3 spots. Sampling was taken vertically from the depth of 10 m to sea surface by using Nasen Botol and phytoplankton net with net diameter 0.52m, length 1.0 m and width of net 0.08 mm or 80 µm.

Water's Physical– Chemical Parameters Measurement

Hydro oceanographic parameters measurement such as temperature, current, brightness, salinity, dissolved oxygen (DO) and nutrients (phosphate-nitrate) were done in surface area (1-10 m).

Analysis and Identification of Plankton Samples

Identification was using identification book of Fujioka (1990), Fukoyo and Borja (1991). Plankton identification was done by using binocular microscope, Olympus microscope with 400 times magnification. Analysis method conducted were:

Plankton Abundance (N)

Using sweeping method on object's glass *Sedgwick Rafter* with individual unit per litre (ind/l). Plankton abundance was calculated based on equation according to APHA[4] as follows:

$$N = Oi/Op \times Vr/Vo \times 1/Vs \times n/p$$

N = amount of individual per liter
Oi = size of preparate cover glass (mm^2)
Op = size of one vision field (mm^2)
Vr = filtered water volume (ml)
Vo = observed water volume (ml)
Vs = filtered water volume (L)
n = amount of plankton in all vision field
p = amount of observed vision field

Shannon-Wiener Index

Shannon-Wiener Index was used to calculate diversity index of types, calculated according to Odum[5]:

$$H' = - \sum_{i=1}^{s} (ni/N) \ln (ni/N)$$

H' = Shannon-Wiener diversity index
ni = amount of individual for I^{th} genus
N = amount of total individual for all general

Gastric Content Analysis of Pearl Oyster

Tools used in this study consist of net, 10 ml measuring glass and a set of surgical tools. For container to preserve gastric samples we use 50 ml sample bottles. For gastric content observation in pearl oyster, microscope was used completed with object glass and cover glass using 400 times magnification.

Toxin Level Analysis – ELISA Test

Enzyme-Linked Immune-Sorbent Assay or ELISA was known as enzymatic immune test. ELISA is relatively inexpensive and safer than RIA (Radio Immuno Assay) which used radioactive and ELISA could be done in small laboratory without gamma radioactive disperser tool. ELISA method (enzyme-linked

immunosorbent assay) was used in this study based on: specific bond between antigen (Ag) – antibody (Ab). To detect PSP we could use direct competitive ELISA method[6].

Results and Discussion

Hydrooceanographic measurement results either in-situ or laboratory analysis in both study sites consist of measurement several hydrooceanographic parameter such as temperature, brightness, current, salinity, pH, dissolved oxygen (DO), chlorophyl-α, and nutrients (phospate and nitrate) that was conducted in surface area < 1 meter and in 10 meter depth *in-situ*. This was done to found out the effect of hydrooceanographic parameters and nutrients distribution toward plankton abundance in an open water. Result could be seen in (Figure 2) below:

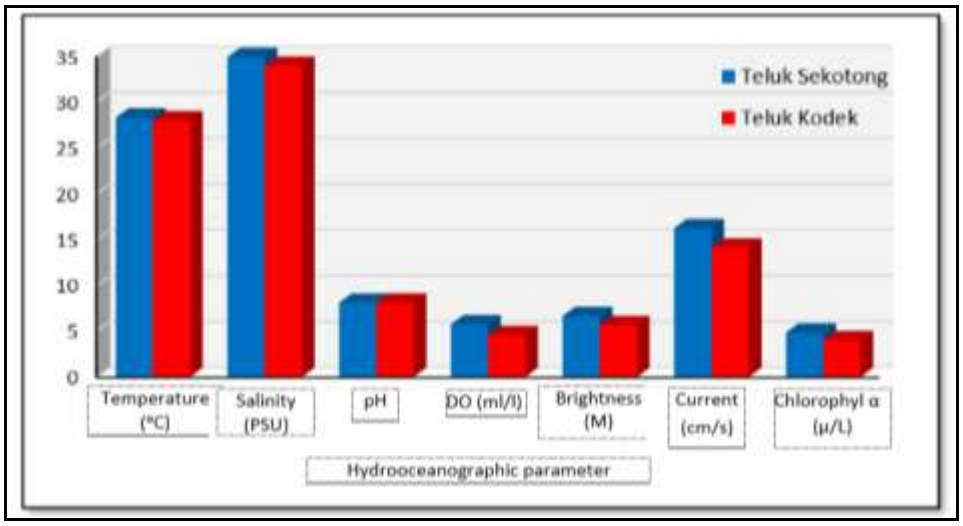

Figure 2. Hydrooceanographic parameter measurement in Teluk Sekotong and Teluk Kodek

Measurement result showed that hydrooceanographic parameters in Teluk Sekotong was higher than Teluk Kodek, consist of temperature, salinity, pH, dissolved oxygen (DO), brightness, current and chlorophyl-α. This was due to each location has different hydrooceanographic parameter characteristic and different nutrients content. Comparison in nutrient parameter between Teluk Sekotong (West Lombok) and Teluk Kodek (North Lombok) could be seen in (Figure 3) below:

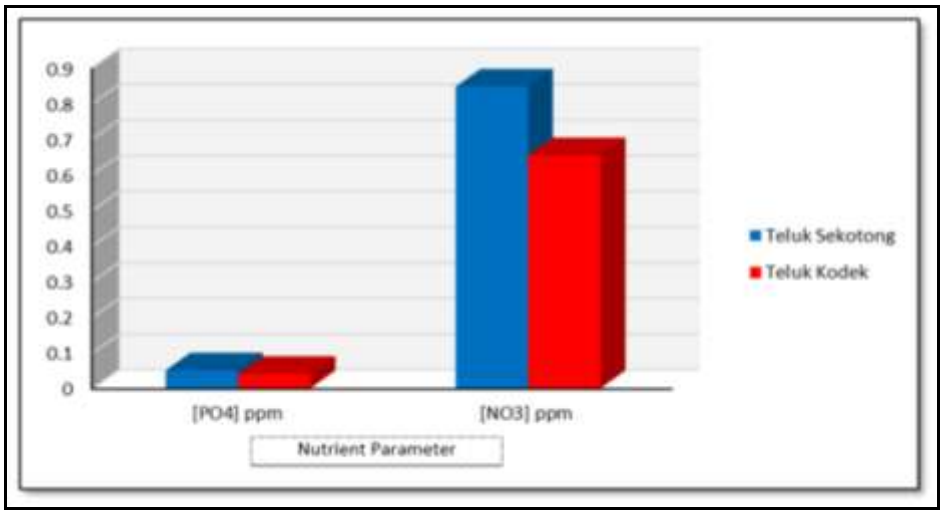

Figure 3. Nutrient parameter measurement between Teluk Sekotong and Teluk Kodek

Measurement result showed that nutrient parameter in Teluk Sekotong was higher than those of Teluk Kodek, consist of phosphate and nitrate. Higher nutrients level (phosphat, nitrate) in Teluk Sekotong (West Lombok) was followed by higher chlorophyl-α content in the area. This was due to different hydrooceanographic parameter characteristic and different nutrient content of each location. It was known that higher chlorophyl value in water would be closely related with prosperous level and it could be seen from high nutrient content in Teluk Sekotong.

From nutrient content analysis (phosphate, nitrate) it could be concluded that Teluk Sekotong water (West Lombok) and Teluk Kodek water (North Lombok) has medium prosperous level (mesotrophic) that could be seen from phosphate and nitrate content value in Teluk Sekotong with 0.050 ppm and 0.846 ppm while in Teluk Kodek it was 0.040 ppm and 0.653 ppm for phosphate and nitrate.

Abundance and Diversity of Phytoplankton in Water

Phytoplankton abundance in Teluk Sekotong water showed that phytoplankton abundance was 12601 cell/L and dinoflagellata was 2107 cell/L and higher than phytoplankton abundance in Teluk Kodek with 9044 cell/L and 1327 cell/L for phytoplankton and dinoflagellata respectively (Figure 4).

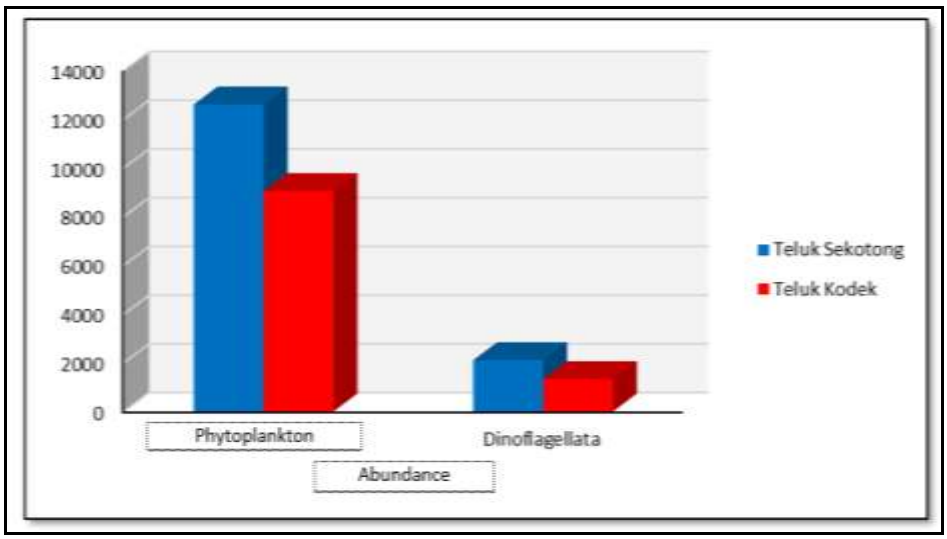

Figure 4. Comparison in Abundance and Diversity of Phytoplankton between Teluk Sekotong (West Lombok) and Teluk Kodek (North Lombok)

Gastric Content Analysis

Abundance of dinoflagellata in gastric of pearl oyster "Pinctada maxima" that was taken from study location (1) of Teluk Sekotong (West Lombok) with pearl oyster "Pinctada maxima" from location (2) of Teluk Kodek (North Lombok) was significantly different. Result of this study was based on option indices which shown that pearl oyster "Pinctada maxima" has eat all type of phytoplankton in its surroundings, or in other word, food of pearl oyster would depend on phytoplankton intensity found in its surrounding. In general, pearl oysters did not choose its type of food or non preferential. It means all type of phytoplankton available in the water was identical with those found in its gastric content.

Abundance of dinoflagellata in the water, dinoflagellata in gastric content of pearl oyster and saxytoxin level within pearl oyster has indicated linear pattern. It could be seen that higher dinoflagellata abundance was followed by high level of this particular group in pearl oyster also relatively high saxytoxin level in pearl oyster (Figure 5).

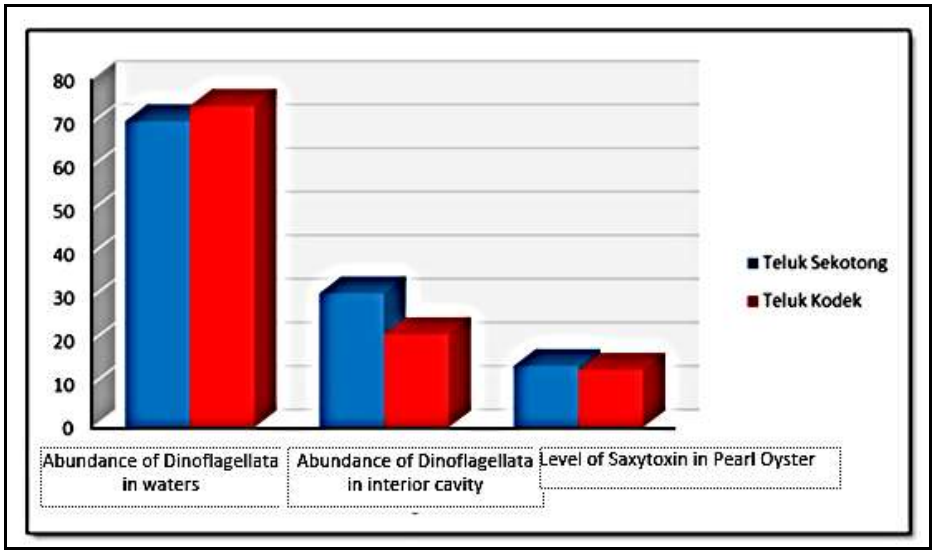

Figure 5. Comparison in dinoflagellata abundance in water, gastric content of pearl oyster and saxytoxin in pearl oyster between Teluk Sekotong and Teluk Kodek

Comparison of Saxytoxin Level

ELISA test result showed that saxytoxin level in the water has linear relationship with those in gastric content of pearl oyster. Figure 6 showed saxytoxin level contained in the water and in gastric content of pearl oysters from Teluk Sekotong with 9.022 µg and 14.145 µg were higher than of Teluk Kodek with 8.272 µg and 13.211 µg

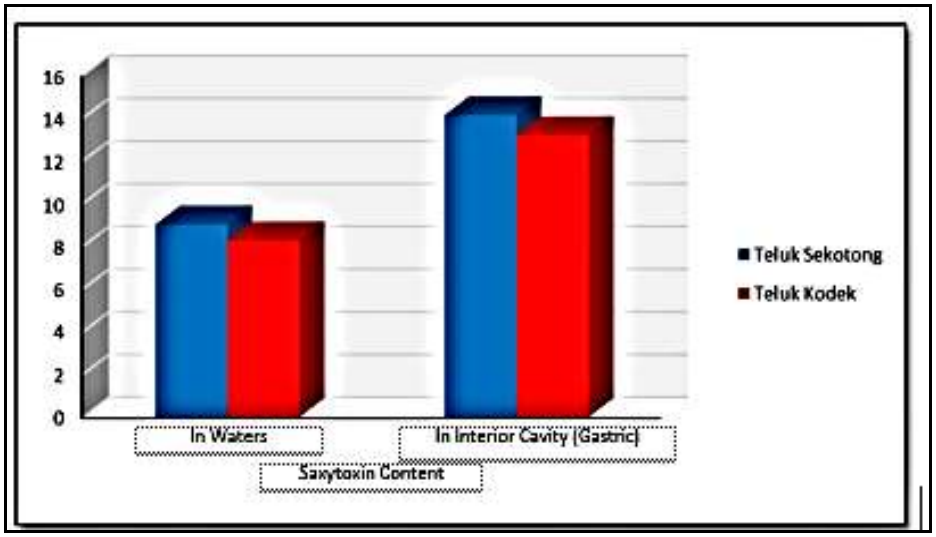

Figure 6. Comparison of Saxytoxin Level in Pearl Oyster of Teluk Sekotong and Teluk Kodek

Conclusion

1. Phytoplankton that was found either in Teluk Sekotong (West Lombok) or Teluk Kodek (North Lombok) consist of 8 species dinoflagellata and 28 types of diatoms. Photoplankton abundance in the water of Teluk Sekotong (West Lombok) was 12601 cell/L, higher than abundance in the water of Teluk Kodek (North Lombok) with 9044 cell/L.
2. In the water of Teluk Sekotong and in gastric content of pearl oyster (*Pinctada maxima*) we found 5 (five) types of dinoflagellata which are *Alexandrium* sp, *Dynophysis* sp, *Genyoulax* sp, *Protoperidinium* sp and *Peridinium* sp for 16.7%, and in Teluk Kodek (North Lombok) there was 3 (three) types of dinoflagellata

which are *Alexandrium* sp, *Dynophysis* sp and *Protoperidinium* sp with composition 12.8%, and test result regarding PSP level in flesh of pearl oyster was 14.145 µg (Teluk Sekotong) and 13.211 µg (Teluk Kodek).

3. Pearl oyster was a *filter feeder* type. It eat all type of phytoplankton in its surroundings. Pearl oyster did not being selective concerning type of food naturally available but it was selective regarding plankton's size and prefer smaller plankton. This was based on analysis result of option indices and identification of gastric content of pearl oysters.

Suggestions

Based on analysis result toward *Paralytic Shellfish Poisoning* (PSP) toxin level in both location of Teluk Sekotong and Teluk Kodek, PSP toxin level was still under the tolerance benchmark and phytoplankton abundance was still in normal condition. This could be used for scientific informational source and policy taking (by stakeholder) in management of coastal areas regarding efforts related with oyster pearl production.

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