Dinoflagellates Habs Potential Responsible For Paralytic Shellfish Poisoning (Psp) In Inner Ambon Bay - Maluku - Indonesia

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ABSTRACT: This research was conducted to clarify whether plankton as the primary food source and fish in Inner Ambon Bay (TAD) could accumulate saxitoxin in PSP (Paralylic Shellfish Poisoning) so the information acquired from this research could be used as a basic reference on oceanography studies especially in Ambon Bay, environmental toxicology, environmental bioremediation and a reference on Ambon Bay sustainable water management concept formulation. The research was conducted in East season (June-August 2015) and West season (December 2015 – February 2016). The method used was descriptive method, in situ sampling and enumeration. Hydro-oceanography parameters measured were temperature, salinity, DO (Dissolved Oxygen), pH, phosphate, nitrate and transparency. Dinoflagellate abundance in east season is higher than in west season. It is followed with abundance of dinoflagellates in anchovy fish (Stolephorus heterolobus) gizzard so saxitoxin level in the fish is also higher. Dinoflagellates species found Alexandrium, Protoperidinium, Genyoulax and Dynophysis. ELISA test result on saxitoxin level in anchovy fish was 12.415 µg (east season) and 5.13µg (west season), the concentration was still below saxitoxin toleration level, 80 µg.

Keyword: Dinoflagellate, HABS, ELISA, Stolephorus heterolobus, saxitoxin

INTRODUCTION

Algal bloom occurrence, or known as the red tide, has increased drastically recently in almost every part of the world, including in Indonesia. It caused destruction of marine environment and also threatened human safety through the food chain. From 5000 identified algae, 300 of them have great potential to grow exponentially and 40% of them has the ability to produce life threatening toxins through the fish, shells or other food source (Hallegraeff, 1993). Generally, toxin produced by algae divided into five groups based on the symptoms, Paralytic Shellfish Poisoning (PSP), Diarhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP), Ciguatera Shellfish Poisoning (CSP) and Neurotoxic Shellfish Poisoning (NSP) (FAO), 2004). PSP Toxin, or known as saxitoxin (STX) is produced by toxic algae such as Alexandrium tamarense, Pyrodinium bahamense var Compressum (PbC), Gymnodinium catenatum and other dinoflagellates (Dam etal., 2009). Toxic algae which produces saxitoxin in Asia Pacific dominated by Alexandrium spp, and in Australia dominated Gymnodium catenatum. Pyrodinium bahemense is dominant in South East Asia and South Pacific including the Philippines, Malaysia and Brunei. In Japan, Alexandrium catenella was firstly found in Owase Bay and spread from morth to south of Japan (Ashley et al., 2005).

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Saxitoxin mostly found in bivalves and gastropods (which prey on the bivalves) and through the food chain reaches human who consumes seafood contaminated with saxitoxin. Saxitoxin with its 20 derivates is the most active toxin in blocking neural tissues and membranes causing from thickening oral area to paralysis to heart muscles which causes death (EFSA, 2009). Saxitoxin connected to sodium channel in the nerve cells, then it blocks sodium ion channel and it is more deadly than sarin nerve gas so it is categorized as biological weapon (Cbwinfo, 2009). Saxitoxin is colorless liquid with very strong odour (like acid) with 1.0 g/ml density. Saxitoxin is toxic and causes irritation on skin, eyes, respiration and mouth. This compound has LD50 value at 263g/kg weight. Saxitoxin is soluble in water and methyl alcohol, less soluble in ethyl alcohol and acetate acid and is not soluble in organic solution (non polar). This compound is easily hydrolyzed in base solution and the toxin is not active after being boiled from 3-4 hours at pH3. Saxitoxin cannot be removed from seafood either with heating process of hydrolysis (Cbwinfo, 2009). As of mid-year of 1994, there were 3,164 cases of algal toxicity reported and caused 148 deaths in Asia Pacific(Corrales &Maclean, 2000). While Ashley (2005) reported that in 1989, in Dongshan China, shell consumption of Venerupisphilippinarum, caused one death and 136 people seriously ill. In 1991, two cases were reported from Pernaviridis consumption which was toxic from Daya Bay, Gandong Province. From 24 shell species found, Chlamys nobilis and P. viridis were the most toxic(Ashley et al., 2005). Migration and dispersion of toxic algae in the water is usually through ocean current and ship ballast water. Toxic algae dispersion in eastern Indonesia is believed to be caused by sea current, while in Inner Ambon Bay which has high traffic of shipping, is assumed to be from ballast water of the ships in the port. Some toxic algae found in Ambon Bay water Protoperidinium spp, Gymnodinium spp, and Alexandrium spp, even though with little amount (Sutomo, 1993). Fertile water area in Ambon Bay should be cautioned as it is highly possible for algal blooming especially toxic algae dinoflagellates. Research on saxitoxin content in fish is rarely conducted, even though the cases of fish toxicity happened a few times in

Indonesia. Saxitoxin concentration measurement is done by Elisa Reader (Lusiastuti, 2003). While Mulyasari (2003) stated that saxitoxin concentration of green mussel samples and blood clams from Tanjung Pasir Tangerang and Cilincing in 2001 ranged from 2.1-2.3 µg STXeq. per 100 g. Saxitoxin content measurement was performed using High Performance Liquid Chromatography Fluorescence Detection (HPLC-FD) and Mouse Bio Assay (MBA) (Mulyasari, et al., 2003). This research was aimed to study saxitoxin concentration in anchovy fish caught in Ambon Bay water during east and west season. Saxitoxin concentration measurement in the fish was a critical point in protecting community health from food chain vulnerability. This research results can be used as a basic information to study algal toxin especially saxitoxin which can be used in the risk management and communication.

METHOD

Location and Period of Research

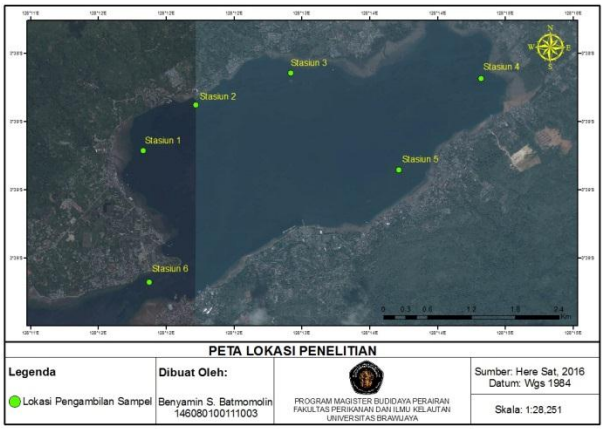

Figure 1. *Research Location Map*

Period of Research

Research was conducted as scheduled below:

Table 1. *Sampling schedule in research location*

No	Period	Location	Activity
1	August 2015 (East Season)	Ambon Bay	Sampling 1
2	January 2016 (West Season)	Ambon Bay	Sampling 2

Table 2. *Parameter and Research Instruments*

Parameter	Unit	Instrument/Material/Method	Information
Physical			
Temperature	°C	CTD	In situ
Chemical			
Salinity	ppt	CTD	In situ
DO	mg/l	CTD	In situ
Posphate	mg/l	Ammonium molybdate/Spectrophotometer λ = 690 nm	Laboratory
Nitrate	mg/l	Phenoldisulfonic acid/Spectrophotometer λ = 410 nm	Laboratory
Organic matter	mg/l	Titration permanganometry	Laboratory
Biological			
Plankton	cell/m^3	Plankton net, sample bottle, lugol 1 %, microscope, Sedgewick rafter	Laboratory
Anchovy gizzard content		Secchioset, microscope, Sedgwick rafter	Laboratory
PSP Toxin		Anchovy fish/ Elisa test	Laboratory

Phytoplankton Sample Collection

Phytoplankton sample was collected using plankton net, close net type with mesh size 30 µm. Phytoplankton sample was collected from 6 sampling locations vertically. The depth was the surface and 10 m. Sea water sample was used to identify phytoplankton, it was filled into plastic bottle (volume 100 ml) and preserved with lugol (1%).

Phytoplankton Enumeration

Phytoplankton identification was done using binocular microscope with 10 x 10 and 10 x 40 magnifications and assisted by hand counter with three times repetition for each sample bottle. Phytoplankton types identification was conducted using references Davis (1955), Yamaji (1979), and Tomas (1997). Phytoplankton abundance was measured by using census method with Sedgewick Rafter Cell (SRC) (APHA 1998), with this formula:

$$N = n \times \frac{V_t}{V_{cg}} \times \frac{1}{V_d}$$

Meaning: N = Phytoplankton abundance (cell/ml)
N = number of cells observed
V_t = Filtered water volume (ml)
V_{cg} = Sedgewick Rafter Cell volume (ml)
V_d = Water volume being filtered (l)

Fish gizzard content analysis

Instruments used for the research were fish net, measuring glass 10 ml and one set of surgical instrument. For gizzard sample preserving container, film bottle 20 ml was used. For observation of gizzard sample content, microscope was used complete with object glass and cover glass. Material used for the fish gizzard sample preservation was 70 % alcohol. Research was conducted using descriptive survey method. Sample collection was done in 5 (five) stations based on different condition around the bay. These stations were determined based on location of lemuru fish catching vertically or linear with the shoreline. From each station, 5 points were determined as the sample collection points. Ten fish were collected from each station. Then food analysis was conducted using count method and frequency of happenings (Effendi, 1992).

ELISA Analysis

Performed by setting standard calibration (0.12 – 30 ppb AFB1) in green mussels extract which does not contain AFB1 and methanol 60%, tested with direct competitive ELISA with antigen reactor. ELISA reader measurement was done at 450 nm wavelength. Then standard calibration for plot between % inhibition versus AFB1 concentration acquired, both being compared and evaluated.

RESULT AND DISCUSSION

The results on hydro oceanography parameter measurement either in situ or laboratory analysis in two seasons were presented in Figure 2. In east season the results of hydro oceanography parameter measurement showed that average temperature, salinity and transparency were lower than in west season, while pH and Do were higher.

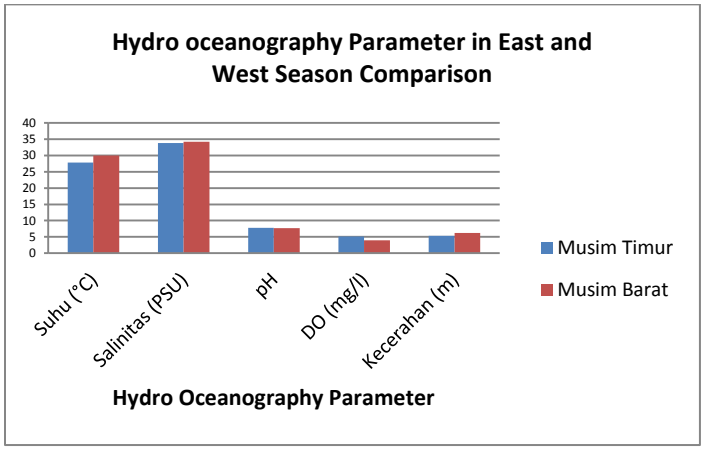

Figure 2. *Ambon Bay Hydro Oceanography Parameter Comparison in East and West Season*

Nutrient measurement (phosphate and nitrate) generally showed that it was higher in east season than in west season. But the nutrients were higher at 10 m depth than on the surface depth (<1m). It showed that each season has different hydro oceanography and nutrient distribution based on its depth. East season is identical with low temperature as in the season there is up welling due to monsoon wind activity (air pressure difference in eastern and western territory). This phenomenon enriches the nutrients in the area so it becomes fertile. It affects the abundance of phytoplankton even the toxic one is increasing in quantity (blooming).

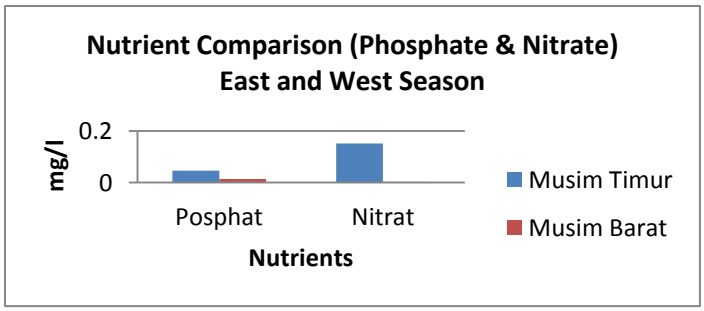

Figure 3. *Nutrient Comparison in East and West Season in Inner Ambon Bay*

Abundance and Diversity of Phytoplankton in the Water

Phytoplankton abundance in east season is different from the abundance in west season (Figure 4), so does dinoflagellates abundance in both seasons. In east season, phytoplankton abundance overall was higher than phytoplankton abundance in west season but it was different with dinoflagellates abundance, in east season this group was found more than in west season. So, it will affect the saxitoxin level in the water or accumulated in anchovy fish. It was presented in this diagram:

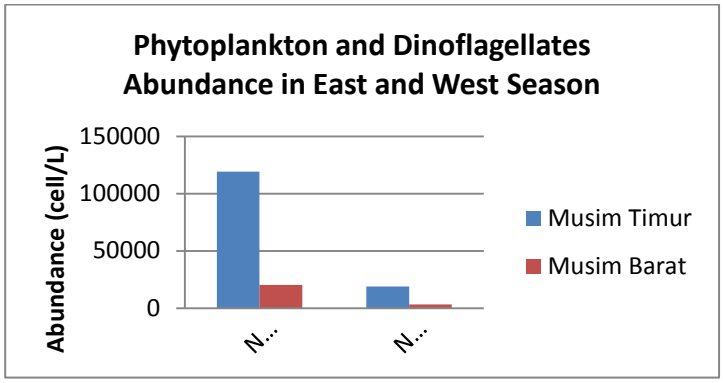

Figure 4. *Total Phytoplankton and Dinoflagellates Abundance Comparison in Inner Ambon Bay in East and West Season*

Gizzard content Analysis

Dinoflagellates abundance in anchovy fish gizzard caught in east season and fish caught in west season were different. According to Ivlev analysis (1961) generally, anchovy fish cannot choose their food or does not have preference. It means all kinds of phytoplankton in the water were the same with food found in the gizzard. Dinoflagellates abundance in the water, dinoflagellates in the fish gizzard and saxitoxin level in anchovy fish (Figure..) showed directly proportional pattern as dinoflagellates abundance in east season followed with excessive findings on the type, as well as the saxitoxin level in the fish which was high. Result of t-test showed $P<0.05$ which means that dinoflagellates abundance in the water affected significantly to dinoflagellates abundance in the anchocy' gizzard. The higher dinoflagellates abundance in the water, the higher dinoflagellates abundance in the fish gizzard, either in east season or west season. Regression analysis (appendix) showed R square 0.713 = 71%, meaning that X variable (dinoflagellates abundance in the gizzard) 88% affected by Y variable (dinoflagellates abundance in the water), and the other 29% was affected by other factors which were not measured.

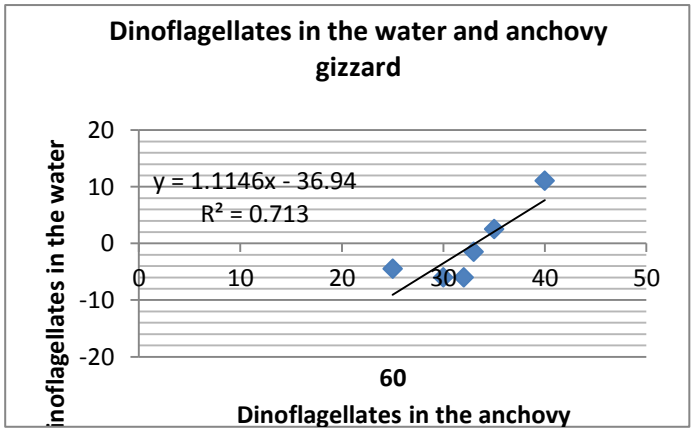

Figure 5. *Regression Analysis on Dinoflagellates abundance in the water (Y) with Dinoflagellates abundance in the anchovy fish gizzard*

Simple linear regression model formulated was Y = -1.114x–36.94 (Figure 5). All X (dinoflagellates abundance in the water) increase one unit, then Y (dinoflagellates in the gizzard) will increase 1.114.

Saxitoxin level comparison in both seasons

Results on ELISA test showed that saxitoxin level in the water was directly proportional with the fish. It means when saxitoxin level in the water was high, and then in the fish also showed linear result. It happened in east season showed in Figure 6 as well as in west season when ELISA test in the water showed low result, the level of saxitoxin in the fish also followed.

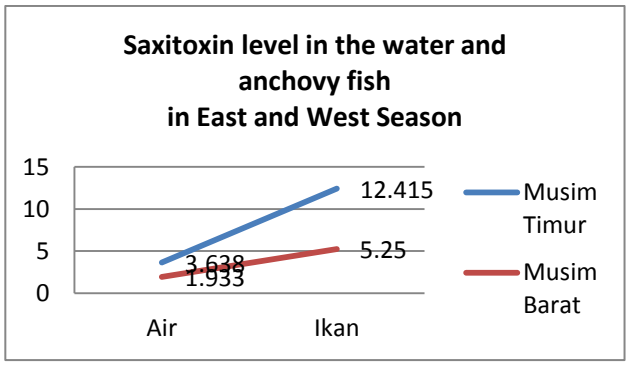

Figure 6. *Saxitoxin Level difference in the water and in anchovy fish in East Season and West Season*

T-test showed P<0.05, it means that saxitoxin level in the water affected significantly to saxitoxin level in the anchovy fish. The higher dinoflagellates abundance in the gizzard, the higher saxitoxin level in the fish, either in East season and West season.

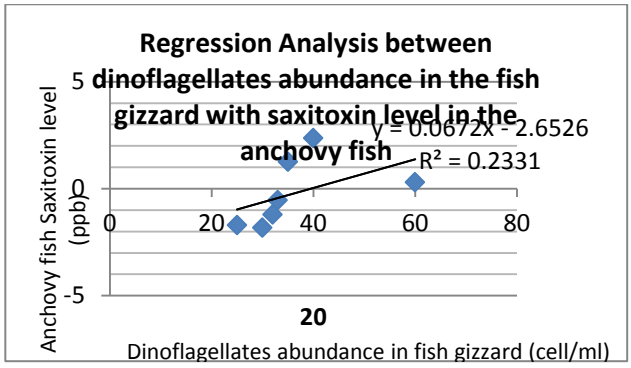

Figure 7. *Regression Analysis between Dinoflagellates abundance in fish gizzard (Y) with SAxitoxin level in the fish*

Regression analysis (appendix) showed R square (R^2) 0.964 = 96 %, it means that X (saxitoxin level in the anchovy fish) affected 97% by variable Y (dinoflagellates abundance in the fish gizzard). The rest 3 % was affected by other factors. Simple linear regression equation model formulated was Y = 0.067x – 2.652. Every X (saxitoxin in the fish) increase one unit, then Y (dinoflagellates abundance in fish gizzard) will increase 2.652. The equation was presented in Figure 7.

CONCLUSION

1. Four kinds of dinoflagellates were found in east season, Alexandrium sp, Genyoulax sp, Dynophysis sp and Peridinium sp while in west season, 3 (three) kinds of dinoflagellates responsible for PSP in Inner Ambon Bay were found Alexandrium sp, Dynophysis sp and Protoperidinium sp.
2. During east season, in anchovy fish gizzard (Stolephorus heterolobus), 4 (four) kinds of dinoflagellates were found, Alexandrium sp, Genyoulax sp, Dynophysis sp and Peridinium sp about 60% and in west season 2 (two) kinds of dinoflagellates were found responsible for PSP, Alexandrium sp and Dynophysis sp with 32% composition from total phytoplankton in the fish gizzard.
3. The results of PSP level in anchovy fish was 12.415 µg (east season) and 5.13µg (west season), saxitoxin concentration in the anchovy fish was still below tolerance limit which is at 80 µg.

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