

Saxitoxin Level Comparison in Bali Sardine (*Sardinella Lemuru*) in Bali Strait in Different Monsoons

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ABSTRACT

Sardinella fish (*Sardinella lemuru*) is a species of important pelagic fish from the family of Clupeidae found in Bali Strait. The dynamic condition of Bali Strait makes it fertile which is identical with plankton blooming. The objective of this research was to understand the potential of saxitoxin in sardinella fish because of dinoflagellates consumption in Bali Strait. The research was conducted in Southeast Monsoon (June - August 2015) and Northwest Monsoon (December 2015 - February 2016). The method used was *in situ* plankton sampling, counting and ELISA test to determine the level of saxitoxin in the fish. Hydro-oceanographic parameters measured were temperatures, salinity, DO (dissolved oxygen), pH level, phosphate level, nitrate level and transparency. They showed average optimum value for phytoplankton growth especially during Southeast Monsoon which its temperature lower and the nutrients (phosphate nitrate) higher than the Northwest Monsoon. This condition caused high richness of phytoplankton in the water and followed with dinoflagellates richness so they accumulated in sardinella fish. It was supported by the analysis of the fish gastric which showed positive correlation between dinoflagellates richness in the water and the fish gastric. ELISA test also showed that saxitoxin level of fish caught in Southeast Monsoon was higher than the one caught in Northwest Monsoon

even though the level of saxitoxin was still in the safe range (less than 80 µg STXeq. per 100 g) but the danger of saxitoxin accumulation should be watched out for.

Keyword: saxitoxin; *Sardinella lemuru*; Bali Strait; Monsoon; ELISA.

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), Diarrhetic 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 et al., 2009). Toxic algae which produces saxitoxin in Asia Pacific dominated by *Alexandrium* spp, and in Australia dominated *Gymnodium catenatum*. *Pyrodinium*

bahemense is dominant in East Asia and South Pacific including the Philippines, Malaysia and Brunei. In Japan, *Alexandrium catenella* was firstly found in Owase Bay and spread from north to south of Japan (Ashley et al., 2005). 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 derivatives 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 263 g/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 (nonpolar). This compound is easily hydrolyzed in base solution and the toxin is not active after being boiled for 3-4 hours at pH3. Saxitoxin cannot be removed from seafood either with heating process of hydrolysis (Cbwinfo, 2009).

As of midyear 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 *Venerupis philippinarum*, 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 South Eastern Indonesia is believed to be caused by sea current, while in Inner Bali Strait 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 Bali Strait Bay water *Protopeiridium spp*, *Gymnodinium spp*, and *Alexandrium spp*, even though with little amount (Sutomo, 1993). Fertile water area in Bali Strait 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 *Sardinella lemuru* fish caught in Bali Straits water during Southeast and Northwest 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.

METHODS

Location and Period of Research.

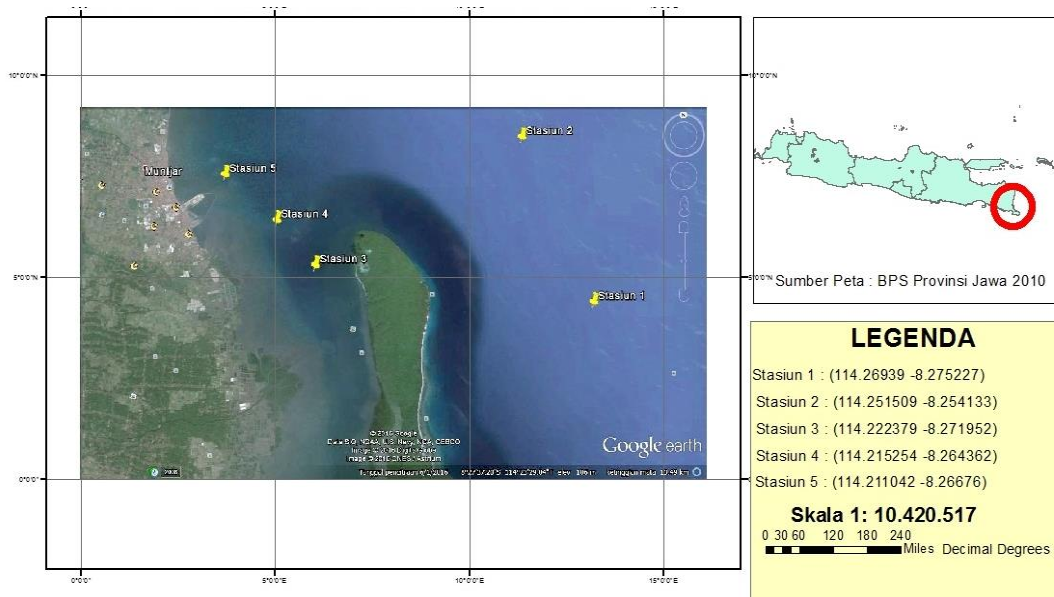


Figure 1. Research Location Map in Bali Province.

Period of Research.

Sampling, location, activities and periods of research were described as Table 1. Parameters and research instruments were explained in Table 2.

Table 1. Sampling schedule in research location

No	Period	Location	Activity
1	August 2015 (Southeast Season)	Bali Strait	Sampling 1
2	February 2016 (Northwest Season)	Bali Strait	Sampling 2

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) (Equation 1).

$$N = n \times \frac{Vt}{Vcg} \times \frac{1}{Vd} \dots\dots\dots 1)$$

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).

Table 2. Parameter and Research Instruments.

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

Fish Gastric Content Analysis.

Instruments used for the research were fish net, measuring glass 10 ml and one set of surgical instruments. For gastric preserving container, film bottle 20 ml was used. For observation of gastric sample content, microscope was used complete with object glass and cover glass. Material used for the fish gastric 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 strait. 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 sardine fish 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 wave length. Then standard calibration for plot between % inhibition versus AFB1 concentration acquired, both being compared and evaluated.

RESULTS AND DISCUSSION

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

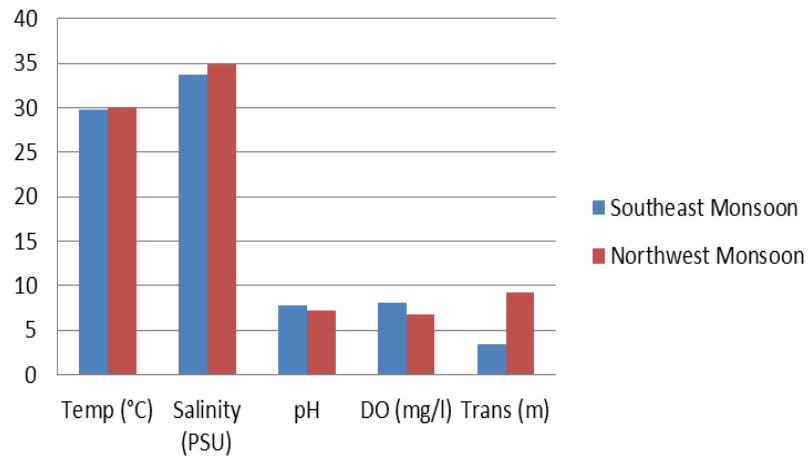


Figure 2. Bali Strait Hydro Oceanography Parameter Comparison in Southeast and Northwest Monsoon

Nutrient measurement (phosphate and nitrate) generally showed that it was higher in Southeast season than in Northwest season (Figure 3). 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.

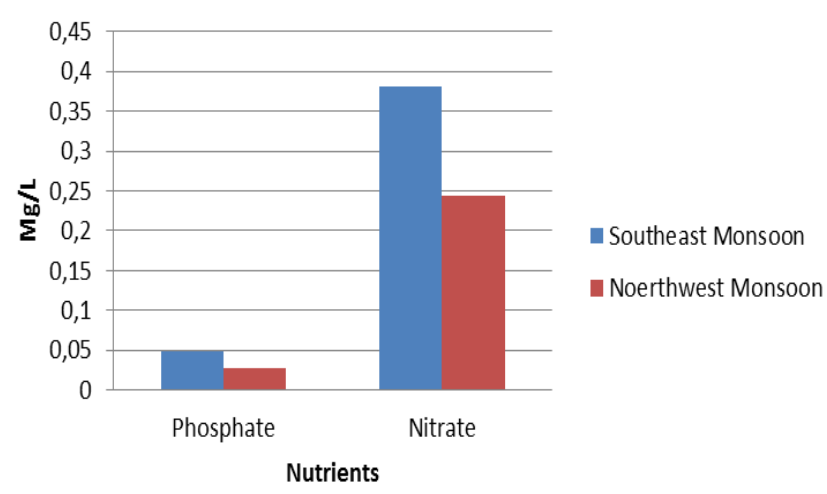


Figure 3. Nutrient Comparison in Southeast and Northwest Monsoon in Bali Strait

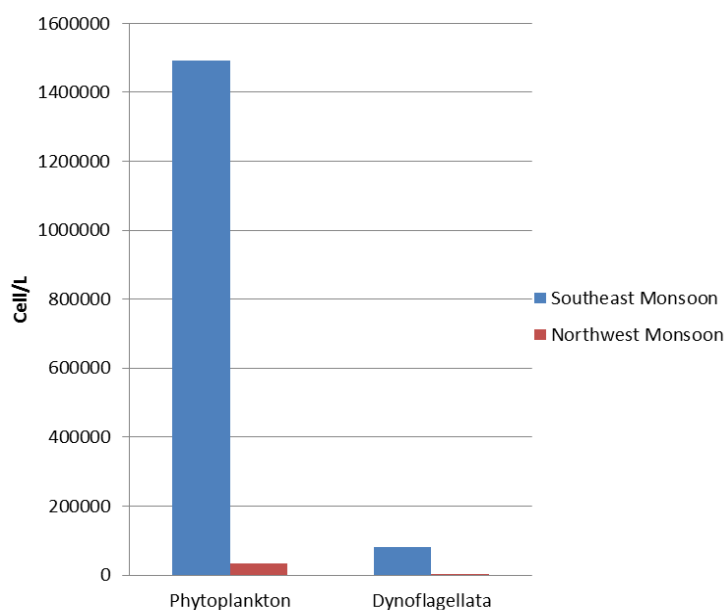


Figure 4. Total Phytoplankton and Dinoflagellates Abundance Comparison in Bali Strait in Southeast and Northwest Monsoon

Southeast season is identical with low temperature as in the season there is upwelling due to monsoon wind activity (air pressure difference in Southeastern and Northwestern 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).

Abundance and Diversity of Phytoplankton in the Water.

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

Gastric Content Analysis.

Dinoflagellates abundance in Sardine fish gastric caught in Southeast season and fish caught in Northwest season were different. According to Ivlev's (1961) analysis generally, sardine 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 gastric.

Saxitoxin level comparison in both seasons.

Results on ELISA test showed that saxitoxin level in the fish was directly proportional with the Dinoflagellates abundance in fish gastric. It means when quantity of dinoflagellates in the fish gastric was high, and then saxitoxin level in the fish also showed linear result. It happened in Southeast season showed in Figure 5 as well as in Northwest season when dinoflagellates abundance showed low in fish gastric, ELISA test in the fish body showed the level of saxitoxin also followed.

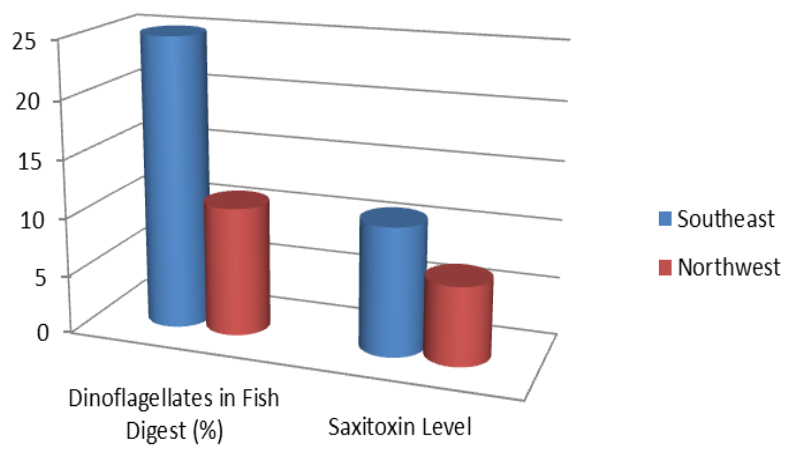


Figure 5. Dinoflagellata Abundance difference in the Fish Digest and Saxitoxin Level in Bali Sardine fish in Southeast Season and Northwest Season.

T-test showed $P < 0.05$, it means that saxitoxin level in the water affected significantly to saxitoxin level in the sardine fish. The higher dinoflagellates abundance in the gastric, the higher saxitoxin level in the fish, either in Southeast season and Northwest season.

The equation of Regression Analysis was presented in Figure 6.

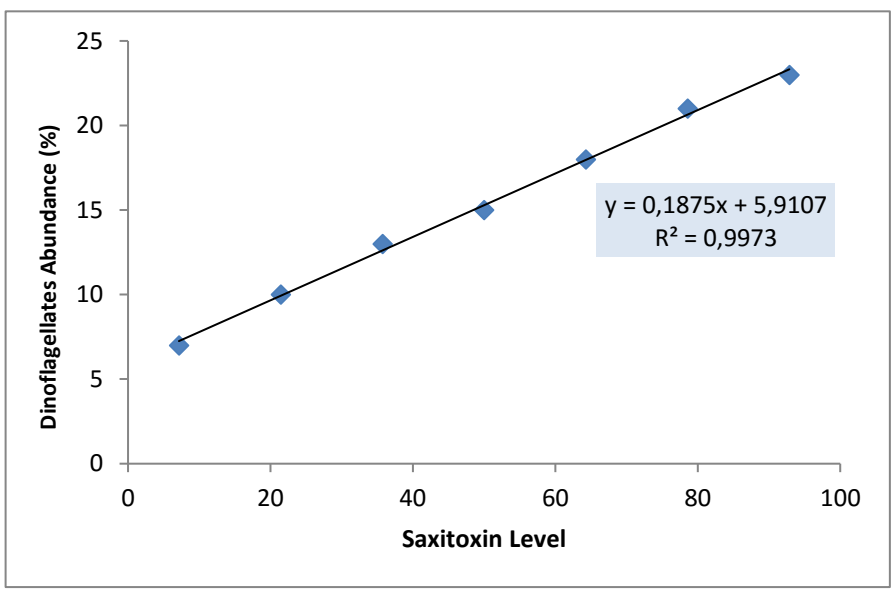


Figure 6. Regression Analysis between Dinoflagellates Abundance in Fish Gastric (Y) with Saxitoxin Level in the Fish.

Regression analysis (appendix) showed R square (R^2) $0.964 = 96\%$, it means that X (saxitoxin level in the sardine fish) affected 99% by variable Y (dinoflagellates abundance in the fish gastric). The rest 1% was affected by other factors.

Simple linear regression equation model formulated was $Y = 0.1875x + 5.9107$. Every X (saxitoxin in the fish) increase one unit, then Y (dinoflagellates abundance in fish gastric) will increase 5.9107.

CONCLUSIONS

The results of saxitoxin level in sardine fish was 10.94 µg (Southeast season) and 6.69 µg (Northwest season), saxitoxin concentration in the sardine fish was still below tolerance limit which is at 80 µg.

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Herawati E. Y. et al.: Saxitoxin Level Comparation In Bali Sardine.....

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