INDONESIA

Mrs Murtiningsih

Head of Laboratory and Inspection Fish Quarantine and Inspection Agency Ministry of Marine Affairs and Fisheries

۲

I. Introduction

()

Monitoring of shellfish and its environment (water quality) is aimed to protect the consumers from chemical and microbiological hazards that may arise from consuming shellfish products. Sufficient data on the safety status of shellfish is also aimed to increase the utilization of its resources for domestic and export markets.

The government of Indonesia has established several regulations, namely Regulation No. PER.01/MEN/2007 and Regulation No. KEP.01/ MEN/2007 which lay down the requirements for Quality and Safety Assurance of Fishery products in general and DG Decree No. 010/DJ-P2HP/2007 with regards to the official control and monitoring of fishery product. These regulations could help to maintain and protect the area of shellfish growth from the domestic and industrial sewage which may contaminate shellfish. The regulation divides the shellfish harvesting area into 4 classes (A-D) based on the several parameters (Table 11). Some observations were done prior to the sampling time, to have an overview of shellfish' and the water quality status. The presence evidence of contamination should be followed by sampling of water and shellfish and laboratory analysis of the present contaminant. The area's status depends on the laboratory results. For example, samples that are taken from an approved area showed results that are not in accordance with the regulatory limits, a reevaluation action should be done and by that time they will be classified as prohibited area.

In Indonesia, the biotoxin monitoring activity which is also known as shellfish sanitation program has been conducted since 1997 as part of a general monitoring program designed to identify and evaluate biological toxins as well as chemical and microbiological contamination of shellfish and the water quality. This program has been scheduled as in Table 12, however the frequency of monitoring might be reduced or terminated when the results achieve satisfactory levels.

Survey of Biotoxins in ASEAN Region

Class	Criteria
A (Approved)	 Not contaminated by domestic waste PSP <80µg/100g DSP negative Most Probable Number (MPN) of faecal coliform <14/100 ml water sample and less than 10% of samples containing faecal coliform and not exceeding 43/100 ml water sample. 5-tube MPN of E.coli < 230/100 g of meat, heavy metals not exceeding the requirements
B (Conditionally Approved)	 Not contaminated by domestic waste PSP <80µg/100g DSP negative MPN of faecal coliform <14/100 ml water sample and less than 10% of samples containing faecal coliform not exceed 43/100 ml water sample. 5-tube MPN of E.coli > 4,600/100 g of meat, heavy metals not exceeding the requirements
C (Restricted)	 Low content of domestic waste and pollutant PSP <80µg/100g DSP negative MPN of faecal coliform 88/100 ml water sample air and less than 10% of samples containing faecal coliform not exceed 260/100 ml water sample. 5-tube MPN of E.coli > 4,600/100 g of meat, heavy metals not exceeding the requirements
D (Prohibited)	 High content of domestic waste and pollutant PSP ≥ 80µg/100g ASP ≥ 2 mg/100g heavy metals do not meet the requirements

No.	Parameter	Regulatory limit	Frequency				
1	Biotoxin :						
	a) PSP	80µg/100g					
	b) DSP	negative by Mouse Bioassay (MBA)	Once every 2 weeks during harvesting period, at sampling poin				
	c) ASP	2 mg/100g					
2	Heavy metals :						
	a) Mercury (Hg)	0.5 mg/kg					
	b) Lead (Pb)	1.0 mg/kg	Once every 3 months during harvesting period, at sampling period,				
	c) Cadmium (Cd)	1.0 mg/kg					

Survey of Biotoxins in ASEAN Region

No.	Parameter	Regulatory limit	Frequency
3	Microbiology:		
	a) E. coli	< 3 MPN	Once every 2 weeks during harvesting period, at sampling point
	b) Salmonella	(-) /25g	

Table 12: Frequency and parameters of water for quality inspection

From the period of 1997-2012, there are several reported cases due to the consumption of contaminated shellfish. On June-September 2010, more than 30 people were hospitalized after consuming clams and fish from Teluk Lasongko and Bau-Bau. They showed similar symptoms, i.e. vomiting, diarrhea, tongue and lips paralysis. The presence of Peridinium sp. was confirmed from the fish caught in those area, however no reported data on the clinical sample of the patient. On 16 July 2010, another case has occurred in Lampung due to contaminated shellfish and fish consumption and cause nausea and vomiting. Phytoplankton of Pirodinium bahamense was identified as a cause of the shellfish poisoning.

Indonesia has two main PSP monitoring programme:

1. PSP Monitoring Conducted by National Center for Fish Quality Control (NCQC)

PSP monitoring has been conducted by NCQC since 1997 and sampling is conducted in the shellfish production area. The samples were analyzed using validated Mouse Bioassay (MBA) method (Limit of Detection, LOD: 40µg STX/100g meat), however, due to limited budget, the sampling frequency could not match up with the procedures performed. In 2011, NCQC conducted PSP monitoring in Sidoarjo, East Java other than the PSP monitoring funded by Japanese Trust Fund II.

2. PSP Monitoring under Japanese Trust

Fund II (JTF II) in Tanjung Balai, Asahan. The selection of this location as the project site because Tanjung Balai Asahan is the main shellfish production both for local consumption and export purpose.

II. Objectives and Goals

The objectives of shellfish sanitation program are to ensure the quality and safety of shellfish harvested in the production area, as well as to provide data and information required for setting policies or regulations in terms of implementation of quality assurance and safety of fishery products.

III. Survey Methodologies

a. Sampling Method, Sampling Site, Target Species, Number of Samples & Sampling Size

Sampling for wild shellfish and water was conducted on sampling site of shellfish monitoring area of ± 7500 ha, where the coordinates of the sampling sites are:

- A1 = 03°06'15" N/ 99°55'00" E
- A2 = 03°04'45" N/ 99°57'30" E
- B1 = 03°04'30" N/ 99°55'00" E
- B2 = 03°04'00" N/ 99°56'15" E
- C1 = 03°03'00" N/ 99°55'00" E
- C2 = 03°02'20" N/ 99°56'00" E

()

21

Survey of Biotoxins in ASEAN Region

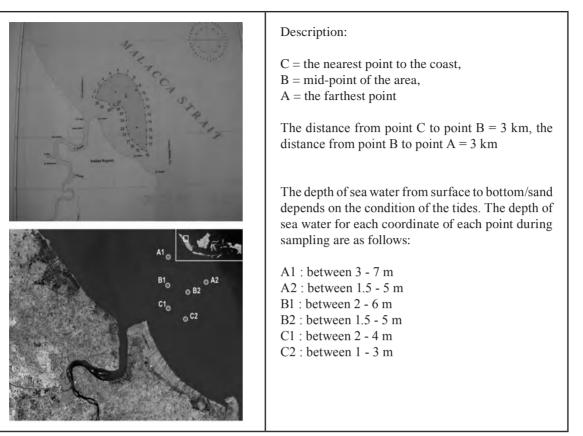


Figure II. Map of shellfish monitoring area and coordinates of sampling site

The targeted species for JTF II project is Baby Clam (*Meritrix meritrix*). However, as the size of Baby Clam does not meet the harvesting size for consumption during January and February, the shellfish cannot be harvested for analysis purpose. Therefore, Anadara inflata is used for samples during January and February considering that *Meritrix meritrix* grows in the same habitat as *Anadara inflate*, so the toxin content is assumed to be equal. The samples were tested for the following:

- Shellfish sampling for PSP analysis is conducted every two weeks in 6 coordinates of sampling site with 6 samples per sampling time
- Shellfish is also analysed for heavy metals (Pb, Cd, Hg) every 2 months
- Sampling of water is conducted every 2 months for additional data such as field condition (weather, depth, temperature, pH, Salinity and DO), plankton identification and heavy metals (Pb, Cd and Hg)

b. Method of Analysis

For time of sampling, it was carried out every two weeks from January to December 2011, as follow:

Time of Sampling	Dominant Sample (s)	Remarks
January:		
• Week 2 (W2): 11 January 2011	kerang bulu (Anadara inflata).	-
• Week 4 (W4): 25 January 2011		

()

Survey of Biotoxins in ASEAN Region

Time of Sampling	Dominant Sample (s)	Remarks		
 February: Week 2 (W2): 8 February 2011 Week 4 (W4): 22 February 2011 	kerang bulu (Anadara inflata).	In Week 4, water was carried out for fitoplankton and heavy metals (Pb, Cd and Hg) identification. In addition, shellfish in Week 4 are also tested for heavy metals.		
March: • Week 2 (W2): 9 March 2011 • Week 4 (W4): 23 March 2011	baby clam (<i>Meritrix meritrix</i>)	-		
April: • Week 2 (W2): 11 April 2011 • Week 4 (W4): 29 April 2011	baby clam (<i>Meritrix meritrix</i>)	In Week 4, water was carried out for fitoplankton and heavy metals (Pb, Cd and Hg) identification. In addition, shellfish in Week 4 are also tested for heavy metals.		
May: • Week 2 (W2): 10 May 2011 • Week 4 (W4): 23 May 2011	baby clam (<i>Meritrix meritrix</i>)	-		
June: • Week 2 (W2): 10 June 2011 • Week 4 (W4): 22 June 2011	baby clam (<i>Meritrix meritrix</i>)	In Week 4, water was carried out for fitoplankton and heavy metals (Pb, Cd and Hg) identification. In addition, shellfish in Week 4 are also tested for heavy metals.		
July: • Week 2 (W2): 12 July 2011 • Week 4 (W4): 25 July 2011	baby clam (<i>Meritrix meritrix</i>).	-		
August: • Week 2 (W2): 9 August 2011 • Week 4 (W4): 22 August 2011	baby clam (<i>Meritrix meritrix</i>)	In Week 4, water was analyzed for field condition, plankton and heavy metals (Pb, Cd and Hg) identification. In addition, shellfish in Week 4 are also tested for heavy metals.		
 September: Week 2 (W2): 12 September 2011 Week 4 (W4): 26 September 2011 	baby clam (<i>Meritrix meritrix</i>).	-		

Survey of Biotoxins in ASEAN Region

Time of Sampling	Dominant Sample (s)	Remarks		
October: • Week 2 (W2): 10 October 2011 • Week 4 (W4): 24 October 2011	baby clam (<i>Meritrix meritrix</i>)	In Week 4, water was analyzed for field condition, plankton and heavy metals (Pb, Cd and Hg) identification. In addition, shellfish in Week 4 are also tested for heavy metals.		
November: • Week 2 (W2): 8 November 2011 • Week 4 (W4): 21November 2011	baby clam (<i>Meritrix meritrix</i>).	-		
December: • Week 1 (W1): 5 December 2011 • Week 3 (W3): 19 December 2011	baby clam (<i>Meritrix meritrix</i>)	In Week 3, water was analyzed for field condition, plankton and heavy metals (Pb, Cd and Hg) identification. In addition, shellfish in Week 3 are also tested for heavy metals.		

Table I3: Time of sampling and dominant sample

The shellfish sampling was conducted through the following steps:

- 1. Sampling was carried out with the scallop shells dredge gear (scallop rakes) which consists of three types namely: *garuk*, *tojok* and *tanktailand*. In this monitoring, *garuk* is most often used.
- 2. After arriving at the area, garuk is lowered to scrape into the sand layer. The shaft is held firmly and then the boat turned around until the *garuk* contains shellfish.
- 3. *Garuk* is then removed from the water and the shellfish are washed to clean off sand and any other foreign matter by shaking the gear several times on the surface of sea water.
- 4. Shellfish are then shed on the deck of the boat waiting to be sorted. The sorted shellfish were washed again with clean sea water and put into a sack. Samples were collected at per sampling point and selected with individual weight of \pm 50g/

shellfish (minimum weight accepted by company/exporter to get \pm 1kg shellfish that yield > 100g of meat.

5. Samples were prepared according to AOAC, 2000. Shells were clean with fresh water and then opened. The inside was cleaned to remove foreign matter. Meat is separated from the shell and it was done carefully to prevent damage or truncation. Meat is then drained and frozen at -20°C until analysis.

Meat of shellfish for saxitoxin (STX) for Paralytic Shellfish Poisoning (PSP) analysis is extracted as follows:

- 1. 0.5g sample was weighed in centrifuge tube, 1ml of 0.1M HCl was added and vortex for 3 minutes at maximum speed
- 2. Heated in waterbath at 85°C for 5 minutes
- 3. Vortex for 1 minute, centrifuge for 10 minutes at 4000rpm

()

- 4. Transferred 0.5ml of clear supernatant to a new tube
- 5. Diluted 20 times with sample extraction buffer prior to testing
- 6. Analyzed using ELISA method (the results are obtained in units of ng/g and converted to μ g/100 g of meat)

For water sampling:

- Sampling site for water is the same as the shellfish sampling site coordinates.
- Water sampling for phytoplankton identification uses plankton net.

• Water sampling for heavy metals done by using Niskin bottle.

c. Limit of Detection & Limit of Quantification

PSP testing uses validated ELISA method:

Limit of Detection (LoD):0.153µg/100g (1.53ng/g)

Limit of Quantification (LoQ):0.236µg/100g (2.36ng/g)

CCβ:< 40µg/100g (400ng/g)

Concentration (ng/g)	Repeatability (%RSD)	Recovery (%)
400	8	121
800	7	115
1200	3	98

۲

Table I4: Repeatability and recovery(%) for various concentration

d. National Regulatory Limits

()

PSP: maximum 80µg STX/100g (800ng/g) of meat

IV. Results and Discussions

a. Participation in Inter-Laboratory Proficiency Testing & Results

In 2011, NCQC, in coordination with National Accreditation Body of Indonesia participated

in PSP proficiency testing provided by Asia Pacific Laboratory Accreditation Cooperation (APLAC). To date, no result is available yet.

b. Survey Results & Discussion

PSP monitoring conducted by NCQC shows that high level of PSP is always detected in Lampung and Ambon and low level in Bangka. Other sites show no detected PSP. Data of PSP conducted by NCQC from 1997 – 2011 is provided in Table 15.

No	Location	Time	Testing method	Samples		(µg/100g of meat)		
				Number	Posi- tive	Mini- mum	Maxi- mum	Aver- age
1	Ambon	May-1997	MBA	8	4	40	128	96.7
2	Banda Aceh	September-1997	MBA	8	-	ND	ND	-
3	Teluk Hanura & Gebang, Lampung	September-1997	MBA	8	3	47	76	61.3

Survey of Biotoxins in ASEAN Region

				Samp	oles	(µg/100g of meat)		
No	Location	Time	Testing method	Number	Posi- tive	Mini- mum	Maxi- mum	Aver- age
4	Bali	October-1997	MBA	8	-	ND	ND	-
5	Jakarta	December-1997	MBA	8	-	ND	ND	-
6	Tj. Balai, North Sumatera	April-1999	MBA	8	-	ND	ND	-
7	Kep. Riau	March-1999	MBA	8	-	ND	ND	-
8	Kep. Babel	March-1999	MBA	8	-	ND	ND	-
9	Teluk Hanura & Gebang, Lampung	April-1999	MBA	8	2	52	60	56.0
10	Jakarta	March-1999	MBA	8	-	ND	ND	-
11	Cirebon	July-1999	MBA	8	-	ND	ND	-
12	Ambon	August-1999	MBA	8	2	130	135	132.5
13	Tj. Balai, North Sumatera	May-2000	MBA	8	-	ND	ND	-
14	Bandar Lampung	June-2000	MBA	8	2	49	61	55.0
15	Tj. Balai, North Sumatera	April-2001	MBA	12	-	ND	ND	-
16	Musi Banyu Asin, South Sumatera	March-2001	MBA	12	-	ND	ND	-
17	Teluk Hanura & Gebang, Lampung	May-2001	MBA	12	-	ND	ND	-
18	Perairan Blurukidul, East Java	September-2001	MBA	12	-	ND	ND	-
19	Pantai Makassar, South Sulawesi	September-2001	MBA	12	-	ND	ND	-
20	Tj. Balai & Belawan, North Sumatera	April-2002	MBA	12	-	ND	ND	-
21	Teluk Hanura & Pangkep, Lampung	May-2002	MBA	12	-	ND	ND	-
22	Kenjeran & Uj.Pangkah, East Java	July-2002	MBA	12	-	ND	ND	-
23	P.Satando & Batang, South Sulawesi	August-2002	MBA	12	-	ND	ND	-
24	Tj. Balai, North Sumatera	June-2003	MBA	12	-	ND	ND	-
25	Tj. Balai, North Sumatera	August-2003	MBA	12	-	ND	ND	-

Survey of Biotoxins in ASEAN Region

			Tagting	Samples		(µg/100g of meat)		
No	Location	Time	Testing method	Number	Posi- tive	Mini- mum	Maxi- mum	Aver- age
26	Perairan Sidoarjo, West Java	July-2003	MBA	12	-	ND	ND	-
27	Perairan Sidoarjo, West Java	September-2003	MBA	12	-	ND	ND	-
28	Tj. Balai, North Sumatera	June-2004	MBA	6	-	ND	ND	-
29	Tj. Balai, North Sumatera	August-2004	MBA	6	-	ND	ND	-
30	Teluk Hanura, Lampung	April-2004	MBA	6	-	ND	ND	-
31	Teluk Hanura, Lampung	August-2004	MBA	6	-	ND	ND	-
32	Bangka Belitung	September-2004	MBA	6	-	ND	ND	-
33	Bangka Belitung	December-2004	MBA	6	-	ND	ND	-
34	Sedati & Uj.Pangkah, East Java	July-2004	MBA	6	-	ND	ND	-
35	Sedati & Uj.Pangkah, East Java	October-2004	MBA	6	-	ND	ND	-
36	Tj. Balai, North Sumatera	August-2005	MBA	6	-	ND	ND	-
37	Teluk Hanura & Tj. Putus, Lampung	May-2005	MBA	10	6	64.2	948.5	747.9
38	Perairan Bangka Belitung	July-2005	MBA	6	-	ND	ND	-
39	Sidoarjo & Uj.Pangkah, East Java	September-2005	MBA	12	-	ND	ND	-
40	Musi Banyu Asin, South Sumatera	October-2005	MBA	6	-	ND	ND	-
41	Per. Mangkang, Central Java	October-2005	MBA	6	-	ND	ND	-
42	Tj. Balai, North Sumatera	May-2006	MBA	9	-	ND	ND	-
43	Tj. Balai, North Sumatera	October-2006	MBA	9	-	ND	ND	-
44	Teluk Hanura, Lampung	September-2006	MBA	9	-	ND	ND	-
45	Teluk Hanura, Lampung	December-2006	MBA	9	-	ND	ND	-
46	Per. Sidoarjo & Pasuruan, East Java	September-2006	MBA	9	-	ND	ND	-

Survey of Biotoxins in ASEAN Region

				Samp	oles	(µg/100g of meat)		
No	Location	Time	Testing method	Number	Posi- tive	Mini- mum	Maxi- mum	Aver- age
47	Per. Sidoarjo & Pasuruan, East Java	December-2006	MBA	9	-	ND	ND	-
48	Per. Baguala, Ambon	May-2006	MBA	9	9	674.9	864.5	778.8
49	Per. Baguala, Ambon	August-2006	MBA	9	9	49.9	76.5	61.1
50	Per. Berau, East Kalimantan	April-2006	MBA	9	-	ND	ND	-
51	Per. Berau, East Kalimantan	September-2006	MBA	9	-	ND	ND	-
52	Per. Kawal, Kep. Riau	May-2006	MBA	9	-	ND	ND	-
53	Per. Kawal, Kep. Riau	October-2006	MBA	9	-	ND	ND	-
54	Tj. Balai, North Sumatera	June-2007	MBA	9	-	ND	ND	-
55	Tj. Balai, North Sumatera	December-2007	MBA	9	-	ND	ND	-
56	Per. Blurukidul, Sidoarjo, East Java	April-2007	MBA	9	-	ND	ND	-
57	Per. Blurukidul, Sidoarjo, East Java	August-2007	MBA	9	-	ND	ND	-
58	Per. Baguala, Ambon	August-2007	MBA	9	7	55.84	70.52	60.8
59	Tj. Balai, North Sumatera	May-2008	MBA	9	-	ND	ND	-
60	Tj. Balai, North Sumatera	September-2008	MBA	9	-	ND	ND	-
61	Per. Desa Itam, Bangka Belitung	May-2008	MBA	9	1	46	-	46
62	Per. Desa Itam, Bangka Belitung	November-2008	MBA	9	-	ND	ND	-
63	Teluk Hanura, Lampung	March-2008	MBA	9	-	ND	ND	-
64	Teluk Hanura, Lampung	November-2008	MBA	9	-	ND	ND	-
65	P. Panggang, Kep. Seribu	March-2008	MBA	9	-	ND	ND	-
66	P. Panggang, Kep. Seribu	September-2008	MBA	9	-	ND	ND	-
67	Per. Blurukidul, Sidoarjo, East Java	April-2008	MBA	9	-	ND	ND	-

Survey of Biotoxins in ASEAN Region

				Samp	oles	(µg/100g of meat)		
No	Location	Time	Testing method	Number	Posi- tive	Mini- mum	Maxi- mum	Aver- age
68	Per. Blurukidul, Sidoarjo, East Java	September-2008	MBA	9	-	ND	ND	-
69	Per. Baguala, Ambon	May-2008	MBA	9	-	ND	ND	-
70	Per. Baguala, Ambon	October-2008	MBA	9	-	ND	ND	-
71	Tj. Balai, North Sumatera	May-2009	MBA	9	-	ND	ND	-
72	Tj. Balai, North Sumatera	August-2009	MBA	9	-	ND	ND	-
73	Teluk Hanura, Lampung	April-2009	MBA	9	-	ND	ND	-
74	Teluk Hanura, Lampung	October-2009	MBA	9	-	ND	ND	-
75	P. Panggang, Kep. Seribu	April-2009	MBA	9	-	ND	ND	-
76	P. Panggang, Kep. Seribu	September-2009	MBA	9	-	ND	ND	-
77	Per. Blurukidul, Sidoarjo, East Java	March-2009	MBA	9	-	ND	ND	-
78	Per. Blurukidul, Sidoarjo, East Java	August-2009	MBA	9	-	ND	ND	-
79	Per. Gerupuk, West Nusa Tenggara	May-2009	MBA	9	-	ND	ND	-
80	Per. Gerupuk, West Nusa Tenggara	October-2009	MBA	9	-	ND	ND	-
81	Per. Baguala, Ambon	May-2009	MBA	9	-	ND	ND	-
82	Per. Baguala, Ambon August-2009		MBA	9	-	ND	ND	-
83	Per. Blurukidul, Sidoarjo, East Java March-2011		ELISA	9	-	ND	ND	-
84	Per. Blurukidul, June-2011 E Sidoarjo, East Java		ELISA	9	1	0.247	ND	0.247
85	Per. Blurukidul, Sidoarjo, East Java	September-2011	ELISA	9	3	0.278	0.303	0.29

ND: Not Detected

۲

Table 15. Data of PSP conducted by NCQC from 1997 – 2011

Survey of Biotoxins in ASEAN Region

۲

Meanwhile, for PSP Monitoring under JTF II, PSP was detected in samples of *Anadara inflata* with results between $0.567 - 1.770 \mu g/100g$ of meat and between $0.635 - 1.334 \mu g/100g$ of meat in January and February respectively. Only one out of whole samples of *Meritrix meritrix* gave detected results in March 2011 with $0.237\mu g/100g$ of meat. The detected results are above Limit of Quantitation (LOQ). Detailed data of PSP funded by JTF II from January – December 2011 is provided in Table I6.

No	Testing Mehod	Period	Sample ID. (species, month, week, coordinat)	Concentration (µg/100g of meat)
1	ELISA	January	Anadara inflata Jan W2 – A1	1.770
			Anadara inflata Jan W2 – A2	1.318
			Anadara inflata Jan W2 – B1	1.136
			Anadara inflata Jan W2 – B2	1.140
			Anadara inflata Jan W2 – C1	1.087
			Anadara inflata Jan W2 – C2	1.152
			Anadara inflata Jan W4 – A1	1.239
			Anadara inflata Jan W4 – A2	1.208
			Anadara inflata Jan W4 – B1	0.977
			Anadara inflata Jan W4 – B2	0.885
			Anadara inflata Jan W4 – C1	0.700
			Anadara inflata Jan W4 – C2	0.567
2	ELISA	February	Anadara inflata Feb W2 – A1	1.314
			Anadara inflata Feb W2 – A2	1.256
			Anadara inflata Feb W2 – B1	0.804
			Anadara inflata Feb W2 – B2	1.165
			Anadara inflata Feb W2 – C1	0.635
			Anadara inflata Feb W2 – C2	0.897
			Anadara inflata Feb W4 – A1	1.247
			Anadara inflata Feb W4 – A2	1.334
			Anadara inflata Feb W4 – B1	1.109
			Anadara inflata Feb W4 – B2	1.030

۲

Survey of Biotoxins in ASEAN Region

۲

۲

No	Testing Mehod	Period	Sample ID. (species, month, week, coordinat)	Concentration (µg/100g of meat)
			Anadara inflata Feb W4 – C1	0.987
			Anadara inflata Feb W4 – C2	0.968
3	ELISA	March	Meritrix meritrix Mar W2 – A1	ND
			<i>Meritrix meritrix</i> Mar W2 – A2	ND
			Meritrix meritrix Mar W2 – B1	ND
			Meritrix meritrix Mar W2 – B2	ND
			Meritrix meritrix Mar W2 - C1	ND
			Meritrix meritrix Mar W2 – C2	ND
			Meritrix meritrix Mar W4 – A1	ND
			Meritrix meritrix Mar W4 – A2	ND
			Meritrix meritrix Mar W4 – B1	0.237
			<i>Meritrix meritrix</i> Mar W4 – B2	ND
			Meritrix meritrix Mar W4 – C1	ND
			Meritrix meritrix Mar W4 – C2	ND
4	ELISA	April	Meritrix meritrix Apr W2 – A1	ND
			Meritrix meritrix Apr W2 – A2	ND
			Meritrix meritrix Apr W2 – B1	ND
			Meritrix meritrix Apr W2 – B2	ND
			Meritrix meritrix Apr W2 - C1	ND
			<i>Meritrix meritrix</i> Apr W2 – C2	ND
			Meritrix meritrix Apr W4 – A1	ND
			Meritrix meritrix Apr W4 – A2	ND
			Meritrix meritrix Apr W4 – B1	ND
			Meritrix meritrix Apr W4 – B2	ND
			Meritrix meritrix Apr W4 – C1	ND
			Meritrix meritrix Apr W4 – C2	ND

Survey of Biotoxins in ASEAN Region

No	Testing Mehod	Period	Sample ID. (species, month, week, coordinat)	Concentration (µg/100g of meat)
5	ELISA	May	Meritrix meritrix May W2 – A1	ND
			Meritrix meritrix May W2 – A2	ND
			Meritrix meritrix May W2 – B1	ND
			Meritrix meritrix May W2 – B2	ND
			Meritrix meritrix May W2 - C1	ND
			Meritrix meritrix May W2 – C2	ND
			Meritrix meritrix May W4 – A1	ND
			Meritrix meritrix May W4 – A2	ND
			Meritrix meritrix May W4 – B1	ND
			Meritrix meritrix May W4 – B2	ND
			Meritrix meritrix May W4 – C1	ND
			Meritrix meritrix May W4 – C2	ND
6	ELISA	June	Meritrix meritrix Jun W2 – A1	ND
			Meritrix meritrix Jun W2 – A2	ND
			Meritrix meritrix Jun W2 – B1	ND
			Meritrix meritrix Jun W2 – B2	ND
			Meritrix meritrix Jun W2 - C1	ND
			Meritrix meritrix Jun W2 – C2	ND
			Meritrix meritrix Jun W4 – A1	ND
			Meritrix meritrix Jun W4 – A2	ND
			Meritrix meritrix Jun W4 – B1	ND
			Meritrix meritrix Jun W4 – B2	ND
			Meritrix meritrix Jun W4 – C1	ND
7	ELISA	July	Meritrix meritrix Jul W2 – A1	ND
			Meritrix meritrix Jul W2 – A2	ND

Survey of Biotoxins in ASEAN Region

No	Testing Mehod	Period	Sample ID. (species, month, week, coordinat)	Concentration (µg/100g of meat)
			<i>Meritrix meritrix</i> Jul W2 – B1	ND
			<i>Meritrix meritrix</i> Jul W2 – B2	ND
			Meritrix meritrix Jul W2 - C1	ND
			Meritrix meritrix Jul W2 – C2	ND
			Meritrix meritrix Jul W4 – A1	ND
			Meritrix meritrix Jul W4 – A2	ND
			Meritrix meritrix Jul W4 – B1	ND
			Meritrix meritrix Jul W4 – B2	ND
			Meritrix meritrix Jul W4 – C1	ND
			Meritrix meritrix Jul W4 – C2	ND
8	ELISA	August	Meritrix meritrix Aug W2 – A1	ND
			Meritrix meritrix Aug W2 – A2	ND
			Meritrix meritrix Aug W2 – B1	ND
			Meritrix meritrix Aug W2 – B2	ND
			Meritrix meritrix Aug W2 - C1	ND
			Meritrix meritrix Aug W2 – C2	ND
			Meritrix meritrix Aug W4 – A1	ND
			Meritrix meritrix Aug W4 – A2	ND
			Meritrix meritrix Aug W4 – B1	ND
			Meritrix meritrix Aug W4 – B2	ND
			Meritrix meritrix Aug W4 – C1	ND
			Meritrix meritrix Aug W4 – C2	ND
9	ELISA	September	Meritrix meritrix Sep W2 – A1	ND
			Meritrix meritrix Sep W2 – A2	ND
			Meritrix meritrix Sep W2 – B1	ND
			Meritrix meritrix Sep W2 – B2	ND

Survey of Biotoxins in ASEAN Region

No	Testing Mehod	Period	Sample ID. (species, month, week, coordinat)	Concentration (µg/100g of meat)
			Meritrix meritrix Sep W2 - C1	ND
			Meritrix meritrix Sep W2 – C2	ND
			Meritrix meritrix Sep W4 – A1	ND
			Meritrix meritrix Sep W4 – A2	ND
			<i>Meritrix meritrix</i> Sep W4 – B1	ND
			Meritrix meritrix Sep W4 – B2	ND
			<i>Meritrix meritrix</i> Sep W4 – C1	ND
			Meritrix meritrix Sep W4 – C2	ND
10	ELISA	October	<i>Meritrix meritrix</i> Oct W2 – A1	ND
			Meritrix meritrix Oct W2 – A2	ND
			<i>Meritrix meritrix</i> Oct W2 – B1	ND
			Meritrix meritrix Oct W2 – B2	ND
			Meritrix meritrix Oct W2 - C1	ND
			Meritrix meritrix Oct W2 – C2	ND
			Meritrix meritrix Oct W4 – A1	ND
			Meritrix meritrix Oct W4 – A2	ND
			<i>Meritrix meritrix</i> Oct W4 – B1	ND
			<i>Meritrix meritrix</i> Oct W4 – B2	ND
			<i>Meritrix meritrix</i> Oct W4 – C1	ND
			<i>Meritrix meritrix</i> Oct W4 – C2	ND
11	ELISA	November	Meritrix meritrix Nov W2 – A1	ND
			Meritrix meritrix Nov W2 – A2	ND
			Meritrix meritrix Nov W2 – B1	ND
			Meritrix meritrix Nov W2 – B2	ND
			Meritrix meritrix Nov W2 - C1	ND
			Meritrix meritrix Nov W2 – C2	ND

Survey of Biotoxins in ASEAN Region

No	Testing Mehod	Period	Sample ID. (species, month, week, coordinat)	Concentration (µg/100g of meat)
			Meritrix meritrix Nov W4 – A1	ND
			Meritrix meritrix Nov W4 – A2	ND
			Meritrix meritrix Nov W4 – B1	ND
			Meritrix meritrix Nov W4 – B2	ND
			Meritrix meritrix Nov W4 – C1	ND
			Meritrix meritrix Nov W4 – C2	ND
12	ELISA	December	Meritrix meritrix Dec W1 – A1	ND
			Meritrix meritrix Dec W1 – A2	ND
			Meritrix meritrix Dec W1 – B1	ND
			Meritrix meritrix Dec W1 – B2	ND
			Meritrix meritrix Dec W1 - C1	ND
			Meritrix meritrix Dec W1 – C2	ND
			Meritrix meritrix Dec W3 – A1	ND
			Meritrix meritrix Dec W3 – A2	ND
			Meritrix meritrix Dec W3 – B1	ND
			Meritrix meritrix Dec W3 – B2	ND
			Meritrix meritrix Dec W3 – C1	ND
			Meritrix meritrix Dec W3 – C2	ND

ND: not detected

()

Table I6. Data of PSP from Tanjung Balai, Medan funded by Japanese Trust Fund II from January – December 2011

PSP monitoring conducted by NCQC during 1997 – 2011 shows that the PSP content of shellfish obtained from Teluk Hanura and Gebang, Lampung and Baguala, Ambon exceeds the requirements several times. Teluk Baguala, Ambon has been closed at 1997, 1999 and 2005 and the shellfish harvesting area has been changed as prohibited. Any shellfish harvesting activity has been prohibited in the areas of Teluk Hanura and Gebang since 2005 and they have been labelled as restricted area in year 1997 and 1999. Both areas experience frequent red tide and the waters are closed to shellfish harvesting area. The government has also banned people for eating shellfish from waters of Teluk Hanura and Gebang, Lampung and Baguala, Ambon.

Sample for PSP analysis sponsored by the JTF II is *Anadara inflate* during January and February 2012 and *Meritrix meritrix* during March until December 2012.

No PSP toxin exceed the requirements found in the 144 shellfish samples obtained from Tanjungbalai Asahan waters in North Sumatra which serve as the sampling location in 2011 in biotoxin monitoring activities sponsored by the JTF II. Even if any PSP toxins were detected by ELISA, it was still far below the requirements in the range of $0.237 - 1.77\mu g/100g$ of meat. There were PSP toxin found in small concentrations in January and February on *Anadara inflata* and one sample of *Meritrix meritrix* in March. *Anadara inflata* has a larger size than *Meritrix meritrix*, so the presence of PSP toxin could be more accumulated in this species.

Sampling history in Tanjungbalai Asahan waters since 1998 showed that there were no samples that exceed the requirements.

c. Corrective Actions

Ð

Some samples give positive results using the ELISA method particularly samples of Anadara inflata. To illustrate, PSP results between 0.567 - 1.770 μ g/100g of meat in January and between 0.635 - 1.334 μ g/100g of meat in February. Only one out of whole sample of Meritrix meritrix give detected results in March 2012 with 0.237 μ g/100g of meat. The detected results are above LOQ, however these results are not confirmed as the results are far below national regulatory standards (maximuml 80 μ g STX/100g (800ng/g) of meat.

V. Problems and Challenges Encountered

No significant problems were encountered except for the sampling process. The long distance between the sampling location and laboratory testing requires the samples to be frozen and sent by air so the testing is conducted within the schedule.

The selection of species that serve as a sample is dependable on the availability in nature at the time of sampling. Shellfish are used as the sample as an individual with the whole weight of $\pm 50g$ (acceptable for canning companies and exporters), so the smaller sizes should be returned back into the water.

VI. Recommendations and Suggestions for Future Follow-Up Action

This project should be continued periodically in the future to build a shared understanding of the potential of PSP toxins in regional waters. It is also important to establish shellfish reference laboratory in ASEAN region in order to strengthen laboratories that have recently developed monitoring shellfish. In addition it is also important to set up a community of Harmful Algae Blooms (HAB) at the regional level as a forum for sharing knowledge and exchanging information related to shellfish toxin and also to learn about other toxins that are present in the shellfish, including the methods of analysis.