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SHORT COMMUNICATION

Monitoring and Risk Assessment of Paralytic Shellfish Poisoning (PSP) Toxins in Two Estuaries at Chanthaburi Province, Thailand

TIDAPORN CHAWEEPAK[1], TATSUYA YURIMOTO[2,*], KAZUMI MATSUOKA[3], KOOLVARA SANGRUNGRUANG[1]
[1]Chanthaburi Coastal Fisheries Research and Development Center, DOF, Chanthaburi, Thailand
[2]Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Ibaraki, Japan
(Present affiliation: Seikai National Fisheries Research Institute, FRA, Nagasaki, Japan)
[3]Institute of East China Sea Research, Graduate School of Fisheries Sciences and Environmental Studies, Nagasaki University, Nagasaki, Japan

*E-mail: yurimoto@outlook.com | Received: 26/07/2019; Accepted: 17/09/2019

Abstract

An enzyme-linked immunosorbent assay (ELISA) kit was introduced to monitor the Chanthaburi region in eastern Thailand to validate the presence of paralytic shellfish poisoning (PSP). This study surveyed palynomorphs from the sediment, dinoflagellate vegetative cells in water column and PSP toxins in two bivalves namely oysters, mixture of *Saccostrea* sp. and *Crassostrea* sp. and blood cockles, *Anadra granosa* (Linnaeus, 1758) from the Chanthaburi and Welu estuaries. The survey did not detect toxic dinoflagellates cysts and the vegetative cells the causative agents of PSP in the two estuaries. In addition, the PSP toxin levels in the bivalves were negligible and thus safe for human consumption. Thus, the two estuaries were considered to be safe of PSP risk.

Keywords: paralytic shellfish poisoning, ELISA, monitoring, palynomorph, Thailand

Introduction

Bivalves accumulate paralytic shellfish poisoning (PSP) toxins in the body by ingestion of toxic dinoflagellates, and poisoning occurs when humans eat toxic bivalve (James et al. 2010). The main symptom of poisoning is paralysis of nervous and respiratory systems and can lead to dyspnea and death in severe cases (James et al. 2010). In recent years, distribution of the toxic plankton has increased in Southeast Asian countries (Lim et al. 2012; Lim and Leaw 2012; Su-Myat et al. 2012a, 2012b), and development of a monitoring system has become an important issue as a countermeasure. In Malaysia, there have been reports of the occurrence of PSP in Northern Borneo and monitoring has been implementing for many years (Wong and Ting 1989). In addition, PSP toxins monitoring has also been conducted in Peninsular Malaysia in recent years in association with the occurrence of toxic dinoflagellate (Su-Myat et al. 2012b; Wan et al. 2016). In Thailand, although the presence of the toxic dinoflagellate species in the Gulf of Thailand was reported in the 1980s (Kodama et al. 1988; Wisessang et al. 1991), yet it has not been a serious problem for bivalve aquaculture. However, it is important to develop a risk assessment and monitoring system for PSP to enable appropriate action. The detection method for the PSP toxins has used mice for many years, however, this is changing as new methods including instrumental analysis or enzyme-linked immunosorbent assay (ELISA) using specific antibodies are being used from the viewpoint of animal protection (Sato et al. 2014; Sato 2015; Mustakim et al. 2016). In this study, the focus was on using the ELISA method, which is simple for PSP toxins analysis because it does not require expensive analytical instruments. Moreover, the method can simultaneously analyse many samples in a short time. And this method seems suitable for field monitoring for rapid inspection covering a wide-area involving many samples. In this study, an ELISA kit for PSP toxins (S-kit) was introduced to the Chanthaburi region in eastern Thailand and toxins and plankton monitoring were conducted in two estuaries. In addition, the risk of PSP was predicted in the areas based on the results obtained from the study.

Materials and Methods

Survey sites

The Chanthaburi region is located in eastern Thailand near the Cambodian border, and the coastal area facing the Gulf of Thailand (Fig. 1A). Mangrove forests are developed along the estuaries of the Chanthaburi and Welu Rivers, and water in both the estuaries is shallow around 2 m. Open-pond shrimp farming is widespread in the mangrove areas, and oyster hanging culture, blood cockle sowing culture and fish cage culture are carried out in the estuary areas. Oyster spats are collected using a thin rope with many small granular pieces of cement hanging from a raft or rack in the estuary (Okoshi 2003). The species of oyster cultured in these estuaries is mainly *Saccostrea* sp. However, Rayong Province, about 100 km north of Chanthaburi Province, cultures *Saccostrea* sp. and *Crassostrea* sp., which are shipped to local markets (Okoshi 2003). For this reason, it is highly likely that the same two oysters species are cultured in the two estuaries of Chanthaburi Province. Additionally, the blood cockle *Anadara granosa* (Linnaeus, 1758) is cultured by sowing in the mud culture technique in the Welu River estuary, using local spat and spat purchased from another province in Thailand.

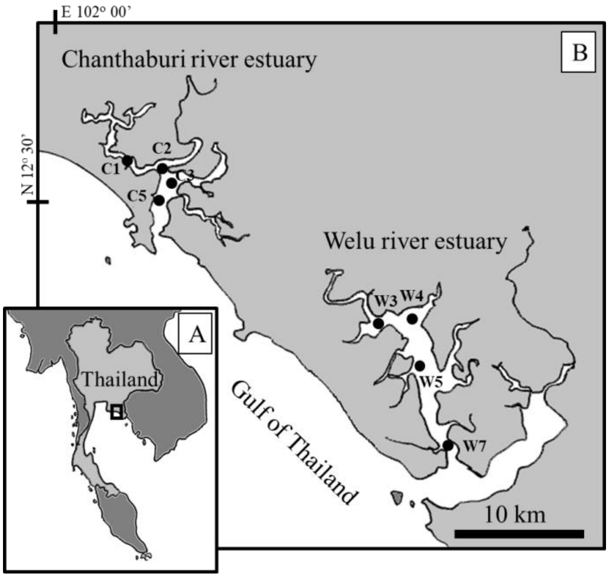

Fig. 1. Survey and monitoring stations in the two estuaries of Chanthaburi (stations C1, C2, C3 and C5) and Welu (stations W3, W4, W5 and W7) Rivers. Map A shows the location of Thailand in Southeast Asia. The enlarged map A indicates the location of map B in Chanthaburi Province. C1, C5, W4, W5 and W7: Surface sediment sampling stations for the palynomorph survey. C2, C3 and W3: PSP toxins monitoring stations of hanging aquaculture oysters *Saccostrea* sp. and *Crassostrea* sp. W3: PSP toxins monitoring station of sown aquaculture blood cockles *Anadra granosa*. C2 and W3: Environmental monitoring stations for water temperature, salinity and phytoplankton of surface water.

Palynomorph survey

Stations for the palynomorph survey were established in the Chanthaburi (stations C1 and C5) and Welu (stations W4, W5 and W7) River estuaries in July 2013 (Fig. 1B). Surface sediment at each station (0–2 cm) was collected from one or two cores of acrylic pipes (inner diameter of 2.6 cm) using a TFO corer Type II and the sediment was preprocessed with the method of Matsuoka et al. (2017, 2018). Observation of palynomorphs was performed under a light microscope (AxioPhot, Zeiss, Germany) with 200 and 400 magnifications. Presence or absence of toxic species cysts was recorded together with other palynomorphs, and the number of individuals was calculated from one-gram of dry sediment.

Environmental observations

Surface-layer water samples and plankton net samples (mesh size 20 μm, diameter 20 cm, vertical hauling) were collected at two monitoring stations (station C2 in Chanthaburi River and W3 in Welu River) every month from July 2013 to November 2016 (Fig. 1B). Temperature and salinity of the water surface layer were measured with a thermometer and a salinometer, respectively, at regular monitoring. These samples were used for common plankton observation under a light microscope at 40～200 magnifications (Hallegraeff et al. 1995; Jitchum et al. 2012; Omura et al. 2012; Yurrimoto et al. 2015). Besides checking for the presence or absence of toxic plankton species and counting the number of individuals or cells of the top three dominant planktons classified into four categories: dinoflagellate, diatom, other phytoplankton and zooplankton.

PSP monitoring

The PSP toxins monitoring was conducted from July 2013 to November 2016. Monitoring stations were set up in the Chanthaburi (C2 and C3) and Welu (W3) estuaries (Fig. 1B). Hanging cultured oysters (shell height about 4 cm) in the surface layer were collected monthly from stations C2, C3 and W3. In addition, sowing cultured blood cockles (shell length about 3 cm) on bottom mud layer were collected monthly with a fisherman's dredge at station W3 in the Welu estuary from July 2013 to January 2016. The collection of samples was not possible during low production periods. Samples of oysters and blood cockles were taken to the laboratory, where the shells were removed, and the edible portion retained. The samples were pooled to a total weight of 25 g or more except when the sample amount was small, and these were stored frozen until analysed. The PSP toxins were then extracted from the thawed samples according to the AOAC method (Hollingworth and Wekell 1990). The ELISA analysis used a commercially available S-kit for PSP (SK: Shin-nihon-kentei-kyokai, Japan), with sample pretreatment and toxins analysis performed according to the kit's manual. The pretreatment of samples was performed using reagents in the kit to convert from PSP toxin analogues to N1-H carbamate toxins before the ELISA analysis, and the concentration measured with standard solution (PD-STX: saxitoxin and pyridylamino group coupled through dithiothreitol) included in the kit with a microplate reader after the coloration (Sato et al, 2014; Sato 2015).

Assessment of PSP risk

The risk level of PSP was classified into five levels based on the presence or absence of causative dinoflagellate cysts, vegetative cells, and bivalve PSP toxins. The five evaluation criteria were the presence of cysts of toxic species in surface sediments and/or vegetative cells in water column, past reports on the occurrence of highly toxic bivalve, the annual occurrence or any recent occurrence.

Results and Discussion

Palynomorph survey

Palynomorphs consisting of dinoflagellates, acritarchs, rhizopods, tintinnomorphs, foraminifers, copepoda and others were observed in Chanthaburi (C1 and C5) and Welu (W4, W5 and W7) estuaries (Fig. 2, Table 1). The community composition and amounts were at equivalent levels to similar surveys in other tropical estuarine areas. However, the numbers were lower than in temperate coastal regions (Matsuoka et al. 2017, 2018). In addition, although dinoflagellate cyst assemblages of three species of Gonyaulacales and six genera of Peridiniales (Table 1) were observed but these were not toxic species. In this survey, the toxic species cysts in both estuaries had very low densities or were almost nonexistent. However, caution is necessary for the possibility of blooms caused by *Peridinium quinquecorne* (Abe, 1927), because this species was detected in both estuaries.

Environmental observations

Monthly changes in water temperature and salinity in both estuaries (stations C2 and W3) during the PSP toxins monitoring period are shown in Fig. 3. Annual variations of water temperature were in the range of 26–34 °C in Chanthaburi and 25–33 °C in Welu. At both sites, temperature changes were similar and minimum water temperature was 25–26 °C during the cooler season in December to January and maximum 33–34 °C during April to May. Salinity fluctuation was high in both estuaries between the dry and rainy seasons. Salinity exceeded 30 ppt in both estuaries during the dry season and decreased significantly below 10 ppt in the Chanthaburi River estuary and to around 10 ppt in the Welu River estuary almost every year in June to October during the rainy season. Although the decrease in salinity has a complex relationship with water flow rate, location, exchange volume of seawater and other factors in each estuary, the data suggested that the water environment changed drastically between the dry and rainy seasons. The top three plankton numbers observed monthly in the Chanthaburi and Welu estuaries are shown in Fig. 4. Plankton density remained below 2,000 individuals (cells.L^{-1}), however, plankton blooms of over 10,000 individuals (cells.L^{-1}) sometimes occurred in both estuaries. It is suggested that nutrient supply from the river and daylight hours affected the blooms because they were mainly observed during November and December and from March to May when seasons changed from rainy to dry and from dry to rainy, respectively. No blooms due to diatoms and dinoflagellate cells related to PSP toxins were observed in both estuaries from July 2013 to November 2016. However, dinoflagellate bloom probably identical to *Peridinium quinquecorne* (Abe, 1927) and known to be non-PSP toxin producing but can cause harmful red tide in tropical coastal waters around the world (Horstmann 1980; Horiguchi and Sotto 1994; Yu et al. 2007; Gárate-Lizárraga and Muñetón-Gómez 2008; Satta et al. 2010; Al-Hashmi et al. 2013), was observed at a density of 4,000 individuals (cells.L^{-1}) in the Welu estuary in June 2016. Furthermore, no toxic dinoflagellate bloom was detected in either estuary. This result was also consistent with the absence of toxic dinoflagellate cysts in the sediment samples.

PSP monitoring

The ELISA results for oysters and blood cockles at stations C2, C3 and W3 in the two estuaries are shown in Fig. 5. The toxicity level in oysters collected at stations C2 (Fig. 5A) and C3 (Fig. 5B) were generally below 1 nM; however, this increased to 1.5 and 2.0 nM in October 2016, respectively. The toxicity levels for stations C2 and C3 were similar, likely because they were only about 2 km apart. The toxicity levels of all oyster samples at station W3 were below 1 nM (Fig. 5C). Blood cockle samples from W3 were also below 1 nM except for December 2014 when it was 1.6 nM; however, toxicity levels in oysters from the same station during the same period were lower than 1 nM (Fig. 5D). The reason for this difference might be due to the water depth of the habitats of these bivalves. The hanging oysters are kept near the surface whereas the blood cockles are sown on the bottom in the mud. It could also be possible that some of the blood cockle spats were introduced from other sites in Thailand. The PSP toxins levels (1.5-2.0 nM) detected were minute traces and close to the detection limit of the highly sensitive ELISA. The self-regulation level of PSP toxins is known to be 4 mouse unit (MU.g^{-1}) or higher in Japan (Onoue et al. 1980; Murakami et al. 1999). In addition, comparison of results of mouse analysis and the ELISA kit (S-kit) by Suzuki (2014) showed that the self-regulation level (4 MU.g^{-1}) was roughly equivalent to more than 20 nM for results obtained with ELISA. Thus, the results for oysters and blood cockles obtained in the present study indicate that they were safe for human consumption.

Table 1. Classification and individual numbers (unit: No.g^{-1} D.W.) of palynomorphs in surface sediments of the estuaries of Chanthaburi (stations C1 and C5) and Welu (stations W4, W5 and W7) Rivers, Thailand.

		C1	C5	W4	W5	W7
Dinoflagellata	Gonyaulacales					
	Spiniferites bulloideus (Deflandre & Cookson, 1955)				50	
	Spiniferites ramosus (Ehrenberg, 1837)		30			
	Operculodinium israelianum (Rossignol, 1962)	170	30	80	150	220
	Peridiniales					
	Brigantedinium spp.	100	110		150	680
	Protoperidinium spp.		30		100	
	Peridinium quinquecorne (Abe, 1927)	30		40		
	Scrippsiella spp.		70			
	Zygabikodinium cf. lenticulatum (Loeblich Jr. & Loeblich III, 1970)	30			50	
	Echinidinium sp.	30		40		
Acritarcha	Spherical cyst		30	160		
	Michystridium sp.			40		
	Pseudoschizaea sp.	30	150	40	50	
Chrysophyceae	*Archeomonas* spp.	60	70			
Tintinnomorph		100	450		420	1360
Foraminifera		1530	1850	200	2380	2270
Rhizopoda	*Arcella* sp.	60	70			
Copepod		60	220		50	
Total		2200	3110	600	3400	4530

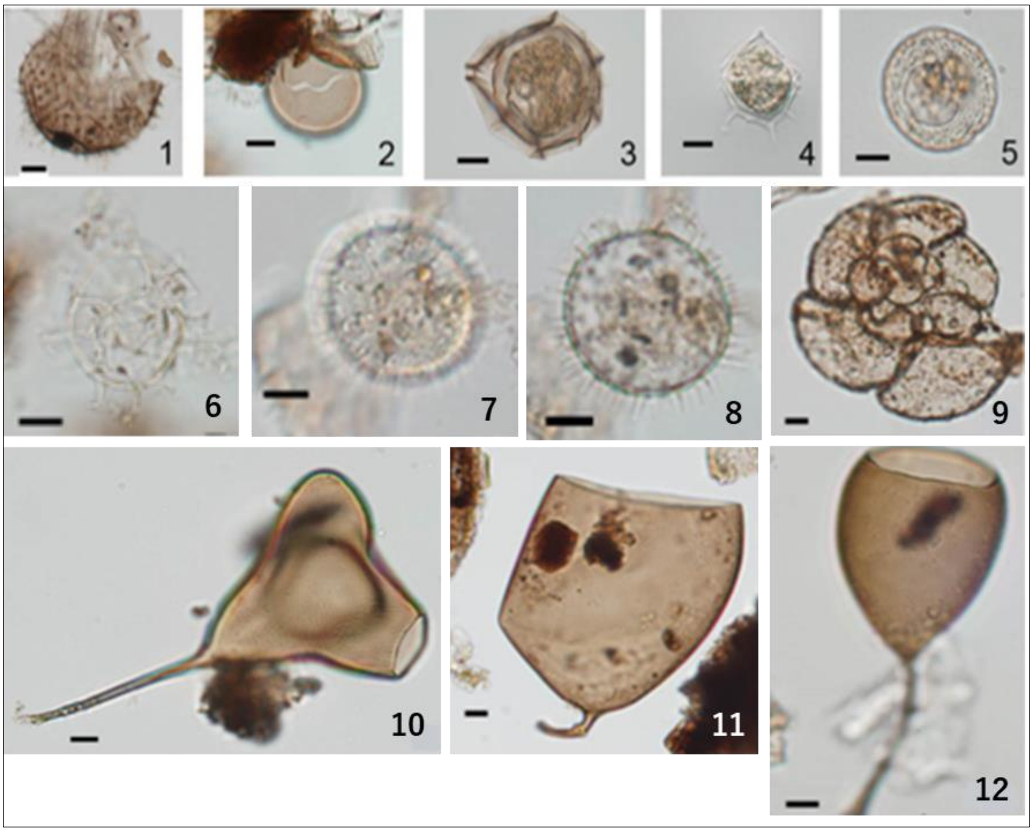

Fig. 2. Palynomorphs observed in surface sediments from the estuaries of Chanthaburi and Welu Rivers. Phytoplankton; Dinoflagellate (heterotrophic species): 1. *Echinidinium* sp. (St. C1); 2. *Brigantedinium* sp. (St. C1) and 3. *Protoperidinium* cf. *parthenopes* (Zingone & Montresor, 1988) (St. W5). Dinoflagellate (autotrophic species): 4. *Peridinium quinquecorne* (Abe, 1927) (St. W4); 6. *Spiniferites bulloides* (Deflandre & Cookson, 1955) (St. W5); and 7, 8. *Operculodinium israelianum* (Rossignol, 1962) (St. C1). Benthic protist; Foraminifera: 9. coiled type (St. W5), Rhizopoda: 5. *Arcella* sp. (St. C1). Zooplankton; resting cyst of tintinnid: 10. (St. C1), 11. (St. C5) and 12. (St. W5). Scale bar: 10 μm.

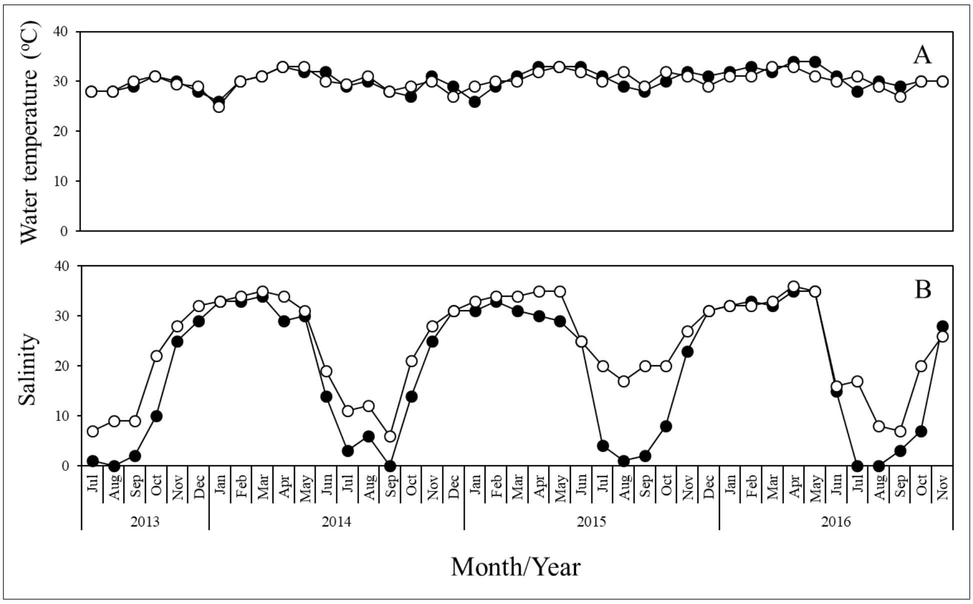

Fig. 3. Changes of surface water temperature (°C) and salinity (ppt) in the estuaries of Chanthaburi (●: station C2) and Welu (○: station W3) Rivers, Thailand. The graphs data was obtained in the regular sampling every month.

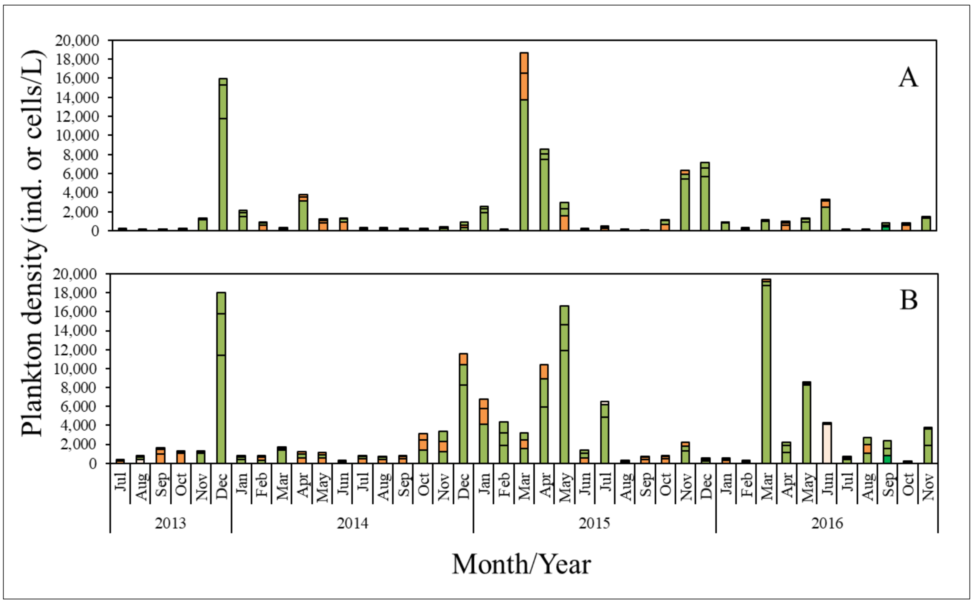

Fig. 4. Changes of plankton densities in the estuaries of Chanthaburi (graph A, station C2) and Welu (graph B, station W3) Rivers. Ivory, dinoflagellate; light green, diatom; deep green, other phytoplankton; and orange, zooplankton. The data was obtained in the regular sampling every month.

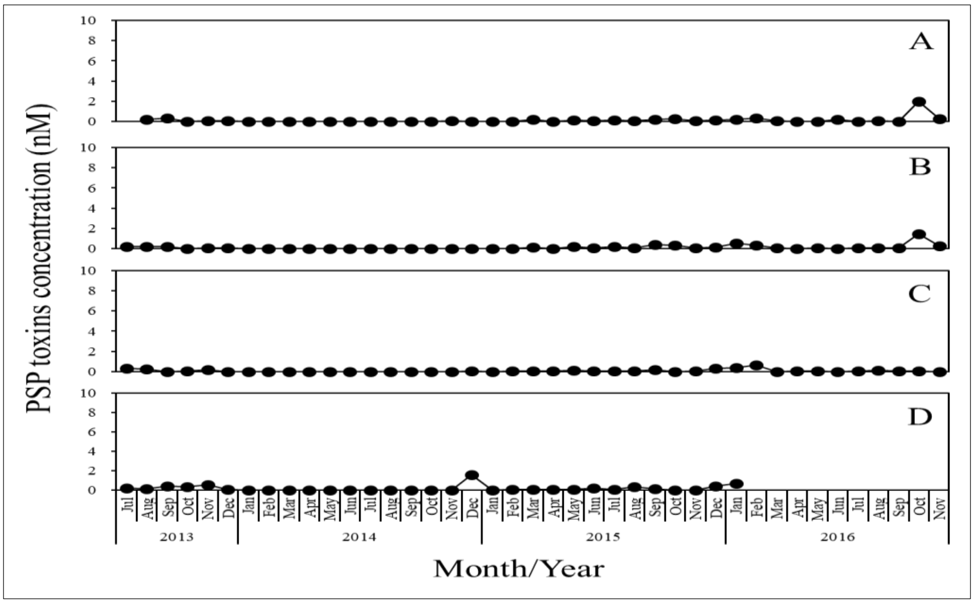

Fig. 5. Changes in PSP toxins concentrations in soft tissues of cultured oyster and blood cockle obtained from the estuaries of Chanthaburi (stations C2 and C3) and Welu (station W3) Rivers. A, B and C: Toxin levels in hanging cultured oysters, mixture of *Saccostrea* sp. and *Crassostrea* sp., in stations C2, C3 and W3, respectively. D: Toxin levels in the sowing cultured blood cockle, *Anadra granosa* (Linnaeus, 1758), in station W3. This sampling was interrupted due to unstable aquaculture production.

Assessment of PSP risk

The risk level of PSP was classified into five levels based on the presence or absence of causative dinoflagellate cysts and vegetative cells, and bivalve PSP toxins (Table 2). The causative agents of PSP, palynomorph sample including dinoflagellates cysts from the sediment and the plankton bloom of dinoflagellate cells were not detected in the two estuaries. Furthermore, the toxins levels in the bivalves were below the harmful levels for consumers in both the estuaries. Thus, the Chanthaburi and Welu estuaries were concluded to be at the safe from PSP risk.

Conclusion

The study revealed that PSP causative palynomorphs and the plankton blooms were not detected in the Chanthaburi and Welu estuaries. And the PSP toxin levels detected in the oysters and blood cockle were in minute traces. The Chanthaburi and Welu estuaries were concluded to be safe of PSP risk. Furthermore, the use of ELISA kit as an alternative method for monitoring PSP toxins was found suitable in this study.

Table 2. Risk level of paralytic shellfish poisoning (PSP) in the monitoring area classified into five levels based on presence or absence of causative dinoflagellate cysts and vegetative cells, and bivalve PSP toxins.

Level	Cysts of toxic species	Vegetative cells of toxic species	Highly toxic bivalve occurred in the past	Highly toxic bivalve occurs every year
I*	×	×	×	×
II	○	×	×	×
	×	○	×	×
III	○	○	×	×
IV	○	○	○	×
V	○	○	○	○

Cysts of toxic species: detection of cysts causative agent of PSP in surface sediments, vegetative cells of toxic species: detection of plankton cells causative PSP in water column, highly toxic bivalve occurred in the past: bivalves have been highly toxic with PSP in the past, highly toxic bivalve occurs annually: bivalves have been highly toxic with PSP every year. *Level I mean the safest in terms of PSP risk; both estuaries showed this level. ×: negative factor, ○: positive factor.

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