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Case Study of Paralytic Shellfish Poisoning (PSP) Monitoring in Tropical Mangrove Estuaries: A Mini Review

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Abstract

In recent years, the incidence of toxic plankton and bivalve poisoning that causes paralytic shellfish poisoning (PSP) has increased in Southeast Asian countries. Thailand is one of the most active bivalve aquaculture fisheries and needs to be prepared for this problem in the future. In this paper, we introduce a case of monitoring efforts on the Chanthaburi coast near the Cambodian border, where experimental PSP monitoring was conducted in water from 2013 to 2016, and a suitable monitoring system was considered in the region. As a result, we concluded that it is unlikely that the PSP problem will soon become serious in the region. However, in the surrounding waters, we must be aware of some invasive issues that may involve toxic plankton and cysts, such as ballast tank water from large vessels and transplantation of bivalves introduced from outside for aquaculture. Even if the waters are safe, PSP monitoring should continue on a small scale and at a low cost for an extended period.

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Introduction

Paralytic Shellfish Poisoning

Paralytic shellfish poisoning (PSP) toxins consist of multiple alkaloid toxins with more than 20 kinds of analogues, including saxitoxins, gonyautoxins, and C toxins (Kodama, 2010). Some specific toxic dinoflagellates produce these toxins (Prakash, 1963; Prakash, 1967; Hallegraeff et al., 1988; Lim & Ogata, 2005), and filter-feeding animals, such as bivalves and ascidians, ingest toxic plankton and accumulate toxins in their bodies (Prakash, 1963; Bourne, 1965; Sekiguchi et al., 2001a; Sekiguchi et al., 2001b; Bougrier et al., 2003). Additionally, humans who consume animals that accumulate toxins may experience numbness of the tongue and lips in mild cases, but can also experience numbness of the hands and feet, shortness of breath, and in severe cases death due to respiratory failure (Lehane, 2001; James et al., 2010; Suleiman et al., 2017). Normally, visually identifying PSP toxins accumulation in animals is difficult, and detoxification by cooking does not effectively detoxify the toxins as they are thermostable (Murakami et al., 1999; Murakami & Noguchi, 2000). Therefore, continuous monitoring of the occurrence of toxic plankton and periodic toxin analysis of bivalves are important to prevent poisoning in humans.

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PSP in Southeast Asia

Bivalve fisheries are important components of coastal fisheries in Southeast Asian countries. Oysters, blood cockles, and mussels inhabit the brackish waters near the coast. These shellfish are actively fished and aquacultured and they have become an important food source (Fong-Oon, 1984; Tookwinas & McCoy, 1985; Nair, 2001; Chalermwat et al., 2003). However, the presence of PSP-producing plankton species in Southeast Asian coastal waters has recently been widely reported (Fukuyo et al., 2011; Furio et al., 2012). Toxin accumulation in bivalves has been widely reported in the Philippines and Malaysia. In the Philippines, poisoning has been documented since the 1980s. Between 1983 and 1998, approximately 2,000 PSP cases were reported, with 5.8% of them resulting in death (Van Dolah et al., 2001). In Malaysia, the coast of the Sabah State has a long history of poisoning. A red tide of the toxic species *Pyrodinium bahamense* has been reported since the 1970s with over 300 cases of poisoning were reported between 1976 and 1988, more than 30 of which were fatal. Many of these poisonings have been linked to the consumption of bivalves like oysters and hard-shelled mussels (Ting & Wong, 1989). Furthermore, the abundance of PSP toxins-producing plankton has increased since 2000. *Alexandrium minutum* first appeared in Tumpat on Peninsular Malaysia's east coast in 2001(Usup et al., 2002). In addition, the presence of various plankton-producing PSP toxins, such as *P. bahamense*, *Alexandrium* spp. (*A. tamiyavanichii*), and *Gymnodinium catenatum*, has been confirmed along the west coast of the Malay Peninsula (Usup et al., 2002; Lim et al., 2012; Su-Myat et al., 2012; Yurimoto et al., 2020). Thus, even in coastal waters where PSP has not been reported in Southeast Asian countries, attention needs to be paid to the possibility of bivalves accidentally accumulating toxins, and countermeasures against poisoning need to be developed to prevent further damage (Etheridge, 2010; Wan Norhana et al., 2016; Chaweepak et al., 2019).

PSP in Thailand

Aquaculture is particularly active in Thailand's mangrove coastal zone with shrimp aquaculture, hanging or pen aquaculture of oysters and mussels, and sowing cultures being the most common (Tookwinas & McCoy, 1985; McCoy & Chongpeepien, 1988; Flaherty & Karnjanakesorn, 1995; Chalermwat et al., 2003). In Thailand, a PSP incident was reported in 1983; 63 people were affected after eating green hard-shelled mussels, one of whom died in Pranburi and Prachuab Kirikhan (Fukuyo et al., 1988; Sukasem, 1996). Protogonyaulax cohorticula (also known as A. tamiyavanichii) was identified as a PSP-producing plankton in the Gulf of Thailand in 1988 (Kodama et al., 1988). However, PSP toxins contamination in Thailand's coastal waters is currently lower than in the Philippines and Malaysia. Furthermore, Thailand's Fish Inspection and Quality Control Division (FIQD) actively promotes regular inspections of bivalve food safety [e.g. Paphia undulata], including PSP toxins contamination and red tide monitoring (Sanguansin, 2011).

Monitoring System

Referred System of Monitoring

First, we present an example of a conventional PSP monitoring system in Japan. PSP damage has a long history in Japan, with the 1948 poisoning of a short-necked clam, *Ruditapes philippinarum*, in Toyohashi City, Aichi, being regarded as the first record (Noguchi, 1983; FSC, 2014). PSP caused by *Chlamys farreri nipponensis* was later reported in Ofunato City, Iwate, in 1961, and by the Pacific oyster *Crassostrea gigas*, in Miyazu City, Kyoto, in 1962 (Sasagawa & Inoue, 1968; Noguchi, 1983; Taguchi et al., 1994; FSC, 2014). Therefore, local governments in Japan have developed monitoring systems for PSPs (Figure 1; APSD, 2015a). Water for bivalve production has been divided into appropriate area sections (zoning with regard to industrial impact), and plankton monitoring and bivalve toxin analysis are periodically performed at each sampling station for every section (APSD, 2015b). When the amount of toxins in the edible portion of bivalves exceeds 4 MU g^{-1} (the lethal toxin amount for a mouse weighing 20 g in 15 min is defined as 1 MU), the shipment of aquaculture filter-feeding animals, such as scallops, oysters, and ascidians, is stopped by self-regulation (APSD, 2015a).

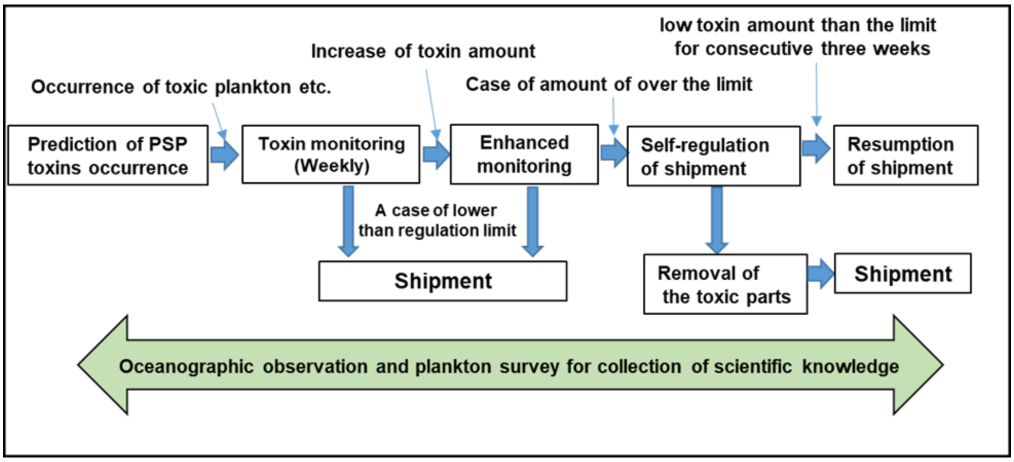

Figure 1. Flow chart of risk management of the paralytic shellfish poisoning (PSP) toxins on the production site in Japan (Modified from a figure of APSD, 2015a).

Toxins Analysis

Conventional PSP toxins analyses were performed using male ddY mice (Nagashima et al., 1991; FSC, 2014). However, this analysis has not been recommended in recent years, considering the labour required to maintain mice and animal protection policies (Yasumoto, 2013). As an alternative simple method that excludes instrumental analysis, PSP toxins-specific antibodies have recently been developed, and PSP toxins have been assessed using an enzyme-linked immunosorbent assay (ELISA) or immunochromatography (Usleber et al., 1997; Jellett et al., 2002). Therefore, in our monitoring project in Thailand, a new PSP toxins ELISA kit developed in Japan (Sato et al., 2014) was introduced to determine its applicability in local situations.

Monitoring Site

Bivalve Aquaculture and Weather in Chanthaburi

The Wang Ta Not, Chanthaburi, and Welu rivers flow from the north to the coastal waters of Chanthaburi Province in Thailand's southeastern gulf, near the Cambodian border (Figure 2). Many open-air shrimp aquaculture ponds can be found in the mangrove forests surrounding these rivers' estuaries (Hazarika et al., 2000). Furthermore, hanging oyster (*Saccostrea* sp. and *Crassostrea* sp.) aquaculture and sowing blood cockle (*Tegillarca granosa*) aquaculture have been performed in estuaries (Figure 3; McCoy & Chongpeepien, 1988; Sungkasem & Tookwinas, 1994; Okoshi, 2003). Hence, the Chanthaburi Coast is an important site for coastal aquaculture in southeastern Thailand. The average monthly air temperature in the region remained relatively stable. However, the temperature dropped to approx. 27°C from December to February (early dry season), and exceeded 29°C from March to May (late dry season) (Figure 4). However, rainfall varied significantly between wet and dry seasons. After May, the monthly rainfall reaches several hundred millimetres, especially from June to September, which is the peak rainy season, and occasionally exceeds 500 mm (Figure 4). Subsequently, rainfall gradually decreased to less than 100 mm from November to April.

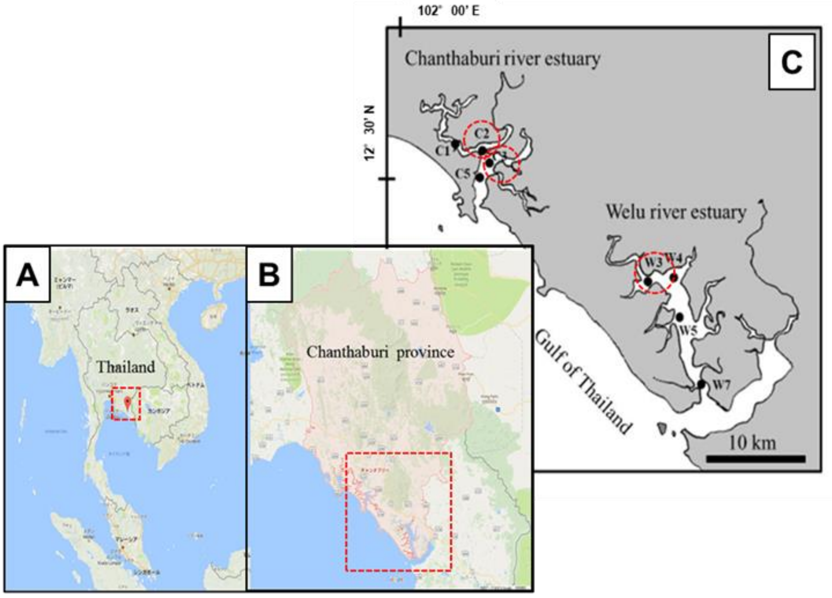

Figure 2. Maps of the Chanthaburi Region in Thailand and monitoring sites. A and B highlight Thailand and Chanthaburi Province (from Google Maps). C shows two monitoring estuaries in the province (from Chaweepak et al., 2019), with stations for monitoring PSP toxins in oysters (C2, C3 and W3) and blood cockles (W3). These stations also monitor water temperature, salinity, and surface water phytoplankton.

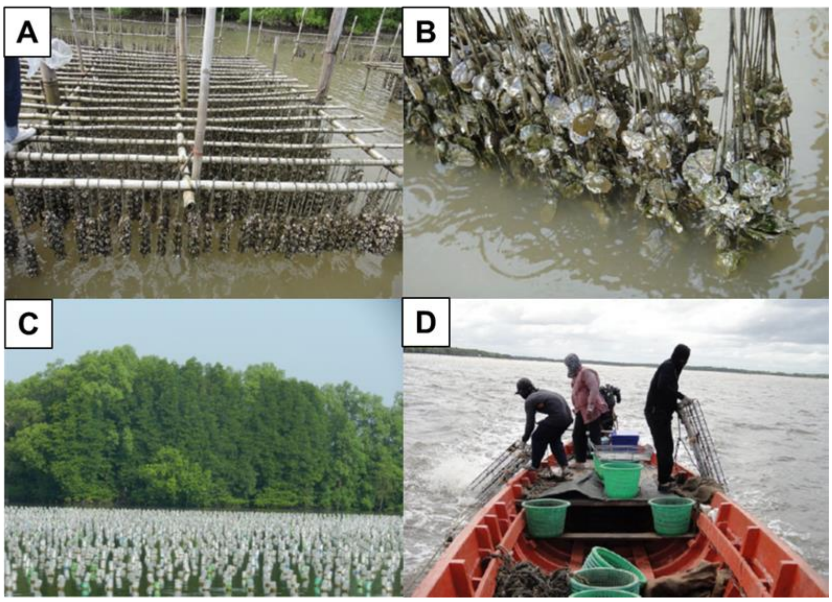

Figure 3. Photographs of bivalve aquacultures in the Chanthaburi region of Thailand. A and B: Oyster farming (a mix of *Saccostrea* sp. and *Crassostrea* sp. from Okoshi, 2003) was performed using cement spat collectors in the Chanthaburi estuary; C: Oyster farming was performed using PET bottle floats in the Welu estuary; D: Harvesting of sown blood cockles (*Tegillarca granosa*) by steel dredges in the Welu Estuary.

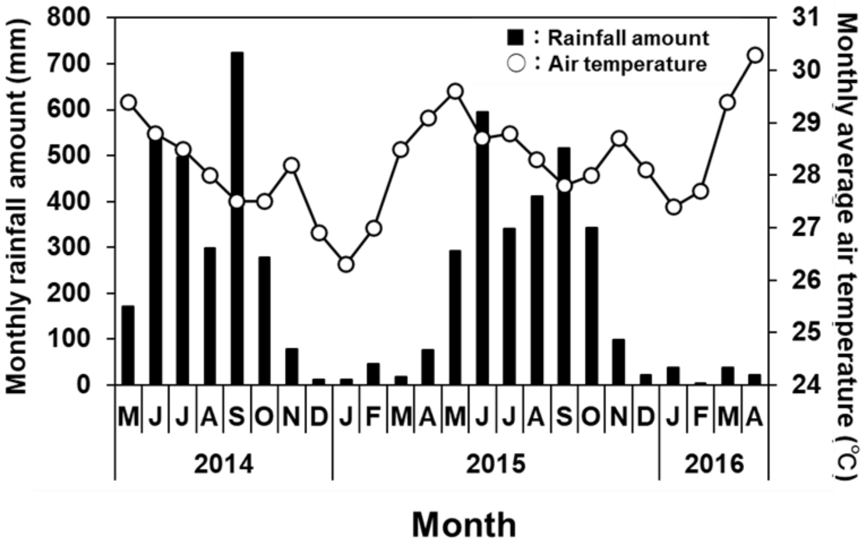

Figure 4. Monthly weather changes in the Chanthaburi region, Thailand, from May 2014 to April 2016. Line graph: monthly average air temperature; bar graph: monthly rainfall. Data were obtained online from the homepage of the Meteorological Agency of Japan (https://www.data.jma.go.jp/gmd/cpd/monitor/climatview/frame.php).

Environment of the Two Estuaries

Environmental monitoring stations were established in both estuaries of the Chanthaburi and Welu Rivers (Figure 2C). The temperature and salinity of the surface water were measured as basic environmental data at each monitoring station. A fixed amount of surface water was sampled using a bucket, and the plankton sample was concentrated using a small plankton net (diameter: 20 cm, side length: 80 cm, mesh size: 20 µm). From the monitoring results, the surface water temperature did not change significantly throughout the year, remaining less than 29°C from the wet to the early dry season, and more than 32°C in the late dry season (Chaweepak et al., 2019). However, monthly salinity varies greatly throughout the year, with more than 30 PSU in the dry season and less than 10 PSU in the wet season, due to the strong influence of freshwater inflow at these sites (Chaweepak et al., 2019). Therefore, the coastal environment and phytoplankton species composition in bivalve aquaculture farms vary significantly between wet and dry seasons. The annual predominance of dinoflagellates is low, whereas that of Diatomaceae is high. Furthermore, PSP-causing plankton rarely appeared throughout the year. Furthermore, hanging-cultured oysters were periodically collected at a station in the Chanthaburi River estuary, and hanging-cultured oysters and sowing-cultured blood cockles collected from the Welu River estuary were monitored for PSP toxins by ELISA. However, the levels of toxins in these bivalves were approx. zero, and they were not toxic to the human body (Chaweepak et al., 2019). Based on the checklist criteria, the risk of damage by PSP on the coast of Chanthaburi Province was evaluated by classifying the degree of risk into five levels. On the coast of Chanthaburi, no cysts of toxic species causing PSP were observed at the bottom of the bivalve culture waters, and a high density of toxic plankton cells was not confirmed at the stations during the wet and dry seasons. Furthermore, according to local fishermen and government officials, no cases of poisoning have been reported in coastal waters. Additionally, ELISA-based PSP monitoring from 2013 to 2016 revealed no bivalve poisoning. Thus, the coast of Chanthaburi is thought to be a low-risk area, and fished bivalves are safe from PSP (Chaweepak et al., 2019).

Monitoring of Shallow Estuary

In severe waters in Japan, plankton monitoring and toxicity tests of bivalves are performed periodically, once or twice a month. Additionally, when the cell numbers of toxic plankton and/or the toxicity level in bivalves increase, regular monitoring increases to once per week (APSD, 2015a). In addition, in important waters for PSP, the monitoring waters are subdivided so that only limited areas have self-imposed restrictions in the event of a PSP. In contrast, the monitoring sites on the Chanthaburi coast had no cases of PSP in the past, and these sites are considered to have a low possibility of PSP in the current situation. Thus, setting a monitoring site as a representative station in the centre of the bivalve aquaculture ground would be considered sufficient. In addition, water quality monitoring is sufficient to understand the conditions of surface water because these sites are shallow. Although the Chanthaburi coast currently has a low risk of PSP, we need to be aware of the occurrence of toxic plankton in the future because there are several invasive factors for toxic plankton, such as ballast water discharge from large vessels passing around and transplanting bivalves for aquaculture introduced from outside (Hallegraeff & Bolch, 1991; Hallegraeff et al., 1998; Bolch & de Salas, 2007; Matsuyama et al., 2010). Therefore, small-scale and long-term PSP monitoring is necessary with minimal effort and cost, while considering the safety of fished bivalves. We believe that a safety check for bivalves by periodic monitoring once a month is ideal at this time. Therefore, it is important to simplify the analysis of toxins and reduce the cost of the analysis.

In our study, ELISA was used as a new method for PSP toxins analysis. Even measuring a few samples took time because the analysis required a standard curve for each test, though the concentration could also be determined. As a result, when there were only a few test samples, many sample-wells for analysis were used to prepare a calibration curve, which made the test inefficient. However, with immunochromatography, there is no need to generate a calibration curve, allowing for rapid and straightforward test results. As a result, this method is making it ideal for regular small-scale monitoring. Although experimentally, toxin analysis was performed using immunochromatography (PSP Rapid Test; Scotia Rapid Testing, Ltd., Canada) was also tested in this study, and we confirmed that it was capable of detecting the toxins from cultured bivalves at the monitoring site that the trace levels did not affect humans (Figure 5). In this test, the test principle uses a specific antibody against PSP toxins, as in ELISA, if an extract sample from the bivalve is obtained, chromatic analysis can be completed in less than 35 min (Laycock et al., 2000). However, the analysis cost was high, with each sample costing approx. 5,000 Japanese yen (JPY) in 2014. Another company recently developed immunochromatographic PSP analysis kits (MT test Immunochromato-PSP; Nissui Pharmaceutical Co. Ltd., Japan), which were sold at a lower price of around 2500 JPY in 2021. If the immunochromatographic method becomes more popular for PSP monitoring, analysis costs will fall further, and monitoring efficiency will improve more. Thus, a field-specific inspection system can be implemented. Immunochromatographic analysis, in particular, is an excellent screening method when dealing with a small number of samples because the analytical procedure is simple. Furthermore, the need for rapid monitoring of aquaculture farms renders the ability to obtain preliminary values that are immediately useful. If a positive sample is identified, the toxin concentration of that sample can be accurately quantified using instrumental analysis, reducing the need for on-site monitoring.

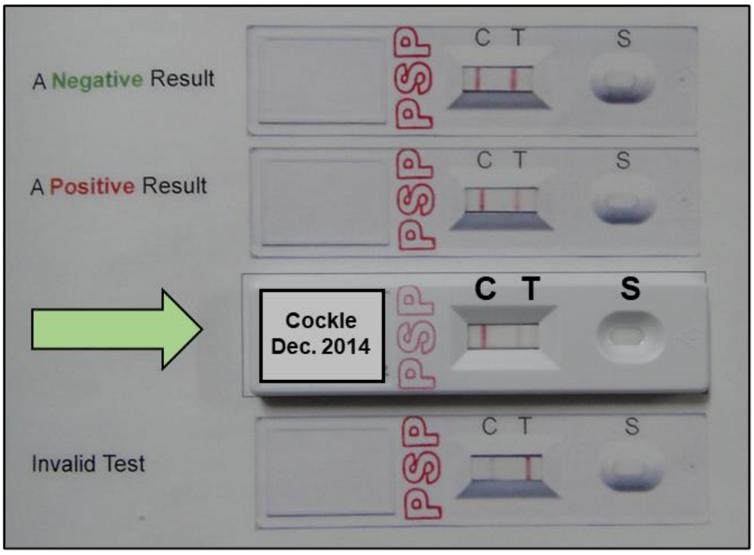

Figure 5. Immunochromatographic analysis result for the PSP toxins (arrow) and the result decision with a test interpretation sheet. A negative result had both C and T red-coloured bands, whereas a positive outcome had only the C band with a thin or missing T band. An invalid result is obtained occurs if the C band is absent. The analyzed sample (arrow) showed a positive outcome, with almost no T band. Extracted from cultured blood cockles in December 2014, the sample had an ELISA-confirmed toxin concentration of 1.6 nM, which is below 1/10 of the regulated value. Where S is the sample well, T is the test band, and C is the control band.

Conclusions

We selected the Chanthaburi coast, an active fishery ground for bivalve aquaculture in Thailand, as the PSP monitoring test site. We assessed the risk of PSP occurrence in water and investigated a PSP toxins monitoring system suitable for the region. Although there are no major issues right now, we must consider the possibility of toxic plankton occurrence factors, such as the discharge of ballast water from large vessels and the transplantation of bivalves for aquaculture obtained from outside in the future. Therefore, although high-frequency monitoring of PSP is currently unnecessary, a cost-effective and labour-saving safety confirmation system with targeted monitoring points and simple PSP toxins analysis, such as immunochromatography, should be adopted for sustainable aquaculture management.

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