Domoic Acid Contamination in Fish from Sriracha Bay, Chonburi Province, Thailand

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ABSTRACT

Contamination of domoic acid, a marine biotoxin that causes amnesic shellfish poisoning (ASP), and could be transferred to humans through consumption of contaminated fish, was investigated in three species of fish (*Siganus canaliculatus*, *Sardinella albella* and *Rastrelliger brachysoma*) from Sriracha Bay, Chonburi Province, Thailand. Fish samples were collected every two months between May 2012 and July 2013. Body tissue and viscera of fish were examined for domoic acid content using enzyme-linked immunosorbent assay (ELISA) method. Results demonstrated contamination in all samples of *S. canaliculatus*, *S. albella* and *R. brachysoma*, with domoic acid content ranging between 0.32 and 48.64, ND-61.43, and ND-84.80 $ng \cdot g^{-1}$ in body tissues, and between 51.66 and 276.29, 7.93 and 235.00, and 2.19 and 283.50 $ng \cdot g^{-1}$ in the viscera, respectively. Domoic acid content of *S. canaliculatus* was higher than *R. brachysoma* and *S. albella*. Average contents of domoic acid in *S. canaliculatus* and *R. brachysoma* during the dry season were significantly higher than during the wet season. Overall results indicated that the average domoic acid content in the body tissue of all fish sampled throughout the year was 13.91% (19.94 $ng \cdot g^{-1}$ wet weight); with domoic acid in the viscera of 152.54 $ng \cdot g^{-1}$ wet weight, an eight-fold increase. However, domoic acid content in the three fish species studied was lower than the safe consumption level of 20 $\mu g \cdot g^{-1}$, originally set in Canada.

Keywords: Domoic acid, Fish, Sriracha Bay

INTRODUCTION

Domoic acid (DA) contamination in marine organisms has been a concern regarding food safety for the last decade. Domoic acid is a neurotoxin produced by several species of phytoplankton, predominantly of the diatom genera *Pseudo-nitzschia* and *Nitzschia* (Zabaglo *et al*., 2016). This toxin is responsible for a human illness known as amnesic shellfish poisoning (ASP). DA enters food webs through feeding interactions and can accumulate in higher trophic levels (Doucette *et al*., 2006). In 1958, DA was first identified in a red marine alga, *Chondria armata* (Takemoto and Daigo, 1958). The first incident of ASP occurred in 1987 on the eastern coast of Prince Edward Island, Canada, when 143 people became ill and at least 4 died after consuming blue mussels (*Mytilus edulis*) contaminated with DA (Perl *et al*., 1990). The plankton species causative for the Canadian incident was identified as the diatom, *Pseudo-nitzschia multiseries* (Bates *et al*., 1989). In

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humans, symptoms of ASP are both gastrointestinal (vomiting and diarrhea) and neurologic effects including short-term memory loss, confusion, seizures, coma, and even death (Perl *et al*., 1990). Accumulation of domoic acid in bivalves was also reported in various parts of the world (Bates and Trainer, 2006) and planktivorous fish accumulate high levels of domoic acid (DA) during toxic *Pseudo-nitzschia* sp. blooms (Lefebvre *et al*., 2002). Recently, the blooms of *Pseudo-nitzschia* sp. have caused widespread concern for human health with the potential to weaken the fisheries. Blooms of *Pseudo-nitzschia* sp. have increased in the past two decades, with observations on the west, east and Gulf of Mexico coasts in the USA and countries in Europe and Asia (Liefer *et al*., 2013). Domoic acid has now been identified in 19 species of *Pseudo-nitzschia* spp. and 2 species of *Nitzschia* spp. (Zabaglo *et al*., 2016). However, the presence or absence of DA contamination in food webs neither reflects the population size of *Pseudo-nitzschia* spp., nor the production of DA, since there is still limited understanding of the environmental and oceanographic conditions that stimulate DA production. Thus, cells of *Pseudo-nitzschia* spp. may have very low concentrations of DA to contaminate planktivores. Moreover, although DA depuration of mussels and fish is normally fairly rapid, it cannot completely eliminate the possibility that wide-ranging fish, birds or mammals obtain the toxin elsewhere (Busse *et al*., 2006).

In Thailand, domoic acid has recently been detected for the first time in *Spondylus versicolor*, albeit at a low concentration (Takata *et al*., 2009), with blooms of phytoplankton species becoming more frequent. The action levels for DA in shellfish, originally set in Canada, is 20 $\mu g \cdot g^{-1}$ DA in shellfish tissue (Anderson *et al*., 2001). From the toxicity perspective, dangerous levels for the California food web are considered to be $5x10^4$ $cells \cdot L^{-1}$ of *Pseudo-nitzschia australis*, the concentration at which mussels and fish reach average toxic levels considered unfit for human consumption (Busse *et al*., 2006). McCarron and Hess (2006) found that cooking shellfish products containing DA at 121°C did not reduce its absolute concentration. Recently, food safety has become a high priority issue. This research contributes important information concerning DA contamination in commercial fish and presents the first evidence of domoic acid accumulation in white sardinella, white-spotted spinefoot and Indo-Pacific mackerel from Thai waters. This data set provides useful information for managing food security concerns in Thailand.

MATERIALS AND METHODS

Study sites and survey period

The study area was located at Sriracha Bay, Chonburi Province, Thailand (Figure 1) where *Pseudo-nitzschia* spp. and *Nitzschia* spp. are frequently found (Yoosamran *et al*., 2006) and with red tide incidents of *Pseudo-nitzschia* spp. recorded (Phuket Marine Biological Center, 2016). Field surveys and sample collection were conducted every two months from May 2012 to July 2013. May 2012, January, March and May 2013 were representative of the dry season while July, September, November 2012 and July 2013 comprised the wet season.

Sampling methodology

Four to ten specimens of the three fish species, namely *Sardinella albella* (white sardinella), *Siganus canaliculatus* (white-spotted spinefoot) and *Rastrelliger brachysoma* (Indo-Pacific mackerel) (Figure 2) were collected using set nets. All specimens were frozen immediately after collection.

Sample preparation and analysis

The body tissue and viscera organs of each fish species were homogenized, then partitioned into 1 g samples, and an aliquot of 4 ml of 50% methanol was added to each sample before centrifugation at 3,500 rpm for 30 min. The supernatants were decanted and filtered onto a Whatman glass-fiber filter (GF/C) to obtain sample extracts for analysis. Extracted samples were analyzed following the enzyme-linked immunosorbent assay (ELISA) method (Dao *et al*., 2006). All samples were measured in $ng \cdot g^{-1}$ tissue (wet weight).

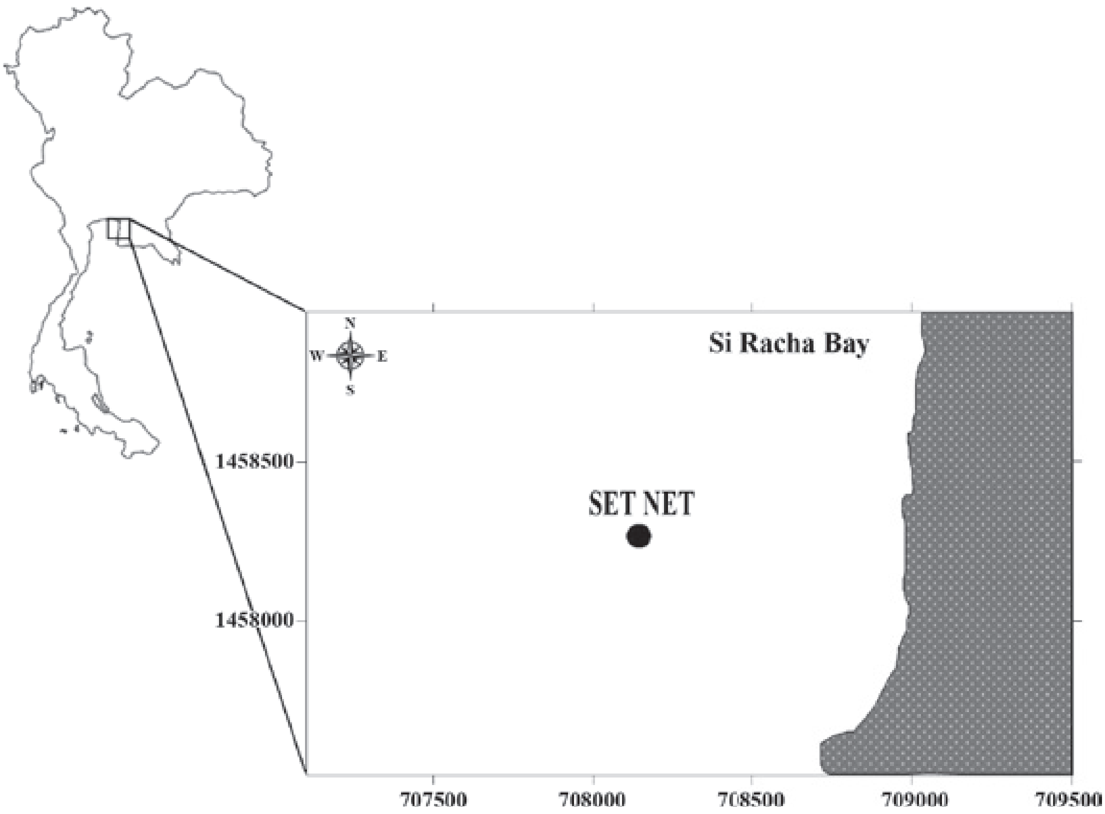

Figure 1. A map of sampling sites in Sriracha Bay, Chonburi Province, Thailand

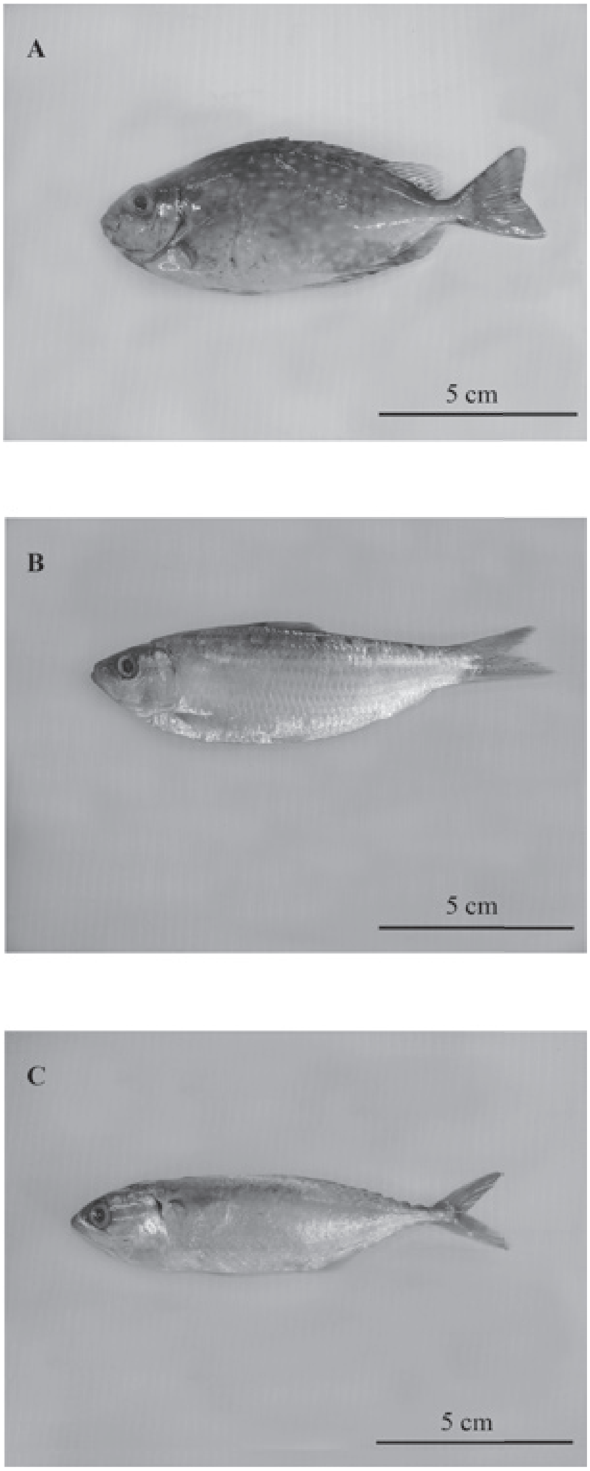

Figure 2. Morphology of fish sampled: *Siganus canaliculatus* (A), *Sardinella albella* (B), *Rastrelliger brachysoma* (C)

RESULTS

This study of domoic acid in fish was conducted in three fish species, *Siganus canaliculatus*, *Sardinella albella* and *Rastrelliger brachysoma*, which are the most abundant in set net fishery in Sriracha Bay, Chonburi Province. These fish species live in different habitats with diverse food requirements and are also popular for human consumption (Department of Fisheries, 1964). Results showed that the domoic acid concentrations in *S. canaliculatus*, *S. albella* and *R. brachysoma* body tissue ranged between 0.32-48.64, ND-61.43 and ND-84.80 ng•g^{-1}, respectively (Figure 3). The highest contamination of domoic acid in the body tissue of *S. canaliculatus* was recorded in May 2012, whereas for *S. albella* and *R. brachysoma*, the highest contamination was recorded in July and January 2013, respectively. Average concentrations of domoic acid in body tissue of *S. canaliculatus* and *R. brachysoma* during the dry season were 14.67 and 32.23 ng•g^{-1}, and 6.28 and 3.10 ng•g^{-1} during the wet season, respectively. For *S. albella*, average concentrations of domoic acid in body tissue during the dry and wet seasons were 23.85 and 46.98 ng•g^{-1}, respectively.

Domoic acid content in the viscera of *S. canaliculatus*, *S. albella* and *R. brachysoma* ranged between 51.66-276.29, 7.93-235.00 and 2.19-283.50 ng•g^{-1}, respectively (Figure 4). Highest contamination of domoic acid in the viscera of *S. canaliculatus* was found in March 2013, whereas *S. albella* and *R. brachysoma* had it highest in September 2012 and January 2013, respectively. Average concentrations of domoic acid in the viscera of *S. canaliculatus* and *R. brachysoma* during the dry season were 223.94 and 162.22 ng•g^{-1}, with 137.84 and 102.92 ng•g^{-1}, respectively, recorded during the wet season. For *S. albella*, average concentrations of domoic acid in the viscera during the dry and wet seasons were 115.33 and 141.83 ng•g^{-1}, respectively.

Results showed that the average domoic acid content in the whole body of fish in Sriracha Bay, Chonburi Province were found in descending order from *S. canaliculatus* > *R. brachysoma* > *S. albella*. Results indicated that domoic acid contamination in the viscera of the three fish species was significantly higher than in body tissue. Figure 5 shows that domoic acid contamination in the viscera of all the fish studied was 70% higher compared to the body tissue throughout the study period.

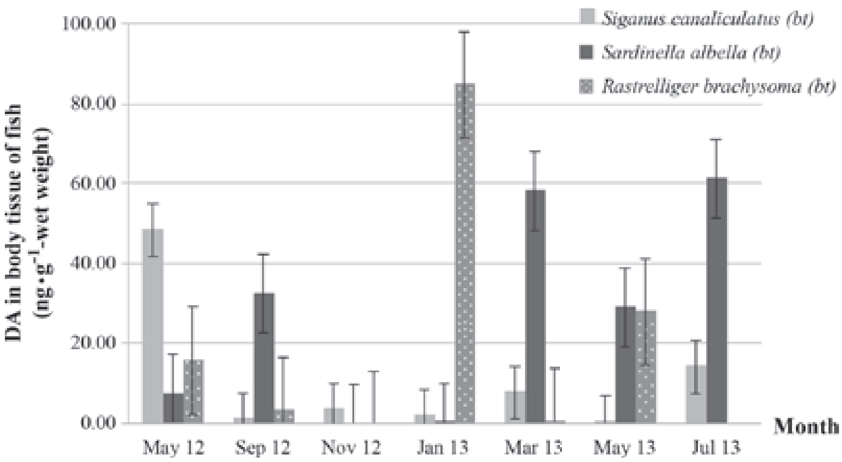

Figure 3. Domoic acid contents in body tissue of fish in set net from Sriracha Bay, Chonburi Province, Thailand, May 2012-July 2013

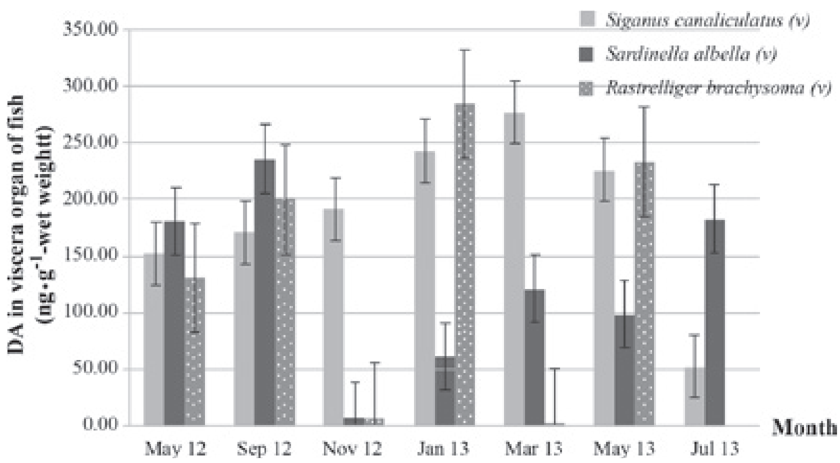

Figure 4. Domoic acid contents in viscera of fish in set net from Sriracha Bay, Chonburi Province, Thailand, May 2012-July 2013

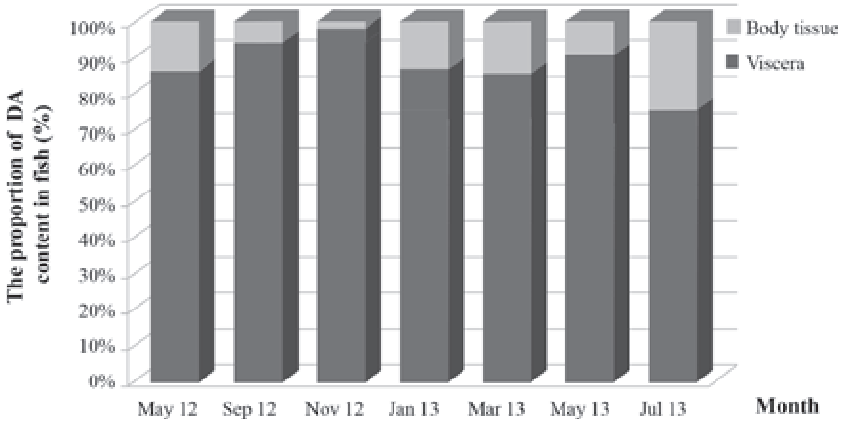

Figure 5. The proportion of domoic acid contents in body tissue and viscera of fish in set net from Sriracha Bay, Chonburi Province, Thailand, May 2012-July 2013

DISCUSSION

Results demonstrated that all species of fish collected from Sriracha Bay, Chonburi Province for this study were contaminated with domoic acid in both body tissue and viscera throughout the year. Lefebvre *et al*. (2002) also found DA in the viscera of fish during the *Pseudo-nitzschia* bloom. They suggested that the increase in *Pseudo-nitzschia* cell densities causing toxin in the viscera was related to the levels of domoic acid in the whole fish. Bates and Trainer (2006) demonstrated that DA production by phytoplankton could be transferred to higher trophic levels. They found that bivalves were the major carriers in transferring domoic acid through the food chain, together with other aquatic organisms e.g. shrimp, crab, fish and benthos. Veschasit (2016) showed that both *Pseudo-nitzschia* sp. and *Nitzschi* sp. had high potential to produce domoic acid. These diatoms are also common species found in Sriracha Bay throughout the year (Veschasit, 2016), implying that fish in this area have a high possibility of contamination with domoic acid.

Our results indicated that *S. canaliculatus* had the highest contamination of domoic acid compared to the other fish species. This was because *S. canaliculatus* lives close to the sea floor and consumes demersal zooplankton and benthic organisms (Department of Fisheries, 1964), which contain high concentrations of domoic acid, since the phytoplankton that produced DA belong to the group of diatoms *Pseudo-nitzschia* spp. and *Nitzschia* spp. (Zabaglo *et al*., 2016). Diatoms are more abundant in bottom water as the silicate inside their cells makes them sink (Wongrat, 1999); they are consumed by demersal zooplankton which are the prey of *S. canaliculatus*. In contrast, *R. brachysoma* and *S. albella* live in surface water and consume both phytoplankton and zooplankton (Department of Fisheries, 1964) thereby accumulating domoic acid directly.

Our results demonstrated that the average concentration of domoic acid in body tissue of *S. albella* during the wet season was 46.98 $ng \cdot g^{-1}$. This level was low when compared with the results of Vale and Sampayo (2001), who found that sardines in Portugal (where the presence of DA affects shellfish resources several times a year) accumulated domoic acid ranging between ND-74.2 $\mu g \cdot g^{-1}$. However, our results concurred with Liefer *et al*. (2013) who examined Gulf Kingfish, striped anchovy and white mullet in the Northern Gulf of Mexico and found levels of domoic acid at 53, 23 and 44 $ng \cdot g^{-1}$, respectively.

Our results also indicated that domoic acid content in the viscera was higher than in body tissue. The average domoic acid content in the body tissue of all fish sampled throughout the year was 13.91% of the viscera (19.94 $ng \cdot g^{-1}$ wet weight), while in the viscera it was 152.54 $ng \cdot g^{-1}$ wet weight, eight-fold higher. Differences found between domoic acid content in viscera and body tissues were low compared to the results from Vale and Sampayo (2001). They recorded average accumulated domoic acid in sardine viscera at 267.2 $\mu g \cdot g^{-1}$, about 103 times higher than in body tissue at only 2.6 $\mu g \cdot g^{-1}$. Moreover, Lefebvre *et al*. (2002) found that levels of domoic acid in the viscera of anchovies and sardines in Monterey Bay, California (where at least two events of planktivorous fish mortality have been recorded from the neurotoxin domoic acid) were higher than in body tissue by 250-1,800 times, with levels in body tissue at 0.2-2.2 $\mu g \cdot g^{-1}$ (0.2±0.1% of viscera). Busse *et al*. (2006) suggested that domoic acid in fish was concentrated in the gut.

Therefore, to increase food safety, toxin concentration can be reduced by removing the viscera before cooking. Domoic acid also accumulated in the brain of sardines ranging between 0.2 and 1.7 $\mu g \cdot g^{-1}$ (Vale and Sampayo, 2001). A comparison of domoic acid contents in the viscera of *S. canaliculatus* (bottom-feeding fish) with *S. albella* and *R. brachysoma* (pelagic fish) showed that the toxic level in *S. canaliculatus* was higher than in *S. albella* and *R. brachysoma* by about 1.5 and 1.3 times, respectively. Domoic acid contamination level in *S. canaliculatus* in our study was lower than that recorded by Busse *et al*. (2006) who found that the viscera of bottom-feeding fish in San Diego, California, where DA was recorded in phytoplankton during a bloom of *P. australis* and *P. multiseries* (50.1 $\mu g \cdot g^{-1}$), contained up to 10 times higher domoic acid content than pelagic fish (5.5 μg•g^{-1}). Lefebvre *et al*. (2002) suggested that the viscera of anchovies accumulated 39 times as much domoic acid than sardines, with the highest content in anchovy at 1,815 μg•g^{-1}.

Seasonal comparison indicated that average domoic acid content in two out of three fish in the set net fishing area in the dry season was higher than in the wet season. This occurred because domoic acid accumulated in fish through consumption of phytoplankton that produced domoic acid, and increasing cell density of phytoplankton occurred during the dry season. The toxin was only present when the fish were feeding or had recently fed on toxic phytoplankton. Generally, the toxin content in planktivorous fish was consistent with the total toxin in the *Pseudo-nitzschia* spp. population in the area, suggesting that toxin was quickly depurated and that fish were only dangerous vectors during the bloom period (Lefebvre *et al*., 2002). However, our results demonstrated that domoic acid content in the three target fish was not high, and lower than regulation levels of 20 μg•g^{-1}. Busse *et al*. (2006) noted that domoic acid depuration of fish was normally fairly rapid, but not completely eliminated. They suggested that because the fish lived in a wide area, they may obtain toxin from elsewhere. Our results indicated that domoic acid accumulated at high concentration in the viscera but low concentration in body tissue at levels that did not threaten human consumers. Moreover, McCarron and Hess (2006) found that cooking fish at 121°C did not reduce the domoic acid concentration. Long-term monitoring of domoic acid contamination in fish regarding food security requires the concern of all stakeholders.

CONCLUSION

Results of the study on domoic acid contamination in three species of fish in set nets in Sriracha Bay, Chonburi Province, indicated that all the three fish species contain domoic acid throughout the year, with the content in visceral organs higher than in body tissues. Fortunately, the contamination of domoic acid in fish in Sriracha Bay is still lower than regulation levels (20 μg•g^{-1}). Our study demonstrated that domoic acid was able to transfer to fish body parts and showed seasonal variation. Average content of domoic acid in *S. canaliculatus* and *R. brachysoma* in the dry season was significantly higher than during the wet season. Long-term monitoring of domoic acid contamination in fish regarding food security requires the concern of all stakeholders especially during the dry season.

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