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Paralytic Shellfish Poisoning Toxin Accumulation in Shellfishes Collected From Various Habitats in Murcielagos Bay, Philippines during Harmful Algal Blooms Occurrence

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ABSTRACT

This study aims to determine whether the habitat of bivalves plays an influence in the occurrence of tropical shellfish toxicity during toxic red tide bloom occurrences in Murcielagos Bay, Misamis Occidental, Philippines. Various shellfish species were collected during the occurrence of red tide blooms. The type of habitat and the shellfish toxicities were investigated. Likewise, the phytoplankton profile in the seawater column was assessed. Results of our study revealed that the occurrence of shellfish toxicities was habitat specific in spite of the fact that the causative organism *Pyrodinium bahamense* var. *compressum* was present in low concentrations in the sampling sites. Shellfish collected from sea grass, coralline area, and seafloor habitats were notably susceptible against the paralytic shellfish poisoning toxin contamination compared to those samples obtained from soil substrate. Continuous monitoring of areas that are affected with shellfish toxicity must be conducted so as to safeguard the general public's welfare dependent on these resources.

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INTRODUCTION

The Philippines has been monitoring for disastrous harmful algal blooms (HABs) or locally known as toxic red tide for several years. These paralytic shellfish poisoning (PSP) cases in the country have been associated to the *Pyrodinium bahamense* var. *compressum* blooms. However, there are reports [1] indicating that there are other marine dinoflagellates that contribute to the occurrence of paralytic shellfish toxins. This is oftentimes reported to affect tropical bivalves, particularly the green mussel *Perna viridis* and the thorny oyster *Spondylus squamosus* in the country. The concern nowadays is the problem associated with toxic blooms, as it results to morbidities and mortalities for those who ingest contaminated seafood and on the losses associated to economic opportunities for shellfish traders who cannot sell the produce in the market systems [2]. Currently, decentralized PSP monitoring is carried out by the national government to help out in preventing the occurrence of poisoning cases in the communities affected by these toxic blooms. Other programs instituted in safeguarding the consumer's health include the imposition of total bans on shellfish harvest during HAB occurrences.

Continuous PSP monitoring of the bivalves are being undertaken to continuously safeguard the concerns of the general populace's health and economy. A study [1] has indicated that the distribution of the toxins in the bivalves varies particularly that a number of factors come into play regarding the occurrence of these toxins in the bivalves. In the Philippines, there is a paucity of literature that investigates the variation of PSP toxin among the bivalves. Likewise, there is limited information pertaining to the environmental conditions where these harmful algal blooms occur in the country. In order to investigate the variation of PSP toxin accumulation among the bivalves in the country, this study was conducted to assess whether the type of shellfish habitat and phytoplankton profile in the seawater column affect the toxin accumulation in the tropical shellfish. Results of this study are vital, as they provide baseline information on the habitat influencing the occurrence of PSP toxin accumulation in the tropical bivalves.

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Methodology:

The study site is in Murcielagos Bay, Misamis Occidental. Murcielagos Bay is recognized as one of those areas in the country that is affected by HABs. The study site is located at the northeastern side of Zamboanga Del Norte and at the boundary of Misamis Occidental. The Murcielagos Bay has three major marine ecosystems: sea grass (2,674.96 ha), mangroves (785.48 ha), and marine sanctuary (27 ha) [3]. It is positioned at the 8°40'0"N and 123°36'0"E. A total of 17 shellfish species obtained from various habitats were collected in Murcielagos Bay, Misamis Occidental. There were three identified sampling sites, as shown in Table 1. In each sampling site identified, different habitats of where the bivalves were situated were noted.

Table 1: Sampling stations and coordinates in Murcielagos Bay, Misamis Occidental.

Sampling stations	Coordinates
1	8°39.167'0"N and 123°35.322'0"E
2	8°39.469'0"N and 123°35.000'0"E
3	8°39.649'0"N and 123°35.730'0"E

Bivalves gathered from the different habitats and sampling sites were properly labeled in zip lock plastic bags, stored at -20°C, and transported to the laboratory for analysis. Bivalves brought in the laboratory were scrubbed and cleaned. Bivalves were measured for their lengths and width and weighed. Bivalves were shucked, and tissues were removed and weighed.

Grab water samples were obtained in areas where the shellfish were obtained. Grab water samples were obtained to determine the physicochemical parameters namely temperature and salinity. Temperature was determined through a thermometer, and the salinity was determined through a refractometer. Both physicochemical parameters were determined in situ. The water samples were collected at the surface and bottom layers using a 2.5-L Niskin bottle (General Oceanics, Canada) to assess the phytoplankton profile in each sampling site. Grab water samples obtained in the sampling sites were fixed with 10% formalin. A 500-mL preserved water sample was concentrated to 20 mL by passing through a sieve with a mesh size of 20 µm for the quantitative determination of phytoplankton cell densities. Phytoplankton cells in 1 mL of concentrated water were counted under light microscopy using the Sedgwick-Rafter chamber. Cell densities were expressed as cells per liter of seawater [4].

Bivalve tissues removed from their shells that were previously weighed were homogenized using a standard blender mixer. Homogenized shellfish tissues were placed into a 50-mL polypropylene centrifuge tube, and 0.1 M HCl was added. The centrifuge tubes containing the mixture were vortex to completely mix the contents. The pH of the mixture was monitored between 3.0 and 4.0. The supernatant liquid was separated by centrifugation at 3,000 rpm for 10 min [5]. The PSP toxin extracts were cleaned up by passing through the column of 10,000 MWCO Amicon-Ultra centrifugal filter units (Millipore, Ireland) to remove the adhering fluorescence-sensitive components that might interfere with the instrumental analysis.

Supernatant extracts were analyzed using the high-performance liquid chromatography (Hitachi-D-7000 HPLC System) with fluorescence detection of the oxidation products. Post column reaction was employed using Merck-LaChrom-L-7350 Reaction Oven at 80°C. All the buffers used for HPLC were freshly prepared and filtered prior to PSP toxin analysis. Aliquots ranging from 10 to 30 µL of the standard solutions of PSP toxins, sample extracts, and blanks were injected into the HPLC system, and the resulting chromatographic peaks as shown in Figure 1 were integrated afterwards [6]. All sample bivalve species were analyzed in triplicates.

PSP toxins namely saxitoxin (STX), neosaxitoxin (neoSTX), decarbamoylsaxitoxin (decSTX), and gaunyotoxin 5 (GTX5) and their specific toxicity relative to the total toxin concentration computation for each tissue were considered accordingly [7]. Results throughout this study were expressed as total toxin concentration in µg STXeq/100 g tissue, which was calculated from µmol/L obtained by HPLC analysis. Dr. Shigeru Sato of Kitasato University, Japan, provided the PSP toxin standards used in the HPLC analyses.

RESULTS AND DISCUSSION

The sampling sites' depth of collection ranges from 0.5 to 4 m in the shallow sea with constant seawater temperature recorded at 30^{o}C during the collection period. The seawater salinities in stations 1 and 2 were 32 and 33 ppt, respectively, whereas the lowest seawater salinity recorded was in station 3, a brackish zone area, at 27 ppt. Stations 1 and 2 were dominated with sea grasses with sandy-muddy substrates, whereas station 3 was purely muddy substrates with mangroves. Results of our study showed that diatoms dominated the phytoplankton profile of the grab seawater samples collected in all the identified sampling stations. Table 2 shows the shellfish samples obtained at the different habitats and sampling stations of Murcielagos Bay, Philippines.

Table 2: Shellfish obtained at different habitats and sampling stations of Murcielagos Bay, Philippines.

Station	Habitats			
	Seafloor (n = 3 per species)	Soil substrate (n = 3 per species)	Sea grass (n = 3 per species)	Coralline area (n = 3 per species)
1	*Strombus urceus* *Tectus fenestratus*	*Tapes dorsatus* *Periglypta lacerata* *Anadara antiquata*	*Atrina vexillum* *Pinna bicolor* *Pinna muricata*	*Spondylus squamosus*
2	*Tectus fenestratus*	*Trachycardium alternatum* *Tapes dorsatus* *Placuna ephippium* *Strombus turturella*	*Pinna bicolor* *Atrina vexillum*	*Spondylus albibarbatus* *Spondylus squamosus*
3		*Telescopium telescopium* *Polymesoda bengalensis* *Lingula unguis* *Katelysia japonica*		

Table 3 shows the shellfish toxicities in all the sampling stations observed. Results showed that the variation in shellfish toxicities were evident at different habitats and among the bivalve species examined. Majority of the shellfish tested exceeded local and international regulation limits of 60 and 80 µg STXeq/100 g tissue, respectively. Highest toxicities were evident in the *Strombus urceus* and *Tectus fenestratus* found in the seafloor of station 1 and in the *Tectus fenestratus* found in the seafloor of station 2. Likewise, high toxicities were also observed in those bivalve species, *Atrina vexillum, Pinna bicolor,* and *Pinna muricata*, obtained from the sea grasses of sites 1 and 2. Those bivalve species, *Spondylus squamosus* and *Spondylus albibarbatus*, obtained in the coralline areas of sites 1 and 2 also showed high toxicities. This occurrence may be attributed to the habitat where the bivalves were obtained, considering that these bivalves are direct filter feeders and the causative organisms suspending in the seawater column may have been eaten by such organisms [8]. On the contrary, the burrowed shellfish species we obtained, namely *Tapes dorsatus, Anadara antiquate, Periglypta lacerata, Trachycardium alternatum, Placuna ephippium, and Strombus turturella*, from both stations 1 and 2 showed low toxicity levels ranging from 3 ± 0.58 to 19 ± 3.1 µg STXeq/100 g tissue. On the other hand, shellfish species burrowing in the soil substrate at station 3 that include *Telescopium telescopium, Polymesoda bengalensis, Lingula unguis,* and *Katelysia japonica* were likewise found to contain minimal toxicities, ranging from 6 ± 1 to 20 ± 1 µg STXeq/100 g tissue. All of these burrowing shellfish species were found to be within the local and international regulation limits for PSP toxin.

It is likely that the low toxicity levels of these bivalves found burrowing in the soil substrate may have been partially exposed to the toxic blooms suspended in the seawater column; hence, low accumulation of the PSP toxins may be accounted for these shellfish species [9]. It is also likely that the variations in the shellfish toxicity observed in various habitats may possibly be the interspecific differences in the toxin accumulation due to the differences in the feeding behavior of the organisms [10].

The highest fraction of 26% of the whole phytoplankton population was that of the *Chaetoceros* sp., and the highest fraction of dinoflagellate was that of the *Noctiluca* sp. Interestingly, the toxic dinoflagellate *Pyrodinium bahamense* var. *compressum* was observed at low fractions in the total phytoplankton profile at only 2% and 3% in sites 1 and 2, respectively. Moreover, it was nil at site 3. Almost similar phytoplankton profiles were evident in all the sampling stations as shown in Table 4. Despite the low fractions of the *Pyrodinium bahamense* var. *compressum* in the seawater column, the presence of the toxic dinoflagellate may heighten the risk of the shellfish in the area to be contaminated with the PSP toxin.

Table 3: Shellfish toxicities at Murcielagos Bay, Philippines.

Habitat/Species	Toxicity level (µg STXeq/100 g tissue)		
	Station 1	Station 2	Station 3
A. Seafloor			
Frilled dogwinkle (*Strombus urceus)*	223.0 ± 15.0	-	-
Top shell (*Tectus fenestratus*)	242.0 ± 17.0	201.0 ± 16.0	-
B. Soil substrate			
Venus clam (*Tapes dorsatus*)	10.0 ± 1.0	11.0 ± 3.0	-
Quahog clam (*Periglypta lacerata*)	5.0 ± 0.6	-	-
Blood cockle (*Anadara antiquata)*	3.0 ± 0.6	-	-
Cockle (*Trachycardium alternatum*)	-	8.0 ± 4.0	-
Windowpane shell (*Placuna ephippium)*	-	14 ± 4.0	-
Dog winged conch (*Strombus turturella*)	-	19.0 ± 3.0	-
Telescope shell (*Telescopium telescopium*)	-	-	8.0 ± 4.0
Mangrove clam (*Polymesoda bengalensis*)	-	-	6.0 ± 1.0
Lamp shell (*Lingula unguis*)	-	-	17.0 ± 4.0
Venus clam (*Katelysia japonica*)	-	-	20.0 ± 4.0
C. Sea grass			
Pen shell (*Atrina vexillum*	352.0 ± 24.0	379.0 ± 10.0	-

Pen shell (*Pinna bicolor*)	502.0 ± 34.0	400.0 ± 10.0	-
Pen shell (*Pinna muricata*)	415.0 ± 21.0	-	-
D. Coralline area			
Thorny oyster (*Spondylus squamosus*)	116.0 ± 5.0	155.0 ± 5.0	-
Thorny oyster (*Spondylus albibarbatus*)	-	134.0 ± 5.0	-

Table 4: Phytoplankton density in Murcielagos Bay, Philippines.

Phytoplankton species	Cell Density (cells/L)		
	Station 1	Station 2	Station 3
Diatoms			
Coscinodiscus sp.	4,000	5,000	5,000
Asterionellopsis glacialis	1,000	2,000	-
Chaetoceros sp.	15,000	16,000	19,000
Rhizosolenia imbricata	2,000	1,000	2,000
Thalassionema nitzschioides	2,000	2,000	-
Navicula sp.	2,000	2,000	6,000
Pleurosigma sp.	6,000	4,000	7,000
Nitzschia sigma	1,000	1,000	-
Pseudonitzschia sp.	1,000	1,000	2,000
Dinoflagellates			
Noctiluca sp.	26,000	24,000	29,000
Ceratium sp.	-	1,000	1,000
Pyrodinium bahamense var. *compressum*	1,000	2,000	-

Conclusion:

The low fractions of *Pyrodinium bahamense* var. *compressum* in the seawater observed indicate that the majority of shellfish present in the area may still be susceptible to contamination of the PSP toxins. Based on the results of our study, habitat greatly influences the toxin contamination of the shellfish. Not all shellfish inhabiting the Murcielagos Bay were contaminated with the PSP toxin.

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