FULL PAPER

Ciguatera in the Philippines: Examining Reef Fish Vectors and Its Causative Benthic Dinoflagellates in Visayan and Sibuyan Seas

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───── A B S T R A C T ─────

Ciguatera Fish Poisoning (CFP) is primarily caused by ingesting reef fishes contaminated with ciguatoxins (CTX) produced by the *Gambierdiscus* species. The unpredictability of this type of food poisoning poses risks to public health and adversely affecting the fish trade industry. This study aimed to provide useful information on ciguatera in the Philippines. Different reef fish species and host-macroalgae for benthic dinoflagellates were collected in Visayan and Sibuyan Seas. Ciguatoxins were extracted from reef fish samples, and toxicity was determined qualitatively using mouse bioassay. Meanwhile, cell density estimation of toxic benthic dinoflagellates isolated from the host-macroalgae was done through microscopy. It was observed that 4.46% of the total reef fish samples were positive with ciguatoxins. Spatially, Carles, Iloilo in Visayan sea had the highest number of toxic specimens belonging to *Epinephelus merra, Lethrinus lentjan, Lutjanus campechanus, Scarus quoyi, Siganus guttatus*, and *Sphyraena barracuda*. Based on data gathered from three sampling sites, fish toxin occurrence is observed to be site-specific. Geographical conditions affect the frequency of toxic samples. Moreover, fish weight is not a good predictor of fish toxicity. For toxic benthic dinoflagellates, *Gambierdiscus* spp. were observed to have the lowest cell density count among other dinoflagellates averaging 7-115 cells per 100 g macroalgae. On the other hand, *Ostreopsis* spp. had the highest average cell density of 118-1,455 cells per 100 g macroalgae, followed by *Prorocentrum* spp. (207-594 cells per 100 g macroalgae). Fish toxicity is directly proportional to the occurrence of benthic dinoflagellates in areas as seen during dry season. Monitoring and management of CFP on identified reef fish vectors and its causative benthic dinoflagellates in the area are necessary to promote food safety and fair trade practice.

1. INTRODUCTION

Ciguatera Fish Poisoning (CFP) is a type of sea-food poisoning primarily caused by the ingestion of reef fishes contaminated with neurotoxins called Ciguatoxins (CTX). The main organism that produces these types of toxin is a dinoflagellate belonging to the genus Gambierdiscus (Adachi and Fukuyo 1979) which colonizes on a variety of coral reef macroalgae commonly found in tropical and temperate waters (between 35°S to 35°N) of the globe (Bagnis et al. 1980). Co-inhabiting with the said dinoflagellate are *Prorocentrum* spp. and *Ostreopsis* spp. In the Pacific, ciguatera has long been recognized as a widely distributed phenomenon affecting many of the island nations (Banner and Helfrich 1964). There are nearly 40,000 reported CFP cases from 17 countries and territories in the Pacific Islands, with an average annual occurrence of about one per 500 individuals from 1998 to 2008 (Skinner et al. 2011).

The Philippines, being an archipelagic

country and part of the biologically diverse Pacific Coral Triangle, is rich in marine resources that include corals, reef fishes, and algae, making it more vulnerable to CFP. The country's susceptibility is heightened by the continuous sustenance of Filipinos on fish as a major protein source. More than 100 CFP and CFP-like cases have been documented in the Philippines from 1988 to 2014. The earliest case recorded was in Basilan, Province in 1988, implicating 19 patients from four families (Chan 2015). In Sibuyan Island, Romblon, 32 cases were suspected as CFP from September 1997 to January 1998, and these were caused by a number of reef fishes locally known as *"manambulao"* (grunt fish), *"isdang bato,"* *"ahaan"* (grunt fish), *"dayang-dayang"* (grunt fish), *"dilis"* (sprat fish), *"lampuyang"* (parrot fish), and *"dugso"* (grunt fish). Patients experienced nausea, diarrhea, vomiting, abdominal pain, muscle weakness, numbness in perioral area and extremities, dysesthesia, ataxia, dysphagia, confusion, protracted pruritus, and athetosis (Montojo et al. 2002). In June 2010, CFP was reported in Iloilo, wherein 22 patients from two families were implicated after consuming red snapper, Lutjanus campechanus (Mendoza et al. 2013). The most recent case was reported in Zamboanga City in Mindanao last 2014, wherein 14 patients experienced shortness of breath, dizziness, and vomiting after eating barracuda fishes (Azanza et al. 2019).

The toxin bioaccumulates in the marine food chain; thus, carnivorous fishes are regarded as higher risk species due to biomagnification. Once humans eat fishes, these lead to CFP. Ingestion of reef fishes contaminated with ciguatoxin produces various gastrointestinal symptoms such as vomiting, diarrhea, nausea, and abdominal pain; neurological symptoms, e.g., tingling of the lips, hands, and feet, and reversal of hot and cold sensations; and cardiac symptoms, e.g., hypotension with bradycardia (Lewis 2006). This type of poisoning is often under-reported and misdiagnosed since there are no visible indicators in the contaminated fishes. Additionally, the toxin is heat stable; thus, cooking the contaminated fish will not eliminate the toxin. Hence, the unpredictability of this type of food poisoning poses risks to the fish trade industry and public health.

The present study documented CFP in the Philippines. Specifically, we detected the presence and seasonality of ciguatoxin in commercially important reef fishes. Additionally, to survey the presence, spatial distribution, and seasonality of toxic benthic dinoflagellates in the targeted areas were determined. Moreover, the study correlated the frequency of positive reef fish samples to the abundance of the toxic benthic dinoflagellates between seasons. Lastly, this study hopes to provide useful information for the regulation and quarantine of commercially important reef fishes in the country.

2. MATERIALS AND METHODS

2.1. Sampling area

The study area (Figure 1) were mainly in the Visayan and Sibuyan seas, specifically in the coastal waters of Carles, Iloilo (CI), Daanbantayan, Cebu (DC), and Cajidiocan, Romblon (CR). The areas were chosen since these are the most productive municipal fishing grounds in the country and where most of the alleged cases of CFP were reported adjacent to these provinces.

2.2. Collection of reef fishes, macrophytes, and toxic benthic dinoflagellates

Monthly collection of commercially important reef fishes from landed catch belonging to eight genera such as barracuda (*Sphyraena barracuda*), parrotfish (*Scarus quoyi*), rabbitfish (*Siganus guttatus*), grouper (*Ephinephelus merra, Plectropomus leopardus, and Variola albimarginata*), snapper (*Lutjanus campechanus*), and emperor fish (*Lethrinus lentjan*) were done thrice during the wet (July to September 2015) and dry seasons (April to June 2016). All fish samples were identified using the book of Motomura et al. (2017). The fishes were photographed and measured for standard length and weight, dissected into flesh, and frozen at -20°C until use.

Simultaneous with reef fish sampling, toxic benthic dinoflagellates were collected, and cell densities were estimated following the procedure described by Tester et al. (2014). Samples of macroalgae, seagrass, and coral rubbles were removed or collected from the substratum and placed inside plastic jars with minimum disturbance. Each seawater sample containing the microalgae collected was shaken vigorously for 5-10 seconds to dislodge the attached dinoflagellates clinging on the macroalgae. Dinoflagellates were collected through a series of sieving (125 μm, 40 μm, and 20 μm). Dinoflagellate cells retained at 20 μm were collected and placed in centrifuge tubes. One to two drops of Lugol's solution were added for preservation. At the laboratory, the collected samples were further diluted to 15 ml to standardize sample volume. The weight of macroalgae
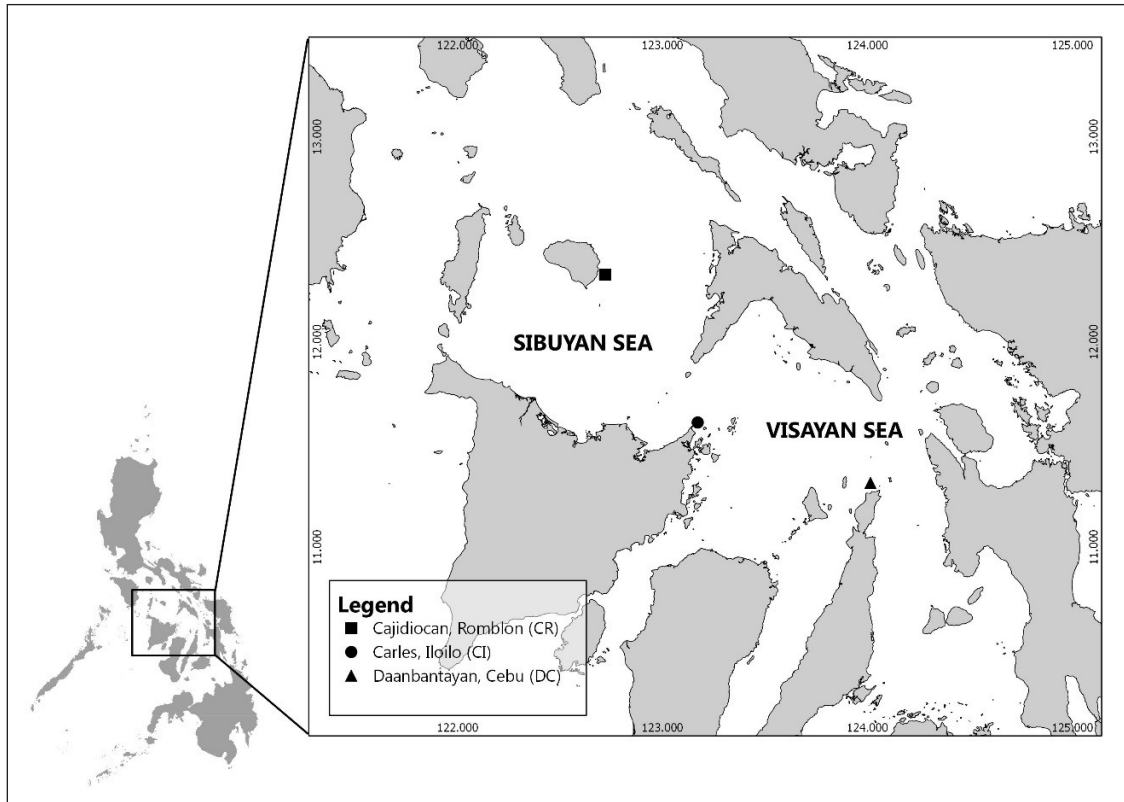
Figure 1. Sampling area

was measured to determine the abundance of dinoflagellates in relation to the macroalgal biomass as number of cells per gram of fresh weight macroalgae.

2.3. Toxicity in reef fishes

Reef fish flesh was extracted and analyzed for CFP using mouse bioassay (MBA) following the procedure described by Oshiro et al. (2010). Flesh sample weighing 120 g was homogenized twice with 350 ml of acetone. The combined acetone extract was evaporated to produce an aqueous concentrate, which was further extracted twice with 100 ml of diethyl ether. The ether extract was evaporated completely and was partitioned between 50 ml hexane and methanol-water (9:1, 25 ml). The aqueous methanol layer was freed entirely of methanol, and the residue was used for mouse bioassay. In all experiments, evaporation of a solution was carried out under reduced pressure using a rotary evaporator.

The standard MBA method was slightly modified to reduce the number of mice used. The extracted residue from fish flesh equivalent to 120 g was suspended in 3 ml of 1% Tween 60 in normal saline solution. Two male mice of ICR strain obtained from the Food and Drug Administration, Department of Health of the Philippines weighing 17-20 g were injected intraperitoneally with 1 ml portion of the suspension. The mice were observed for 24 hours. The survival of the mice determined sample toxicity, such that (1) survival of two mice means that the suspension containing fish flesh extract is nontoxic, (2) death of both mice means it is toxic to the experimental mice, and (3) death of only one mouse implies an indefinite result requiring injection of the remaining 1 ml of the sample suspension into another mouse. The toxicity of the samples with the indefinite result was assessed by the survival or death of the third mouse. Standards for ciguatoxin are still not available in the market; thus, judging the sample as positive will be based on the symptoms stated by FAO (2004) for P-CTX-1 such as piloerection, diarrhea, lachrymation, hypersalivation, dyspnea, wobbly upright gait and gasping. Quantitative analysis of screened positive samples was not possible due to the insufficient amount of fish flesh obtained.

2.4. Estimation of toxic benthic dinoflagellates

The cell densities of *Gambierdiscus* spp., *Ostreopsis* spp. and *Prorocentrum* spp. in macroalgal

samples were determined using microscopy. Samples were processed based on the method described by Tester et al. (2014). One ml aliquot of the filtrate was examined under the light microscope with 10x and 40x total magnification for morphological identification and quantification of toxic benthic dinoflagellates. Each sample was counted thrice for better accuracy. Cell densities were determined using the average abundance of cells in each sample and an appropriate volumetric conversion factor. Concentrations were normalized to the wet weight of the macrophyte, and dinoflagellate-algal substrate relationship was expressed as the number of cells per 100 g of algae. Cell densities were determined using the following formula:

$$\text{Cell density} = \frac{\text{Cell count} \times \left(\frac{\text{Initial Volume of sea water (mL)}}{\text{Final Volume of sea water (mL)}}\right) \times \text{Volume in Centrifuge Tube (mL)}}{\text{Wet weight of macroalgae (grams)}} \times 100$$

2.5. Statistical analysis

Data analyses and all of the descriptive graphs were completed using functions incorporated in the Statistical Package for the Social Sciences (SPSS) version 21. Significant differences were assessed using Student's t-test (normal data) or Mann-Whitney rank-sum test (non-normal data). Shapiro-Wilk test was used to assess the normality of values. Fish weight as a predictor of fish toxicity was assessed using Binary Logistic Regression. Moreover, significant differences in fish toxicity between two seasons and the test for independence of reef fishes to the area were assessed

using Chi-square. A comparison of dinoflagellate cell densities in areas by species between two seasons was assessed using Kruskal-Wallis test.

3. RESULTS

3.1. Fish toxicity and size

Table 1 shows the toxicity profile of reef fish samples collected from three sampling sites. Of the total reef fishes collected, 25 fishes out of 561 samples or 4.46% tested positive with ciguatoxins. *V. albimarginata* (585±49.50 g) ranked first for having the highest toxic proportion among all positive reef fishes collected. This was followed by *E. merra* (443.75 ± 140.81g), *L. lentjan* (535.00 ± 77.78 g), *S. guttatus* (508.00 ± 273.17 g), *L. campechanus* (480.00 ± 28.28 g), *S. quoyi* (400.00 ± 106.77 g), and *S. barracuda* (645.00 ± 77.78 g). No *P. leopardus* sample tested positive for ciguatoxins. Overall, the smallest and largest sample observed belonged to *S. guttatus* weighing 150.00 g and 870.00 g, respectively.

3.2. Spatial distribution and seasonality of fish toxin

Spatially, CI had the highest number of toxic specimens followed by DC in the Visayan Sea and CR in the Sibuyan Sea (Table 2). Among the species examined in CI, *E. merra* (423.33 ± 131.71 g) ranked first for having the highest toxic proportion followed by *S. barracuda* (645.00 ± 77.78 g), *L. lentjan* (535.00 ± 77.78 g), *L. campechanus* (460.00 g), *S. quoyi*

Table 1. List of reef fishes screened for Ciguatoxin

Species	Number of specimens		Proportion of toxic specimen (%)
	Examined (n)	Toxic	
Variola albimarginata	12	2	16.67
Epinephelus merra	99	8	8.08
Lethrinus lentjan	34	2	5.88
Siganus guttatus	129	5	3.88
Lutjanus campechanus	67	2	2.99
Scarus quoyi	134	4	2.99
Sphyraena barracuda	79	2	2.53
Plectropomus leopardus	7	0	0.00
Total	561	25	4.46

(375 ± 77.78 g), and *S. guttatus* (260.00 ± 155.56 g). On
the contrary, in DC, *S. guttatus* (673.33 ± 186.10 g)
had a higher toxic proportion than *E. merra* (505.00
± 205.06 g). Lastly, in CR, *V. albimarginata* (585.00
± 49.50 g) had the highest toxic proportion, followed
by *L. campechanus* (500.00 g) and *S. quoyi* (425.00 ±
176.78 g).

Table 3 shows the seasonality of fish toxins.
A higher number of positive samples was observed
during the dry season. *E. merra* (418.33 ± 146.34
g) had the highest toxic proportion among the reef
fish samples collected, followed by *V. albimarginata*
(585.00 ± 49.50 g), *S. guttatus* (620.00 ± 353.55 g),
S. barracuda (645.00 ± 77.78 g), *L. lentjan* (535.00 ±
77.78 g), and *S. quoyi* (375.00 ± 21.21 g). In the wet
season, *L. campechanus* (480.00 ± 28.28 g) had the
highest toxic proportion, followed by *S. quoyi* (425.00

± 176.78 g) and *S. guttatus* (433.33 ± 256.58 g).

3.3. Distribution and seasonality of toxic benthic dinoflagellates

Table 4 shows the presence of toxic benthic
dinoflagellates in host-macroalgae collected in
the Visayan and Sibuyan seas. Three groups of
dinoflagellates were observed, such as *Gambierdiscus*,
the primary causative organism of CFP, *Ostreopsis*,
and *Prorocentrum*. As seen from Table 4, most of
the host-macroalgae collected were predominantly
phaeophytes (n = 128) followed by rhodophytes (n =
79), chlorophytes (n = 63), coral rubbles, seagrasses
and other macrophytes (n = 87).

Cell densities of different toxic benthic
dinoflagellates were examined in each host-

Table 2. Frequency of toxic samples in all areas

Area	Daanbantayan, Cebu (DC)			Carles, Iloilo (CI)			Cajidiocan, Romblon (CR)		
	Number of specimens								
Species	Examined (n)	Toxic	Proportion (%)	Examined (n)	Toxic	Proportion (%)	Examined (n)	Toxic	Proportion (%)
Epinephelus merra	33	2	6.06	38	6	15.79	28	0	0.00
Lethrinus lentjan	4	0	0.00	29	2	6.90	1	0	0.00
Lutjanus campechanus	28	0	0.00	21	1	4.76	18	1	5.56
Plectropomus leopardus	0	0	0.00	2	0	0.00	5	0	0.00
Scarus quoyi	46	0	0.00	43	2	4.65	45	2	4.44
Siganus guttatus	43	3	6.98	46	2	4.35	40	0	0.00
Sphyraena barracuda	51	0	0.00	17	2	11.76	11	0	0.00
Variola albimarginata	0	0	0.00	0	0	0.00	12	2	16.67
	205	5		196	15		160	5	

Table 3. Seasonality of Ciguatoxin

Species	Dry season		Toxic specimens	Wet season		Toxic specimens
	Examined	Toxic		Examined	Toxic	
Epinephelus merra	21	6	28.57	78	2	2.56
Lethrinus lentjan	34	2	5.88	0	0	0.00
Lutjanus campechanus	17	0	0.00	50	2	4.00
Plectropomus leopardus	7	0	0.00	0	0	0.00
Scarus quoyi	58	2	3.45	76	2	2.63
Siganus guttatus	43	5	11.63	86	0	0.00
Sphyraena barracuda	24	2	8.33	55	0	0.00
Variola albimarginata	12	2	16.67	0	0	0.00
Total	216	19		345	6	

Table 4. Average cell density of toxic benthic dinoflagellates per host-macroalgae. Different ranges of cell densities are shown such as 0 cells per 100 gram (-), 0-250 cells per 100 gram (+), 251-500 cells per 100 gram (++), 501-750 cells per 100 gram (+++), 751-1,000 cells per 100 gram (++++), 1001-5,000 cells per 100 gram (+++++), and ≥ 5,001 cells per 100 gram (++++++).

Division	Genus	Average Cell Density								
		Daanbantayan, Cebu (DC)			Carles, Iloilo (CI)			Cajidiocan, Romblon (CR)		
		Gambierdiscus spp.	*Prorocentrum* spp.	*Ostreopsis* spp.	*Gambierdiscus* spp.	*Prorocentrum* spp.	*Ostreopsis* spp.	*Gambierdiscus* spp.	*Prorocentrum* spp.	*Ostreopsis* spp.
Chlorophyta	*Boodlea* sp.	-	++	+	-	-	-	-	-	-
	Bornetella spp.	-	+	++++	++	++	+	-	-	-
	Caulerpa spp.	+	++	+++	+	+	+	++	+++	+++++
	Codium sp.	+	+	+	+	+	+	++	+++++	++
	Dictyosphaeria sp.	+	++	+++	-	-	-	-	-	-
	Halimeda spp.	+	+	+	+	++	++	+	++	+++
	Halophila sp.	-	-	-	-	-	-	-	+	+
	Halycoryne sp.	-	-	-	-	+	+++++	-	+++++	++++++
	Ulva sp.	-	+	+	+	+	++	-	-	-
	Valonia sp.	-	-	-	-	-	-	-	-	-
Phaeophyta	*Dictyota* spp.	-	+	-	+	++	+	+	+	+++++
	Hormophysa spp.	-	+++++	+	+	+	+	-	-	-
	Jania sp.	-	+++	++	-	-	-	-	-	-
	Leathesia sp.	-	+++++	+++	-	-	-	-	-	-
	Lobophora sp.	-	-	-	-	-	-	+	+++++	++
	Padina sp.	+	++	+	+	+++	+++++	+	++	+++++
	Sargassum spp.	+	++	+	+	+	++++	+	+++	+++
	Turbinaria spp.	-	++	-	+	+	+++	-	-	-
Rhodophyta	*Acanthophora* spp.	+	+	++	+	+++	+++++	-	-	-
	Actinotrichia sp.	+	++	+	+++++	-	+	-	-	-
	Amphiroa sp.	-	-	-	-	-	-	+	+++	++
	Ceratodictyon spp.	-	-	-	+	+	+	-	-	-
	Chondria sp.	-	-	-	-	-	-	-	-	+
	Chondrococcus sp.	-	-	-	+	+	+++++	-	-	-
	Galaxaura spp.	+	++	+++++	+	+	+	-	+	+
	Gelidiella sp.	-	-	-	-	-	-	-	+++	++
	Gracilaria spp.	+	+	+	+	+	++	+	+	++
	Halymenia spp.	-	-	-	+	+	+	+	+	++
	Hypnea sp.	-	-	-	-	+	-	-	-	-
	Kappaphycus sp.	-	++	++++	+	+	+	-	-	-
	Laurencia spp.	+	-	++	-	-	-	+	+++++	++
	Liagora sp.	-	-	-	-	+	+	-	-	-
	Mastophora sp.	+	+	+	-	-	-	+	++	+
	Titanophora sp.	-	-	-	-	-	-	-	+++	++++++
Coral rubbles, sea grasses and other macrophytes	*Bryophyte*	-	-	-	-	-	-	-	-	++
	Coral	+	+	+	+	+	+	++	++	++
	Seagrass	-	-	-	-	-	-	+	+++	+++++
	Sponge	+	+	+	-	-	-	+	+	+
	Thalassia sp.	-	+	+	-	-	-	-	-	-
	Unknown	+	++	++	+	+	++	+	++	++

macroalgae. First, in DC, *Prorocentrum* spp. thrived most on *Hormophysa* spp. having an average cell density of 2,833 cells per 100 g macroalgae. *Ostreopsis* spp. thrived most on *Galaxaura* spp. (1,959 cells per 100 g macroalgae). Comparatively, in CI, *Prorocentrum* spp. thrived most on *Acanthophora* spp. (571 cells per 100 g macroalgae) while *Ostreopsis* spp. thrived most on *Chondrococcus* spp. (3763 cells per 100 g macroalgae). Lastly, in CR, *Prorocentrum* spp. and *Ostreopsis* spp. thrived most on *Halycoryne* spp. (3,146

and 28,173 cells per 100 g macroalgae), respectively. In all areas, *Gambierdiscus* spp. had the lowest cell density counts among all toxic benthic dinoflagellates, but it was observed to have a high density of 1,583 cells per 100 g macroalgae on *Actinotrichia* sp.

Overall, higher cell densities of toxic benthic dinoflagellates were observed during the dry season (Figure 2). *Ostreopsis* spp. had the highest average cell density (118-1,198 cells per 100 g macroalgae) followed by *Prorocentrum* spp. (207-594 cells per

100 g macroalgae). On the other hand, *Gambierdiscus* spp. had an average cell density of 7-116 cells per 100 g macroalgae.

4. DISCUSSION

4.1. Fish toxicity and size

Fish weight is not a good predictor of fish toxicity ($p < 0.05$). This only means that bigger fish does not always test positive. The concept of the food chain theory by Randall (1958), indicating that larger reef fishes are more toxic than smaller fishes, is not always true (Gaboriau et al. 2014). In a recent study by Dao et al. (2018), one family experienced a confirmed Ciguatera poisoning from a two (2) kg body weight of red snapper Lutjanus bohar last June 2016. This study, together with previously conducted studies (Bienfang et al. 2012; Caillaud et al. 2012; Dierking and Campora 2009; O'Toole et al. 2012), revealed that the size of reef fishes is not correlated with fish toxicity. Gaboriau et. al (2014) stated that restricting the consumption to small individuals only, or to individuals below a given size, does not appear to be an effective strategy in managing CFP. Although the total length and weight are useful in describing the morphology of the fish, it does not indicate the actual age, past growth trajectory, and actual physiological condition of the fish. From the data, it can be inferred that larger fishes have higher risks of containing ciguatoxins, but it should be noted that smaller fishes may be toxic as well.

4.2. Spatial distribution and seasonality of fish toxin

According to Lehane and Lewis (2000), ciguatoxins are found mostly and frequently in selected fish species that thrive in warmer subtropical and tropical regions. Samples belonging to genera *Epinephelus* and *Scarus* tested positive in both seasons. While *Lethrinus, Siganus, Sphyraena,* and *Variola* are
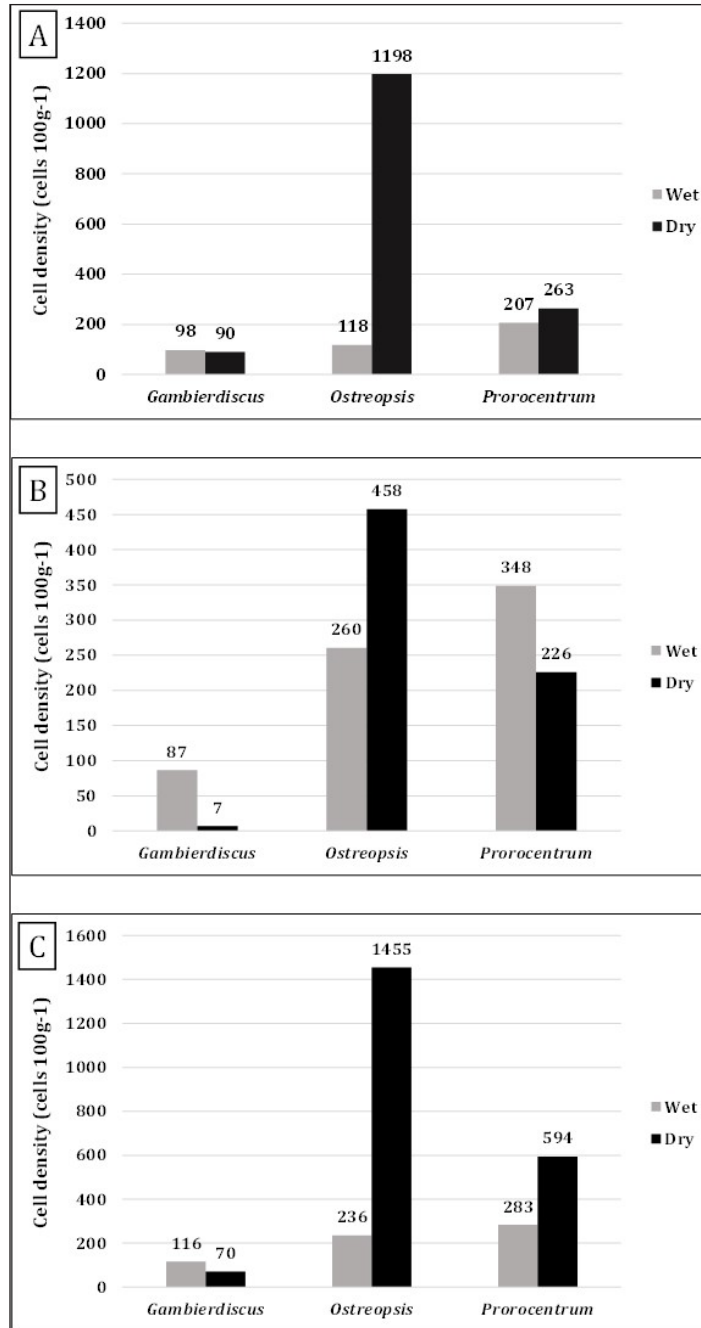

Figure 2. Distribution of toxic organisms in wet and dry seasons. (A) Carles, Iloilo, (B) Daanbantayan, Cebu, and (C) Cajidiocan, Romblon

positive in dry season. Lastly, *Lutjanus* is positive in wet season. Nutrient enrichment and warming sea surface temperatures can stimulate *Gambierdiscus* growth and result in higher cell densities (Parsons et al. 2010). It is evident that the incidence of contamination in reef fish samples from dry season is greater than in the wet season ($p < 0.01$). Since CFP increases dramatically

with increasing sea surface temperatures, it is expected to see more positive samples in the dry season than in wet season.

Based on data from the three sampling sites, fish toxin occurrence is site-specific (p < 0.05). Geographical conditions of the sampling sites affect the frequency of toxic samples. It can be inferred that CI has a higher proportion of toxic fish samples using multiple comparisons of proportion between sampling sites. As observed during sampling, dead corals were present from sampling sites in CI. Yasumoto et al. (1980) pointed out that the dead coral surfaces covered with filamentous or calcareous macroalgae provide a favorable environment for the proliferation of the causative organism of CFP, *Gambierdiscus* spp. Moreover, Lewis and King (1996) reported that *G. toxicus* favors growth following both natural and human-made disturbances of coral reefs. High cell density counts of the toxic organisms in a particular area suggests that the area has a higher risk of CFP.

4.3. Distribution and seasonality of toxic benthic dinoflagellates

It was observed that there is a significant difference between seasons and species of toxic benthic dinoflagellates. A comparison of seasons in all sampling areas was shown in Figure 2 A-C. *Gambierdiscus* spp. and *Ostreopsis* spp. have significant differences between two seasons in CI and CR. On the other hand, all species of toxic benthic dinoflagellates had significant differences in DC. Cell densities of *Gambierdiscus* spp. were highly significant during dry season in all areas while *Prorocentrum* spp. was highly significant during wet season in all areas. Lastly, *Ostreopsis* spp. were found to have a significant difference between seasons.

Several macroalgal samples contained zero cells of dinoflagellates. It was noted that a high number of zero cell counts were observed in *Gambierdiscus* spp. during dry season in all areas. This was in contrast to cell density counts of *Ostreopsis* spp. wherein it was found that the species dominated the other two toxic benthic dinoflagellates in all areas during the dry season followed by *Prorocentrum* spp. Moreover, *Gambierdiscus* spp. had the lowest cell density in all areas. This may be due to the allelopathic activity of the dinoflagellate that inhibits the growth of the other two co-occurring species. This agrees with Skinner et al. (2013), wherein they hypothesized that the three genera of benthic dinoflagellates could be keeping populations of each other in check by allelopathy and species competition.

Cell densities of the toxic benthic dinoflagellates were also determined in each species of macroalgae. Similar to the observations by Litaker et al. (2010), there is no consistent preference for the three toxic benthic dinoflagellates for particular macroalgae. *Gambierdiscus* spp., *Ostreopsis* spp., and *Prorocentrum* spp. thrived on different macroalgae species. The dinoflagellate cell density estimated in this study is relatively lower compared to the findings of Yasumoto et al. (1980), where 400,000 cells per gram of *Gambierdiscus* sp. were found in Gambier Island. Briggs and Leff (2007) implied that controlling the macroalgal population in marine areas exposed to continual human disturbance will be critical in maintaining lower base densities of toxic dinoflagellates. Low cell densities present in the three sampling sites may be attributed to the high level of anthropogenic disturbance, amongst other factors as most selected sampling sites were near the shore. Moreover, using the substrate method as an estimation of the abundance of the toxic benthic dinoflagellates in a specific area does not promote standard sampling procedures, thus acquiring variable observations on benthic dinoflagellates. Tester et al. (2014) demonstrated that the effectiveness of an artificial substrate in observing benthic dinoflagellates is more effective and has numerous advantages over the natural substrate method. This method should be done in future studies to standardize sampling as well as eliminate destructive macroalgal collection in samplings.

4.4. Correlation of frequency of toxic fishes to the abundance of benthic dinoflagellates

As observed, a higher number of contaminated fishes were collected during the dry season (Table 3). Moreover, higher cell densities of toxic benthic dinoflagellates were also observed during the dry season. Fish toxicity is directly proportional to the occurrence of benthic dinoflagellates in areas as seen during dry season. Nevertheless, it should be noted that since high cell density of *Ostreopsis* spp. were observed, the toxicity of the reef fishes can be attributed to other related toxins that have a similar symptom with ciguatoxin.

5. CONCLUSION

Ciguatera and ciguatera-like toxin are present in the Visayan and Sibuyan Seas. Out of 561

reef fish samples, 25 or 4.46% were found positive that belonged to *E. merra, L. lentjan, L. campechanus, S. quoyi, S. guttatus, S. barracuda* and *V. albimarginata.* Moreover, the toxicity of reef fishes is site-specific with Carles, Iloilo having more toxic samples compared to other selected areas. Also, fish weight is not a good predictor of fish toxicity; hence a bigger fish does not always indicate a positive sample. The concept of the food chain theory indicating that larger reef fishes are more toxic than smaller fishes is not always true. As for the causative organism of CFP, *Gambierdiscus* spp. is present in all sampling sites, although observed in low densities (7-1,455 cells per 100 g). Also, other toxic benthic dinoflagellates such as *Ostreopsis* spp. and *Prorocentrum* spp. are present and co-inhabiting with *Gambierdiscus* spp. The main causative organism of CFP, *Gambierdiscus* spp. had the lowest cell density count among all the benthic dinoflagellates. *Ostreopsis* spp. dominated other benthic dinoflagellates in the sampling sites. A higher number of contaminated reef fish samples and benthic dinoflagellates were collected in the dry season. This suggests that fish toxicity is directly proportional to the occurrence of benthic dinoflagellates in areas by season. It should be noted that the toxicity of reef fishes can be attributed to other related toxins due to the observed high number of *Ostreopsis* spp. Lastly, monitoring and management of CFP on seven identified reef fish vectors in the areas are necessary to regulate CFP. This could also support competent authorities, producers, and traders in assuring consumer protection from the standpoint of health and safety and fair practices in the food trade.

6. ACKNOWLEDGMENT

7. REFERENCES

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