

Possible Presence of Ciguatera in Red Lionfish from the Cayman Islands

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Introduction

Ciguatera is an illness which inflicts humans after they consume ciguatoxic fish. The primary source of the toxin in fish is from a marine dinoflagellate, *Gambierdiscus toxicus* which produces a lipid-soluble ciguatoxin and a water-soluble maitotoxin (Caillaud et al. 2010). The dinoflagellate is an epiphyte on marine algae, which is consumed by herbivorous fish (Randall 1958). The toxin builds in their flesh, and when the herbivores are consumed, passes up the food chain. Toxic fish and the toxin, are found circumglobally on tropical reefs from 35° N to 35° S (Randall 1958).

In the Caribbean, the incidence of ciguatera has been reported to be highest in St. Barthelemy, Puerto Rico, and the Virgin Islands, with only a mild incidence in the Cayman Islands and Guadeloupe. Additionally, studies have shown that ciguatoxic fish poisoning has the highest incidence rates in the Eastern Caribbean where surface waters are warmest and stable (Tester et al. 2010).

The Red Lionfish (*Pterois volitans*) is an invasive species, which was first found off Florida's coast in 1992, possibly due to aquarium or accidental releases (Whitfield et al. 2002). Once released, they spread rapidly due to a lack of natural predators and an ample food supply. The lionfish is carnivorous, feeding off of native fishes and arthropods. Since its discovery off Florida, it has spread up the Eastern Seaboard and throughout the Caribbean (Schofield 2009). This spread has prompted many groups to work to help control lionfish numbers through the implementation of culling programs. "Save a Reef, Eat a Lionfish" is a quote often used to promote eating lionfish in order to lower their numbers. Lionfish roundup derbies and a lionfish cookbook are also used as encouragement to catch and eat the fish (Weintraub 2007).

Lionfish with detectable levels of ciguatoxin were first reported in July 2010 from St. Maarten's waters, where native fish tend to have high levels of the ciguatoxin. NOAA/FDA researchers have captured 186 lionfish from the Virgin Islands, Puerto Rico, Antilles, and St. Maarten waters. To date, 76 of the 186 fish caught have been tested; 26% contain levels exceeding the FDA threshold (McFadden 2011). The FDA established action level for Caribbean ciguatera fish poisoning: it has been set at a level of 0.1 ppb (Food and Drug Administration 2011). For this study, lionfish from the Cayman Islands were tested for the presence of detectable levels of ciguatoxin using the brine shrimp nauplii bioassay (Davin 1986).

Methods

In this study, twenty lionfish were collected from all three sister islands making up the Cayman Islands and returned to the lab for analysis. Two freshwater largemouth bass were used as negative controls. The extraction process of the samples followed the procedures of Dickey et al. (1984). Due to their high sensitivity to extracts of ciguatoxic fish, a brine shrimp bioassay was conducted using the extracted samples (Granade et al. 1976).

Tissue Collection Procedure

Supplied samples were evaluated and cataloged based on weight, length, and location of capture. From each fish a 100g tissue sample was taken. The sample was made up of approximately 50% visceral and 50% muscle tissue. After being labeled and freeze dried, the sample was powdered in a coffee grinder, and stored at -20°C.

Isolation Procedure

The freeze dried samples were refluxed at approximately 70°C for one hour. The first reflux used 500mL of reaction grade methanol. The sample was filtered under vacuum using 1.6 micron fiberglass filter paper, saving the methanol. The sample underwent a second reflux in 300mL of methanol for an additional hour. The tissue was then filtered off and discarded. The methanol extracts were combined and then allowed to cool. Once back to room temperature, the filtrate was again filtered through a 1.6 micron fiberglass filter under a vacuum then concentrated in a rotary evaporator. The resulting concentrate was diluted in 25mL of deionized water and approximately 100mL of diethyl ether was added to the sample fraction and mixed, to ensure full inclusion of the sample. The solution was placed in a 200mL separation funnel, and the ether fraction was drawn off and saved. This was repeated for a total of three extractions and the ether fractions were combined and concentrated using rotary evaporation. 25ml of 80% methanol solution was added to the concentrated ether sample. The sample was then washed three times with 50 ml of hexane in a separation funnel and the methanol fractions were collected and subsequently combined, then concentrated using rotary evaporation until light oil formed. The extract was then washed with 50ml of HPLC grade acetone and then heated to 70°C for approximately 15 minutes. The precipitate was filtered off using 1.6 micron fiberglass filter paper under a vacuum. The collected liquid was quantitatively transferred to a previously weighed 35mL vial and concentrated using rotary evaporation under high vacuum until a thick, typically dark brown/orange, oil formed. The complete sample was allowed to cool and weight was recorded and then stored at -20°C.

Brine Shrimp Assay Procedure

Twenty-four-hour-old brine shrimp nauplii were used for the preliminary assay. The freshly hatched nauplii were diluted in a 3.5% instant ocean solution to a concentration of approximately 100 nauplii per milliliter and keep suspended in solution on a stir plate. One-

milliliter aliquots were drawn out and plated in 1 ml cells on a ceramic spot plate (12 cells per plate).

Ten milligram subsamples of each fish extract were drawn off for each trial. The pre-measured subsamples, which had been stored in HPLC grade methanol at -20°C, were warmed to room temperature and dried using a rotary evaporator. The resulting 10mg of extract was then re-suspended in 200µL of a 2.5% Tween 60 solution using a Aquasonic® ultrasonic water bath, resulting in a 50mg/ml Tween suspension.

Each fish sample was tested in triplicate on the spot plate at a concentration of 1mg/ml. To achieve this concentration, 20µL aliquots were drawn off using a micropipette and placed in the cell with the 1 ml solution containing the brine shrimp. This was repeated three times for each sample. One Tween (2.5%) control was run on every test plate (3 cells) and then three fish samples, including the non-toxic control largemouth bass. Typically five plates were run at once accounting for 15 cells of Tween 60 control, 6 cells of bass control, and 39 cells of lionfish extract (13 different fish extracts). The plates were covered to prevent any evaporation and stored in the dark at room temperature for 24 hours.

Each cell was examined after 24 hours using a dissection microscope. The percent of dead shrimp was assessed and when possible the actual number of dead shrimp was counted. The values from all three test cells were average to obtain a single mortality value. Most of the lionfish samples were tested multiple times, in which case all the values were averaged together. The percent mortality of the brine shrimp nauplii were then used to assess the potential presence of ciguatoxin (bioactivity) by comparing the results to the toxicity scale reported by Granade et al. (1976). Extracts resulting in a 40 to 50% mortality at 0.5mg/ml would be classified as mildly toxic and extracts killing more than 50% of the shrimp at 0.5mg/ml or lower would be considered toxic. Any sample that was classified as toxic or mildly toxic would then be tested using the standard mouse bioassay (Hoffman et al. 2002). Since none of the samples tested approached that level of toxicity, the mouse assay was not utilized in this study.

Results

A total of 20 red lionfish were tested from the three sister islands of the Cayman Islands, BWI. Twelve of the fish were caught off of Cayman Brac at unknown locations. These fish had a mean weight of 403g (range 265 to 625g) and a mean total length (TL) of 310mm (273-368mm TL). Three of these fish were female, the sexes of the other nine were not determined. On average, 20.2 grams of viscera and 56.4 grams of flesh were removed from each fish for testing.

Two fish were captured off Little Cayman. Both of these fish were caught at a 35 m depth, and the sex was not known for either. These two fish were small, having a TL of 139 and 153mm and weighing only 93 and 114g, respectively. Due to the small size of these fish, less than 40g of tissue and viscera could be obtained from the fish (less than half the desired amount).

Five fish were caught off Grand Cayman; one off of White Stroke Canyon Wall (70 ft depth), one off of No Dive Zone- Northside (70ft depth), one off of Delwins Delight, two off of the Breakers, and one off South Prospect Beach (40ft depth). These five fish had a mean weight of 295g (161-513g) and a mean TL of 188mm (169-242mm). All of these fish were of unknown sex. An average 17.2 grams of viscera and 62.2 grams of flesh were removed from each fish for testing.

Only three fish samples showed any level of bioactivity from the bioassays; two of these samples were collected from areas off of the south side of Grand Cayman, one on South Prospect Reef and the other on Breakers. The third was collected off Little Cayman. None of the fish caught off of Cayman Brac showed any significant levels of bioactivity. The fish caught off of the Breakers (184g) and the other caught off of Little Cayman (114g) both had weights below the average weight of 341.1 grams for all the samples. Only the lionfish caught off of South Prospect Reef (513g) both showed bioactivity and also had a weight above the average.

T-tests were performed to determine statistical significance, comparing the percent killed in each sample to the percent killed in the Tween control groups. All three samples had a p-value < 0.05, indicating statistically significant difference in the percent killed between these three samples and the control.

Table 1. Brine shrimp bioassay results

Sample	# tests run	Mean kill %	T-test values
Tween	n=12	1.03	x
Neg A	n=2	5	0.14
Neg B	n=3	7.67	
1-13,15-18	n=18	5.33	0.39
14	n=3	19	0.03
19	n=2	36.17	3.83E-05
20	n=2	17.83	2.48E-04

Discussion

None of the lionfish tested from the Cayman Islands showed levels of bioactivity high enough to be classified as ciguatoxic. Only three of the fish showed bioactivity levels significantly higher than the controls, but still well below the threshold to be considered toxic (Davin 1986, Granade et al. 1976). The low levels of bioactivity were measured at a concentration of 1 mg/ml and an LC₅₀ at 0.5 mg/ml is needed to classify the fish as ciguatoxic (Davin 1986). While the United States FDA has established the action level for Caribbean ciguatoxin at 0.1 ppb (Food and Drug Administration 2011), this level may be well below the concentration needed to cause ciguatera poisoning. Ciguatera poisoning does occur from fishes captured around the Cayman Islands; however, there have not been any documented cases of poisoning from the consumption of lionfish. While the lionfish is a predator, it does not feed

nearly as high up on the food chain as other fishes commonly associated with ciguatera poisoning (barracuda, jacks, and grouper) therefore it is not as likely to accumulate sufficient amounts of ciguatoxin to cause ciguatera. Juvenile and young adult lionfish feed mostly upon crustaceans, while mature adults feed upon small fish and crustaceans. Since the toxin bio-accumulates, smaller predators tend to be less toxic than larger adults of that same species (Randall 1961). The largest fish caught and tested was 625 grams in weight and 368 mm in total length. The maximum reported size for a mature lionfish is about 380 mm TL, and so our largest lionfish was near the maximum size. This fish did not test positive, nor did any of the other lionfish caught off Cayman Brac, all of which weighed 200 or more grams. Two of the fish that showed some bioactivity were less than 200 g and while the third weighed 513 g, due to their size, it would be assumed that the principal component of their diet would be crustaceans. Ciguatoxin can be passed through invertebrates to the fishes. Ciguatera was first coined by the Spanish to describe an illness contracted after eating the gastropod *Turbo pica* which was also known as *Cigua* (Scheuer 1976). The Hogfish (*Lachnolaimus maximus*) has been found to be ciguatoxic, even though its diet consists of mollusks, echinoderms, gastropods, and crustaceans (Randall 1958). Tester et al.(2000) showed how brevetoxin, another dinoflagellate produced, lipid-soluble toxin, was able to be passed from copepods to juvenile fish, without causing any harm to the fish as it accumulated in the tissue. Since the diet of smaller and juvenile lionfish is crustacean based, it is possible that smaller lionfish can be ciguatoxic if they are feeding in a location with high concentrations of *G. toxicus*.

Summary

None of the fish tested showed levels of ciguatoxin to be classified as ciguatoxic based solely on the results of the brine shrimp bioassay. Larger fish from suspected hot spots should be examined as an additional safety measure.

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Appendix A.

Fish #	weight (g)	Location	Date	Depth (ft)	% kill
1	360	Cayman Brac			9.33
2	570	Cayman Brac			4.00
3	505	Cayman Brac			2.33
4	295	Cayman Brac			9.33
5	625	Cayman Brac			12.00
6	570	Cayman Brac			4.67
7	535	Cayman Brac			6.33
8	300	Cayman Brac			6.00
9	200	Cayman Brac			1.33
10	310	Cayman Brac			5.67
11	310	Cayman Brac			3.00
12	265	Cayman Brac			2.33
13	93	Little Cayman	7/2/2011	100	3.33
14	114	Little Cayman	7/2/2011	100	19.00
15	277	Grand Cayman- White Stroke Wall	6/30/2011	70	3.67
16	265	Grand Cayman- No Dive Zone, Northside	6/21/2011	70	2.67
17	370	Grand Cayman- Delwins Delight	6/23/2011		8.67
18	161	Grand Cayman- Breakers	6/24/2011		6.00
19	184	Grand Cayman- Breakers	6/24/2011		36.17
20	513	Grand Cayman-South Prospect Reef	6/23/2011	40	17.83
Control LBM #1	211	Tryon, NC	10/9/2011	surface	5.00
Control LMB #2	324	Tryon, NC	10/9/2011	surface	7.67