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Ciguatoxin Prevalence in 4 Commercial Fish Species Along an Oceanic Exposure Gradient in the US Virgin Islands

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Abstract: Ciguatera fish poisoning is a seafood-toxin illness resulting from consumption of fish contaminated with ciguatoxins. Managing ciguatera fish poisoning is complex. It is made easier, however, by local fishers from endemic areas reporting regional predictability for local fish species' ciguatera fish poisoning risk, which the present study then tested. We investigated the prevalence of ciguatoxins in 4 commonly marketed and consumed species (Balistes vetula, Haemulon plumierii, Ocyurus chrysurus, and Epinephelus guttatus) across an oceanic gradient (north, south, east, and west) from the US Virgin Islands. Fish muscle extracts were analyzed for Caribbean ciguatoxins using an in vitro mouse neuroblastoma (N2a) cytotoxicity assay and confirmed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Fish collected from the north location had 0 fish with detectable ciguatoxins; this site also had the greatest wave energy. Caribbean ciguatoxins in fish ranged from 0.01 to 0.11, 0.004 to 0.10, and 0.005 to 0.18 ng Caribbean ciguatoxin-1 eq/g, from the west, east, and south respectively. Ciguatoxin-like activity was detectable by the N2a assay in 40, 41, 50, and 70% of H. plumierii, O. chrysurus, B. vetula, and E. guttatus, respectively. Of the fish collected, 4% had Caribbean ciguatoxin levels exceeding the US Food and Drug Administration guidance of 0.1 ng Caribbean ciguatoxin-1 eq/g fish. These findings concurred with spatial ciguatera fish poisoning prevalence information provided by local fishers in the US Virgin Islands and demonstrate how partnerships between researchers and fishers can aid the improvement of science-based ciguatera fish poisoning management. *Environ Toxicol Chem* 2018;37:1852–1863. Published 2018 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

Keywords: Ciguatera fish poisoning; Ciguatoxins; Caribbean; Fisheries management; Cytotoxicity

INTRODUCTION

Ciguatera fish poisoning is a food-borne illness, endemic to many tropical and subtropical regions of the world. Poisoning occurs after consuming reef fish contaminated with phytoplankton-borne neurotoxins, which are classed as ciguatoxins [1]. The clinical syndrome of "ciguatera" is diagnosed based on a history of eating reef fish and on a clinical presentation of complaints that are defined by gastrointestinal, neurologic symptoms, or a mixed pattern of symptoms [2]. Ciguatoxins are derived from tertiary metabolite precursors produced by benthic dinoflagellates in the genus *Gambierdiscus*. When *Gambierdiscus* spp. cells are ingested by primary consumers, the toxins contained within are bioaccumulated and biotransformed in marine biota, occurring as lipid soluble ciguatoxins in fish. Ciguatera fish poisoning occurs after the consumption of fish containing sufficient concentrations of ciguatoxins [3]. The US Food and Drug Administration (USFDA) established guidance levels for the United States and its territories regarding ciguatoxins originating in the Caribbean (Caribbean ciguatoxins; 0.1 ng Caribbean ciguatoxin-1/g) and Pacific (P-ciguatoxins; 0.01 ng P-ciguatoxin-1/g) [3]. These guidance levels propose nonbinding recommendations for the control of ciguatoxins, such as advising against the consumption of certain species of fish and avoiding purchasing fish from established and emerging areas of concern [4,5].

Ciguatoxic fish from the Caribbean and Pacific regions have been difficult to avoid, both when fishing and purchasing,

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because of the lack of available premarket testing (and thus lack of warning labels). There is an inherent natural spatiotemporal variability of ciguatoxins in fish across species, and among and within islands [6,7]. This spatial variability persists around individual islands where discrete sites or coastlines can be associated with the majority of ciguatera fish poisoning events in a region [8–11]. Based on these observations studies have postulated that environmental conditions and biological processes may influence the abundance and distribution of *Gambierdiscus* and the subsequent trophic transfer of ciguatoxins through the food web [12–15].

To mitigate the occurrence of ciguatera fish poisoning regionally, local fishers have implemented voluntary harvest restrictions based on species and locations. However, fishers and consumers alike need more reliable and accurate data regarding the spatial distribution and frequency of toxic fish. A hyperendemic region for ciguatera fish poisoning is the islands of St. Thomas and St. John, of the northern United States Virgin Islands. The eastern and southern regions are considered higher risk whereas the north coast of St. Thomas is considered by local fishers to be safe [9,11,16–18]. Despite attempts by fishers to avoid ciguatoxic fish from higher risk areas in the United States Virgin Islands, the annual illness rates are significant, at more than 20 times higher than in the state of Florida [19,20]. Annual estimates of ciguatera fish poisoning for Puerto Rico and the United States Virgin Islands range from 20 000 to 40 000 illnesses per year [21].

Some of the most commonly consumed fish species in St. Thomas and St. John are *Balistes vetula* (queen triggerfish), *Epinephelus guttatus* (red hind), *Ocyurus chrysurus* (yellowtail snapper), and *Haemulon plumierii* (white grunt) [19]. They represent approximately 23% of the total artisanal fisheries landings by weight (~72 000 kg of 309 513 kg total) and approximately 22% of the total landed value (USD $793 000 of $3 648 000 in 2012) [22]. The popularity of *B. vetula*, *E. guttatus*, *O. chrysurus*, and *H. plumierii* is likely the result of several factors including: 1) generational knowledge of low-risk species for ciguatera fish poisoning, 2) population abundance of the fish (availability), 3) fishing gear selection, 4) consumer preference, and 5) market value. These species can be found in the same habitats, and they maintain relatively similar mid-trophic consumption levels, but they differ in their ecological niches [19,23]. The home range of these 4 fish species is relatively small and therefore can be considered representative of the local ciguatoxin burden for their consumptive food webs [24–34]. Despite local preference, *E. guttatus* and *B. vetula* have been associated with ciguatera fish poisoning illnesses [35]. Therefore, it is important to understand the spatial variability of ciguatoxin prevalence in these and related species to reduce the ciguatera fish poisoning risk.

In the present study, we selected 4 fish species, *B. vetula*, *E. guttatus*, *O. chrysurus*, and *H. plumierii*, from 4 localized regions, and tested them for ciguatoxins to determine the prevalence and spatial distribution. The ciguatoxin testing procedure chosen for the present study and used by the USFDA for analyses includes a 2-tiered protocol involving: 1) in vitro mouse neuroblastoma (N2a) assay as a semiquantitative screen for neurotoxicity consistent with the ciguatoxin mode of action at voltage gated sodium channels; and 2) liquid chromatography–tandem mass spectrometry (LC-MS/MS) for molecular confirmation of ciguatoxin. All species, except for *O. chrysurus*, were considered to have a variable risk of ciguatera fish poisoning based on prior reports [9]. We examined the relationships between ciguatoxin prevalence in fish with the dominant physical oceanographic properties present at each location. We also tested relationships between fish size and ciguatoxin body burden in the 4 species collected.

MATERIALS AND METHODS

Materials and reagents

All solvents were high performance liquid chromatography grade and purchased from Fisher Scientific, and included acetone, methanol, hexane, chloroform, water, and acetonitrile. Consumables, including serological pipettes, filter capped culture flasks, 96-well polystyrene plates (Corning™ 3596), and Whatman filter paper #4 and #5, were also sourced from Fisher Scientific. Bond-elute silica and amino solid phase extraction (SPE) cartridges were obtained from Agilent Technologies. Mouse (*Mus musculus*) neuroblastoma cells (Neuro-2a, CCL-131) were purchased from the American Type Culture Collection. Culture media and supplements (heat-inactivated fetal bovine serum, glutamine, sodium pyruvate, penicillin-streptomycin, trypsin) and reagents for N2a assay (ouabain octahydrate, veratridine hydrochloride, phosphate buffered saline, dimethyl sulfoxide, and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide [MTT] solution) were obtained from Sigma Aldrich. Caribbean ciguatoxin-1 standards used in the cytotoxicity assay were prepared at the USFDA Gulf Coast Seafood Laboratory, Dauphin Island, AL, and purity was verified by LC-MS/MS before use.

Sample location and collection

St. Thomas and St. John are unincorporated United States insular areas and are located within the Caribbean among the Leeward Islands of the Lesser Antilles. The annual air temperatures range from approximately 22 to 32 °C with water temperatures ranging seasonally from 25 to 30 °C. The annual precipitation is approximately 116 cm^3 (windward sides of the islands tend to receive ~20 cm^3 less rainfall) [36–38].

To examine the potential relationship between ciguatoxin levels in fish and the environmental differences among sites, physical properties dominating these environments were recorded. These included wave properties (e.g., wave power, height, and period), salinity, and temperature. The spatial distribution of the annually averaged wave power was computed for the region surrounding the United States Virgin Islands, where the wave power density ([P] per meter) of wave crest is given by:

$$P = \frac{1}{16}\rho g H_3^2 C_g$$

where ρ is the seawater density, g is the gravitational acceleration, H_s is the significant wave height, and C_g is the

wave group speed, which is a function of the wave period and the water depth at each grid point. Model predictions for significant wave height and wave period were obtained from the CariCOOS Nearshore Wave Model (CNWM), an operational model based on the Simulating Waves Nearshore (SWAN) spectral wave model [39]. The wave model is forced with 2-dimensional spectral wave data from the United States National Oceanic and Atmospheric Administration's National Centers for Environmental Protection Multigrid WaveWatch III Model [40]. Surface wind forcing is provided to the model from the National Weather Service (USA) National Digital Forecast Database, which consists of wind data from the Weather Research and Forecasting wind model run operationally by the National Weather Service, San Juan Office. The CNWM consists of a parent grid and several nested grids with spatial resolution ranging from 1.1 km to 30 m in nearshore regions. For the present study area, data from the parent 1-km grid and the United States Virgin Islands high-resolution grid at 240 m resolution were used. The model has been validated using buoy data, and further details of the validation of the CNWM have been previously described [41,42]. Another example of a previous application of how the wave model has been applied in the United States Virgin Islands is found in Manger et al. [43].

Figure 1 shows the average wave power per unit wave crest computed using model output from August 6, 2013 to August 4, 2014. This dataset was not captured during sample collection but is representative of the prevailing annual conditions of the region.

Fish collection

The present study utilized 3 fish species considered to have some risk of ciguatera fish poisoning based on fishers and hospital reports (*B. vetula*, *E. guttatus*, and *H. plumieri*) and 1 species (*O. chrysurus*) that was considered safe. All fish were caught by licensed fishers using artisanal fish traps inspected by the Department of Planning and Natural Resources (St. Croix, US Virgin Islands) and uniquely labeled before deployment in compliance with Virgin Islands Code Title 12, Chapter 9A, §321 (d). The fish traps had a minimum square mesh size of 5 cm (per side) and escape panels to limit biological impact if the traps were lost (all were recovered). Fish were collected in the United States Virgin Islands' territorial sea, except for the north site located in the United States' marine exclusive economic zone, over a 40-d period between February and March 2011. Fish traps were deployed and samples collected at 4 distinct locations. The location coordinates represent the endpoint of a trap string that was approximately 750 m in length: (see Figure 1 and Table 1). Five individuals of each species were captured from each location with one exception at the eastern site where only 2 *O. chrysurus* were obtained. Depths at the sites were approximately 46 m (north), 29 m (west), 26 m (south), and 35 m (east) and comparable to the range of commonly targeted fishing depths (30–50 m) by St. Thomas fishers [44]. Physical oceanographic differences exist between the sites and are described in Table 1. Following collection, whole fish were placed immediately on ice until they could be stored at –20 °C in secured storage in St. Thomas. Frozen samples were subsequently shipped to the USFDA Gulf Coast Seafood Laboratory in Dauphin Island, Alabama and were received and stored frozen (–20 °C) until extraction and analysis.

Sample preparation and toxin extraction

Fish were thawed and standard morphometrics taken including weight, total length, fork length, and standard length (Table 2). For *E. guttatus* and *B. vetula*, total length was taken at

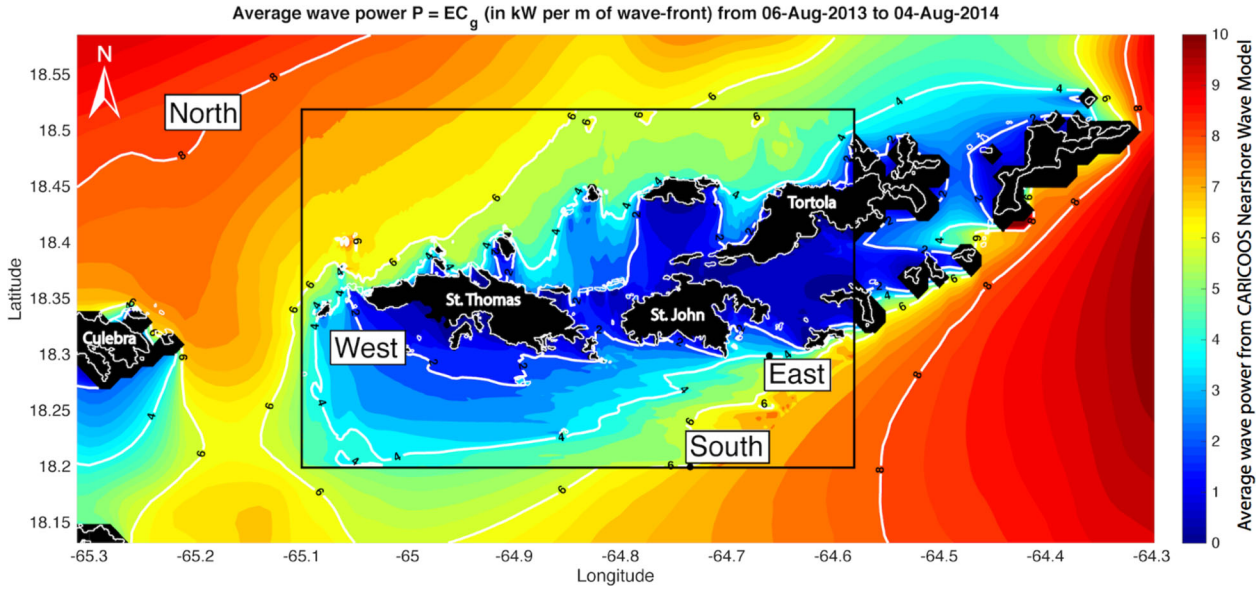

FIGURE 1: Average wave power per unit wave crest from August 6, 2013 to August 4, 2014 computed using model output from the CariCOOS Nearshore Wave Model [36]. The black rectangle shows the boundary of the United States Virgin Islands high-resolution grid, nested within the 1-km resolution parent grid. Site location titles are within the white boxes.

TABLE 1: Sampling locations and the dominant physical oceanographic properties present at each location from 2013–2014[a]

Sampling location	East	South	West	North
Maximum wave power (kW/m)	33.0	47.6	39.8	65.2
Standard deviation wave power (kW/m)	3.51	6.62	2.75	7.64
Mean wave height (m)	0.98	1.25	0.93	1.31
Mean peak period (s)	6.61	7.57	6.10	7.87
Maximum wave height (m)	2.70	3.25	2.96	3.18
Average SST (°C)	28.2	28.2	28.0	27.7
Average salinity (ppt)	35.1	35.1	35.2	35.5
Average concentration (ng/g C-CTX-1 eq)	0.028	0.027	0.018	0.00
Average total toxin (ng)	0.007	0.005	0.003	0.00

[a]Average sea surface temperature and salinity from April–December 2013, including the average concentration and total toxin content of ciguatoxins.
SST = sea surface temperature; C-CTX = Caribbean ciguatoxin.

the broad side of the tail. Muscle tissue (100 g) was excised and skin removed. Total edible flesh was divided by the total weight of the fish to yield a proportion of edible flesh to total weight. The fish muscle samples were extracted as previously described [45]. Importantly, these extraction methods remove potential interferences (e.g., nonpolar lipids, proteins, and hydrophilic compunds) and enrich potential ciguatoxins in a final chloroform extract. Solid phase extraction (Agilent) was then performed to further clean extracts prior to toxicity assessment. An SPE step was performed prior to LC-MS/MS analysis using a 500 mg aminopropyl (NH_2) cartridge (Agilent) as previously described [7] that removed additional matrix interferences causing ionization suppression.

In vitro N2a cytotoxicity assay

An in vitro N2a cytotoxicity assay was used to screen for sodium channel–specific toxins in fish extracts as previously described [45,46]. This functional assay exploits the binding of ciguatoxins to voltage-gated sodium channels, which, in the presence of ouabain and veratridine, results in a dose-dependent loss in cellular viability. Using an MTT reduction assay, viable cells with active metabolism convert MTT into a purple formazan product that can be measured by absorbance at 570 nm. Although not specific to ciguatoxin alone, this N2a assay can distinguish between toxins that activate and inhibit voltage gated sodium channels and is also effective in the assessment of neurotoxic components with modes of action unrelated to the sodium channel (e.g., okadaic acid, which inhibits protein phosphatases). As a screening method, the N2a offers an assessment of composite toxicity, measuring any component in the fish extract affecting neuronal cells, and sensitive detection of sodium channel–specific toxins such as ciguatoxin and brevetoxins. The N2a assay is more sensitive than the traditional mouse bioassay and current analytical methods [47]. In addition, many potentially toxic, but unknown, secondary metabolites may be present in environmental samples, so this method allows a nontargeted estimation of risk that is not possible with selective analytical methods alone.

Neuroblastoma cells were propagated from cryostorage and maintained in Roswell Park Memorial Institute (RPMI-1640) medium supplemented with antibiotics (50 μg/mL streptomycin, 50 units/mL penicillin), glutamine (2 mM), sodium pyruvate (1 mM), and heat-inactivated fetal bovine serum (10% v/v). Cells were harvested for assay when cultures were approximately 85 to 90% confluent and seeded at 4×10^4 cells/well (200 μL volume) into sterile 96-well plates. Dose–response curves (8-dilutions) of Caribbean ciguatoxin-1 standards and sample extracts were prepared with sensitized (+OV) and nonsensitized (–OV) cells. Sensitized cells were used to determine the concentration of extract required to reduce cell viability by 50% (LC50) and compared with a Caribbean ciguatoxin-1 standard (see Supplemental Data, Figure S1). Controls, standard, and sample dilutions were analyzed in triplicate across 2 separate cell passages. Results are expressed as nanogram Caribbean ciguatoxin-1 equivalents wet weight^{-1} (g). The limit of detection (estimated at LC30) for the assay using *O. chrysurus* (species with the lowest detected toxin levels) was determined to be 0.002 ng Caribbean ciguatoxin-1 eq/g [48]. The limit of quantification (where a full dose–response curve was evident without matrix interference) for spiked negative tissue controls was 0.004, 0.005, 0.006, and 0.006 ng Caribbean ciguatoxin-1/g for *E. guttatus*, *O. chrysurus*, *H. plumierii*, and *B. vetula*, respectively. The maximum sample tissue equivalent dose used for quantification was 250 mg tissue equivalent (Supplemental Data, Figure S1D). All samples (including those deemed negative during screening) were further analyzed by LC-MS/MS. Total toxin content expressed in nanogram Caribbean ciguatoxin-1 equivalent was calculated by multiplying the total edible flesh weight for each fish by its respective Caribbean ciguatoxin-1 equivalent concentration per gram of flesh.

Analysis of Caribbean ciguatoxin-1 by LC-MS/MS

Confirmation of Caribbean ciguatoxin-1 in fish extracts was performed by LC-MS/MS using an Agilent 1260 LC system coupled to a QTRAP 4000 mass spectrometer (Applied Biosystems) as previously described [49], with minor modifications. Briefly, analytes were separated by gradient elution using a Kinetex C8 column (75 × 2.1 mm; 2.6 μM; Phenomenex) held at 40 °C. The mobile phase consisted of (A) water and (B) 95:5 acetonitrile: water, both with 0.1% formic acid. The chromatographic conditions were as follows: column equilibration of 10% B (1 min), a gradient from 10 to 95% B at 1.5 min, followed by an isocratic hold at 95% B for 3.5 min, after which the conditions were returned to 10% B for 0.2 min. Between each sample, the column

TABLE 2: Range of fish morphometrics, ciguatoxin concentration, and total toxin content determined by N2a assay for species sampled around the islands of St. Thomas and St. John, US Virgin Islands

Species	Location	Weight (kg)	Edible flesh (kg)	Standard length (cm)	Age (yr)	Concentration (ng/g C-CTX-1 eq)	Total toxin (ng)
Balistes vetula	East	0.86–1.14 ($\bar{x}$ = 0.998)	0.23–0.31 ($\bar{x}$ = 0.272)	28–32.1 ($\bar{x}$ = 30.06)	5–6[a]	0–0.10 ($\bar{x}$ = 0.059)	0–50 ($\bar{x}$ = 16)
	South	0.78–1.02 ($\bar{x}$ = 0.931)	0.20–0.33 ($\bar{x}$ = 0.262)	27.5–29.5 ($\bar{x}$ = 28.6)	4–5[a]	0–0.11 ($\bar{x}$ = 0.049)	0–50 ($\bar{x}$ = 14)
	West	0.74–1.29 ($\bar{x}$ = 1.04)	0.20–0.33 ($\bar{x}$ = 0.248)	26.5–32.0 ($\bar{x}$ = 29.3)	4–6[a]	0–0.03 ($\bar{x}$ = 0.009)	0–10 ($\bar{x}$ = 2)
	North	0.31–0.40 ($\bar{x}$ = 0.350)	0.09–0.11 ($\bar{x}$ = 0.099)	20.5–23.2 ($\bar{x}$ = 21.7)	2–3[a]	0–0 ($\bar{x}$ = 0)	0–0 ($\bar{x}$ = 0)
Epinephelus guttatus	East	0.37–0.66 ($\bar{x}$ = 0.554)	0.13–0.20 ($\bar{x}$ = 0.175)	25.4–30.3 ($\bar{x}$ = 28.2)	5–9[b]	<0.01–0.09 ($\bar{x}$ = 0.033)	<10–30 ($\bar{x}$ = 0.006)
	South	0.35–0.41 ($\bar{x}$ = 0.378)	0.10–0.12 ($\bar{x}$ = 0.107)	23.0–25.0 ($\bar{x}$ = 23.8)	4–5[b]	0.02–0.03 ($\bar{x}$ = 0.023)	≤10 ($\bar{x}$ = 2)
	West	0.41–0.74 ($\bar{x}$ = 0.559)	0.10–0.22 ($\bar{x}$ = 0.173)	24.0–29.8 ($\bar{x}$ = 27.3)	5–9[b]	0–0.18 ($\bar{x}$ = 0.051)	0–30 ($\bar{x}$ = 7)
	North	0.26–0.31 ($\bar{x}$ = 0.283)	0.08–0.10 ($\bar{x}$ = 0.088)	23.0–23.8 ($\bar{x}$ = 23.2)	4–5[b]	0–0 ($\bar{x}$ = 0)	0–0 ($\bar{x}$ = 0)
Haemulon plumierii	East	0.29–0.60 ($\bar{x}$ = 0.380)	0.09–0.17 ($\bar{x}$ = 0.111)	21.0–27.0 ($\bar{x}$ = 23.4)	2–5[c]	0–0.01 ($\bar{x}$ = 0.003)	0–≤10 ($\bar{x}$ = 0.3)
	South	0.31–0.52 ($\bar{x}$ = 0.383)	0.08–0.15 ($\bar{x}$ = 0.106)	22.0–25.5 ($\bar{x}$ = 23.4)	2–5[c]	0–0.09 ($\bar{x}$ = 0.023)	0–10 ($\bar{x}$ = 2)
	West	0.36–0.70 ($\bar{x}$ = 0.523)	0.10–0.18 ($\bar{x}$ = 0.138)	23.0–29.0 ($\bar{x}$ = 26.0)	3–7[c]	0–0.02 ($\bar{x}$ = 0.007)	0–<10 ($\bar{x}$ = 1)
	North	0.26–0.32 ($\bar{x}$ = 0.291)	0.07–0.10 ($\bar{x}$ = 0.083)	21.0–23.2 ($\bar{x}$ = 22.3)	2–3[c]	0–0 ($\bar{x}$ = 0)	0–0 ($\bar{x}$ = 0)
Ocyurus chrysurus	East (n = 2)	0.37–0.37 ($\bar{x}$ = 0.373)	0.15–0.18 ($\bar{x}$ = 0.165)	26.2–26.4 ($\bar{x}$ = 26.3)	5[a]	0–0 ($\bar{x}$ = 0)	0–0 ($\bar{x}$ = 0)
	South	0.38–0.76 ($\bar{x}$ = 0.584)	0.15–0.30 ($\bar{x}$ = 0.201)	25.5–32.0 ($\bar{x}$ = 28.7)	5–10[a]	0–0.02 ($\bar{x}$ = 0.011)	0–<10 ($\bar{x}$ = 2)
	West	0.71–1.10 ($\bar{x}$ = 0.856)	0.29–0.43 ($\bar{x}$ = 0.333)	34.1–38.1 ($\bar{x}$ = 35.42)	10–13[a]	0–0.01 ($\bar{x}$ = 0.004)	0–<10 ($\bar{x}$ = 1)
	North	0.30–0.43 ($\bar{x}$ = 0.370)	0.12–0.19 ($\bar{x}$ = 0.153)	23.0–27.5 ($\bar{x}$ = 25.2)	4–6[a]	0–0 ($\bar{x}$ = 0)	0–0 ($\bar{x}$ = 0)

[a]Estimates based on Manooch and Drennon [68].
[b]Estimates based on Sadovy et al. [70].
[c]Estimates based on Murie and Parkyn [67].
C-CTX = Caribbean ciguatoxin.

was re-equilibrated to starting conditions for 4.3 min to ensure no carryover between samples and standards. The mass spectrometer was operated in positive electrospray ionization mode. Principal instrument settings were: ionspray voltage 5500 V, declustering potential 75 V, entrance potential 10 V, cell exit potential 15 V; collision energy 35 eV; temperature 400 °C; curtain gas 20 psi, GS1 and GS2 both at 60 psi, and collision gas medium. Caribbean ciguatoxin-1 was confirmed by selecting the dehydrated Caribbean ciguatoxin-1 ion $(M + H - H_2O)^+$ as a precursor for the following selected ion transitions: m/z 1123.6 > 1105.6; m/z 1123.6 > 1087.6; and 1123.6 > 1069.6 as previously described [50]. Analyst 1.6.1 software (Applied Biosystems) was utilized for instrument control and data acquisition, and XY data was used to reconstruct chromatograms at high resolution using SigmaPlot (Ver 13, Systat Software).

Statistical analyses

Statistical analyses were performed using JMP software (Ver 9, SAS Institute). The differences between Caribbean ciguatoxin-1 equivalent concentration and total toxin content of ciguatoxins in muscle tissue were investigated with a two-way factorial analysis of variance using ranked data (Friedman's rank test) with harvest locations and fish species as crossed factors (Table 3). A Spearman Rank Order Correlation was performed to investigate the relationship between total toxin content, toxin concentration, total fish weight, and standard fish length.

RESULTS

Concentration and total ciguatoxin content by location and species

Mean Caribbean ciguatoxin-1 equivalent concentration (ng Caribbean ciguatoxin-1 eq g^{-1} fish tissue) in fish from St. Thomas and St. John had distinct spatial and species distribution (Figure 2). The factors of location and species were both significantly different, including the interaction among these factors; indicating that the differences in ciguatoxins around the islands were different among species (Table 3). The percentage of fish containing ciguatoxins at the north, west, east, and south, locations were 0, 60, 65, and 80% at concentrations ranging

TABLE 3: Results of two-way analysis of variance (Friedman's rank test)[a]

	C-CTX-1 concentration			Total toxin content		
Effect test	*df*	*F*	*p*	*df*	*F*	*p*
Whole model	15	48.79	<0.0001***	15	51.90	<0.0001***
Species	3	12.27	0.0065**	3	13.33	0.0040**
Location	3	25.53	<0.0001***	3	26.06	<0.0001***
Species *x* location	9	19.92	0.0184*	9	24.25	0.0039**

[a]To assess the effects of species, location, and the interaction of species and location on the mean concentrations of C-CTX-1 pg eq from fish and the total toxin content in μg C-CTX-1 eq from fish.
*, **, and *** indicate significance at $p < 0.05$, 0.01, and 0.001 probability levels, respectively.
C-CTX = Caribbean ciguatoxin.

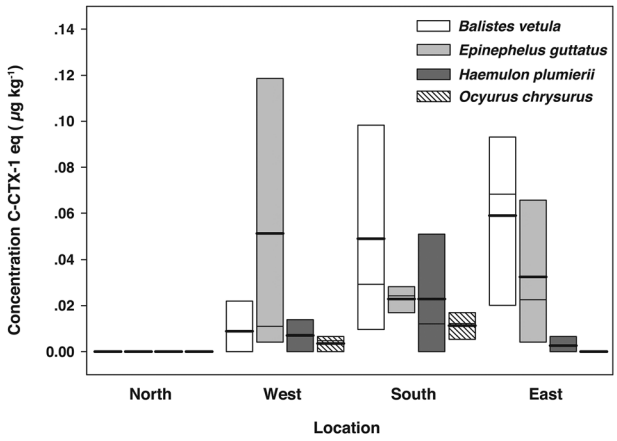

FIGURE 2: Box plots of Caribbean ciguatoxin-1 (C-CTX-1) concentration by location and fish species. Bold horizontal lines indicate the mean concentration, and thin horizontal lines indicate the median. Box top and bottom indicate the 75th and 25th percentiles, respectively, which are the same as the 95th and 5th percentiles in these plots. Individuals of *Balistes vetula* from the south and east, and *Epinephelus guttatus* from the west, exceeded the US Food and Drug Administration guidance level of 0.1 ng Caribbean ciguatoxin-1 eq/g. Results for all box plots represent $n = 5$ except in the case of *Ocyurus chrysurus* from the east location where $n = 2$.

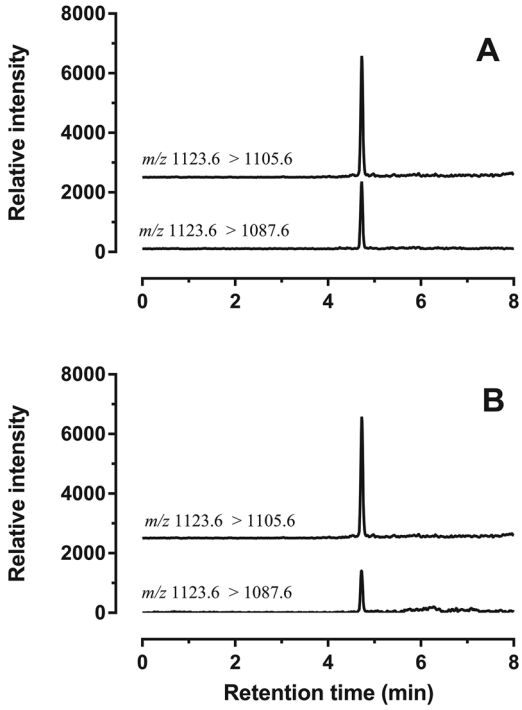

FIGURE 3: (A) Extracted ion chromatogram of a Caribbean ciguatoxin-1 standard, (B) confirmed *Epinephelus guttatus* sample from south location. 1123.6 > 1087.6 in each case was adjusted 2500 units on the *y*-axis for clarity.

from 0 to 0, 0.005 to 0.182, 0.004 to 0.101, and 0.011 to 0.111, ng Caribbean ciguatoxin-1 eq/g fish tissue, respectively. A gradation of mean Caribbean ciguatoxin-1 equivalent concentration by location was identified with highest levels found in fish from the east (0.028 ng/g ± 0.008; mean ± standard error [SE]) and south (0.027 ng/g ± 0.008), and lower Caribbean ciguatoxin-1 levels in fish from the west (0.018 ng/g ± 0.007; Table 1); Tukey post hoc: south (A), east (A), west (A), north (B). Variability in Caribbean ciguatoxin-1 equivalent across the 4-fish species were identified, with the highest in *B. vetula* (0.029 ng/g ± 0.0087; mean ± SE), then *E. guttatus* (0.027 ng/g ± 0.0098), and lower Caribbean ciguatoxin-1 equivalent concentrations in *H. plumierii* (0.008 ng/g ± 0.0043) and *O. chrysurus* (0.004 ng/g ± 0.0015). By species, ciguatoxins were detected in 70% of *E. guttatus*, 50% of *B. vetula*, 41%*O. chrysurus*, and 40% of the *H. plumierii* (with ranges from 0.004–0.182, 0.006–0.111, 0.005–0.019, and 0.006–0.086 ng/g Caribbean ciguatoxin-1 equivalent, respectively, see Table 3).

The total toxin content (ng Caribbean ciguatoxin-1 equivalent) for the muscle tissue of fish from around St. Thomas and St. John had a distinct spatial distribution similar to the concentration of ciguatoxins (Table 3). The gradation of mean total toxin content by species was *B. vetula* (mean ± SE; 7.98 ng ± 1.49), *E. guttatus* (3.79 ng ± 1.49), *O. chrysurus* (0.94 ng ± 1.61), and *H. plumierii* (0.85 ng ± 1.49).

Caribbean ciguatoxin content in relation to size and weight

A series of Spearman rank-order correlations were conducted to determine if there were any relationships between the total ciguatoxin content, ciguatoxin concentration, fish weight, and fish length. There was a weak to moderate monotonic positive relationship between the total toxin content of a fish and the weight. The heavier the fish, the higher the predicted toxin concentration r_s (77) = 0.31, $p = 0.0059$ and total toxin content r_s (77) = 0.38, $p = 0.0007$. The longer the fish the higher the predicted toxin concentration r_s (77) = 0.29, $p = 0.0119$ and total toxin content r_s (77) = 0.37, $p = 0.0009$. There was a strong, positive monotonic correlation between the total toxin content and the measured toxin concentration r_s (77) = 0.99, $p < 0.0001$. A strong positive monotonic correlation between the total weight and the standard-length measurement r_s (77) = 0.88, $p < 0.0001$ was also observed.

Confirmation of Caribbean ciguatoxin-1 by LC-MS/MS

Confirmation of Caribbean ciguatoxin-1 in fish extracts was based on comparison of retention time and the presence of the multiple ion transitions relative to a Caribbean ciguatoxin-1 reference standard (Figure 3). Of the 77 samples: 39 fish samples exhibited positive sodium channel activity by N2a, with 29 (74%) having a composite cytotoxicity ≥ 0.005 ng Caribbean ciguatoxin-1 eq/g. Among them, only 13 (45%) samples were confirmed to contain Caribbean ciguatoxin-1, representing 2 fish species, *E. guttatus* ($n = 12$) and *H. plumierii* ($n = 1$). All samples found to be negative by N2a, were negative by LC-MS/MS. The reduced level of confirmations are likely due to the much lower limits of detection by the N2a assay compared with the LC-MS/MS. Although *B. vetula* was one of the fish species

that exhibited the highest composite cytotoxicity levels, ranging 0.01 to 0.11 ng Caribbean ciguatoxin-1 eq/g, none of these specimens could be confirmed to contain Caribbean ciguatoxin-1 by LC-MS/MS. This is likely a result of the high selectivity gained by the LC-MS and lack of standards for other metabolites and knowledge of Caribbean ciguatoxin metabolites at present. Future investigations will focus on ciguatoxin metabolite formation and elucidation in this species to determine if the sodium channel activity observed in the n2a assay was caused by the presence of ciguatoxin metabolites. In contrast, we could not confirm Caribbean ciguatoxin-1 by LC-MS/MS in any *O. chrysurus* samples because these samples had composite cytotoxicity levels below the detection limits of the LC-MS/MS method at the time of analysis. Efforts to improve detection by LC-MS/MS will be the focus of future studies.

DISCUSSION

The present investigation identified and characterized significant regional and species-specific differences in Caribbean ciguatoxins of marketable fish from the St. Thomas and St. John reef complex. For all sites and samples, 51% of the fish presented cytotoxic activity by the N2a cell assay and 4% (3 of 77 fish) of all the fish sampled during the present study were at or above the USFDA guidance level of 0.1 ng Caribbean ciguatoxin-1 eq/g. Our results were consistent with the locations surrounding St. Thomas and St. John (located in eastern and southern sampling site) described by fishers and historically recognized as harboring ciguatoxic fish [9]. Our LC-MS/MS results confirmed the presence of Caribbean ciguatoxin-1 in a selection of fish from the south, west, and east locations and our N2a cytotoxicity data indicated that some species from these sites exceeded the USFDA guidance level. The highest average concentrations of Caribbean ciguatoxin-1 equivalent was among fish captured from the south and east locations (Figure 1). No fish collected from the north location presented any detectable levels of Caribbean ciguatoxins by the N2a assay. Total toxin content from muscle tissue per fish was significantly different among locations, where average amounts were highest in the south and east locations. These study regions are subject to different wave energy regimes, and *Gambierdiscus* spp. is likely susceptible to being removed from host macroalgae by disturbances. The northern region coincides with the highest energy environment investigated in the present study and contained no detectable levels of ciguatoxins.

The fish with the highest Caribbean ciguatoxin concentration (almost 2-fold above the USFDA guidance level) was *E. guttatus* from the west location (0.18 ng Caribbean ciguatoxin-1 eq/g). All *O. chrysurus* specimens (irrespective of location) were found to be below ($\leq$ 0.02 ng Caribbean ciguatoxin-1 eq/g) the USFDA guidance level of 0.1 ng Caribbean ciguatoxin-1 eq/g. The average total toxin content available per fish was significantly different among species, as *B. vetula* (largest species by weight) had $>$ 2 times more average total toxin content per fish than the next highest, *E. guttatus* (Table 2). Significant relationships between total toxin content, toxin concentration, and fish morphometrics (standard length and weight) were observed, highlighting the potential for toxin estimates based on fish size. The significant differences in ciguatoxin levels observed among sites and species suggest regional and species-specific variability related to their natural habitats, life history, and diet within a ciguatera fish poisoning–endemic region.

Effects of location on ciguatoxins

The United States Virgin Islands is considered a hyperendemic region for ciguatera fish poisoning [51], but as expected from the observations of fishers, considerable spatial variability exists. This was confirmed by the absence of any detectable levels of Caribbean ciguatoxins in fish of all species collected from the northern site of St. Thomas. The benthic habitat in the north is not well characterized; the nearest benthic habitat maps [52] show small amounts of reef/colonized pavement around some of the nearest islands (~21 km to the southeast). Recent work by Groves (S. Groves, 2016, Master's thesis, University of the Virgin Islands, St. Thomas, US Virgin Islands) describes areas in the north that consist of large carbonate banks where coral cover was generally sparse ($<$ 4%), but ranged from 0 to 18% (for depths between 27 and 67 m depth), and in some places approximately 60% of the benthos consisted of macroalgae. The north site is subject to greater wave power and a more intense energy regime (higher maximum wave power and standard deviation) than the other sampling sites (Figure 1, Table 1), because winter swell predominately originates in the Atlantic Ocean, north of the islands. The 3 other sites are significantly sheltered from long-period North Atlantic swells. Wave energy has been documented to influence the abundance and distribution of *Gambierdiscus* because they are not particularly adept at swimming and are susceptible to being dislodged from their host surfaces in higher energy environments [13]. Therefore, if the energy regime in the north is sufficient to remove *Gambierdiscus* cells, then their presence on potential algal food sources and subsequent toxin bioavailability may be reduced in the process. All sites with detectable levels of toxins had at least 4% of fish above the USFDA guidance level (0.1 ng Caribbean ciguatoxin-1 eq/g fish.). Benthic habitat maps for an area near the western site (~7 km east), showed the presence of coral reef/colonized pavement with channels and a large zone (~2 km^2) classified as macroalgae/patchy 50 to 90% [52]. This area has been previously described to host *Gambierdiscus* at approximately 4 cells cm^{-2} when open to grazing and 18 cells cm^{-2} when protected from grazers, and where wave energy regimes observed were insufficient to effectively cause significant differences in *Gambierdiscus* populations between depths of 10 and 20 m [15]. In the present study, toxins were readily detected by N2a in fish collected from the west location at levels averaging 0.018 ng ciguatoxin-1/g and up to 1.8 times above guidance. This site was an energetically more stable environment for the putative source *Gambierdiscus* to inhabit, experiencing less than half the mean wave power of the north (Table 1). We did note that the waters around these islands are subject to different wave regimes, temperatures, and other contrasts inherent in the environmental

conditions due to exposure to Atlantic Ocean water (north and a portion of the west site) or Caribbean Sea water (south and east sites; Figure 1, Table 1). Wave-dominated energetic sites contained fish without ciguatoxins (north site) compared with calmer locations that harbored fish with elevated ciguatoxins levels, which could be related to alterations in the benthic community structure (i.e., the source) and/or fish movements.

The average total toxin content estimated from harvested fish was 2-fold greater in the east (0.006 μg Caribbean ciguatoxin-1 equivalent) than the west site (0.003 μg Caribbean ciguatoxin-1 equivalent). The major benthic substrate around St. John (~75% of the 53.44 km^2 study area) is algae [53]. *Gambierdiscus* is predominately epiphytic [reviewed in [54,55]] and therefore most available benthic surfaces are suitable for colonization (e.g., macroalgae, turf algae, sandstone tiles, and artificial substrates) [15,56–59]. These host surfaces for *Gambierdiscus* are also important food sources for many organisms, creating an introduction point where precursor ciguatoxins enters the food web. Differences in biological benthic cover, composition, and wave energy regime will likely influence the abundance and distribution of *Gambierdiscus*, as is potentially the case in the northern site. *Gambierdiscus*' toxin production can be species- and/or strain-specific, with inherently different toxin consequences for ciguatera fish poisoning [60]. A fluctuation in species composition may play a role in the ciguatoxin variability observed between locations close in relative proximity, such as the west and east sites.

Ciguatera is a serious intoxication that can lead to hospitalization and will continue to have impacts on vulnerable coastal populations until improved management strategies can be implemented [61]. In certain harvest locations, multiple fish containing ciguatoxins were identified, indicating that fish toxicity and harvest location may be related, because fish are likely acquiring toxins within the implicated region. Information provided about ciguatera fish poisoning hotspots by fishers may help to guide studies investigating the regional prevalence of ciguatoxin, benefiting harvesters through increased accuracy and confirmation of historical knowledge. In the present study, areas identified by fishers to contain ciguatoxic fish were confirmed. In addition, these areas more accurately conformed to historic generalizations of toxicity predictors such as size and species, because data collected from fish harvested in these areas were more reliable predictors of ciguatoxin content. A practical example of this method, where size limits are in place for specific species from defined regions, is employed as a response to historical ciguatera fish poisoning events in the fish market in Sydney, Australia [62]. Significant positive relationships between ciguatoxins (both total toxin and toxin concentration) and the size of fish (by standard length and weight) were evident from the data generated in the present study. However, when fish were collected at fringe sites toxin levels were less predictable based on size. For instance, in all species collected from the north site, an area approximately 80 km away from our most toxic site, no ciguatoxin was detected. Therefore, in the north, no predictions related to toxin content of fish based on species, size, or age could be made (besides the fact that ciguatoxins were absent).

Effects of species

All species examined demonstrated cytotoxicity by the N2a assay, indicating their consumptive habits result in ciguatoxin exposure. The species sampled in the present study have historically been shown to consume a diverse assortment of plants and animals inhabiting the northern United States Virgin Islands tropical marine food web, including algae (*B. Vetula*), plankton (*O. chrysurus*), and invertebrates and vertebrates (*O. chrysurus*, *H. plumierii*, *B. vetula*, and *E. guttatus*) [26]. Because of the epiphytic nature of *Gambierdiscus*, many surfaces of the benthic marine food web can harbor *Gambierdiscus* and their inherent toxins. Therefore, the grazing mechanisms and prey items targeted by each of the fish in the present study can lead to unique trophic exposure routes to ciguatoxins and their congeners [63–65]. The highest apex order predator represented in the present study, and which had the highest percentage of individuals containing ciguatoxins at 93% (14 of 15; excluding the north site), was *E. guttatus*. The highest overall concentration of ciguatoxins was found in an *E. guttatus* individual from the west location, exceeding the USFDA guidance level 1.8-fold. Only one *E. guttatus* from the west site tested negative, a possible outlier, because this site is adjacent to a spawning aggregation site and these samples were collected near the spawning season (December–February) [31]. To reach the southwestern spawning area near St. Thomas, *E. guttatus* travel up to 33 km [31]. Therefore, it is possible that this individual may not represent the resident population and the local ciguatoxin burden as found in all species tested. Another species that tested positive for ciguatoxins, *B. vetula*, feeds mainly on primary consumers and has a preference for the long-spined sea urchin *Diadema antillarum* [26]. Almost 70% of *B. vetula* contained ciguatoxins (excluding the north site) with some individuals showing levels $\geq$ 0.1 ng Caribbean ciguatoxin-1 eq/g, indicating that fish feeding on primary consumers (such as *D. antillarum*) in this region are exposed to ciguatoxins and can acquire them at levels that, if consumed, would be concerning for human health. The total ciguatoxin content within muscle tissue was significantly higher in *B. vetula*, exceeding all other species tested by as much as 8 times the total found in *O. chrysurus* and *H. plumierii*, and by >3 times that found in *E. guttatus*. A total of 7.5% of the *E. guttatus* and *B. vetula* were at or above the USFDA guidance level of 0.1 ng Caribbean ciguatoxin-1 eq/g. *Ocyurus chrysurus* and *H. plumierii* have been reported to have a diverse diet [26], feeding on herbivores as well as detritivores, and in the present study were found to have a 58% and 53% probability of containing ciguatoxins, respectively (excluding the north site). Although not tested in the present study, *O. chrysurus*'s and *H. plumierii*'s potentially diverse prey selectivity may prevent them from ingesting excessive amounts of ciguatoxin precursors from *Gambierdiscus* especially when they feed as benthic detritivores or planktivores, where they may ingest prey items that are independent or semiremoved from the trophic routes most commonly associated with ciguatoxins.

Ciguatoxins are lipid-soluble and can bioaccumulate in the flesh over time [1]. The fish surveyed in the present study can

reach maximum ages that exceed 10 yr and lengths in excess of 30 cm [23,24,66–70]. These species have a wide geographical range throughout the greater Atlantic with relatively small spatial home ranges. They typically inhabit a home reef and have a small hunting range (typically <10 km), with occasional spawning aggregation migrations (6–33 km for *E. guttatus*) [24–34]. Because these species are relatively localized in their foraging range, they may serve as indicators of the local ciguatoxin burden for mid-trophic–level finfish. An understanding of the species and the locations where ciguatoxins persist could improve the resolution of ciguatera fish poisoning risk across a wider endemic region.

Fisheries and bidirectional engagement

Currently, there are no rules, regulations, or restrictions on fishing locations formally recognized for ciguatera fish poisoning within the United States Virgin Islands territory; however, fishers have a working knowledge regarding areas of ciguatera fish poisoning risk and facilitated the present study through bidirectional engagement. In St. Thomas, fishers avoid many fish from the south side of the island (e.g., barracuda, mackerel, and hogfish) due to local knowledge of this region as the most ciguatoxic zone (now verified in the present study). These fish are only sold if harvested from the north. The exception to this is yellowtail snapper, which has been harvested safely for many years by local fishers and is considered safe to eat even in the south (consistent with the low toxicity determined for this species in the present study). In general, St. Thomas fishers sell their catch directly to customers, and if poisoned, that person will inform the source fishers. Based on many interactions (with fresh wholesome fish and those fish occasionally causing illness), each fisher makes his or her own decision about ciguatera fish poisoning risk. Local fish consumers reduce their risk of ciguatera fish poisoning by purchasing from the fishers they trust, and fishers meet that responsibility by not selling "risky" fish. The impact of these choices can result in reduced landings due to the avoidance of some areas and selection of smaller fish, both resulting in reduced sales but also benefiting the consumer and the reputation of the fisher. The locations closest to the Virgin Islands Coral Reef National Monument (south and east) produced more fish containing ciguatoxins, and at higher average levels, than regions located farther away (west and north). These data are consistent with observations by fishers (D. Olsen, personal communication). Fishers targeting fish at the border of the marine protected area may be more likely to capture a fish containing ciguatoxins as opposed to other harvest locations examined in the present study. In addition, from a fishery management perspective, the fish from these protected waters show a higher exposure to ciguatoxins, and a potential impact on the fish's fecundity and survival [71]. Based on a trap fishery report for the northern United States Virgin Islands referencing commercial catch reports, the western sampling site was inside the most commonly fished area in the territory, the northern site was located within the second, and the southern and eastern sites were located within the fourth most commonly fished area [44]. Therefore, it appears that fishers of the region have already adapted their harvest efforts to areas found to be lower in ciguatoxin prevalence. However, it is important to note that the north sampling site for the present study (within the second most fished region of the territory) is in a relatively distant part of this region and is specifically described as less heavily fished compared with other locations within this region. Public outreach warnings from the Florida Department of Health for gamefish commonly associated with ciguatera fish poisoning state that "in general, the larger the fish, the greater the potential for poisoning," and data from the present study also showed this relationship in 3 of 4 species tested [72].

Human health impact

Prior research indicated that there had been no increase in ciguatera fish poisoning incidence in the United States Virgin Islands between a 1980 survey and a more recent telephone-based survey conducted in 2010 [19], despite an increase in seawater temperatures over the same period. Radke et al. [19] hypothesized that ciguatera fish poisoning incidences would increase with seawater temperature; however, they observed stability in the occurrence rate. This may be due to the shift in species preference for consumption between 1980 and 2010, during which period fewer ciguatoxin-associated species were harvested, or because imported species have been substituted for the higher risk species reported in the 1980 survey. However, ciguatera fish poisoning incidence levels have not decreased. Therefore, the target species in the present study (*B. vetula* and *E. guttatus*) may still be responsible for some of the incidences of ciguatera fish poisoning described [19]. Because water temperatures are predicted to increase in this area, and seasons for favorable growth conditions extend, species of *Gambierdiscus* may respond favorably, increasing the risk for ciguatera fish poisoning in lower risk species [73–76].

Technical barriers

Many *B. vetula* samples examined in the present study were deemed positive for ciguatoxin-like activity by the N2a assay (Figure 2, Table 2, Supplemental Data S1-C), but could not be confirmed by LC-MS/MS. This suggests that Caribbean ciguatoxin-1 may not be the best biomarker to confirm the presence of Caribbean ciguatoxins in all species from this region and highlights the need for an improved methodology that incorporates additional toxin congeners. Although to date no ciguatera fish poisoning outbreaks have been reported to the USFDA from the consumption of *B. vetula*, ongoing work by the authors of the present study has found evidence of ciguatoxin metabolites that are associated with sodium channel activity by N2a from *B. vetula* (A. Robertson and C.R. Loeffler; unpublished data). Additional studies have found that multiple Caribbean ciguatoxins, even from within the same fish, contribute to the fish toxicity [3,77,78]. Although diet was not assessed in the present study, *B. vetula* is thought to feed directly on macroalgae as well as macroalgal herbivores such as *Diadema antillarum*. Both food items are directly associated with *Gambierdiscus*, the primary source of Caribbean ciguatoxin congeners. The sodium channel

activity in *B. vetula* could be explained by the presence of other minor toxic components resulting in sodium channel activity, including possible congeners of Caribbean ciguatoxin-1. This information highlights the importance of using a tiered analysis approach that incorporates an assessment of composite toxicity (such as the N2a assay used in the present study) in determining the risk of fish consumption and emphasizes the need to improve detection methods for ciguatoxin congeners, derivatives, and precursors.

CONCLUSIONS

Predicting ciguatera fish poisoning using species, size, and location information to prevent human health impacts has been the goal of consumers and harvesters alike. In regions where ciguatera fish poisoning is endemic (e.g., the United States Virgin Islands), contracting ciguatera fish poisoning is not guaranteed, and local fish sources as food are in demand. Therefore, fish and local waters are subject to fishing pressures to meet demand, putting consumers at risk for ciguatera fish poisoning. Historical knowledge has been used to guide local fishers when choosing harvest locations and selecting fish species in an effort to decrease the occurrence of ciguatera fish poisoning in the United States Virgin Islands, with varying degrees of success. Local fishers are aware of locations where ciguatoxic fish occur, often reporting knowledge of discrete regions and species of ciguatoxic fish. We worked with local fishers in an endemic region for ciguatera fish poisoning and largely confirmed their predictions of ciguatoxic locations. In the present study, we evaluated sites in 4 regions fishers described as having a gradient of ciguatoxins, and we found no evidence of ciguatoxins from the only site historically recognized as being safe (the northern site). The water regime in this northern location was different from the other harvest locations in that it was the most energetic. These conditions inhibit the growth and attachment of *Gambierdiscus*, potentially resulting in undetectable levels of ciguatoxins in fish for this region. All species investigated were impacted by ciguatoxins, and a majority of fish tested had detectible levels of ciguatoxins. This indicates that the food webs of these 4 species directly interact, to a varying degree, with precursor molecules of ciguatoxins produced by *Gambierdiscus*. We identified significant positive relationships between ciguatoxins and the easily observable morphometrics of fish (size and weight), indicating that efforts in other regions to visually screen suspect species from historic ciguatera fish poisoning regions may have merit. These methods could be further explored in endemic regions that do not currently employ these screening techniques to test their validity and how spatially conserved these relationships are. Although this positive correlation was identified, the small sample sizes represented across field sites and species limit our ability to make conclusions associated with size, and it is highly likely that age, diet, and associated trophic level are also important, and so should be tested in a wider array of species in future studies.

At this point, it is unknown what the human health implications are for long-term low dose exposures to ciguatoxins. However, ciguatoxin levels greater than the USFDA guidance level were detected in representatives of 2 of the species, which raises concern for the acute risk of ciguatera fish poisoning for local consumers. This is particularly true because these species are commercially targeted and commonly consumed. The exact underlying cause of spatial and species differences observed with ciguatoxins remains unknown and additional research is needed to identify the cause of the observed differences in ciguatoxin content (e.g., wave energy, temperature, prey items, etc.). Based on our modest data set, the present study showed that species, size, and physical oceanic energy exposure are significant factors in the accumulation and distribution of ciguatoxins, but we cannot discount other factors. Given the confirmation of local knowledge regarding ciguatoxin prevalence during the present study, and given that these trends have persisted through time, it may be possible for managers and fishers to use historical information and data (e.g., species, size, and physical environment of specific harvest locations) to support temporary decisions to reduce the ciguatera fish poisoning incident rate in a hyperendemic region.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4137.

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Data Availability—Data are available from the corresponding author (Chrisrloeffler@gmail.com).

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