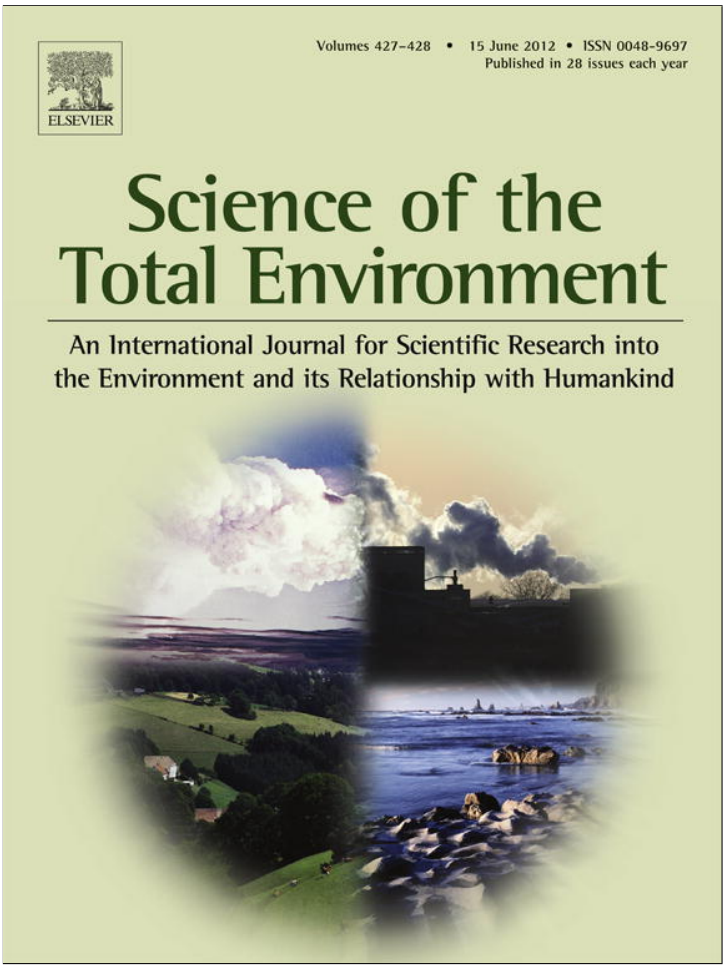

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Linking ciguatera poisoning to spatial ecology of fish: A novel approach to examining the distribution of biotoxin levels in the great barracuda by combining non-lethal blood sampling and biotelemetry

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ABSTRACT

Ciguatera in humans is typically caused by the consumption of reef fish that have accumulated Ciguatoxins (CTXs) in their flesh. Over a six month period, we captured 38 wild adult great barracuda (*Sphyraena barracuda*), a species commonly associated with ciguatera in The Bahamas. We sampled three tissues (i.e., muscle, liver, and blood) and analysed them for the presence of ciguatoxins using a functional *in vitro* N2A bioassay. Detectable concentrations of ciguatoxins found in the three tissue types ranged from 2.51 to 211.74 pg C-CTX-1 equivalents/g. Blood and liver toxin concentrations were positively correlated ($\rho = 0.86$, $P = 0.003$), indicating that, for the first time, blood sampling provides a non-lethal method of detecting ciguatoxin in wild fish. Non-lethal blood sampling also presents opportunities to couple this approach with biotelemetry and biologging techniques that enable the study of fish distribution and movement. To demonstrate the potential for linking ciguatoxin occurrence with barracuda spatial ecology, we also present a proof-of-concept case study where blood samples were obtained from 20 fish before releasing them with acoustic transmitters and tracking them in the coastal waters using a fixed acoustic telemetry array covering 44 km^2. Fish that tested positive for CTX may have smaller home ranges than non-toxic fish (median distance travelled, $U = 2.21$, $P = 0.03$). Results presented from this study may help identify high risk areas and source-sink dynamics of toxins, potentially reducing the incidence and human health risk of ciguatera fish poisoning. Moreover, development of the non-lethal sampling approach and measurement of ciguatera from blood provide future opportunities to understand the mechanistic relationship between toxins and the spatial ecology of a broad range of marine fish species.

1. Introduction

Ciguatera fish poisoning is a type of human poisoning caused by the consumption of reef fish contaminated with ciguatoxins (CTXs) that occurs in tropical and subtropical regions of the Caribbean Sea, Pacific Ocean, and Indian Ocean (Lehane and Lewis, 2000; Dickey and Plakas, 2010). It is estimated that more than 25,000 cases of ciguatera fish poisoning are reported worldwide every year (Lewis, 2001) and may cause severe and potentially long-lasting human health effects including gastrointestinal distress, neurological disturbances, and occasional cardiovascular problems (Swift and Swift, 1993; Lehane and Lewis, 2000; Lewis, 2001). CTX is produced by dinoflagellates (genus *Gambierdiscus*) often found inhabiting warm, shallow waters in association with various macroalgal species or in recently disturbed or altered marine habitats (Yasumoto et al., 1977; Bagnis et al., 1980; Lewis, 1986; Ruff, 1989; Grzerbyk et al., 1994). CTX produced by *Gambierdiscus* spp. biomagnifies through marine food webs reaching peak concentrations in apex predators such as great barracuda (*Sphyraena barracuda*), snappers

Abbreviations: CTX, ciguatoxin; C-CTX, Caribbean ciguatoxin; MDT, median distance travelled; MLD, minimum linear distance; TE, tissue equivalents.

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(*Lutjanus* spp.), moray eel (*Gymnothorax* spp.) or jacks (*Caranx* spp., Vernoux et al., 1985; Randall, 1958; Lewis and Holmes, 1993). The toxin is typically most concentrated in visceral tissue (Vernoux et al., 1985; Swift and Swift, 1993), yet relative distribution of CTXs in liver versus muscle may vary significantly within and between fish species and between tissues within an individual fish. For example, in the Pacific, moray eels with high toxicity detected in liver present low to non-significant toxin concentrations in their flesh (Yasumoto and Satake, 1996; Helfrich et al., 1968). As well, the average toxin concentration ratio of liver to flesh is found as high as 43:1 in green moray eels (*Gymnothorax funebris*) collected in the French Caribbean (Vernoux et al., 1985). In contrast, other groups including Scombridae and Carangidae showed a liver to flesh ratio for CTX of around 2:1, and those fish generally accumulated ciguatoxins mainly in their muscle (Vernoux et al., 1985).

Many challenges are associated with the prediction, diagnosis, and treatment of ciguatera poisoning, especially within island and coastal communities that are highly affected due to reliance on reef fish as a main protein source (Lewis, 1986, 2001). Ciguatoxin is odourless and colourless, and the origin of a particular fish at the time of sale or ingestion is often unknown (Lewis, 1986; de Sylva, 1994). Moreover, low concentrations often make its detection difficult and there remains a need for a reliable method to help identify and prevent consumption of contaminated fish. Laboratory analyses have been developed to effectively detect the presence and concentration of CTXs, however these methods traditionally require invasive procedures and lethal sampling of tissues such as liver and muscle (Pottier et al., 2003; Bottein Dechraoui et al., 2005; 2007; Lewis et al., 1999, 2009). Thus, there is a need to develop a non-lethal sampling technique that will enable researchers to understand the ecological correlates associated with the accumulation of CTXs in marine fish.

Very little is known about the behavioural ecology (i.e., spatial ecology and foraging habits) of great barracuda and currently there is a lack of ecological studies conducted on fish contaminated with CTXs. Non-lethal sample collection combined with behavioural studies (i.e., telemetry) is a reasonably recent innovation that has the potential to elucidate the ecology of free-living wild fish (Cooke et al., 2008). Great barracuda are commonly associated with ciguatera fish poisoning due to their ecological position as apex reef predators and nearshore accessibility for anglers and subsistence fishers (Lewis and Holmes, 1993; de Sylva, 1994; Dunaway, 2008). Monitoring the behaviour of high-risk species such as great barracuda in the wild, while simultaneously determining if they have been exposed to CTXs will help improve the understanding of the occurrence and geographical distribution of CTX. Various anecdotal reports have led to the belief that great barracuda caught in some areas are unsuitable for consumption, whereas fish captured in adjacent areas are considered safe to eat by humans (e.g., inside of a reef versus outside, on one side of an island versus the other). Without an explicit understanding of the links between toxin accumulation and fish spatial ecology, it is questionable whether ciguatoxins are accumulated locally or if it is acquired elsewhere by migratory fish that move into the local area. The primary objective of the study was to identify a non-lethal sampling method for detection of ciguatoxin in wild fish. We also present a case study to demonstrate the potential for using non-lethal tissue sampling to link great barracuda spatial ecology to the occurrence of CTXs. Given the novelty of this approach, we consider this to be a proof-of-concept study as opposed to an exhaustive study. It is our hope that this work will stimulate future research using the techniques developed and tested here.

2. Material and methods

2.1. Field sampling

Between August 2008 and February 2009, adult great barracuda ($n = 38$) were captured off the coast of Cape Eleuthera, The Bahamas (24°54′N; 76°20′W). Located along the edge of the Grand Bahama Bank at the junction of the deep Exuma Sound, the waters surrounding Cape Eleuthera support a range of habitat types including tidal flats, mangrove creeks, patch reefs, seagrass beds, and deeper offshore environments. All barracuda were captured by trolling with artificial lures and heavy action recreational fishing gear (i.e., 14 kg [30 lb] test fishing line; details in O'Toole et al., 2010a, 2010b), apart from two barracuda liver samples obtained from a local fisherman who had captured the fish within the study area. Upon landing, the fish were held in a large cooler and placed into a supine position while a 2 ml blood sample was obtained via caudal venipuncture using a 3 ml vacutainer (lithium heparin coated; B-D Inc, New Jersey) and 3.8 cm (1.5″) 21 gauge needle (Fig. 1). The blood sampling process typically required <1 min to complete. Blood samples were immediately placed in an ice-water slurry and transferred to Eppendorf tubes (in replicate) upon return from the field (<3 h post-collection). One replicate was refrigerated and the other was frozen in an ultracold −80 °C freezer. All fish were measured for total length. Of the 38 great barracuda included in the study, 30 were sampled for blood and 11 barracuda were lethally sampled to obtain whole liver and/or muscle (≥1 g) samples. Liver and muscle biopsies were not taken from released individuals. All liver and muscle samples were frozen in an ultracold −80 °C freezer until ready for toxin analysis.

2.2. CTX analysis

Blood aliquots (100 μl) were vortex-mixed for 1 min with three volumes of acetonitrile then stored for 15 min at −20 °C to facilitate protein precipitation. Samples were then centrifuged for 15 min at 3000 × *g*, at 4 °C. The supernatant was collected, dried under nitrogen flux, and resuspended in 100% methanol. This method of ciguatoxin extraction from whole blood samples was found to recover 62% of the Caribbean ciguatoxin C-CTX-1 (Bottein Dechraoui et al., 2007).

Ciguatoxin from tissue samples were extracted as previously described (Murata et al., 1990; Lewis et al., 1991; Bottein Dechraoui et al., 2005) with some modifications. Tissues were minced and extracted in acetone (3 ml/g of tissue) by homogenization, probe sonication on ice (1 min), and centrifugation for 5 min at 3000 × *g*. Supernatants were collected and pellets were again extracted by vortex mixing for 30 s, sonication for 1 min and centrifugation as described above. Both supernatants were combined in glass test tubes and dried under nitrogen flux at 50 °C. The dried residue was dissolved in 3 ml of 90% aqueous methanol by sonicating and vortex mixing and the aqueous methanol was extracted twice with 3 ml n-hexane by vortex mixing and phase separation by

Fig. 1. Photograph of a blood sample being obtained from a great barracuda (*S. barracuda*) via caudal venipuncture using a 3 ml lithium heparin vacutainer and 3.8 cm (1.5″) 21 gauge needle.

gravity. Both methanol fractions were combined and dried under nitrogen as described above. The dried residue was dissolved in 3 ml of 25% aqueous ethanol by sonication and vortex mixing, and extracted twice with an equal volume of diethyl ether. The diethyl ether layers were combined and evaporated under gentle nitrogen flux and resuspended in methanol in Varian screw cap vials at a concentration of 2 g tissue equivalents/ml, and stored at −20 °C until analysis. Toxin recovery from fish using similar method was estimated at 63% (Lewis, 2003; Lewis and Sellin, 1993).

Methanolic extracts were analysed using the functional bioassay N2A cytotoxicity assay following a modified version of the method developed by Manger et al., in, 1993 (Dechraoui et al., 1999; Bottein Dechraoui et al., 2005). C-CTX-1 standard (generously given by R. Dickey, FDA) or samples, resuspended in MeOH, were directly added to ouabain/veratridine (0.5/0.05 mM) pretreated mouse neuroblastome cells (N2A) plated in a 100 µl total volume of medium (RPMI supplemented with 5% FBS). Sodium channel dependent toxic activity was assessed to ensure specificity of the assay by additionally testing each sample in the absence of ouabain/veratridine (results not shown). After an 18–20 h incubation cell viability was assessed using MTT tetrazolium salt. With the exception of the analysis of four blood samples (for which sample size was limited), assays were repeated at a minimum of three-times with each run in duplicate, as previously described (Dechraoui et al., 1999; Bottein Dechraoui et al., 2005). Data were analysed with GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA) and sample concentration was expressed as C-CTX-1 equivalents. The detection limit in tissue was estimated to be 0.32 pg/gTE.

2.3. Acoustic telemetry

An acoustic telemetry array consisting of 53 autonomous receivers (VR2 and VR2W; Vemco/Amirix Systems, Shad Bay, NS) was deployed off the coast of Cape Eleuthera. Receiver stations were established in a range of habitat types, positioned in three curtains projecting out from Powell Point with receivers placed in a net formation between the curtains and along the edge of the continental shelf (Fig. 2A). The entire array covered an area of approximately 44 km^2. Range tests indicated that receivers deployed in shallow, coastal habitats (>1 m deep) had an average detection radius of 250 m and receivers placed in deeper areas (i.e., mid-array) had an average coverage radius of 500–600 m.

A subset of the barracuda that were captured and blood sampled were tagged with acoustic transmitters (n = 20; V16, V9AP; Vemco/Amirix Systems, Shad Bay, NS). Not all barracuda captured during the study were implanted and released with transmitters due to small body size of some fish or extreme water temperatures (>29 °C may have increased the probability of surgical or post-release mortality). Following blood sampling, these individuals were transferred to a 100 l cooler infused with a dosage of 3-aminobenzoic acid ethyl ester (MS222) that would cause the fish to lose equilibrium (approximately 100 mg/l). A small 2–3 cm incision was cut along the ventral midline of the fish, midway between the pelvic and anal fins. The disinfected transmitter was placed intracoelomically and the incision closed using 2–3 simple interrupted sutures (PDS II 3/0 absorbable sutures, Ethicon Ltd, NJ; Cooke et al., 2003). Fresh seawater was added to dilute the anaesthetic concentration in the cooler beginning halfway through the surgery in order to begin the revival process and each fish was allowed to recover for 30–60 min with consistent water changes. Once recovered (i.e., able to independently maintain equilibrium), the barracuda were released back into the array. Fish were tracked until the last receiver download was completed on October 27, 2009 (up to 321 days). All handling and surgical procedures conformed to the guidelines of the Canadian Council for Animal Care administered through Carleton University, Ottawa, ON.

2.4. Data analysis

A Spearman rank correlation analysis was conducted to test for association between ranked CTX concentrations of the three tissue types (i.e., liver, muscle, blood) and with total length of each fish. To determine the duration each barracuda was present within the confines of the array, a residency index (I_R) was calculated by dividing the number of days an individual was detected by the number of days at large. I_R values range from 0 to 1, with 0 indicating low residency and 1 indicating 100% residency within the array. Home range was estimated by determining the median distance travelled (MDT) and the minimum linear distance (MLD) for each individual barracuda (Chapman et al., 2005). MDT was calculated by finding the median value of the distances between the most frequently visited receiver to all other receivers visited within the array. MLD was calculated by measuring the distance between the two most distant receivers that each individual visited. Spearman rank correlation analysis was used to determine if blood CTX was associated with spatial movement (i.e., I_R, MDT, MLD). A Mann Whitney U test was used to compare median spatial movement metrics (i.e., I_R, MDT, MLD) between fish that tested positive for toxin presence and fish that tested negative (fish U48, U53, U54, and U55 were not included in the analysis because they were not detected after 24 h post-release, Table 2). All statistical testing was completed using JMP 8.0 (SAS Institute, SC) and results were assessed at $\alpha = 0.05$.

3. Results

3.1. Ciguatoxin assay

Of the 12 liver samples collected, 11 samples tested positive for CTX, ranging from 4.96 to 167.77 pg/g_{TE} with a median value of 21.58 pg/g (mean, 31.7 ± 14.1 pg/g_{TE}). The only liver sample that was below the detection limit (0.32 pg/g_{TE}) was obtained from fish U58 (Table 1). Eight out of twelve muscle samples tested positive for CTX, with concentrations ranging between 2.51 and 98.83 pg/g_{TE} and a median value of 5.58 pg/g_{TE} (mean, 19.06 ± 11.6 pg/g_{TE}). When sampled for both tissues, individual barracuda had equivalent or lower toxin concentrations in muscle than in the associated liver sample (Table 1). Over the entire sampling period, 30 blood samples were drawn from barracuda, and 18 of these samples tested positive for CTX. Blood toxin concentrations from the positive samples ranged from 3.07 to 211.74 pg/ml and the median value was 13.75 pg/ml (mean, 25.26 ± 11.2 pg/ml, Table 1). Fish U70 had the highest concentration of toxin in blood (211.74 pg/ml). Spearman rank correlation analysis showed that CTX concentrations in fish blood and liver were positively correlated ($\rho = 0.86$, $P = 0.003$). Although muscle and liver toxin concentration correlations were not statistically significant ($\rho = 0.58$, $P = 0.078$), a potential association may exist had there been a larger sample size available for these tissues. Mean size of sampled barracuda was 86.5 ± 2.2 cm (total length ranging from 60 to 120 cm, Table 1). Toxin concentrations were not correlated with fish total length ($P > 0.05$).

3.2. Spatial ecology

All barracuda were captured within the footprint of the telemetry array, although the majority of individuals (n = 14) that showed detectable levels of CTX in their tissues were generally captured within the western portion of the array, in deeper water along the edge of the continental shelf (Fig. 2B). Tagged barracuda with detectable CTX concentrations in blood tended to stay within the western section of the array, along the coast and just around the end of Powell Point (Fig. 2A).

Of all barracuda released with transmitters during the study period, I_R values ranged from 0.02 to 0.72, with a median value of 0.08 (n = 14) and a mean value of 0.17 ± 0.06 (median I_R: CTX positive fish, 0.06; CTX

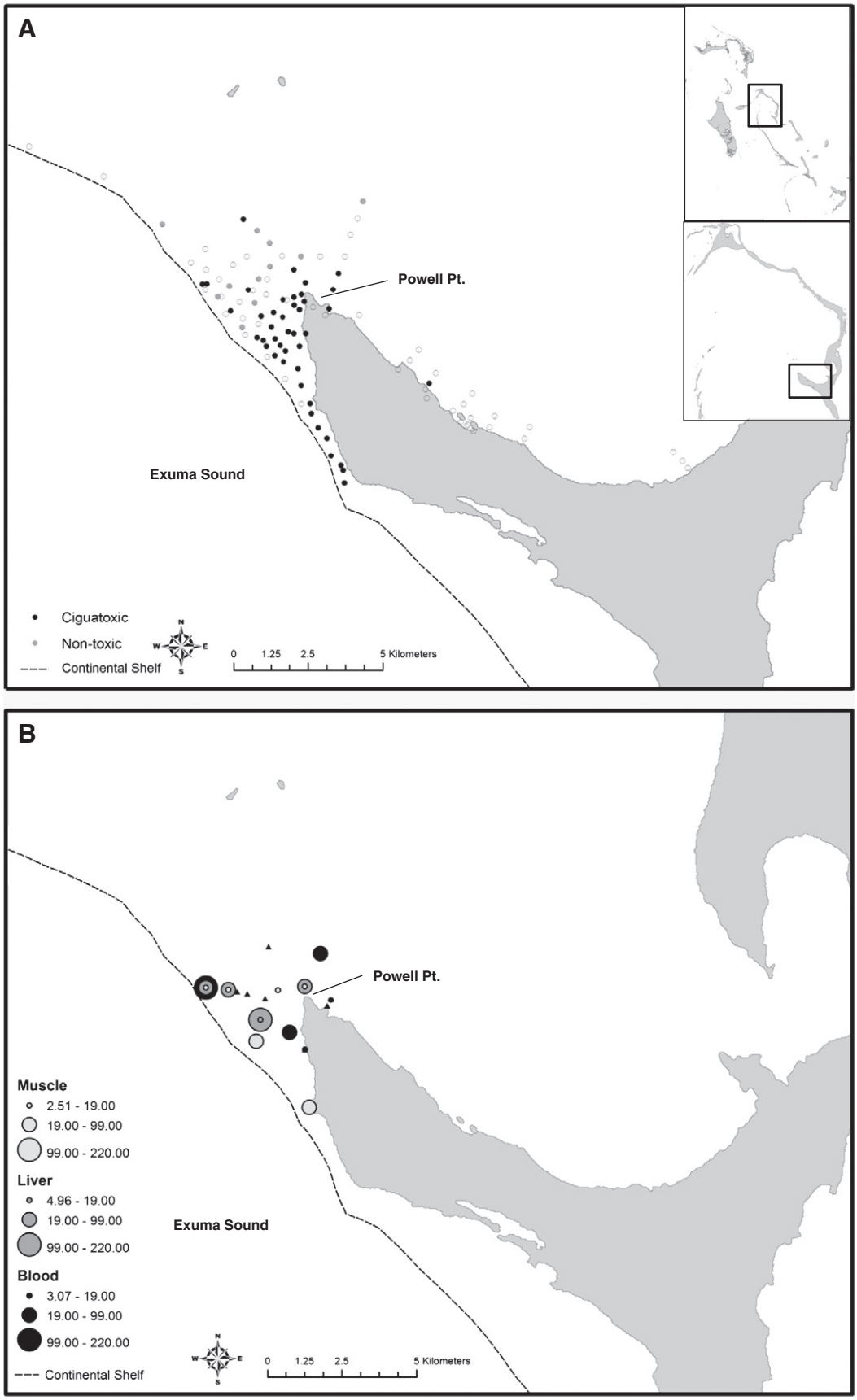

Fig. 2. Spatial distribution and ecology of *S. barracuda* for ciguatoxin analysis near Cape Eleuthera, The Bahamas. A) Receiver stations at which telemetered barracuda were detected during the study period. Black circles represent stations that were visited by fish that tested positive for blood ciguatoxin and grey circles represent stations that had fish which did not exhibit ciguatoxin like activity. Open circles represent receiver stations included in the telemetry array during the study period that were not visited by tagged barracuda. B) Individual barracuda capture locations with associated muscle, liver, and blood CTX concentrations (pg/ml). Black filled triangles represent capture locations for barracuda that did not test positive for CTX.

Table 1
Summary of mean ± SE C-CTX-1 equivalents (pg/ml or pg/g tissue equivalents) detected in wild *S. barracuda* tissue (liver, muscle, and blood) with associated capture date, capture location (UTM, WGS84, Zone 18), and size information for tagged and un-tagged fish off the coast of Cape Eleuthera, The Bahamas between August 2008 and January 2009. The detection limit was estimated at 0.32 pg/gTE.

Fish ID	Capture date	TL (cm)	Capture location		Liver mean ± SE	N	Muscle mean ± SE	N	Blood mean ± SE	N	Tagged
			X	Y							
U22	Aug-23-2008	74	364,258	2,744,213	N/A		21.74 ± 1.76	4	9.85 ± 1.7	4	N
U23	Aug-26-2008	86	364,986	2,747,818	N/A		N/A		16.36 ± 1.38	3	N
U24	Aug-26-2008	66	364,227	2,744,741	N/A		N/A		<dl		N
U25	Aug-26-2008	109	364,110	2,746,151	N/A		N/A		15 ± 0.84	3	N
U26	Aug-26-2008	92	363,915	2,747,613	N/A		N/A		<dl		N
U35	Aug-26-2008	84	362,464	2,746,422	N/A		98.83 ± 14.09	4	N/A		N
U37	Aug-25-2008	98	364,106	2,748,277	41.05 ± 7.46	3	11.43 ± 0.65	4	9.7 ± 1.63	3	N
U38	Dec-10-2008	101	362,763	2,747,876	N/A		N/A		<dl		Y
U39	Dec-12-2008	120	364,855	2,747,621	N/A		N/A		N/A		Y
U40	Dec-12-2008	94	362,168	2,748,033	N/A		N/A		<dl		Y
U41	Dec-12-2008	83	362,168	2,748,033	N/A		N/A		<dl		Y
U42	Dec-13-2008	96	361,534	2,748,166	N/A		N/A		5.87 ± 0.33	3	Y
U43	Dec-13-2008	62	361,534	2,748,166	N/A		N/A		<dl		Y
U44	Dec-13-2008	80	361,534	2,748,166	N/A		N/A		14.87 ± 1.42	4	Y
U45	Dec-13-2008	94	361,534	2,748,166	N/A		N/A		12.7 ± 0.79	3	Y
U46	Dec-13-2008	79	361,534	2,748,166	N/A		N/A		9.84 ± 2.5	3	Y
U47	Dec-13-2008	81	361,534	2,748,166	N/A		N/A		15.96 ± 3.96	3	Y
U48	Dec-13-2008	103	361,832	2,748,105	N/A		N/A		N/A		Y
U49	Dec-13-2008	92	362,880	2,749,613	N/A		N/A		<dl		Y
U50	Dec-13-2008	79	362,464	2,746,422	N/A		N/A		7.65 ± 1.3	3	Y
U51	Dec-13-2008	99	363,601	2,746,718	N/A		N/A		48.89 ± 0.48	2	Y
U52	Dec-14-2008	79	360,637	2,748,227	N/A		N/A		<dl		Y
U53	Dec-14-2008	60	364,258	2,744,213	N/A		N/A		<dl		Y
U54	Dec-14-2008	99	364,110	2,746,151	N/A		N/A		8.48 ± 1.32	4	Y
U55	Dec-15-2008	112	364,633	2,749,380	N/A		N/A		N/A		Y
U56	Dec-15-2008	81	364,633	2,749,380	N/A		N/A		N/A		Y
U57	Dec-15-2008	85.5	364,633	2,749,380	N/A		N/A		24.83 ± 2.81	4	Y
U58	Dec-13-2008	80	361,534	2,748,166	<dl		<dl		N/A		N
U69	Jan-14-2009	84	360,783	2,748,241	23.55 ± 3.09	3	2.6 ± 0.53	3	18.47 ± 4.16	4	N
U70	Jan-14-2009	94	360,783	2,748,241	27.86 ± 1.51	3	5.62 ± 1.43	3	211.74 ± 109.31	2	N
U71	Jan-18-2009	72	361,534	2,748,166	21.58 ± 4.17	4	<dl		6.6 ± 0.1	2	N
U72	Jan-18-2009	68.5	361,534	2,748,166	26.69 ± 1.93	6	2.51 ± 0.25	3	14.8 ± 3.32	2	N
U73	Jan-22-2009	80	361,534	2,748,166	5.22 ± 0.84	3	<dl		<dl		N
U74	Jan-22-2009	103	361,534	2,748,166	5.6 ± 0.59	3	<dl		<dl		N
U75	Jan-23-2009	85	363,187	2,748,150	4.96 ± 1.07	4	5.54 ± 1.42	5	<dl		N
U76	Jan-24-2009	71	361,534	2,748,166	6.25 ± 1.19	4	4.2 ± 0.35	5	3.07 ± 0.84	3	N
U77	Jan-17-2009	80	362,603	2,747,144	18.22 ± 2.89	3	N/A		N/A		N
U78	Jan-17-2009	80	362,603	2,747,144	167.77 ± 26.72	3	N/A		N/A		N

<dl: below detection limit; N/A: no samples available; tagged: yes (Y) or no (N).

Table 2
Summary of residency data for *S. barracuda* tagged with acoustic transmitters detected within a fixed acoustic receiver array off the coast of Cape Eleuthera, The Bahamas. Residency index (I_R) was calculated by dividing the # days detected by the # days at large. Individuals that tested positive for C-CTX-1 equivalents are represented in bold.

Fish ID	Tag ID	Date tagged	TL (cm)	Total detections[a]	# days in array[b]	# days detected[b]	# days at large	I_R
U38	79	Dec-10-2008	101	161	9	7	321	0.02
U39	222	Dec-12-2008	120	843	10	10	65	0.15
U40	228	Dec-12-2008	94	355	5	5	65	0.08
U41	152	Dec-12-2008	83	823	116	80	160	0.50
U42	**9523**	**Dec-13-2008**	**96**	**4568**	**318**	**50**	**318**	**0.16**
U43	204	Dec-13-2008	62	129	105	20	105	0.19
U44	**9529**	**Dec-13-2008**	**80**	**234**	**168**	**38**	**318**	**0.12**
U45	**80**	**Dec-13-2008**	**94**	**2226**	**304**	**21**	**318**	**0.07**
U46	**150**	**Dec-13-2008**	**79**	**88**	**4**	**4**	**160**	**0.03**
U47	**148**	**Dec-13-2008**	**81**	**30**	**117**	**8**	**160**	**0.05**
U48	1440	Dec-13-2008	103	0	1	1	318	0.00
U49	220	Dec-13-2008	92	2297	75	47	65	0.72
U50	**206**	**Dec-13-2008**	**79**	**1**	**101**	**2**	**101**	**0.02**
U51	**1441**	**Dec-13-2008**	**99**	**13,761**	**317**	**106**	**318**	**0.33**
U52	224	Dec-14-2008	79	151	4	3	65	0.05
U53	202	Dec-14-2008	60	0	1	1	95	0.01
U54	**226**	**Dec-14-2008**	**99**	**0**	**1**	**1**	**65**	**0.02**
U55	9522	Dec-15-2008	112	0	1	1	316	0.00
U56	254	Dec-15-2008	81	2414	22	20	95	0.21
U57	**208**	**Dec-15-2008**	**85.5**	**67**	**4**	**4**	**95**	**0.04**

[a] Detections during the first 24 h are not included in the total detections tally.
[b] Number of days in the array and number of days detected include the first 24 h post-release.

negative fish, 0.14). Median MDT for all tagged barracuda was 1006 m (n = 14; mean, 1259 ± 355 m), with the largest range of movement of up to 4989 m (median MDT: CTX positive fish, 506 m; CTX negative fish, 1773 m). Overall, tagged barracuda had a median MLD value of 3314 m (mean, 3248 ± 701 m) and some fish moved up to 7460 m across the array area (median MLD; CTX positive fish, 1115 m; CTX negative fish, 5176 m) (Table 3).

Spearman correlation analysis showed that I_R, MLD, and MDT were not correlated with blood CTX concentrations (I_R, $\rho = -0.05$, $P = 0.85$; MLD, $\rho = -0.13$, $P = 0.62$; MDT, $\rho = -0.37$, $P = 0.16$). Median MDT values between fish that did and did not test positive for CTX were significantly different ($U = 2.21$, $P = 0.03$). Thus, when compared to fish that did not have detectable blood CTX, tagged barracuda that tested positive for the blood toxin presence may have had smaller home range sizes within the confines of the telemetry array, although larger sample sizes will be required to confirm this result (CTX positive fish, n = 8, CTX negative fish n = 6). Median I_R and MLD values were similar between fish with detectable amounts of toxin and fish without detectable tissue toxin amounts (I_R, $U = 0.97$, $P = 0.33$; MLD, $U = 1.49$, $P = 0.12$).

4. Discussion

For the first time, ciguatoxin-like activity was detected from a non-lethal blood sample extracted from a wild fish. Past studies have shown C-CTX concentrations extracted from barracuda muscle and liver range from below the detection limit to >50 ppb C-CTX-1 equivalents (Pottier et al., 2003; Bottein Dechraoui et al., 2005; Villareal et al., 2007). We found that great barracuda blood toxin concentrations are also comparable to concentrations detected in liver samples, where a high blood toxin level, such as that documented in the present study, may be an indicator of recent exposure due to consumption of toxic prey and consequent distribution and accumulation of ciguatoxin within other tissues. Within a given region, it is not unusual for reef fish to have variable toxin levels that may reflect metabolism, accumulation rate, detoxification rate, or foraging behaviour of an individual fish (Tosteson et al., 1988; Lewis and Holmes, 1993).

Great barracuda with higher toxin concentrations in our study were captured along the portion of the array that lies west of Cape Eleuthera, typically along the continental shelf. These areas are typically characterised by deeper water and a mosaic of fringing reefs, large patch reefs, and sandy substrate and are also highly accessible to local artisanal fishers, potentially elevating the risk of ciguatera within the local community. Local residents within the region believe that some of the reef areas along south Eleuthera (i.e., west side of the telemetry array) represent high risk regions for fish harbouring CTX and that consumption of larger fish is more likely to be associated with ciguatera fish poisoning (A. O'Toole and A. Danylchuk, personal observation). The same belief that barracuda from deeper reef areas are more likely to result in ciguatera fish poisoning is also common on other islands in The Bahamas and Caribbean (A. Danylchuk, personal observation). However, we did not detect a relationship between toxin concentration and fish size (size distribution in the present study was similar to historical size distributions reported from Bimini, The Bahamas and Miami, FL; de Sylva, 1963). The most toxic samples from each of the three tissue types were obtained from fish that ranged from 80 to 94 cm in total length, a size range that is often regarded locally as safe for consumption (O'Toole, personal observation). Although the majority of the samples extracted from fish during this study contained concentrations too low to intoxicate humans (in the U.S., action levels for ciguatera are being listed at 0.01 ppb for Pacific ciguatoxin and 0.1 ppb for Caribbean ciguatoxin; Fish and Fishery Products Hazards and Controls Guidance April, 2011), there remains a potential risk due to successive low doses that may lead to intoxication or result in intensification or reoccurrence of previous symptoms (Lewis, 1986; Dickey and Plakas, 2010).

In general, very little is known about great barracuda basic biology and only recently has the extent of barracuda behaviour and spatial ecology been assessed using telemetry devices (O'Toole et al., 2010a, 2010b, 2011). Apart from a study by Villareal et al. (2007) that evaluated the presence of *Gambierdiscus toxicus* on oil platforms in the Gulf of Mexico and compared barracuda movement information garnered from course-scale mark-recapture data, researchers have not, until now, attempted to link the occurrence of ciguatoxin with spatial ecology of high-risk species. For the first time, with the ability to detect toxin concentrations in non-lethal blood samples that are comparable to other tissues (i.e., liver), we were able to tag and thus study barracuda spatial ecology in the wild while at the same time provide a potential link to ciguatoxin occurrence. Barracuda with detectable concentrations of CTX may have smaller home ranges within the confines of the telemetry array. Variability in fish toxicity within and between sampling sites is common (Dierking and Campora, 2009) and home ranges of individual fish may overlap with high-risk areas. In our study, toxin accumulation may have occurred along the edge of the continental shelf in the

Table 3
Summary of *S. barracuda* movement within an acoustic telemetry array off the coast of Cape Eleuthera, The Bahamas. Total receivers visited, median distance travelled (MDT), minimum linear distance (MLD), minimum, maximum, and mean ± SE receivers visited per day are represented. All spatial variables do not include the first 24 h post-release. Individuals that tested positive for C-CTX-1 equivalents are bolded.

Fish ID	Tag ID	# receivers	X	Y	MDT (m)	MLD (m)	Min receivers	Max receivers	Mean receivers ± SE
U38	79	9	18,362,763	2,747,876	1926	5037	1	5	2.50 ± 0.67
U39	222	5	18,364,855	2,747,621	1870	2724	1	5	2.89 ± 0.35
U40	228	7	18,362,168	2,748,033	449	1407	1	5	3.25 ± 0.76
U41	152	14	18,362,168	2,748,033	1173	2897	1	6	1.59 ± 0.11
U42	**9523**	**3**	**18,361,534**	**2,748,166**	**563**	**898**	**1**	**2**	**1.10 ± 0.04**
U43	204	5	18,361,534	2,748,166	1621	5318	1	1	1.00 ± 0.00
U44	**9529**	**1**	**18,361,534**	**2,748,166**	**0**	**0**	**1**	**1**	**1.00 ± 0.00**
U45	**80**	**5**	**18,361,534**	**2,748,166**	**1577**	**5711**	**1**	**3**	**1.15 ± 0.11**
U46	**150**	**6**	**18,361,534**	**2,748,166**	**449**	**1331**	**3**	**5**	**3.67 ± 0.67**
U47	**148**	**1**	**18,361,534**	**2,748,166**	**0**	**0**	**1**	**1**	**1.00 ± 0.00**
U48	1440	0	18,361,832	2,748,105	0	0	0	0	0
U49	220	12	18,362,880	2,749,613	2477	5949	1	6	1.98 ± 0.19
U50	**206**	**1**	**18,362,464**	**2,746,422**	**0**	**0**	**1**	**1**	**1.00 ± 0.00**
U51	**1441**	**37**	**18,363,601**	**2,746,718**	**1558**	**7460**	**1**	**10**	**4.71 ± 0.27**
U52	224	3	18,360,637	2,748,227	4989	5729	1	3	2.00 ± 1.41
U53	202	0	18,364,258	2,744,213	0	0	0	0	0
U54	**226**	**0**	**18,364,110**	**2,746,151**	**0**	**0**	**0**	**0**	**0**
U55	9522	0	18,364,633	2,749,380	0	0	0	0	0
U56	254	20	18,364,633	2,749,380	1392	4395	1	14	7.32 ± 1.00
U57	**208**	**10**	**18,364,633**	**2,749,380**	**838**	**3730**	**1**	**8**	**4.67 ± 2.03**

western portion of the array, an area that is characterised by a mosaic of deep patch reefs that could possibly harbour toxin-producing organisms. Ciguatera-causing dinoflagellates have also been found in association with drift algae samples, further illustrating the potential for spatial distribution of ciguatoxin throughout The Bahamas, Caribbean Sea, and Floridian waters (Bomber et al., 1988). However, toxin origin and accumulation remain unclear as tagged barracuda are able to move outside of the range of the receivers (as evidenced by a 5% median residency estimation) and are known to travel great distances (Villareal et al., 2007) including forays to other islands within the Bahamian Archipelago before returning near to the site of original capture (O'Toole et al., 2011) and to locations in southern Florida (O'Toole and Danylchuk, unpublished data).

5. Conclusions and future directions

Our study is the first report of non-lethal sampling of wild fish for ciguatoxin analysis and will enable future research to address a variety of questions that currently make it difficult to fully characterise the risks of ciguatera. For example, non-lethal blood sampling provides opportunities to identify temporal aspects of ciguatoxin dynamics because fish can be serially sampled (e.g., once a month over the course of a year) if they can be recaptured in the wild or held in the lab or field enclosures. Blood CTX concentrations may be a good indicator of recent exposure to ciguatoxins and thus, could provide a useful application for fishing area surveillance and ciguatera risk prevention. Our study also represents one of the first attempts to combine toxin measures with fish behaviour. Cooke et al. (2008) advocated for using biotelemetry and biologging techniques as the backbone for integrative studies that incorporate other aspects of fish condition including those that can be obtained from non-lethal blood samples. Non-lethal biopsy of fish for blood (or other tissues including small muscle and gill samples) prior to electronic tagging has been used to evaluate correlates of migration failure in Pacific salmon (e.g., Cooke et al., 2006; Crossin et al., 2009) and the correlates of catch-and-release mortality and behavioural impairments in largemouth bass (Thompson et al., 2008). In addition, biopsy approaches have been validated and such non-lethal sampling as we conducted here has been shown to not influence mortality or behaviour of large, adult teleosts (i.e., Pacific salmon, Cooke et al., 2005). Our work serves as a proof-of-concept study that we hope will stimulate future research that combines non-lethal biopsies to evaluate biotoxin concentrations with biotelemetry or biologging tools to evaluate the spatial ecology of fish. Given the inherent variability in both biotoxin concentrations and movements among individuals, we suggest that future studies should attempt to use larger sample sizes based on a priori power analyses.

Additionally, seasonal fluctuations of ciguatoxin in barracuda not only have been reported by Tosteson et al. (1988) in Puerto Rico, but also refuted by de Sylva (1994) in data collected from southern Florida. Seasonal association between water temperature and toxic fish was not apparent in the present study, although we did not sample barracuda year-round and such a result should be considered inconclusive at this time. Clearly additional work is needed to understand the temporal and seasonal patterns of ciguatoxin dynamics in wild fish.

Due to the spatial limitations of a fixed telemetry array in our study (i.e., fish could swim outside of the array footprint), a more in-depth evaluation of barracuda movement and behaviour could potentially be provided by using pop-up satellite tags. Information obtained from future electronic tagging studies that incorporate non-lethal biopsy to quantify ciguatoxin should provide insight to regional and species-specific risk evaluation and prevention. Combining knowledge of fish movement with toxin levels and blooms of *Gambierdiscus* spp. will contribute to the future management and use of marine resources and local risk assessment.

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