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An investigation into ciguatoxin bioaccumulation in sharks

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ABSTRACT

Ciguatoxins (CTXs) produced by benthic *Gambierdiscus* dinoflagellates, readily biotransform and bioaccumulate in food chains ultimately bioconcentrating in high-order, carnivorous marine species. Certain shark species, often feeding at, or near the top of the food-chain have the ability to bioaccumulate a suite of toxins, from both anthropogenic and algal sources. As such, these apex predators are likely sinks for CTXs. This assumption, in conjunction with anecdotal knowledge of poisoning incidents, several non-specific feeding trials whereby various terrestrial animals were fed suspect fish flesh, and a single incident in Madagascar in 1994, have resulted in the widespread acceptance that sharks may accumulate CTXs. This prompted a study to investigate original claims within the literature, as well as investigate CTX bioaccumulation in the muscle and liver of 22 individual sharks from nine species, across four locations along the east coast of Australia. Utilizing an updated ciguatoxin extraction method with HPLC-MS/MS, we were unable to detect P-CTX-1, P-CTX-2 or P-CTX-3, the three primary CTX congeners, in muscle or liver samples. We propose four theories to address this finding: (1) to date, methods have been optimized for teleost species and may not be appropriate for elasmobranchs, or the CTXs may be below the limit of detection; (2) CTX may be biotransformed into elasmobranch-specific congeners as a result of unique metabolic properties; (3) 22 individuals may be an inadequate sample size given the rare occurrence of high-order ciguatoxic organisms and potential for CTX depuration; and (4) the ephemeral nature and inconsistent toxin profiles of *Gambierdiscus* blooms may have undermined our classifications of certain areas as CTX hotspots. These results, in combination with the lack of clarity within the literature, suggest that ciguatoxin bioaccumulation in sharks remains elusive, and warrants further investigation to determine the dynamics of toxin production, accumulation and transformation throughout the entire food-web.

1. Introduction

Ciguatera Fish Poisoning (CFP) is a debilitating human neurointoxication caused by consumption of tropical marine organisms, contaminated with bioaccumulated ciguatoxins (CTXs) (Donati, 2006). Although past estimates suggest that CFP affects between 50,000 and 500,000 people annually (Fleming and Easom, 1998), epidemiological data remains unreliable, given that only ~20% of cases are properly diagnosed and reported (Laurent et al., 2005). Whilst initially elusive, by 1977 the causative agent and the origin of the toxin was determined, and found to be the dinoflagellate *Gambierdiscus* (Yasumoto et al., 1977), a predominately shallow water epiphytic organism (Yasumoto et al., 1979). In the 30 years that followed this discovery, more than 400 fish species have been implicated in poisoning incidents (Tester et al., 2010), most of which are high-order carnivores (Lehane and Lewis, 2000; Lewis, 2006).

Having established the link between trophic position and CTX bioaccumulation, sharks occupying high trophic positions have long been considered carriers of the toxin (Clark, 1915; Banner, 1966; Halstead, 1978; Dawson, 1977; Habermehl et al., 1994; Robinson et al., 1999; Froese and Pauly, 2011; Villareal et al., 2006; Zaccaroni and Scaravelli, 2008; Henrich and Henrich, 2010). When considered in conjunction with their propensity to bioaccumulate similarly lipophilic compounds, such as HPBs (halogenated bipyrroles); PCBs (polychlorinated biphenyls); DDTs (dichlorodiphenyltrichloroethane); and a range of heavy metals (Serrano et al., 2000; Fisk et al., 2002; Gelsleichter et al., 2008; Gelsleichter and Walker, 2010; Mull et al., 2012, 2013; Lyons et al., 2013), the aforementioned understanding of their role as CTX carriers goes unquestioned.

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However, despite numerous statements throughout the literature, ciguatoxins within sharks have evaded detection by modern chemical analysis. Nearly all available information has been gleaned from a combination of local knowledge, studies utilizing unrelated fish and animal feeding assays (Banner, 1966; Randall, 1980), and a single incident in Madagascar in 1994, whereby a shark caused a mass poisoning which was attributed to chemically distinct "carchatoxins A and B" (Boisier et al., 1995; Yasumoto, 1998).

Despite many recent breakthroughs illuminating key aspects of CFP and the nature of CTX distribution and bioaccumulation, the role sharks play in accumulating these toxins is largely unknown and has not been thoroughly investigated. In light of these unknowns, this study aims to: 1) discuss our current understanding of CTXs and CFP as it relates to sharks; 2) investigate CTX concentrations in 22 sharks from Australia's east coast using modern analytical tools; and 3) discuss the uncertainties associated with CTX bioaccumulation in sharks.

1.1. Toxin production and trophic transfer

Ciguatoxins (CTX) are a group of highly stable, lipophilic, cyclic polyether compounds with strong skeletal structures (Murata et al., 1989, 1990) that facilitate toxin retention during cooking, freezing, and exposure to mild basic and acidic conditions, thus making them prone to bioaccumulation through trophic transfer (Lehane and Lewis, 2000; Dickey and Plakas, 2010). To date, more than 29 CTX congeners have been identified and grouped according to geographic distribution: Indian Ocean CTXs (I-CTX), Caribbean CTXs (C-CTX) and most investigated, Pacific CTXs (P-CTX) (Hamilton et al., 2002). These regional-specific congeners exhibit vast differences in potency, trophic transfer, and associated CFP symptoms, thus this work focuses on Pacific CTX congeners only.

Although the distribution of CFP largely mirrors that of *Gambierdiscus,* the causative ciguatoxins are not directly produced by these algae, but instead are products of bioconversion as a result of trophic transfer (Randall, 1958). These biotransformations are a product of a range of chemical pathways, but primarily occur through oxidative metabolism and carbon-chain backbone spiroisomerisations in both the stomach and liver (Lewis and Holmes, 1993). This results in highly stable compounds, which, depending on the biochemical conditions within various consumers, may undergo further oxidative transformations with subsequent increases in associated toxicity (Table 1).

In the case of the Pacific CTXs, these subsequent transformations result in the highly stable P-CTX-1 that dominates the toxin profiles of CFP causing fish (Lewis et al., 1991). Furthermore, the relationship between different digestive capabilities and CTX toxicity (Murata et al., 1990), congener-specific retention (Banner, 1966), pharmacokinetics and potential elimination through metabolic inactivation (Dechraoui et al., 2011) and/or previously uncharacterized final metabolites beyond P-CTX-1, remains elusive (Lewis and Holmes, 1993).

Table 1
Fish and dinoflagellates from the Pacific Ocean (P-) and potency of structurally defined ciguatoxins (CTX) and precursor gambiertoxins (GTX) (After Lewis and Holmes, 1993).

Ciguatoxin	Source	Potency (μg/kg)[a]
P-CTX-1	Carnivorous fish	0.25
P-CTX-2	Carnivorous fish	2.3
P-CTX-3	Carnivorous fish	0.9
P-CTX-3C	*Gambierdiscus toxicus*	2
GTX-4A	*Gambierdiscus toxicus* Herbivorous fish	2
GTX-4B	*Gambierdiscus toxicus* Herbivorous fish	4

[a] Intraperitoneal (i.p.) LD_{50} potency to mice.

1.2. Ciguateric fishes

More than 400 fish species are considered high risk for CFP (Tester et al., 2010), most of which are high-order carnivores (Lehane and Lewis, 2000; Lewis, 2006). Although herbivores and omnivores as well as lower-order carnivores play key roles in the trophic transfer of these toxins, they usually contain relatively low levels of CTXs with toxin profiles often dominated by P-CTX-2 and P-CTX-3 (Chan et al., 2011). However, the lipophilic nature coupled with high stability, especially of P-CTX-1, makes these toxins persistent within the food chain, readily biomagnifying to dangerous levels within higher order carnivores (Lehane and Lewis, 2000; Chan et al., 2011). Owing to this lipophilic nature, CTXs also demonstrate tissue-specific distributions, concentrating in visceral tissue (Vernoux et al., 1985; Swift and Swift, 1993; Lehane and Lewis, 2000; Lewis, 2006; Chan et al., 2011) with liver: flesh CTX concentration ratios ranging from 2:1 (Scombridae and Carangidae) to 43:1 (Muraenidae *Gymnothorax funebris*, Vernoux et al., 1985). Compounding bioaccumulation effects, the detoxification or elimination of CTXs in fishes is a slow process (Banner, 1966), with laboratory fish often remaining ciguatoxic for up to 30 months after toxin ingestion (Banner, 1966), leading to higher ciguatoxicity in older and larger fishes (Caillaud et al., 2010). Furthermore, the marked increase in potency associated with P-CTX amplifies the effective levels of CTX accumulated in fish (Lewis, 2006).

With biomagnification of CTX, there is a parallel increase in CTXs with increasing trophic levels, reaching peak concentrations in apex predators (Randall, 1958; Vernoux et al., 1985; Lewis and Holmes, 1993). This is evident when examining CFP occurrences with associated fractional trophic levels (TL) which correspond to positions within the food web (in which: 1 = primary producers; 2 = primary consumers such as herbivores; 3 = secondary consumers, and so on). Accordingly, it is high trophic level species such as mackerel (Scombridae or Scomberomorus spp. TL = 4.5), coral trout (*Plectropomus* spp., average TL = 4.2), barracuda (*Sphyraena jello,* TL = 4.5), red emperor (*Lutjanus sebae,* TL = 4.3), grouper (*Epinephelus lanciolatus,* TL = 4.0), red bass (*Lutjanus bohar* TL = 4.1), trevally (*Caranx* spp. average TL = 4.0), Maori wrasse (*Chelinus trilobatus* TL = 3.5), kingfish (*Seriola* spp. TL = 4.1), chinaman fish (*Symphorus nematophorus* TL = 4.1) and other tertiary consumers that have most frequently been implicated in ciguatera outbreaks within Australia (Fenner et al., 1997; Mitchell, 1976; Gillespie et al., 1986; Payne, 1994; Froese and Pauly, 2011; Sparrow et al. in review).

With these factors in mind, it calls into question CTX biomagnification in other high-order carnivores, especially regional top predators occupying the highest trophic levels, i.e. sharks, with an average TL of 3.65, with many large species well above a TL of 4 (Cortes, 1999). Additionally, new techniques (including stable isotope analysis) have revealed these are likely under-estimates with many sharks feeding well above previously reported TLs (Hussey et al., 2014), suggesting an even greater potential for CTX bioaccumulation.

1.3. Biomagnification in sharks

As marine top-order predators, sharks are particularly susceptible to bioaccumulation and biomagnification due to their high lipid content, long life span, high trophic level, slow growth and metabolism (Van der Oost et al., 2003; Endo et al., 2008; Gelsleichter and Walker, 2010; Mull et al., 2012). This is evident as many large, long lived, high trophic-level shark species readily