In Vitro Metabolism of Caribbean Ciguatoxins

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Introduction

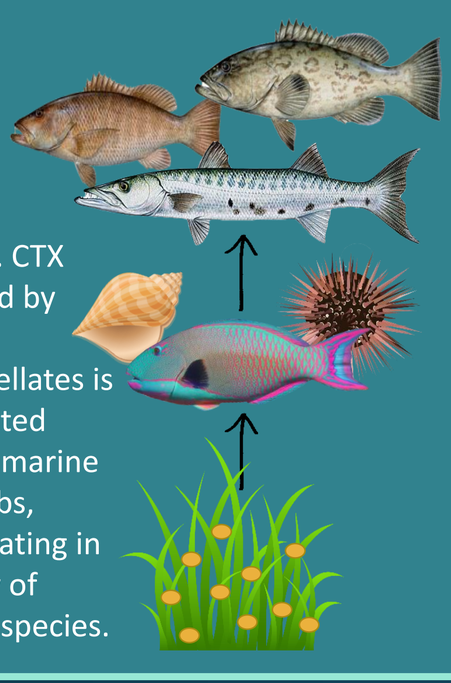


Figure 1. CTX produced by benthic dinoflagellates is transported through marine food webs, accumulating in a variety of seafood species.

Ciguatoxins (CTX) are potent marine neurotoxins produced by benthic dinoflagellates (*Gambierdiscus* and *Fukuyoa* spp.) that inhabit a variety of reef substrates. These toxins are bioaccumulated in marine organisms (**Fig. 1**) and undergo biotransformation to a variety of structurally and toxicologically related metabolites. Human exposure to CTX causes ciguatera poisoning – one of the most prevalent seafood borne illnesses worldwide, which is endemic to tropical and subtropical regions.

Understanding CTX biotransformation is critical to predicting its fate in marine food webs. Limited studies on the metabolism of Pacific CTX (P-CTX) have been done, showing *in vitro* oxidation by Phase I cytochrome P450 oxidase (CYP) enzymes and induction of Phase II pathways involving UDP-glucuronosyltransferase (UGT). However, the metabolic pathways of Caribbean CTX (C-CTX) (**Fig. 2**) remain unknown.

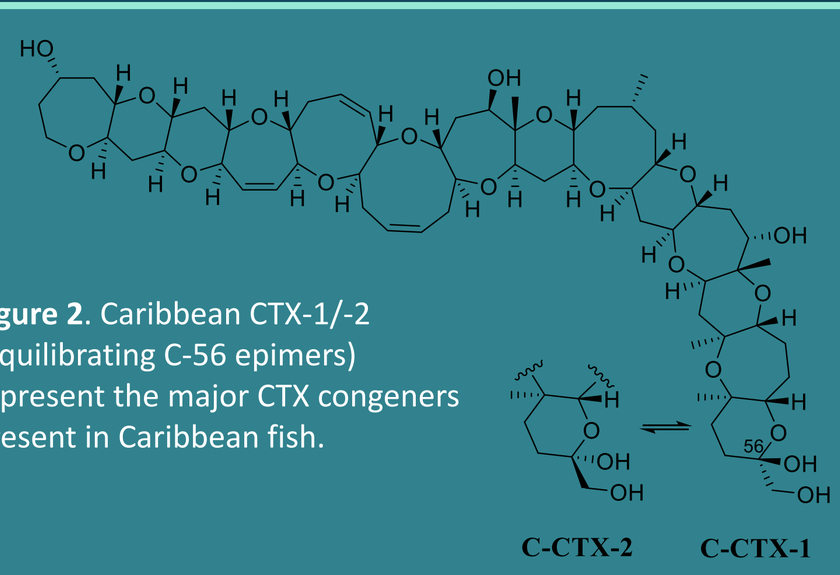


Figure 2. Caribbean CTX-1/-2 (equilibrating C-56 epimers) represent the major CTX congeners present in Caribbean fish.

Research Objectives

1. Identify Phase I and II metabolites of C-CTX-1/-2
2. Compare *in vitro* C-CTX-1/-2 metabolism across marine fish and mammalian species

Methods

Fish Collection

- Northern Gulf of Mexico along MS, AL, and FL panhandle
- Fish euthanized by immersion in ice-slurry
- Livers immediately dissected and flash frozen in liquid N_2
- Fish maintained and/or euthanized following IACUC protocols 1562086, 1384468, 1442738, and 1380906

Lutjanus campechanus
Red snapper
Bottom long-line

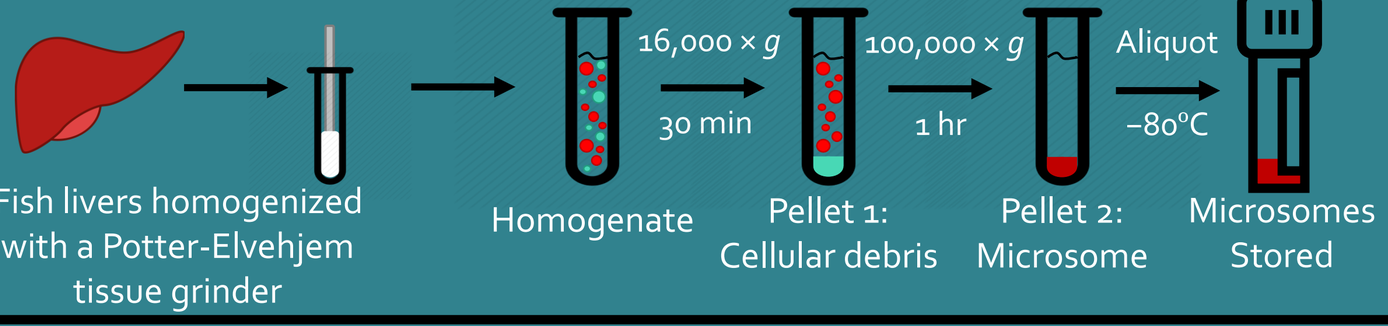
Lutjanus griseus
Grey snapper
SCUBA, spear

Pterois volitans
Red lionfish
SCUBA, spear

Nicholsina usta
Emerald parrotfish
Shallow seagrass trawl

Archosargus probatocephalus
Sheepshead
Hook-and-line

Liver Microsome Preparation

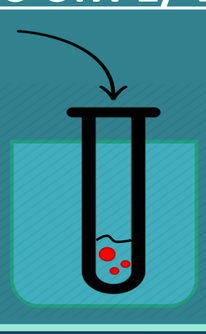


Fish liver microsomes characterized using:
- Lowry method (total protein concentration)
- *In vitro* metabolism of probe substrates* (to confirm major CYP and UGT enzyme activities)

*Confirmed activities related to CYP2D6 and -3A4, as well as UGT1A1, -1A9, -2B7; kinetics somewhat variable among species

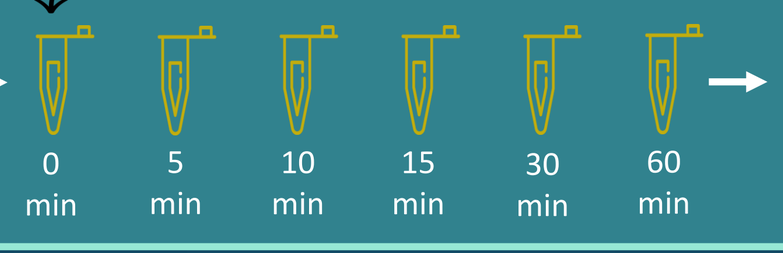
In vitro Metabolism of C-CTX-1/-2

Microsomes (2 mg mL^{-1})
+
CYP or UGT specific cofactors
+
C-CTX-1/-2 substrate



Incubations sampled at several time points

Reactions terminated by adding ice-cold acetonitrile (MeCN)



Instrumental analysis:
- UHPLC – Vanquish Horizon, Thermo
- HRMS(/MS)* – Q-Exactive, Thermo

* for untargeted, high resolution detection of known substrate (C-CTX-1/-2) and unknown metabolites

Results

Overview

No Phase I (CYP) metabolites of C-CTX-1/-2 were detected (*data not shown*).

However, we were able to detect and confirm two Phase II (UGT) metabolites across fish species.

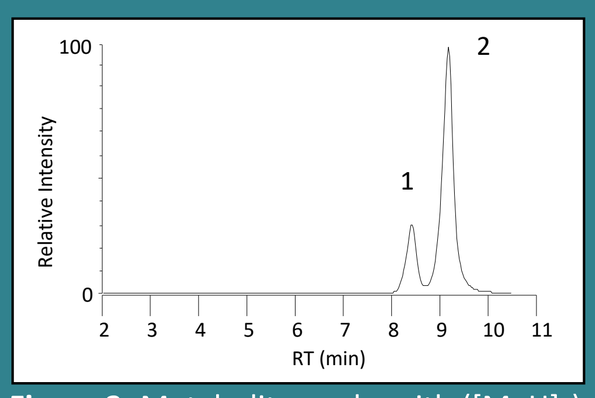


Figure 3. Metabolite peaks with ($[M-H]^-$) *m/z* 1315.6481 corresponding to two mono-glucuronic acid (GlcA) products of C-CTX-1/-2 were detected.

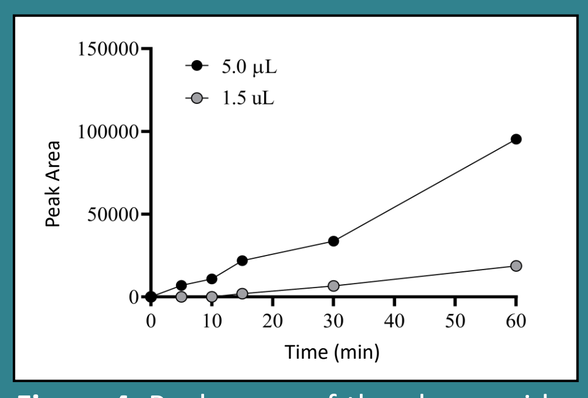


Figure 4. Peak areas of the glucuronide metabolites increased linearly over time throughout 60 min incubation with *L. campechanus* microsomes.

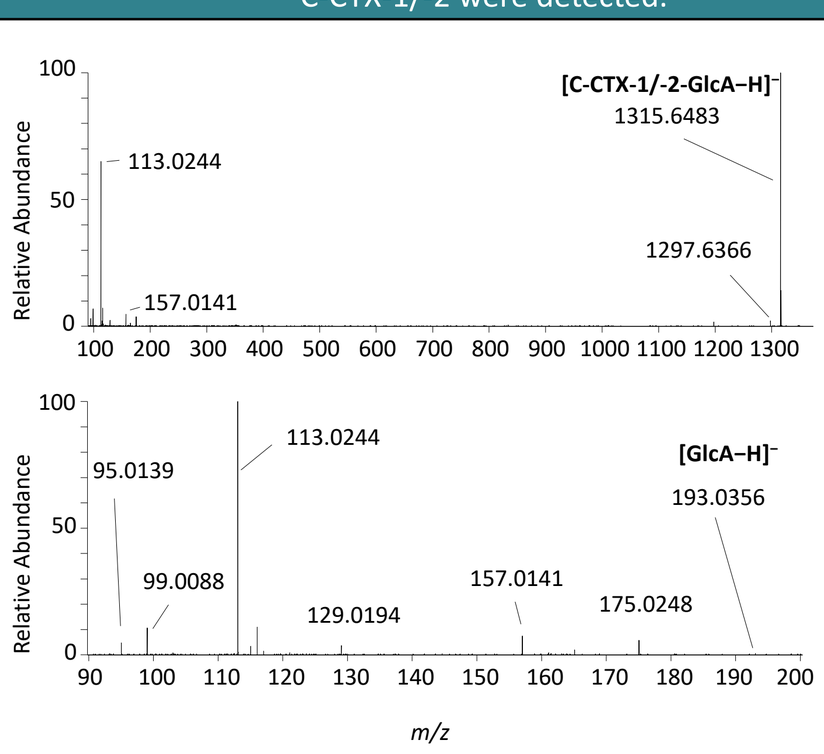
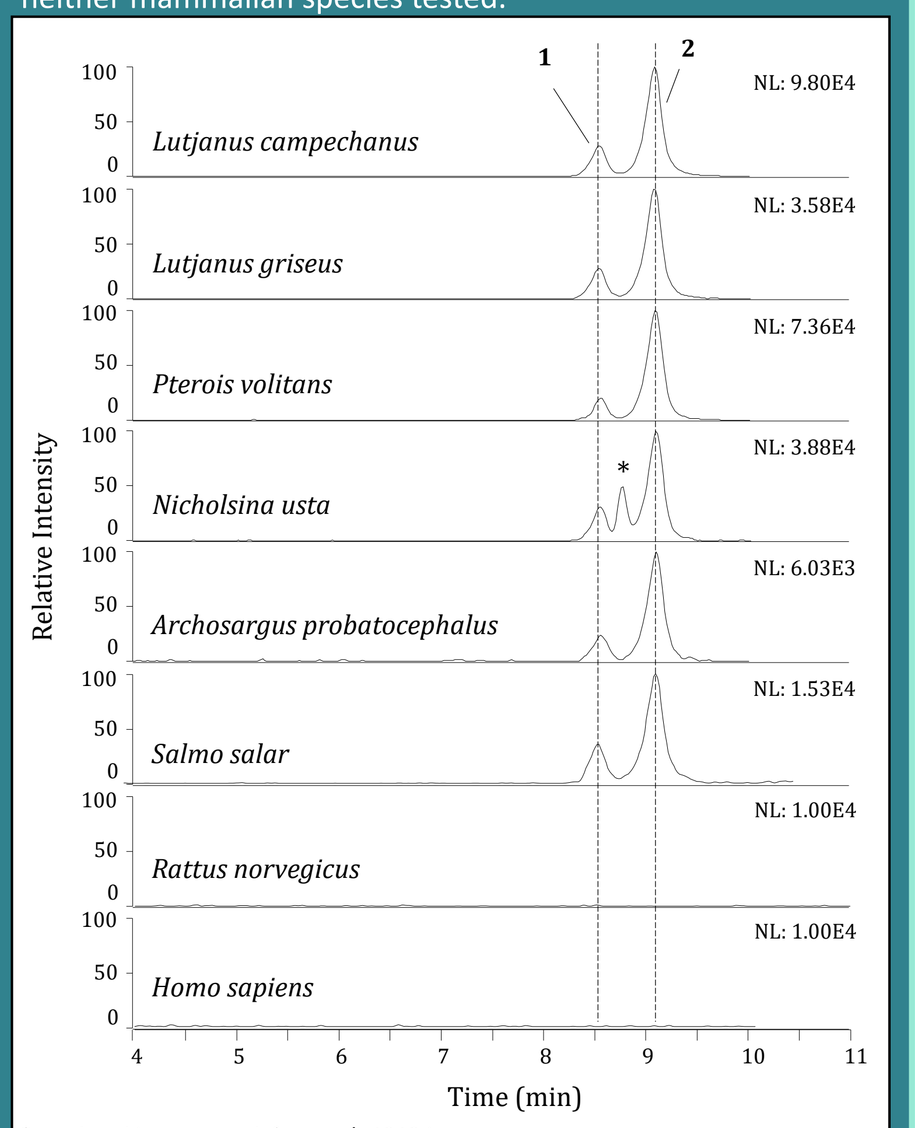


Figure 5. Negative HRMS/MS of C-CTX-1-2 glucuronide $[M-H]^-$ ions, showing full range from *m/z* 90-1350 (top) and the zoomed range of GlcA fragments from *m/z* 90-200 (bottom).

Table 1. Annotated fragments from negative HRMS/MS of C-CTX-1/-2 glucuronide, confirming GlcA attachment.

m/z	Ion Formula	Identity
1315.6482	$[C_{68}H_{99}O_{25}]^-$	$[M-H]^-$
1297.6366	$[C_{68}H_{97}O_{24}]^-$	$[M-H-H_2O]^-$
193.0356	$[C_6H_9O_7]^-$	$[GlcA-H]^-$
175.0248	$[C_6H_7O_6]^-$	$[GlcA-H-H_2O]^-$
157.0141	$[C_6H_5O_5]^-$	$[GlcA-H-(H_2O)_2]^-$
129.0194	$[C_5H_5O_4]^-$	$[GlcA-H-H_2O-CO]^-$
113.0244	$[C_5H_5O_3]^-$	$[GlcA-H-H_2O-CO_2]^-$
99.0088	$[C_4H_3O_3]^-$	$[GlcA-H-H_2O-CO-CH_2O]^-$
95.0139	$[C_5H_3O_2]^-$	$[GlcA-H-(H_2O)_2-CO_2]^-$

Figure 6. Comparison of C-CTX-1/-2 glucuronide across northern Gulf of Mexico fish microsomes (top five panels), as well as microsomes from Atlantic salmon (*Salmo salar*), Wistar rat (*Rattus norvegicus*), and human (*Homo sapiens*). Both glucuronide metabolites were produced by all fish, but neither mammalian species tested.



*unrelated isotope peak from m/z 1313 ion

Conclusions

No *in vitro* Phase I metabolism: CYP pathways not important in C-CTX-1/-2 biotransformation (contrary to what's been reported for P-CTX)

Found two C-CTX-1/-2 glucuronide metabolites: novel Phase II (UGT) pathway not yet described for CTX & first report of C-CTX metabolism

Comparison of C-CTX-1/-2 glucuronides across species: C-CTX glucuronidation may be specific to and potentially widespread across fish

Toxicokinetics & ecotoxicology: glucuronidation has been shown to reduce toxicity and improve elimination for other xenobiotics – could C-CTX glucuronidation drive toxicity in fish, potentially affecting the risk of contracting ciguatera from seafood species?

Acknowledgements: Special thanks to Clay Bennett, Sean Collins, Grant Lockridge, Cpt. Tom Guoba, Dottie Byron-David, Alex Rodriguez, Ed Kim, Merritt McCall, Crystal Hightower, DISL AAUS divers, and Drs. Ken Heck & Sean Powers for assistance with fish collections. Also thank you to Dr. Chris Miles for discussion on metabolism data.

Funding: This work was funded in part by the National Science Foundation (NSF) Partnerships in International Research and Education Program (CiguaPIRE; 1743802) and the contributes to the NSF and NIEHS Center for Oceans and Human Health: Greater Caribbean Center for Ciguatera Research (NSF: 1841811 and NIH: 1P01ES028949-01) Research Project 2. NVI contributions are supported by the supplemental PIRE award through the Research Council of Norway. JG was supported by a graduate research assistantship funded by NSF and NIH (Robertson), and received research support from the Bullard Fund (Univ. S. AL) and partial travel support to Norway from the Society of Toxicology and Chemistry Student Training Exchange Opportunity.

Collection permits and approvals: Fish were collected following permits F/SER24:RM and SAL-18-1230-SR, and were maintained and/or euthanized according to IACUC protocols 1562086, 1384468, 1442738, and 1380906.