

Supplementary Materials: Evaluating Age and Growth Relationship to Ciguatoxicity in Five Coral Reef Fish Species from French Polynesia

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Table S1. Occurrence of ciguatoxins (CTXs) analogs in marine food webs. Only formal identifications of CTXs by chemical methods (i.e., liquid chromatography tandem mass spectrometry, LC-MS/MS) were considered.

Trophic stage	Pacific CTXs	Caribbean CTXs	Indian CTXs
<i>Gambierdiscus</i>	CTX3C, CTX3B, CTX4A, CTX4B, 2-hydroxyCTX3C, 51-hydroxyCTX3C, 54-deoxy-CTX1B, 52- <i>epi</i> -54-deoxy-CTX1B, M- <i>seco</i> -CTX3C, M- <i>seco</i> -CTX3Cmethyl acetal, M- <i>seco</i> -CTX4A/B, 2-OH-CTX3C, CTX3B/C isomers 2-3 [64,65,88,114–127]	ND*	ND
Fish	CTX1B, CTX1A, CTX4A, CTX4B, CTX4C, 52- <i>epi</i> -54-deoxyCTX1B, 54-deoxyCTX1B, 7-oxo-CTX1B, 7-hydroxyCTX1B, 4-hydroxy-7-oxoCTX1B, 54-deoxy-50-hydroxyCTX1B, 51-hydroxyCTX3C, 2,3-dihydro-2,3-dihydroxyCTX3C, 2,3-dihydro-2-hydroxyCTX3C, 2,3-dihydro-51-hydroxy-2-oxoCTX3C, 2,3-dihydro-2,3,51-trihydroxy-CTX3C, A- <i>seco</i> -2,3-dihydro-51-hydroxyCTX3C, M- <i>seco</i> -CTX4A/4B, M- <i>seco</i> -CTX3C, CTX3C, CTX3B [17,18,32–34,50,51,69,86,88,110,114,117–119,121,127–134,136–146]	C-CTX1, C-CTX-2, C-CTX3, C-CTX4, C-CTX5-12** [2,3,36,52,53,56,87,111,135,147–159]	I-CTX-1-6** [160,161]
Marine invertebrates	CTX3C, CTX3B, CTX4A, CTX4B, 51-hydroxyCTX3C, CTX1B, M- <i>seco</i> -CTX3C, 54-deoxyCTX1B, 52- <i>epi</i> -54-deoxyCTX1B [18,88,95,162]	ND	ND
Sharks	ND	ND	I-CTX1-6** [163]

*ND : not documented. **Structure not elucidated.

Table S2. Relationship between biological or environmental factors and the CTX concentration or ciguatoxic status of fish samples. Only studies reporting statistical results based on a number of samples with $n \geq 20$ both by species (or families) and by factors were considered.

	Size	Weight	Trophic level	Lipid content	Fishing site	Season
Absence or weak to moderate relationship	Pacific Region Acanthuridae <i>Acanthurus leucopareius</i> , <i>Ctenochaetus striatus</i> , <i>Naso unicornis</i> Scaridae <i>Chlorurus microrhinos</i> ^a , <i>Leptoscarus vaigiensis</i> , <i>Scarus altipinnis</i> , <i>S. rubroviolaceus</i> Kyphosidae <i>Kyphosus cinerascens</i> Serranidae <i>Cephalopholis argus</i> , <i>Epinephelus polyphkadion</i> ^a , <i>Plectropomus laevis</i> [18,47,50,51]	Acanthuridae <i>Acanthurus leucopareius</i> , <i>Ctenochaetus striatus</i> , <i>Naso unicornis</i> Scaridae <i>Chlorurus microrhinos</i> ^a , <i>Leptoscarus vaigiensis</i> , <i>Scarus altipinnis</i> , <i>S. rubroviolaceus</i> Kyphodidae <i>Kyphosus cinerascens</i> Serranidae <i>Cephalopholis argus</i> , <i>Epinephelus polyphkadion</i> ^a , <i>Plectropomus laevis</i> Muraenidae <i>Gymnothorax flavimarginatus</i> [18,47,49–51]	Herbivores Scaridae, Acanthuridae Omnivores Kyphosidae Carnivores Lutjanidae, Serranidae, [47]	Serranidae <i>Cephalopholis argus</i> Muraenidae <i>Gymnothorax flavimarginatus</i> , <i>Gymnothorax javanicus</i> [18]	ND	ND
	Asia region ND	Scaridae <i>Scarus quoyi</i> Siganidae <i>Siganus guttatus</i> Lutjanidae <i>Lutjanus campechanus</i> Lethrinidae <i>Lethrinus letjan</i> Serranidae <i>Epinephelus merra</i> Sphyraenidae <i>Sphyraena barracuda</i> [48] ^c	ND	ND	ND	Scaridae <i>Scarus quoyi</i> Siganidae <i>Siganus guttatus</i> Lutjanidae <i>Lutjanus campechanus</i> Lethrinidae <i>Lethrinus letjan</i> Serranidae <i>Epinephelus merra</i> Sphyraenidae <i>Sphyraena barracuda</i>

						[48] ^c
	Caribbean region Scorpionaeidae <i>Pterois sp.</i> Balistidae <i>Balistes vetula</i> Serranidae <i>Epinephelus guttatus</i> Haemulidae <i>Haemulon plumierii</i> [52,57]	Scorpionidae <i>Pterois sp.</i> Balistidae <i>Balistes vetula</i> Carangidae <i>Caranx bartholomaei</i> Serranidae <i>Epinephelus guttatus</i> Haemulidae <i>Haemulon plumierii</i> [52,54,57]	ND	ND	ND	ND
	Atlantic region Sphyracidae <i>Sphyracna barracuda</i> [55]	Carangidae <i>Caranx latus</i> <i>Seriola fasciata</i> Serranidae <i>Epinephelus marginatus</i> [5,37,53,56] ^c	ND	ND	ND	Carangidae <i>Seriola dumerilli</i> , <i>S. rivoliana</i> [5,37] ^c
Significant relationship	Pacific region Lutjanidae <i>Lutjanus bohar</i> Muraenidae <i>Gymnothorax flavimarginatus</i> , <i>Gymnothorax javanicus</i> [18,47]	Muraenidae <i>Gymnothorax javanicus</i> [18]	ND	ND	Serranidae <i>Cephalopholis argus</i> [164] ^c	ND
	Asia region ND	ND	ND	ND	ND	Scaridae <i>Scarus quoyi</i> Siganidae <i>Siganus guttatus</i> Lutjanidae <i>Lutjanus campechanus</i>

						Lethrinidae <i>Lethrinus letjan</i> Serranidae <i>Epinephelus merra</i> Sphyraenidae <i>Sphyraena</i> <i>barracuda</i> [48] ^c
	Caribbean region ND	Carangidae <i>Caranx latus</i> [54]	ND	ND	ND	ND
	Atlantic region ND	Carangidae <i>Seriola dumerilli</i> , <i>S. rivoliana</i> [5,37] ^c	ND	ND	Carangidae <i>Seriola dumerilli</i> , <i>Seriola rivoliana</i> Serranidae <i>Epinephelus</i> <i>marginatus</i> [5,37,53] ^c	ND

^aSame fish species as the one selected in our study. ^bND: Not documented. ^cCiguatoxin data were established according to ciguatoxicity status from percentages of cell viability using neuroblastoma cell-based assay (CBA-N2a) or symptoms observed in mice using mouse bioassay (MBA).

Table S3. Number of specimens analyzed per species and site for their ciguatoxic status (N_{tox}), size (cm, fork length), weight (g), and age (y) estimation (N_{age}).

Species	Ciguatoxic status*	Kaukura					Mangareva					Total	
		N_{tox}	N_{age}	Size (cm)	Weight (g)	Age (y)	N_{tox}	N_{age}	Size (cm)	Weight (g)	Age (y)	N_{tox}	N_{age}
<i>C. microrhinos</i>	Negative						44	43	23–51	240–2820	5–9	44	43
	Suspect						5	5	30–44	505–1690	6–8	5	5
	Positive						21	20	36–49	680–2940	5–9	21	20
Total Cmic		0	0	0	0		70	68	70	70	68	70	68
<i>S. forsteni</i>	Negative	9	9	31–40	477–1054	4–7						9	9
	Suspect	4	4	30–41	410–1240	4–9						4	4
	Positive	0	0									0	0
Total Sfor		13	13	13	13	13	0	0	0	0		13	13
<i>S. ghobban</i>	Negative	3	3	24–30	215–412	5–6						3	3
	Suspect	4	4	28–36	326–642	5–7						4	4
	Positive	8	7	26–36	332–681	5–7						8	7
Total Sgho		15	14	15	15	14	0	0	0	0		15	14
<i>N. lituratus</i>	Negative	9	7	23–30	247–571	6–18	42	41	17–36	120–950	3–15	51	48
	Suspect	5	2	29	488	7–9	9	8	26–32	415–870	5–10	14	10
	Positive	1	0				11	10	25–35	350–1070	5–9	12	10
Total Nlit		15	9	15	15	9	62	59	62	62	59	77	68
<i>E. polyphekadion</i>	Negative	0					1	1	33	510	7	1	1
	Suspect	0					4	3	38–45	800–1290	6–11	4	3
	Positive	8	8	34–48	429–1680	6–12	32	25	36–64	830–3650	9–27	40	33
Total Epol		8	8	8	8		37	29	37	37	29	45	37
Total		51	44	51	51	44	169	156	169	169	156	220	200

*The ciguatoxic status was established according to the neuroblastoma cell-based assay (CBA-N2a) results (see § 3.4). Of note, all these samples were also tested using the radioactive receptor binding assay (rRBA) (data not shown).

Table S4. Planned comparisons of ciguatoxicity levels in two-way (species × ciguatoxicity) permutational multivariate analysis of variance (PERMANOVAs) of size (fork length) and weight of five coral reef fish species (*Epinephelus polyphekadion*, *Scarus ghobban*, *Chlorurus microrhinos*, *Scarus forsteni*, *Naso lituratus*) and three levels of ciguatoxicity (negative, suspect, positive).

Response	Comparison	Factor	DF	Sum Sq	Mean Sq	Iter	p-value
Size	Negative - Suspect	Species	4	2134.8	533.71	10 ⁷	<2 × 10 ⁻¹⁶ ***
		Ciguatoxicity	1	50.6	50.64	10 ⁷	0.161
		Species:Ciguatoxicity	4	68.9	17.23	10 ⁷	0.6155
		Residuals	129	3268.9	25.34		
	Negative - Positive	Species	4	4662.6	1165.64	10 ⁷	<2.2 × 10 ⁻¹⁶ ***
		Ciguatoxicity	1	337.5	337.52	10 ⁷	0.0005101***
		Species:Ciguatoxicity	3	188.1	62.69	10 ⁷	0.0988557
		Residuals	180	5482.1	30.46		
	Suspect - Positive	Species	4	3170.8	792.7	10 ⁷	<2 × 10 ⁻¹⁶ ***
		Ciguatoxicity	1	163.7	163.72	10 ⁷	0.01774*
		Species:Ciguatoxicity	3	93.2	31.05	10 ⁷	0.356
		Residuals	103	2920.6	28.36		
Weight	Negative - Suspect	Species	4	11071231	2767808	10 ⁷	1 × 10 ⁻⁷ ***
		Ciguatoxicity	1	107299	107299	10 ⁷	0.4483
		Species:Ciguatoxicity	4	234158	58540	10 ⁷	0.87
		Residuals	129	25432305	197150		
	Negative - Positive	Species	4	31747716	7936929	10 ⁷	<2.2 × 10 ⁻¹⁶ ***
		Ciguatoxicity	1	2403490	2403490	10 ⁷	0.004543**
		Species:Ciguatoxicity	3	1358031	452677	10 ⁷	0.193043
		Residuals	180	52274663	290415		
	Suspect - Positive	Species	4	15197253	3799313	10 ⁷	<2.2 × 10 ⁻¹⁶ ***
		Ciguatoxicity	1	2206487	2206487	10 ⁷	0.007699**
		Species:Ciguatoxicity	3	1694638	564879	10 ⁷	0.133591
		Residuals	103	30788970	298922		

No planned comparisons were made for age since this response variable showed no significant effects (see Table 1 in main text). Significance codes: <0.001 ***, <0.01 **, <0.05 *.

Table S5. Non-linear mixed-effects (NLME) estimates and 95% confidence intervals (CI) of von Bertalanffy growth coefficients in five coral reef fish species

Species	Fixed-effects	Lower 95% CI*	Estimate	Upper 95% CI
<i>Chlorurus microrhinos</i>	L_{∞}	57.9	60.3	62.7
<i>Scarus forsteni</i>	L_{∞}	47.5	53.5	59.6
<i>Scarus ghobban</i>	L_{∞}	37.1	42.5	47.8
<i>Naso lituratus</i>	L_{∞}	30.6	32.5	34.5
<i>Epinephelus polyphekadion</i>	L_{∞}	49.7	52.3	54.8
<i>Chlorurus microrhinos</i>	K	0.174	0.191	0.207
<i>Scarus forsteni</i>	K	0.157	0.198	0.239
<i>Scarus ghobban</i>	K	0.179	0.223	0.267
<i>Naso lituratus</i>	K	0.321	0.342	0.363
<i>Epinephelus polyphekadion</i>	K	0.170	0.191	0.212

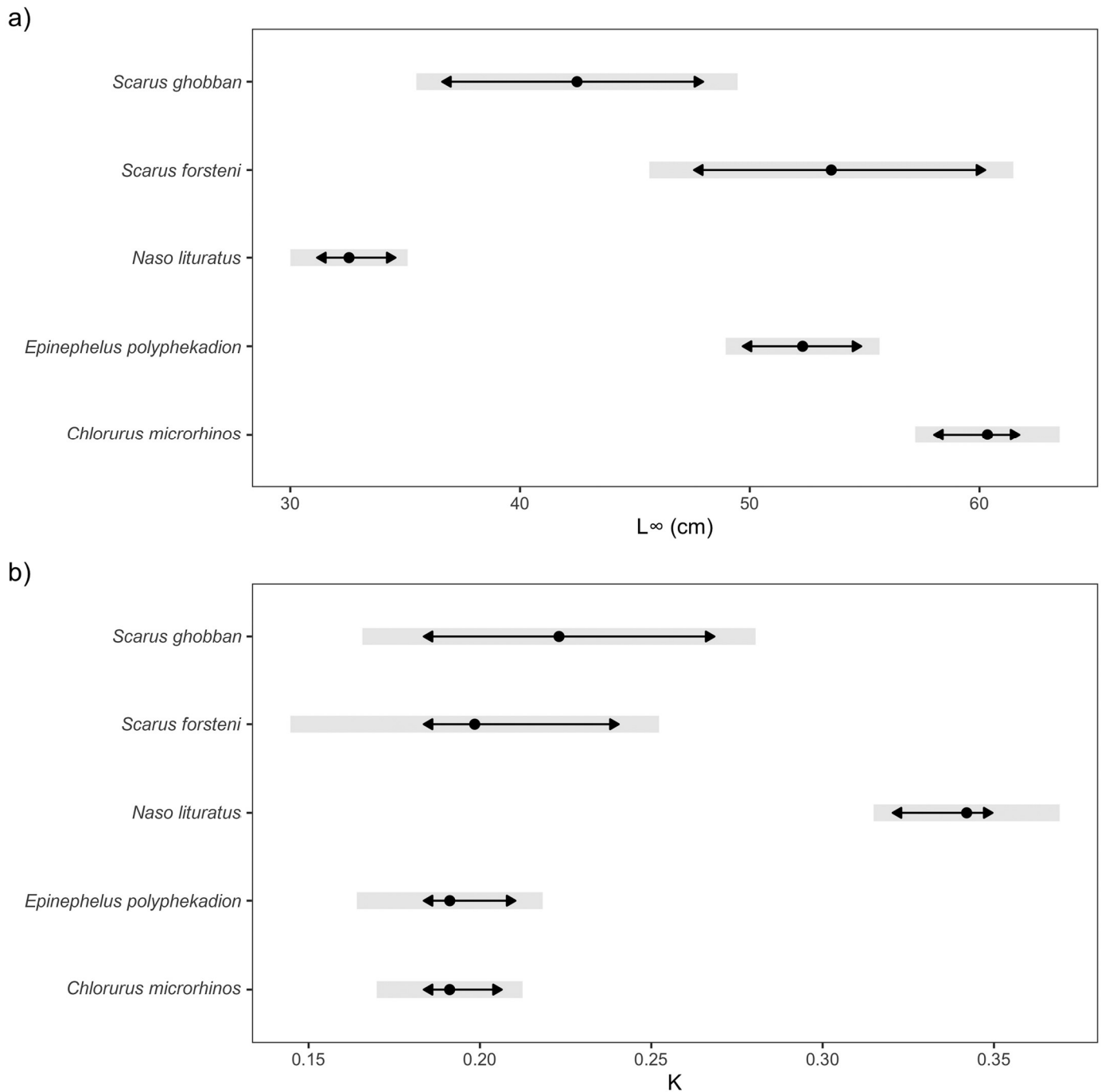


Figure S1. Post-hoc comparisons of species fixed-effects in the best non-linear mixed-effects (NLME) modeling of growth trajectories using the von Bertalanffy growth equation. **(A)** Asymptotic body length (L_{∞}). **(B)** Growth rate coefficient (K). Grey boxes represent 95% confidence intervals in species fixed-effect estimates. Non-overlapping arrows indicate significant differences in species estimates with Tukey post-hoc tests.

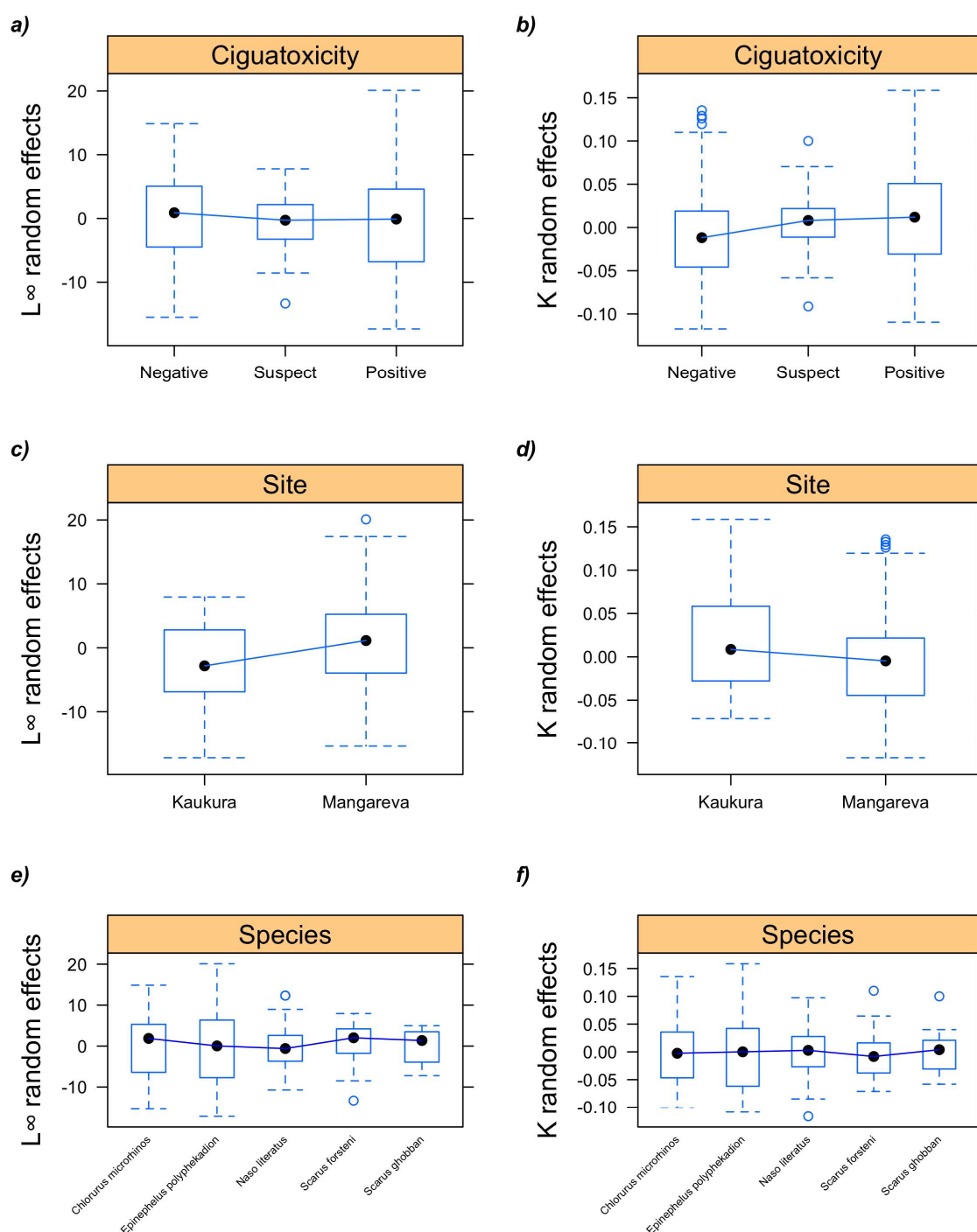


Figure S2. Distribution among ciguatoxicity classes (A,B), sites (C,D), and species (E,F) of individual variation in growth, i.e., random effects of non-linear mixed-effects (NLME) modeling of growth) for both von Bertalanffy growth rate coefficient (K) and asymptotic body length (L_{∞}). The plots indicate no detectable relationship between growth and ciguatoxicity or site, and that species effect was well accounted for by the model. See Table 2 for model specification.