

An Extensive survey of ciguatoxins on grouper *Variola louti* from the Ryukyu Islands, Japan, using liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Naomasa Oshiro ^{1,*}, Hiroya Nagasawa ^{1,2}, Miharuru Watanabe ^{1,2}, Mio Nshimura ^{3,†}, Kyoko Kuniyoshi ¹, Naoki Kobayashi ², Yoshiko Sugita-Konishi ^{2,‡}, Hiroshi Asakura ¹, Katsunori Tachihara ^{4,*} and Takeshi Yasumoto ⁵

Table S1. The *V. louti* specimens analyzed in this study and their CTXs levels.

Specimen ID	Collection date ¹	Age (y) ²	SL ³ (cm)	BW ⁴ (g)	CF ⁵	Sex ⁶	Collection Area ⁷	CTXs level (µg/kg) ⁸			
								1B	epi-deoxy1B	deoxy1B	Total
141065	06/Aug/2013	6	36.5	1330	2.74	F	East, Okinawa	0	0	0	0
141069	14/Aug/2013	3	30.2	910	3.30	F	South West, Okinawa	0.003	0.003	0.004	0.010
141070	09/Sep/2013	9	46.5	3240	3.22	M	South West, Okinawa	0.005	0.006	0.007	0.017
141071	09/Sep/2013	6	38.8	1680	2.88	F	South West, Okinawa	0.021	0.019	0.026	0.066
141072	10/Sep/2013	3	26.7	615	3.23	F	North West, Okinawa	0	0	0	0
141073	10/Sep/2013	5	33.9	1260	3.23	F	North West, Okinawa	0.003	0.003	0.003	0.009
141074	10/Sep/2013	3	33.3	1090	2.95	F	North West, Okinawa	0	0	0	0
141075	24/Sep/2013	2	20.1	248	3.07	F	North West, Okinawa	0	0	0	0
141076	30/Sep/2013	7	46.8	3325	3.24	F	North West, Okinawa	0.047	0.047	0.073	0.167
141077	03/Oct/2013	2	25.3	505	3.14	F	South West, Okinawa	0	0	0	0
141078	11/Oct/2013	5	41.2	1850	2.65	F	South West, Okinawa	0.005	0.003	0.006	0.013
141079	12/Oct/2013	2	21.2	300	3.17	F	North West, Okinawa	0.003	0.003	0	0.006
141080	19/Oct/2013	3	36.0	1385	2.97	F	North West, Okinawa	0.016	0.022	0.029	0.067
141081	19/Oct/2013	5	38.8	1630	2.79	F	North West, Okinawa	0	0	0	0
141082	19/Oct/2013	5	39.8	1600	2.54	F	North West, Okinawa	0	0	0	0
141083	19/Oct/2013	9	41.5	2020	2.83	F	South West, Okinawa	0	0	0	0
141084	31/Oct/2013	10	49.1	3540	2.99	M	East, Okinawa	0.015	0.010	0.012	0.037
141085	31/Oct/2013	5	38.6	1620	2.82	F	East, Okinawa	0.028	0.028	0.034	0.090
141086	02/Nov/2013	4	34.1	1100	2.77	F	North West, Okinawa	0.008	0.008	0.008	0.024
141087	02/Nov/2013	4	31.9	826	2.54	F	North West, Okinawa	0	0	0	0
141088	02/Nov/2013	4	31.0	917	3.08	F	North West, Okinawa	0	0	0	0
141089	06/Nov/2013	3	29.4	900	3.54	F	North West, Okinawa	0	0	0	0
141090	15/Nov/2013	3	29.2	900	3.61	F	South West, Okinawa	0.005	0.003	0.003	0.011
141091	15/Nov/2013	2	27.8	673	3.15	F	South West, Okinawa	0	0	0	0
141092	16/Nov/2013	5	44.0	2800	3.29	M	North West, Okinawa	0.003	0.003	0.007	0.013
141093	16/Nov/2013	4	31.9	1010	3.11	F	North West, Okinawa	0.003	0	0	0.003
141094	02/Dec/2013	3	26.8	667	3.48	F	South West, Okinawa	0	0	0	0
141095	02/Dec/2013	3	29.3	960	3.84	F	East, Okinawa	0.007	0.006	0.006	0.018
141098	07/Dec/2013	5	35.7	1290	2.84	F	East, Okinawa	0	0	0	0
141108	24/Jan/2014	8	44.5	2700	3.06	M	South West, Okinawa	0.015	0.003	0.006	0.024
141109	29/Jan/2014	10	47.9	3200	2.91	M	South West, Okinawa	0.049	0.032	0.047	0.128
141116	08/Feb/2014	NO	22.6	465	4.03	F	North West, Okinawa	0	0	0	0
141117	18/Feb/2014	2	24.4	474	3.26	F	North West, Okinawa	0	0	0	0
150090	16/Nov/2015	NO	53.0	4800	3.22	NI	unknown	0	0	0	0
150091	16/Nov/2015	NO	48.0	3300	2.98	NI	unknown	0	0	0	0
150092	16/Nov/2015	NO	46.0	2700	2.77	NI	unknown	0	0	0	0
153001	01/Sep/2014	4	35.0	1335	3.11	F	East, Okinawa	0.006	0	0	0.006
153002	01/Sep/2014	3	28.6	703	3.01	F	East, Okinawa	0.011	0.003	0.003	0.017
153003	01/Sep/2014	2	23.4	339	2.65	F	East, Okinawa	0.012	0.003	0.003	0.018
153004	13/Sep/2014	5	37.5	1345	2.55	F	Yaeyama	0.026	0.018	0.021	0.065
153005	13/Sep/2014	4	33.4	971	2.61	F	Yaeyama	0.013	0.003	0.003	0.019
153006	19/Sep/2014	3	28.4	777	3.39	F	South West, Okinawa	0.014	0.003	0.003	0.020

¹ UK: unknown, ² y: years; NO: not observed, ³ SL: standard length, ⁴ BW: body weight, ⁵ CF: condition factor, CF = 100 × BW/SL², ⁶ F: female; M: male; bi: bisexual; NI: not identified, ⁷ the locations of collection areas are shown in Figure 2, ⁸ 1B: CTX1B; epi-deoxy1B: 52-epi-54-deoxyCTX1B; deoxy1B: 54-deoxyCTX1B; 0: less than LOD; 0.003: detected but less than LOQ.

Table S1. Continued.

Specimen ID	Collection date ¹	Age (y) ²	SL ³ (cm)	BW ⁴ (g)	CF ⁵	Sex ⁶	Collection Area ⁷	CTXs level (µg/kg) ⁸			
								1B	epi-deoxy1B	deoxy1B	Total
153007	19/Sep/2014	NO	32.4	1152	3.39	F	South West, Okinawa	0.011	0.006	0.003	0.020
153008	UK	1	18.5	178	2.81	NI	South West, Okinawa	0.003	0	0	0.003
153009	29/Sep/2014	NO	13.5	92	3.74	F	South West, Okinawa	0.006	0	0	0.006
153010	20/Oct/2014	5	39.4	1548	2.53	F	South West, Okinawa	0.008	0.003	0.003	0.014
153011	20/Oct/2014	NO	25.0	379	2.43	F	South West, Okinawa	0.007	0	0	0.007
153012	22/Oct/2014	5	41.8	1915	2.62	F	South West, Okinawa	0.021	0.017	0.026	0.064
153013	22/Oct/2014	2	25.8	566	3.30	F	South West, Okinawa	0.009	0	0	0.009
153014	22/Oct/2014	2	24.6	400	2.69	F	South West, Okinawa	0.010	0	0	0.010
153015	22/Oct/2014	3	26.2	543	3.02	F	South West, Okinawa	0.003	0	0	0.003
153016	04/Nov/2014	4	43.3	1993	2.45	M	South West, Okinawa	0.006	0	0	0.006
153017	04/Nov/2014	4	34.6	1235	2.98	F	South West, Okinawa	0.003	0	0	0.003
153018	12/Nov/2014	3	32.9	975	2.74	F	North West, Okinawa	0	0	0	0
153019	13/Nov/2014	2	30.1	941	3.45	F	North West, Okinawa	0.006	0	0	0.006
153020	22/Nov/2014	2	27.1	705	3.54	F	Amami	0.008	0	0	0.008
153021	03/Dec/2014	NO	48.2	2585	2.31	bi	Amami	0.017	0.005	0.007	0.029
153022	27/Jan/2015	3	33.3	1145	3.10	bi	South West, Okinawa	0.003	0.003	0.003	0.009
153023	19/Dec/2014	NO	22.2	397	3.63	F	Amami	0.003	0	0	0.003
153056	18/Apr/2015	6	45.3	2622	2.82	NI	South West, Okinawa	0	0	0	0
153057	18/Apr/2015	2	22.3	368	3.32	F	South West, Okinawa	0	0	0	0
153058	07/May/2015	2	18.8	184	2.77	F	South West, Okinawa	0.005	0.003	0	0.008
153059	18/May/2015	NO	16.8	135	2.85	F	South West, Okinawa	0	0	0	0
153060	18/May/2015	NO	16.7	125	2.68	F	South West, Okinawa	0	0	0	0
153061	28/May/2015	7	47.3	3720	3.52	M	South West, Okinawa	0.020	0.019	0.026	0.065
153062	28/May/2015	3	26.4	515	2.80	F	South West, Okinawa	0.005	0	0	0.005
153063	28/May/2015	2	27.8	699	3.25	F	South West, Okinawa	0.058	0.037	0.047	0.142
160024	25/May/2016	NO	49.0	2934	2.49	NI	Miyako	0.103	0.042	0.104	0.249
160085	UK	NO	41.0	1734	2.52	NI	unknown	0.023	0.007	0.016	0.045
160135	19/Dec/2016	NO	49.0	2594	2.20	NI	Miyako	0.050	0.021	0.054	0.125
160136	19/Dec/2016	NO	45.0	2430	2.67	NI	Miyako	0.042	0.174	0.067	0.283
163015	14/Dec/2015	NO	28.0	649	2.96	NI	South West, Okinawa	0	0	0	0
163016	14/Dec/2015	2	26.0	540	3.07	F	South West, Okinawa	0	0	0	0
163017	UK	2	27.3	588	2.89	F	North West, Okinawa	0	0	0	0
163018	30/Jan/2016	2	31.0	833	2.80	F	South West, Okinawa	0	0	0	0
163019	29/Jan/2016	7	47.6	2540	2.36	NI	North West, Okinawa	0.019	0.014	0.026	0.059
163020	29/Jan/2016	5	41.2	1798	2.57	F	North West, Okinawa	0	0	0	0
163021	30/Jan/2016	5	41.0	1750	2.54	bi	North West, Okinawa	0.005	0.003	0.003	0.011
163022	30/Jan/2016	6	44.0	2320	2.72	F	North West, Okinawa	0.010	0.006	0.009	0.025
163023	30/Jan/2016	4	26.3	491	2.70	F	North West, Okinawa	0.003	0	0	0.003
163024	05/Feb/2016	4	37.2	1606	3.12	F	South West, Okinawa	0.003	0.003	0.003	0.009
163025	05/Feb/2016	5	29.5	772	3.01	F	South West, Okinawa	0.009	0.003	0.003	0.015
163026	19/Feb/2016	4	43.0	2375	2.99	M	South West, Okinawa	0.012	0.006	0.011	0.030
163027	19/Feb/2016	3	36.5	1547	3.18	F	South West, Okinawa	0.007	0.003	0.003	0.013

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Table S1. Continued.

Specimen ID	Collection date ¹	Age (y) ²	SL ³ (cm)	BW ⁴ (g)	CF ⁵	Sex ⁶	Collection Area ⁷	CTXs level (µg/kg) ⁸			
								1B	epi-deoxy1B	deoxy1B	Total
163028	19/Feb/2016	NO	36.3	1345	2.81	F	South West, Okinawa	0	0	0	0
163029	19/Feb/2016	5	32.0	1087	3.32	F	South West, Okinawa	0.003	0	0.003	0.006
163030	19/Feb/2016	4	34.5	1236	3.01	F	South West, Okinawa	0.007	0.006	0.006	0.019
163031	19/Feb/2016	NO	27.0	564	2.87	F	South West, Okinawa	0.006	0.003	0.003	0.012
163039	18/Mar/2016	4	32.1	1061	3.21	F	South West, Okinawa	0.005	0	0	0.005
163040	18/Mar/2016	NO	37.6	1524	2.87	F	South West, Okinawa	0.012	0	0	0.012
163041	18/Mar/2016	4	34.0	1360	3.46	M	South West, Okinawa	0.012	0	0	0.012
163046	12/Dec/2015	7	40.7	2216	3.29	M	South West, Okinawa	0.014	0.003	0.008	0.025
163047	12/Dec/2015	7	40.0	1826	2.85	F	South West, Okinawa	0.012	0.006	0.009	0.027
163048	15/Dec/2015	5	38.2	1391	2.50	NI	South West, Okinawa	0.020	0.019	0.029	0.068
163049	UK	NO	31.7	897	2.82	F	North West, Okinawa	0.010	0.003	0.003	0.016
163050	UK	4	40.6	1872	2.80	M	North West, Okinawa	0.007	0.003	0.003	0.013
163051	05/Feb/2016	NO	31.8	1089	3.39	F	South West, Okinawa	0.009	0.003	0.003	0.015
163052	05/Feb/2016	4	35.2	1611	3.69	F	South West, Okinawa	0.010	0.003	0.006	0.019
163053	19/Feb/2016	4	38.4	1596	2.82	M	South West, Okinawa	0.005	0	0	0.005
163062	13/Oct/2015	1	20.2	291	3.53	F	South West, Okinawa	0.003	0	0	0.003
163063	05/Aug/2015	12	50.4	4100	3.20	NI	Amami	0.063	0.035	0.056	0.154
163064	21/May/2015	14	47.5	2880	2.69	M	Amami	0.045	0.027	0.036	0.108
163065	16/Oct/2015	13	49.6	3040	2.49	M	South West, Okinawa	0.093	0.039	0.103	0.235
163067	31/Mar/2016	4	33.7	1385	3.62	F	North West, Okinawa	0.011	0.008	0.011	0.031
163068	31/Mar/2016	2	31.2	1050	3.46	F	North West, Okinawa	0	0	0	0
163069	31/Mar/2016	5	39.0	1606	2.71	F	North West, Okinawa	0	0	0	0
163070		10	40.2	2072	3.19	F	Amami	0.005	0.003	0.003	0.011
163071	不明	14	46.2	3060	3.10	M	Amami	0.047	0.028	0.058	0.133
163072	27/May/2016	12	44.6	2861	3.22	F	Amami	0.015	0.003	0.006	0.024
163073	05/Sep/2016	4	32.2	1004	3.01	F	Miyako	0.008	0.009	0.010	0.028
163074	05/Sep/2016	NO	40.3	1728	2.64	F	Miyako	0.014	0.015	0.014	0.043
163075	05/Sep/2016	4	31.3	933	3.04	F	Miyako	0	0	0	0
163076	30/Sep/2016	4	30.4	934	3.32	F	Miyako	0	0	0	0
163077	07/Oct/2016	12	45.6	2719	2.87	M	South West, Okinawa	0.141	0.078	0.157	0.376
163078	07/Oct/2016	3	30.0	795	2.94	F	South West, Okinawa	0	0	0	0
163095	07/Oct/2016	2	25.5	570	3.44	F	South West, Okinawa	0	0	0	0
163096	29/Mar/2016	3	26.6	644	3.42	F	South West, Okinawa	0	0	0	0
163097	29/Mar/2016	2	32.7	1227	3.51	F	South West, Okinawa	0.003	0.003	0.003	0.009
163098	29/Mar/2016	2	26.9	721	3.70	F	South West, Okinawa	0	0	0	0
163099	29/Mar/2016	2	23.7	413	3.10	F	South West, Okinawa	0	0	0	0
163100	11/Apr/2016	3	30.8	1055	3.61	F	South West, Okinawa	0	0	0	0
163101	11/Apr/2016	4	35.2	1438	3.30	F	South West, Okinawa	0	0	0	0
163102	15/Apr/2016	8	37.9	1510	2.77	F	South West, Okinawa	0.003	0.003	0.003	0.009
163103	18/Apr/2016	7	36.6	1318	2.69	F	South West, Okinawa	0.003	0.003	0.005	0.011
163104	18/Apr/2016	NO	31.0	1017	3.41	F	South West, Okinawa	0	0	0	0
163105	18/Apr/2016	4	30.1	830	3.04	NI	South West, Okinawa	0.005	0.005	0.003	0.012

¹ UK: unknown, ² y: years old; NO: not observed, ³ SL: standard length, ⁴ BW: body weight, ⁵ CF: condition factor, CF = 100 × BW/SL², ⁶ F: female; M: male; bi: bisexual; NI: not identified, ⁷ the locations of collection areas are shown in Figure 2, ⁸ 1B: CTX1B; epi-deoxy1B: 52-epi-54-deoxyCTX1B; deoxy1B: 54-deoxyCTX1B; 0: less than LOD; 0.003: detected but less than LOQ.

Table S1. Continued.

Specimen ID	Collection date ¹	Age (y) ²	SL ³ (cm)	BW ⁴ (g)	CF ⁵	Sex ⁶	Collection Area ⁷	CTXs level (µg/kg) ⁸			
								1B	<i>epi</i> -deoxy1B	deoxy1B	Total
163106	26/Apr/2016	5	33.9	1185	3.04	F	unknown	0	0	0	0
163107	06/May/2016	5	38.1	1737	3.14	F	unknown	0.009	0.009	0.012	0.031
163108	06/May/2016	6	40.9	2037	2.98	F	unknown	0	0	0	0
163109	06/May/2016	6	39.7	1928	3.08	F	unknown	0.007	0.005	0.010	0.022
163110	06/May/2016	7	40.0	1997	3.12	F	unknown	0.003	0.003	0.003	0.009
163111	06/May/2016	4	24.4	447	3.08	F	unknown	0	0	0	0
163112	06/May/2016	18	46.4	2760	2.76	M	unknown	0.003	0.003	0.003	0.009
163113	06/Jun/2016	NO	31.8	871	2.71	F	unknown	0	0	0	0
163114	06/Jun/2016	6	34.8	1280	3.04	F	unknown	0.003	0.003	0.003	0.009
163115	06/Jun/2016	4	34.1	1153	2.91	F	unknown	0.003	0.003	0.003	0.009
163116	06/Jun/2016	5	31.2	981	3.23	F	unknown	0	0	0	0
163117	04/Jul/2016	4	28.7	756	3.20	F	South West, Okinawa	0	0	0	0
163118	06/Jun/2016	11	47.0	3300	3.18	M	unknown	0.005	0.003	0.005	0.013
163119	13/Jun/2016	4	36.8	1735	3.48	F	unknown	0.006	0.006	0.010	0.023
163120	13/Jun/2016	NO	35.0	1413	3.30	F	unknown	0	0	0	0
163121	22/Jun/2016	NO	33.0	1191	3.31	F	unknown	0.003	0.003	0.003	0.009
163122	13/Jun/2016	NO	32.2	1042	3.12	F	unknown	0	0	0	0
163123	06/Aug/2016	4	29.2	775	3.11	F	unknown	0.003	0.003	0.003	0.009
163124	05/Sep/2016	3	16.3	134	3.09	F	Miyako	0	0	0	0
163125	30/Sep/2016	6	37.9	1533	2.82	M	Miyako	0	0	0	0
163126	30/Sep/2016	4	31.7	1032	3.24	F	Miyako	0	0	0	0
163127	30/Sep/2016	5	34.5	1317	3.21	F	Miyako	0	0	0	0
163128	30/Sep/2016	6	36.4	1295	2.69	NI	Miyako	0.005	0.003	0.006	0.014
163129	11/Oct/2016	NO	42.0	2441	3.29	NI	North West, Okinawa	0.010	0.007	0.013	0.030
163130	11/Oct/2016	NO	38.0	1604	2.92	NI	North West, Okinawa	0.003	0.003	0.003	0.009
163131	31/Oct/2016	NO	20.3	273	3.26	NI	North West, Okinawa	0	0	0	0
163132	31/Oct/2016	NO	21.2	303	3.18	NI	North West, Okinawa	0.003	0.003	0.003	0.009
163155	31/Mar/2016	3	38.8	1094	1.87	F	North West, Okinawa	0.003	0.003	0.003	0.009
Maximum		18.0	53.0	4800	4.03		Maximum	0.141	0.174	0.157	0.376
Minimum		1.0	13.5	92	1.87						
Average		4.8	34.3	1399	3.03						
Median		4.0	34.0	1209	3.04						
SD		3.0	8.3	907	0.3						
RSD		61%	24%	65%	12%						

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Table S2. LC-MS/MS condition for CTX analysis**LC**

Equipment: Agilent 1290 Infinity (Agilent Technologies)
 Column: Zorbax Eclipse Plus C18
 (1.8 μ m, 2.1 x 50 mm, Agilent Technologies)
 Column Temp.: 40 °C
 Injection volume: 5 μ L
 Eluent A: 5 mM ammonium formate in 0.1% formic acid
 Eluent B: methanol
 Flow rate: 0.4 mL/min
 Gradient condition: 0 min (60%B)–0.25 min (60%B)–0.5 min (75%B)–
 12 min (90%B)–14 min (90%B)–14.1 min (100%B)

MS

Equipment: 6460 Triple Quad LC/MS (Agilent Technologies)
 Ionization: ESI with Agilent Jet Stream
 Nebulizer gas: N₂, 50 psi
 Sheath gas: N₂, 380 °C, 11 L/min
 Dry gas: 300 °C, 10 L/min
 Capillary voltage: 5000 V
 Fragmentor voltage: 300 V
 Collision energy: 40 eV
 Monitor ions: [M+Na]⁺ > [M+Na]⁺
m/z 1045.6: CTX3C and 49-*epi*CTX3C
m/z 1061.6: 2-hydroxyCTX3C and 51-hydroxyCTX3C
m/z 1079.6: 2,3-dihydroxyCTX3C
m/z 1083.6: CTX4A and CTX4B
m/z 1095.6: 2,3,51-trihydroxyCTX3C
m/z 1117.6: 52-*epi*-54-deoxyCTX1B and 54-deoxyCTX1B
m/z 1133.5: CTX1B

Table S3. The CTXs levels in the flesh of *V. louti* by age collected from the Ryukyu Islands, Japan.

Age (years old)	Total CTXs levels (µg/kg)					
	Total	<0.003 (LOD) ¹		0.003-0.010 ²		0.011-0.17 ³
1	2			2	100%	
2	21	12	57%	7	33%	2 10%
3	20	9	45%	5	25%	6 30%
4	29	8	28%	10	34%	11 38%
5	20	8	40%	2	10%	10 50%
6	9	4	44%	1	11%	4 44%
7	7			1	14%	6 86%
8	2			1	50%	1 50%
9	2	1	50%			1 50%
10	3					3 100%
11	1					1 100%
12	3					2 67%
13	1					1 100%
14	2					2 100%
15						
16						
17						
18	1			1	100%	
Total	123	42	34%	30	24%	49 40%

¹ All CTX analogues were less than LOD (0.003 µg/kg).

² CTXs were detected but the levels were below FDA guidance (0.01 µg/kg) [64] [61].

³ The levels were above the FDA guidance but less than the minimum levels that caused CFP as described below.

⁴ The CTXs levels may cause CFP by the consumption of the 400 g or less amount of the flesh. The level was estimated based on the minimum amount of CTX1B cause CFP (70 ng CTX1B/person, 70 ng CTX1B/400 g flesh = 0.18 µg/kg) [10].

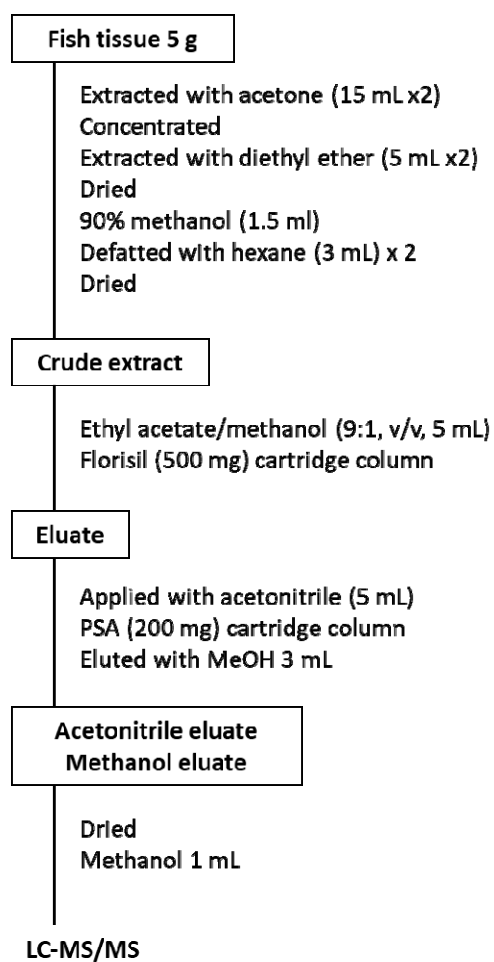


Figure S1. Sample preparation from flesh for LC-MS/MS.

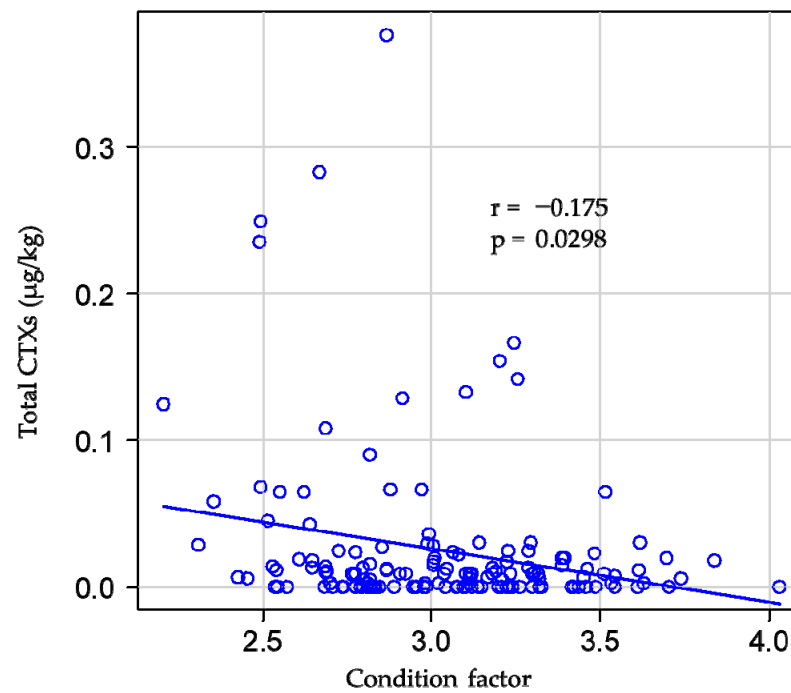


Figure S2. The relationship between the condition factors and the total CTX levels in the flesh of *V. louti* specimens collected from the Ryukyu Island, Japan.

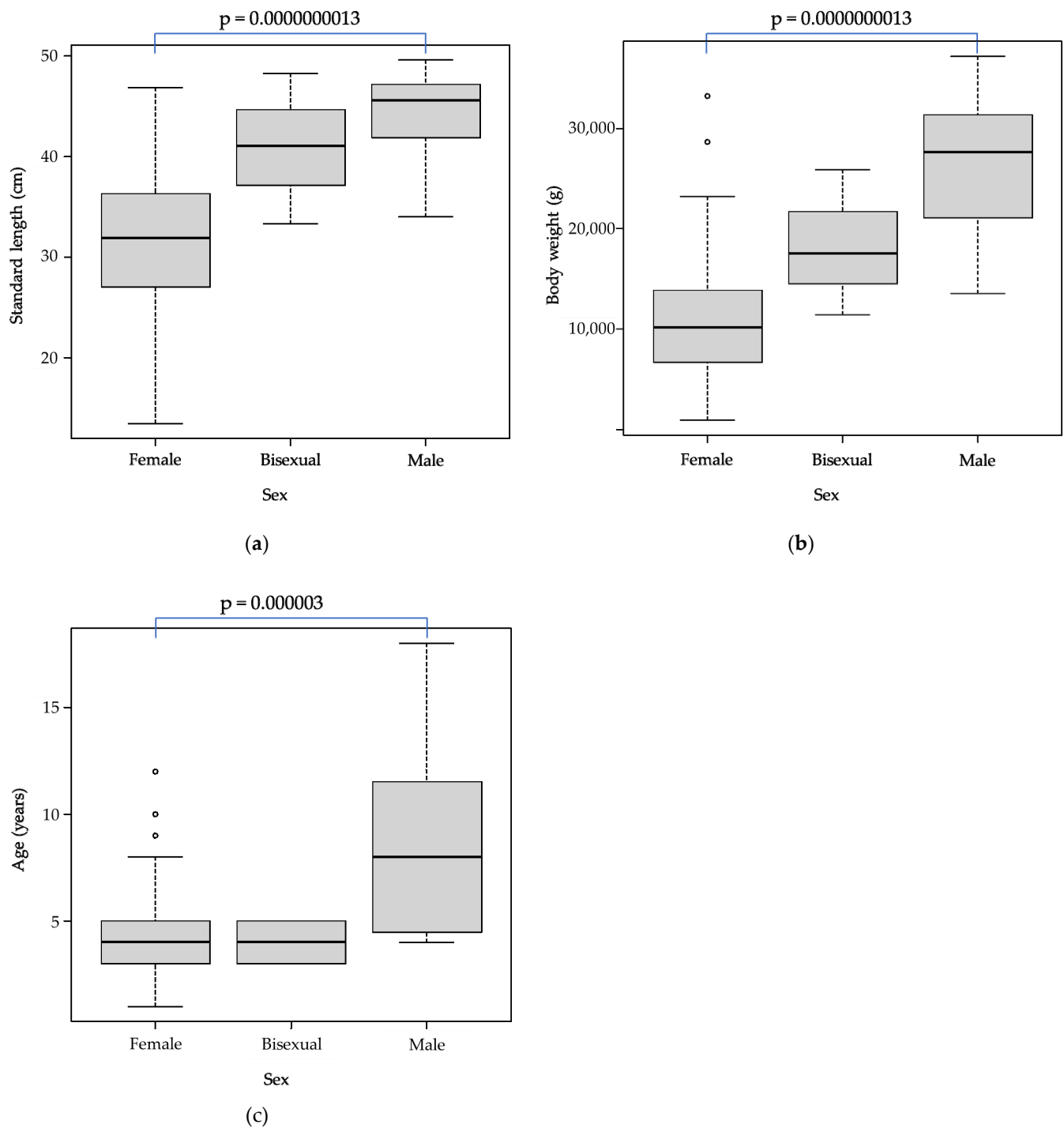


Figure S3. Relationship between the genders and other biological data (a: total length, b: body weight, and c: age) of *V. louti* specimens collected from the Ryukyu Islands, Japan.

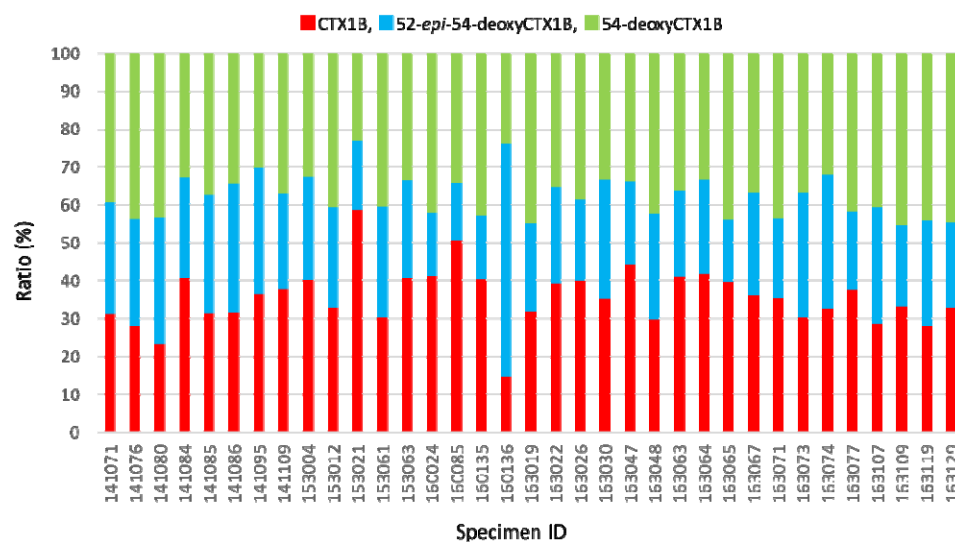


Figure S4. CTXs profiles of *V. louti* specimens collected from Ryukyu Island, Japan, from which quantifiable levels of CTXS were detected (n = 36).