Examination of Stomach Contents in Xanthid Crabs

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In order to elucidate the infestation mechanism of toxic crabs with paralytic shellfish poison (PSP), three xanthid crabs (*Zosimus aeneus*, *Atergatis floridus* and *Platypodia granulosa*) collected from Ishigaki Island and Miura Peninsula, were surveyed for stomach contents. Stomach contents of the three toxic crabs from Ishigaki Island consisted of red algae, animal tissues, corals, sand, etc., irrespective of their PSP toxicity levels. The same stomach contents composition was encountered in a nontoxic crab *Daira perlata* examined for comparison. Stomach contents of *A. floridus* specimens collected from Miura Peninsula showed a somewhat different composition, in which *Hypnea* sp., bivalves, gastropods, animal tissues and sand were dominant.

No definite conclusion was drawn from these data, as to the association of the eating habit of these toxic crabs with their PSP toxification.

Three xanthid crabs, *Zosimus aeneus*, *Atergatis floridus*, and *Platypodia granulosa*, which inhabit the Ryukyu and Amami Islands, are known to be highly infested with paralytic shellfish poison (PSP).¹⁾ In the previous paper,²⁾ we reported that *A. floridus* possesses a much stronger PSP and tetrodotoxin resistibility than nontoxic crabs. This, together with that the eggs of *A. floridus* were toxic, and that the toxicity level hardly changed during a long feeding period with nontoxic diet,³⁾ suggests that the PSP involved in toxic crabs is endogenous.

On the other hand, the individual and local variations of toxicity in those crabs, seem to support that the toxin is rather exogenous.*³

The present study was undertaken to elucidate the PSP infestation mechanism. This paper deals with the eating habits of the above xanthid crabs, as examined from the composition of their stomach contents.

Materials and Methods

Toxic Crabs

Thirty-seven *Z. aeneus* specimens, twenty-eight *A. floridus* specimens, and two *P. granulosa* specimens, were collected from Ishigaki Island,

Okinawa Prefecture, in 1979-1981. The specimens were immediately frozen, transported to the Laboratory of Marine Biochemistry, the University of Tokyo, and kept frozen until used. Besides, 75 specimens of *A. floridus* were collected from some localities of Miura Peninsula, Kanagawa Prefecture, and kept frozen similarly. The details of all these crab specimens are shown in Tables 1-5.

The specimens were examined for PSP toxicity and subsequently for stomach contents, as described below.

Nontoxic Crab

Twenty specimens of *Daira perlata* were collected from Ishigaki Island, and similarly examined after transportation to the University of Tokyo.

Assay of Toxicity

A leg or legs were torn off from each crab specimen and determined for PSP toxicity by the usual method.³⁾

Examination of Stomach Contents

The contents were squeezed out from the stomach of each crab specimen, weighed, and examined microscopically.

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Table 1. Composition of stomach contents and toxicity of *Zostimus aeneus* specimens collected from Ishigaki Island

Specimen No.	Date of catch	Sex	Body weight (g)	Toxicity*2 (MU/g)	Weight of stomach contents (g)	Composition of stomach contents*1											
						<i>Codium</i> sp.	<i>Ectocarpus</i> sp.	<i>Jania</i> sp.	<i>Polysiphonia</i> sp.	<i>Galaxaura</i> sp.	<i>Hypnea</i> sp.	Poriferans	Corals	Bivalves	Gastropods	Crustacea	Sand
1	June 12, 1979	M	50	1,150	1.5		+	t	t		##	##	##		t		+
2	"	"	62	1,500	2.1			##	##		##	##	+	t	t		+
3	"	"	59	1,580	1.4			##			+	+	+	+	+		+
4	"	"	25	4,140	0.8			##			+	+	t		t		t
5	"	"	15	2,100	0						Empty						
6	"	"	29	115	0.6			##			t	+		##	t		##
7	"	F	33	1,100	1.8			##				+	##	##		t	+
8	June 13, 1979	M	26	4,400	0.4			##			+	+	+	##	t		##
9	"	"	99	330	2.3			+			+	##	##	+	t		##
10	"	F	95	630	2.6			+	t		+	##	##	+	t	t	+
11	"	"	135	460	1.0			##			##	+	+	+	t	t	+
12	"	"	45	16,500	0.8			##	t		##	t	t	##	t	t	##
13	"	"	40	16,500	1.0			t			t	t	##	##	t	t	##
14	July 15, 1979	M	15	5,750	0.6			t			t	t	##	##			##
15	"	"	15	30	0.4			t			t	t	##	##	t		##
16	"	"	20	660	0.5		t	##	t		+	##	##	##	t	t	##
17	"	"	25	770	1.0			+	t	t	t	##	##	##	t	t	##
18	"	"	25	1,650	0.7			+	t	t	t	##	##	##	t	t	##
19	"	"	120	1,100	2.8			+			t	##	t	##	t	t	##
20	"	"	142	330	0.5			t				t	t	##	t	t	##

21	July 15, 1979	F	60	1,150	1.9	t	t	t	+	##	++	t	+
22	"	"	65	2,200	1.2	t	t	t	t	##	++	t	+
23	"	"	30	110	0.6	t	t	t	+	##	+	t	+
24	June 16, 1980	M	38	1,500	0			Empty					
25	"	F	9	2,000	0			Empty					
26	"	"	24	10	0.4	t	t	t	+		t	+	+
27	June 18, 1980	M	20	1,350	0.8	t	t	t	+	+	+	+	+
28	"	"	7	85	0.3	t	t	t	t	+	+	+	+
29	"	"	7	480	0.4	t	t	t	+	t	+	t	+
30	"	"	43	150	0.1	t	t	t		t	+	t	+
31	"	F	28	40	1.3	t	+	t	t	+	+	t	+
32	"	M	58	2,000	1.6	t	t	t	+	+	+	t	+
33	"	"	62	1,000	1.1	t	t	t	t	+	+	t	+
34	"	F	20	5,000	0.7	+	+	t	t	+	+	t	+
35	"	"	30	1,000	0.3	t	t	t	t	t	+	t	+
36	"	"	17	2,600	0.5	t	t	t	t	+	+	t	+
37	"	"	17	1,000	0.3	t	t	t	+	t	t	t	+

*1 ##: main, ++: moderate, +: minor, t: trace

*2 Toxicity of leg(s).

Results and Discussion

Stomach Contents in Zosimus aeneus

The composition of stomach contents in the *Z. aeneus* specimens are shown in Table 1, together with their toxicity scores.

The contents consisted of a green alga (*Codium* sp.), a brown alga (*Ectocarpus* sp.), red algae (*Jania* sp., *Polysiphonia* sp., *Galaxaura* sp., and *Hypnea* sp.), poriferans, corals, bivalves, gastropods, and sand. As shown in Table 1, *Jania* sp., *Hypnea* sp., corals and sand were dominant in stomach contents of all the specimens collected in June, 1979. In several specimens, poriferans were also dominant. In the specimens collected in July 1979 and June 1980, corals, sand, poriferans and *Hypnea* sp. were dominant, followed by *Jania* sp. The composition of stomach contents of these three lots of different collection dates were, however, roughly comparable to each other.

SAISHO and USHIO⁴⁾ examined stomach contents in six toxic *Z. aeneus* specimens caught at Amami Is. in June to November 1967, and found the debris of various species of algae: *Codium* sp., *Chlorodesmis comosa*, *Ectocarpus* sp., *Sphacelaria* sp., *Jania* sp., *Hypnea* sp. and *Polysiphonia* sp. Poriferans and corals were also detected.

The *Z. aeneus* specimens collected from Ishigaki Is. this time were very similar to the Amami specimens in stomach contents composition except that a brown alga *Sphacelaria* sp. was present in the latter.⁴⁾ The toxicity levels of the *Z. aeneus* specimens ranged from 10 to 16,500 MU/g. However, no clear relationship was observed between the toxicity value and the composition of stomach contents.

From the above results, *Z. aeneus* is postulated to be rather omnivorous than herbivorous, since parts of animal tissues, though not so much, were always found in the stomach.

Stomach Contents in Atergatis floridus

The results with *A. floridus* are summarized in Tables 2 (Ishigaki Is.) and 3 (Miura Peninsula).

Some local variations of the composition of stomach contents were recognized between the Ishigaki and Miura specimens.

Ishigaki specimens: In the specimens collected in June 1979, *Hypnea* sp., sand, shells, animal tissues, poriferans and fish fragments were detected. No parts of *Jania* sp., *Polysiphonia* sp., *Galaxaura* sp., were found. The toxicity scores

of the crab specimens ranged from 78 to 3,600 MU/g. In the specimens collected in June 1980, *Codium* sp., *Ectocarpus* sp., *Jania* sp., *Galaxaura* sp., *Hypnea* sp., annelids, animal tissues, corals and sand were identified or confirmed. The toxicity scores of the *A. floridus* specimens ranged from 10 to 100 MU/g. The composition of stomach contents is more similar to that of the *Z. aeneus* specimens collected in 1979 to 1980, than that of the *A. floridus* specimens collected in June 1979.

Jania sp., *Polysiphonia* sp., *Galaxaura* sp., *Hypnea* sp., poriferans, corals, bivalves, gastropods, animal tissues, shells and sand appeared in the specimens collected through April to July, 1981. *Hypnea* sp., animal tissues, corals and sand were found in most of the specimens. This composition is similar to that of the specimens collected in June 1980. Toxicity values were fairly high, ranging from 200 to 9,000 MU/g.

Miura specimens: The stomachs of the specimens collected at Enoshima in November 1981, contained *Hypnea* sp., bivalves, gastropods, animal tissues and sand. Sand was common in all the specimens. The toxicity value was 80 MU/g on the average. In the specimens collected at Arazaki through May to September 1981, *Hypnea* sp., red algae, poriferans, bivalves, animal tissues and sand were identified. The toxicity values ranged from 10 to 300 MU/g. As described above, the stomachs of the *A. floridus* specimens from Miura Peninsula contained animal tissues abundantly, followed by algae. No coral pieces were detected.

Stomach Contents in Platypodia granulosa

The results with two *P. granulosa* specimens collected in July 1981, are summarized in Table 4.

Poriferans and corals were dominant, while *Hypnea* sp., other red algae, bivalves and nemerteans were minor. Their toxicity values were 1,000 and 3,000 MU/g.

The composition of stomach contents is similar to that of the *A. floridus* specimens collected from Miura Peninsula.

Stomach Contents in Daira perlata

The results with twenty *D. perlata* specimens collected from Ishigaki Is. are summarized in Table 5. No toxicity was detected in any of them. Main stomach contents were *Jania* sp., *Hypnea* sp., *Ectocarpus* sp. and corals, while *Codium* sp., *Caulerpa* sp., other brown algae, *Galaxaura* sp., *Gelidium* sp., other crustacea, and sand were also

Table 2. Composition of stomach contents and toxicity of *Atergatis floridus* specimens collected from Ishigaki Island

Specimen No.	Date of catch	Sex	Body weight (g)	Toxicity* ² (MU/g)	Weight of stomach contents (g)	Composition of stomach contents* ¹																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
						<i>Codium</i> sp.	<i>Ectocarpus</i> sp.	<i>Jania</i> sp.	<i>Polysiphonia</i> sp.	<i>Galaxaura</i> sp.	<i>Hypnea</i> sp.	Poriferans	Corals	Annelids	Bivalves	Gastropods	Carapace of Crustacea	Fish	Egg	Animal tissue	Sand																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
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*1,*2 See footnotes in Table 1.

Line	Account	Amount	Balance	Debit	Credit	Balance
47	Empty	0.1	t			t
48	Empty	0				
49						
50						
51						
52	Empty	0				
53	Empty	0.1				
54						
55	Empty	0				
56						
57						
58						
59-61						
62						
63-65						
66						
67-69						
70						
71						
72						
73						
74						
75						

***1,*2** See footnotes in Table 1.

*3 Collected at Enoshima.

Table 4. Composition of stomach contents and toxicity of *Platypodia granulosa* specimens collected from Ishigaki Island

Specimen No.	Date of catch	Sex	Body weight (g)	Toxicity* ² (MU/g)	Weight of stomach contents (g)	Composition of stomach contents* ¹
1	May 7, 1981	M	4.5	1,000	0.1	<i>Codium</i> sp. <i>Ectocarpus</i> sp. <i>Jania</i> sp. <i>Polysiphonia</i> sp. <i>Galaxaura</i> sp. <i>Hypnea</i> sp. Other red algae Poriferans Corals Nemerteans Annelids Bivalves Gastropods Crustacea Fish eggs Egg Animal tissue Sand
2	"	F	10.5	3,000		

#1,*2 See footnotes in Table 1

Table 5. Composition of stomach contents and toxicity of *Daira perlata* specimens collected from Ishigaki Island

Specimen No.	Date of catch	Sex	Body weight (g)	Toxicity* ² (MU/g)	Weight of stomach contents (g)	Composition of stomach contents* ¹																	
						<i>Codium</i> sp.	<i>Caulerpa</i> sp.	<i>Ectocarpus</i> sp.	<i>Sphaecelaria</i> sp.	Other brown algae	<i>Jania</i> sp.	<i>Polysiphonia</i> sp.	<i>Galaxaura</i> sp.	<i>Hypnea</i> sp.	<i>Gelidium</i> sp.	Other red algae	Corals	Bivalves	Gastropods	Carapace of Crustacea	Animal tissue	Sand	
1	Apr. 9, 1981	M	29.0	ND* ³	0.1																		
2	"	"	23.5	"	0.2																		
3	"	"	26.5	"	0.3																		
4	"	"	30.0	"	0.3																		
5	"	"	13.5	"	0.2																		
6	"	F	41.0	"	0.8	t		+															
7	"	"	22.5	"	0.2			+		t													
8	"	"	11.0	"						t													
9	"	"	13.0	"	0.1	t		+		t													
10	"	"	19.0	"	0.4			+															
11	"	"	36.5	"						+													
12	"	"	15.5	"	0.2			t		+													
13	"	"	24.5	"	0.4			t		+													
14	"	"	16.0	"	0.2			+		t													
15	"	"	21.5	"	0.4			t		t													
16	"	"	27.5	"	0.4					t													
17	"	"	36.5	"	1.0	t		+	t	t													
18	"	"	19.0	"	0.3			+		t													
19	"	"	9.5	"	0.1			+		t													
20	"	"	13.0	"	0.1					t													

*1,*2 See footnotes in Table 1.

*3 Less than 2 MU/g.

detected.

This composition agrees well with that of the *Z. aeneus* specimens which were collected from Ishigaki Is. through 1979 to 1980 and showed high toxicity values as described above.

The stomach contents in *Z. aeneus*, *A. floridus* and *D. perlata*, all collected at Ishigaki Is., were very similar to each other. They inhabit coral reefs and are likely to have the same eating habits. However, the specimens of *Z. aeneus* and *A. floridus* generally showed high toxicity values while those of *D. perlata* no toxicity. On the other hand, *P. granulosa* at Ishigaki Is., also inhabit coral reefs and the two specimens examined here showed high toxicity values, though their stomach contents composition differed from those of the above species. It rather resembles that of *A. floridus* specimens collected from Miura Peninsula, although the *P. granulosa* specimens and the *A. floridus* specimens from Miura Peninsula were significantly different from each other as far as the toxicity scores are concerned (Tables 3 and 4). As common stomach contents of *Z. aeneus* and *A. floridus* collected from Ishigaki Is., *Jania* sp., *Polysiphonia* sp., *Galaxaura* sp., *Hypnea* sp., poriferans, corals, bivalves, gastropods, carapace of crustacea and sand were listed up. When the results with *P. granulosa* are also taken in, *Hypnea* sp., poriferans, bivalves, carapace of crustacea, and sand are listed up as common stomach contents. However, those were also almost common to the *D. perlata* specimens which did not show any toxicity.

Thus, the origin of PSP in those toxic crabs has not been found in their stomach contents. As reported before,²⁾ toxic crab species possess an extremely strong PSP resistibility in comparison with nontoxic crabs. Considering also that the

eggs of *A. floridus* were toxic, and that toxic crabs maintained a fairly high toxicity level for a long period when fed nontoxic diets, PSP in toxic crabs may be endogenous, some portion being inherited from their parents and the rest being synthesized *in vivo*. On the other hand, wide individual and local variations of toxicity value were observed in the same species (Table 1-4), suggesting that PSP in toxic crabs is related to some environmental factor(s), or that it comes from a food. Even in this case, the mechanism may not be a simple food chain. Toxicity assay of the foods to be ingested by toxic crabs, and of the crabs fed the diets of above compositions will be needed to elucidate the PSP-infestation mechanism in those toxic crabs.

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