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The Effects of Hydrogen Cyanide on *Asellus communis* and *Gammarus pseudolimnaeus* and Changes in Their Competitive Response when Exposed Simultaneously[1,2]

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Most studies of the long-term effects of toxicants have exposed a single species in each chamber. This study was designed to determine the effect of the presence of a second organism on the reaction of a test animal to cyanide. The first organism *Gammarus pseudolimnaeous* Bousfield was chosen because of its aggressive and competitive behavior (HYNES 1954, ANDERSON AND RAASVELDT 1974, OSEID 1977) and the second, *Asellus communis* Say, because it has a passive nature (ALLEE 1929, OSEID 1977[3]). *Asellus communis* has also been shown in previous short-term tests (OSEID AND SMITH 1974[4]) to be much more resistant to hydrogen sulfide than *Gammarus*, and it was thought that a similar reaction would result with exposure to hydrogen cyanide. In the present study, short-term tests were done on each species alone and then full-life-cycle tests were done on each species alone and then with the two together.

MATERIALS AND METHODS

Field collections of *Gammarus* were taken about 10 days prior to the start of each test from a small, spring-fed stream entering the Saint Croix River at Marine-on-Saint Croix, Washington County, Minnesota. Days in the laboratory, length and weight of organisms at start of test, and test duration are noted in Table 1. The *Asellus* were cultured from an initial group collected from Rainy Lake near Rainier, Koochiching County, Minnesota, on November 19, 1974. *Asellus* and *Gammarus* were kept in the laboratory at 18°C and O_2 saturation and were fed presoaked deciduous tree leaves and dead fish. Exposures were made with the

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[3]Keyed out to *Asellus militaris* Hay in PENNAK (1953) but to *Asellus communis* Say in WILLIAMS (1974).

[4]*Ibed.*

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test water, apparatus and chemical procedures described by SMITH *et al.* (1977). Three acute tests were performed on *Gammarus* and two on *Asellus*, and both 96-hr LC50 and lethal threshold concentrations were calculated. For each series of chronic exposures (*Asellus* alone, 1 and 2; *Gammarus* alone; and *Asellus* and *Gammarus* together in the same test tank), two simultaneous but separate tests with interspersed treatment concentrations were conducted.

Nominal test conditions for both acute and chronic exposures were 18°C, 8.0 pH, and 6 mg/*l* dissolved oxygen. Two 20-watt "Vita-Lite" fluorescent tubes above each tank provided approximately 500 lux at the water surface for 16 hr of light and 8 hr of darkness. Chronic tests were started by placing 40 individuals at random into each of the test tanks. From the time of the start of reproduction, the tests were continued 45 days. At that time all test specimens were preserved, measured, checked for fecundity, and weighted as a group for each tank.

RESULTS AND DISCUSSION

The 96-hr LC50 for *Asellus* was 2,295 µg/*l* HCN and for *Gammarus* was 169 µg/*l*. The respective LTC values (10-12 days) were 1,895 and 74 µg/*l*. The ranges of the means and the standard deviations for individual test tanks within each test series of the chronics are shown in Table 1. Tables 2, 3, 4, and 5 list the mean HCN (molecular) concentration and standard deviation of the biological indices for each test tank in the four test series.

For the first test series on *Asellus* (1) there was a large reduction of numbers and weight at the lowest tested concentration, 51 µg/*l* (Table 2). The highest concentration with survival and reproduction was 317 µg/*l* with no survival or reproduction in the range 432 to 2,108 µg/*l*. For the second series (Table 3) there were survival and reproduction at all concentrations tested (5-100 µg/*l*). The numbers of free individuals, eggs and young were variable, making evaluation on these indices questionable. The standard deviation for total weight was relatively low, and concentrations of 40 µg/*l* and higher were lower than the mean of the two controls by more than two times the standard error of the control mean. The mean number of eggs per gravid female was reduced only at 100 µg/*l*. All other indices were within two standard errors of the control mean. The highest no-effect concentration lies between 29 and 40 µg/*l*, Table 6.

In the series on *Gammarus* (Table 4) numbers were variable, but weight was more consistent and, except for a low value for 16 µg/*l*, concentrations of 32 µg/*l* and higher were reduced more than two standard errors of the mean. The "effect-no-effect" concentrations were 16 and 21 µg/*l* based on total number of eggs or young in the brood pouch and mean number of eggs or young per gravid female.

The effect of exposing the *Asellus* and *Gammarus* in the same

TABLE 1

Test conditions for *Gammarus* and *Asellus* bioassays

	Asellus[a] (1)		*Asellus*[a] (2)		*Gammarus*[a]		*Asellus* and *Gammarus* together	
							Asellus	*Gammarus*
Days in laboratory before testing	300		429		12		360	17
Size at start of test								
Mean length (mm)	6.3		6.7		8.7		6.1	9.7
Length range (mm)	4.0-8.0		5.0-9.5		3.0-14.0		3.5-8.0	2.5-17.0
Mean weight (mg)	7.7		9.2		10.7		7.1	14.1
Duration (days)	115		112		83		98	98
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Temperature (°C)	18.1-18.2	0.1-0.2	18.0-18.1	0.1-0.5	18.0-18.1	0.4-0.8	18.0-18.2	0.1-0.2
pH	7.98-8.13	0.03-0.06	7.92-7.06	0.05-0.10	7.99-8.02	0.04	7.92-7.98	0.04-0.05
Dissolved O_2 (mg/ℓ)	6.03-6.52	0.26-0.42	5.88-6.14	0.26-0.40	6.77-7.00	0.50-0.69	5.70-6.09	0.30-0.41
Total alkalinity (mg/ℓ)	238	3	236	2	236	2	236	1
Light intensity (lux)	481-660	--	506-657	--	452-667	--	431-603	--

[a] Alone

TABLE 3

Hydrogen cyanide (molecular) means and standard deviations, and biological indices for each test tank in the chronic series on *Asellus* alone (2), both tests included[a]

	Diluter number											
	3	4	4	3	4	4	3	4	3	4	3	3
HCN (µg/l) Mean	0	0	5	10	19	29	40	47	58	70	77	100
HCN (µg/l) S.D.	0	0	1	3	2	5	4	10	8	7	12	12
Number of free individuals[b]	758	2551	1622	2023	1091	1158	184	183	1335	376	453	655
Eggs or young in brood pouch	154	732	67	578	198	394	0	72	621	124	123	51
Free individuals plus eggs and young	912	3283	1689	2601	1289	1552	184	255	1956	500	576	706
Weight (g) free individuals plus eggs and young	2.495	3.221	6.918	5.466	3.646	3.146	0.488	0.520	1.454	1.418	0.867	0.973
Mean eggs or young per gravid female	19.2	22.2	22.3	22.2	24.8	21.9	--	18.0	20.7	31.0	20.5	17.0

[a] Underlined values are lower than the mean of the controls by more than two times the standard error of the mean.

[b] Those individuals living separate from the brood pouch.

TABLE 2

Hydrogen cyanide (molecular) means and standard deviations and biological indices for each test tank in the chronic series on *Asellus* alone (1), both tests included[a/]

	Tank number						
	6	6	3	3	5	4	2
HCN (µg/*l*) mean	0	0	51	110	220	317	432
HCN (µg/*l*) S.D.	0	0	9	16	19	36	43
Number of free individuals[b/]	1612	2467	262	109	85	56	0
Number of eggs or young in brood pouch	626	855	72	0	60	17	0
Total number of free individuals plus eggs and young in brood pouch	2238	3322	334	109	145	73	0
Total weight (g) of free individuals plus eggs and young in brood pouch	3.703	4.612	0.725	0.292	0.106	0.142	--
Mean number of eggs or young per gravid female	35	34	18	--	20	17	--

[a/]Underlined values are lower than the mean of the controls by more than two times the standard error of the mean.

[b/]Those individuals living separate from the brood pouch.

test chamber was twofold (Table 5). The *Gammarus* almost eliminated the *Asellus* from the controls and low treatments, and there was a shift downward of the "effect-no-effect" concentrations for *Gammarus*. On the basis of all the indices for *Gammarus* the highest no-effect concentration lies between 4 and 9 µg/*l*, values which are lower than when *Gammarus* was exposed alone. The highest no-effect concentration for *Asellus* in the same test was between 41 and 55 µg/*l*, approximately the same as on the two series for *Asellus* alone.

TABLE 4

Hydrogen cyanide (molecular) means and standard deviations and biological indices for each test tank in the chronic series on *Gammarus* alone, both tests included[a/]

	Diluter number									
	3	4	4	3	4	4	3	4	3	4
Mean (µg/l) Mean	0	0	5	11	16	21	32	42	52	64
HCN (µg/l) S.D.	0	0	2	2	3	6	5	7	14	10
Number of free individuals[b/]	137	554	764	331	82	335	214	20	6	0
Eggs or young in brood pouch	6	135	113	13	16	0	0	0	0	0
Free individuals plus eggs and young	143	689	877	344	98	335	214	20	6	0
Weight (g) free individuals plus eggs and young	0.795	1.343	1.705	0.726	0.255	0.629	0.273	0.110	0.033	0
Mean eggs or young per gravid female	3	27	19	13	16	-	-	-	-	-

[a/] Underlined values are lower than the mean of the controls by more than two times the standard error of the mean.

[b/] Those individuals living separate from the brood pouch.

TABLE 5

Hydrogen cyanide (molecular) means and standard deviations and biological indices for each test tank in the chronic series with *Asellus* (A) and *Gammarus* (G) together, both tests included[a/]

		Diluter number									
	Species	1	2	1	2	1	2	1	2	1	2
HCN (µg/l) Mean		0	0	4	9	21	31	41	55	62	76
HCN (µg/l) S.D.		0	0	1	1	2	5	5	5	11	6
Number of free individuals[b/]	A	27	52	34	519	625	903	644	132	114	437
	G	354	591	745	212	24	3	2	0	0	0
Eggs or young in brood pouch	A	0	0	0	257	470	363	697	127	34	224
	G	334	386	359	25	0	0	0	0	0	0
Free individuals plus eggs and young	A	27	52	34	776	1095	1266	1341	259	148	661
	G	688	977	1104	237	24	3	2	0	0	0
Weight (g) free individuals plus eggs and young	A	0.216	0.419	0.314	2.227	1.734	1.552	1.522	0.568	0.369	1.176
	G	1.965	3.142	3.852	0.941	0.086	0.017	0.011	--	--	--
Mean eggs or young per gravid female	A	--	--	--	19.8	23.5	25.9	25.8	15.9	17.0	18.7
	G	17.6	16.8	17.1	8.3	--	--	--	--	--	--

[a/] Underlined values are lower than the mean of the controls by more than two times the standard error of the mean.

[b/] Those individuals living separate from the brood pouch

TABLE 6

Comparisons of the "effect/no-effect" concentrations (µg/*l*) of hydrogen cyanide for *Asellus* and *Gammarus*

	Asellus			*Gammarus*	
	Exposed alone, Series 1	Exposed alone, Series 2	Exposed with *Gammarus*	Exposed alone	Exposed With *Asellus*
Number of free individuals[a/]	0-51	-	41-55	32-42	4-9*
Number of eggs or young in brood pouch	0-51*	-	41-55	16-21	4-9*
Total number of free individuals plus eggs and young	0-51*	-	41-55	32-42	4-9*
Total weight (g) of free individuals plus eggs and young	0-51*	29-40*	41-55	21-32*	4-9*
Mean number of eggs or young per gravid female	0-51*	77-100	41-55	61-21	4-9*
Highest concentration with any survival	317	-	-	52	41
Highest concentration with any reproduction	317	-	-	16	9

*Those marked with an asterisk were lower than the mean of the two controls for the respective test by at least two times the standard error of the mean.

[a/] Those individuals living separate from the brood pouch.

The attributes for survival of *Asellus* in a mixed community are their much greater resistance for short periods (10-12 day LTC value of 1,895 µg/*l* HCN) and the ability of some fraction of the population to survive and reproduce at high concentrations (up to 317 µg/*l* HCN) after a continuous exposure of 115 days. By comparison, *Gammarus* have a 25-fold lower 10-day LTC, a 6-fold lower concentration which permits any survival and a 19-fold lower for reproduction. Therefore, the presence of low concentrations (9 to 30 µg/*l* HCN) probably shifts the competitive advantage from

the aggressive *Gammarus* to the more passive *Asellus*. *Gammarus* are probably excluded from cyanide polluted areas, satisfactory to them if alone, because they are unable to compete with more resistant species whether typically predator or prey. *Asellus* would be benefited by reduced competition and predation.

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