A Comparison of the Variability of Asellus communis (Crustacea: Isopoda) and Gammarus pseudolimnaeus (Crustacea: Amphipoda) and Suitability for Joint Bioassays^{1, 2}

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INTRODUCTION

One of the important considerations in selecting a test organism for bioassays is its variability in response. An organism with low variability will give greater precision and make comparisons of test results more reliable. Also, researchers interested in doing tests on two species for studies of competition, predatorprey and other interactions require test organisms which can both do well under the same test conditions.

The experiments reported here were conducted to compare the variability of *Asellus communis* Say and *Gammarus pseudolimnaeus* Bousfield and to determine if both species produced viable populations under the same conditions when tested in separate tanks.

MATERIALS AND METHODS

The Asellus used in the test were the second generation from adults hand picked from bottom material scooped from Rainy Lake near Ranier, Koochiching County, Minnesota. They were abundant along the shoreline in sheltered coves where decomposing organic material had accumulated. The Gammarus were collected with a drift net from a small, spring-fed stream entering the Saint Croix River at Marine-on-Saint Croix, Washington County, Minnesota.

Prior to the start of the tests Asellus and Gammarus were held separately in 25-l (50 x 25 x 25 cm high; water depth, 20 cm) tanks constructed with double strength glass and silicone adhesive. Well water (Table 1) flowed through the aerated tank at 700 ml/min. The dissolved oxygen was maintained at saturation and the temperature at $18^{\circ}C$. A constant supply of fall-dropped leaves and dead minnows was provided as food. The leaves also provided cover for the test animals. The leaves were soaked in flowing, aerated water for about 30 days prior to use and were a mixture of white

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TABLE 1. Analysis of well water $^{\mathrm{l}}$

Item	Concentration (mg/l)
Total hardness as CaCO ₃	220
Calcium as CaCO ₃	140
Iron	0.02
Chloride	<1
Sulfate	<5
Sulfide	0.0
Fluoride	0.22
Total phosphates	0.03
Sodium	6
Potassium	2
Copper	0.0004
Manganese	0.0287
Zinc	0.0044
Cobalt, nickel	<0.0005
Cadmium, mercury	<0.0001
Ammonia nitrogen	0.20
Organic nitrogen	0.20

¹Water taken from well head before aeration and heating; pH 7.5.

poplar, hackberry, and cottonwood. The dead fish were freshly killed or frozen, and it appeared that little feeding was done until some decomposition and fungus growth had developed.

Water for the tests was adjusted to test temperature and saturated with oxygen in a head tank and then flowed by gravity to a water supply system (BRUNGS and MOUNT 1970) which distributed the water to the five individual test chambers. The 20-7 glassilicone chambers were 50 X 25 x 21 cm, and a water depth of 16 cm was maintained. The flow-through system provided 95% water replacement in 3 hr.

At the start of the *Asellus* test, 40 individuals were randomly placed in each of 5 test chambers, and 40 were preserved to determine mean and range of total length and the mean weight of the individuals. The same procedure was followed for *Gammarus* in five

separate tanks (Table 2). During the test, temperature and pH

TABLE 2

Test conditions in Gammarus and Asellus experiments

Item	Asell	us	Gcommo	rus
Date collected	19 Nov.	1974	3 Mar.	1975
Laboratory holding (days)	114		10	
Test duration (days)	109		71	
Size at Start Mean total length (mm)	4.2		13.3	
Range (mm)	2.0-6.0)	6.0-18.	0
Mean weight	3.1		23.4	
(mg)	Means 1	$\underline{\text{s.p.}}^1$	Means 1	<u>s.D.</u> 1
Temperature (°C)	17.96-18.02	0.13-0.15	18.15-18.23	0.70-0.72
pН	8.01-8.03	0.03-0.04	8.03-8.04	0.03
Dissolved oxygen (mg/l)	7.06-7.33	0.34-0.41	7.53-7.69	0.24-0.30
Total alkalinity (mg/l)	236	2	235	4
Light intensity (lux)	420–506	-	538-646	-

¹Range of means and standard deviations are for all tanks in each experiment.

were determined three times and total alkalinity and dissolved oxygen once per week. Each week all tanks were checked for the appearance of young produced by the original stock. The procedure was to gently disturb the fine silt accumulations on the bottom with a plastic probe and watch for the scurrying young. was terminated 45 days after the first young were observed, and the contents of each tank were passed through three screens (square mesh openings of 6.0, 2.0, and 0.4 mm) to separate debris and organisms into four size groups. The group that passed through the smallest screen contained no organisms and was discarded. three larger groups were then preserved and stained with a mixture of 10% formalin and rose-bengal (0.6 g/l). Separating the samples into size groups and staining the organisms greatly enhanced their visibility and facilitated picking and measuring, especially of the smaller individuals. After a few weeks in the stain the organisms were separated from debris and measured. The eggs and

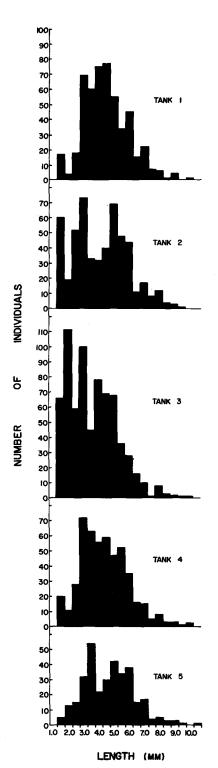
young were removed from gravid females and counted. The entire population of adults, eggs, and young was centrifuged at a low speed to remove excess water and weighed.

RESULTS AND DISCUSSION

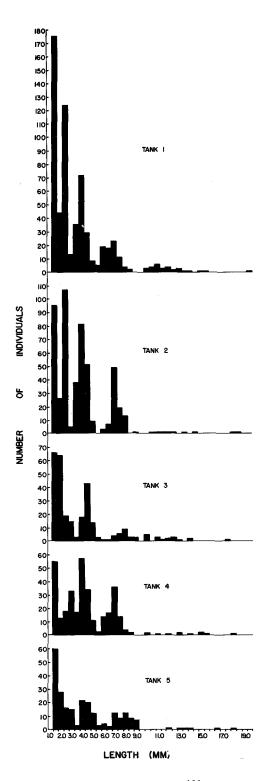
Pretest and test conditions are noted in Table 2. Length-frequency diagrams of all organisms in each test chamber were made (Figures 1 and 2). Table 3 lists the population parameters for each test chamber. The term "free individuals" refers to organisms not contained in the brood pouch. Table 4 compares Asellus and Gammarus on the basis of the mean, standard deviation and coefficient of variability for each of the population indices for the five tanks combined.

The similarity of the general shape of the length-frequency diagrams within each species indicates that the five tanks were fairly well synchronized in terms of reproduction time and growth rates (Figures 1 and 2). Comparison of the mean values for the five tanks of each species (Table 4) shows that the number of free individuals, number of eggs and young in the brood pouch, total number, and total weight were similar for the two species. The values of Asellus are all about 25% higher than for Gammarus, but the coefficients of variability are about half as high as those for Gammarus. One exception was the number of eggs and young in the brood pouch. Tank 3 of the Asellus test reproduced slightly earlier, and there were many females with empty brood pouches, and the number of free individuals in the 1.5 to 3.0 mm length group In the Asellus culture tanks in this laboratory where stocks have been maintained from 19 November 1974 to the present, densities are much greater than in the test tanks. Attempts to culture Gammarus at similar densities have failed. ALLEE (1929) found that the Asellus can occur at high densities and remarks that in general they are tolerant of many individuals in a limited space. By contrast, papers by ANDERSON and RAASVELD (1974) and HYNES (1954) report predation and cannibalism for species of Gammarus. OSEID (1977) studying the effect of Asellus militaris! and Gammarus pseudolimnaeus on fish eggs found that Gammarus consumed live fry and eggs of fish whereas Asellus did not. The control tanks of toxicity bioassays of NEBEKER and PUGLISI (1974) and OSEID and SMITH (1974) had mean total individuals of 254 and 433, respectively, where test conditions other than water supply and tank size were similar. The later work had water with a higher mineral content and larger tanks. Mean weight and mean number of eggs of young per gravid female for Asellus and Gammarus were very similar, but again the coefficient of variability was greater for Gammarus. The mean length of gravid females was much smaller for Asellus. The growth rates for both species appeared to be similar so that maturity at a small size allowed shorter

¹Keyed out to Asellus militaris Hay in Pennak (1953) but to Asellus communis Say in Williams (1974).



Length-frequency diagrams of all Asellus in each test chamber. Figure 1.



Length-frequency diagrams of all Gammarus in each test chamber. Figure 2.

TABLE 3. Values for population parameters in Asellus and Gammarus tests

			Asellus				ণ্ড	Gammarus		
		ŀ	Tank Number				ì	Tank Number		
		2	3	4	2	1	2	3	4	2
Number of free individuals	510	526	702	967	334	612	513	289	340	237
Number of eggs or young in brood pouch	162	120	0	163	103	9	09	117	7.7	31
Total number of free individuals plus eggs and young in brood pouch	672	979	702	659	437	677	573	907	417	268
Total weight of free individuals plus eggs and young in brood pouch (g)	1.766	1,538	1.387	1.539	1.026	1.246	1.352	0.617	1.225	0.602
Mean weight of free individuals (mg)	3.46	2.92	1.98	3.10	3.07	2.04	2.64	2.13	3.60	2.54
Mean number of eggs or young per gravid female	23.1	20.0	1	20.4	20.6	21.7	30.0	16.7	19.2	15.5
Mean length of gravid females (mm)	5.50	5.58	1	5.50	5.50	11.8	13.0	11.6	11.8	12.5

TABLE 4.

Comparison of the means for the population indices of the five replications

		Asellus			Ganmarus	
	ı×	S.D.	C.V.	ı×	S.D.	c.v.
Number of free individuals	514	131	25	298	158	40
Number of eggs and young in brood pouch	110	67	61	70	31	77
Total number of free individuals plus eggs and young in pouch	624	106	17	468	159	34
Total wieght of free individuals plus eggs and young in pouch (g)	1.451	0.274	19	1.008	0.367	36
Mean weight of free individuals (mg)	2.91	0.55	19	2.59	0.62	24
Mean number of eggs or young per gravid female	21.0	1.4	7	20.6	5.8	28
Mean length of gravid females (mm)	5.52	0.04	H	12.14	0.59	ŗ,
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generation time. After 45 days from the first observation of young the F_1 generation of Asellus had many gravid females, and tanks 2 and 3 had large numbers of F_2 . After the same time Gammarus F_1 had not grown to the size of maturity, and only the original stock individuals, all 10.0 mm or larger, were gravid.

These tests indicate that as an individual test organism Asellus has several advantages. It has a lower variability, therefore fewer replications would be required to get the same precision. It can be easily cultured in high numbers in the laboratory and possibly the time to complete a generation is shorter, thereby reducing the time required to do full generation tests. However, apart from variability the tests also showed that in terms of the population parameters (Tables 3 and 4) the two species produced fairly similar populations. Therefore, when tested in the same test chamber where the two species can interact, both species should have an equal chance to develop a population apart from factors such as competition, predator-prey relations, etc.

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