SALINITY TOLERANCES OF TWO MARYLAND CRAYFISHES[1, 2]

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The recent population expansion and the ecological tolerances of the introduced crayfish, *Orconectes virilis* (Hagen), within the Patapsco River watershed of Maryland have been demonstrated by Schwartz, Rubelmann, and Allison (1963). We wish to determine the salinity tolerances of this species prior to its possible expansion into other watersheds of the state and to compare them with a typical freshwater form, *Cambarus b. bartonii* (Fabricius), which is not known to have inhabited saline waters.

Few data are available on the tolerance of the euryhalinity of freshwater crayfishes. Herrmann (1931) found *Astacus astacus* (= *Potamobius astacus*) would survive for three months in 50 per cent seawater while Bogucki (1934) found that the same species, which he designated *Astacus fluviatilis*, would tolerate 66 per cent sea water for one month. Clemens and Jones (1954) found an Oklahoma crayfish (species unknown, personal communication August 15, 1963) tolerant of oil well brine solutions which were diluted to 8.7 per cent by volume of the original brine. Three species, *Rhithropanopeus*, *Callinectes*, and *Platychirograpsus*, of North American marine or fresh water crabs have been known to invade or inhabit freshwaters (Chace et al., 1959). Most observations, however, have dealt with tissue osmoregulation, ion concentration, osmotic receptor sites, influences of molt cycle, etc. (Krogh, 1939; Prosser, 1950; and Waterman, 1960, 1961). Schleiper (1935) stated that no freshwater invertebrate could maintain a normal concentration of its internal medium when placed in seawater. Presson (1957) noted that temperature had no effect on the ionic concentration of the blood of *O. virilis* when it was placed in solutions containing calcium and potassium ions; the blood concentrations of sodium were different at various temperatures. Gross (1957) found that *Procambarus clarkii* (= *Cambarus clarkii*) had the least permeable exoskeleton among various marine and aquatic decapods and reasoned that this permitted the species to regulate to osmotic stress.

METHODS

Orconectes virilis specimens were seined from above a dam on the 100-ft wide and 3-ft deep Patapsco River in the Patapsco State Park, 1.5 mile W. of U. S. Rt. 1 near Relay, Maryland. The river water was fresh, non-tidal, and heavily polluted by industrial and domestic waste (Schwartz, Rubelmann, and Allison, 1963). The exoskeleton and appendages of many of the 80- to 110-mm long specimens were overcoated with a filamentous-hairy growth (*Epistylis?*) (fig. 1). The specimens were placed in a styrene ice chest containing ice and river water and transported 90 miles to the Chespeake Biological Laboratory at Solomons by car.

Cambarus b. bartonii were similarly collected from the Casselman River near U. S. Rt. 40, Garrett County, Maryland. The habitat, in contrast to the slow, silt-and sewage-ladened Patapsco River, was a rapidly flowing, shallow, rocky, mountain stream. A similar double-trayed ice chest permitted safe conduct of the crayfish during the seven-hour trip to the laboratory.

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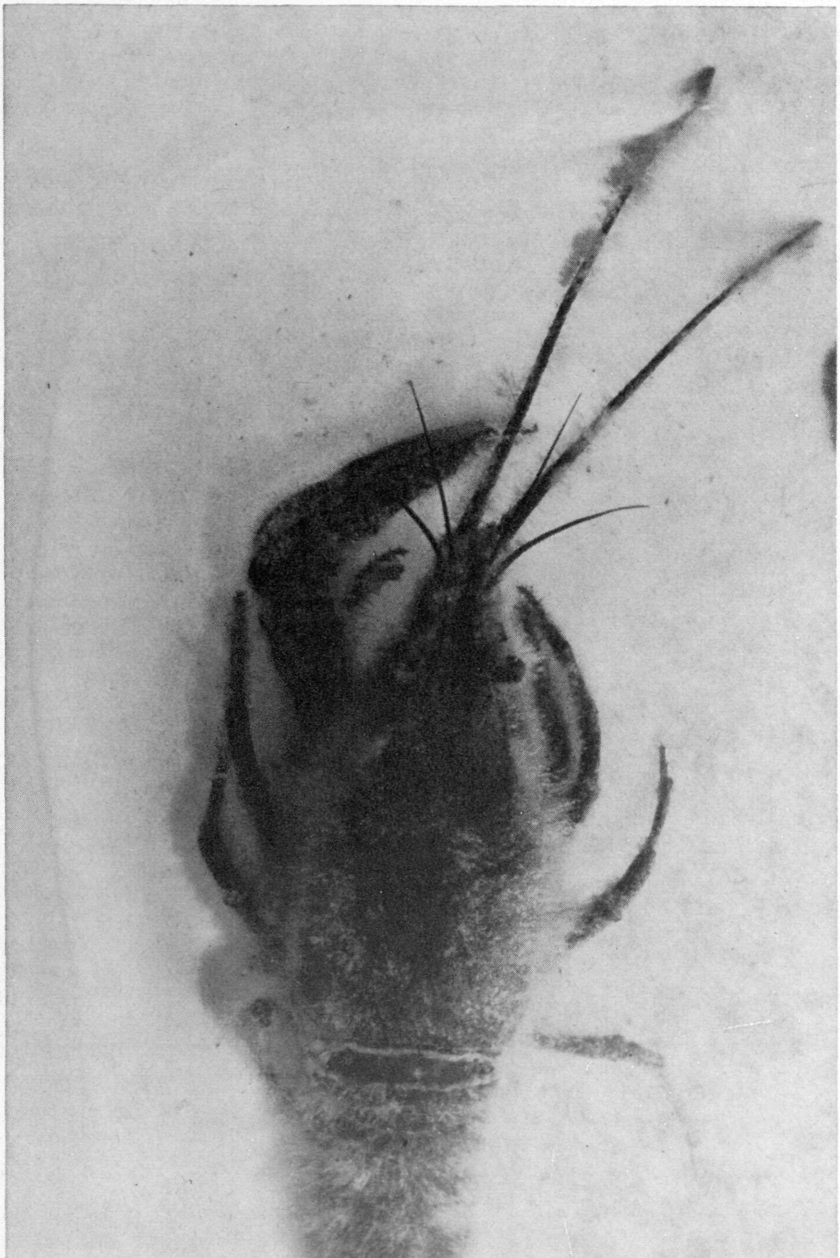

FIGURE 1. Typical specimen of *O. virilis* collected in June and August, 1962, in the Patapsco River, Maryland.

Experimentally, four lots (10♂♂, 10♀♀) of each species were placed in separate aquaria. Running fresh well water and approximately 6 ppt (parts per thousand), 14 ppt, and 27 to 33 ppt saline waters were the test media (table 1).

TABLE 1

Longevity of two species of crayfishes subjected to varying salinities and temperatures

	Fresh	6 ppt	14 ppt	30 ppt
	Orconectes virilis			
Expt. I				
Total days survived by any or all specimens	13+	9	12	10
Salinity				
Mean	0.8	6.1	13.1	27.4
Range	0.4–1.7	3.4–9.7	12.8–13.7	26.9–28.5
Temperature (C)				
Mean	21.3	22.4	24.0	22.2
Range	19.7–23.0	20.4–24.0	21.9–25.4	20.7–23.5
Expt. II				
Total days survived by any or all specimens	31+	31+	23	5
Salinity				
Mean	1.2	5.8	15.2	33.0
Range	0.7–1.6	1.6–8.9	14.4–15.8	32.7–33.3
Temperature (C)				
Mean	22.7	23.2	24.1	24.5
Range	19.9–25.1	19.4–24.8	19.0–26.3	23.4–25.9
	Cambarus b. bartonii			
Expt. I				
Total days survived by any or all specimens	11+	11	5	8
Salinity				
Mean	1.4	5.2	13.6	27.5
Range	0.8–1.7	3.4–9.4	12.8–14.2	26.7–28.4
Temperature (C)				
Mean	21.9	22.9	24.9	24.9
Range	19.9–25.1	22.1–24.8	22.8–25.9	22.3–26.3
Expt. II				
Total days survived by any or all specimens	31+	10	27	5
Salinity				
Mean	1.2	5.9	15.4	33.6
Range	0.7–1.6	2.1–8.9	14.5–15.8	33.0–34.0
Temperature (C)				
Mean	22.8	23.5	24.0	24.7
Range	19.9–25.1	22.1–25.0	19.0–26.1	22.4–25.9

The 6 ppt solution was obtained by mixing nearly equal amounts of fresh well water with that of the Patuxent River (14 ppt). The 14 ppt test solution was simply Patuxent River water which was pumped into the aquarium. A closed recirculating system of water from the Atlantic Ocean was maintained near 27 or 33 ppt. Plexiglass dividers separated the species within each aquarium. Holes drilled in the plexiglass permitted the water to flow freely between the two halves.

Twelve or 24-hour observations consisted of noting the number of animals alive in each lot, the water temperature, and the salinity (determined by a hydrometer). Each experiment was run until all but the controls succumbed or until an individual or a particular species tolerated a given salinity for some prolonged period of time without further deaths. The $LD_{50's}$ for each species were determined by simply noting (fig. 2 and 3) the elapsed time interval when 50 per cent of the test animals had succumbed.

RESULTS AND DISCUSSION

Orconectes virilis

Identical salinity tolerance tests begun July 2 and August 13, 1962, revealed that *O. virilis*, when temporarily subjected to saline waters, as during a tidal flush, could tolerate salinities up to 33 ppt. However, if the influences were prolonged (fig. 2), one-half of the population so subjected would have died within

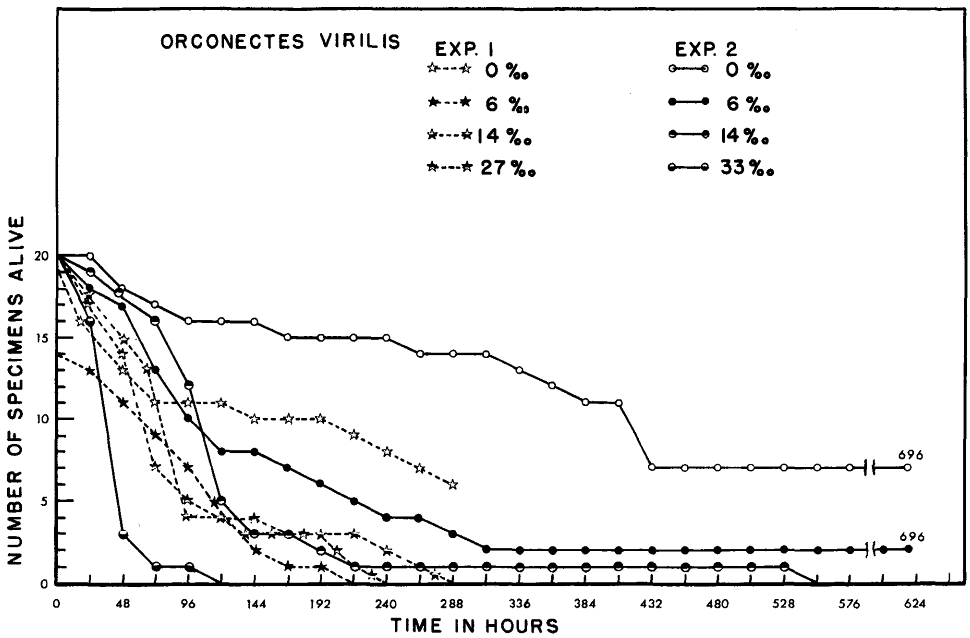

FIGURE 2. Survival tolerances of *O. virilis* subjected to salinities of approximately 0, 6, 14 and 27 to 33 ppt.

96 hours. Specimens of *O. virilis*, regardless of sex, tolerated salinities of approximately 0, 6, 14, and 30 ppt for a maximum of 288, 216, 288, and 240 hours, during the first test, while survival periods of 696, 696, 528, and 120 hours were noted during the second test (fig. 2). Water temperatures, throughout the experiment, approximated that of the ambient temperature of the laboratory room (table 1) and were insignificant as death causal agents. A test of the survival-salinity data substantiated that no statistical differences existed in the rates of mortality, except in experiment 2, when the 33 ppt salinity was a serious delimiting factor killing 17 of the 20 test individuals in 48 hours and the remainder in 120 hours (fig. 2). The removal, statistically (Snedecor, 1950) of the effects of the 33 ppt salinity on the remaining data indicated comparable mortality patterns among the specimens subjected to 0 to 14 ppt salinities ($F = 1.03$). The regressions of

salinity and survival were best shown by the formulae Y = 1.437–0.189X (Experiment 1) and Y = 1.641–0.347X (Experiment 2).

Higher salinities seemed to increase the incidence of molting. Likewise, those specimens, regardless of species, that molted seemed to die sooner than those that did not shed. Molted specimens were found to have greatly deformed chelae, antennae, antennules, and other appendages.

Cambarus b. bartonii

Two similar experiments, begun July 11 and August 16, 1962, employing a seemingly fresh water specific species, *C. b. bartonii*, produced several startling results. Individuals of the *C. b. bartonii* population collected from a clear mountain stream with no previous saline history were able to withstand salinities of 0, 5.2, 13.6, and 27.5 ppt for 240, 252, 108, and 192 hours (fig. 3), respectively. Again, one-half of the test population died within 72 to 96 hours (fig. 3). Repeating the experiment with 0, 5, 9, 15.4, and 33.6 ppt salinities, overall survival periods were

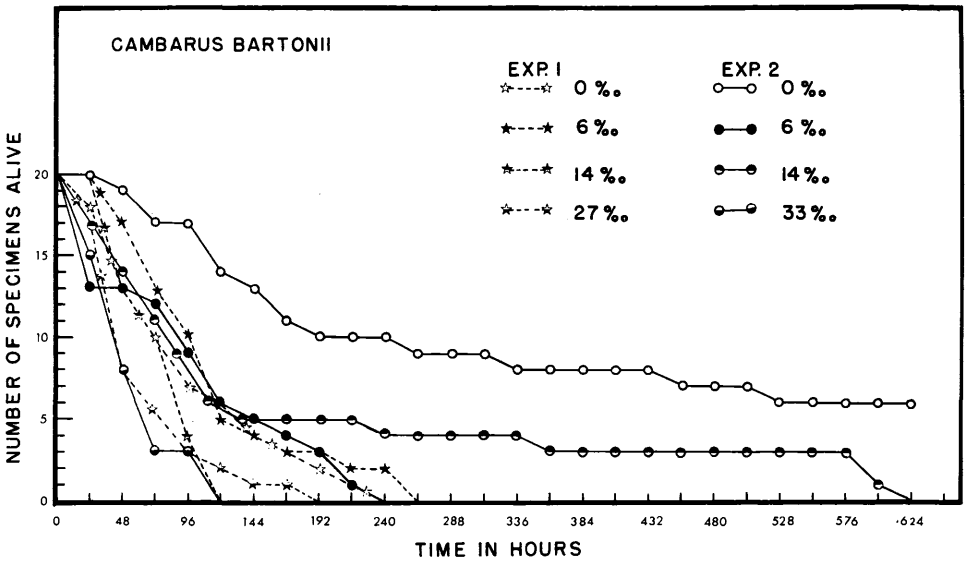

FIGURE 3. Survival tolerances of *C. b. bartonii* subjected to approximately 0, 6, 14, and 27 to 33 ppt salinities.

624, 612, 228, and 120 hours, with one-half the test specimens dying within 96 hours. Both sets of data were comparable statistically (table 2). Salinities of 6 to 15 ppt were readily tolerated for a period of 27 days, while higher concentrations were detrimental. These salinity-survival relationships (fig. 2) were best expressed by the regression formulas Y = 1.314–0.073X for Experiment 1 and Y = 1.473–0.191X for Experiment 2.

These data were interesting, as they suggested new fields for exploration. The ability of both *O. virilis* and *C. b. bartonii* to withstand varying degrees of salinity might permit migrations or dispersals of these and other species (Meredith and Schwartz, 1960) across salinity barriers. It has been shown that one-half of the test population could survive high saline conditions for at least four days. This would permit a species to transgress a salinity barrier and travel into adjacent watersheds. Perhaps disjunct populations of seemingly fresh water species of crayfishes are a result of migrations across salinity barriers. Increased downstream salinities might further isolate the populations or hold them in check. Although we know little of the rate or extent of crayfish movements in fresh-waters as we do for marine Portunids (Cargo, 1958) or Homarids (Templeman, 1935; Wilder, 1963), observations suggest that they often undertake sizeable migrations (Black, 1963). Experiments should be conducted to see what other species tolerate saline waters. Conclusions concerning disjunct crayfish zoo-

TABLE 2

Analysis of covariance and test of significance of the effects of various salinities on the survival of two species of crayfishes[1]

	Source of variation	d.f.	Sum of squares and products Sx²	Sxy	Sy²	Errors of estimate Sum of squares	d.f.	Mean square	
O. virilis	Total	46	1.9093	−0.3613	0.9883	0.9200	45		
Expt. I	salinity	3	1.8821	−0.3578	0.1277				
	within lots (error)	43	.0272	−0.0035	0.8606	0.8570	42	0.0204	
	For test of significance of adjusted means					0.0630	3	0.0210	F=1.0294
O. virilis	Total	88	2.75944	−0.95826	2.12622	1.79345	87		
Expt. II	salinity	3	2.65609	−0.96429	1.49324				
	within lots (error)	85	0.10335	0.00603	0.63298	0.62360	84	0.00753	
	For test of significance of adjusted means					1.16085	3	0.38695	F=51.39**
C. bartonii	Total	38	1.56039	−0.11338	1.05706	1.04882	37		
Expt. I	salinity	3	1.54353	−0.07494	0.03498				
	within lots (error)	35	0.01686	−0.03844	1.02208	0.93430	34	0.02748	
	For test of significance of adjusted means					0.11452	3	0.03817	F=1.389
C. bartonii	Total	70	2.6996	−0.5146	1.1547	0.6986	69		
Expt. II	salinity	3	2.6543	−0.6235	0.2192				
	within lots (error)	67	0.0453	0.1089	0.9355	0.6729	66	0.0101	
	For test of significance of adjusted means					0.0257	3	0.0085	F=0.84

[1]Data in logs and coded by adding 10 throughout for ease of analysis.

geographies as either the result of extripated populations, gradient, ecological changes or interspecific competition (Penn and Fitzpatrick, 1963) should be reviewed or cautioned. Likewise, in the light of the above, the role of the saline water areas of the central and southwestern United States should be investigated as dispersal agents or barriers to crayfish populations.

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