Adaptation of *Cambarus bartonii cavatus* (Hay) (Decapoda: Cambaridae) to Acid Mine–Polluted Waters[1]

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ABSTRACT. **Juvenile crayfish [*Cambarus bartonii cavatus*] were taken from two nearby sites on Big Four Creek, Vinton County, OH. Water from upstream (UpS) and downstream (DnS) sites had total conductivities of 250 µS and 600 µS (µS = µmho/cm^2) at 25° C. Non–carbonate conductivity was largely made up of sulfuric acid and heavy metals. Carbonates represented 40% of the conductivity at UpS but were absent from DnS. With only 100 µS of carbonate buffering capacity, water from UpS had little ability to neutralize acidic input, and this was easily overcome. Tests in seven solutions ranging between 250 µS and 5000 µS showed that: 1) mortality of crayfish was effected by the conductivity of test solutions, 2) DnS crayfish survived longer than UpS crayfish in all test conditions except the clean UpS water, and 3) there was no interaction term between source area and strength of conductivity on longevity. Attempts to acclimate crayfishes to higher levels of mine acid over short time periods were unsuccessful. Crayfish mortality under low acid conditions was not increased by addition of iron precipitate, though deaths were associated with ecdysis at intermediate and higher concentrations of mine acid. If DnS, but not UpS, crayfishes of this subspecies have acclimatized to intermediate concentrations of mine acid, then we infer a regime of semi-isolated reproduction over a short distance of streambed. Such an adaptation might have evolved following long–term exposure to low level, naturally – occurring acid seepage from coal outcrops, with success depending on the pre-adaptation of this subspecies to life in waters that are naturally low in carbonate buffering capacity.**

OHIO J. SCI. 91 (4): 167–171, 1991

INTRODUCTION

Prior to laws requiring cleanup of mining residues, the severity of acid mine pollution in southeastern Ohio tended to be directly proportionate to coal mining activity. Ground water leached pyritic materials, containing iron, manganese, and sulfur compounds, from coal seams and wastes. On exposure to air, bacteria oxidize the sulfur compounds to produce ferric hydroxide [$Fe(OH)_3$] and sulfuric acid [H_2SO_4], the later being formed at a 4:1 ratio from the original pyritic material (Cairns et al. 1971). Ferric hydroxide forms a dense, yellow–orange, flocculent precipitate known as "yellow boy," which blankets the stream bottom and prevents periphyton growth (Warner 1971, Herricks and Cairns 1974). As acidity of the water increases, so does the solubility of other metallic ions, mainly aluminum and molybdenum, which then also leach into the running water (Cairns et al. 1971, Warner 1971). To survive, an organism must tolerate these chemicals as well as elevated acidity (Campbell and Stokes 1985). Acid mine pollution also varies widely from place to place, from season to season, and from year to year.

We concur with Kimmel and Hales (1973) in finding pH to be of limited value as an index of acid mine pollution. Waters of the same pH can vary considerably with respect to their loading capacity for mine acids. Even an abrupt decrease in benthic species diversity, as noted by Warner (1971) at pH 4.0–4.4, is related to overcoming natural carbonate buffering capacity of the water. In natural waters, Dills and Rogers (1974) found correlations between pH and total conductivity in µS (µS = µmho/cm^2) at 25° C (r = –0.72) to be lower than those between total conductivity and iron (r = +0.78), or manganese (r = +0.84), or sulfate (r = +0.94), or hardness (r = +0.96). Brezina et al. (1970) had previously used total conductivity as an index to acid sulfate discharge into a reservoir. Since conductivity relates to the geo–chemical character (ionic solute) of water and to the chemical weathering (dissolved solids) of sediments consequent to acid mine drainage (Pickering and Musser 1970), it may be the best single index of acid mine pollution. A combination of total conductivity and carbonate conductivity [1.65 x total alkalinity as mg/l] appears to be even more preferable (Faucon and Hummon 1976). It is a combination from which both non–carbonate conductivity and loading capacity can be assessed (Hummon et al. 1978). Each is of biological significance in meiobenthic Gastrotricha, since mine acidity can reduce both longevity and reproductive rate, and both of these population parameters can largely be restored by a reconstitution of the carbonate buffering capacity of the water (Faucon and Hummon 1976, Hummon and Hummon 1979).

As part of a study to monitor the effectiveness of the Lake Hope Mine Drainage Abatement Project during the period 1980–83, Big Four Creek, located 6 km north of Lake Hope, Vinton County, OH, was sampled. The Project, physically completed in 1979, was expected to reduce by 40% the overall acid input to Lake Hope from Big Four Creek (Nicholson 1979). Ground water seepage into the stream was to be reduced by means of an 0.5 km–long clay dam that was constructed parallel to the stream, extending from soil–surface to bedrock. Hand–dug drift mines were capped using a double bulkhead seal and other mines were capped using a surface seal. Water filling the sealed mines, by reducing exposure of pyritic matter to oxidization, would lessen the amount of acidity pro-

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[1] Manuscript received 27 June 1990 and in revised form 14 June 1991 (#90–18).

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duced. Reduction of total acidic input did not result in the rapid recovery of Big Four Creek (Hummon 1982, 1986), the ability of a stream to assimilate mine acid being proportionate to stream flow and carbonate alkalinity (Herricks and Cairns 1974, Hummon et al. 1978). While rain often produces mild to severe flooding, low flow rates (seldom exceeding 10 dm^3/sec) combined with low levels of carbonate conductivity (not exceeding 100 µS) characterize Big Four Creek.

Ortmann (1909), Francois (1959), and Warner (1971) had found crayfish to be among the most sensitive macrobenthos to acid pollution. By contrast, we found that juvenile crayfish [*Cambarus bartonii cavatus*] were the most abundant macroinvertebrate stream fauna in the sections of Big Four Creek moderately polluted by mine acid. Earlier, Schwartz and Meredith (1962) had shown that *C. bartonii* of a different subspecies than ours had been eliminated from those portions of the Cheat River drainage, West Virginia, where mine acid had seriously reduced water quality, and were restricted to the cleaner headwater portions of the system. In light of this seeming discrepancy, we decided to compare tolerance and rates of acclimation of juvenile *C. b. cavatus* from a downstream site (DnS) with those from a site 100 m upstream (UpS) with respect to various concentrations of acid mine pollution.

Juvenile crayfish used in the study were collected in late spring 1983 from downstream, near the upper end of the clay dam, and from upstream, above nearly all acid inputs. Water from upstream has a total conductivity of 250 µS at 25° C, of which only 40% (or 100 µS) represents carbonate conductivity. Total conductivity is always higher downstream and carbonate conductivity negligable or absent; downstream water being polluted by runoff seepage from a reclaimed strip mine and from an uncapped drift mine. While the acid input is not of high volume, its potency is sufficient to nullify the loading capacity of the stream with respect to the buffering of acid by carbonates. Juvenile by crayfish enter the stream after spring rains have leached acid from the area. In the dry summer season, those downstream may encounter conductivity of 175–1000 µS, usually reaching 400–600 µS, but lacking carbonates. Downstream from our downstream site, larger additions of acid from capped mines and an outflow from the clay dam further increase the pollution level, such that no benthic macrofauna could be found.

MATERIALS AND METHODS

Crayfishes used for the experiments had a mean total length of 2.77 (s = 0.48) cm, carapace lengths of 1.26 (s = 0.23) cm, and blotted weights of 0.55 (s = 0.29) g. Analysis by MANOVA (Dixon 1981), indicates that animals from the two collecting sites did not differ significantly from one another in size or weight ($P > 0.05$).

Survivorship was determined (a static bioassay) for animals from each of the two collection sites, with 11 animals placed in each of seven test solutions (2 sites x 7 solutions x 11 individuals = 154 total animals). Test solutions (Table 1) ranged from upstream (UpS) water through downstream (DnS) water to highly acidic water exiting from a capped mine (SP). Each animal was blotted, measured, and weighed; it was then immersed for 30 min each in two consecutive washes of its test solution, so as to dilute residual water from its integument and gill spaces, before a test began. Tests were conducted in 50 ml of solution contained in 100 ml jars, with caps fitted loosely to minimize evaporation. Animals were fed weekly *ad libitum* with Hartz® freeze–dried *Tubifex*, excess food being removed after 30 min. Mortality was determined at 6 hr intervals, death being defined as cessation of response following physical stimulation. Raw data for animals originating from the two collection sites were analyzed by Bartlett's test for homogeneous variances, followed by non–parametric Kruskal–Wallis tests on groups of solutions for which variances were homogeneous. Student–Newman–Keuls (SNK) *a posteriori* tests were performed, following a two–way analysis of variance of $\log_{10}$–transformed data, to determine the success with which animals from different collection sites survived in test solutions of 2000 µS conductivity or less.

TABLE 1

Test solutions, their components, and their conductivities.

Solutions	Component Waters	Total conductivity in µS at 25° C	Alkaline conductivity as a % of total cond.
1	Upstream (UpS) alone	250	40
2	Downstream (DnS) alone	600	0
3*	6:1 UpS:SP	1200	0
4*	3:1 Ups:SP	2000	0
5*	1:1 UpS:SP	3300	0
6*	1:1 DnS:SP	3400	0
7	Capped mine (SP) alone	5000	0

UpS – milieu water from the upstream study site; DnS – milieu water from the downstream study site; SP – water coming directly from a capped mine; * – dilutions.

Crayfishes ($n = 7$) from upstream were tested using the same protocol to determine whether the iron precipitate reduced their survivorship. The heavy precipitate, decanted from water of 4800 µS conductivity, was added to upstream water (resultant being water with 370 µS, 250 µS plus that associated with the precipitate). In this test solution, the floc settled into a thick layer unless the animal moved; rapid or continued movement would suspend the floc so that the animal was no longer visible.

As mortality often coincided with ecdysis, both were recorded throughout the bioassays. Cumulative percent curves of animals successfully completing one or more ecdyses in solutions of increasing total conductivity were compared by site, using a Kolmogorov-Smirnov test.

Acclimation experiments were conducted on 20–24 crayfish from each of the two collection sites. Animals, prepared as described above, were moved through a series of solutions at 4– or 8–day intervals, beginning with 600 µS and ending with 2000 µS. Results, in days of survival at 2000 µS, were compared by means of Kruskal–Wallis tests. Six animals, collected just below the upstream site, and henceforth referred to as the middle site (MdS), were also tested at 2000 µS. This middle site, during the summer drought of 1983, was a pool in the otherwise dry streambed

TABLE 2

Days of survival for animals collected from upstream and downstream collection sites when subjected to test solutions of various concentrations of conductivity.

Conductivity of test solutions in µS at 25° C		250	600	1200	2000	3300	3400	5000
Animals from Upstream Site	Mean	51.4	54.1	44.6	19.6	3.5	3.2	4.2
	Std. Dev.	26.3	21.2	33.6	23.6	2.8	2.0	1.8
Animals from Downstream Site	Mean	44.7	67.3	64.7	34.4	4.6	5.5	6.8
	Std. Dev.	34.3	14.2	24.2	25.1	2.3	3.3	3.0

n = 11 for each concentration

that lay between the two uncapped mine openings; it received a seepage from above. Water in the pool had 1000 µS of total conductivity without carbonate conductivity.

RESULTS

Downstream (DnS) crayfishes outlived those from upstream (UpS) in all cases, except in upstream milieu water (Table 2). Bartlett's test indicated that the data were subdivisible into two groups, whose variances differed from one another though each was homogeneous within itself. The first group included solutions exceeding 2000 µS total conductivity; the second included those of 2000 µS or less. A Kruskal–Wallis test showed that downstream animals in the test solutions that exceed 2000 µS had far greater survivorship than their upstream counterparts ($P < 0.001$), whereas pooled survivorships for the two sites did not differ in test solutions of 2000 µS or less ($P > 0.05$). While the ANOVA revealed differences which occurred among animals with respect to test solution and collection site, no interaction between the two factors was found. The SNK test (Table 3) indicated that, while animals from upstream survived well in solutions of 250 µS (their milieu water) to 1200 µS, they suffered reduced longevity at 2000 µS. Animals from downstream, conversely, survived even better than those from upstream in solutions of 600 µS (their milieu water) to 1200 µS, and nearly as well at 250 µS and 2000 µS as upstream animals did in 250–1200 µS water. However, for downstream crayfishes, a decrease in conductivity below the 600–1200 µS range was as detrimental as an increase above, so that upstream water of 250 µS (40% carbonates) had the same effect on the survivorship of downstream crayfishes as 2000 µS water (lacking carbonates).

Animals from upstream survived no worse in 370 µS water containing a 2 cm–thick layer of "yellow–boy" precipitate than the same animals living in 250 µS upstream milieu water or 600 µS downstream water (Kruskal–Wallis: $P > 0.25$). The Kolmogorov–Smirnov test of cumulative curves indicated that mortality at 1200 µS total conductivity was associated with ecdydis in animals from upstream, but not in those from downstream ($P < 0.001$). Mortality during ecdysis increased in both groups at conductivity levels up to 3300 µS, but actually preceeded ecdysis at higher levels (3400–5000 µS).

No differences were seen between the 4- and the 8-day acclimation series, or between those and the survivorship series in 2000 µS water (Kruskal–Wallis: $P > 0.05$). The animals collected under stressed conditions in the field at the middle site did not differ statistically ($P > 0.10$) from animals from upstream in their inability to withstand acid stress, but both of these populations showed less tolerance than animals from downstream ($P < 0.001$).

TABLE 3

Student – Newman – Keuls a posteriori *test for association. Groups of test conditions in which survival data do not differ significantly are underlined.*

Conductivity (µS at 25° C)	600	250	1200	2000
Animals from Upstream Site Log mean d	1.70	1.65	1.43	1.04

Conductivity (µS at 25° C)	600	1200	250	2000
Animals from Downstream Site Log mean d	1.83	1.80	1.65	1.58

n = 11 for each concentration

DISCUSSION

Berrill et al. (1985) suggest that there is a generic difference in tolerance to low pH between crayfishes belonging to the genera *Orconectes* and *Cambarus*. There are correlated differences in life histories between the two genera, *Orconectes* spp. breeding in early spring in southern Ontario (Berrill 1978; for additional data on *Orconectes* cf. Fielder 1972; France 1985, 1987a,b; Malley 1980; and Wood and Rogano 1986). *Cambarus bartonii* and *C. robustus*, on the other hand, breed throughout spring and summer (Hamr and Berrill 1985), coinciding with the time of lowest pH of the waters in that region.

Cambarus bartonii appears to have originated in the southern Appalachian Plateau, and then migrated northeasterly into Ohio (Turner 1926, Ortmann 1931), where it is found mainly in the Ohio River drainage. This species inhabits headwater streams which have cold, oxygenated water and a bottom of stones mixed with sand and gravel

(Ortmann 1913, 1931; Francois 1959; Berrill 1978). There it "lives under stones, excavating holes and often becomes more or less a burrowing form" (Ortmann 1931), though Crocker and Barr (1968) do not consider it to be primarily a burrower. Such habitat preferences lead to localized isolation, morphological variation, and sub–speciation (Francois 1959). Forerunners of *Cambarus bartonii cavatus* may have inhabited the Marietta River, a tributary of the pre–glacial Teays River (Jezerinac 1983). The Big Four Creek watershed would have been connected to this system by way of the ancient Zaleski Creek. In the Raccoon Creek drainage of southeastern Ohio, of which Big Four Creek is a part, the subspecies *C. bartonii cavatus* is the only stream–inhabiting cambarid (Jezerinac and Thoma 1984). Juveniles reside in the stream proper, while adults resort to burrowing into its banks, where they were found in the present study as well.

Too little is known of the mechanisms that cause invertebrate populations to show reduced survivorship in acidic waters (Malley 1980). Acidity and heavy metals are both implicated, the former lysing the gills of aquatic organisms or causing acidosis, and the latter being directly toxic to the organism (Cairns et al. 1971, Warner 1971, Wood and Rogano 1986). Iron precipitates may work by reducing the benthic food supply (Warner 1971, Nichols and Bulow 1973), but they are not likely to operate by clogging gill spaces of crayfish, though this was suggested by Warner (1971).

Kimmel and Hales (1973) noted that aquatic insects may show increased sensitivity to low pH during ecdysis, and Malley (1980) had confirmed this for crayfish, linking it to Ca^{++} metabolism. Our results confirm that crayfishes had problems completing ecdyses at intermediate levels (1200–2000 µS) of conductivity. Animals molted frequently and successfully in the 250–1200 µS solutions, the range of conductivity that gives optimal survival for both upstream and downstream crayfishes and the range that they are most likely to encounter in the field. Several crayfishes were found during the summer of 1983 that had died in mid–molt or had stressful molts while in isolated pools of 800–1920 µS conductivity. Following a stressful molt, they would lose chelae and/or periopods and often had a splayed out carapace, which left the posterior–most pair of gills extruded from the branchial chambers and the remainder more than normally exposed. Ecdysis thus represents a vulnerable stage in the life cycle; pollution levels that can be tolerated under other circumstances become lethal during this period. At higher levels of conductivity (3400–5000 µS), however, mortality tends to preceed ecdysis.

Hazlett et al. (1974) showed that while adults tended to stay in place, when they did move it was over distances of 100 m or more, often when they were molting. Avoidance behavior (France 1985), especially during times of molting, may play a role in the survival of crayfishes in the downstream site relative to periodically high concentrations of mine acid.

Crayfish from any of the three sites (downstream, middle, or upstream) would die within a week, if trapped in a summer drought where the only available water was polluted by mine acids of 3000+ µS conductivity. At intermediate conductivities of 1000–2000 µS, the differential response shown by downstream over upstream crayfish would determine which would survive. Upstream crayfish would tend to be eliminated from such polluted sections of the stream, downstream crayfish being more likely to survive. Under competition for upstream habitat, the downstream crayfish would have an advantage in that there would be alternative stream habitat refugia available for their use.

Our tests indicated that short–term acclimation of this sort was unlikely, but also confirmed differences in tolerances between upstream and downstream crayfishes. Upstream crayfishes not only have a lesser probability of surviving critical periods, but they also showed reduced survivorship at 2000 µS, whereas there was no reduction in the ability of downstream crayfish to survive in a solution of 2000 µS conductivity. Field observations on crayfishes from the middle site corroborate these experiments. These animals apparently were UpS crayfishes that were caught by the summer drought in a pool with 1000 µS conductivity, since their response to a 2000 µS test solution was similar to that of the upstream crayfish.

The importance of differential survival becomes even more apparent when rains follow drought conditions. Additional acid is washed into the streams, resulting in increased rather than decreased acid loads (Seibert 1966, Dugan 1975). This is usually a short–term phenomenon; but, if the additional pollution was sufficiently severe and the condition persisted for more than several days, all of the upstream crayfishes would be eliminated, while the downstream crayfishes would remain largely unaffected.

The apparent lack of short–term acclimation in any of these animals to increased stress from mine acid would point to the likelihood of long–term adaptation to elevated acid pollution levels by downstream crayfish. If downstream, but not upstream, crayfishes of this subspecies have acclimatized to high levels of mine acid pollution, and our data suggest that this is the case, then we infer a regime of semi–isolated reproduction over a short distance of streambed. Such an adaptation might have evolved following long-term exposure to low–level, naturally–occurring seepage pollution from coal outcrops, with success depending in part on the pre–adaptation of this subspecies to life in waters that are naturally low in carbonate buffering capacity, as has been claimed by Jezerinac (1983).

The geomorphology of Big Four Creek, with coal seams sloping down into the valley on both sides, is such that exposed veins of high sulfur coal and natural acid seepages have been present for a long time. Openings to the hand-dug mines in this valley were accessed through the same exposed veins in the hollows of the hills (Farnsworth, in Nicholson 1979). Crayfishes in parts of this drainage would have been exposed to moderate levels of acid mine pollution for long periods of time (on the order of hundreds to thousands of years), already possessing a high tolerance for life in waters that were virtually lacking in carbonate buffers. Thus, these individuals would have had both the selective pressure and the pre–adaptative character that in combination could have lent itself to an evolutionary adaptation such as this.

The main evidence that we have for such a scenario is the tolerance of both upstream and downstream animals to lower–intermediate levels of mine acid, and the lack of acclimatization in either population. If our inference is valid, then we should expect other such populations to be present in headwater streams that are located in regions of high sulfur coal, where acid mine pollution occurs in localized seeps and outflows.

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