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High Tolerance of Alderfly Larvae (*Sialis* spp: Megaloptera) to Metals Is Not Affected by Water pH

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Elevated levels of heavy metals, such as Al, Cd, Cu, Fe, Mn, Pb, and Zn are often associated with acid mine drainage (AMD), a particularly widespread environmental problem in coal mining regions. AMD-impacted streams are also characterized by high acidity and increased sediment and flocculate loads. The combination of these pollutants severely reduces the abundance and diversity of benthic macroinvertebrates (Rosemond *et al.* 1992, Clements *et al.* 1994).

Although there is an extensive literature on the biological effects of heavy metals on aquatic communities at circumneutral pH, predicting biological responses to metals in AMD impacted streams is complicated by a variety of abiotic and biotic factors. First, most metals are present in the water column and sediment in several different forms, depending on the pH and other physicochemical features of the water, and these forms vary in their toxicity to organisms. Second, the bioavailability and toxicity of metals differs among taxa, and can be either increased or decreased by the presence of inorganic and organic ligands, such as humic acid. Thirdly, the biological response elicited by a dissolved metal can be altered by other ions, including Ca^{++}, Mg^{++}, and H^{+}, which reduce the availability of binding sites on the gill surface for toxic metals (Campbell and Stokes 1985).

Most laboratory bioassays and field studies are not designed to investigate synergistic or sublethal effects of metals and low pH, even though such effects are likely important in structuring biological communities at sites moderately impacted by AMD. It is generally assumed that metals are more toxic to organisms in acidic than near-neutral waters, because ligand-metal complexes tend to dissociate and increase free metal ion concentrations at low pH, and organisms may be additionally stressed by low pH. However, the interactions between pH and metal toxicity can be positive or negative, depending on the metal. The toxicity of some metals (Cd, Cu and Zn) to fish and *Daphnia* is decreased by acidity, while in others it is increased (Pb). Evidence for the influence of pH on Al toxicity is mixed (Campbell and Stokes 1985, Wren and Stephenson 1991).

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In this paper, we document the unusually high tolerance of alderfly larvae (*Sialis* spp.: Megaloptera) to four metals (Al, Cu, Mn and Zn) and demonstrate that this tolerance is not influenced by pH in acute exposure (8 day) trials. High concentrations of Al (> 300 μg/L), Zn (5000 μg/L) and Mn (> 500 μg/L) are characteristic of acid mine drainage (FWPCA 1968). Cu is frequently associated with other types of mining activities. Although *Sialis* larvae are extremely tolerant of AMD pollution and are often the most abundant taxa at heavily impacted sites, their physiological tolerance to metals has not been reported in the literature. Their general abundance and wide pH tolerance (pH 2.8-8.3) (Tarter and Woodrum 1972) make them an ideal model organism for investigation of pH-metal interactions.

MATERIALS AND METHODS

Sialis sp. larvae were collected from a tributary of Monday Creek in Athens Co. OH using kick and D-ring dip nets, and were tentatively identified as *S. aequalis* (Canterbury 1978). The pH of the water at this AMD impacted site typically ranges from 3.6 to 4.0. Animals were transported to the laboratory and either used immediately in the assays, or fed small *Drosophila* larvae for several days until assays were conducted. The individual and combined effects of acidity (pH 2.0-4.0) and metal concentration were tested for four metals (Al, Cu, Mn and Zn) in four factorial experiments, each lasting eight days.

Bioassays were conducted in an artificial stream water (2.26 grams of Kent Marine® R/O Right™ to 8 L of deionized water) that provided a balanced mixture of essential salts plus minor and trace minerals to the water while ensuring a neutral pH and no added heavy metals. The pH of the water was altered by adding sulfuric acid (H_2SO_4). Stock solutions of $AlCl_2$,$CuCl_2$, $MnSO_4$, or $ZnCl_2$ were added to water of differing pH (2.0, 3.0, and 4.0) to obtain the final total metal concentrations. Both metal sulfates and metal chlorides are commonly used in studies like this (Clements 1999). Al was tested at 0, 300, 600, 900 μg/L, Cu at 0, 50, 100, 150 μg/L, Mn at 0, 4000, 8000, and 16,000 μg/L, and Zn at 0, 100, 200, 300 μg/L. In a preliminary assay, the effects of varying pH from 1.0- 5.0 in increments of one on *Sialis* survival was tested, but the metals were tested only at pH 2.0, 3.0 and 4.0.

Sialis larvae were placed individually in 30 ml plastic cups (eight to ten per pH-metal treatment) containing 20 ml of test water, then randomized and placed in a growth chamber at 26° C on a 16:8 light/dark cycle. Because the copper/pH assay was performed later in the year than the others (late Nov., compared to Sept-Oct.), the temperature was lowered to 20° C to minimize mortality due to temperature stress. Survival was checked every twelve hours for the first five days and then every 24 hours until day eight. Individuals were recorded as dead if they did not respond to a sharp tap to the outside of the cup with any visible movement. All individuals were measured from the tip of the mandibles to the tail (excluding caudal filaments) at the end of the experiment to test for the possible influence of body size on treatment effects. After an analysis of covariance showed that body size did not significantly affect pH toxicity, the effects of pH and metal concentration on larval survival time were tested using one-way ANOVA (Jandel® Sigma Stat™ software).

Table 1. ANOVA results for metal and pH bioassays using *Sialis* larvae

Aluminum					
Source	DF	SS	MS	F	P
pH	2	344541.38	172270.692	53.212	<0.001
Al	3	7697.506	2565.835	0.793	0.501
pH x Al	6	25111.000	4185.167	1.293	0.267
Error	106	343166.40	3237.419		
Copper					
Source	DF	SS	MS	F	P
pH	2	85052.039	42526.020	21.586	<0.001
Cu	3	830.080	276.693	0.140	0.936
pH x Cu	6	10401.686	1733.614	0.880	0.512
Error	108	212764.509	1970.042		
Manganese					
Source	DF	SS	MS	F	P
pH	2	776817.98	388408.991	322.649	<0.001
Mn	3	2768.389	922.796	0.767	0.515
pH x Mn	6	7609.632	1286.272	1.054	0.395
Error	107	128808.000	1203.813		
Zinc					
Source	DF	SS	MS	F	P
pH	2	617042.753	308521.376	144.93	<0.001
Zn	3	5836.593	1945.531	0.914	0.437
pH x Zn	6	4078.258	679.710	0.319	0.926
Error	107	227756.945	2128.570		

RESULTS AND DISCUSSION

Lower water pH significantly reduced survival time ($p < 0.001$) in all the metal trials, although most animals survived a pH as low as 3.0 (Figure 1). While no animals survived a pH of 2.0 or below, some survived for 2-4 days at pH 2.0. The LC_{50} from the pooled data from all the trials was graphically estimated to be pH 2.4.

None of the metals tested (Al, Cu, Mn, or Zn) affected survival of *Sialis*, even at the highest concentrations, which are considerably higher than USEPA criterion levels and those observed to kill many sensitive taxa. There were no statistically significant effects of metal concentration, nor were there significant interactions between pH and copper, aluminum, manganese, or zinc (Table 1).

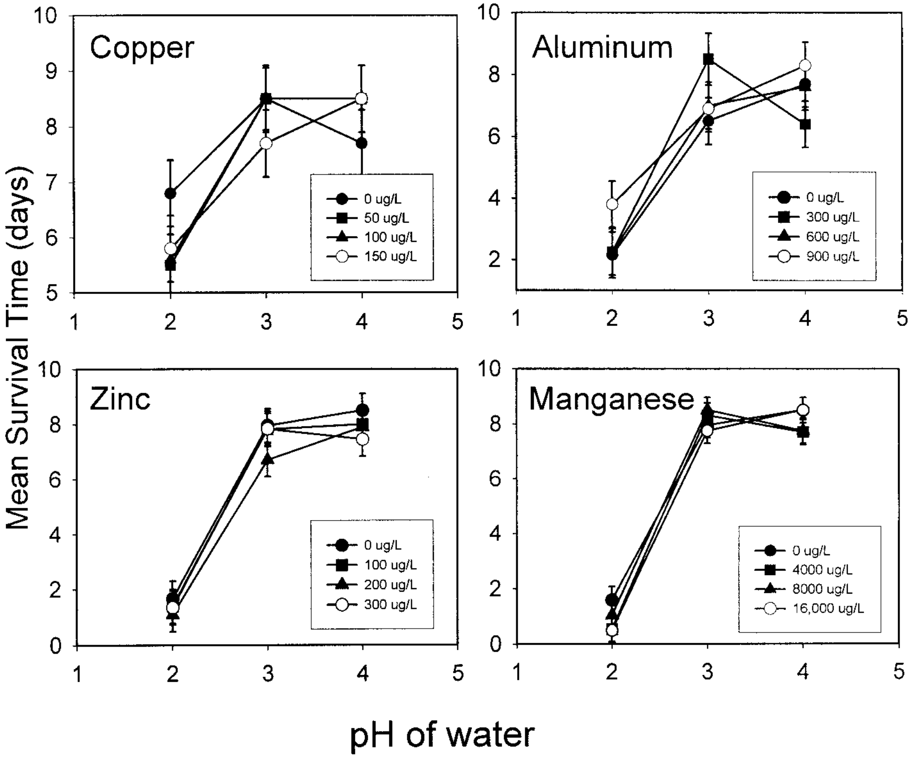

Figure 1. Acute effects of metals and pH on survival of *Sialis* larvae. Each point represents the mean and standard error of 8-10 replicates.

In the field, the abundance of *Sialis* larvae at heavily AMD impacted sites clearly indicated that they were tolerant of acid conditions and some metals, but we nonetheless expected that they would be adversely affected by very high metal concentrations. Individual metal concentrations are not as frequently measured as pH or specific conductivity in field studies of AMD waters, so there was little existing data on the upper tolerance level of this organism. Our results show that larval *Sialis* are broadly tolerant of several metals (Al, Cu, Mn and Zn) and that the tolerance levels surpass those of more sensitive taxa, such as the Ephemeroptera, by several-fold.

The high tolerance to Al, Cu, Mn and Zn was uniform, even though these metals differ in their mechanisms of uptake and toxicity in more sensitive taxa. Cu is generally regarded as the most toxic of the four, with a USEPA chronic criterion level of 12 µg/L. Concentrations of 5-30 µg/L are sufficient to reduce species richness and invertebrate densities of major orders (Ephemeroptera, Plecoptera, Coleoptera, Trichoptera and Diptera) in natural communities (Clements *et al.*

1994). Our highest concentration (150 µg/L) exceeded this dose by at over seven-fold but had no observable effect on *Sialis* larvae. The tolerance of *Sialis* appears to exceed that of the pollution tolerant chironomid midges, which have EC50's from 50-59 µg/L or lower (Campbell and Stokes 1985, Fargasova 1997, Timmermans *et al.* 1992).

Zinc is less toxic than copper, (USEPA chronic criterion level of 110 µg/L), but also appears to be better tolerated by *Sialis* than even chironomids found in heavily metal-contaminated sites (Timmermans *et al.* 1992). In our experiments, *Sialis* was tolerant of 300 µg/L of zinc, considerably higher than lethal concentrations to more sensitive species (200 µg/L and lower).

Manganese has been less thoroughly investigated than other metals, but chironomids are sensitive to concentrations as low as 55 µg/L (Fargasova 1997), well below the lowest concentration (4000 µg/L) used in our experiments.

The effects of aluminum on aquatic macroinvertebrates appear to be species specific and highly variable (Wren and Stephensen 1991 and references within). Differences in toxicity to aluminum may be due to the different chemical forms of Al, different pH levels, or mode of action. Many midges (*Chironomus* and *Chaoborus*) tolerate concentrations as high as 1000 µg/L total Al, and high tolerance is also reported for dragonfly nymphs. Most Ephemeroptera are sensitive whereas Trichoptera are intermediate, with considerable species-level variation in sensitivity. Vuori (1995) found a nearly two-fold difference in Al sensitivity in a comparison of three Trichopteran species, but all three demonstrated anal papillae deformities at total Al concentrations around 1000 µg/L. *Hydropsyche angustipennis* can tolerate brief exposures to acidity (pH 4.4) and extremely high Al (> 10,000 µg/L) (Vuori 1995), but concentrations of 500 µg/L are toxic to most other Trichoptera and Plecoptera (Burton and Allen 1986). In our assays, *Sialis* larvae tolerated up to 900 µg/L with no apparent ill effects.

We expected to see significant interactions between metal toxicity and water pH in *Sialis*, since biological responses to Al and Cu in laboratory settings have been correlated with the concentrations of free metal ion in laboratory studies (Burton and Allen 1986). The effect of water pH on Al toxicity in particular has received attention in recent years. Aluminum exists primarily as the trivalent cation ($Al(OH)6^{3+}$) below pH 4.0. Between pH 4.0 and 6.5, it exists as a mixture of $Al(OH)^{2+}$, $Al(OH)^{2+}$ and $Al(OH)^{3+}$ (Campbell and Stokes 1985). Laboratory studies have shown that Al toxicity in water with pH > 6.0 is low, ranging from 300-800 µg/L (reveiwed in Wren and Stevenson 1991). For several taxonomic groups (*Daphnia*, *Chaoborus* and *Chironomus*), the H^+ ion is more toxic than Al (Havas and Likens 1985), and low pH synergizes Al at concentrations of 100 – 300 µg/L (Ormerod et al. 1987). In several studies, Al toxicity was magnified by a lower water pH. Rockwood et al. (1990) reported that the addition of 3000 µg/L or more of aluminum caused a significant depression in oxygen consumption in dragonfly nymphs. In our bioassays, however, even the lowest water pH treatments (pH 2.0) did not synergize the toxicity of aluminum or any of the other metals. The unusual tolerance of *Sialis* for acidity appears to be well-matched by a strong tolerance to high metal concentrations.

The physiological basis for the alderfly's tolerance to acidity and metals is not known. The mode of action of metals varies depending on the metal and route of entry. Some are accumulated through the gut, while others adsorb onto outer surfaces or penetrate through tissues of high permeability, such as gill epithelial cells (Hare *et al.* 1991). Roy and Hare (1998) and Hare *et al.* (1991) found that Cd accumulation in *Sialis velata* under both natural and experimental conditions occurred primarily in gut tissues. While the metal may enter the insect via gills or some other body region, food was considered the most important source for Cd uptake. Measures of metal exchange rates indicate that Zn uptake is also primarily through the gut, not the gills in mayflies and *Sialis* (Hare *et al.* 1991), predatory mites (Acarini) and caddisflies (Timmermans *et al.* 1992). Copper uptake, on the other hand, occurs via both food and water in Trichoptera and herbivorous mayfly nymphs (Gower and Darlington 1990). Active transport of Cu and Zn across membranes is carrier-mediated, as they are essential nutrients in trace amounts, and in cytosol they are bound to metal-transporting proteins (metallothioneins). These proteins are inducible and appear to be an important mechanism of tolerance to some metals (Hare 1992). The ability to sequester metals in intracellular granules also appears to be important for tolerance to Cu, Zn, Mn and Fe (Hare 1992).

The uniformity in the tolerance of *Sialis* to acidity and the four metals we tested suggests that a common mechanism, such as the low permeability of the cuticle and/or gill epithelium may be involved. Many taxonomic groups appear to have similar patterns of tolerance for acidity and metals; for example, Ephemeroptera tend to be highly sensitive to both, while Plecoptera and Trichoptera are intermediate in their responses to metals (Clements 1994) and acidity (Rosemond *et al.* 1992). Several workers have observed that variation in metal tolerance among invertebrate groups appears to be related to the permeability of the body covering (Hodgson 1979). Because metals often enter the insect by ion pumps or transport mechanisms involving ion exchange, the propensity to exchange ions with surrounding water also appears to be an important factor for metal toxicity (Hodgson 1979, Hare 1992), as well as sensitivity to acidity (Havas and Advokaat 1995). In *Sialis*, water is probably absorbed primarily through the gut, because the cuticle is highly impermeable to both water and ions (Shaw 1955). This trait may help prevent disruption of ion balance and respiratory processes caused by H^+ and metals in more sensitive taxa.

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