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Survival, reproduction, and arsenic body burdens in *Chironomus riparius* exposed to arsenate and phosphate

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ABSTRACT

Despite the increasing awareness of arsenic (As) contamination in surface waters worldwide, little is known about how As alone and in the presence of other chemicals affects aquatic insects. Larvae of *Chironomus riparius* were exposed in a laboratory investigation to factorial combinations of 0, 0.13, 2.0, 5.3, and 13 μmol As l^{-1} and 0, 0.15, and 15 μmol PO_4 l^{-1} throughout development from first instar to pupal emergence. The time between male and female emergence increased from 1.8 ± 0.17 days to 2.9 ± 0.34 days with exposure at higher As levels. The highest As exposure also decreased the number of eggs per egg mass, which may affect population maintenance. For these parameters, there was no effect from PO_4, and no interaction between As and PO_4. Total As determination of larval and adult tissues was conducted using Hydride Generated Atomic Absorption Spectroscopy (HGAAS) and revealed concentrations ranging from 2.48 ± 0.363 to 30.5 ± 0.473 μg/g and 1.03 ± 0.286 to 8.97 ± 0.662 μg/g, respectively, indicating elimination of approximately 72% of total As body burdens between the fourth instar and adult stages. There was no effect of PO_4, indicating PO_4 does not alter uptake of As in *C. riparius*. The potential for movement of As to terrestrial systems exists, though trophic transfer may be more likely during the aquatic larval stage.

1. Introduction

Background concentrations of arsenic (As) in the environment can be elevated as a result of both natural (geothermal and weathering processes) and anthropogenic contamination. Smedley and Kinniburgh (2002) review worldwide concentrations of arsenic in natural waters, which range from near zero up to 10,000 μg l^{-1} in naturally enriched areas, and up to 850,000 μg l^{-1} in anthropogenically disturbed areas. However, concentrations up to 1000 μg l^{-1} are more typical. In the US, As is considered a priority toxic pollutant of natural waters, and the US Environmental Protection Agency (US EPA) has set the maximum safe concentration for chronic exposure at 150 μg l^{-1} for freshwater life (US EPA, 2006). Despite this, how As affects freshwater life, specifically aquatic insects, is still not well understood.

Arsenic is unique among the common metal and metalloid contaminants given its solubility at neutral pH (Tamaki and Frankenberger, 1992). Arsenic exists in several oxidative states, but inorganic arsenite [As(III)] and arsenate [As(V)] are most common in natural waters. Distinguishing species in environmental analyses is crucial to fully understanding toxicity, as As(III) and As(V), in addition to organic forms, have different modes of action and varying bioavailabilities. Arsenate, the less toxic of the two (Hughes, 2002; Irving et al., 2008; Jeyasingham and Ling, 2000) but more environmentally prevalent (Tamaki and Frankenberger, 1992), replaces phosphate in biochemical reactions, thus disrupting glycolysis by altering the structures of molecular intermediates and inhibiting ATP synthesis (Hughes, 2002).

Given the widespread nature of As, there is a dearth of information regarding effects on aquatic life and potential interactions with other pollutants. Arsenate and phosphate are chemical analogues, and have been shown to compete for the same uptake carriers in the plasmalemma of plant roots (Meharg and Hartley-Whitaker, 2002). The resulting uptake of As can be variable, however, due to phosphate affecting As solubility by competitive adsorption reactions. Creger and Peryea (1994) documented increased uptake of As(V) from soils when apricot rootstocks were exposed to PO_4 in fertilizers. This synergistic interaction could be particularly devastating in parts of Southeast Asia where groundwater exceeding safe levels is used for crop irrigation (250–500 μg l^{-1}; Meharg and Rahman, 2003). Rahman et al. (2008) documented the aquatic macrophyte, *Spirodela polyrhiza*, as having a negative correlation between As(V) and PO_4 uptake. Although this interaction may be variable in plants as a result of competitive adsorption, this relationship has not been evaluated in animals.

Many toxicity studies do not explore the possible sublethal effects of metals and metalloids (Mogren and Trumble, 2010), and instead rely on death as a toxicological endpoint (Stark and Banks, 2003). The few studies that focus on As effects in aquatic insects report on

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development of LC_{50}s (Canivet et al., 2001; Jeyasingham and Ling, 2000; Liber et al., 2011) or accumulation of As by insects collected from contaminated streams (Burghelea et al., 2011; Lavilla et al., 2010). While presenting valuable information with regards to As accumulation at a single point in time, these studies do not provide information on how insects respond throughout their life cycles, which has the potential to inform upon population level effects. We examine how As(V) and PO_4 alone and combined affect the chronic survival of *Chironomus riparius* Meigen (Diptera: Chironomidae), a ubiquitous aquatic insect. We also evaluate how exposure as larvae affects elemental As concentrations in the terrestrial adults. Finally, we discuss how adults exposed to As(V) as larvae could transfer As to higher trophic levels.

2. Methods

2.1. Chironomid survival assay

Egg masses of *C. riparius* maintained in a colony were purchased from Environmental Consulting and Testing, Inc. (Superior, WI). After two days at 23 °C, the eggs began hatching. First instar larvae (14–20 individuals per beaker) were transferred to 600 ml glass beakers containing 300 ml of reconstituted water (described below) and factorial combinations of As(V) (at 0, 0.13, 2.0, 5.3, and 13 μmol l^{-1}, as sodium hydrogenarsenate heptahydrate, 99.998% (Sigma-Aldrich, St. Louis, MO, USA) and PO_4 (at 0, 0.15, and 15 μmol l^{-1}, as potassium dihydrogen phosphate, 99.99% (Sigma-Aldrich, St. Louis, MO, USA)). The As concentrations were chosen because they represent the World Health Organization's recommendation for drinking water (10 μg l^{-1}, or 0.13 μmol l^{-1}) (WHO, 2008); the US Environmental Protection Agency's recommended maximum concentration for indefinite exposure of aquatic life (150 μg l^{-1}, or 2.0 μmol l^{-1}) (US EPA, 2006); the median As concentration in Hot Creek (Mono Co., CA, USA) (400 μg l^{-1}, or 5.3 μmol l^{-1}), a geologically active stream (Mariner and Willey, 1976), and; the LC_{50} of *Baetis tricaudatus* nymphs after chronic exposure (1000 μg l^{-1}, or 13 μmol l^{-1}) (Irving et al., 2008). All of these concentrations are environmentally relevant and fall below the maximum values reported elsewhere (Smedley and Kinniburgh, 2002). The PO_4 levels chosen represent low concentrations typical in aquatic systems (0.15 μmol l^{-1}) (Rahman et al., 2008), or high concentrations such as a pulse of phosphate as a result of agricultural runoff (15 μmol l^{-1}).

A thin layer of pre-rinsed quartz sand (Repti Sand, Zoo Med Laboratories, Inc., San Luis Obispo, CA, USA) was added to the beakers to cover the bottom, which provided a substrate for the immatures while facilitating counting of the larvae. Reconstituted water was prepared using calcium chloride hexahydrate, 98%, sodium bicarbonate ACS reagent, 99.7–100.3%, calcium sulfate, ≥99.9% trace metals basis, magnesium sulfate heptahydrate, 98 + %, ACS reagent (Sigma-Aldrich, St. Louis, MO, USA), and potassium chloride (Fisher Scientific, Pittsburgh, PA, USA), mixed in Milli-Q HPLC grade water. The chemistry of this water follows the recommendations established by the US EPA (1994) (Table 1). A single batch of this reconstituted water was mixed initially and 300 ml was transferred to each of the 15 beakers. Amounts of PO_4 and/or As(V) were added from premixed stock solutions to create and maintain the required treatment concentrations.

Table 1
Water chemistry used during chironomid larval survival assays.

Alkalinity	Cations				Anions	
	Ca	K	Mg	Na	SO_4	Cl
mEq						
1.14	1.64	0.05	0.50	1.14	1.23	1.64

Beakers were covered with cheesecloth and placed in an environmental rearing chamber at 23 °C and 16L:8D with constant aeration. Compressed air was filtered through a one-way glass microfiber Whatman air filter before reaching the test beakers. Water temperature throughout the experiment averaged 22.4 ± 0.5 °C, and pH was maintained between 7.1 and 7.8. Larvae were fed a slurry of TetraMin® Tropical Fish Flakes (United Pet Group, Inc., Cincinnati, OH, USA) made by adding 1 g of flakes to 10 ml of deionized water at a rate of 3 drops every other day, through pupation. Thus, *C. riparius* was exposed chronically in all treatments from first instar larva through pupal emergence, approximately two weeks.

Evaporative water loss was replenished daily by adding Milli-Q HPLC-grade water to maintain a 300 ml total volume in the beakers. Beginning on day 5, one third of the water in the beakers was replaced daily and As(V) and/or PO_4 added to maintain the test concentrations. Water replacement was delayed until day 5 to minimize injury to early instars. Arsenic concentrations in the 0 and 0.13 μmol l^{-1} As(V) treatments were validated using Hydride Generated Atomic Absorption Spectroscopy (HGAAS). Actual concentrations were within 15% agreement of the target concentrations. Arsenic concentrations in the 2.0, 5.3, and 13 μmol l^{-1} As(V) treatments were validated using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and all were within 5% agreement of the target concentrations. Phosphorus concentrations were also validated using ICP-OES, and actual concentrations fell within 1% agreement for 15 μmol l^{-1}. Actual concentrations for the 0.15 μmol l^{-1} treatments were 4× higher than expected, possibly resulting from residual dissolved fish flakes present in the analyzed solution.

Larval survival and pupation were monitored daily starting on day five. Adults were counted and sexed as they emerged. Egg masses were removed daily and the number of eggs per egg mass counted. At 72 h post oviposition, egg masses were monitored for hatch percentage. Using these observations, the net reproductive rate (R_0), generation time (G), and intrinsic rate of increase (r, estimated using the equation $r \approx (\ln R_0)/G$) (Gotelli, 2008) were calculated for each of the 15 treatments. This experiment was replicated five times through time.

2.2. Arsenic analysis

As adults expired in the survival assay, they were removed from the treatments and stored in 1.5 ml centrifuge tubes. Prior to digestion, the chironomid adults were washed with 1 ml of 0.25 M KH_2PO_4 solution and rinsed twice with 1 ml ultra pure water to remove any adsorbed As. This rinse procedure effectively removed surface bound As from biological tissues in a concurrent experiment. The chironomid adults were then oven-dried at 50 °C to constant mass.

The digestion procedure was modified from Ringmann et al. (2002). Preliminary digestions of oyster tissue standard reference material (NIST 1566b, Gaithersberg, MD, USA) resulted in only 10% recovery of As using the published protocol of US EPA Method 200.8 (US EPA, 1999). The arsenobetaines (AB) that comprise the majority of As in oyster tissue are incapable of being broken down without extended hold periods at high temperatures and pressures (Fecher and Ruhnke, 1998), which exceeded the operating limits of our equipment. Though Andrahennadi and Pickering (2008) reported that insects are not likely to create AB to sequester As, detoxification mechanisms are still unknown and could involve the production of hard-to-breakdown organoarsenicals. To validate the As values for unknowns, we therefore adapted a protocol (Ringmann et al., 2002) that would breakdown all putative As species in the standard reference material.

All glassware used for digestions and analysis was acid washed prior to use. Digestions were carried out using Microwave Accelerated Reaction System (MARS) 5.0 (CEM Corporation, Matthews, NC, USA) HP-500 Teflon PFA digestion vessels. The maximum operating