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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

temperature and pressure for these vessels are 210 °C and 2413 kPa (350 psi), respectively. The digestion chemistry and microwave programs are shown in Table 2. Saturated solutions of sodium persulfate ≥98% and sodium fluoride ≥99% (Sigma-Aldrich, St. Louis, MO, USA) were mixed by adding 55 g of $Na_2O_8S_2$ and 5 g NaF to 100 ml of Milli-Q HPLC grade water, respectively.

After the second digestion, sample digestates were diluted to a 25 ml final volume using Milli-Q HPLC-grade water. An aliquot of 6 ml of this diluted digestate was then transferred to 15 ml transport tubes and prereduced overnight using 2 ml concentrated HCl and 2 ml 5%/5% w/w KI/L-ascorbic acid solution. Preliminary digestion trials of chironomid adults revealed very small concentrations in composite samples, and thus a larger digestate volume was analyzed for the unknowns to maximize the likelihood of As detection. The masses of oven dried adults that were digested for analysis were determined using a microbalance accurate to 0.00001 g (Sartorius model 1712 MP 8, Goettingen, Germany), and ranged between 1.30 and 5.12 mg. There were between 7 and 19 adults analyzed per sample, with an average of 13.

In order to determine whole body As concentrations in larvae, a separate cohort was reared to the 4th larval instar as described above with three replicates of each of the five As(V) treatments. Preliminary data from adult As accumulation revealed no significance of PO_4 additions, and thus larvae were reared in the As(V) treatments alone. When larvae reached the 4th instar, they were frozen and stored in 1.5 ml centrifuge tubes until they were digested and prereduced as described for the adults. Eleven to 17 individuals were analyzed per sample.

The digested samples of adults and larvae were analyzed using a Perkin-Elmer (Waltham, MA, USA) Analyst 800 Atomic Absorption Spectrophotometer, with a Perkin-Elmer FIMS 400 flow injection mercury system coupled with an As-90 autosampler. The minimum detection limit of the HGAAS was determined by analyzing five samples of 1 μg As l^{-1} and multiplying the standard deviation of the results by the one-sided t-distribution. This was calculated to be 0.050 μg l^{-1} for As. Digestion and prereduction blanks (containing only prereduction solutions and 6 ml Milli-Q HPLC-grade water) were also included for analysis, as well as digestions of 10 mg NIST oyster tissue standard reference material for validation. Arsenic bioaccumulation factors were calculated by subtracting any As recovered in controls from the concentration recovered in tissues and dividing by the original exposure concentrations.

2.3. Statistical analysis

All statistical analyses were conducted using SAS v. 9.2 (SAS Institute, 2008). Data were assessed for normality (Kolmogorov–Smirnov test) and homoscedasticity (Bartlett's or Levene's test) prior to analysis. Data upholding these assumptions were analyzed using two-way ANOVA (PROC GLM procedure) with As(V) and PO_4 as the independent variables. When data violated these assumptions and could not be corrected using a transformation, Friedman's non-parametric test was used. When necessary, Wilcoxon two sample tests and two sample Kolmogorov–Smirnov tests were applied for comparisons between treatments, Wilcoxon two sample tests being applied when data were not normal but had equal variances and two sample Kolmogorov–Smirnov being applied when data were not normally distributed and had unequal variances. For post hoc comparisons of ANOVA results, Tukey's test was applied. Sidak's correction was applied to adjust the α value for post hoc comparisons (Abdi, 2007).

Table 2
Digestion chemistry and microwave programs for first and second digestions, following Ringmann et al. (2002).

	Digestion 1	Digestion 2[a]
Digestion chemistry	6 ml $Na_2O_8S_2$	
	2 ml NaF	2 ml $Na_2O_8S_2$
	0.2 ml HNO_3	1 ml NaF
Microwave program	10 min ramp to 200° C	10 min ramp to 200° C
	Hold at 200° C for 15 min	Hold at 200° C for 15 min
	5 min cool down	5 min cool down

[a] Ringmann et al. (2002) uses a second digestion step in which an additional 3 ml of digestion reagents is added after the digestate has cooled completely.

3. Results

3.1. Chironomid survival assay

Survival to adult emergence averaged 84 ± 4% in controls, which exceeds Environment Canada's (1997) requirement for 70% survival of controls for a replicate to be valid. Thus, all replicates were valid and included in the analysis. There was a significant difference in the average time between male and female emergence (calculated as the first day to female emergence minus the first day to male emergence) for the As(V) treatments (Friedman's test controlling for PO_4, $F = 13.6$, $p = 0.0086$). Because post hoc analyses showed no significant difference between the 0 and 5.3 μmol As l^{-1} treatments, they were pooled and compared to the 13 μmol As l^{-1} treatment. There was a significant increase in the average time between male and female emergence in the highest As(V) treatment (Wilcoxon, $T = 783$, $p = 0.0035$) as a result of female emergence being delayed (Fig. 1). However, there was no significant differences detected between PO_4 treatments (Friedman's test controlling for As, $F = 5.33$, $p = 0.0697$) (Table 3). There was also no difference in the proportion of adults emerging from each of the As(V) and PO_4 treatments, and no interaction of As(V) and PO_4.

With regard to the reproductive potential of females, there was again no significant difference between the 0–5.3 μmol As l^{-1} treatments so they were pooled for analysis. The numbers of eggs per egg mass from these treatments were consistent with values reported for *C. riparius* elsewhere (Péry et al., 2002), although there were significantly fewer eggs per egg mass in the 13 μmol As l^{-1} treatment (Kolmogorov–Smirnov two-sample test, $D = 0.19$, $p = 0.0023$) (mean ± SE: 0–5.3 μmol As l^{-1} treatments: 299.3 ± 6.0 eggs; 13 μmol As l^{-1} treatment: 270.6 ± 11.1 eggs). There was no significant difference between PO_4 treatments (Friedman's test controlling for As, $F = 0.25$, $p = 0.8832$) (Table 3).

Analysis of the various life history parameters revealed no significant differences between treatments for R_0 (2-way ANOVA, $F = 2.20$, $df = 4,2$, $p = 0.0952$), G (2-way ANOVA, $F = 0.55$, $df = 4,2$, $p = 0.8892$),

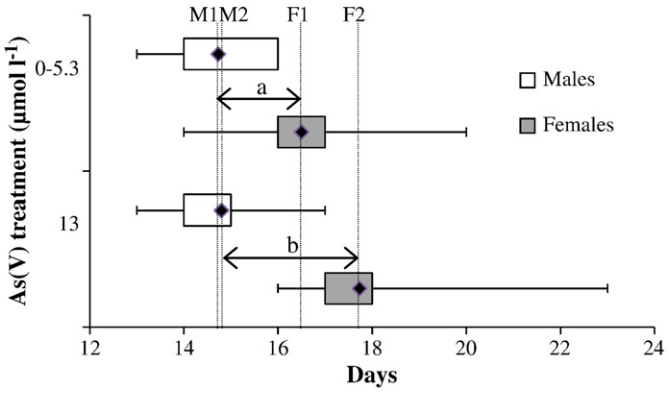

Fig. 1. Box and whisker plot of the first day to emergence for males and females. The second quartile box values for the females and the third quartile box values for the males are zero. The black diamonds represent the respective means. The lower whisker value represents the earliest day that an individual emerged, and the upper whisker represents the longest it took for an individual to emerge. There was a significant difference between the first day to male and female emergence (females–males) between treatments, represented here by a (F1 minus M1) and b (F2 minus M2). $N = 60$ for 0–5.3 μmol l^{-1} and $n = 15$ for 13 μmol l^{-1}.

Table 3
Means and standard errors for parameters measured during the chironomid survival assay for the PO_4 treatments.

PO_4 treatment (μmol l^{-1})	Days between male and female emergence		Number of eggs per egg mass	
	N	Mean ± SE (d)	n	Mean ± SE
0	25	5.64 ± 0.36	215	290.4 ± 9.56
0.15	25	4.84 ± 0.23	207	295.7 ± 8.75
15	25	4.60 ± 0.14	169	295.8 ± 9.26

and r (2-way ANOVA, F = 0.69, df = 4,2, p = 0.7794) (Table 4), although R_0 approaches significance. This may be due to the negative effect of As on female fecundity. There was no difference between PO_4 treatments and no significant interaction.

3.2. Arsenic analysis

Digestion and analysis of the NIST oyster tissue validated the protocol used to extract As from the digested chironomid tissues, with 111.8 ± 3.5% recovery. Analysis of As accumulation in adults revealed significant differences between As(V) treatments (Friedman's test, F = 56.2, df = 4, p<0.001) when controlling for PO_4. The presence of PO_4 did not affect As accumulation and there was no interaction between the treatment variables. Comparisons between As(V) treatments showed a significant increase in As accumulation by adults with an increase in As(V) exposure concentration (Fig. 2). Adults bioaccumulated approximately 8.2 times the As in which they were exposed as larvae. Significant As accumulation was also observed in larvae (ANOVA, F = 918.3, df = 4, p<0.0001) (Fig. 3). Larvae bioaccumulated approximately 51.7 times the As in which they were reared. Based on the average differences between As concentrations recovered in adults and larvae for each As(V) treatment, it is apparent that *C. riparius* eliminates approximately 72% of As body burdens between the 4th instar and adult stages.

There was no detectable As in the digestion or prereduction blanks; therefore, the presence of As in the controls for both the adults and larvae is the result of As contamination encountered prior to digestion. This may be due to small amounts of As being present in the TetraMin slurry fed to larvae. Our analysis of the fish flakes detected the presence of 3.690 ± 0.70 μg As g^{-1} (n = 3).

4. Discussion

4.1. Arsenate and phosphate interaction

This is the first study to examine the interaction between arsenate and phosphate in an insect. Unlike in plants, As(V) and PO_4 did not interact to alter reproduction, survival, or As accumulation in *C. riparius*. This suggests that the interaction may be important only to autotrophs. However, this interaction may still be of importance for herbivorous insects consuming plants containing both of these elements (e.g. *Azolla* sp. (Zhang et al., 2008), *Hydrilla verticillata* (Srivastava et al., 2007), *S. polyrhiza* (Rahman et al., 2008), and *Wolffia globosa* (Zhang et al., 2009)).

Table 4
Results from life history parameter analysis.

		As(V) Treatment, μmol l^{-1}				
		0[a]	0.13	2.0	5.3	13
R_0	Mean	148.54	138.38	164.28	139.59	119.87
	SE	5.27	5.66	8.99	6.61	5.49
G	Mean	22.2	21.6	21.5	21.5	22.5
	SE	0.14	0.08	0.13	0.12	0.17
r	Mean	0.223	0.226	0.236	0.223	0.202
	SE	0.00079	0.0014	0.0040	0.0013	0.0021

[a] N = 15 for each variable in each treatment.

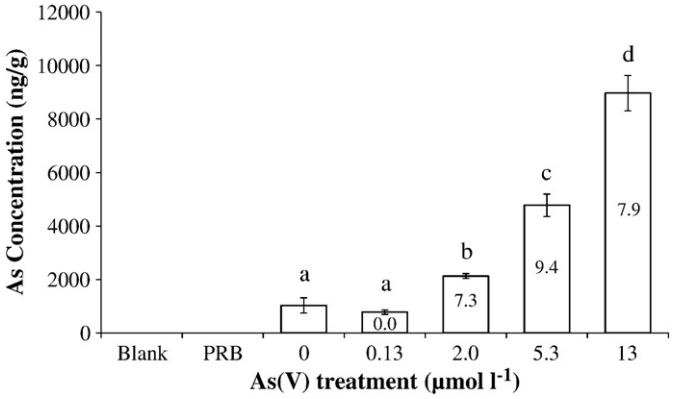

Fig. 2. The mean (±SE) concentration of As recovered from digestion of chironomid adults. Letters indicate significant differences, with α = 0.0102 (Sidak's correction for 10 contrasts). There was no As detected in either digestion or prereduction blanks (PRB) (n = 8 for each). Numbers inside the bars indicate the As bioaccumulation factor for that treatment.

4.2. Survival and reproduction

This is the first time that the effects of chronic As exposure have been evaluated throughout the entire life cycle of an aquatic insect. Though studies have focused on acute effects of arsenic in aquatic systems, they report on alderflies (Croisetière et al., 2006), caddisflies (Canivet et al., 2001), dragonflies (Lavilla et al., 2010), and mayflies (Canivet et al., 2001; Irving et al., 2008), in addition to midges (Croisetière et al., 2006; Jeyasingham and Ling, 2000; Liber et al., 2011; Martinez et al., 2006). Certain studies have investigated how effluents containing metals induce mentum deformities in midges (e.g. Martinez et al., 2002, 2006), though the effects of As alone are impossible to deduce when working with metals mixtures. However, these do not address how chronic exposure affects survival and reproduction in aquatic insects.

We have shown that even though relatively high, ecologically relevant concentrations of As(V) exposure will not affect larval survival or the proportion of adults emerging between treatments, the highest As(V) level did increase the time between male and female emergence in *C. riparius* by delaying female emergence. In a study investigating survival of chironomids, Liber et al. (2011) found the LC_{50} for *Chironomus dilutus* exposed for 96 h to As-spiked water to be 7.1 mg l^{-1}, approximately 7× the highest concentration tested here, though an acute toxicity threshold was reached at 3.31 mg l^{-1}. LC_{50} values for *Chironomus zealandicus*, *C.* sp. a, and *Polypedilum pavidus*

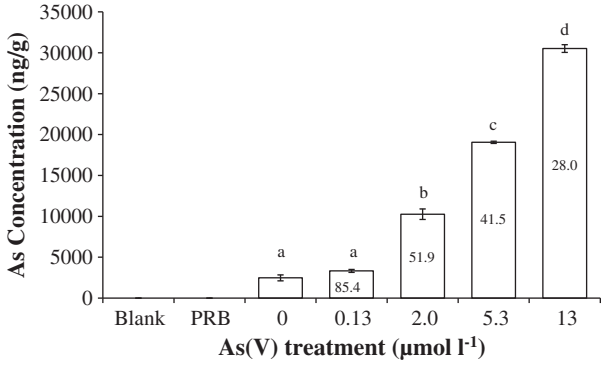

Fig. 3. The mean (±SE) concentration of As recovered from digestion of chironomid larvae. Letters indicate significant differences using Tukey's test, with α = 0.05. There was no As detected in either digestion or prereduction blanks (PRB) (n = 2 for each). Numbers inside the bars indicate the As bioaccumulation factor for that treatment.

were found to increase with age in 96 h bioassays, and ranged from 33.1 to 4176 μg l^{-1}. (Jeyasingham and Ling, 2000). These elevated LC_{50} values highlight the potential unsuitability of using short term LC_{50}s (96 h or less) to gauge the long term effects of As on chironomids, particularly when assays do not evaluate potential sublethal effects.

In chironomids, males emerge before females to form mating swarms that ensure access to females (Ferrington et al., 2008). Because these are relatively short lived insects as adults, delayed female emergence as found in this study could result in males dying before females become receptive, particularly as adults do not feed. This may then lead to local extinctions of populations, as suggested for *Megaselia scalaris* (Diptera: Phoridae) exposed to selenium (Jensen et al., 2005). Reproduction as measured by the number of eggs per egg mass was also significantly reduced in the 13 μmol As l^{-1} treatment, indicating that this concentration may be at or above a threshold for *C. riparius* at which sublethal effects become significant.

4.3. Arsenic accumulation

Arsenic accumulation within adults and larvae showed a significant dose response to increases in As(V). The majority of what has been published examines accumulation in predators, with only a single study monitoring accumulation of As in *C. riparius* as a prey item (Croisetière et al., 2006). The authors transported laboratory reared 2nd instars to a contaminated lake containing 1.45 nmol As l^{-1}, where they were held for 1 wk before being fed to *Sialis velata* (Megaloptera: Sialidae). Larvae accumulated 17.2 μg As g^{-1} dry weight during this time, with an accumulation factor of 1.1×10^3. Studies examining As accumulation in other aquatic insects found concentration factors of 327 for *S. velata* when fed prey exposed to 1.56 μg As l^{-1} (Croisetière et al., 2006), 131 for *Pteronarcys dorsata* (Plecoptera: Pteronarcyidae) exposed to 100 μg As l^{-1} and 33 when exposed to 1000 μg As l^{-1} (Spehar et al., 1980), 1.22 and 1093 for *Heptagenia sulphurea* and *Hydropsyche pellucidula*, respectively, when exposed to 100 μg As l^{-1} (Canivet et al., 2001), and 1, 1.28, and 1 for *Hydroglyphus pusillus*, *Laccophilus minutus*, and *Rhantus suturalis* (Coleoptera: Dytiscidae) when exposed to 0.32 μg As l^{-1} (Burghelea et al., 2011). However, the Croisetière et al. (2006) and Burghelea et al. (2011) studies were also field based, and analyzed As in addition to numerous other elements at the same time. Because of this, As accumulation may have been higher or lower as a result of undocumented synergistic or antagonistic interactions. The high variability between and within species highlights the need for caution when using bioaccumulation factors to assess the ability of an organism or group of organisms to accumulate As. When comparing between organisms or feeding guilds, only bioaccumulation factors from the same exposure concentration should be compared.

Interestingly, within *C. riparius* bioaccumulation factors change depending on the life stage being analyzed. In our study, bioaccumulation factors for adults and larvae were 8.2 and 51.7, respectively. This species clearly has the capacity to excrete large amounts of As body burdens between the last larval instar and the adult stage. Whether this is the result of As being shed as a meconium, in the pupal exuvia (e.g. as seen with selenium in *Cotesia marginiventris* (Hymenoptera: Braconidae) (Vickerman et al., 2004)), or through some other mechanism is still unknown. In this study, HGAAS analysis would not detect As in pupal exuvia because the sample mass was too small. Future studies are planned, however, to investigate As in pupal exuvia using micro X-ray Atomic Spectroscopy. Chironomids have been documented elsewhere as excreting metals during the transition to the pupal and adult stages (e.g. cadmium (Groenendijk et al., 1999; Timmermans and Walker, 1989), uranium (Muscatello and Liber, 2009), and zinc (Groenendijk et al., 1999; Timmermans and Walker, 1989).

Chironomids are known in certain areas of the world to emerge en masse and when they do so, transport significant quantities of nitrogen and carbon to the surrounding terrestrial environment (Gratton et al., 2008). In their Icelandic study system at Lake Mývatn, 189 kg ha^{-1} d^{-1} of midge infall occurred over a 1 wk period. Based on the concentrations of As found in *C. riparius* adults in our study, it is possible for 1.70 g As ha^{-1} to be deposited onto terrestrial systems or consumed by terrestrial predators in a single day. This deposition could have substantial negative consequences over time (Lamberti and Chaloner, 2010; Morrissey et al., 2007), such as significant accumulation and trophic transfer of As within the food chain. Though there are small quantities of As per individual, As could also be bioconcentrated at the population level during periods of high emergence (Green, 2008). Higher concentrations recovered in larvae also indicate that trophic transfer of As to aquatic predators is highly likely, particularly in areas where chironomid larvae reach high densities.

4.4. Conclusions

Often the aquatic insects chosen for toxicity assays are those able to survive and reproduce readily in a laboratory setting, which may be because they are tolerant to variable environmental conditions. *C. riparius* is a widely used toxicity assay organism and ranks as one of the more tolerant species, with regional tolerance values between 8.1 and 10, on a scale of 1 (low tolerance) to 10 (high tolerance) (Barbour et al., 1999). We found significantly reduced reproduction in females and a significant increase in the difference between male and female emergence times at the highest As concentration tested. Both of these may have significant effects on population maintenance in wild populations of *C. riparius*. Arsenic accumulation increased significantly with increasing exposure concentrations in larvae and adults, and *C. riparius* is able to eliminate 72% of As body burdens before reaching the adult stage. However, given the generally high tolerance of *C. riparius* to pollution, this may be unrepresentative of other aquatic insects, and more research is needed to determine the sublethal effects of As for these less tolerant species.

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