



Water Resources Research Institute
of The University of North Carolina

Report No. 465

COAL ASH CONSTITUENTS AT THE BASE OF AQUATIC FOOD WEBS: PROCESSES
AFFECTING BIOACCUMULATION AND TROPHIC TRANSFER

By

Adeline R. Lopez¹, Dean R. Hesterberg², David H. Funk³ and David B. Buchwalter⁴

¹Department of Biological Sciences

³Department of Soil Science

⁴Department of Environmental and Molecular Toxicology

North Carolina State University

Raleigh, NC

²Stroud Water Research Center

Avondale, PA

UNC-WRRI-465

The research on which this report is based was supported by funds provided by the North Carolina General Assembly and/or the US Geological Survey through the NC Water Resources Research Institute.

Contents of this publication do not necessarily reflect the views and policies of WRRI, nor does mention of trade names or commercial products constitute their endorsement by the WRRI, the State of North Carolina, or the US Geological Survey.

This report fulfills the requirements for a project completion report of the Water Resources Research Institute of The University of North Carolina. The authors are solely responsible for the content and completeness of the report.

WRRI Project No. 15-04-W
May 2016

ABSTRACT.

Project Title: Coal ash constituents at the base of aquatic food webs: Processes affecting bioaccumulation and trophic transfer.

The goal of this research was to investigate arsenic bioaccumulation at the base of aquatic food webs, including uptake of arsenic from solution and depuration kinetics by benthic invertebrates, uptake and bioconcentration of arsenic by periphyton, and potential trophic transfer to primary consumers. To better understand arsenate bioaccumulation dynamics in lotic food webs we used a radiotracer approach to characterize accumulation in periphyton and subsequent trophic transfer to benthic grazers. Flux rates from solution for a variety of benthic invertebrates are also described. Our results show that over an 8 day period periphyton concentrated As from environmentally realistic exposures 3,200–9,700-fold on a dry weight basis without reaching steady state. These As-enriched diets resulted in negligible accumulation of As in *Neocloeon triangulifer* relative to the concentration in periphyton after a full lifecycle exposure. Other dietary studies with invertebrate grazers showed that the assimilation efficiency of As from periphyton is generally quite low, ranging from 22% in the mayfly *N. triangulifer* to 75% in the mayfly *Isonychia sp.*, suggesting factors controlling bioavailability limit the amount of As that is transferred to grazers. We propose that two such mechanisms may be the role of As adsorption to iron oxides in periphyton, and biotransformation of As by periphyton. Data showing relatively low uptake rate constants (K_u) from solution in benthic invertebrates ranging from $0.063 \pm 0.04 \text{ L g}^{-1} \text{ d}^{-1}$ in *Psephenus herricki*, to $0.001 \pm 0.003 \text{ L g}^{-1} \text{ d}^{-1}$ in *M. pudicum*. Efflux (K_e) was generally high ranging from $0.15 \pm 0.03 \text{ d}^{-1}$ in *Maccaffertium sp.* to $0.03 \pm 0.03 \text{ d}^{-1}$ in *Pleurocera sp.* Together these results have broad implications for monitoring programs by highlighting the role of periphyton as a sink for arsenate as well as interspecies differences in As bioaccumulation.

Acknowledgements

We thank Justin Conley for editorial assistance, Shane Scheibener (NCSU), Vinicius Richardi (UFP), Allison Camp (NCSU) for editorial assistance and help with field sampling. Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research, and by the National Institutes of Health, National Institute of General Medical Sciences (including P41GM103393). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH. We thank Sam Webb (SSRL) for technical guidance and support. This work was graciously funded by the North Carolina Water Resources Research Institute.

1.0 Introduction:

A growing body of literature highlights the importance of bioaccumulation of potentially toxic trace elements at the base of freshwater food webs (e.g.,(Patrick 1978; Farag et al. 1998; Ancion et al. 2010; Cain et al. 2011)) and the importance of dietary exposure routes in dictating accumulation.(Luoma and Rainbow 2005) Periphytic biofilms comprise different types of diatoms, algae, bacteria, fungi, and detritus that are often the predominant food resource at the base of aquatic food webs. Periphyton can significantly bioconcentrate trace elements and act as a dietary vector for metal exposures to grazing fauna. For example, cadmium,(Bradac et al. 2009; Xie et al. 2010) zinc,(Kim et al. 2012) copper,(Cain et al. 2011) and selenium(Conley et al. 2009; Conley et al. 2013) have all been shown to accumulate in periphytic biofilms and are trophically transferred to invertebrate grazers. In contrast, less is known about periphytic uptake, bioconcentration, and trophic transfer of arsenic.

Arsenic is the 20th most abundant element in the Earth's crust,(Woolson 1975) and is a common contaminant in aquatic ecosystems as well as an EPA priority pollutant.(U.S. EPA 2014) The mineral co-localization of As with geologic resources such as metal rich ores and coal often result in As contamination associated with the extraction and use of these natural resources (e.g. mining, smelting, and coal combustion). Background concentrations of As in rivers are reported to range from 0.02 $\mu\text{g L}^{-1}$ to 2 $\mu\text{g L}^{-1}$, while contaminated rivers typically range from 1-280 $\mu\text{g L}^{-1}$ but have been reported as high as 79,000 $\mu\text{g L}^{-1}$.(Smedley and Kinniburgh 2002) While arsenate is expected to be the dominant chemical species of As in lotic systems,(Smedley and Kinniburgh 2002) the biogeochemistry of As is complex. As exists in different oxidation states in the environment (-3, 0, +3, and +5) and can be converted biologically to several organic forms(Smedley and Kinniburgh 2002; Rahman et al. 2012) or converted between inorganic oxidation states (e.g.,(Kulp et al. 2004; Levy et al. 2005)). These chemical forms dictate how As behaves in the environment and its potential to cause toxicity.(Akter et al. 2005; Sharma and Sohn 2009) Less is understood about the dynamics of As at the base of freshwater food webs, particularly with respect to accumulation into periphytic biofilms and its availability to invertebrate grazers.

Field studies indicate that As accumulation in periphytic biofilms is potentially important since measured concentrations have been shown to exceed those for water or sediment (e.g.,(Ramelow et al. 1987; Drndarski et al. 1993; Koch et al. 1999)). Similar observations have been reported for algae,(Koch et al. 1999; Schaeffer et al. 2006) bryophytes,(Culioli et al. 2009) and aquatic plants,(Favas et al. 2012) though the complexity and variability in natural systems complicates quantifying accumulation dynamics for As. Laboratory studies similarly demonstrate that As is accumulative in a variety of aquatic plants (e.g.,(N.-X. Wang et al. 2013; Y. Wang et al. 2013; Sibi 2014)), algae (e.g.,(N.-X. Wang et al. 2013; Y. Wang et al. 2013; Sibi 2014)), and bacteria.(Y. Wang et al. 2013; Z. Wang et al. 2013) While this accumulation is highly variable(Jasrotia et al. 2014; Sibi 2014; Srivastava et al. 2014) several species are such strong As accumulators that they have been proposed for use in As bioremediation.(Yin et al. 2012; Jasrotia et al. 2014; Srivastava et al. 2014; Islam et al. 2015) In comparison to these single-species evaluations, much less is known about the accumulation dynamics of As in environmentally realistic and complex assemblages of periphyton.

Accumulation of As by primary producers at the base of the food web may have important implications for trophic transfer, though there is conflicting evidence in the literature regarding which route of exposure drives As accumulation in primary consumers. For example, field studies report that tissue concentrations of As in organisms are better correlated with the concentration in their food than with water.(Aida M Farag et al., 2007) Dietary exposure has also been suggested to drive As accumulation in several laboratory studies. For example Maeda(Maeda et al., 1990) found that benthic grazers accumulated an order of magnitude greater As from food than from water, and Williams et al.(Williams, Dutton, Chen, & Fisher, 2010) reported that ingested microalgae could be responsible for more than 80% of accumulated As in suspension/deposit feeding amphipods. Slightly lower dietary contributions of 30–60% were reported by Casado-Martinez et al.,(Casado-Martinez, Smith, Luoma, & Rainbow, 2010) but this was still an important pathway of accumulation. Contradictory findings have been reported by Kalman et al.(Kalman, Smith, Bury, & Rainbow, 2014) who used a biokinetic approach to determine that dissolved exposure was responsible for 50–90% of acquired As in an estuarine bivalve. Similarly, a field study conducted by Hare et al.(Hare, Tessier, & Campbell, 1991) reported that 95% of measured As was associated with the exoskeleton of invertebrates rather than the gut (3%), and Spehar et al.(Spehar, Fiandt, Anderson, & DeFoe, 1980) reported between 100–200 fold increase in As concentration relative to dissolved concentrations for several aquatic invertebrates. Interspecies variability in accumulation of As from solution has also been reported; Canivet et al.(Canivet, Chambon, & Gibert, 2001) noted that two thirds of investigated species accumulated As from solution while the other third did not. Together these inconsistencies point to the need for a broader fundamental understanding of the dynamics and behavior of As at the base of aquatic food webs as well as the factors driving accumulation and trophic transfer.

In this study we used a radiotracer approach to quantitate the bioconcentration of arsenate by natural periphyton assemblages at environmentally realistic concentrations. Lab-reared parthenogenetic *Neocloeon triangulifer* larvae were then raised on these differentially contaminated diets for a full life cycle experiment to investigate trophic transfer. These dietary bioaccumulation studies were combined with assays that examined As assimilation efficiency from food, uptake from solution, and efflux for a variety of benthic invertebrates to better understand As accumulation dynamics at the base of the aquatic food web. Finally, XANES and XRF microprobe analyses of As in periphyton were conducted to better understand As accumulation dynamics at the base of the aquatic food web.

2.0 Methods:

2.1 Reagents:

Arsenate ($\text{HAsNa}_2\text{O}_4 \bullet 7\text{H}_2\text{O}$) was obtained from Alfa Aesar (MA, USA). ^{73}As was obtained from the National Isotope Development Center (U.S. Dept. of Energy) as As(V) in 0.1 M HCl. Working secondary stock solutions were prepared in 0.1 N Omnitrace™ nitric acid (EMD Chemicals, Darmstadt, Germany). American Society for Testing and Materials (ASTM) artificial soft water (ASW) (mM: 0.57 NaHCO_3 , 0.17 $\text{CaSO}_4 \bullet 2\text{H}_2\text{O}$, 0.25 MgSO_4 , and 0.03 KCl) was also used for all experiments.

2.2 Test animals:

N. triangulifer (WCC-2 clone originally obtained from culture at Stroud Water Research Center [SWRC], Avondale, PA) were reared in the lab at room temperature with ambient light. Other larval insects and benthic invertebrates were field collected from the Eno River (Efland, NC and Durham, NC) and Basin Creek (Sparta, NC), and allowed to acclimate without food for at least 48 hours to the laboratory cold room (approximately 15°C).

2.3 Natural periphyton communities:

Natural periphyton assemblages were obtained from SWRC, where they were cultivated by allowing fresh water from White Clay Creek, PA to flow continuously over acrylic plates (6.5 x 23 x 0.15 cm; see Appendix 3 for historical taxonomic data). Periphyton plates were shipped overnight on ice and were subsequently aerated and held at room temperature until experimental use. Background concentrations of As in periphyton ($4.5 \pm 1.2 \text{ mg kg}^{-1}$ dry wt) were determined using nitric acid digestion and ICP-MS at the Environmental and Analytical Testing Services lab at North Carolina State University. All experiments measuring As in periphyton characterize newly acquired As only.

2.4 Radioactivity measurement:

All measurements of radioactivity in water, periphyton, and invertebrates were performed with a Perkin-Elmer Wallac Wizard 1480 automatic gamma counter. All samples were programmed to be counted for three minutes to achieve counting errors generally <5% (errors >10% were not included in analysis). All As concentrations are reported accounting for radioactive decay (half life = 80.5 days), counting efficiency, and mass specific activity.

2.5 Experimental design:

2.5.1 pH experiment:

Arsenic uptake rates in periphyton were studied across a range of environmentally relevant pHs by collecting small scrapings of similar wet weight ($0.1165 \pm 0.0077 \text{ g}$) and transferring them into individual exposure cups with 25 mL of pH-adjusted (6.5, 7.0, 7.5, 8.0, and 8.5) ASW (5mM bis-tris propane used as a buffer) at a nominal concentration of 10 µg/L arsenate along with ^{73}As as a radiotracer. Three replicates were prepared for each time point. To ensure proper aeration, exposure cups were held on mixer tables. Uptake was measured at 3, 6, and 9 hours. At each time point, samples were rinsed with 300 mL of concentration-matched stable arsenate (no radioisotope) to remove superficially adsorbed arsenic, vacuum filtered on to dried and pre-weighed filter paper, and dried overnight at 65°C. Dried samples were weighed and assayed for radioactivity.

2.5.2 Periphyton enrichment and food preparation for the full life cycle experiment with *Neocloeon triangulifer*:

Two separate batches of periphyton plates (referred to as trial 1 and trial 2) were used to assess arsenate bioconcentration. These labeled periphyton plates were then used as a food source for developing mayfly larvae. The experiments were staggered 16 days apart to supply sufficient food for developing larvae and were conducted in the same manner. Immediately upon arrival, individual periphyton plates were placed in aerated exposure jars at room temperature with 1.8 L

of pH-adjusted (7.6 ± 0.04) ASW at nominal exposure concentrations of 0, 1, 5, 10, and $20 \mu\text{g L}^{-1}$ total As in addition to As-73 as a radiotracer ($0.002 \mu\text{Ci mL}^{-1}$). Three replicates per concentration were allowed to accumulate As for 8 days for the first round of plates and 10 days for the second round of plates. Nominal exposure concentrations corresponded to measured concentrations of stable arsenate (ICP-MS) of <0.1 , 1.09, 4.97, 10.4, and $20.7 \mu\text{g L}^{-1}$ in trial 1 and <0.1 , 1.28, 6.85, 16.0, and $24.7 \mu\text{g L}^{-1}$ in trial 2.

During the initial 8 day loading period (trial 1) 1 mL water samples were collected daily from each replicate for radioactivity measurement and periphyton scrapings of similar weight (~ 0.05 – 0.07 g) were collected daily from each replicate onto dried, pre-weighed filter paper. Scrapings were dried at 65°C , weighed, and measured for radioactivity to determine As content. Sample collection from this trial was discontinued on day 8 when newly hatched mayfly larvae were introduced to the chambers (see below). In trial 2, periphyton sampling was conducted less frequently to maintain a high food level for larvae (samples collected on days 4, 5, 6, 7, and 10). These trial 2 periphyton plates were added to the chambers containing the trial 1 plates and mayfly larvae (day 11 of trial 2, day 18–19 of larval development).

After hatching (1–2 days), 15 *N. triangulifer* individuals were randomly placed in each experimental replicate with As-enriched periphyton as described above. Larvae were reared on arsenate-enriched periphyton plates along with residual aqueous concentrations (see SI for detailed exposure characterization) until adult emergence. Periphyton plates from trial 1 were the food source for mayfly rearing days 1–18, though these plates remained in the experimental chambers throughout the study. Beginning day 18 the periphyton plates for trial 2 were also available as a food source for mayfly rearing through study termination. Larvae were assayed for radioactivity on days 25–26 prior emergence as subimagos (days 26–34) to determine the average As content in larvae for each exposure concentration. Larvae were not weighed to minimize handling stress and were returned to their exposure chambers to allow them to complete development to adulthood. Subimagos were assayed for radioactivity beginning on day 26 and were then transferred to molting jars with moist paper towels overnight until final molting. Adults were placed in individual microcentrifuge tubes, which were first frozen at -20°C , then dried at 65°C for 48 hours before being weighed to the nearest 0.001 mg.

2.5.3 Assimilation efficiency (AE%):

Periphyton samples were labeled with ^{73}As for 3–4 days in 150 mL ASW ($0.004 \mu\text{Ci mL}^{-1}$ for all experiments). No stable As was used in the labeling process. Labeled periphyton was then rinsed twice with ASW and added to exposure cups containing ASW only. Field collected benthic grazers ($n=10$ – 20) were allowed to consume radio-labeled periphyton ad libitum for 4–6 hours before being transferred to individual containers with ASW and clean food for 15 hours. Animals were assayed for radioactivity immediately following consumption of radiolabeled periphyton, and again following consumption of clean food. AE was calculated as the percent of radioactivity remaining after consumption of clean food compared to initial radioactivity measured after consumption of radio labeled periphyton.

2.5.4 Microscale elemental associations and biotransformation of As:

Periphyton plates were exposed to nominal concentration of 20 $\mu\text{g L}^{-1}$ stable arsenate in pH-adjusted (6.5 ± 0.02) ASW for 4 days. To maximize As uptake, solutions were refreshed daily. On the final day of exposure, periphyton samples (~0.2 g wet weight) were rinsed and suspended in 10 mL ASW and vacuum filtered onto a 0.2 μm Millipore Isopore polycarbonate filter membrane. The filter was immediately mounted on a 4 \times 2.5 cm acrylic window using Kapton tape. The periphyton mount was then quickly placed in an air-tight glove box covered in aluminum foil to eliminate light exposure and dried with N_2 gas for 5 hours before being packaged for overnight shipment to Stanford Synchrotron Radiation Lightsource (SSRL). Microscale spatial distributions of As, Si, P, S, K, Ca, Ti, Mn, Fe, Cu, and Zn were mapped on an approximately 4800 \times 1100 μm region of the periphyton sample using micro X-ray fluorescence (μXRF) at Beamline 2-3. The beam spot size was nominally 1 \times 1 μm^2 , and images were collected with a step size of 0.01 μm and dwell time of 50 milliseconds. XANES spectra were additionally collected on regions of interest.

2.5.5 Dissolved uptake and efflux:

For aqueous uptake and efflux experiments, field collected invertebrates were transferred to individual acid-washed exposure cups (n=5 per exposure concentration; see Appendix 3 for full taxonomic classification) with a small square PTFE substrate, filled with 25 mL pH-adjusted (7.2 ± 0.2) ASW at nominal concentration of 10 $\mu\text{g L}^{-1}$ arsenate along with As-73 as a radiotracer (volume of isotope adjusted to account for decay and achieve final specific activity in exposure chambers of 0.003–0.005 $\mu\text{Ci mL}^{-1}$ for all experiments). To obtain initial uptake rates from solutions, animals were analyzed *in vivo* for radioactivity at 3, 6, and 9 hours following a rinse with concentration-matched stable As (no radioisotope) solution to remove any superficially adsorbed radiotracer. After the 9 hour time point, animals were returned to their exposure solutions for an additional 4-5 days of loading before being transferred to clean water to measure efflux. Efflux was measured daily for 5-10 days by assaying individuals for radioactivity. Clean ASW was provided each day to reduce re-uptake of radiotracer. Rough estimates of bioconcentration factors (BCFs) were obtained by dividing the average K_u by the average K_e for a given species.

2.6 Data Analysis:

Periphyton bioconcentration of As from water was calculated by dividing the mean measured As in periphyton on the final day of loading by the average measured As concentration in water (on a mass basis where 1 L water = 1 kg) across all days of the periphyton loading phase. Comparisons of As accumulated in larvae were calculated by averaging the measured mass of As in all individuals in each replicate and all replicates per exposure, which was then compared to the average final mass of As accumulated per gram of dry weight of periphyton.

Uptake rate constants (K_u) were estimated as the slope of the measured As concentration over time (linear regression) divided by the exposure concentration. Efflux rate (K_e) was estimated as:

$$C_t = C_i \times e^{K_e \times t}$$

where C_t = tissue concentration at time t, C_i = tissue concentration at time 0 d, K_e = efflux rate constant, and t = time in days.

X-ray microprobe images of As and other element spatial distributions were processed using Sam's Microprobe Analysis Toolkit (SMAK, developed by Samuel Webb, Stanford Synchrotron Radiation Lightsource, Palo Alto, CA). (Webb 2011) A blur filter of 5 (Stdev = 0.85) was applied to images before plotting spatial correlations of As with the other elements imaged. Pearson correlation coefficients were derived by taking the square root of the R^2 value reported in SMAK. XANES data were analyzed using SixPack.

Data are expressed as mean \pm standard error unless otherwise specified and analyzed using GraphPad Prism (V6).

3.0 Results

Uptake rate constants (K_u) in periphyton at pH of 6.5, 7.0, 7.5, 8.0, and 8.5 were 1.147 ± 0.1114 , 0.6626 ± 0.08733 , 0.7897 ± 0.08332 , 0.4934 ± 0.1071 , and $0.1573 \pm 0.06517 \mu\text{g As g}^{-1} \text{ ww day}^{-1}$, respectively (Fig 1). Uptake rates were statistically significantly different ($p < 0.05$) at all pH levels except for 7 and 7.5 ($p = 0.3104$). At the same concentration of arsenic, the periphyton at pH 6.5 concentrated almost 3 times more arsenic from solution than the periphyton at pH of 8.5.

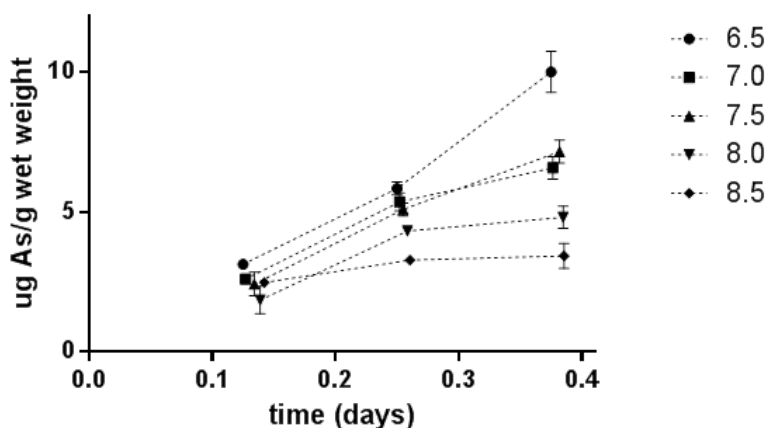


Figure 1. Uptake of arsenate in periphyton across different pH levels over 3, 6, and 9 hours of exposure

Two independent trials of periphyton exposed to a single pulse of 1, 5, 10 and 20 $\mu\text{g L}^{-1}$ arsenate resulted in consistent decreases in dissolved As concentrations. Dissolved As concentrations dropped rapidly over the first few days of exposure and stabilized at roughly 50% of their initial concentrations (Fig 2A,C) by days 2–4. Uptake of As into periphyton was less consistent and did not always mirror dissolved As concentrations. For example in trial 1, periphyton As concentrations generally increased over time but not in a monotonic fashion (Fig 2B). Periphyton As appeared to decrease briefly at days 4–5 before increasing again thereafter, most notably at the highest exposure level. Similarly, in trial 2, periphyton appeared to decrease in concentration after an initial rapid uptake at the higher exposure concentration; in this trial As in periphyton continued to decrease over days 4–10 (Fig 2D).

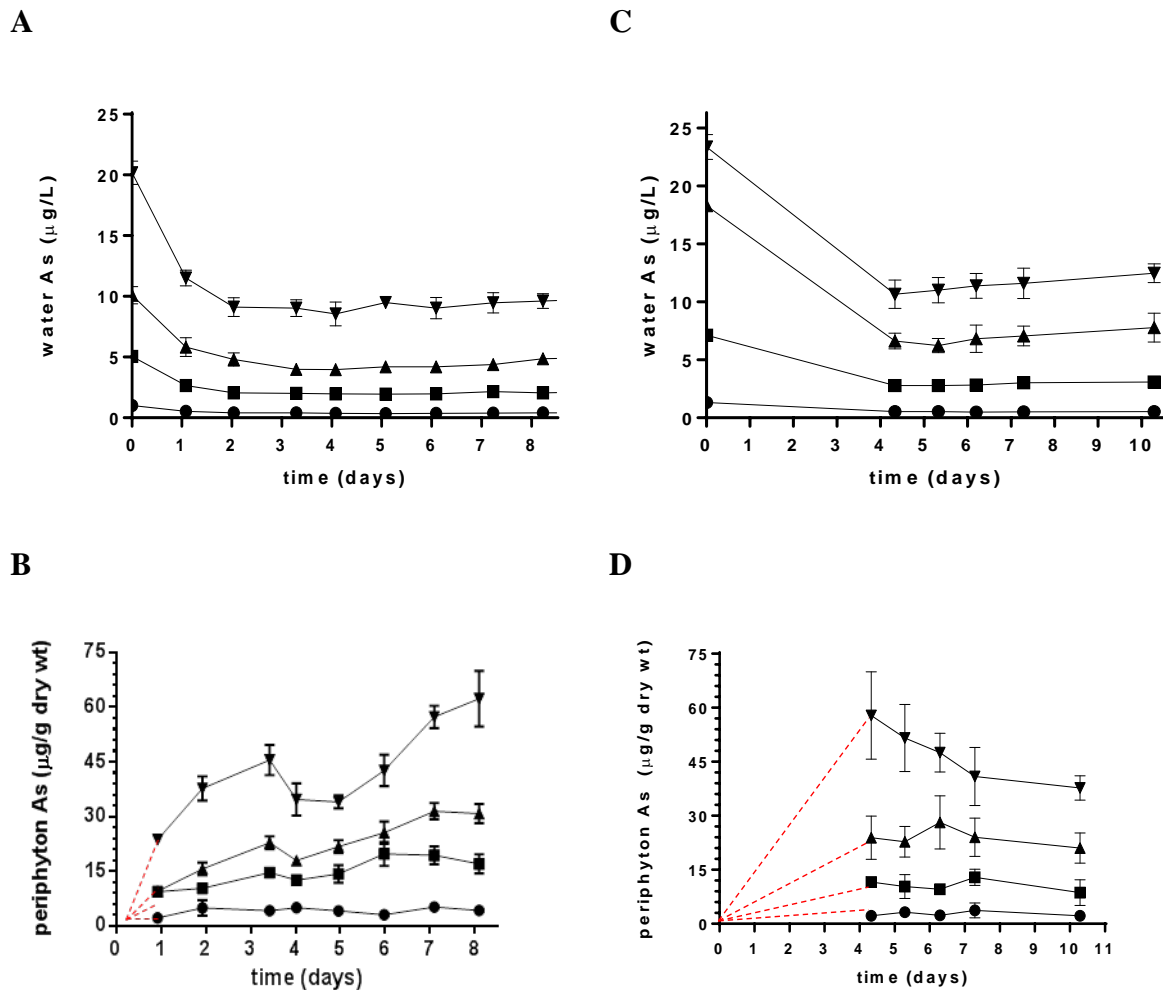
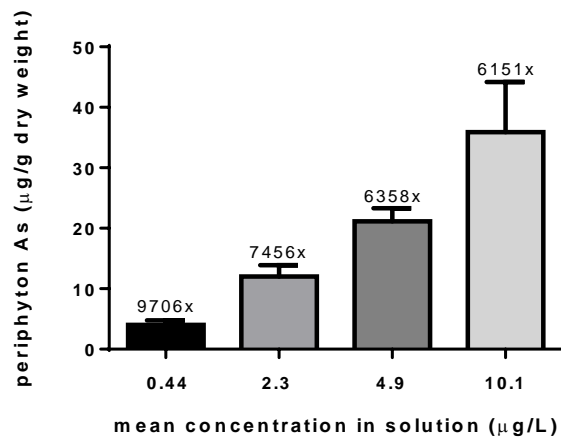


Figure 2. Temporal trends in dissolved As concentrations (A) and newly acquired As in periphyton (B) during 8 day loading period (trial 1). Measured concentration of As in solution (C) and newly acquired As in periphyton (D) during the 10 day loading period (trial 2). Red dashed lines (B,D) indicate intervals where samples were not taken. N=3 for each treatment at each time point. Symbols (low to high) represent initial nominal dissolved As concentrations of 1, 5, 10, and 20 $\mu\text{g L}^{-1}$ arsenate, respectively. Values plotted are mean \pm SEM.

After 8 days of exposure to a single pulse of arsenate (1, 5, 10, or 20 $\mu\text{g L}^{-1}$), the concentration of As in periphyton (dry weight basis) was compared to the average dissolved As concentration over the 8 day exposure period to quantify As bioconcentration in periphyton. In trial 1, periphyton bioconcentrated As 6,000-9,000-fold (Fig 3A). Ratios of periphyton As to mean dissolved As decreased with increasing dissolved As concentrations. In trial 2, periphyton bioconcentrated As 3,200-4,200-fold (Figure 3B) after 10 days of exposure to a single pulse of arsenate. Ratios of periphyton As to mean dissolved As were more consistent in this trial and did not trend with dissolved concentrations.

A



B

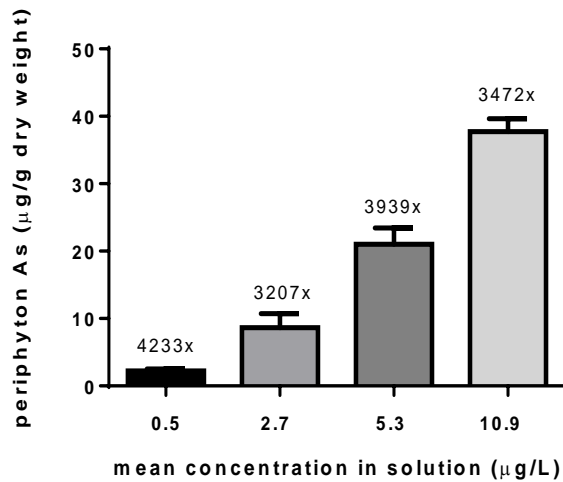


Figure 3. Periphyton bioaccumulation of arsenate after 8 days of loading for the first round of plates (A) and after 10 days of loading in the second round of plates (B). Values plotted are mean \pm SEM; n=3 for final measured arsenic concentration in each exposure group. Numbers above each bar represent fold increase of As concentrations in periphyton compared to the average concentration in solution. Initial nominal concentrations in solution were 1, 5, 10, or 20 $\mu\text{g L}^{-1}$ As.

To test whether periphyton-bioconcentrated As was trophically available to an invertebrate grazer, we reared the mayfly *N. triangulifer* on the periphyton diets described above (see SI for full exposure characterization). Very low radioactivity was measured in the larvae corresponding to 0.0006–0.005 μg As per individual (Fig. 4). While these individuals were not weighed to avoid handling stress, if we assume approximately 1 mg dry weight (average for developmental stage) we estimate that tissue As concentrations were 18–35% lower than As concentrations in periphyton, suggesting significant biodilution. When assayed again as subimagos radioactivity could not be detected.

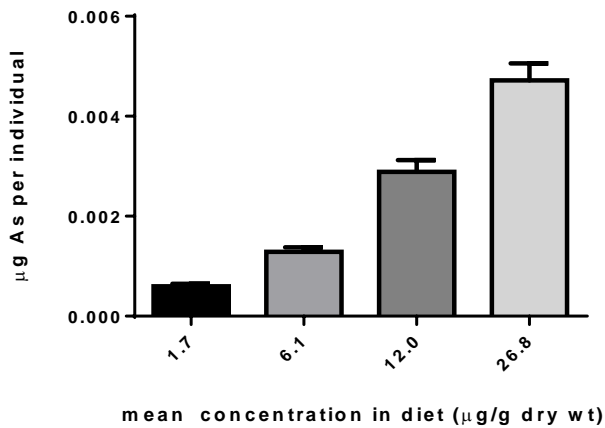


Figure 4. Bioaccumulation of As in *N. triangulifer* larvae fed on differentially contaminated periphyton plates in full lifecycle exposures. Values plotted are mean \pm SEM; n=26–34.

Assimilation efficiencies (AE) of As from periphyton in *N. triangulifer* and other invertebrate grazers varied among species tested (Fig. 4). *N. triangulifer* had the lowest assimilation efficiency ($22\pm 8.5\%$) followed by *Pleurocera sp.* ($28\pm 10\%$), *Corbicula fluminea* ($57\pm 12.7\%$), *Maccaffertium sp.* ($60\pm 13.2\%$), and *Isonychia sp.* ($75\pm 8.5\%$). *Hydropsyche betteni* did not acquire enough radioactivity from labeled periphyton until 15 hours of exposure, and therefore only had 8 hours on clean food for excretion. Thus our estimate of $71\pm 6\%$ as an AE for this species may be an over-estimate and is not included in Fig. 5.

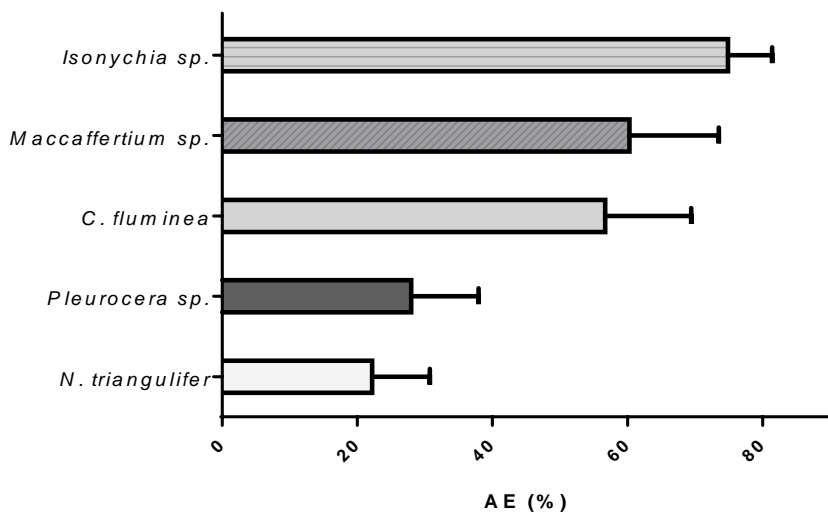


Figure 5. Assimilation efficiencies (AE) from dietary arsenic exposure in periphyton for several species of aquatic invertebrates. Data shown are mean \pm SD; n=6–15.

Periphyton treated with arsenate by static-renewal for 5 days and analyzed using X-ray fluorescence mapping (XRF) revealed that As was not strongly correlated with Si, P, S, K, Ca, Ti, Mn, Cu, or Zn (data not shown). Conversely, As and Fe were largely co-localized across the sample area analyzed (Fig. 6A) and showed a strong correlation ($R=0.92$). This sample also showed evidence of bioreduction of arsenate to arsenite using XANES (Fig. 6B). Arsenic was not adequately measured by XRF in mayflies that had eaten As-enriched periphyton for 10 days, therefore no speciation or elemental associations could be evaluated (data not shown).

A



B

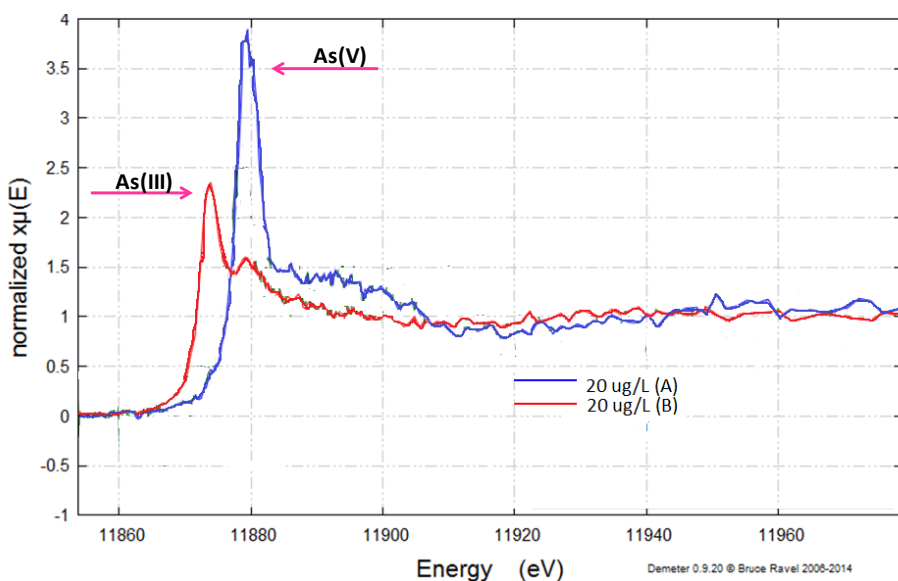


Figure 6. (A) X-ray fluorescence microprobe image showing co-localization of arsenic (red) and iron (blue) in a periphyton sample. The scale bar represents 500 μm , and areas of purple and magenta represent As-Fe associations. Correlation of As and Fe in periphyton $R=0.92$; $n=54,432$ from the image. (B) XANES spectra showing distinct peaks at the arsenate energy and at the arsenite energy.

To explore aqueous As bioaccumulation pathways, we estimated uptake rate constants (K_u) and efflux rate constants (K_e) in several aquatic invertebrates (see SI for taxonomic characterization and raw data). Mean K_u varied across multiple benthic invertebrate species, but were generally low (Fig. 7). *Tallopserla sp.* did not absorb enough measurable As during the experiment to be included. *Ephemerella sp.*, *Maccaffertium sp.*, *N. triangulifer*, and *M. pudicum* all had K_u values of $\sim 0.001 \text{ L g}^{-1} \text{ d}^{-1}$. *C. fluminea*, *Pleurocera sp.*, and *P. immarginata* all had K_u values of $\sim 0.01 \text{ L g}^{-1} \text{ d}^{-1}$. *A. abnormis* and *H. betteni* were $0.02\text{--}0.03 \text{ L g}^{-1} \text{ d}^{-1}$, and *Corydalus sp.*, *Isonychia sp.*, and *P. herricki* had K_u values of $\sim 0.05\text{--}0.06 \text{ L g}^{-1} \text{ d}^{-1}$.

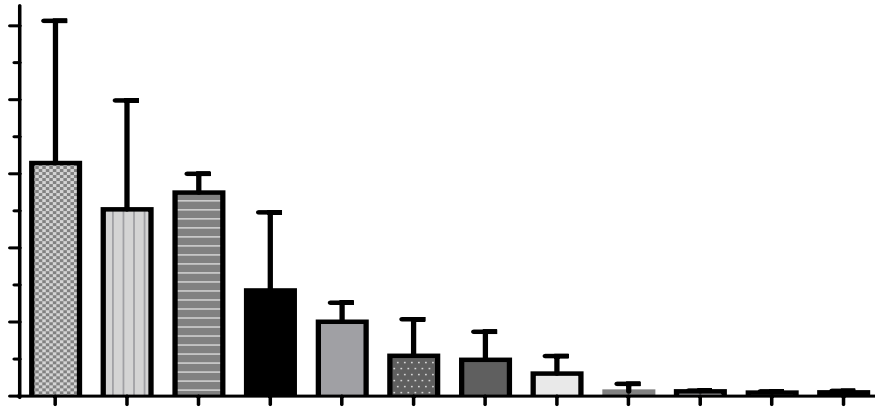


Figure 7. Dissolved arsenic uptake rate constants (K_u) in several species of aquatic invertebrates; $n=5$. Error bars represent the standard errors of the slope for each regression line (uptake measured at 3, 6, and 9 hours).

In contrast to the relatively low uptake rate constants we observed, mean K_e tended to be relatively high (Fig 8). Some of the tested species eliminated As too rapidly (24–48 hours) to be included in this study (*N. triangulifer*, *M. pudicum*). On the slower end, *C. fluminea*, *Pleurocera sp.*, and *P. herricki* had K_e values of $\sim 0.03 \text{ d}^{-1}$. *H. betteni*, *Ephemerella sp.*, and *A. abnormis* had K_e values between 0.06 and 0.09 d^{-1} . The highest K_e values reported were for *Maccaffertium sp.* and *Isonychia sp.*, which were $\sim 0.15 \text{ d}^{-1}$ and 0.29 d^{-1} , respectively.

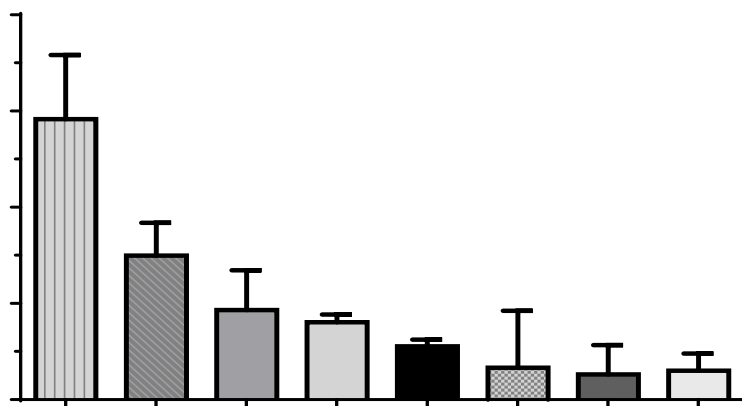


Figure 8. Efflux rate constant (K_e) in several species of aquatic invertebrates after 4-5 days of aqueous exposure; $n=5$. Error bars represent the standard errors of the slope for each regression line (efflux measured daily for up to 10 days).

For the subset of organisms for which both K_e and K_u could be derived, BCF estimates were similar (200–250) for *Isonychia sp.*, *C. fluminea*, *A. abnormis*, and *Pleurocera sp.* The highest estimated BCF was approximately 960 for *P. herricki*, while the lowest estimated BCF was approximately 7 for *Maccaffertium sp.* (data not shown).

4.0 Discussion:

4.1 Periphyton uptake of As across pH

The influence of protonation state on uptake of AsV in primary producers has been characterized in laboratory studies for a few species. Generally there is good agreement between studies that lower pH facilitates greater concentration of AsV into primary producers including algae (Pawlik-Skowrońska et al. 2004; Sibi 2014), and aquatic plants (Tu & Ma 2003). There is some variability however; Favas et al. (2012) reported that of several aquatic plant species investigated, only two had highly significant negative correlations between tissue arsenic and pH while one species had a significant positive correlation. Chen et al. (Chen et al. 2014) similarly report a positive correlation between pH and total arsenic accumulation in an aquatic plant exposed to AsV, which was proposed to be due to the fact that phosphate transporters, which are tricked into transporting As due to structural similarities (Zhao et al. 2009), have a higher affinity for the more electronegative AsO_4^{3-} species than for the more protonated forms dominating

lower pHs. In this study, pH had an inverse relationship on As uptake in periphyton, which could result in higher or lower As bioconcentration by periphyton under different site specific environmental conditions. Our results suggest that AsV uptake in periphyton as a function of pH is more similar to what is reported for other aquatic algae species.

4.2 Periphyton loading and enrichment:

Arsenic concentrations in lotic ecosystems vary widely ($\leq 2 \mu\text{g L}^{-1}$ in reference streams to $>300 \mu\text{g L}^{-1}$ in contaminated streams)(Smedley and Kinniburgh 2002). Thus the range of dissolved As concentrations used in our experiments ($1\text{--}20 \mu\text{g L}^{-1}$) represent environmentally common exposures. After a single pulse of such concentrations, periphyton rapidly removed and concentrated As from solution, suggesting that periphyton communities are an important sink for arsenate. For example, in this experiment periphyton exposed to $20 \mu\text{g L}^{-1}$ As for 8–10 days accumulated approximately $30\text{--}60 \text{ mg kg}^{-1}$ As on a dry weight basis. This observation is supported by several field studies showing accumulation of As by periphyton (e.g.,(Ramelow et al. 1987; Drndarski et al. 1993; Koch et al. 1999)). When measured water samples from differentially impacted streams averaged $\sim 20\text{--}40 \mu\text{g L}^{-1}$ As, periphyton was found to have As concentrations ranging from $0.6\text{--}50 \text{ mg kg}^{-1}$ on a dry weight basis.(Ramelow et al. 1987) When industrially impacted water concentrations were approximately $32 \mu\text{g L}^{-1}$ As, periphyton were reported to range from 46 to 57 mg kg^{-1} dry weight.(Drndarski et al. 1993) Similarly, naturally elevated water concentrations ranging from approximately $250\text{--}300 \mu\text{g L}^{-1}$ resulted in microbial mats with $82\text{--}290 \text{ mg kg}^{-1}$ As on a dry weight basis.(Koch et al. 1999) Thus the measured As in periphyton reported here is approximately representative of sites with mild to moderate contamination from field studies, though variations in environmental conditions limit broader generalizations. Similar findings of As accumulation by other primary producers are also reported from laboratory investigations (e.g.,(N.-X. Wang et al. 2013; Sibi 2014; Islam et al. 2015)). For example, after exposure to $1000 \mu\text{g L}^{-1}$ As, the aquatic plant *Micranthemum umbrosum* was found to accumulate 1219 mg kg^{-1} dry weight As,(Islam et al. 2015) and different species of microalgae accumulated $3,000\text{--}17,000 \text{ mg kg}^{-1}$ As on a dry weight basis when exposed to a range of concentrations from $10,000\text{--}50,000 \mu\text{g L}^{-1}$ As;(Sibi 2014) however, these exposure concentrations are representative of only the most extreme As-impacted waters.(Smedley and Kinniburgh 2002) To our knowledge this is the first study to characterize accumulation of As by ecologically realistic periphyton assemblages at environmentally relevant exposure concentrations in a laboratory setting.

We have conducted several iterations of time-course investigations that have shown uptake into periphyton is non-monotonic. This is most apparent from the first trial of periphyton loading at the highest initial concentration of $20 \mu\text{g L}^{-1}$, but is also demonstrated by the 5 and $10 \mu\text{g L}^{-1}$ treatments in this study as well as in two smaller scale pilot studies (data not shown). In all instances periphyton generally increase in As concentration over time, but appear to have a notable decrease in As content between the third and fifth days of exposure before continuing to increase. Similarly, we observed a slight reduction in As periphyton concentration on days 4-10 in trial 2. One possible explanation is that certain species within the periphyton may be detoxifying and excreting As,(Wang et al. 2015) or that surficial cells accumulate As, die and slough off the periphyton surface.

In laboratory studies, BCFs have been reported as 527–4,000 in macrophyte shoots and roots,(Xue and Yan 2011) and 220–360 for several submerged plant species.(Chen et al. 2015) In the field, As bioconcentration by primary producers is highly variable (BCF of 152 reported for bryophytes in a contaminated tributary,(Culioli et al. 2009) and a range of values from 107 to 52,000 across several species of aquatic plants)(Favas et al. 2012). We could not find any laboratory studies of periphyton to compare our results to. While our periphyton values are not BCFs (steady state not reached in trial 1 for example) they appear to be on the higher end of values reported from the field, which vary from 30–1,250 (along a pollution gradient),(Ramelow et al. 1987) 300–1,062.(Koch et al. 1999), and 1,438–1,781.(Drndarski et al. 1993) One possible explanation for this discrepancy is that the periphyton we worked with did not come from a contaminated setting and may contain highly bioaccumulative taxa that may be extirpated from more highly contaminated settings. It is difficult, however to directly compare BCF results from both field data and laboratory studies since As accumulation can vary with different environmental factors,(Sibi 2014) seasonality,(Ramelow et al. 1987) exposure concentrations,(Sibi 2014) and exposure durations(Chen et al. 2015) in addition to the broad variability observed between species.(Sibi 2014; Chen et al. 2015) Differences in the thickness or density(Rosemond 1994; Alam et al. 1997; Kanavillil and Kurissery 2013) of periphyton growth may also play a significant role in differential accumulation and may have contributed to the differences observed in our two trials.

4.3 As content in *N. triangulifer* larvae and adults

N. triangulifer has been a useful laboratory species to study trace element bioaccumulation for zinc,(Kim et al. 2012) cadmium,(Xie et al. 2010) and selenium.(Conley et al. 2009) Here, after being reared for a full lifecycle with both As-enriched diet (and residual aqueous exposure), *N. triangulifer* larvae were found to have minimal measureable As, and As was not detectable in adults. Larvae of this species had very low uptake from solution and after several trials, we were unable to quantify efflux due to rapid elimination (24-48 hours) of any radiolabel obtained (data not shown). In the current experiment larvae were rinsed in concentration-matched solution to reduce the contribution of externally adsorbed radiotracer, however it is possible that not all superficially adsorbed As was removed in this process. In addition, gut contents of larvae were not purged prior to analysis. Thus, As adsorption to the exoskeleton(Hare et al. 1991; Cain et al. 1992; Mason et al. 2000; Lavilla et al. 2010) and As associated with gut contents(Smith et al. 2015 Sep 21) in the larvae could have contributed greatly to measured radioactivity in larvae.

Intraspecific variation in As content across different life stages appears common for insects. For example, 72% of As was found to be eliminated between the fourth instar and adult stages in *C. riparius*,(Mogren et al. 2012) which was proposed to be accomplished through the meconium.(Mogren et al. 2013) Similar findings are reported for a terrestrial moth *Agrotis infusa*,(Andrahennadi and Pickering 2008) and aquatic mayfly *Ephoron virgo*,(Cid et al. 2010) however a specific removal mechanism was not proposed in either case. These observations are in good agreement with our data where virtually no measureable As was detected in emerged *N. triangulifer* adults, although it is important to note that assayed larvae were not purged overnight and therefore the small amount of radioactivity detected could also be contributed solely from gut content. Assimilation efficiency and bioavailability

4.4 Assimilation efficiency:

While several studies emphasize dietary exposure as the driver of As accumulation,(Maeda et al. 1990; Suhendrayatna and Maeda 2001; Williams et al. 2010) there appears to be a great deal of variation in AE among and between aquatic invertebrate taxa. While our results indicate a broad range from 22–75%, these values are on the upper end of what is reported in the literature. For example, AEs for primary consumers are reported as 7.8% in *Arenicola marina*,(Casado-Martinez et al. 2010) 11% in *Leptocheirus plumulosus*,(Williams et al. 2010) 25.3% in *Scrobicularia plana*,(Kalman et al. 2014) 29% in *Nereis diversicolor*,(Rainbow 2011) and 72% in *Alitta succinea*.(Baumann et al. 2012) For secondary consumers, AEs are similarly low, for example 9.4% in killifish fed amphipods,(Dutton and Fisher 2011) and 46–61% in two species of *Hydropsyche*.(Awhrahman et al. 2015) Our AE estimates may be biased high because we did not use stable As in the preparation of labeled periphyton for these experiments. Taken together, these results are in agreement with our findings of generally low AE for As in benthic invertebrates as well as the observed inter-and intra-species variability in assimilation.

4.5 Elemental associations and speciation

Arsenate is known to have strong associations with iron oxides in soils (e.g.,(Maji et al. 2007; Miretzky and Cirelli 2010 Jan 28)) as well as in aquatic environments (e.g.,(Meng et al. 2002)). This association has been leveraged in treatment of As-contaminated water as a removal mechanism (e.g.,(Driehaus et al. 1998; Guan et al. 2008)). Iron (Fe) is an essential trace element for primary producers that can be involved in photosynthesis, chlorophyll biosynthesis, and respiratory electron transport.(Street and Paytan 2005; Raven et al.) In some cases Fe may be a limiting factor much like nitrogen or phosphorus, and Fe limitation has been linked to decreased primary production.(Vrede and Tranvik 2006) Although Fe is typically associated with small colloids or organic ligands in freshwater, it can be taken up directly by plant cells if it is in dissolved form or it can be solubilized from particles and colloids.(Street and Paytan 2005) Excess Fe can also form plaques externally (e.g.,(Robinson et al. 2006a; Rahman et al. 2008; Taggart et al. 2009a)). Together these observations indicate that As uptake by plants is complex with direct uptake through phosphate transporters(Oremland and Stolz 2003; Robinson et al. 2006b; Zhao et al. 2009; Rahman and Hasegawa 2011) (shown to be positively correlated to Fe uptake),(Rahman et al. 2008) indirect uptake through solubilization of Fe colloids and therefore any As associated with those colloids,(Street and Paytan 2005) or through external sorption of As on Fe plaques.(Rahman et al. 2008; Letovsky et al. 2011)

Several studies have noted As associations with Fe in aquatic plants (e.g.,(Zhao et al. 2009; Taggart et al. 2009b; Xing 2011)), terrestrial plants (e.g.,(Zhao et al. 2009)), and fungi,(González-Chávez et al. 2014) however no studies were identified that investigated co-localization of As and Fe in periphyton. Here we observed a strong correlation between arsenate and Fe distributions in As enriched periphyton using XRF. The implications of As-Fe associations for trophic transfer are not fully understood. There are conflicting views on the bioavailability of metals associated with Fe oxides. Newman and McIntosh(Newman and McIntosh 1989) suggest that Fe association reduces bioavailability, which is supported by data reported by Baumann et al.(Baumann et al. 2012) where the highest AE for As was reported from radiolabeled pure diatoms (72%) while hardly any As associated with Fe oxide was assimilated (2%). Conversely, others show evidence that Fe content of sediments(Sharma and Sohn 2009) and biofilms(Farag et al. 2007) drives As accumulation in deposit feeders and benthic grazers, respectively. In fact, Farag et al.(Farag et al. 2007) suggest this association is a

critical link in trophic transfer. More work should be done to characterize the role of As-Fe associations in dictating arsenate bioavailability from freshwater periphyton to benthic grazers.

Laboratory studies have demonstrated that a variety of aquatic microalgae and bacteria species are capable of oxidizing AsIII to AsV (e.g., (Levy *et al.* 2005; Qin *et al.* 2009; Zhang B, Wang LH 2011)), reducing AsV to AsIII (e.g., (Hasegawa *et al.* 2001; Hellweger *et al.* 2003)), biomethylating As (e.g., (Hasegawa *et al.* 2001; Ye *et al.* 2012)), or synthesizing complex arsenosugars or arsenolipids (e.g., (Murray *et al.* 2003; Levy *et al.* 2005; Xue *et al.* 2014)). While there is general consensus on the biotransformation capabilities of primary producers, there is a great deal of variability in what is reported as the dominant arsenic species in tissues compared to which As species primary producers were exposed to in solution. For example, there is some evidence that when exposed to AsV or AsIII solutions the predominant arsenic species in plant tissues is AsIII in submerged macrophytes (Xue *et al.* 2012) and duckweed (Zhang *et al.* 2009). Others have reported that AsV is the dominant species in tissues after exposure to either AsV or AsIII in submerged macrophytes (Zheng *et al.* 2003), cyanobacteria (Wang *et al.* 2013) and blue-green algae (Yin *et al.* 2012). Interestingly, (Wang *et al.* 2013) reported that cyanobacteria accumulated more AsV from AsIII treatment than from AsV treatment. In light of this conflicting evidence, our results of distinct AsIII regions in the AsV-treated periphyton from the first experiment is not surprising, but does not fully answer the question of which arsenic species would dominate in natural conditions.

The results presented here along with those reported by others support the idea that the most significant step in As accumulation occurs from water to primary producers with a much smaller step, or even biodilution occurring from primary producers to invertebrate grazers (Fig. 9). Most accumulation of As therefore occurs at the base of the aquatic food web and then is biodiminished through subsequent trophic transfer to primary and secondary consumers, as supported by laboratory (e.g.,(Maeda *et al.* 1990; Cheng *et al.* 2013)) and field studies (e.g.,(Chen *et al.* 2000; Chen and Folt 2000; Farag *et al.* 2007; Culioli *et al.* 2009; Dovick *et al.* 2016)).

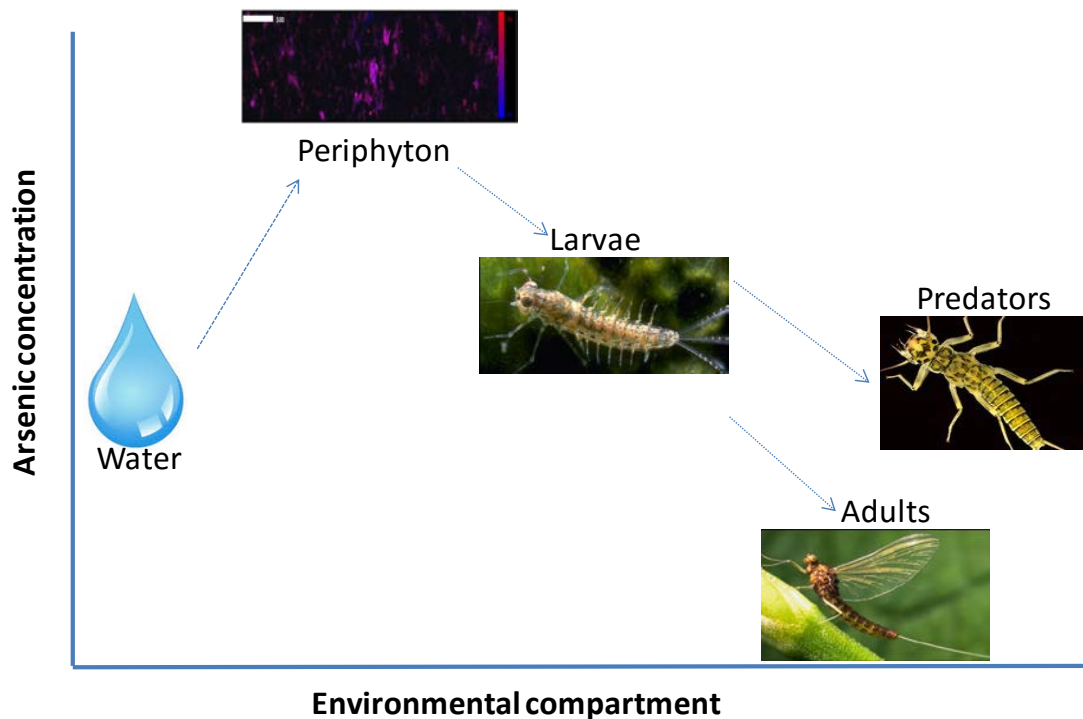


Figure 9. Food web conceptual model of arsenic bioaccumulation dynamics in riverine ecosystems

4.6 Dissolved uptake/efflux

Our results show a great deal of variation in uptake from solution (K_u) between species (ranging from 0.001 ± 0.003 to $0.063 \pm 0.04 \text{ L g}^{-1} \text{ d}^{-1}$; see Appendix 3 for full taxonomic information and raw data). Efflux of As acquired from solution (K_e) was also variable (ranging from 0.03 ± 0.03 to $0.15 \pm 0.03 \text{ d}^{-1}$). In addition, several species tested lost measureable As too quickly (24–48 hours) to be included in analysis (*N. trianfulifer*, *M. pudicum*, data not shown). Our findings of relatively low uptake from solution and relatively fast efflux rates are supported by other studies in the literature. For example, Williams et al. (Williams et al., 2010) reported K_u and K_e constants for estuarine amphipods of $0.028 \text{ L g}^{-1} \text{ d}^{-1}$ and 0.091 d^{-1} , respectively. K_u and K_e values were reported as $0.057 \text{ L g}^{-1} \text{ d}^{-1}$ and 0.049 d^{-1} respectively for *Nereis diversicolor* (PS Rainbow, 2011) and $0.165 \text{ L g}^{-1} \text{ d}^{-1}$ and 0.045 d^{-1} , respectively for *Arenicola marina*. (Casado-Martinez et al., 2010) Higher K_u values were reported for two different *Hydropsyche sp.* (0.350 ± 0.049 and $0.435 \pm 0.054 \text{ L g}^{-1} \text{ d}^{-1}$), though K_e were comparable to what is reported here for *H. betteni*. (mean 0.0731 and 0.0532 d^{-1}). (Awraahman et al., 2015) Interestingly, estuarine bivalves were reported to have rapid K_u of $0.807 \pm 0.129 \text{ L g}^{-1} \text{ d}^{-1}$, but the efflux rate for As was the highest reported ($0.06 \pm 0.001 \text{ d}^{-1}$) among the trace metals investigated (As, Ag, Zn). (Kalman et al., 2014) In addition, some species tested in our experiment did not acquire enough measureable As after 2–5 days to quantitate uptake (*Peltoperla sp.*, *Chironomus dilutus*, data not shown). This is in agreement with other laboratory observations where some organisms did not acquire As from solution (e.g., (Canivet et al., 2001; Dutton & Fisher, 2011)). These results from the literature are generally consistent with the degree of interspecies variability reported here,

pointing to the need for better characterization of As flux rates in benthic invertebrates, particularly for species or taxa that are commonly used for biomonitoring.

Rough estimates of bioconcentration factors (BCFs) can be obtained by dividing the average K_u by the average K_e for a given species. In the small subset of organisms for which both K_e and K_u could be derived in this study, BCFs were generally consistent across different taxa (~250) with the exception of *Maccaffertium sp.* which was much lower (~7). Several other studies also support minimal accumulation from solution. For example Spehar et al. (Spehar et al., 1980) found that fish and amphipods exposed to As for 28 days had the same tissue concentration as controls, and stoneflies and snails had tissue concentrations that resulted in generally low BCFs ranging from 16—131. EPA (U.S. EPA, 2003) reports BCFs from the literature ranging from 0.048 in the common carp to 14 in stoneflies. Culioli et al. (Culioli et al., 2009) derived BAFs for different trophic linkages for field collected biota starting from 0.713 for primary producers to primary consumers and decreasing with each trophic level to 0.005 for invertebrates to trout. Similarly, data reported by Canivet et al. (Canivet et al., 2001) can be used to estimate 10 day exposure concentration factors for several aquatic invertebrates ranging from 1.2 in larval mayflies to 1094 in larval caddisflies, though half of the species tested were on the order of 200–300-fold above the average concentration in solution. Generally BCFs reported for benthic invertebrates are lower than those reported for primary producers and estimated here for periphyton. While there is a great deal of variability across benthic invertebrate species for K_u and K_e , and generally modest BCFs, there is still uncertainty and conflicting evidence (e.g., (Kalman et al., 2014)) regarding the importance of aqueous exposure in As accumulation.

5.0 Summary:

Aquatic invertebrates have been widely used for assessing and monitoring environmental disturbances, (Hodkinson and Jackson 2005) particularly contamination of aquatic ecosystems with trace metals and metalloids. (Hare et al. 1991; Cain et al. 1992; Rainbow 2002) In many cases, only a single species or a handful of species are used for assessment. Our data along with other research shows there is a great deal of variability in flux rates and assimilation efficiency not only between species, but among closely related taxa or species with similar feeding strategies, making it particularly important to identify which species may be best suited for As monitoring. Our data has also identified periphyton, which has been proposed for biomonitoring efforts (e.g., (Ramelow et al. 1987; Rhea et al. 2006)), as an important sink for arsenate. Unlike for other trace elements that are trophically available from natural periphytic biofilms, As bioremediation by periphyton may be a viable strategy since there is only modest apparent trophic transfer and evidence in the literature of biodiminution. (Spehar et al. 1980; Maeda et al. 1990; Chen and Folt 2000; Mason et al. 2000; Dutton and Fisher 2011; Rahman et al. 2012) The results presented here provide data for the accumulation dynamics of As in periphyton and invertebrate grazers, which is critical to understanding the behavior of As at the base of aquatic food webs and potential impacts at higher trophic levels.

Recommendations:

We suggest that field biomonitoring studies should carefully consider interspecific differences in arsenic accumulation dynamics when selecting monitoring species. Further, site-specific environmental variables should be consistently measured and reported, including concentrations

of other important minerals (i.e., Fe) and pH that influence As mobility and bioavailability. Consistent reporting of these variables and continued efforts to characterize accumulation dynamics in a broader range of benthic invertebrates will continue to shed more light on interspecies variability, potential contributions to body burden from food and water, and other environmental factors that have not yet been investigated thoroughly. This knowledge is critical to interpreting existing biomonitoring data, as well as understanding the behavior of As at the base of aquatic food webs and potential impacts at higher trophic levels.

References:

- Akter KFK, Owens G, Davey DDE, Naidu R. 2005. Arsenic Speciation and Toxicity in Biological Systems. In: Ware GW, Albert LA, Crosby DG, de Voogt P, Hutzinger O, Knaak JB, Mayer FL, Morgan DP, Park DL, Tjeerdema RS, et al., editors. *Reviews of Environmental Contamination and Toxicology*. Vol. 184. New York, NY, United States: Springer New York. (Reviews of Environmental Contamination and Toxicology). p. 97–149. [accessed 2016 Feb 10]. <http://link.springer.com/10.1007/0-387-27565-7>
- Alam MA, Khan AA, Alam A, Gaur RK. 1997. Seasonal variation in periphyton density in a tropical pond receiving effluents from medical college complex. *J. Ecotoxicol. Environ. Monit.* 7:135–138.
- Ancion P-Y, Lear G, Lewis GD. 2010. Three common metal contaminants of urban runoff (Zn, Cu & Pb) accumulate in freshwater biofilm and modify embedded bacterial communities. *Environ. Pollut.* 158:2738–45. [accessed 2016 Feb 3]. <http://www.sciencedirect.com/science/article/pii/S026974911000165X>
- Andrahennadi R, Pickering IJ. 2008. Arsenic accumulation, biotransformation and localisation in bertha armyworm moths. *Environ. Chem.* 5:413–419.
- Awrahaman ZA, Rainbow PS, Smith BD, Khan FR, Bury NR, Fialkowski W. 2015. Bioaccumulation of arsenic and silver by the caddisfly larvae *Hydropsyche siltalai* and *H. pellucidula*: A biodynamic modeling approach. *Aquat. Toxicol.* 161:196–207. [accessed 2015 Feb 23]. <http://www.sciencedirect.com/science/article/pii/S0166445X15000053>
- Baumann Z, Koller A, Fisher NS. 2012. Factors influencing the assimilation of arsenic in a deposit-feeding polychaete. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 156:42–50. [accessed 2016 Mar 2]. <http://www.sciencedirect.com/science/article/pii/S1532045612000403>
- Bradac P, Behra R, Sigg L. 2009. Accumulation of cadmium in periphyton under various freshwater speciation conditions. *Environ. Sci. Technol.* 43:7291–7296.
- Cain D, Croteau M-N, Luoma S. 2011. Bioaccumulation dynamics and exposure routes of Cd and Cu among species of aquatic mayflies. *Environ. Toxicol. Chem.* 30:2532–41. [accessed 2016 Feb 3]. <http://www.ncbi.nlm.nih.gov/pubmed/21898563>
- Cain D, Luoma SN, Carter JL, Fend S V. 1992. Aquatic insects as bioindicators of trace element contamination in cobble-bottom rivers and streams. *Can. J. Fish. Aquat. Sci.* 49:2141–2154.
- Casado-Martinez MC, Smith BD, Luoma SN, Rainbow PS. 2010. Bioaccumulation of arsenic from water and sediment by a deposit-feeding polychaete (*Arenicola marina*): A biodynamic modelling approach. *Aquat. Toxicol.* 98:34–43. [accessed 2016 Jan 6]. <http://www.sciencedirect.com/science/article/pii/S0166445X10000275>
- Chen CY, Folt CL. 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. *Environ. Sci. Technol.* 34:3878–3884. [accessed 2015 Dec 3]. <http://dx.doi.org/10.1021/es991070c>

- Chen CY, Stemberger RS, Klaue B, Blum JD, Pickhardt PC, Folt CL. 2000. Accumulation of heavy metals in food web components across a gradient of lakes. *Limnol. Oceanogr.* 45:1525–1536. [accessed 2015 Dec 8]. <http://doi.wiley.com/10.4319/lo.2000.45.7.1525>
- Chen G, Liu X, Brookes P, Xu J. 2015. Opportunities for phytoremediation and bioindication of arsenic contaminated water using a submerged aquatic plant: *Vallisneria spiralis*. *Int. J. Phytoremediation* 17:249–255. [accessed 2015 Jan 6]. <http://tripsaver.lib.ncsu.edu/pdf/761321.pdf>
- Cheng Z, Chen K-C, Li K-B, Nie X-P, Wu SC, Wong CK-C, Wong M-H. 2013. Arsenic contamination in the freshwater fish ponds of Pearl River Delta: Bioaccumulation and health risk assessment. *Environ. Sci. Pollut. Res. Int.* 20:4484–95. [accessed 2014 Sep 3]. <http://www.ncbi.nlm.nih.gov/pubmed/23247527>
- Cid N, Ibáñez C, Palanques A, Prat N. 2010. Patterns of metal bioaccumulation in two filter-feeding macroinvertebrates: exposure distribution, inter-species differences and variability across developmental stages. *Sci. Total Environ.* 408:2795–806. [accessed 2016 Feb 11]. <http://www.sciencedirect.com/science/article/pii/S0048969710003013>
- Conley JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. *Environ. Sci. Technol.* 43:7952–7957.
- Conley JM, Funk DH, Hesterberg DH, Hsu L-C, Kan J, Liu Y-T, Buchwalter DB. 2013. Bioconcentration and biotransformation of selenite versus selenate exposed periphyton and subsequent toxicity to the mayfly *Centroptilum triangulifer*. *Environ. Sci. Technol.* 47:7965–7973. [accessed 2015 Oct 9]. <http://pubs.acs.org.prox.lib.ncsu.edu/doi/abs/10.1021/es400643x>
- Culioli J-L, Fouquoire A, Calendini S, Mori C, Orsini A. 2009. Trophic transfer of arsenic and antimony in a freshwater ecosystem: A field study. *Aquat. Toxicol.* 94:286–93. [accessed 2015 Nov 26]. <http://www.sciencedirect.com/science/article/pii/S0166445X09002525>
- Dovick MA, Kulp TR, Arkle RS, Pilliod DS. 2016. Bioaccumulation trends of arsenic and antimony in a freshwater ecosystem affected by mine drainage. *Environ. Chem.* 13:149–159.
- Driehaus W, Jekel M, Hildebrandt U. 1998. Granular ferric hydroxide — A new adsorbent for the removal of arsenic from natural water. *J. Water Supply Res. Technol. - Aqua* 47:30–35. [accessed 2016 Feb 29]. <http://aqua.iwaponline.com/content/47/1/30.abstract>
- Drndarski N, Stojic D, Markov Z. 1993. Stable isotopes in periphyton and sediments from the Kolubara River and its tributaries. *Environ. Pollut.* 80:287–92.
- Dutton J, Fisher NS. 2011. Bioaccumulation of As, Cd, Cr, Hg(II), and MeHg in killifish (*Fundulus heteroclitus*) from amphipod and worm prey. *Sci. Total Environ.* 409:3438–47. [accessed 2015 Nov 26]. <http://www.sciencedirect.com/science/article/pii/S0048969711005250>
- Farag AM, Nimick DA, Kimball BA, Church SE, Harper DD, Brumbaugh WG. 2007. Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish in the

- Boulder River watershed, Montana, and the role of colloids in metal uptake. *Arch. Environ. Contam. Toxicol.* 52:397–409. [accessed 2015 Oct 19].
<http://www.ncbi.nlm.nih.gov/pubmed/17219028>
- Farag AM, Woodward DF, Goldstein JN, Brumbaugh W, Meyer JS. 1998. Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. *Arch. Environ. Contam. Toxicol.* 34:119–127. [accessed 2015 Dec 8]. <http://link.springer.com/10.1007/s002449900295>
- Favas PJC, Pratas J, Prasad MN V. 2012. Accumulation of arsenic by aquatic plants in large-scale field conditions: opportunities for phytoremediation and bioindication. *Sci. Total Environ.* 433:390–7. [accessed 2015 Dec 8].
<http://www.sciencedirect.com/science/article/pii/S0048969712009242>
- González-Chávez M del CA, Miller B, Maldonado-Mendoza IE, Scheckel K, Carrillo-González R. 2014. Localization and speciation of arsenic in *Glomus* intraradices by synchrotron radiation spectroscopic analysis. *Fungal Biol.* 118:444–52. [accessed 2016 Mar 1].
<http://www.sciencedirect.com/science/article/pii/S1878614614000439>
- Guan X-H, Wang J, Chusuei CC. 2008. Removal of arsenic from water using granular ferric hydroxide: Macroscopic and microscopic studies. *J. Hazard. Mater.* 156:178–85. [accessed 2016 Feb 29]. <http://www.sciencedirect.com/science/article/pii/S0304389407017578>
- Hare L, Tessier A, Campbell PGC. 1991. Trace Element Distributions in Aquatic Insects: Variations among Genera, Elements, and Lakes. *Can. J. Fish. Aquat. Sci.* 48:1481–1491. [accessed 2015 Jun 8]. <http://www.nrcresearchpress.com/doi/abs/10.1139/f91-176#.VXXW7fm6daR>
- Hodkinson ID, Jackson JK. 2005. Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. *Environ. Manage.* 35:649–666.
- Islam MS, Saito T, Kurasaki M. 2015. Phytofiltration of arsenic and cadmium by using an aquatic plant, *Micranthemum umbrosum*: Phytotoxicity, uptake kinetics, and mechanism. *Ecotoxicol. Environ. Saf.* 112:193–200. [accessed 2015 Jan 5].
<http://www.sciencedirect.com/science/article/pii/S0147651314005119>
- Jasrotia S, Kansal A, Kishore VVN. 2014. Arsenic phyco-remediation by *Cladophora* algae and measurement of arsenic speciation and location of active absorption site using electron microscopy. *Microchem. J.* 114:197–202. [accessed 2015 Jan 5].
<http://www.sciencedirect.com/science/article/pii/S0026265X1400006X>
- Kalman J, Smith BD, Bury NR, Rainbow PS. 2014. Biodynamic modelling of the bioaccumulation of trace metals (Ag, As and Zn) by an infaunal estuarine invertebrate, the clam *Scrobicularia plana*. *Aquat. Toxicol.* 154:121–30. [accessed 2015 Dec 15].
<http://www.sciencedirect.com/science/article/pii/S0166445X14001702>

- Kanavillil N, Kurissery S. 2013. Temporal variation of periphyton communities: a 3-year study from northwest Lake Simcoe, Ontario, Canada. *Inl. Waters* 3:473–486. [accessed 2016 Jan 27]. <https://www.fba.org.uk/journals/index.php/IW/article/view/525>
- Kim KS, Funk DH, Buchwalter DB. 2012. Dietary (periphyton) and aqueous Zn bioaccumulation dynamics in the mayfly *Centropilum triangulifer*. *Ecotoxicology* 21:2288–2296.
- Koch I, Feldmann J, Wang L, Andrewes P, Reimer KJ, Cullen WR. 1999. Arsenic in the Meager Creek hot springs environment, British Columbia, Canada. *Sci. Total Environ.* 236:101–117. [accessed 2015 Dec 2]. <http://www.sciencedirect.com/science/article/pii/S0048969799002739>
- Kulp TR, Hoeft SE, Oremland RS. 2004. Redox transformations of arsenic oxyanions in periphyton communities. *Appl. Environ. Microbiol.* 70:6428–34.
- Lavilla I, Rodríguez-Liñares G, Garrido J, Bendicho C. 2010. A biogeochemical approach to understanding the accumulation patterns of trace elements in three species of dragonfly larvae: evaluation as biomonitors. *J. Environ. Monit.* 12:724–30. [accessed 2015 Aug 31]. <http://pubs.rsc.org/en/content/articlehtml/2010/em/b920379f>
- Letovsky E, Heal K V., Carvalho L, Spears BM. 2011. Intracellular versus extracellular iron accumulation in freshwater periphytic mats across a mine water treatment lagoon. *Water, Air, Soil Pollut.* 223:1519–1530. [accessed 2015 Nov 24]. <http://link.springer.com/10.1007/s11270-011-0961-z>
- Levy JL, Stauber JL, Adams MS, Maher WA, Kirby JK, Jolley DF. 2005. Toxicity, biotransformation, and mode of action of arsenic in two freshwater microalgae (*Chlorella* sp. and *Monoraphidium arcuatum*). *Environmental Toxicol. Chem.* [accessed 2015 Mar 22]. <http://search.proquest.com.prox.lib.ncsu.edu/docview/210345655?pq-origsite=summon>
- Luoma SN, Rainbow PS. 2005. Why is metal bioaccumulation so variable? *Biodynamics as a unifying concept.* *Environ. Sci. Technol.* 39:1921–1931.
- Maeda S, Inoue R, Kozono T, Tokuda T, Ohki A, Takeshita T. 1990. Arsenic metabolism in a freshwater food chain. *Chemosphere* 20:101–108. [accessed 2015 Mar 22]. <http://www.sciencedirect.com/science/article/pii/004565359090090G>
- Maji SK, Pal A, Pal T, Adak A. 2007. Sorption kinetics of arsenic on laterite soil in aqueous medium. *J. Environ. Sci. Heal. Part A* 42:989–996.
- Mason RP, Laporte J, Andres S. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Arch. Environ. Contam. Toxicol.* 38:283–97. [accessed 2015 Oct 16]. <http://www.ncbi.nlm.nih.gov/pubmed/10667925>
- Meng X, Jing C, Korfiatis G. 2002. Chapter 6 - A review of redox transformation of arsenic in aquatic environments. In: Cai Y, Braids OC, editors. *Biogeochemistry of Environmentally Important Trace Elements*. Vol. 835. Washington, DC: American Chemical Society. (ACS

Symposium Series). p. 70–83. [accessed 2016 Feb 26]. <http://dx.doi.org/10.1021/bk-2003-0835.ch006>

Miretzky P, Cirelli AF. 2010 Jan 28. Remediation of Arsenic-Contaminated Soils by Iron Amendments: A Review. *Crit. Rev. Environ. Sci. Technol.* [accessed 2016 Feb 10]. <http://www.tandfonline.com/doi/abs/10.1080/10643380802202059>

Mogren CL, von Kiparski GR, Parker DR, Trumble JT. 2012. Survival, reproduction, and arsenic body burdens in *Chironomus riparius* exposed to arsenate and phosphate. *Sci. Total Environ.* 425:60–5. [accessed 2014 Aug 30]. <http://www.sciencedirect.com/science/article/pii/S0048969712003415>

Mogren CL, Webb SM, Walton WE, Trumble JT. 2013. Micro x-ray absorption spectroscopic analysis of arsenic localization and biotransformation in *Chironomus riparius* Meigen (Diptera: Chironomidae) and *Culex tarsalis* Coquillett (Culicidae). *Environ. Pollut.* 180:78–83. [accessed 2015 Mar 9]. <http://www.ncbi.nlm.nih.gov/pubmed/23733012>

Newman MC, McIntosh AW. 1989. Appropriateness of aufwuchs as a monitor of bioaccumulation. *Environ. Pollut.* 60:83–100. [accessed 2016 Feb 11]. <http://www.sciencedirect.com/science/article/pii/0269749189902224>

Oremland RS, Stolz JF. 2003. The ecology of arsenic. *Science* (80-.). 300:939–944.

Patrick R. 1978. Effects of trace metals in the aquatic ecosystem: The diatom community, base of the aquatic food chain, undergoes significant changes in the presence of trace metals and other alterations in water chemistry. *Am. Sci.* 66:185–191.

Rahman MA, Hasegawa H. 2011. Aquatic arsenic: Phytoremediation using floating macrophytes. *Chemosphere* 83:633–46. [accessed 2016 Feb 11]. <http://www.sciencedirect.com/science/article/pii/S0045653511001913>

Rahman MA, Hasegawa H, Lim RP. 2012. Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. *Environ. Res.* 116:118–135.

Rahman MA, Hasegawa H, Ueda K, Maki T, Rahman MM. 2008. Arsenic uptake by aquatic macrophyte *Spirodela polyrhiza* L.: Interactions with phosphate and iron. *J. Hazard. Mater.* 160:356–61. [accessed 2016 Feb 26]. <http://www.sciencedirect.com/science/article/pii/S0304389408003518>

Rainbow P. 2002. Trace metal concentrations in aquatic invertebrates: Why and so what? *Environ. Pollut.* 120:497–507.

Rainbow P. 2011. Biodynamic modelling of the bioaccumulation of arsenic by the polychaete *Nereis diversicolor*. *Environ. Chem.* 8.

Ramelow GJJ, Maples RSS, Thompson RLL, Mueller CSS, Webre C, Beck JNN. 1987. Periphyton as monitors for heavy metal pollution in the Calcasieu River estuary. *Environ. Pollut.* 43:247–61. [accessed 2015 Jun 17]. <http://www.ncbi.nlm.nih.gov/pubmed/15092788>

- Raven JA, Evans MCW, Korb RE. The role of trace metals in photosynthetic electron transport in O₂-evolving organisms. *Photosynth. Res.* 60:111–150. [accessed 2016 Feb 26]. <http://link.springer.com/article/10.1023/A%3A1006282714942>
- Rhea DT, Harper DD, Farag AM, Brumbaugh WG. 2006. Biomonitoring in the Boulder River Watershed, Montana, USA: metal concentrations in biofilm and macroinvertebrates, and relations with macroinvertebrate assemblage. *Environ. Monit. Assess.* 115:381–93. [accessed 2015 Oct 19]. <http://www.ncbi.nlm.nih.gov/pubmed/16648955>
- Robinson B, Kim N, Marchetti M, Moni C, Schroeter L, van den Dijssel C, Milne G, Clothier B. 2006a. Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand. *Environ. Exp. Bot.* 58:206–215. [accessed 2016 Mar 1]. <http://www.sciencedirect.com/science/article/pii/S0098847205001619>
- Robinson B, Kim N, Marchetti M, Moni C, Schroeter L, van den Dijssel C, Milne G, Clothier B. 2006b. Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand. *Environ. Exp. Bot.* 58:206–215.
- Rosemond AD. 1994. Multiple factors limit seasonal variation in periphyton in a forest stream. *J. North Am. Benthol. Soc.* 13:333–344.
- Schaeffer R, Francesconi KA, Kienzl N, Soeroes C, Fodor P, Váradi L, Raml R, Goessler W, Kuehnelt D. 2006. Arsenic speciation in freshwater organisms from the river Danube in Hungary. *Talanta* 69:856–865. [accessed 2015 Dec 2]. <http://www.sciencedirect.com/science/article/pii/S0039914005007575>
- Sharma VK, Sohn M. 2009. Aquatic arsenic: Toxicity, speciation, transformations, and remediation. *Environ. Int.* 35:743–59. [accessed 2014 Oct 30]. <http://www.sciencedirect.com/science/article/pii/S0160412009000051>
- Sibi G. 2014. Biosorption of arsenic by living and dried biomass of fresh water microalgae - potentials and equilibrium studies. *J Bioremed Biodeg* 5. [accessed 2015 Jun 17]. <http://search.proquest.com/docview/1640763198?pq-origsite=summon>
- Smedley P., Kinniburgh D. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochemistry* 17:517–568. [accessed 2014 Jul 11]. <http://www.sciencedirect.com/science/article/pii/S0883292702000185>
- Smith JG, Baker TF, Murphy CA, Jett RT. 2015 Sep 21. Spatial and temporal trends in contaminant concentrations in *Hexagenia* nymphs following a coal ash spill at the Tennessee Valley Authority's Kingston Fossil Plant. *Environ. Toxicol. Chem.* [accessed 2016 Mar 7]. <http://www.ncbi.nlm.nih.gov/pubmed/26387560>
- Spehar RL, Fiandt JT, Anderson RL, DeFoe DL. 1980. Comparative toxicity of arsenic compounds and their accumulation in invertebrates and fish. *Arch. Environ. Contam. Toxicol.* 9:53–63. [accessed 2015 Dec 8]. <http://link.springer.com/10.1007/BF01055499>

- Srivastava S, Sounderajan S, Udas A, Suprasanna P. 2014. Effect of combinations of aquatic plants (*Hydrilla*, *Ceratophyllum*, *Eichhornia*, *Lemna* and *Wolffia*) on arsenic removal in field conditions. *Ecol. Eng.* 73:297–301. [accessed 2015 Jan 5].
<http://www.sciencedirect.com/science/article/pii/S0925857414004406>
- Street JH, Paytan A. 2005. Iron, phytoplankton growth, and the carbon cycle. *Met. Ions Biol. Syst.* 43:153–93. [accessed 2016 Feb 26]. <http://www.ncbi.nlm.nih.gov/pubmed/16370118>
- Suhendrayatna O, Maeda S. 2001. Biotransformation of arsenite in freshwater food-chain models. *Appl. Organomet. Chem.* 15:277–284. [accessed 2015 Mar 22].
<http://doi.wiley.com/10.1002/aoc.139>
- Taggart MA, Mateo R, Charnock JM, Bahrami F, Green AJ, Meharg AA. 2009a. Arsenic rich iron plaque on macrophyte roots--an ecotoxicological risk? *Environ. Pollut.* 157:946–54. [accessed 2016 Mar 1]. <http://www.sciencedirect.com/science/article/pii/S0269749108005526>
- Taggart MA, Mateo R, Charnock JM, Bahrami F, Green AJ, Meharg AA. 2009b. Arsenic rich iron plaque on macrophyte roots--An ecotoxicological risk? *Environ. Pollut.* 157:946–54.
- U.S. EPA. 2014. Priority pollutant list. [accessed 2015 Jan 12].
<http://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf>
- Vrede T, Tranvik JL. 2006. Iron constraints on planktonic primary production in oligotrophic lakes. *Ecosystems* 9:1094–1105.
- Wang N-X, Li Y, Deng X-H, Miao A-J, Ji R, Yang L-Y. 2013. Toxicity and bioaccumulation kinetics of arsenate in two freshwater green algae under different phosphate regimes. *Water Res.* 47:2497–506. [accessed 2015 May 13].
<http://www.sciencedirect.com/science/article/pii/S0043135413001383>
- Wang Y, Wang S, Xu P, Liu C, Liu M, Wang Y, Wang C, Zhang C, Ge Y. 2015. Review of arsenic speciation, toxicity and metabolism in microalgae. *Rev. Environ. Sci. Bio/Technology* 14:427–451. [accessed 2015 Aug 31]. <http://link.springer.com/10.1007/s11157-015-9371-9>
- Wang Y, Zhang C, Wang S, Shen L, Ge Y. 2013. Accumulation and transformation of different arsenic species in nonaxenic *Dunaliella salina*. *Environ. Sci.* 34:4257–4265.
- Wang Z, Luo Z, Yan C. 2013. Accumulation, transformation, and release of inorganic arsenic by the freshwater cyanobacterium *Microcystis aeruginosa*. *Environ. Sci. Pollut. Res. Int.* 20:7286–95. [accessed 2015 Jun 18]. <http://www.ncbi.nlm.nih.gov/pubmed/23636594>
- Webb SM. 2011. The MicroAnalysis Toolkit: X-ray fluorescence image processing software. *AIP Conf. Proc.* 1365.
- Williams JJ, Dutton J, Chen CY, Fisher NS. 2010. Metal (As, Cd, Hg, and CH₃Hg) bioaccumulation from water and food by the benthic amphipod *Leptocheirus plumulosus*. *Environ. Toxicol. Chem.* 29:1755–61. [accessed 2015 Nov 26].

<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3087379&tool=pmcentrez&rendertype=abstract>

Woolson EA. 1975. Arsenical Pesticides. American Chemical Society ACS Symposium Series.

Xie L, Funk DH, Buchwalter DB. 2010. Trophic transfer of Cd from natural periphyton to the grazing mayfly *Centroptilum triangulifer* in a life cycle test. *Environ. Pollut.* 158:272–7. [accessed 2014 Sep 3]. <http://www.ncbi.nlm.nih.gov/pubmed/19647355>

Xing W. 2011. Iron biogeochemistry and its environmental impacts in freshwater lakes. *Fresenius Environ. Bull.* 20:1339.

Xue P, Yan C. 2011. Arsenic accumulation and translocation in the submerged macrophyte *Hydrilla verticillata* (L.f.) Royle. *Chemosphere* 85:1176–81. [accessed 2015 Jun 19]. <http://www.sciencedirect.com/science/article/pii/S0045653511011374>

Yin X-X, Wang LH, Bai R, Huang H, Sun G-X. 2012. Accumulation and transformation of arsenic in the blue-green alga *synechocysis* sp. PCC6803. *Water, Air, Soil Pollut.* 223:1183–1190. [accessed 2015 Jun 18]. <http://link.springer.com/10.1007/s11270-011-0936-0>

Zhao FJ, Ma JF, Meharg AA, McGrath SP. 2009. Arsenic uptake and metabolism in plants. *New Phytol.* 181:777–94. [accessed 2015 May 11]. <http://www.ncbi.nlm.nih.gov/pubmed/19207683>

Appendix 1: abbreviations, symbols

AE	Assimilation efficiency
As	Arsenic
Fe	Iron
Ke	Efflux rate (proportional daily loss)
Ku	Uptake rate constant
XANES	X-ray absorption near edge structure
XRF	X-ray fluorescence

Appendix 2: List of presentations and publications (including thesis)

All presentations and publications resulting from this research are listed below.

Harris, A; Buchwalter, D. Coal fly ash constituents at the base of aquatic food webs: Uptake and efflux of arsenic. Carolina Area Biologists Workgroup. April 1 - 3, 2015. Hot Springs, North Carolina.

Harris, A; Buchwalter, D; Hesterberg, D. Dynamic behavior and speciation of arsenic at the base of aquatic food webs. Society for Environmental Toxicology and Chemistry Annual Meeting. November 1 - 5, 2015. Salt Lake City, Utah

Lopez, A; Buchwalter, D; Hesterberg, D.; Webb, S. Trace elements from coal ash at the base of aquatic food webs: Dynamic behavior or arsenic. WRRRI poster March 17 - 18, 2016. Raleigh North Carolina.

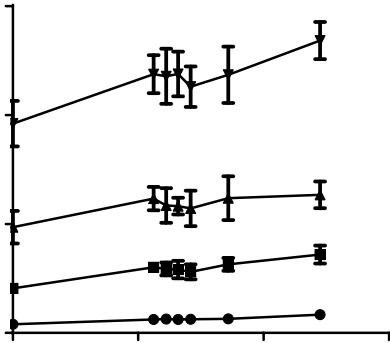
Lopez, A; Buchwalter, D. Periphyton Bioconcentration and Biotransformation of Arsenic: Implications for Trophic Transfer. Carolina Area Biologists Workgroup. April 25 - 27, 2016. Hot Springs, North Carolina.

Lopez, A; Hesterberg, D; Silverman, J; Buchwalter, D. Thesis Title TBD. Graduate Thesis. June 10th, 2016. Raleigh, NC.

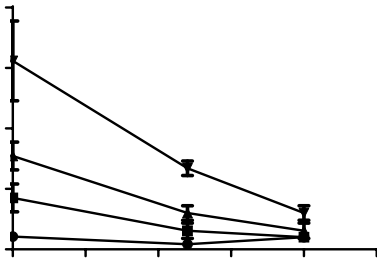
Lopez, A; Buchwalter, D. Arsenic dynamics at the base of aquatic food webs. Society for Freshwater Science, Annual Meeting, May 21-25 2016. Sacramento, CA.

Appendix 3: Supplemental Information

A



B



Supplemental Figure 1. Aqueous (A) and dietary (B) exposure conditions for *N. triangulifer* larvae reared for a full lifecycle. Values plotted are mean \pm SEM; n=3 for each treatment at each time point. Symbols represent

initial nominal dissolved As concentrations of 1, 5, 10, and 20 $\mu\text{g L}^{-1}$ arsenate, respectively.

Supplemental Table 1. Species composition of periphyton plates evaluated June 2009 – December 2009

	Month		
	June–July	October	December
Diatoms¹	<i>Melosira varians</i>	<i>Melosira varians</i>	<i>Melosira varians</i>
	<i>Diatoma vulgare</i>	<i>Cymbella sp.</i>	<i>Gomphonema sp.</i>
	<i>Synedra sp.</i>	<i>Synedra sp.</i>	<i>Nitzschia sp.</i>
	<i>Nitzschia sp.</i>	<i>Nitzschia sp.</i>	<i>Synedra sp.</i>
	<i>Cymbella sp.</i>	<i>Navicula sp.</i>	<i>Fragilaria sp.</i>
	<i>Gomphonema sp.</i>	<i>Achmanthidium sp.</i>	<i>Cymbella sp.</i>
	<i>Fragilaria sp.</i>	<i>Planothidium sp.</i>	<i>Navicula sp.</i>
	<i>Navicula sp.</i>	<i>Frustulia sp.</i>	<i>Diatoma sp.</i>
	<i>Achmanthidium sp.</i>	<i>Cocconeis sp.</i>	<i>Achmanthidium sp.</i>
	<i>Diademesmis sp.</i>	<i>Fragilaria sp.</i>	<i>Asterionella sp.</i>
	<i>Diatoma sp.</i>	<i>Diatoma sp.</i>	<i>Meridion sp.</i>
	<i>Brachysira sp.</i>	<i>Gomphonema sp.</i>	<i>Cyclotella sp.</i>
	<i>Rhoicosphenia sp.</i>	<i>Rhoicosphenia sp.</i>	<i>Planothidium sp.</i>
	<i>Nedium sp.</i>		<i>Cocconeis sp.</i>
	<i>Cyclotella sp.</i>		
Green Algae	<i>Gongrosira</i> or <i>Apatococcus sp.</i>	<i>Spirogyra</i>	<i>Stigeoclonium</i>
	<i>Scenedesmus sp.</i>		
	<i>Monoraphidium sp.</i>		
	<i>Ankistrodesmus sp.</i>		
	<i>Unidentified colonial sp.</i>		
Blue-green Algae	<i>Oscillatoria sp.</i>	<i>Oscillatoria sp.</i>	<i>Oscillatoria sp.</i>
	<i>Psuedanabaena sp.</i>		<i>Psuedanabaena sp.</i>

	<i>Leptolyngbya sp.</i>		
	<i>Phormidium sp.</i>		
	<i>Merismopedia sp.</i>		
	<i>Small unidentified colonial sp.</i>		
Desmids	<i>Staurastrum sp.</i>	<i>Cosmarium sp.</i>	<i>Cosmarium sp.</i>
		<i>Closterium sp.</i>	<i>Closterium sp.</i>
			<i>Staurastrum sp.</i>

¹diatoms listed generally from most abundant to least abundant

Note: Species composition included in this table is for reference only. Taxonomy was not conducted on periphyton plates used in the experiments presented here, however these compositions are fairly stable by season when periphyton plates of are similar gestation period.

Supplemental Table 2. Taxonomic classification of aquatic invertebrates used to measure uptake from solution (K_u), and efflux (K_e) along with average body weights

Class	Order	Scientific Name	Average Wet Weight (g)	K_u ($Lg^{-1}d^{-1}$)	K_e (d^{-1})
Insecta	Ephemeroptera	<i>Isonychia sp.</i>	0.02±0.004	0.05±0.03	0.29±0.07
		<i>Maccaffertium pudicum</i>	0.009±0.003	0.001±0.003	NA
		<i>Maccaffertium sp.</i>	0.06±0.03	0.001±0.004	0.15±0.03
		<i>Ephemerella sp.</i>	0.05±0.007	0.001±0.002	0.08±0.01
		<i>Neocloeon triangulifer</i>	0.003±0.001	0.001±0.0002	NA
	Trichoptera	<i>Hydropsyche betteni</i>	0.035±0.003	0.03±0.2	0.055±0.01
	Coleoptera	<i>Psephenus herricki</i>	0.016±0.008	0.06±0.04	0.03±0.06
	Megaloptera	<i>Corydalus sp.</i>	0.18±0.05	0.06±0.01	-
	Plecoptera	<i>Acroneuria abnormis</i>	0.16±0.05	0.02±0.01	0.09±0.04
		<i>Paragnetina immarginata</i>	0.08±0.02	0.01±0.01	-
Gastropoda	Neotaenioglossa	<i>Pleurocera sp.</i>	0.3±0.08	0.009± 0.01	0.026±0.03
Bivalvia	Veneroidea	<i>Corbicula fluminea</i>	0.9±0.3	0.006±0.004	0.03±0.02

NA = data could not be determined; “-“= not evaluated