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

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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±0.04 L g⁻¹ d⁻¹ in *Psephenus herrickii*, to 0.001±0.003 L g⁻¹ d⁻¹ in *M. pudicium*. Efflux (K_e) was generally high ranging from 0.15±0.03 d⁻¹ in *Maccaffertium sp.* to 0.03±0.03 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.
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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 µg L⁻¹ to 2 µg L⁻¹, while contaminated rivers typically range from 1-280 µg L⁻¹ but have been reported as high as 79,000 µg 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. (Aïda 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 (HAsNa₂O₄●7H₂O) was obtained from Alfa Aesar (MA, USA). ⁷³As 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 NaHCO₃, 0.17 CaSO₄*2H₂O, 0.25 MgSO₄, 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±1.2 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± 0.0077 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 µg L⁻¹ total As in addition to As-73 as a radiotracer (0.002 µCi 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 µg L⁻¹ in trial 1 and <0.1, 1.28, 6.85, 16.0, and 24.7 µg 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-weighted 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 ⁷³As for 3–4 days in 150 mL ASW (0.004 µCi 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 µg 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 × 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 × 1100 µm region of the periphyton sample using micro X-ray fluorescence (µXRF) at Beamline 2-3. The beam spot size was nominally 1 × 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 µg 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 µCi mL\(^{-1}\) for all experiments). To obtain initial uptake rates from solutions, animals were analyzed \textit{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). 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 ± 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 $0.1573 ± 0.06517$ µg As g$^{-1}$ 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](image)

Two independent trials of periphyton exposed to a single pulse of 1, 5, 10 and 20 µg 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 µg L⁻¹ arsenate, respectively. Values plotted are mean ± SEM.

After 8 days of exposure to a single pulse of arsenate (1, 5, 10, or 20 µg 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.
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 ± 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 µg 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 ± 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±8.5%) followed by *Pleurocera sp.* (28±10%), *Corbicula fluminea* (57±12.7%), *Maccaffertium sp.* (60±13.2%), and *Isonychia sp.* (75±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±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 ± 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 arsensate 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).
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). Talloperla sp. did not absorb enough measureable As during the experiment to be included. Ephemerella sp., Maccaffertium sp., N. triangulifer, and M. pudicum all had K_u values of ~0.001 L g⁻¹ d⁻¹. C. fluminea, Pleurocera sp., and P. immarginata all had K_u values of ~0.01 L g⁻¹ d⁻¹. A. abnormis and H. betteni were 0.02–0.03 L g⁻¹ d⁻¹, and Corydalus sp., Isonychia sp., and P. herricki had K_u values of ~0.05–0.06 L g⁻¹ 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 ~0.03 d$^{-1}$. *H. betteni, Ephemeraella 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 ~0.15 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. (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 AsO43- 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 (~2 µg L⁻¹ in reference streams to >300 µg L⁻¹ in contaminated streams)(Smedley and Kinniburgh 2002). Thus the range of dissolved As concentrations used in our experiments (1–20 µg 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 µg L⁻¹ As for 8–10 days accumulated approximately 30–60 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 ~20–40 µg L⁻¹ As, periphyton was found to have As concentrations ranging from 0.6–50 mg kg⁻¹ on a dry weight basis.(Ramelow et al. 1987) When industrially impacted water concentrations were approximately 32 µg 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 250–300 µg L⁻¹ resulted in microbial mats with 82–290 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 µg 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 3,000–17,000 mg kg⁻¹ As on a dry weight basis when exposed to a range of concentrations from 10,000–50,000 µg 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 µg L⁻¹, but is also demonstrated by the 5 and 10 µg 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, \(Xue \text{ and Yan 2011}\) and 220–360 for several submerged plant species, \(Chen \text{ et al. 2015}\). In the field, As bioconcentration by primary producers is highly variable (BCF of 152 reported for bryophytes in a contaminated tributary, \(Culioli \text{ et al. 2009}\) and a range of values from 107 to 52,000 across several species of aquatic plants) \(Favas \text{ et al. 2012}\). 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), \(Ramelow \text{ et al. 1987}\) 300–1,062, \(Koch \text{ et al. 1999}\), and 1,438–1,781. \(Drndarski \text{ et al. 1993}\). 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, \(Ramelow \text{ et al. 1987}\) exposure concentrations, \(Sibi 2014\) and exposure durations \(Chen \text{ et al. 2015}\) in addition to the broad variability observed between species. \(Sibi 2014; Chen \text{ et al. 2015}\) Differences in the thickness or density \(Rosemond 1994; Alam \text{ et al. 1997; Kanavillil and Kurissery 2013}\) 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 \(N. \text{ triangulifer}\) larvae and adults

\(N. \text{ triangulifer}\) has been a useful laboratory species to study trace element bioaccumulation for zinc, \(Kim \text{ et al. 2012}\) cadmium, \(Xie \text{ et al. 2010}\) and selenium, \(Conley \text{ et al. 2009}\). Here, after being reared for a full lifecycle with both As-enriched diet (and residual aqueous exposure), \(N. \text{ triangulifer}\) larvae were found to have minimal measureable As, and As was not detectible 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 \(Hare \text{ et al. 1991; Cain \text{ et al. 1992; Mason \text{ et al. 2000; Lavilla \text{ et al. 2010}}\) and As associated with gut contents \(Smith \text{ et al. 2015 Sep 21}\) 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 \(C. \text{ riparius}\), \(Mogren \text{ et al. 2012}\) which was proposed to be accomplished through the meconium. \(Mogren \text{ et al. 2013}\) Similar findings are reported for a terrestrial moth \(Agrotis \text{ infusa}\), \(Andrahennadi \text{ and Pickering 2008}\) and aquatic mayfly \(Ephoron \text{ virgo}\), \(Cid \text{ et al. 2010}\) 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 \(N. \text{ triangulifer}\) 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*. (Awrahman 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)), biomethylation 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)).
4.6 Dissolved uptake/efflux

Our results show a great deal of variation in uptake from solution ($K_u$) between species (ranging from $0.001\pm0.003$ to $0.063\pm0.04$ L g$^{-1}$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 $0.03\pm0.03$ to $0.15\pm0.03$ 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 $0.028$ L g$^{-1}$d$^{-1}$ and $0.091$ d$^{-1}$, respectively. $K_u$ and $K_e$ values were reported as $0.057$ L g$^{-1}$d$^{-1}$ and $0.049$ d$^{-1}$ respectively for $Nereis diversicolor$ (PS Rainbow, 2011) and $0.165$ L g$^{-1}$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. ($0.350\pm0.049$ and $0.435\pm0.054$ L g$^{-1}$d$^{-1}$), though $K_e$ were comparable to what is reported here for $H. betteni$. (mean 0.0731 and 0.0532 d$^{-1}$). (Awrahman et al., 2015) Interestingly, estuarine bivalves were reported to have rapid $K_u$ of $0.807\pm0.129$ L g$^{-1}$d$^{-1}$, but the efflux rate for As was the highest reported (0.06±0.001 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:


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...


http://www.tandfonline.com/doi/abs/10.1080/10643380802202059


http://doi.wiley.com/10.1002/aoc.139


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].


### Appendix 1: abbreviations, symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Assimilation efficiency</td>
</tr>
<tr>
<td>As</td>
<td>Arsenic</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>Ke</td>
<td>Efflux rate (proportional daily loss)</td>
</tr>
<tr>
<td>Ku</td>
<td>Uptake rate constant</td>
</tr>
<tr>
<td>XANES</td>
<td>X-ray absorption near edge structure</td>
</tr>
<tr>
<td>XRF</td>
<td>X-ray fluorescence</td>
</tr>
</tbody>
</table>
Appendix 2: List of presentations and publications (including thesis)

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


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

Appendix 3: Supplemental Information

Supplemental Figure 1. Aqueous (A) and dietary (B) exposure conditions for *N. triangulifer* larvae reared for a full lifecycle. Values plotted are mean ±SEM; *n*=3 for each treatment at each time point. Symbols represent
initial nominal dissolved As concentrations of 1, 5, 10, and 20 µg L\(^{-1}\) arsenate, respectively.

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

<table>
<thead>
<tr>
<th>Month</th>
<th>June–July</th>
<th>October</th>
<th>December</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diatoms(^1)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melosira varians</td>
<td>Melosira varians</td>
<td>Melosira varians</td>
<td></td>
</tr>
<tr>
<td>Diatoma vulgaris</td>
<td>Cymbella sp.</td>
<td>Gomphonema sp.</td>
<td></td>
</tr>
<tr>
<td>Syedra sp.</td>
<td>Syedra sp.</td>
<td>Nitzsizia sp.</td>
<td></td>
</tr>
<tr>
<td>Nitzsizia sp.</td>
<td>Nitzsizia sp.</td>
<td>Syedra sp.</td>
<td></td>
</tr>
<tr>
<td>Cymbella sp.</td>
<td>Navicula sp.</td>
<td>Fragilaria sp.</td>
<td></td>
</tr>
<tr>
<td>Gomphonema sp.</td>
<td>Achnanthidium sp.</td>
<td>Cymbella sp.</td>
<td></td>
</tr>
<tr>
<td>Fragilaria sp.</td>
<td>Planothidium sp.</td>
<td>Navicula sp.</td>
<td></td>
</tr>
<tr>
<td>Navicula sp.</td>
<td>Frustalia sp.</td>
<td>Diatoma sp.</td>
<td></td>
</tr>
<tr>
<td>Achnanthidium sp.</td>
<td>Cocconeis sp.</td>
<td>Achnanthidium sp.</td>
<td></td>
</tr>
<tr>
<td>Diadesmis sp.</td>
<td>Fragilaria sp.</td>
<td>Asterionella sp.</td>
<td></td>
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<tr>
<td>Diatoma sp.</td>
<td>Diatoma sp.</td>
<td>Meridion sp.</td>
<td></td>
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<tr>
<td>Brachysira sp.</td>
<td>Gomphonema sp.</td>
<td>Cyclotella sp.</td>
<td></td>
</tr>
<tr>
<td>Rhoicosphenia sp.</td>
<td>Rhoicosphenia sp.</td>
<td>Planothidium sp.</td>
<td></td>
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<tr>
<td>Nedium sp.</td>
<td></td>
<td>Cocconeis sp.</td>
<td></td>
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<tr>
<td>Cyclotella sp.</td>
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<td></td>
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</tr>
<tr>
<td><strong>Green Algae</strong></td>
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<td></td>
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<tr>
<td>Gongrosora or Apatococcus sp.</td>
<td>Spirogyra</td>
<td>Stigeosclonium</td>
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<tr>
<td>Scenedesmus sp.</td>
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<tr>
<td>Monoraphidium sp.</td>
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<tr>
<td>Ankistrodesmus sp.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified colonial sp.</td>
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<td></td>
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<tr>
<td><strong>Blue-green Algae</strong></td>
<td></td>
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<tr>
<td>Oscillatoria sp.</td>
<td>Oscillatoria sp.</td>
<td>Oscillatoria sp.</td>
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<tr>
<td>Psuedanabaena sp.</td>
<td></td>
<td>Pseudanabaena sp.</td>
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<tr>
<td>Species</td>
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<td>---------------------------------</td>
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<td></td>
<td></td>
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<tr>
<td>Leptolyngbya sp.</td>
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<tr>
<td>Phormidium sp.</td>
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<td>Merismopedia sp.</td>
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<td></td>
</tr>
<tr>
<td>Small unidentified colonial sp.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Desmids</td>
<td></td>
<td></td>
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<tr>
<td>Staurastrum sp.</td>
<td></td>
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<td></td>
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<tr>
<td>Cosmarium sp.</td>
<td></td>
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<tr>
<td>Closterium sp.</td>
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<td></td>
</tr>
<tr>
<td>Staurastrum sp.</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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.

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

NA = data could not be determined; “–“= not evaluated.