

# Selenium Bioaccumulation and Maternal Transfer in the Mayfly *Centroptilum triangulifer* in a Life-Cycle, Periphyton-Biofilm Trophic Assay

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Selenium contamination in aquatic ecosystems provides management challenges because bioaccumulation in animals is largely a function of dietary exposure, whereas regulatory entities have traditionally focused on direct water to organism interactions. Selenium is known to be readily absorbed by primary producers and can potentially biomagnify in food webs and elicit adverse effects in higher trophic levels. However, selenium bioaccumulation in the invertebrate prey of many predatory animals is poorly understood. Here, we used <sup>75</sup>Se (as selenite) as a radiotracer to characterize Se bioaccumulation into natural periphyton biofilms and subsequent dietary and maternal transfer in the mayfly, *Centroptilum triangulifer*, in a life-cycle assay. On average periphyton bioconcentrated selenium 1113 (±430)-fold following 7–9 days of exposure to a range of environmentally relevant dissolved concentrations (2.4–13.9 μg L<sup>-1</sup>). Mayflies grown to adulthood on these diets further biomagnified Se with trophic transfer factors averaging 2.2 (±0.4)-fold in postpartum maternal tissues. Adults then transferred 46.5 (±8.8) % of their body burdens to eggs with an observed reduction in fecundity for mayflies fed on diets greater than ~11 μg g<sup>-1</sup>. These results suggest that at environmentally feasible dietary Se concentrations insects are potentially affected by Se exposure, and that the current presumption that insects are simply conduits of Se to higher trophic levels is inaccurate.

## Introduction

Selenium is a nonmetal trace element that exhibits a narrow window between essential and toxic concentrations (1). Selenium can be an important contaminant in aquatic environments as a result of human activities, particularly those involving mining and burning of coal for power generation (2–9). Despite historic, high profile examples of Se causing significant ecological damage (e.g., Kesterson Reservoir, CA (10, 11), Belews Lake, NC (12, 13), surprisingly little data are available for Se dynamics in lotic ecosystems and the invertebrates therein. In lotic systems insects are

typically the dominant invertebrate faunal group, contribute vastly to ecological function, and serve as a major food source for stream predators (14). Therefore to understand the potential impacts of Se contamination in lotic environments, it is essential to understand the dynamics of Se movement into primary producers and subsequent transfer to insects.

Two paradigms dominate current understanding of potential Se impacts in aquatic ecosystems. The first is that diet is the predominant route of exposure for organisms in aquatic food webs (5, 15–17), with dissolved Se concentrations being poor predictors of bioaccumulation and toxic effects (4, 18). This understanding has prompted the U.S. Environmental Protection Agency to move toward a tissue based standard for Se (19). A second dominant paradigm is that invertebrates act primarily as conduits of Se from primary producers to higher trophic level animals (e.g., fish and birds), but themselves are not adversely affected by Se exposure (6, 20).

Here we used a laboratory test system to examine Se (as selenite, [SeO<sub>3</sub><sup>2-</sup>]) enrichment in natural periphyton biofilms and the subsequent transfer to the mayfly *Centroptilum triangulifer* (Ephemeroptera: Baetidae) in a life cycle assay. The use of <sup>75</sup>Se as a radiotracer allowed us to quantify transfers of Se from water to periphyton, from periphyton to larval mayflies, and from adult mayflies to their eggs over a wide range of dietary Se exposure concentrations. We further report on the influence of Se bioaccumulation on mayfly fecundity.

## Materials and Methods

**Test Animals.** The mayfly *Centroptilum triangulifer* (Ephemeroptera: Baetidae) was obtained from culture at the Stroud Water Research Center (Avondale, PA). Originally described as *Cloeon triangulifer* by McDunnough (21), this parthenogenetic species typically inhabits marginal areas of lotic systems. Negligible flow requirements make this species particularly amenable to laboratory use as a test species. *C. triangulifer* has previously been used in studies of temperature and development (22), chlordane (23), and aluminum (24). More recently *C. triangulifer* has been used to examine the trophic transfer of cadmium from periphyton (25).

**Radioactivity Measurement.** All measurements of radioactivity in water, periphyton, *C. triangulifer* adults and eggs were performed using a Perkin-Elmer Wallac Wizard 1480 automatic gamma counter (Shelton, CT). Samples were counted for 3 min and all counting errors were generally <5%. Selenium concentrations are reported incorporating appropriate corrections for radioactive decay, counting efficiency, and ratio of <sup>75</sup>Se:stable Se.

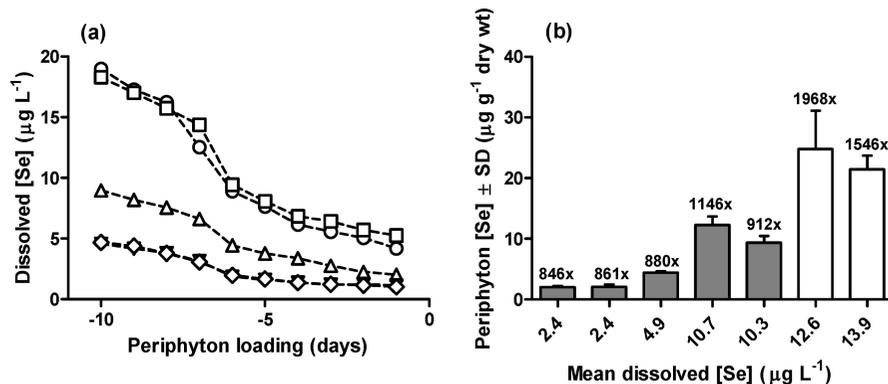
**Labeling Periphyton with Se.** To create differentially Se contaminated mayfly diets, acrylic plates (6.5 × 23 × 0.15 cm) were colonized by natural periphyton biofilms by allowing fresh streamwater from White Clay Creek, PA (39°51'47"N, 75°47'07"W) to flow continuously over the plates in a greenhouse as described previously (25, 26). Periphyton was grown in November 2008 and January 2009 for two distinct sets of experiments. Colonization was complete when the periphyton reached a thickness of approximately 1–2 mm. At this stage, periphyton consisted primarily of diatoms with some blue-green and green algae, along with some naturally colonizing consumers (predominantly micro- and meiofauna (26)).

In each study, the colonized plates were placed in 2.0 L glass bottles holding 1.8 L of American Society for Testing and Materials (ASTM) artificial soft water (48 mg L<sup>-1</sup> NaHCO<sub>3</sub>, 30 mg L<sup>-1</sup> CaSO<sub>4</sub> · 2H<sub>2</sub>O, 30 mg L<sup>-1</sup> MgSO<sub>4</sub>, and 2 mg L<sup>-1</sup> KCl, pH 7.4). The initial pilot study plates (November 2008) were

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**FIGURE 1.** Dissolved Se concentrations during Se loading of periphyton biofilms for Jan 2009 plates (a), and periphyton bioaccumulation of Se at the end of the loading phase in both November 2008 and January 2009 experiments (b). Symbols (a) represent initial ambient concentrations of  $20 \mu\text{g L}^{-1}$  (replicates 20C  $\circ$  and 20D  $\square$ ),  $10 \mu\text{g L}^{-1}$  (10A  $\triangle$ ), and  $5 \mu\text{g L}^{-1}$  (replicates 5A  $\nabla$  and 5B  $\diamond$ ). Gray bars (January 2009) and white bars (November 2008) (b) represent mean periphyton Se concentrations. Numbers above each bar represent periphyton bioconcentration relative to mean dissolved Se concentration over the course of the loading phase in each replicate.

either left unexposed to Se (controls) or exposed to dissolved Se at a nominal concentration of  $20 \mu\text{g L}^{-1}$  with two replicates per treatment. Each Se treatment received equal amounts (0.34 mL) of  $^{75}\text{Se}$  as  $\text{H}_2\text{SeO}_3$  (specific activity:  $0.315 \mu\text{Ci mL}^{-1}$ ; concentration:  $0.275 \text{ ng mL}^{-1}$ ), with stable  $\text{Na}_2\text{SeO}_3$  providing the remainder of the ambient Se in solution. During periphyton labeling, Se concentration in the water for each replicate was measured (10 mL sample) initially and at the end of a seven-day loading period. Triplicate periphyton samples (3–5 mg dry wt) were then collected from each bottle, dried, weighed and analyzed for radioactivity. Plates were then removed from their respective solutions and placed in bottles containing 1.8 L uncontaminated ASTM soft water and allowed to equilibrate for one day.

A second experiment with expanded Se concentrations was conducted. Plates (January 2009) were either unexposed (controls) or exposed to dissolved Se at nominal concentrations of 5, 10, and  $20 \mu\text{g L}^{-1}$ . Each concentration had two replicates, except for the  $10 \mu\text{g L}^{-1}$  exposure, which was unreplicated due to limited plate availability. As was the case in the previous experiment, identical amounts (3.4 mL) of  $^{75}\text{Se}$  as  $\text{H}_2\text{SeO}_3$  (specific activity:  $0.227 \mu\text{Ci mL}^{-1}$ ; concentration:  $0.275 \text{ ng mL}^{-1}$ ) were added to each Se exposure, with stable  $\text{Na}_2\text{SeO}_3$  providing the remainder of the ambient Se in solution. Plates were exposed for nine days and duplicate 1 mL water samples were collected daily to monitor the loss of dissolved Se due to periphyton uptake. At the end of the loading phase triplicate periphyton samples were collected from each exposure and analyzed for radioactivity. Plates were then moved to new bottles containing uncontaminated ASTM soft water and allowed to equilibrate for one day.

**Mayfly Life Cycle Exposure to Dietary Se.** After the periphyton plates were loaded with Se, sampled, and equilibrated in clean water for 24 h, *C. triangulifer* larvae (4–6 days old) were added to each bottle. Each bottle was gently aerated, and the light:dark cycle was natural for the given season with ambient light provided by large laboratory windows. Laboratory temperatures ranged from 19–22 °C in all experiments. Selenium concentrations in the water (duplicate 1 mL samples) and periphyton (triplicate ~6 mg dry wt samples) were measured weekly, however periphyton was only collected for the first three weeks of exposure in order to not deplete the food available for proper mayfly growth and development. Moreover, as early instar larvae are not visible to the naked eye, we wanted to limit the risk of inadvertently removing animals. Exposures lasted for 4.5–6 weeks with subimagos emerging over a 10 day period in both experiments.

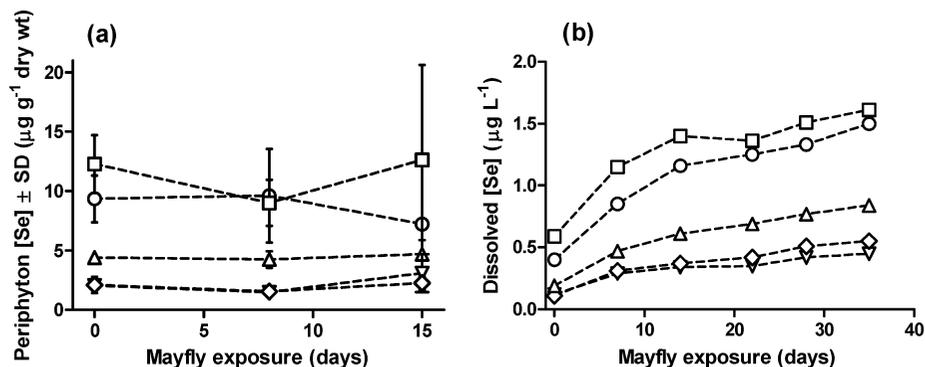
Subimagos emerged into mesh-lined collection lids during mid to late afternoon and were kept overnight in humid chambers containing moist paper towels to facilitate the final molt to adulthood. The following day adults were analyzed for radioactivity twice: before and after oviposition. Gravid adults were assayed for radioactivity then stimulated to release eggs by wetting the abdomen in 3.5 cm Petri dishes containing autoclaved streamwater. Postpartum adults were then assayed again for radioactivity and stored frozen ( $-20 \text{ }^\circ\text{C}$ ) in individual microcentrifuge tubes and subsequently oven-dried at  $60 \text{ }^\circ\text{C}$  for approximately 48 h to obtain a constant weight. The dry weight of each adult was measured on a Sartorius CP225D microbalance to the nearest 0.01 mg.

**Determination of Mayfly Clutch Size.** Petri dishes containing egg masses were divided into four quadrants and each quadrant was photographed using a Leica DFC480 digital camera ( $0.63\times$  magnification) attached to a Leica MZ16F stereoscope ( $0.71\times$  magnification) fitted with a Planapo 1.0 $\times$  objective lens. Images were captured and analyzed using SimplePCI (v6.0.0 Compix Inc. Imaging Systems). Clutch sizes were measured by adding the total number of eggs in each of the quadrants as determined by Image enhancement and Object identifier processing via the SimplePCI interface.

**Statistical Analysis.** Data are expressed as mean  $\pm$  standard deviation and all data analyses were performed using GraphPad Prism (v5.02). Bioconcentration of Se from water to periphyton was determined by dividing the mean measured Se concentrations in periphyton ( $\mu\text{g g}^{-1}$ ) by mean Se in water ( $\mu\text{g L}^{-1}$ ), assuming 1 L of soft water weighs 1000 g. Note that these are not steady state bioconcentration factors (BCFs) as they represent periphyton concentrations in a nonequilibrium scenario. Trophic transfer factors (TTFs) were determined by dividing Se body burden in *C. triangulifer* (postpartum) by mean Se concentrations in periphyton over the duration of the exposure. Maternal transfer of Se was analyzed using correlation by comparing the total Se content of the postpartum adult to the total Se content of the gravid adult. Maternal transfer (%) was calculated by dividing the total mass of Se in the eggs by the total mass of Se in gravid adults. Fecundity effects were analyzed by observing departure of exposed mayflies from the linear regression  $\pm 95\%$  CI of the control mayflies for egg production versus postpartum adult body mass.

## Results

**Periphyton Se Bioconcentration.** To assess the dynamics of Se enrichment in periphyton, we first characterized the loss of Se from solution (Figure 1a) and the accompanying



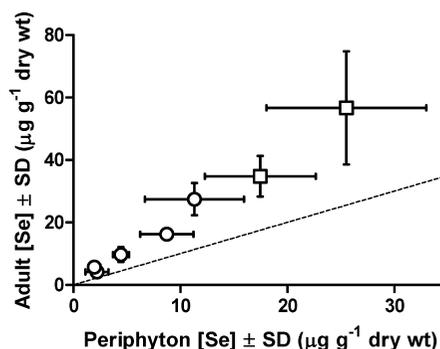
**FIGURE 2.** Mean periphyton (a) and dissolved (b) Se concentrations measured during mayfly exposure for the January 2009 experiment. Symbols (a,b) represent initial ambient concentrations of  $20 \mu\text{g L}^{-1}$  (replicates 20C  $\circ$  and 20D  $\square$ ),  $10 \mu\text{g L}^{-1}$  (10A  $\triangle$ ), and  $5 \mu\text{g L}^{-1}$  (replicates 5A  $\nabla$  and 5B  $\diamond$ ). Each periphyton data point (a) represents the mean of triplicate periphyton samples. Each dissolved concentration (b) represents the mean of duplicate samples taken over the duration of mayfly exposures.

enrichment of Se into periphyton after loading periods of 7 and 9 days (Figure 1b). Dissolved Se decreased continually over the nine day loading period in all Jan 2009 treatment groups with a trend toward, but not reaching uptake equilibrium into the periphyton. Initial dissolved concentrations were very close to nominal, while mean dissolved concentrations over the course of the loading phase were  $12.6$  and  $13.9 \mu\text{g L}^{-1}$  for 20A and 20B, respectively (November 2008), and  $2.4$ ,  $2.4$ ,  $4.9$ ,  $10.3$ , and  $10.7 \mu\text{g L}^{-1}$  for 5A, 5B, 10A, 20C, and 20D, respectively (January 2009). Se bioconcentration by periphyton is reported relative to average measured dissolved concentrations during the loading phase.

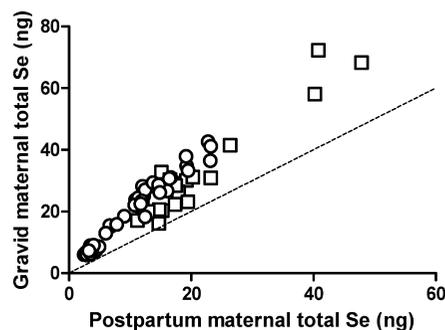
Bioconcentration of Se from water to periphyton averaged  $1113 \pm 430$ -fold (range 804–2025) across all treatments at the end of both experimental loading phases (Figure 1b). The pilot study (November 2008) had a higher average bioconcentration (mean 1642-fold) than the second experiment (January 2009, mean 902-fold). This difference is likely due to the fact that periphyton was grown in different seasons and may reflect slightly different species compositions. Further, the plates were exposed for different time periods (November 2008, 7 days; January 2009, 9 days), and previous research has shown that Se bioconcentration into freshwater primary producers tends to peak from 6–14 days of exposure, and then declines slowly (27). Similar declines in Se content have also been observed in a marine algal species (Martin Grosell, personal communication).

**Selenium Trophic Transfer Experiments.** Periphyton Se concentrations remained relatively constant over the course of mayfly development (Figure 2a). Mean periphyton concentrations for November 2008 plates were  $25.5 \pm 7.5$  and  $17.5 \pm 5.2 \mu\text{g g}^{-1}$  for 20A and 20B, respectively. Mean periphyton concentrations for January 2009 plates were  $2.2 \pm 1.1$ ,  $2.0 \pm 0.6$ ,  $4.4 \pm 0.8$ ,  $8.7 \pm 2.5$ , and  $11.3 \pm 4.6$  for 5A, 5B, 10A, 20C, and 20D, respectively. Dissolved Se displayed a gradual increase in concentration over the exposure period. This rise was most likely due to mayfly dietary efflux and periphyton desorption of selenite (28). For November 2008 plates, dissolved Se concentrations reached a maximum of  $2.8$  and  $2.4 \mu\text{g L}^{-1}$  after 28 days for the two replicates. For January 2009 plates, dissolved Se concentrations increased with increasing periphyton concentrations, with maxima of  $0.4$ ,  $0.5$ ,  $0.8$ ,  $1.5$ , and  $1.6 \mu\text{g L}^{-1}$  after 35 days for replicates 5A, 5B, 10A, 20C, and 20D, respectively. A separate experiment demonstrated that dissolved uptake of selenite by *C. triangulifer* was negligible (data not shown).

Transfer of Se to adult *C. triangulifer* was highly correlated with dietary periphyton concentrations across both experiments (Pearson  $r > 0.99$ ,  $p < 0.0001$ ) (Figure 3). For the pilot study (November 2008), adult mayflies accumulated mean body burdens of  $34.8 \pm 6.5$  and  $56.7 \pm 18.1 \mu\text{g g}^{-1}$  on diets of  $17.5 \pm 5.2$  and  $25.5 \pm 7.5 \mu\text{g g}^{-1}$ , respectively. For January



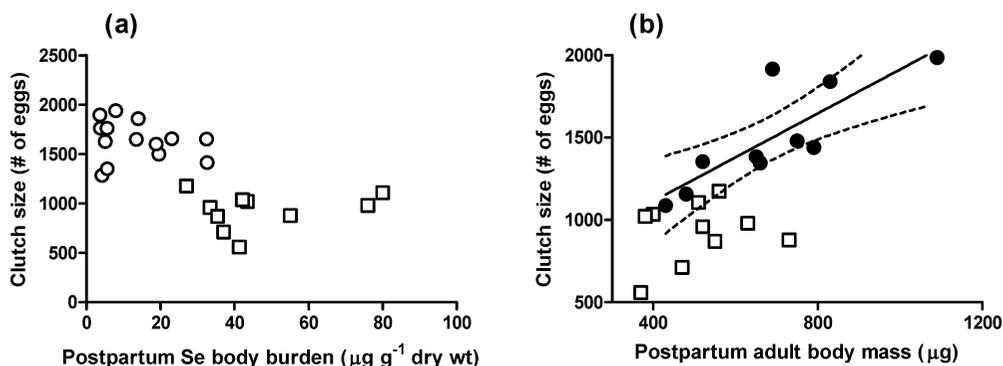
**FIGURE 3.** Trophic transfer of Se from periphyton into adult mayflies (postpartum). Symbols represent Jan 2009 exposures ( $\circ$ ) and November 2008 exposures ( $\square$ ). Concentrations were significantly correlated (Pearson  $r > 0.99$ ,  $p < 0.0001$ ). A 1:1 line (dashed) is provided for reference only. Data points above the dashed line represent biomagnifications of Se.



**FIGURE 4.** Maternal transfer of Se plotted as total Se (ng) in postpartum adults versus gravid adults for both experiments. Symbols represent Jan 2009 exposures ( $\circ$ ) and Nov 2008 exposures ( $\square$ ). Data points are significantly correlated (Pearson  $r > 0.93$ ,  $p < 0.0001$ ). A 1:1 line (dashed) is provided for reference only. Data points above the dashed line represent maternal transfer of Se.

2009 plates, adult mayflies accumulated mean body burdens of  $4.2 \pm 0.1$ ,  $5.7 \pm 0.2$ ,  $9.7 \pm 1.1$ ,  $16.2 \pm 0.5$ ,  $27.5 \pm 1.5 \mu\text{g g}^{-1}$  on diets of  $2.2 \pm 1.1$ ,  $2.0 \pm 0.6$ ,  $4.4 \pm 0.8$ ,  $8.7 \pm 2.5$ , and  $11.3 \pm 4.6 \mu\text{g g}^{-1}$ , respectively. Trophic transfer factors (TTFs) to adult tissues (postpartum) were very similar across experiments, averaging  $2.1 \pm 0.2$  for November 2008 plates and  $2.3 \pm 0.4$  for January 2009 plates. The combined mean TTF for all experiments was  $2.2 \pm 0.4$  (range 1.9–2.9).

**Maternal Transfer.** Mothers transferred a significant proportion of their Se body burdens to eggs (Figure 4). We report maternal transfer as a function of total Se mass in postpartum adults versus the total Se mass in gravid adults



**FIGURE 5.** Fecundity of adult mayflies associated with postpartum adult Se body burden (a) and body mass (b). In panel (a) symbols represent Jan 2009 exposures (○) and Nov 2008 exposures (□). Body burden and fecundity were negatively correlated across both experiments ( $r = -0.69$ ,  $p = 0.0002$ ) (a). In panel (b) symbols represent Nov 2008 exposures (□) and controls (●). Fecundity is associated with body mass in controls ( $R^2 > 0.68$ , slope = 1.33), but is not associated with body mass in treated animals (b).

(Pearson  $r = 0.93$ ,  $p < 0.0001$ ) because it was not possible to obtain dry weights of adults both before and after oviposition. Maternal transfer (% of total Se) was significantly different between experiments ( $p < 0.0001$ ) with November 2008 insects transferring  $36.0 \pm 8.3\%$  of their Se, and January 2009 insects transferring  $51.1 \pm 6.4\%$ . Across both experiments, a decrease in % transferred to eggs was associated with increases in maternal body burden (Pearson  $r = -0.71$ ,  $p < 0.0001$ , data not shown).

**Se Effects on Mayflies.** We observed a decrease in fecundity associated with increasing maternal (postpartum) Se body burden across all exposures (Pearson  $r = -0.69$ ,  $p = 0.0002$ ) (Figure 5a). This decrease was most pronounced in mayflies exposed to diets with the two highest periphyton Se concentrations. While fecundity in control animals exhibited an expected growth dependence with an increase of  $\sim 135$  eggs associated with an increase of  $100 \mu\text{g}$  in total mass ( $R^2 = 0.68$ ,  $p = 0.0032$ ), many mayflies grown on the most contaminated diets (November 2008) were below the lower 95% CI (Figure 5b). Here, adult mayflies did not necessarily produce more offspring as they increased in body mass when they were exposed to diets  $> \sim 11 \mu\text{g g}^{-1}$ . We also note a reduction in growth (adult body mass) associated with elevated Se body burdens (Pearson  $r = -0.45$ ,  $p < 0.0001$ , data not shown).

## Discussion

Selenium concentrations reported for lotic reference sites in freshwater ecosystems are typically  $< 1 \mu\text{g L}^{-1}$  (6, 8, 9), but concentrations can be as high as  $36 \mu\text{g L}^{-1}$  in sites associated with mountaintop removal/valley fill coal mining (8). Thus, the range of dissolved Se concentrations we used to enrich periphyton represents environmentally realistic exposures (3, 4, 6, 8, 9, 29). Similarly, the amount of Se accumulated by periphyton was comparable to literature values reported for primary producers in Se contaminated field sites, which tend to be from  $1\text{--}10 \mu\text{g g}^{-1}$  (2, 4, 6, 29) and as high as  $22 \mu\text{g g}^{-1}$  (29). Overall, the goal of creating Se contaminated periphyton diets that spanned a range of environmentally realistic concentrations was achieved during the 7 and 9 day loading experiments.

Despite the apparent importance of Se bioconcentration into freshwater primary producers, surprisingly few data are available to reference. Field-based measures in lotic sites include BCFs ranging from 200-fold for algae to 550-fold for moss in coal mine impacted lotic systems in Alberta, Canada (6), 300–1900-fold for aquatic macrophytes in Se exposed stream mesocosms (30), and an average of 1400-fold for Se contaminated agricultural drainage waters into aquatic microphytes in the San Joaquin Valley, California (29). Thus, our measures of Se bioconcentration by periphyton are

generally in the higher end of the range of reported BCF values. Together, these field and laboratory measures support the fact that in Se contaminated areas the largest increase in Se tissue content occurs from the enrichment of dissolved Se into primary producers (i.e., periphyton).

Variability in Se bioconcentration by primary producers in nature could be a result of both physiological differences among species, as well as the predominant geochemical form of Se in solution. In our experiments, exposures were initiated with dissolved selenite which has been shown to accumulate more readily into algae than selenate (15, 28). Selenite and selenate constitute the predominant species of Se known to exist in freshwater ecosystems with organo-selenium forms making up a smaller fraction (31). Relative contributions of individual Se species to total Se content (dissolved selenite: selenate) are driven by site-specific factors (i.e., pH, DO, source of contamination, and biological activity). In lotic sites (highly aerated) it has been speculated that selenate would be the most dominant species, however the large majority of studies report values only for total Se concentration, so there is much yet to learn about these differences, particularly with respect to periphyton. Interestingly, a recent speciation study of a lotic ecosystem found selenite in all field samples of biofilms (14–16%) and insects (2–12%) but not selenate (2). Regardless of speciation, once absorbed by primary producers, both forms seem to be largely converted to organic selenides (e.g., R–Se–R) that associate with proteins and are bioavailable for trophic transfer (2, 15, 29).

Trophic transfer factors here compared well to TTFs that have been calculated in both field surveys of aquatic insects (4, 5, 29) and a laboratory experiment with *Daphnia magna* (32). TTFs from field sites average 1.9 (29), 1.7 (4), and 4.7-fold (5). In the lab, Guan and Wang (32) reported TTFs of Se from two green algal species into *D. magna* at a range of 2–4-fold. The agreement between the TTF data reported here and those cited for additional species in the lab and the field lend further support to the contention that the largest step in biomagnification of Se in aquatic food webs is from water to primary producers (500–2000-fold), with a smaller increase from primary producers to aquatic invertebrates (1.7–4.7-fold).

Trophic transfer resulted in postpartum adult mayfly body burdens that were within the range of aquatic invertebrate body burdens found in Se contaminated lotic field sites (2, 3, 6, 7, 9). Even the highest mean body burdens obtained in this study ( $34.8$  and  $56.7 \mu\text{g g}^{-1}$ ) were not outside the realm of environmentally relevant invertebrate body burdens. Hamilton and Buhl (3) reported Se body burdens as high as  $75.2 \mu\text{g g}^{-1}$  from streams below a phosphate mining site in Idaho. Typically, however, values tend to fall in the range of  $4.2\text{--}8.8 \mu\text{g g}^{-1}$  for aquatic invertebrates, which is above the

recommended threshold of  $3 \mu\text{g g}^{-1}$ , suggested by Lemly (20) for protection of fish and wildlife. All mayflies in the current study accumulated body burdens greater than  $3 \mu\text{g g}^{-1}$ . Additionally, upon further analysis it was noted that dietary Se concentration was a much better predictor ( $R^2 = 0.98$ ,  $p < 0.0001$ ) than dissolved Se concentration ( $R^2 = 0.71$ ,  $p = 0.02$ ) of adult body burden, supporting the argument that dissolved Se concentration is less reliable than dietary exposure route in field assessment and management of Se contaminated sites (33).

Selenium readily transfers from mother to offspring because it is an essential element, however high maternal body burdens do not necessarily result in greater maternal transfer of Se. Additional data analysis from the current study showed that at increasing postpartum maternal Se body burdens there was a trend of decreasing maternal transfer (Pearson  $r = -0.71$ ,  $p < 0.0001$ , data not shown). Guan and Wang (32) reported maternal transfer of Se from 37 to 40% in *D. magna* exposed to dietary Se concentrations ranging from 1 to  $30 \mu\text{g g}^{-1}$ . In a later study by Lam and Wang (34), maternal transfer of Se in *D. magna* was reduced to between 19 and 24% after exposure to a dietary concentration of  $115.6 \mu\text{g g}^{-1}$ . This reduction in transfer of Se at increasing dietary exposure levels suggests that Se transfer to eggs is not a detoxification strategy, but may be an indirect result of decreased reproductive output. If a mother partitions a certain amount of Se to each egg (35), reduced fecundity associated with Se exposure may result in an overall decrease in maternal transfer.

Although limited in number, laboratory studies focused on invertebrate exposure to Se have reported adverse effects similar to the reduction in fecundity found here. Ingersoll et al. (36) reported significant reductions in total young, young per available female reproductive days, and intrinsic rate of natural increase ( $r$ ) in *D. magna* at Se body burdens of  $\geq 31.7 \mu\text{g g}^{-1}$ . In that study, however, *D. magna* were exposed to high dissolved Se concentrations, with dietary exposure not considered. Adverse effects were also reported in a study by Malchow et al. (37) where reduction in larval growth of *Chironomus decorus* occurred at body burdens  $\geq 2.55 \mu\text{g g}^{-1}$  after exposure to Se contaminated algae at  $\geq 2.11 \mu\text{g g}^{-1}$ . Therefore, contrary to the belief that invertebrates are largely unaffected by elevated Se exposure (6, 20), the current study and others have shown that growth, fecundity, or both may be affected by Se in aquatic invertebrates. It is possible that Se uptake by periphyton renders the food unpalatable resulting in reduced consumption, growth, and fecundity. Alternatively, the mechanistic action of Se on reproductive deficit may be related to oxidative stress, although this has largely been studied in vertebrate species (38–40).

The present results provide data for the dietary dynamics of Se in freshwater periphyton and insects, which is critical to understanding the impacts at higher trophic levels that have been highlighted in the implementation of a tissue-based water quality criterion for Se (19). The vast majority of biomagnification of Se occurs at the level of absorption by primary producers, followed by small, but important, increases into aquatic insects. As follows, all mayflies in the present study accumulated Se body burdens greater than that recommended for the protection of fish and wildlife (20). Finally, this study is one of the few to report adverse impacts on an aquatic insect from exposure to environmentally realistic concentrations of Se via diet, refuting the argument that aquatic insects are not impaired by Se exposure.

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## Literature Cited

- Painter, E. P. The chemistry and toxicity of selenium compounds, with special reference to the selenium problem. *Chem. Rev.* **1941**, *28*, 179–213.
- Andrahennadi, R.; Wayland, M.; Pickering, I. J. Speciation of selenium in stream insects using x-ray absorption spectroscopy. *Environ. Sci. Technol.* **2007**, *41*, 7683–7687.
- Hamilton, S. J.; Buhl, K. J. Selenium in water, sediment, plants, invertebrates, and fish in the Blackfoot River drainage. *Water Air Soil Pollut.* **2004**, *159*, 2–34.
- Muscatello, J. R.; Belknap, A. M.; Janz, D. M. Accumulation of selenium in aquatic systems downstream of a uranium mining operation in northern Saskatchewan, Canada. *Environ. Pollut.* **2008**, *156*, 387–393.
- Muscatello, J. R.; Janz, D. M. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. *Sci. Total Environ.* **2009**, *407*, 1318–1325.
- Orr, P. L.; Guiguer, K. R.; Russel, C. K. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. *Ecotox. Environ. Safe.* **2006**, *63*, 175–188.
- Peterson, M. J.; Smith, J. G.; Southworth, G. R.; Ryon, M. G.; Eddlemon, G. K. Trace element contamination in benthic macroinvertebrates from a small stream near a uranium mill tailings site. *Environ. Monit. Assess.* **2002**, *74*, 193–208.
- Pond, G. J.; Passmore, M. E.; Borsuk, F. A.; Reynolds, L.; Rose, C. J. Downstream effects of mountaintop coal mining: comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. *J. N. Am. Benthol. Soc.* **2008**, *27*, 717–737.
- Wayland, M.; Crosley, R. Selenium and other trace elements in aquatic insects in coal mine-affected streams in the Rocky Mountains of Alberta, Canada. *Arch. Environ. Contam. Toxicol.* **2006**, *50*, 511–522.
- Presser, T. S.; Sylvester, M. A.; Low, W. H. Bioaccumulation of selenium from natural geologic sources in western states and its potential consequences. *Environ. Manage.* **1994**, *18*, 423–436.
- Ohlendorf, H. M.; Hoffman, D. J.; Saiki, M. K.; Aldrich, T. W. Embryonic mortality and abnormalities of aquatic birds - apparent impacts of selenium from irrigation drainwater. *Sci. Total Environ.* **1986**, *52*, 49–63.
- Finley, K. A. Observations of bluegills fed selenium-contaminated *Hexagenia* nymphs collected from Belews Lake, North Carolina. *Bull. Environ. Contam. Toxicol.* **1985**, *35*, 816–825.
- Sorensen, E. M. B.; Cumbie, P. M.; Bauer, T. L.; Bell, J. S.; Harlan, C. W. Histopathological, hematological, condition-factor, and organ weight changes associated with selenium accumulation in fish from Belews Lake, North Carolina. *Arch. Environ. Contam. Toxicol.* **1984**, *13*, 153–162.
- Wallace, J. B.; Webster, J. R. The role of macroinvertebrates in stream ecosystem function. *Annu. Rev. Entomol.* **1996**, *41*, 115–139.
- Besser, J. M.; Canfield, T. J.; Lapoint, T. W. Bioaccumulation of organic and inorganic selenium in a laboratory food-chain. *Environ. Toxicol. Chem.* **1993**, *12*, 57–72.
- Stewart, A. R.; Luoma, S. N.; Schlegel, C. E.; Doblin, M. A.; Hieb, K. A. Food web pathway determines how selenium affects aquatic ecosystems: a San Francisco Bay case study. *Environ. Sci. Technol.* **2004**, *38*, 4519–4526.
- Dubois, M.; Hare, L. Selenium assimilation and loss by an insect predator and its relationship to Se subcellular partitioning in two prey types. *Environ. Pollut.* **2009**, *157*, 772–777.
- Hillwalker, W. E.; Jepson, P. C.; Anderson, K. A. Selenium accumulation patterns in lotic and lentic aquatic systems. *Sci. Total Environ.* **2006**, *366*, 367–379.
- USEPA. *Draft aquatic life water quality criteria for selenium*, 2004; <http://www.epa.gov/seleniumcriteria/pdfs/complete.pdf>.
- Lemly, A. D. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. *Environ. Monit. Assess.* **1993**, *28*, 83–100.
- McDunnough, J. New species of North American Ephemeroptera. *Can. Entomol.* **1931**, *63*, 82–93.
- Sweeney, B. W.; Vannote, R. L. Influence of food quality and temperature on life-history characteristics of the parthenogenetic mayfly *Cloeon triangulifer*. *Freshwater Biol.* **1984**, *14*, 621–630.

- (23) Sweeney, B. W.; Funk, D. H.; Standley, L. J. Use of the stream mayfly *Cloeon triangulifer* as a bioassay organism - life-history response and body burden following exposure to technical Chlordane. *Environ. Toxicol. Chem.* **1993**, *12*, 115–125.
- (24) Tabak, L. M.; Gibbs, E. Effects of aluminum, calcium and low pH on egg hatching and nymphal survival of *Cloeon triangulifer* McDunnough (Ephemeroptera, Baetidae). *Hydrobiologia* **1991**, *218*, 157–166.
- (25) Xie, L.; Funk, D. H.; Buchwalter, D. B. Trophic transfer of Cd from natural periphyton biofilms to the grazing mayfly *Centropilum triangulifer* in a life cycle test. *Environ. Pollut.* **2009**, DOI: 10.1016/j.envpol.2009.07.10.
- (26) Xie, L.; Flippin, J. L.; Deighton, N.; Funk, D. H.; Dickey, D. A.; Buchwalter, D. B. Mercury(II) bioaccumulation and antioxidant physiology in four aquatic insects. *Environ. Sci. Technol.* **2009**, *43*, 934–940.
- (27) Dobbs, M. G.; Cherry, D. S.; Cairns, J. Toxicity and bioaccumulation of selenium to a three-trophic level food chain. *Environ. Toxicol. Chem.* **1996**, *15*, 340–347.
- (28) Riedel, G. F.; Ferrier, D. P.; Sanders, J. G. Uptake of selenium by fresh-water phytoplankton. *Water Air Soil Poll.* **1991**, *57–58*, 23–30.
- (29) Fan, T. W. M.; Teh, S. J.; Hinton, D. E.; Higashi, R. M. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. *Aquat. Toxicol.* **2002**, *57*, 65–84.
- (30) Swift, M. Stream ecosystem response to, and recovery from, experimental exposure to selenium. *J. Aquat. Ecosyst. Stress Recov.* **2002**, *9*, 159–184.
- (31) Simmons, D. B. D.; Wallschlager, D. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. *Environ. Toxicol. Chem.* **2005**, *24*, 1331–1343.
- (32) Guan, R.; Wang, W. X. Dietary assimilation and elimination of Cd, Se, and Zn by *Daphnia magna* at different metal concentrations. *Environ. Toxicol. Chem.* **2004**, *23*, 2689–2698.
- (33) Luoma, S. N.; Johns, C.; Fisher, N. S.; Steinberg, N. A.; Oremland, R. S.; Reinfelder, J. R. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. *Environ. Sci. Technol.* **1992**, *26*, 485–491.
- (34) Lam, I. K. S.; Wang, W. X. Transgenerational retention and maternal transfer of selenium in *Daphnia magna*. *Environ. Toxicol. Chem.* **2006**, *25*, 2519–2525.
- (35) Heinz, G. H.; Hoffman, D. J.; Krynitsky, A. J.; Weller, D. M. G. Reproduction in mallards fed selenium. *Environ. Toxicol. Chem.* **1987**, *6*, 423–433.
- (36) Ingersoll, C. G.; Dwyer, F. J.; May, T. W. Toxicity of inorganic and organic selenium to *Daphnia magna* (Cladocera) and *Chironomus riparius* (Diptera). *Environ. Toxicol. Chem.* **1990**, *9*, 1171–1181.
- (37) Malchow, D. E.; Knight, A. W.; Maier, K. J. Bioaccumulation and toxicity of selenium in *Chironomus decorus* larvae fed a diet of seleniferous *Selenastrum capricornutum*. *Arch. Environ. Contam. Toxicol.* **1995**, *29*, 104–109.
- (38) Hoffman, D. J. Role of selenium toxicity and oxidative stress in aquatic birds. *Aquat. Toxicol.* **2002**, *57*, 11–26.
- (39) Kaushal, N.; Bansal, M. P. Diminished reproductive potential of male mice in response to selenium-induced oxidative stress: Involvement of HSP70, HSP70–2, and MSJ-1. *J. Biochem. Mol. Toxicol.* **2009**, *23*, 125–136.
- (40) Hoffman, D. J.; Marn, C. M.; Marois, K. C.; Sproul, E.; Dunne, M.; Skorupa, J. P. Sublethal effects in avocet and stilt hatchlings from selenium-contaminated sites. *Environ. Toxicol. Chem.* **2002**, *21*, 561–566.

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