Assessing selenium dynamics at the base of aquatic food webs: Application of a novel periphyton-mayfly test system

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Introduction

- Selenium contamination is a major concern in aquatic ecosystems due to well documented impacts on upper trophic level organisms, such as fish and birds, including reproductive impairment and teratogenic deformities (*Lemly, 1993*).
- Se exposure is largely a function of diet and dissolved concentrations are not necessarily predictive of the eventual body burdens found in aquatic organisms (*Luoma and Presser, 2009*).
- Currently, aquatic invertebrates are viewed as being relatively tolerant to Se exposure and act merely as trophic conduits of Se from primary producers, which readily bioconcentrate Se, to fish and birds.
- Dissolved Se is dominated by the inorganic forms selenite (SeO_3) and selenate (SeO_4), however aquatic organisms largely possess organo-Se species (i.e. selenocysteine and selenomethionine) (*Fan et al., 2002*).
- Field studies have correlated Se concentration with loss of abundance and diversity of mayflies, however little to no laboratory studies on aquatic insects are available to reference (*Pond et al., 2008*).
- The parthenogenetic mayfly *Centroptilum triangulifer* can be reared on periphyton plates in the lab creating a novel test system for assessing chemical exposures.

Larval and adult *Centroptilum triangulifer* (photos by David Funk)

Objectives

- Quantify the movement of Se from water to periphyton to mayflies to eggs
- Determine what adverse effects, if any, dietary Se exposure has on mayfly fitness and reproduction
- Preliminary assessment of:
  - Second generation effects of dietary Se exposure on mayfly development
  - How initial dissolved Se speciation affects resulting Se speciation in periphyton and larval mayflies

Methods

- *Centroptilum triangulifer* eggs and periphyton plates were provided by Stroud Water Research Center
- Full life cycle dietary Se exposures were conducted using ^{75}Se (as Na_2SeO_3) as a radiotracer with stable Na_2SeO_3 providing the remainder of Se in solution
- ^{75}Se radioactivity was measured using a Perkin-Elmer Wallac Wizard 1480 automatic gamma counter
- Two rounds of experiments were conducted with two replicates per treatment of the following Se concentrations (initial dissolved):
  - Nov 08: 0 and 20 µg L^{-1}
  - Jan 09: 0, 5, 10*, and 20 µg L^{-1} (*one replicate due to periphyton availability)
- Periphyton plates were loaded with ^{75}Se for 7-9 d then moved to clean artificial soft water, 20 mayflies were added to each replicate, and exposures were conducted for ~35 d
- Concentrations are reported in dry wt as measured using a microbalance. Digitally photographed egg clutches were counted using Compix Simple PCI Image Processing
- Statistical calculations were performed using GraphPad Prism 5.0 software.
- Second generation mayflies were raised using periphyton exposed to 0 and 20 µg L^{-1} Se.
- Speciation analysis was conducted at the National Synchrotron Light Source on periphyton and mayflies differentially exposed to 0 and 20 µg L^{-1} SeO_3 or SeO_4 (non-radioactive) for ~21 days.

Results and Discussion

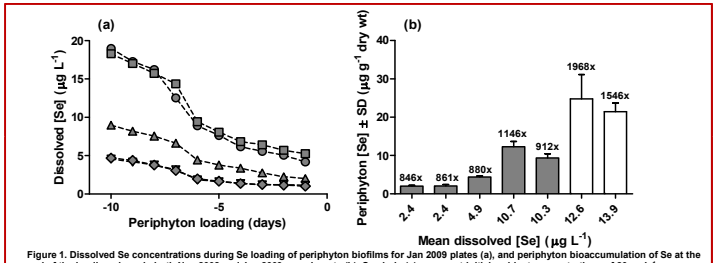

Figure 1. Dissolved Se concentrations during Se loading of periphyton biofilms for Jan 2009 plates (a), and periphyton bioaccumulation of Se at the end of the loading phase in both Nov 2008 and Jan 2009 experiments (b). Symbols (a) represent initial ambient concentrations of 20 µg L^{-1} (replicates 20C(●) and 20D(□)), 10 µg L^{-1} (10A(△)), and 5 µg L^{-1} (replicates 5A(▽) and 5B(◇)). Gray bars (Jan 2009) and white bars (Nov 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 (source: *Conley et al., 2009*).

- Mean dissolved Se concentrations during the loading phase ranged from 2.4 – 13.9 µg L^{-1} resulting in periphyton concentrations ranging from 2.0 – 25.5 µg g^{-1}
- On average, periphyton bioconcentrated Se 1113 ± 430 -fold above dissolved levels

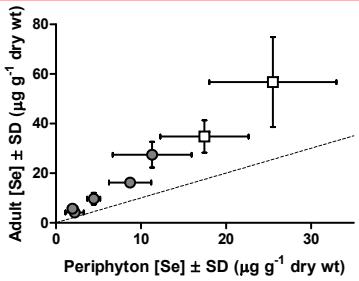

Figure 2. Trophic transfer of Se from periphyton into adult mayflies (postpartum). Symbols represent Jan 2009 exposures (●) and Nov 2008 exposures (□). A 1:1 line (dashed) is provided for reference only. Data points above the dashed line represent biomagnifications of Se (source: *Conley et al., 2009*).

- **Pearson r > 0.99**
- **p-value < 0.0001**
- **Larvae n (from left to right): 15, 12, 5, 13, 12, 17, 6**
- **Periphyton n: 9 (Jan 09), 6 (Nov 08)**

- Mayflies accumulated mean body burdens ranging from 4.2 – 56.7 µg g^{-1}
- Trophic transfer factors ranged from 1.9 – 2.9 fold with a mean of 2.2 ± 0.4 -fold

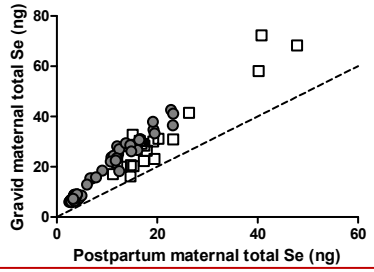

Figure 3. Maternal transfer of Se plotted as total Se (ng) in postpartum adults versus gravid adults for both experiments. Symbols represent Jan 2009 exposures (●) and Nov 2008 exposures (□). A 1:1 line (dashed) is provided for reference only. Data points above the dashed line represent maternal transfer of Se (source: *Conley et al., 2009*).

- **Pearson r > 0.93**
- **p-value < 0.0001**
- **n = 79**

- On average mayflies transferred 46.5% of their body burden to eggs.

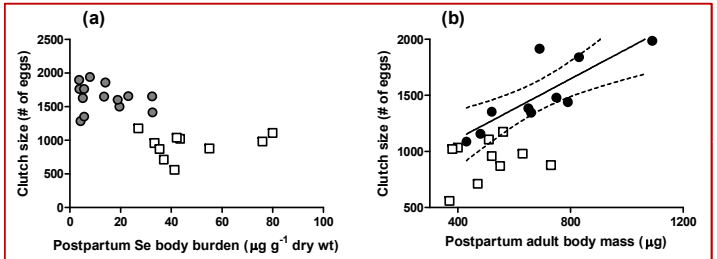

Figure 4. Fecundity of adult mayflies associated with postpartum adult Se body burden (a) and body mass (b). In panel (a) symbols represent Jan 2009 exposures (●) and Nov 2008 exposures (□). Body burden and fecundity were negatively correlated across both experiments ($r = -0.69$, $p = 0.0002$) (a). 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) (source: *Conley et al., 2009*).

- Body burdens > ~11 µg g^{-1} resulted in decreased reproductive performance for many adult mayflies.

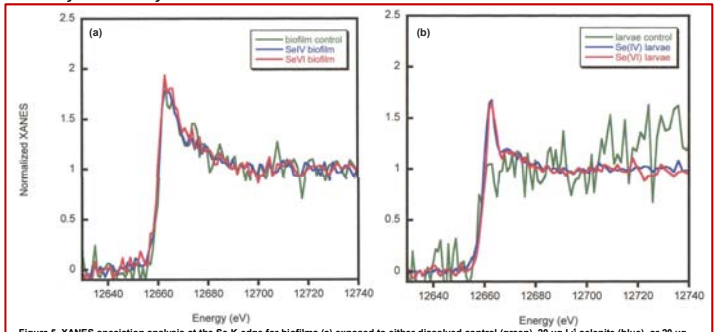

Figure 5. XANES speciation analysis at the Se K-edge for biofilms (a) exposed to either dissolved control (green), 20 µg L^{-1} selenite (blue), or 20 µg L^{-1} selenate (red) and for mayfly larvae (b) exposed to those biofilms under the same dissolved conditions. Periphyton spectra were nearly identical following exposure to each anionic Se form, and displayed enrichment of organo-Se species. Larval speciation was different than that of periphyton, but was also characterized by a high proportion of organo-Se species.

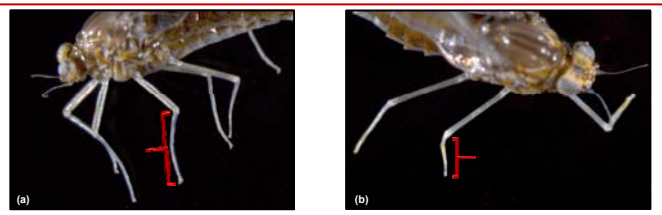

Figure 6. Example images of second generation *C. triangulifer* adults raised on control diet (a) and Se contaminated diet (b, ~ 25 µg g^{-1}). Control adults display normal tibial and tarsal development of limbs, whereas Se adults display teratogenic deformities of both the tibial and tarsal segments. Images were generated under different magnifications, however the critical aspect is the relative length ratios of limb segments.

Research Gaps and Priorities

- Determine the relative uptake kinetics and maximum steady state Se BCFs of selenite vs. selenate in representative communities of primary producers.
  - Se toxicity may be dictated by the kinetics and extent of Se bioconcentration at the base of aquatic food webs.
- Confirm similarity of Se speciation in periphyton after exposure to selenite vs. selenate using XANES analysis
- Determine dietary Se effect concentration levels on the formation of teratogenic defects in second generation mayflies.
  - Maternal transfer results in elevated body burdens for second generation mayflies, potentially making them even more sensitive to effects than first generation exposures.

Citations

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