R.E. Casey, S.M. Lev, J.W. Snodgrass. Diet vs. Sediment: Relative importance in trace metal trophic transfer to grazing larval amphibians.

Determining the relative contributions of different routes of exposure to trace element uptake in grazing aquatic organisms is important when evaluating the dominant pathways connecting contaminated environmental media with the biosphere. Biofilms growing on contaminated sediments may represent a distinct route of transfer as the biofilm itself may accumulate trace elements with differing availability for uptake than the contaminated sediment itself. Many larval amphibians forage by consuming biofilm growing on the sediment surface; as a result sediment is co-ingested along with the biofilm. Many studies have evaluated the relative contributions of water column and dietary metal levels to metal accumulation in primary consumers (e.g. Roy and Hare 1999, Guan and Wang 2004) and several have evaluated sediment versus water column contributions (Warren et al. 1998, Hare et al. 2001) to metal accumulation in aquatic biota. However, no studies have yet described the relative contributions of co-ingested sediment and biofilm to metal accumulation. In part, this is due to the technical difficulties of physically separating biofilm from contaminated sediment in the field or in laboratory exposures. This project describes experiments in which the relative contributions of these different media can be measured, specifically with regards to Zn accumulation from roadway-derived particulate matter (as described in Project 1).

Earlier experiments in our laboratory utilized coal combustion waste (CCW) as a contaminated sediment to assess the relationship between route of exposure and the accumulation of trace elements in larval amphibians. Results indicated that ingestion of contaminated biofilm was the dominant mechanism for Se accumulation in larval wood frogs; contaminated sediment contributing no additional Se to the body burden. Both biofilm and sediment were significant routes of transfer for As; sediment alone explained the uptake observed for Cr and Ni.

In the current experiments, we will evaluate the role of biofilm versus sediment for the transfer of Zn from contaminated sediments to larval amphibians. As with Project 1, we will utilize a stable isotope approach to increase our ability to attribute accumulated Zn in biota to specific sources. Algal biofilms will be cultured in aquaria on plastic tiles with two levels of ^{68}Zn-enriched nutrient media to obtain isotopically distinct biofilms, one with high and one with low total Zn. Dose-dependent accumulation of Zn in algae has already been demonstrated (Guan and Wang 2004, De Schamphelaere et al. 2004). Tiles will be scraped weekly for several weeks and the resulting biofilm dried, homogenized and refrigerated. We will collect roadway particulate debris using a forensic vacuum with a 0.2 μm filter and sieve the dust to obtain the < 63 μm size fraction. Earlier work in our laboratory showed that this fraction contains the highest Zn levels (Camponelli 2006). Amplexed pairs of Fowlers toads (*Bufo fowleri*) or American toads (*Bufo americanus*), depending on availability, will be transported to the laboratory where they will be allowed to deposit eggs. Newly hatched tadpoles will be utilized in the experiments.

As in previous experiments, treatments will consist of four exposures involving varying Zn levels and the presence or absence of a contaminated sediment (sieved road dust): 1) low Zn algae; 2) low Zn algae with road dust; 3) high Zn algae; 4) high Zn algae with road dust. In this case, low Zn algae is representative of an uncontaminated site and high Zn algae represents a contaminated site. After 2 weeks of exposure, larvae will be euthanized, gut coils excised and bodies and digestive tracts analyzed separately for total Zn body burdens and $^{68}Zn/^{66}Zn$ ratios, as described in Project 1. Previous work has demonstrated that road dust has a background isotopic composition and we will confirm that the same is true of the larval amphibians. Biofilm is the only source of enriched ^{68}Zn, so elevation of this ratio in amphibian larvae will indicate the uptake of Zn from the biofilm diet. Comparison of the levels of accumulated Zn between treatments will allow us to determine the dominant routes of exposure as with our previous experiments. However, with the isotopic data, we can use a mixing model (as in Project 1) to quantify the mass of Zn accumulated from contaminated diet in comparison with contaminated sediment and determine the proportion of accumulated Zn coming from each source. No data differentiating these pathways quantitatively yet exist in the literature.