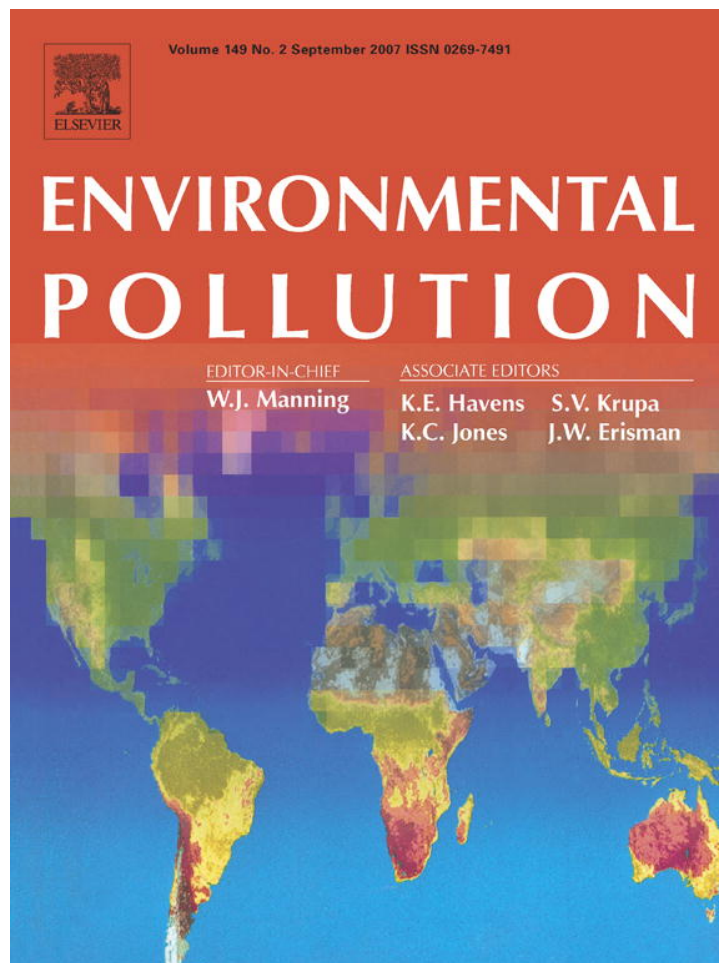


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# Bioaccumulation of trace elements in omnivorous amphibian larvae: Implications for amphibian health and contaminant transport

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Received 14 August 2006; received in revised form 3 January 2007; accepted 7 January 2007

*Omnivorous amphibian larvae can be efficient accumulators of trace elements.*

## Abstract

Despite the influence that amphibians have on the flow of energy and nutrients in ecological systems, the role that amphibians play in transporting contaminants through food webs has received very little attention. This study was undertaken to investigate bioaccumulation of trace elements in amphibians relative to other small aquatic organisms in a contaminated wetland. We collected bullfrog larvae (*Rana catesbeiana*) along with three other species of small vertebrates and four species of invertebrates from a site contaminated with a wide array of trace elements and analyzed them for trace element concentrations and stable nitrogen and carbon isotope composition. We found that amphibian larvae accumulated the highest concentrations of most trace elements, possibly due to their feeding ecology. These results suggest that omnivorous amphibian larvae can serve as a critical link for trace element trophic transfer. Their propensity to accumulate trace elements may have important implications for amphibian health in contaminated environments and should be further investigated.

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*Keywords:* Trace element; Amphibian; Bioaccumulation; Coral combustion waste; Stable isotope

## 1. Introduction

Amphibians are critical components of aquatic and terrestrial ecosystems. Frogs and salamanders can exceed average terrestrial and aquatic densities of 40,000 individuals/hectare (Stewart and Woolbright, 1996; Harris et al., 1988; Jaeger, 1980), often representing the single most abundant vertebrate in natural systems. A recent study indicates that a single 10 ha isolated wetland produced >360,000 metamorphic amphibians (>1400 kg of biomass) in 1 year (Gibbons et al., 2006), suggesting that energy and nutrient subsidies may be greater than previously realized in some habitats. Such high abundance, coupled with the voracious appetites of many amphibian species

(Taylor et al., 1988) and their high conversion efficiency (Burton and Likens, 1975; Grayson et al., 2005), enables amphibians to have a profound influence on the flow of energy and key nutrients through food webs. For example, experimental exclusion of anurans in a subtropical forest reduced concentrations of dissolved organic carbon and nitrogen, as well as Ca, Fe, Mg, Mn, P, K, and Zn by 60–100% in leaf wash (throughfall) and reduced K and P availability in decomposing leaf litter by 12–14% (Beard et al., 2002). Salamander predation on leaf litter fragmenters can reduce leaf litter decomposition rates by as much as 17%, potentially altering forest carbon dynamics (Wyman, 1998). Amphibians can also subsidize aquatic and terrestrial habitats with substantial quantities of energy and nutrients as they move across habitat boundaries (Seale, 1980; Regester et al., 2006).

Despite the influence that amphibians have on the flow of energy and nutrients in ecological systems, the role that

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amphibians play in transporting environmental contaminants through food webs has received little attention. A propensity to bioaccumulate contaminants may enhance their risk of experiencing deleterious effects. Anuran larvae are often omnivorous, ingesting everything from sediment and phytoplankton to conspecifics and carrion (Hoff et al., 1999). Thus, it is possible that the feeding habits of some anuran larvae expose them to a wide variety of contaminants, some of which may not be available to more narrow dietary specialists. That anuran larvae can accumulate high concentrations of contaminants when only polluted sediments are present (Snodgrass et al., 2004) also suggests that they may be critical in transforming contaminants from less bioavailable forms to those that are bioavailable to higher trophic level consumers such as fish, birds, and reptiles. Finally, the complex life cycles of many amphibians may make them important for transferring contaminants from aquatic to terrestrial food chains (Roe et al., 2005), and vice versa (Hopkins et al., 2006).

The current study was undertaken to determine whether amphibians can bioaccumulate trace elements to a greater extent than other organisms in a trace-element contaminated ecosystem. Some amphibians are known to accumulate high concentrations of metalloids and organic contaminants in their tissues (Sparling, 2000; Hopkins et al., 2006; Roe et al., 2006), but how their accumulation relates to bioaccumulation in other organisms from the same community has seldom been evaluated (Phaneuf et al., 1995). To accomplish our objective, we sampled bullfrog larvae (*Rana catesbeiana*) along with three other species of small vertebrates and four species of invertebrates from a site contaminated with a wide array of trace elements. Because trophic position can in some cases influence contaminant accumulation in aquatic organisms (Fagerstrom, 1991; Cabana and Rasmussen, 1994; Kiriluk et al., 1995; Jarman et al., 1996; Pokarzhevskii and vanStraalen, 1996; Reinfelder et al., 1998), abundant animal species were selected for comparison that represent a wide range of trophic niches (Table 1). To further understand the relative trophic position of each species, we analyzed the stable isotopes of carbon (as  $\delta^{13}\text{C}$ ) and nitrogen (as  $\delta^{15}\text{N}$ ) in dried tissue samples. Little trophic isotopic fractionation of C occurs ( $<1\%$ ) during

assimilation of foods, making  $\delta^{13}\text{C}$  values useful in attributing various C sources to organisms where such C sources differ in their carbon isotope ratios due to differences in carbon fixation pathways (DeNiro and Epstein, 1978). On the other hand, N isotopes are more fractionated during amino acid and protein metabolism, leading to an increase in  $\delta^{15}\text{N}$  values of 1–5‰ in each successive trophic level (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001; Post, 2002). This makes  $\delta^{15}\text{N}$  useful as an indicator of trophic level (Cabana and Rasmussen, 1994). Stable isotope ratios in consumers provide temporally and spatially integrated information about their diet. Therefore, isotope analysis can often compliment traditional techniques, such as stomach content analyses, which only provide data on foraging activity that are specific to a location and point in time.

## 2. Materials and methods

### 2.1. Site description and sampling

The study site was a 2 ha drainage swamp located near the D-area power and steam generation facility on the U.S. Department of Energy's Savannah River Site (SRS) on the upper coastal plain of South Carolina (33.1°N, 81.3°W). The SRS is a 778 km<sup>2</sup> area established in the early 1950s by the United States Atomic Energy Commission for the production of nuclear materials. The D-area power plant disposes of sluiced ash from a coal-fired power plant into a series of settling basins, which eventually drain into a swamp (the study site) and then into Beaver Dam Creek, a tributary of the Savannah River. The swamp is a shallow basin (generally  $<0.5$  m) characterized by a deeper central channel ( $<1.5$  m) leading from the inflow to the outflow, and larger, shallow, and generally more stagnant peripheral areas that are dominated by emergent macrophytes. The small spatial extent of the swamp (2 ha), and the fact that the swamp has one small inlet and one small outlet acted as restrictions for migration of biota and minimized potential influences of spatial heterogeneity and movement of organisms on contaminant accumulation and stable isotope values. Continual input of ash particles and frequent re-suspension of sediment following inclement weather result in a thick coating of ash/sediment particles on submerged surfaces.

Bullfrog larvae (*R. catesbeiana*), snails (*Heliosoma trivolvis*), clams (*Corbicula fluminea*), odonate larvae (*Tramea* sp. and *Erythemis* sp.), eastern mosquitofish (*Gambusia holbrooki*), juvenile spotted sunfish (*Lepomis punctatus*), and juvenile largemouth bass (*Micropterus salmoides*) were collected by hand or in minnow traps from random locations throughout the swamp during September and October, 2002. The size of the bullfrog larvae indicated that they

Table 1

Summary of species collected for analysis of trace element concentrations and stable isotope ratios for nitrogen and carbon from a drainage swamp contaminated by coal combustion waste from a coal-fired power plant on the U.S. Department of Energy Savannah River Site near Aiken SC USA

Species	Common name	Trophic classification <sup>a</sup>	<i>n</i> <sup>b</sup>	Body mass (mean $\pm$ SD; g dry mass)	Standard length (mean $\pm$ SD; mm)	References for trophic classification
<i>Heliosoma trivolvis</i>	Marsh ramshorn	Herbivore	55(3)	0.01 $\pm$ 0.006		Lombardo and Cooke (2002)
<i>Corbicula fluminea</i>	Asian clam	Herbivore (detritivore)	24(3)	0.02 $\pm$ 0.006	19 $\pm$ 3	Hakenkamp et al. (2001)
<i>Lepomis punctatus</i>	Spotted sunfish (juvenile)	Carnivore (omnivore)	15	1.47 $\pm$ 0.395	53 $\pm$ 5	Lattimore and Gibbons (1976)
<i>Gambusia holbrooki</i>	Eastern mosquitofish	Omnivore	12	0.24 $\pm$ 0.048	37 $\pm$ 2	Garcia-Berthou (1999)
<i>Rana catesbeiana</i>	Bullfrog tadpole	Omnivore	16	0.27 $\pm$ 0.265		Hoff et al. (1999)
<i>Tramea</i> sp.	Saddlebags dragonfly nymph	Carnivore	122(5)	0.02 $\pm$ 0.017		Westfall (1984)
<i>Erythemis</i> sp.	Pondhawk dragonfly nymph	Carnivore	62(5)	0.03 $\pm$ 0.014		Westfall (1984)
<i>Micropterus salmoides</i>	Largemouth bass (juvenile)	Carnivore	16	0.72 $\pm$ 0.349	54 $\pm$ 8	Carlander (1977)

<sup>a</sup> Occasional minor component of trophic classification shown in parentheses.

<sup>b</sup> Number of organisms collected, when individuals were pooled for analyses number of pooled samples shown in parentheses.

were most likely <1-year-old and they had not yet developed hindlimbs (Table 1.). The organisms were held in dechlorinated tap water for 48 h to clear their gut contents (Burger and Snodgrass, 1998). They were then sacrificed, weighed and stored at  $-70^{\circ}\text{C}$  until preparation and analysis. Only soft tissues were retained for snails and clams. Grab samples of sediment and water were also obtained from random locations in the swamp and stored at  $-4^{\circ}\text{C}$  until preparation and analysis. The number of replicates, size, general trophic classification, and references for trophic classification for the organisms are given in Table 1. Some organisms (i.e. snails, clams, and odonate larvae) were too small for individual trace element analyses and were therefore pooled. These animals were first weighed and grouped into pooled samples of similar size (Table 1) so that potential allometric effects on contaminant accumulation and stable isotope values could be accounted for. A sufficient number of animals for both stable isotope and trace element analysis within each size class were then pooled. Odonate larvae were divided into five size classes while clams and snails were divided into three size classes. All organisms were lyophilized and finely pulverized using a cryogenic mill. For two of the fish species (*L. punctatus* and *M. salmoides*), only juveniles were captured because the size of adult fish would preclude meaningful comparisons to small amphibian larvae.

## 2.2. Trace element analyses

Sediment and tissue samples were either digested in trace metal grade  $\text{HNO}_3$  using a MARS-5 microwave digestion system (CEM, Matthews, NC, USA) or trace metal grade  $\text{HNO}_3$  and ACS grade 30%  $\text{H}_2\text{O}_2$  in an MDS-2000 microwave digestion system (CEM, Matthews, NC, USA). Analytical method blanks and standard reference materials (SRMs) were included in each digestion batch. Trace element (Fe, Mn, V, As, Se, Zn, Hg, Cd, Cu, Pb) analyses were performed using a Perkin–Elmer Sciex Elan DRC Plus inductively coupled plasma mass spectrometer (ICP-MS; Norwalk, CT, USA) operating in standard mode. Method detection limits (MDLs) for dry samples depended on sample mass and were calculated separately for each observation based on the dry mass of sample analyzed. Average MDLs ranged from  $0.015\ \mu\text{g g}^{-1}$  dry mass for mercury (Hg) to  $8.06\ \mu\text{g g}^{-1}$  dry mass for iron (Fe). Tissue trace element concentrations generally exceeded detection limits with the exception of three observations for (As) in *M. salmoides*. The calculated concentrations for these observations were replaced with one half of the detection limit of each observation for statistical purposes. The analytical error expressed as relative percent difference (RPD) for independent analyses of replicate dilutions of a subsample ranged from 2.9% for Fe to 11.4% for As. The sub-sampling variation expressed as RPD between replicate subsamples ranged from 31% for Pb to 7.4% for Fe. Mean recovery of trace elements for the reference material TORT-2 (lobster hepatopancreas; National Research Council of Canada, Ottawa, ON, Canada) were in good agreement with the certified values and ranged from 77% for Fe to 120% for Pb. Mean analytical spike recovery at approximately 2–5 $\times$  the unspiked sample concentration ranged from 77% for Hg to 101% for Pb ( $n = 10$ ).

## 2.3. Stable isotope analyses

Lyophilized and ground tissue samples of approximately 2–7 mg were weighed to the nearest micrograms into pre-cleaned tin capsules. Small muscle samples were taken from fish and bullfrog larvae and subsamples of homogenized, pooled whole organisms were used for other organisms. We previously determined that variances in stable isotope ratios among pooled samples of invertebrates from the swamp were not significantly different from variances among individuals and that stable isotope ratios in whole fish were equivalent to stable isotope ratios in muscle. Lipids are depleted in  $^{13}\text{C}$  relative to proteins and carbohydrates (DeNiro and Epstein, 1977), so lipids were pre-extracted from animal samples using a 2:1 chloroform:methanol mixture for 24 h to avoid complications arising from differences in lipid content among or within species. Stable isotope ratios were then determined using an elemental analyzer (Carlo Erba NC2500 Elemental Analyzer, Milan, Italy) coupled to a continuous flow isotope ratio mass spectrometer (Finnigan Delta<sup>plus</sup> XL, San Jose, CA, USA). Stable isotope ratios are reported in per mill units (‰) using  $\delta$  notation ( $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1]10^3$ ), where  $X = ^{13}\text{C}$  or  $^{15}\text{N}$  and  $R =$  the

ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  (Fry, 1991). Values were calibrated to the internal standards AIR and V-PDB through an external working standard, DORM-2 (dogfish muscle; National Research Council of Canada, Ottawa, ON, Canada), which has an assigned  $\delta^{13}\text{C}$  of  $-16.94\text{‰}$  and a  $\delta^{15}\text{N}$  value of  $14.01\text{‰}$ . Isotopic compositions were reproducible to  $\pm 0.1\text{‰}$  ( $\pm 1$  standard deviation) for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

We applied a two source mixing model as described by Post (2002) in order to estimate trophic positions. This model requires that the  $\delta^{15}\text{N}$  values for basal resources ( $\delta^{15}\text{N}_{\text{base}}$ ) be known or estimated from  $\delta^{15}\text{N}$  values of primary consumers and that these basal resources have distinct  $\delta^{13}\text{C}$  values. Two end-member primary consumers (i.e. primary consumers with the most extreme  $\delta^{13}\text{C}$  values) are used in the model to estimate  $\delta^{15}\text{N}_{\text{base}}$  for each of two primary carbon sources. In lentic systems, snails and bivalves can be used as end-member primary consumers in a two source mixing model (Post, 2002). The trophic levels of other consumers, which may feed from food webs based on each of the two basal resources, are calculated as: trophic position =  $\lambda + (\delta^{15}\text{N}_{\text{consumer}} - [\delta^{15}\text{N}_{\text{base1}}\alpha + \delta^{15}\text{N}_{\text{base2}}(1 - \alpha)]/\Delta_n$ , where  $\alpha$  is the proportion of N in the consumer derived from the base of food web one,  $\lambda$  is the trophic position of the organisms used to estimate  $\delta^{15}\text{N}_{\text{base}}$  and  $\Delta_n$  is the increase in  $\delta^{15}\text{N}$  per trophic level, for which we used  $3.4\text{‰}$  (Post, 2002). Assuming that movement of N and C through the food web is similar,  $\alpha = (\delta^{13}\text{C}_{\text{consumer}} - \delta^{13}\text{C}_{\text{base2}})/(\delta^{13}\text{C}_{\text{base1}} - \delta^{13}\text{C}_{\text{base2}})$ . When the number of important basal resources ( $n$ ) is  $>2$ , isotope ratios from a minimum of  $n - 1$  elements are required to resolve the system (Peterson and Fry, 1987; Post, 2002). Therefore, an assumption that is required by this model is that only two primary carbon sources are present because use of a single isotope ratio ( $\delta^{13}\text{C}$ ) in the model cannot provide a unique solution when there are more than two possible carbon sources. In addition to using  $\delta^{13}\text{C}$  to fit a two source mixing model for estimating trophic positions, we interpreted the variation in  $\delta^{13}\text{C}$  within a species to determine how broadly they fed from different basal resources or food webs based on these resources. Species that feed from multiple food webs having basal resources with different  $\delta^{13}\text{C}$  values would be expected to have higher variation in their  $\delta^{13}\text{C}$  values than organisms feeding on single food webs with only one important basal resource.

## 2.4. Statistical analyses

Analysis of variance (ANOVA) was used to test for significant variation in stable isotope values and trace element concentrations. Trace element concentrations and stable isotope values tended not to be normally distributed and the variances were unequal among species, so the data were rank-transformed to better satisfy the assumptions of ANOVA. Post hoc multiple comparisons were made using the Student–Neuman–Keuls (SNK) procedure.

The multivariate statistical approach used here is similar to that used in a previous study of trace elements in *R. catesbeiana* larvae exposed to coal ash (Snodgrass et al., 2003) although a few modifications were made. Preliminary analyses of the data indicated that co-linearity existed among concentrations of many trace elements which made it possible to reduce the dimensionality of the data set using principal components analysis (PCA). Principal components analysis identifies a few orthogonal axes in multivariate distributions that summarize the majority of variation in the data set and expresses these axes as linear combinations of the original variables (Everitt and Dunn, 2001). Dry mass was included with trace element concentrations in the PCA to control for potential confounding allometric relationships between body mass and trace element concentrations. First, a matrix of Pearson's correlation coefficients with associated significance tests was constructed for trace element concentrations and body mass. Principal components axes were computed from the correlation matrix. We retained principal component axes with eigenvalues greater than one for further analyses. A varimax rotation was applied to the PCA axes to facilitate their interpretation. We then interpreted the principal components by examining loading of each variable on the rotated axes using factor analysis. Variation in PCA scores was then compared among species using analysis of variance (ANOVA). Principal component scores were rank-transformed to satisfy homoscedasticity assumptions for ANOVA. Post hoc multiple range tests were performed using the SNK procedure. All statistical analyses were performed using SAS version 9.00 (SAS Institute, Cary, NC USA).

### 3. Results

#### 3.1. Trace element concentrations

Significant variation in trace element concentrations was observed among species for every element ( $p < 0.05$ ; Fig. 1). A high degree of co-linearity was observed in the data set as evidenced by Pearson's correlation matrix (Table 2). A Scree plot of the principal components (PC) computed from the Pearson's correlation matrix suggested that the first three PCs should be retained for further analyses since they had eigenvalues greater than one and explained a cumulative 90% of the variation in the data set. The first principal component (PC I) explained 56% of the variation in the data set (Table 3). Factor analysis revealed that this axis was positively related to V, Mn, Fe, Zn, As, Se, and Pb concentrations. The second axis (PC II) explained 20% of the variation in the data set and had a strong positive relationship with Cu and Cd concentrations (Table 2). The third axis (PC III) explained 14% of the variation in the data set and was positively related to Hg concentrations and body mass (Table 3).

Significant differences in mean PC scores were observed among species for each PC axis (Fig. 2). The bullfrog (*R. catesbeiana*) and the snail (*H. trivolvis*) had the highest average scores for PC I (Fig. 2A;  $F_{7,70} = 26.30$ ,  $p < 0.001$ ). The clam (*C. fluminea*) had the highest average scores for PC II (Fig. 2B;  $F_{7,70} = 39.68$ ,  $p < 0.001$ ), with *R. catesbeiana* and the two dragonfly species having the next highest scores. The scores for PC III varied greatly among species (Fig. 2C;  $F_{7,70} = 22.88$ ,  $p < 0.001$ ). Principal component III scores were high in *R. catesbeiana* and *C. fluminea* but much lower in the primary consumer *H. trivolvis*. The scores increased from the dragonfly larvae (*Erythemis* sp. and *Tramea* sp.) to the mosquitofish (*G. holbrooki*), largemouth bass (*M. salmoides*) and spotted sunfish (*L. punctatus*).

To explain the PC groupings of the elements based on their physicochemical properties, a plot of ionization potential versus ionic charge density was constructed and it revealed that the principal components axes were qualitatively related to ionic/covalent bonding tendencies of the elements (Fig. 3). The elements associated with PC I tended to be in the borderline region between "hard" (class A) ions, which have a tendency to form bonds of more ionic character and have greater affinity for O bearing ligands, and "soft" (class B) ions, which have a tendency to form bonds of more covalent character and have greater affinity for S and N bearing ligands (Frausto da Silva and Williams, 2001). The elements associated with PC II had more of a class B tendency. Mercury was the only PC III element and it is well within the class B region. Arsenic and Se were not included in this analysis because they readily hydrolyze to form oxyanions in oxic surface waters rather than cations.

Trace element concentrations in water averaged  $7.0 \pm 0.44$  (V),  $66.6 \pm 4.76$  (Fe),  $7.6 \pm 0.42$  (Cu),  $15.0 \pm 1.77$  (As),  $2.5 \pm 0.23$  (Se),  $5.9 \pm 3.42$  (Mn),  $0.78 \pm 0.062$  (Cd), and  $2.3 \pm 0.24$  (Pb)  $\mu\text{g L}^{-1}$ . Aqueous Zn and Hg concentrations were below their MDLs. Sediment concentrations averaged

$63.8 \pm 7.69$  (V),  $5309 \pm 979.1$  (Fe),  $231.9 \pm 62.18$  (Cu),  $249.5 \pm 34.06$  (Zn),  $100.6 \pm 18.25$  (Mn),  $79.2 \pm 14.45$  (As),  $16.6 \pm 1.95$  (Se),  $8.4 \pm 1.68$  (Cd),  $0.23 \pm 0.029$  (Hg) and  $22.2 \pm 2.63$  (Pb)  $\mu\text{g g}^{-1}$  dry mass.

#### 3.2. Stable isotope values

Significant variation in  $\delta^{13}\text{C}$  values existed among species ( $F_{7,72} = 12.28$ ,  $p < 0.001$ ), ranging from  $-20.0\text{‰}$  to  $-27.5\text{‰}$  (Fig. 4). Body mass generally had no relationship with  $\delta^{13}\text{C}$  value ( $p > 0.107$ ), except for *R. catesbeiana* ( $p = 0.037$ ,  $r^2 = 0.22$ ); however, this relationship was heavily influenced by one very large individual. In the absence of this individual, no significant relationship existed ( $p = 0.173$ ). *R. catesbeiana* had a mean  $\delta^{13}\text{C}$  value of  $-24.6\text{‰}$ . Significant variation in  $\delta^{15}\text{N}$  values also existed among species ( $F_{7,72} = 116.61$ ,  $p < 0.001$ ). The range of  $\delta^{15}\text{N}$  values among individual samples was  $1.7\text{‰}$  (in *H. trivolvis*) to  $11.5\text{‰}$  (in *L. punctatus*). Species mean  $\delta^{15}\text{N}$  values in animals ranged from  $1.9\text{‰}$  for *H. trivolvis* to  $10.1\text{‰}$  for *L. punctatus* (Fig. 4). *Rana catesbeiana* had a mean  $\delta^{15}\text{N}$  value of  $4.5\text{‰}$ . Based upon an assumption of an average increase  $3.4\text{‰}$  in  $\delta^{15}\text{N}$  per trophic level (Post, 2002), our sample ranged over three trophic levels; this generally agrees with trophic classifications provided in the literature (Table 1). No significant linear relationship between body mass and  $\delta^{15}\text{N}$  was observed in any species ( $p > 0.07$  in all cases).

### 4. Discussion

Principal components analysis of these multivariate data indicated that amphibian larvae may be highly susceptible to trace element bioaccumulation relative to other small aquatic organisms in this swamp. *R. catesbeiana* accumulated higher concentrations of most PC I elements (V, Mn, Fe, Zn, As, Se and Pb) than any other species, including small aquatic predators. The single exception to this being Mn where *H. trivolvis* had the highest mean concentration and *R. catesbeiana* had the second highest mean concentration. In addition, *R. catesbeiana* consistently accumulated concentrations of elements loading on PC II (Cd and Cu) and PC III (Hg) that were greater than or equal to those accumulated by higher trophic level organisms in the community. Only *C. fluminea* surpassed *R. catesbeiana* in accumulating any of these elements. With the exception of Hg, the only trace element that consistently biomagnifies, lower trophic level organisms (*R. catesbeiana*, *H. trivolvis*, and *C. fluminea*) accumulated higher concentrations of trace elements than higher trophic level organisms (*L. punctatus*, *G. holbrooki*, *Tramea* sp., *Erythemis* sp. and *M. salmoides*). This suggests that the relatively low trophic status of *R. catesbeiana* partially contributed to their propensity to bioaccumulate trace elements.

Although the PC I trace elements bioaccumulated to the greatest extent in *R. catesbeiana* larvae, not all of the accumulated elements may be assimilated into tissues and/or completely retained through metamorphosis. Snodgrass et al. (2003) demonstrated that concentrations of some trace elements, namely V, As, Al, Cr, Cu and Ni can decrease significantly

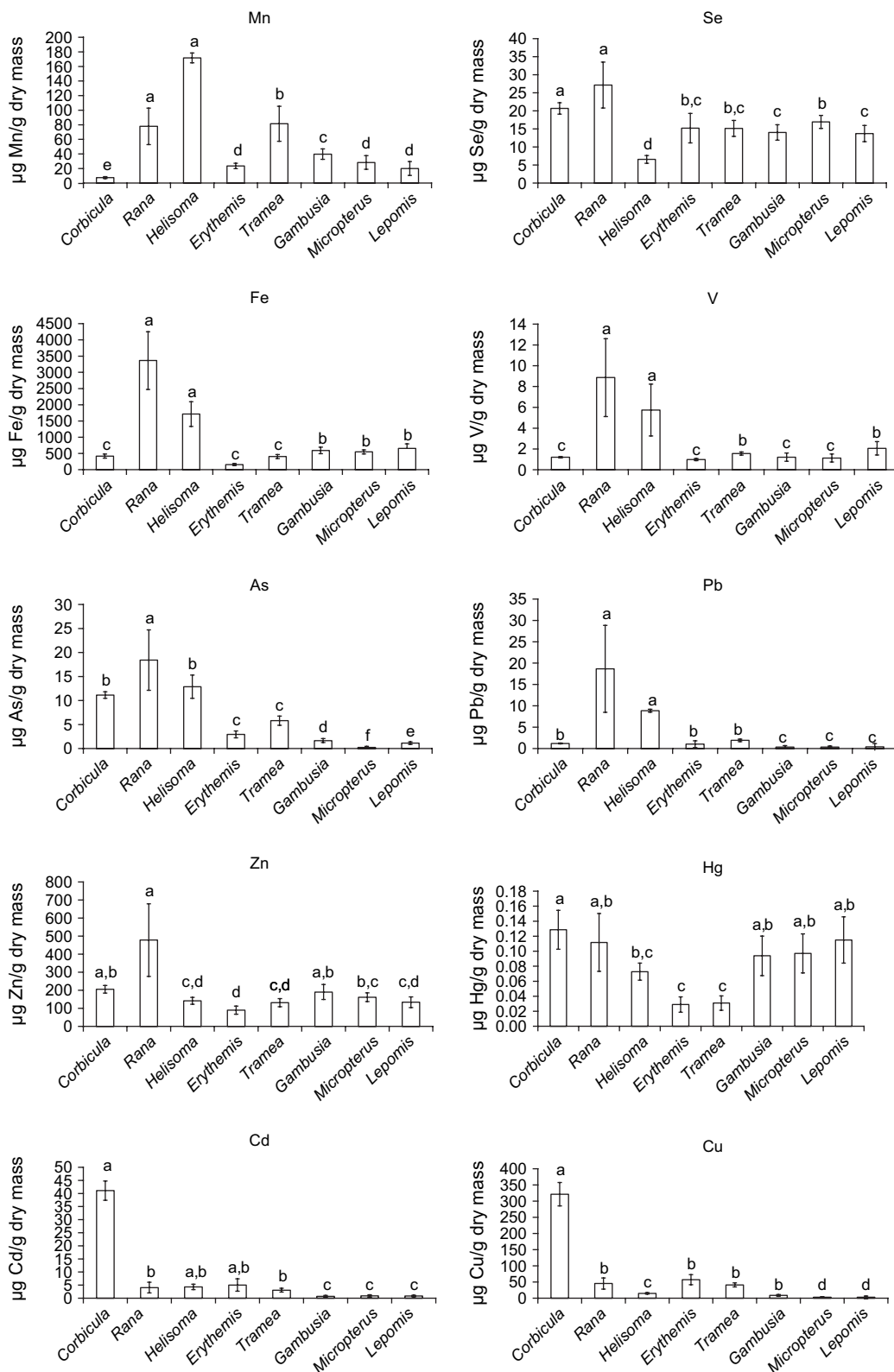


Fig. 1. Trace element concentrations in *Corbicula fluminea*, *Rana catesbeiana*, *Helisoma trivolvis*, *Tramea* sp., *Erythemis* sp., *Gambusia holbrooki*, *Lepomis punctatus*, and *Micropterus salmoides* collected from a coal ash impacted swamp on the U.S. Department of Energy Savannah River Site near Aiken, SC, USA. The species are indicated in the graphs by their generic name. Error bars represent standard errors of the means and significantly different means are indicated by different letters above the bars. The species are arranged according to their trophic classifications with trophic status increasing from left to right.

Table 2

Pearson's correlation matrix for trace element concentrations in whole bodies of aquatic organisms from a swamp contaminated by coal combustion in South Carolina, USA

	Mass	V	Mn	Fe	Cu	Zn	As	Se	Cd	Hg
V	-0.19									
Mn	<b>-0.46</b>	<b>0.60</b>								
Fe	-0.22	<b>0.89</b>	<b>0.62</b>							
Cu	<b>-0.37</b>	0.11	-0.04	0.09						
Zn	<b>-0.28</b>	<b>0.73</b>	<b>0.44</b>	<b>0.85</b>	0.18					
As	<b>-0.41</b>	<b>0.90</b>	<b>0.66</b>	<b>0.89</b>	<b>0.37</b>	<b>0.75</b>				
Se	-0.21	<b>0.66</b>	<b>0.25</b>	<b>0.74</b>	<b>0.28</b>	<b>0.73</b>	<b>0.71</b>			
Cd	<b>-0.31</b>	0.02	-0.07	0.02	<b>0.97</b>	0.11	<b>0.28</b>	0.21		
Hg	<b>0.35</b>	<b>0.31</b>	-0.03	<b>0.37</b>	0.14	<b>0.46</b>	<b>0.27</b>	<b>0.27</b>	0.16	
Pb	<b>-0.30</b>	<b>0.79</b>	<b>0.62</b>	<b>0.94</b>	0.15	<b>0.83</b>	<b>0.88</b>	<b>0.67</b>	0.07	<b>0.35</b>

Correlation coefficients shown in bold are significantly different from zero ( $p < 0.05$ ).

through metamorphosis in *R. catesbeiana* larvae. They hypothesized that these elements may be bound to exterior cell surfaces of the gut epithelium in tadpoles and are lost as these cells are shed during metamorphosis when the gut is remodeled. In contrast, some potentially toxic elements such as Cd, Ag, Se and Sr were retained through metamorphosis. In another study, Snodgrass et al. (2005) observed decreases of Cd, Cu, Fe, Sr, and V concentrations through metamorphosis in *Rana clamitans* larvae while Zn, As, and Se were retained in terrestrial metamorphs. Mercury was not included in either of these studies. However, evidence from a controlled feeding study and a field study of *Rana sphenocephala* suggests that little Hg is lost through metamorphosis (Unrine and Jagoe, 2004; Unrine et al., 2005). Furthermore, some studies have demonstrated that although a large fraction of trace elements may be associated with the gut coil, concentrations in carcasses with gut removed still have concentrations that are high compared to other organisms (Unrine et al., 2005; Hall and Mulhern, 1984). Differential elimination and retention of trace elements through metamorphosis has important implications

for their potential effects on juvenile life stages, as well as contaminant transport from the aquatic to terrestrial environment. However, organisms preying on the larval stages would be exposed at a minimum to the pre-metamorphic concentrations presented in this study, highlighting the importance of amphibian larvae in trophic transfer and bioavailability within the aquatic community. That Hg and Se are retained through metamorphosis has important implications for the terrestrial environment and for amphibian health as these elements can be efficiently transmitted through food webs and have been documented to cause wide spread adverse effects in wildlife (Ohlendorf, 2003; Wiener et al., 2003).

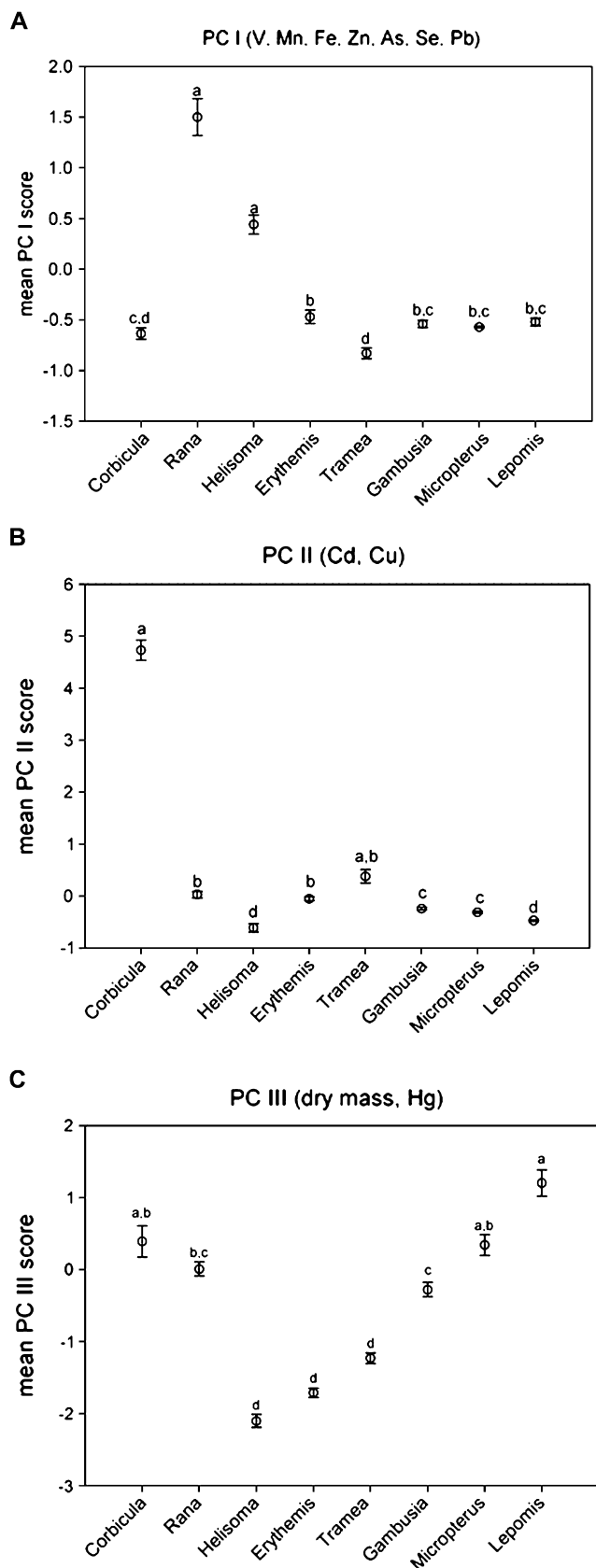
We hypothesize that *R. catesbeiana* and *H. trivoltis* accumulated such high concentrations of PC I elements (Fe, Mn, Pb, V, Zn, As and Se) because both species graze periphyton, detritus, and other biotic and abiotic materials from submerged surfaces (or procedurally defined *aufwuchs* sensu Newman et al., 1985; Newman, 1989 hereafter referred to as *aufwuchs*). Previous studies in this coal ash contaminated wetland have shown that high concentrations of trace elements are associated with Fe and Mn oxides which can comprise a major fraction of the abiotic material in *aufwuchs* (Newman et al., 1985; Newman, 1989). The cations  $Pb^{2+}$ ,  $VO_2^{2+}$ , and  $Zn^{2+}$  can bind to hydrous Mn oxides as these oxides would be expected to have a net negative surface charge at circumneutral pH due to their low pH of zero charge ( $pH_{zpc}$ ; 1.5–5.6) while the oxyanions As and Se could be associated with Fe oxides which would be expected to have a net positive surface charge under these conditions because of their relatively high  $pH_{zpc}$  values (6.5–8.7) (Newman et al., 1985). Furthermore, cations such as  $Pb^{2+}$  and  $Cu^{2+}$  can be bound directly to Fe oxides by inner sphere mechanisms, which can occur below the  $pH_{zpc}$  (Templeton et al., 2003; Peacock and Sherman, 2005). If *R. catesbeiana* is able to liberate and assimilate metals bound to ingested mineral particles, this may partially explain why *R. catesbeiana* accumulated concentrations of the PC I elements far exceeding those of other organisms. The fact that the elements loaded on each of the principal components could be grouped according to chemical bonding tendencies (Fig. 3) supports the contention that the physico-chemical properties of the elements played an important role in their patterns of bioaccumulation among species. For example the PC I elements had the most "Class A" character of the

Table 3

Loading of trace elements on the first three principal component axes from a principal component analysis (PCA) of trace element concentrations in aquatic biota from a swamp contaminated by coal combustion waste on the U.S. Department of Energy Savannah River Site in South Carolina, USA

Variable	Rotated PC axis		
	I	II	III
Mass	-0.26	-0.35	<b>0.79</b>
V	<b>0.91</b>		
Mn	<b>0.65</b>		-0.54
Fe	<b>0.98</b>		
Cu		<b>0.98</b>	
Zn	<b>0.89</b>		
As	<b>0.92</b>		
Se	<b>0.77</b>		
Cd		<b>0.98</b>	
Hg	0.41		<b>0.77</b>
Pb	<b>0.94</b>		
% of variance	56	20	14

Axes were rotated using a varimax rotation to aid in interpretation. Only loadings  $>0.25$  or  $<-0.25$  are shown for clarity and the heaviest loading for a given element is shown in bold. Percent of variance in the data set summarized by each of the axes is also given.



elements analyzed and would therefore be preferentially bound to the O bearing ligands found on the metal oxide surfaces to which *R. catesbeiana* would be exposed through grazing on sediment and *aufwuchs*. The observed difference in accumulation between the two grazers (*R. catesbeiana* and *H. trivolvis*) likely relates to differences in their foraging ecology. Although both species graze *aufwuchs*, *R. catesbeiana* larvae also directly ingest sediments, which have high trace element concentrations and can concentrate trace elements from the PC I grouping due to the presence of Fe and Mn oxides. *H. trivolvis* tend not to rely on detritus from sediment as a food resource (Table 1), which is supported in this study by the relatively narrow range of  $\delta^{13}\text{C}$  values for *H. trivolvis*, which are much higher than those of the sediment.

*R. catesbeiana* had concentrations of the PC II elements (Cd and Cu) that were greater than or equal to all other organisms besides *C. fluminea*. *C. fluminea* may have accumulated such high concentrations of Cd and Cu because of physiological mechanisms specific to this species. For example, extreme accumulation of Cu in *C. fluminea* has been observed previously and was related to extremely slow elimination processes (Croteau et al., 2004).

Mercury concentrations (PC III) in *R. catesbeiana* larvae were as high as those in the predatory fish *L. punctatus* and *M. salmoides*. Although Hg is the only metal known to consistently biomagnify (Wiener et al., 2003), speciation of Hg must be taken into consideration because, while methylmercury (MMHg) concentrations tend to increase with trophic level, inorganic mercury (Hg (II)) concentrations show the opposite pattern (Hill et al., 1996; Houserova et al., 2007). A recent study (Unrine et al., 2005) demonstrated that Hg concentrations in *R. sphenoccephala* tadpoles are comparable to higher trophic level fish in isolated wetlands contaminated by atmospheric Hg deposition because of elevated concentrations of Hg (II) in the tadpoles' diet. Thus, bioaccumulation of high concentrations of Hg in amphibian larvae may be consistent among species and habitat types. Mercury in *aufwuchs* and in larval anuran gut contents from these habitats is primarily Hg (II), and although total Hg concentrations decrease through trophic transfer from *aufwuchs* to larvae, the relative % MMHg increases (Unrine and Jagoe, 2004; Unrine et al., 2005). Thus, anuran larvae may also help to enrich MMHg content, thereby enhancing Hg bioavailability and trophic transfer in wetlands.

Bioaccumulated Hg concentrations in *R. sphenoccephala* larvae from isolated wetlands contaminated by atmospheric deposition of Hg can often exceed concentrations at which

Fig. 2. Comparison of mean principal components (PC) scores for aquatic organisms collected from a coal ash impacted swamp on the U.S. Department of Energy Savannah River Site near Aiken, SC, USA. Data are presented for *Corbicula fluminea*, *Rana catesbeiana*, *Helisoma trivolvis*, *Tramea* sp., *Erythemis* sp., *Gambusia holbrooki*, *Lepomis punctatus*, and *Micropterus salmoides*. The species are indicated in the graphs by their generic name. Error bars represent standard errors of the means and significantly different means are indicated by different letters above the bars. Panel A shows PC I scores which is associated with V, Mn, Fe, Zn, As, Se and Pb concentrations, panel B shows PC II scores which are related to Cd and Cu concentrations and panel C shows PC III scores which are related to Hg concentrations and dry body mass.



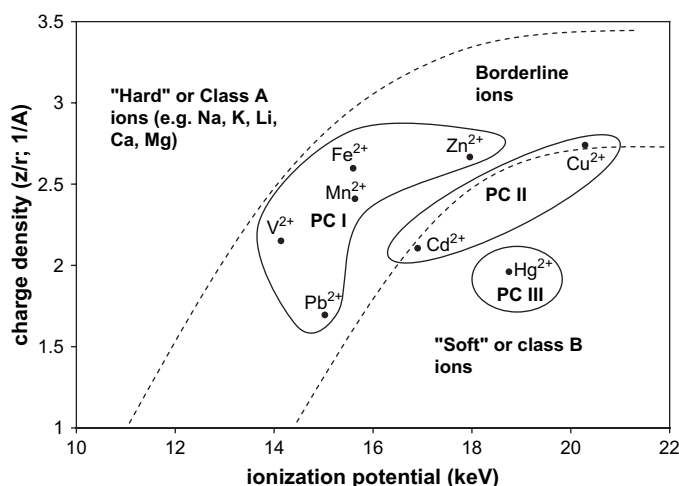


Fig. 3. Second ionization potential versus ionic charge density for elements analyzed in aquatic organisms collected from a coal ash impacted swamp on the U.S. Department of Energy Savannah River Site near Aiken, SC, USA. Groupings that are circled are based on a factor analysis of principal components scores computed from a Pearson's correlation matrix of trace element concentrations in the aquatic organisms.

adverse effects have been observed in the larvae fed diets containing realistic combinations of Hg species (Unrine et al., 2004; Unrine et al., 2005). This highlights the need to consider amphibians as a potential target receptor of Hg in aquatic environments, despite their relatively low trophic status. Accumulation of trace elements has also been associated with adverse effects of coal ash in amphibian larvae including malformations (Burger and Snodgrass, 2000; Hopkins et al., 2000), endocrine disruption (Hopkins et al., 1997), decreased swimming performance (Raimondo et al., 1998), elevated metabolic rates due to increased maintenance costs (Rowe et al., 1998) and altered growth and development (Snodgrass et al., 2004). Some or all of these adverse effects can translate to

the population level and ultimately lead to failed recruitment creating a population sink in coal ash polluted habitats (Rowe et al., 2001).

We have shown that amphibian larvae in this impacted wetland tend to accumulate relatively high concentrations of a great number of elements. This observation suggests that amphibians may serve as a critical link in biotransference of these elements within aquatic communities and that some of these elements (e.g., Se and Hg) may also be transported by metamorphic amphibians to terrestrial communities. Past studies support this contention. For example, banded water snakes (*Nerodia fasciata*) experimentally reared on a diet of fish collected from the D-area coal ash polluted wetland bioaccumulated concentrations of trace elements that were only a fraction of those actually observed in *N. fasciata* collected from the field. *Rana catesbeiana* and other anuran larvae were a component of the snakes' diet in the field and some of these larvae accumulate trace element concentrations three to five times higher than fish at the same site. Further, snakes from the field site with elevated tissue residues also had elevated metabolic rates while the snakes fed only fish in the laboratory did not exhibit this response, illustrating the potential toxicological significance of selecting amphibians as prey (Hopkins, 2006). Although, *Rana catesbeiana* larvae may not be palatable to all potential predators, there is no reason to assume that high concentrations observed in this species are unique among amphibians. Elevated concentrations of trace elements have also been observed in more palatable species exposed to coal ash such as *R. sphenoccephala* and *R. clamitans* (Snodgrass et al., 2004; Roe et al., 2005) and Hg in *R. sphenoccephala* from isolated wetlands (Unrine et al., 2005).

Although trace element accumulation in amphibian larvae can vary among species within aquatic systems and within species among aquatic systems (Sparling and Lowe, 1995), to our knowledge few studies have explicitly compared accumulation in an amphibian species to other small aquatic organisms. A few studies that have made such comparisons are summarized by Hall and Mulhern, 1984. Several authors have concluded that various anuran amphibian species tended to accumulate concentrations of many trace elements that were relatively higher than other aquatic organisms in polluted habitats and attributed this to the tendency of anuran larvae to feed on fine sediments (Brungs, 1963; Jennett et al., 1977).

Stable isotope analysis revealed that more than two C sources likely existed within the swamp, precluding the use of a two-element stable isotope approach to estimate trophic positions. This was easily observed when a two source mixing model was applied to the data. The regression line connecting *C. fluminea* and *H. trivolvis* in Fig. 4 illustrates how  $\delta^{15}\text{N}_{\text{base}}$  at trophic level one varies as a function of  $\delta^{13}\text{C}$  according to the two source mixing model. In the present study, the two source mixing model calculates trophic levels for *R. catesbeiana* and *Traimea* sp. which are less than one (primary consumers are defined as trophic level one). This is obviously a paradoxical result given that *R. catesbeiana* are omnivores and *Traimea* sp. are carnivores. Further, *Erythemis* sp. fell on the baseline, but it is known that these odonate larvae are also carnivores (Table 1).

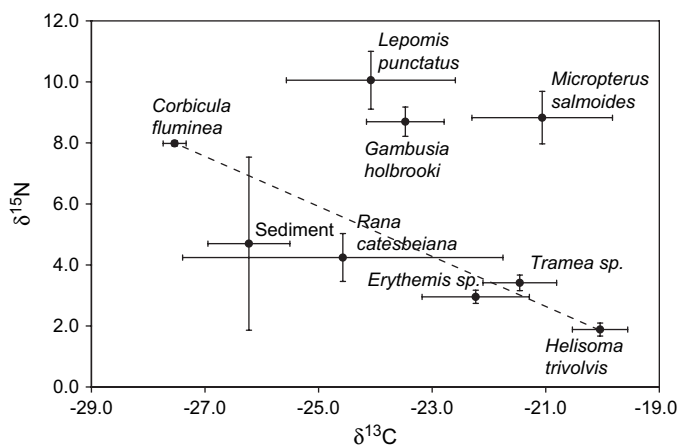


Fig. 4. Relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for aquatic organisms collected from a coal ash impacted swamp on the U.S. Department of Energy Savannah River Site near Aiken, SC, USA. Error bars indicate standard deviations. The dotted line shows the baseline  $\delta^{15}\text{N}$  values predicted from a two source mixing model derived from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the snail *Helisoma trivolvis* and the clam *Corbicula fluminea*. Stable isotope values are expressed in per mil units.

The most plausible explanation for this result is that more than two important basal resources exist in the swamp for food webs that exist there. It is difficult or impossible to differentiate among more than two important basal resources using only  $\delta^{13}\text{C}$ . The relative contribution of these resources to the  $\delta^{15}\text{N}_{\text{base}}$  values of each food web must be known to estimate trophic levels of consumers.

Another complicating factor may involve input of inorganic N from the Savannah River, the water source for the power plant waste disposal system. The  $\delta^{15}\text{N}$  values of the N pool may vary seasonally and annually due to fluctuations in N inputs from sewage and fertilizers used in agricultural operations as well as the  $\delta^{15}\text{N}$  values of those sources. Given that the species collected have different residence times in the swamp, they may have been integrating these fluctuating  $\delta^{15}\text{N}$  values over different time scales, resulting in considerable variation in  $\delta^{15}\text{N}$  values among species at a given trophic level.

Although stable isotope analysis using only C and N isotopes and a two source mixing model could not provide reliable estimates of trophic position using  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  values did provide valuable information regarding primary consumers. The results indicate that *R. catesbeiana* larvae were feeding very broadly from basal resources while the other lower trophic level consumers (*C. fluminea* and *H. trivolis*) fed relatively narrowly from the ecosystem. This is evidenced in that *R. catesbeiana* had  $\delta^{13}\text{C}$  values that nearly spanned the range of those observed in the ecosystem while *C. fluminea* and *H. trivolis* had relatively narrow ranges of  $\delta^{13}\text{C}$  values. The results also suggest that *C. fluminea* may function primarily as detritivores (Hakenkamp and Palmer, 1999; Hakenkamp et al., 2001), while snails primarily fed on submerged macrophytes and algae, which tend to have less negative  $\delta^{15}\text{N}$  values similar to those observed in snails (J. Unrine, unpublished data). Indeed, our  $\delta^{13}\text{C}$  values are consistent with gut contents of tadpoles indicating that they feed on a diverse array of ecosystem components including but not limited to sediment, bacteria, protists, fungi, algae and plants (both living and dead), small multicellular animals, vertebrate tissue (including heterospecific and conspecific larvae and eggs) and fecal material (Hoff et al., 1999). It is possible that this breadth of dietary preferences predisposes the *R. catesbeiana* in this swamp to accumulation of a wide variety of trace elements that concentrate in various basal resources. Species such as *H. trivolis* and *C. fluminea* that feed more narrowly from the available resources only accumulated the highest concentrations of one or two elements.

In summary, our study suggests that omnivorous amphibian larvae can efficiently bioaccumulate trace elements relative to other small aquatic organisms in a trace-element contaminated wetland, perhaps placing these larvae at elevated risk of toxicity. This finding is significant and merits further investigation in light of concerns regarding global decline of amphibian populations for which contaminants have been hypothesized as a potential contributing factor (Corn, 2000; Houlahan et al., 2000). Combined with the observation that considerable biomass of amphibians can be produced in and exported from wetlands (Gibbons et al., 2006), we suggest that amphibians may play an important role in cycling of trace elements in this swamp,

including transport from the aquatic to the terrestrial environment and conversion of elements to more bioavailable forms. These processes have important implications for both aquatic and terrestrial predators that prey on amphibians in trace-element contaminated wetlands. Further studies are warranted to determine if our observations are consistent in other amphibian species and in other polluted aquatic ecosystems.

## Acknowledgement

This research was supported by the U.S. Department of Energy, through Financial Assistance Award Nos. DE-FC09-96-SR18546 and DE-FC09-07SR22506. B. Taylor, A. DeBiase, D. Harrelson, L. Paddock, and B. Staub provided technical assistance. This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

## References

- Beard, K.H., Vogt, K.A., Kulmatiski, A., 2002. Top-down effects of a terrestrial frog on forest nutrient dynamics. *Oecologia* 133, 583–593.
- Brungs, W.A., 1963. The Relative Distribution of Multiple Radionuclides in a Freshwater Pond. PhD thesis, Ohio State University, Columbus, OH. (As cited in Hall and Muhlhorn, 1984).
- Burger, J., Snodgrass, J., 1998. Heavy metals in bullfrog (*Rana catesbeiana*) tadpoles: effects of depuration before analysis. *Environ. Toxicol. Chem.* 17, 2203–2209.
- Burger, J., Snodgrass, J.W., 2000. Oral deformities in several species of frogs from the Savannah River Site, USA. *Environ. Toxicol. Chem.* 19, 2519–2524.
- Burton, T.M., Likens, G.E., 1975. Energy flow and nutrient cycling in salamander populations in the Hubbard Brook Experimental Forest, New Hampshire. *Ecology* 56, 1068–1080.
- Cabana, G., Rasmussen, J.B., 1994. Modeling food-chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* 372, 255–257.
- Carlander, K.D., 1977. Life History Data on Centrarchid Fishes of the United States and Canada. In: Handbook of Freshwater Fishery Biology, vol. II. The Iowa State University Press, Ames, IA, USA.
- Corn, P.S., 2000. Amphibian declines: review of some current hypotheses. In: Sparling, D.W., Linder, G., Bishop, C.A. (Eds.), *Ecotoxicology of Amphibians and Reptiles*. SETAC Press, Pensacola, FL, USA, pp. 663–696.
- Croteau, M.N., Luoma, S.N., Topping, B.R., Lopez, C.B., 2004. Stable metal isotopes reveal copper accumulation and less dynamics in the freshwater bivalve *Corbicula*. *Environ. Sci. Technol.* 38, 5002–5009.
- DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid-synthesis. *Science* 197, 261–263.
- DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. et Cosmochim. Acta* 42, 495–506.

- Everitt, B., Dunn, G., 2001. Applied Multivariate Data Analysis. Oxford University Press, New York.
- Fagerstrom, T., 1991. Biomagnification in food-chains and related concepts. *Oikos* 62, 257–260.
- Fry, B., 1991. Stable isotope diagrams of fresh-water food webs. *Ecology* 72, 2293–2297.
- Garcia-Berthou, E., 1999. Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *J. Fish Biol.* 55, 135–147.
- Gibbons, J.W., Winne, C.T., Scott, D.E., Willson, J.D., Glaudas, X., Andrews, K.M., Todd, B.D., Fedewa, L.A., Wilkinson, L., Tsaliagos, R.N., Harper, S.J., Greene, J.L., Tuberville, T.D., Metts, B.S., Dorcas, M.E., Nestor, J.P., Young, C.A., Akre, T., Reed, R.N., Buhlmann, K.A., Norman, J., Croshaw, D., Hagen, C., Rothermel, B.B., 2006. Remarkable amphibian biomass and abundance in an isolated wetland: implications for wetland conservation. *Conserv. Biol.* 20, 1457–1465.
- Grayson, K.L., Cook, L.W., Todd, M.J., Pierce, D., Hopkins, W.A., Gatten, R.E., Dorcas, M.E., 2005. Effects of prey type on specific dynamic action, growth, and mass conversion efficiencies in the horned frog, *Ceratophrys cranwelli*. *Comp. Biochem. Physiol. A* 141, 298–304.
- Hakenkamp, C.C., Palmer, M.A., 1999. Introduced bivalves in freshwater ecosystems: the impact of *Corbicula* on organic matter dynamics in a sandy stream. *Oecologia* 119, 445–451.
- Hakenkamp, C.C., Ribblett, S.G., Palmer, M.A., Swan, C.M., Reid, J.W., Goodison, M.R., 2001. The impact of an introduced bivalve (*Corbicula fluminea*) on the benthos of a sandy stream. *Freshwat. Biol.* 46, 491–501.
- Hall, R.J., Mulhern, B.M., 1984. Are anuran amphibians heavy metal accumulators? In: Seigel, R.A., Hunt, L.E., Knight, J.L., Malaret, L., Zuschang, N.L. (Eds.), *Vertebrate Ecology and Systematics – A Tribute to Henry S. Fitch*. The University of Kansas Museum of Natural History, Lawrence, KS, USA.
- Harris, R.N., Alford, R.A., Wilbur, H.M., 1988. Density and phenology of *Notophthalmus viridescens dorsalis* in a natural pond. *Herpetologica* 44, 234–242.
- Hill, W.R., Stewart, A.J., Napolitano, G.E., 1996. Mercury speciation and bioaccumulation in lotic primary producers and primary consumers. *Can. J. Fish Aquat. Sci.* 53, 812–819.
- Hoff, K.v., Blaustein, A.R., McDiarmid, R.W., Altig, R., 1999. Behavior: interactions and their consequences. In: McDiarmid, R.W., Altig, R. (Eds.), *Tadpoles: The Biology of Anuran Larvae*. The University of Chicago Press, Chicago, IL, USA, pp. 215–239.
- Hopkins, W.A., Mendonca, M.T., Congdon, J.D., 1997. Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. *Gen. Comp. Endocrinol.* 108, 237–246.
- Hopkins, W.A., Congdon, J., Ray, J.K., 2000. Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal-burning power plant. *Environ. Toxicol. Chem.* 19, 862–868.
- Hopkins, W., 2006. Use of tissue residues in reptile ecotoxicology: a call for integration and experimentalism. In: Gardner, S., Oberdorster, E. (Eds.), *Toxicology of Reptiles*. Taylor and Francis, Boca Raton, FL USA, pp. 35–62.
- Hopkins, W.A., DuRant, S.E., Staub, B.P., Rowe, C.L., Jackson, B.P., 2006. Reproduction, embryonic development, and maternal transfer of contaminants in the amphibian *Gastrophryne carolinensis*. *Env. Health. Persp.* 114, 661–666.
- Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Meyer, A.H., 2000. Quantitative evidence for global amphibian population declines. *Nature* 404, 752–755.
- Houserova, P., Kuban, V., Kracmar, S., Sitko, J., 2007. Total mercury and mercury species in birds and fish in an aquatic ecosystem in the Czech Republic. *Environ. Pollut.* 145, 185–194.
- Jarman, W.M., Hobson, K.A., Sydeman, W.J., Bacon, C.E., McLaren, E.B., 1996. Influence of trophic position and feeding location on contaminant levels in the gulf of the farallones food web revealed by stable isotope analysis. *Environ. Sci. Technol.* 30, 654–660.
- Jaeger, R.G., 1980. Microhabitats of a terrestrial forest salamander. *Copeia* 1980, 265–268.
- Jennett, J.C., Wixson, B.G., Lowsley, I.H., Purushothaman, K., Bolter, E., Hemphill, D.D., Gale, N.L., Tranter, W.H., 1977. Transport and distribution from mining, milling, and smelting operations in a forest ecosystem. In: Boggess, W.R. (Ed.), *Lead in the Environment*. National Science Foundation RANN Program NSF/RA 770214 (As cited in Hall and Mulhern, 1984).
- Kiriluk, R.M., Servos, M.R., Whittle, D.M., Cabana, G., Rasmussen, J.B., 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. *Can. J. Fish Aquat. Sci.* 52, 2660–2674.
- Lattimore, R.E., Gibbons, J.W., 1976. Body condition and stomach contents of fish inhabiting thermally altered areas. *Am. Mid Nat.* 95, 215–219.
- Lombardo, P., Cooke, G.D., 2002. Consumption and preference of selected food types by two freshwater gastropod species. *Archiv. fur Hydrobiol.* 155, 667–685.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of N-15 along food-chains – further evidence and the relation between Delta-N-15 and animal age. *Geochim. et Cosmochim. Acta* 48, 1135–1140.
- Newman, M.C., 1989. Appropriateness of aufwuchs as a monitor of bioaccumulation. *Environ. Pollut.* 60, 83–100.
- Newman, M.C., Alberts, J.J., Greenhut, V.A., 1985. Geochemical factors complicating the use of aufwuchs to monitor bioaccumulation of arsenic, cadmium, chromium, copper and zinc. *Water Res.* 19, 1157–1165.
- Ohlendorf, H.M., 2003. Ecotoxicology of Selenium. In: Hoffman, D.J., Ratner, B.A., Burton, G.A., Cairns, J. (Eds.), *Handbook of Ecotoxicology*. Lewis Publishers, Boca Raton, FL, USA, pp. 465–501.
- Peacock, C.L., Sherman, D.M., 2005. Copper(II) sorption onto goethite, hematite, and lepidocrocite: a surface complexation model based on ab initio molecular geometries and EXAFS spectroscopy (vol. 68, pg 2623, 2004). *Geochim. et Cosmochim. Acta* 69, 5141–5142.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* 18, 293–320.
- Phaneuf, D., DesGranges, J.L., Plante, N., Rodrigue, J., 1995. Contamination of local wildlife following a fire at a polychlorinated biphenyls warehouse in St. Basile le Grand, Quebec, Canada. *Arch. Environ. Contam. Toxicol.* 28, 145–153.
- Pokarzhevskii, A.D., vanStraalen, N.M., 1996. A multi-element view on heavy metal biomagnification. *Appl. Soil Ecol.* 3, 95–98.
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* 83, 703–718.
- Raimondo, S.M., Rowe, C.L., Congdon, J.D., 1998. Exposure to coal ash impacts swimming performance and predator avoidance in larval bulldogs (*Rana catesbeiana*). *J. Herpetol.* 32, 289–292.
- Regeher, K.J., Lips, K.R., Whiles, M.R., 2006. Energy flow and subsidies associated with the complex life cycle of ambystomatid salamanders in ponds and adjacent forest in southern Illinois. *Oecologia* 147, 303–314.
- Reinfelder, J.R., Fisher, N.S., Luoma, S.N., Nichols, J.W., Wang, W.X., 1998. Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. *Sci. Tot. Environ.* 219, 117–135.
- Roe, J.H., Hopkins, W.A., Jackson, B.P., 2005. Species- and stage-specific differences in trace element tissue concentrations in amphibians: implications for the disposal of coal-combustion wastes. *Environ. Pollut.* 136, 353–363.
- Roe, J.H., Hopkins, W.A., DuRant, S.E., Unrine, J.M., 2006. Effects of competition and coal-combustion wastes on recruitment and life history characteristics of salamanders in temporary wetlands. *Aquat. Toxicol.* 79, 176–184.
- Rowe, C.L., Hopkins, W.A., Coffman, V.R., 2001. Failed recruitment of southern toads (*Bufo terrestris*) in a trace element-contaminated breeding habitat: direct and indirect effects that may lead to a local population sink. *Arch. Environ. Contam. Toxicol.* 40, 399–405.
- Rowe, C.L., Kinney, O.M., Nagle, R.D., Congdon, J.D., 1998. Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. *Physiol. Zool.* 71, 27–35.
- Seale, D.B., 1980. Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. *Ecology* 61, 1531–1550.
- Silva, J.J.R. Frausto da, Williams, R.J.P., 2001. *The Biological Chemistry of the Elements*, second ed. Oxford University Press, Oxford, UK.
- Snodgrass, J.W., Hopkins, W.A., Jackson, B.P., Baionno, J.A., Broughton, J., 2005. Influence of larval period on responses of overwintering green frog (*Rana clamitans*) larvae exposed to contaminated sediments. *Environ. Toxicol. Chem.* 24, 1508–1514.
- Snodgrass, J.W., Hopkins, W.A., Broughton, J., Gwinn, D., Baionno, J.A., Burger, J., 2004. Species-specific responses of developing anurans to coal combustion wastes. *Aquat. Toxicol.* 66, 171–182.

- Snodgrass, J.W., Hopkins, W.A., Roe, J.H., 2003. Relationships among developmental stage, metamorphic timing, and concentrations of elements in bullfrogs (*Rana catesbeiana*). *Environ. Toxicol. Chem.* 22, 1597–1604.
- Sparling, D.W., Lowe, T.P., 1995. Metal concentrations of tadpoles in experimental ponds. *Environ. Pollut.* 91, 149–159.
- Sparling, D.W., 2000. Ecotoxicology of organic contaminants to amphibians. In: *Ecotoxicology of Amphibians and Reptiles*. SETAC Press, Pensacola, FL USA, pp. 461–494.
- Stewart, M.M., Woolbright, L.L., 1996. Amphibians. In: Reagan, D.P., Waide, R.B. (Eds.), *The Food Web of a Tropical Rain Forest*. University of Chicago Press, Chicago, pp. 363–398.
- Taylor, B.E., Estes, R.A., Pechmann, J.H.K., Semlitsch, R.D., 1988. Trophic relations in a temporary pond: larval salamanders and their microinvertebrate prey. *Can. J. Zool.* 66, 2191–2198.
- Templeton, A.S., Spormann, A.M., Brown, G.E., 2003. Speciation of Pb(II) sorbed by *Burkholderia cepacia*/goethite composites. *Environ. Sci. Technol.* 37, 2166–2172.
- Unrine, J.M., Jagoe, C.H., 2004. Dietary mercury exposure and bioaccumulation in larvae of the southern leopard frog, *Rana sphenocephala*. *Environ. Toxicol. Chem.* 23, 2956–2963.
- Unrine, J.M., Jagoe, C.H., Hopkins, W.A., Brant, H.A., 2004. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. *Environ. Toxicol. Chem.* 23, 2964–2970.
- Unrine, J., Jagoe, C., Brinton, A.C., Brant, H., Garvin, N., 2005. Dietary mercury exposure and bioaccumulation in amphibian larvae inhabiting Carolina bay wetlands. *Environ. Pollut.* 135, 245–253.
- Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in delta N-15 and delta C-13 trophic fractionation: implications for aquatic food web studies. *Limnol. Oceanogr.* 46, 2061–2066.
- Westfall, J.M., 1984. Odonata. In: Cummins, K.W. (Ed.), *An Introduction to the Aquatic Insects of North America*. Kendall/Hunt Publishing Company, Dubuque, IA, USA.
- Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H., Scheuhammer, A.M., 2003. Chapter 16: ecotoxicology of mercury. In: Hoffman, D.J., Rattner, B.A., Burton, G.A., Cairns, J. (Eds.), *Handbook of Ecotoxicology*. Lewis Publishers, Boca Raton, FL, USA, pp. 409–463.
- Wyman, R.L., 1998. Experimental assessment of salamanders as predators of detrital food webs: effects on invertebrates, decomposition, and the carbon cycle. *Biodivers. Conserv.* 7, 641–650.