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Abstract: The Department of Energy's Savannah River Site is a former nuclear weapon material production and current research facility located in South Carolina, USA. Wastewater discharges from a fuel and nuclear reactor target manufacturing facility released depleted and natural U, as well as other metals into the Tims Branch-Steed Pond water system. We investigated the current dynamics of this system for the purposes of environmental monitoring and assessment by examining metal concentrations, bioavailability, and trophic transfer of contaminants in seven ponds. Biofilm, detritus, and Anuran and Anisopteran larvae were collected and analyzed for stable isotopes (δ15N, δ13C) and contaminants of potential concern (COPC) with a focus on Ni, U, and Hg, to examine metal mobility. Highest levels of Ni and U were found in biofilms U (147 and 332 mg kg−1 DW, respectively), while highest Hg levels were found in tadpoles (1.1 mg kg−1 DW). We found intraspecific biomagnification of COPCs as expressed through stable isotope analysis. Biofilms were the best indicators for contamination and Anuran larvae with the digestive tract removed were the best indicators of the specific bioavailability of the focal metals. Monitoring data showed that baseline δ15N values differed between ponds, but within a pond, values were stable throughout tadpole Gosner stage, strengthening the case to use this species for monitoring purposes. It is likely that there still is risk to ecosystem integrity as COPC metals are being assimilated into lower trophic organisms and even low levels of this mixture has shown to produce deleterious effects to some wildlife species.

Keywords: Anuran larvae, Biofilms, Hg, Ni, Stable isotopes, Trophic transfer, U

Introduction

The Department of Energy's (DOE) Savannah River Site (SRS) is a 777 km2 former nuclear weapon material production and current research facility located along the Savannah River in west-central South Carolina (Fig. 1). In 1954, direct environmental discharges were released from "M-Area", a fuel and aluminum-clad uranium (U) nuclear reactor target manufacturing facility, in the northwest region of the SRS (Fig. 1) through a drainage ditch into the Tims Branch-Steed Pond water system (Sowder et al. 1996). Approximately 1 kmdownstream of the drainage ditch is Steed Pond, a farm pond prior to construction of the SRS, whichwas dammed and converted to the main settling basin for the M-Area effluent between 1954 and 1985 (Pickett et al. 1987). The wastewater discharges released into Tims Branch contained large volumes of inorganic wastes including depleted and natural U, aluminum (Al), and nickel (Ni) along with smaller quantities of lead (Pb), copper (Cu), chromium (Cr), and zinc (Zn) (Pickett et al. 1987). An estimated 43,500 kg of U entered this system during this time period (1954–1985) with the majority of releases occurring from1966 to 1968 (Evans et al. 1992). Similar quantities of Ni are thought to have been released, although the exact amount is unknown

(Sowder et al. 1996). In 1979, large releases into Tims Branch ceased and all subsequent inputs were redirected into theM-Area settling basin. However, Tims Branch still received minor inputs until 1982 when all untreated effluent was routed to the M-Area basin. In 1984, the wooden spillway within Steed Pond ruptured, releasing sediment-bound metals downstream through Tims Branch (Evans et al. 1992). However, contamination transport down gradient still occurs during episodic storm events (Batson, et al. 1996). Following the rupture, pond 25 (another farm pond in Tims Branch) acted as a settling basin for the released sediment-bound contaminants. As with Steed Pond, this pond was also breached, releasing contaminants (Arnett and Spitzer 1994). Both Tims Branch and Steed Pond are part of the Upper Three Runs Integrator Operable (stream system) under investigation through the CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) (SRS Index no. 510,456, CERCLIS no. 70, WSRC-OS-94-42; SRS FFA 1993)

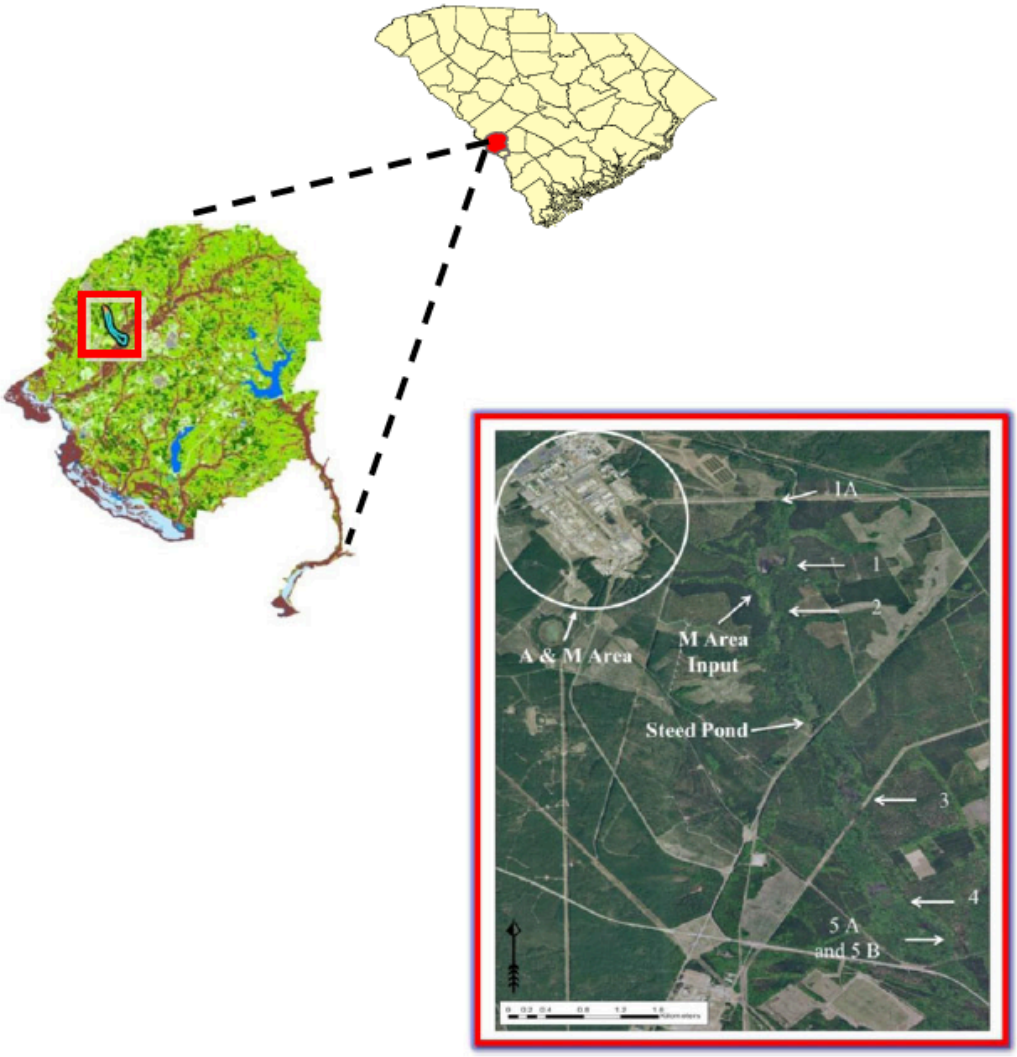


Fig. 1 Map of the U.S. Department of Energy's (DOE) Savannah River Site (SRS) located in western South Carolina, USA, highlighting the Tims Branch study area. Included is a schematic of the Tims Branch study area, a second-order stream system

consisting of impounded ponds and braided streams, including the location of the A & M Area, the M Area drainage ditch contaminant input, all ponds sampled (1A, 1, 2, 3, 4, 5A, and 5B) as well as Steed Pond (currently unimpounded)

The primary focus for this study was to better understand the current trace metal dynamics in Tims Branch and determine whether remedial actions should be pursued by the DOE for the contaminants of potential concern (COPCs). Specifically, Al, Cr, Cu, Ni, Pb, and U are considered COPCs for watershed due to bioavailability and the transfer factors previously observed (e.g., Pickett et al. 1987; Pickett 1990; Batson et al. 1996; Punshon et al. 2003a, b; Murray et al. 2010). Additionally, Hg is considered a COPC due to the history of Hg inputs into the Tims Branch stream system (Looney et al. 2012). Although previous work has indicated that COPCs have not entered the terrestrial food web associated with Tims Branch (Reinhart 2003), other ecotoxicological studies have shown that the contamination is present in both the aquatic and transitional wetland communities (Murray et al. 2010; O'Quinn 2005; Punshon et al. 2003a, b; Sowder et al. 1996). While these studies indicated that there are potential risks to both wildlife communities, as well as humans who may consume aquatic organisms that move off the SRS, the exposure pathways, bioavailability, and trophic transfer of contaminants is not well understood.

Biofilm/periphyton matrices (hereafter biofilm), detritus, and Anuran and Anisopteran larvae were sampled in 2010–2011 and fish and crayfish data collected from 2005 to 2006 (before remediation efforts) were utilized to investigate the Tims Branch system dynamics. Anuran larvae are widely known as ideal receptor species when studying ecosystem level contamination (Birdsall et al. 1986; Burger and Snodgrass 1998; Cooke 1981; Freda 1991; Gillilland et al. 2001), and trace metal effects on these organisms as focal endpoint bioindicators have been documented extensively, especially on the SRS, including oral deformities, reduced growth, suspended metamorphosis, and alteration of antipredatory behavior (Burger and Snodgrass 2000; Lefcort et al. 1998; Marques et al. 2008; Marques et al. 2009; Rowe et al. 2009; Snodgrass et al. 2004; Sparling et al. 2006). Tadpoles are opportunistic generalists with diets dominated by biofilms and detritus (Altig et al. 2007; Altig and Kelly 2012). Based on these life history characteristics, Anuran larvae are excellent indicator species for the Tims Branch system because they represent a pathway from aquatic to terrestrial systems due to their amphibious nature. Moreover, their physiology and behavior allow them to very quickly assimilate the biogeochemistry of their environment becoming a lower trophic indicator of the ecosystem. Anisopteran larvae were chosen for their omnivory (Blois 1985; Johansson 2010; Johnson et al. 2010; Logan et al. 2000;Wellborn and Robinson 2010) as they feed on multiple trophic levels including detritus and biofilm (Solomon et al. 2007). These invertebrates have been widely used as an indicator species of trace metal contamination due to their diet, minimal spatial movement, and deleterious effects from exposure to metals have been well documented (Casey et al. 2006; Farag et al. 1999; Mason et al. 2000).

Biofilms are a focus in this study not only because they are an important food source for both the Anuran and Anisopteran larvae, but they also play a very important role in the

fate of trace metals, especially in their ability to accumulate and convert specific metals into a bioavailable form giving rise to trophic movement (Hill et al. 2005; Hill et al. 1996; Krawczyk-Bärsch et al. 2011; Newman et al. 1985; Serra and Guasch 2009). Therefore, biofilms and detritus both provide the baseline to understand the trophic structure of the system. We use fish and crayfish data from previous monitoring activities to gain insight into upper trophic pathways. An important tool for baseline food web determination are stable isotopes (Hecky et al. 2011; Jardine et al. 2006), as the stable isotope composition of biological materials provides insights into the life histories of wildlife species to help better understand and reconstruct trophic systems (Hobson andWassenaar 1997; Gannes et al. 1998; Kelley 2000). Therefore, based on the information from previous studies suggesting that Ni, U, and Hg are still a potential risk, the specific objectives and hypotheses of this study were: (1) to quantify the amount of trace metals acquired by (purged) Anuran and Anisopteran larvae, biofilms, and detritus samples within seven pond beaver impoundments located within the Tims Branch tributary of the SRS; (2) determine if metal concentration in sample matrices can be better predicted using carbon and nitrogen stable isotopes; (3) determine if nitrogen isotope signatures differ between sequentially hydrologically linked ponds; (4) determine if removing the digestive tracts of purged tadpoles (e.g., sediment/detritus/undigested biota) changes whole body metal concentration for COPCs in this system; (5) determine whether a relationship exists between tadpole stage of development (Gosner stage) and trophic structure as expressed by δ15N; and (6) determine if trace metal concentrations in Ranidae tadpoles are influenced by stage of development.

Methods

Study site

The Tims Branch-Steed Pond water system is hydrologically dynamic. Steed Pond has dried (except for the braided Tims Branch stream channel) and ponded several times throughout the last decade. The entire Tims Branch watershed undergoes periods of drought and high beaver activity results in ponding, which makes summarizing and predicting the ecotoxicological components of this system a challenge. Although some of the pollution has washed downstream, much remains bound to sediments and the Tims Branch watershed remains under investigation.

Concurrent to the releases into Tims Branch, Hg inputs were discharged into the system from the 1950s to 2000s via ground water pumped into the A11 outfall which discharges into the M-Area input tributary leading into Tims Branch, including a pulse of Hgcontaminated groundwater in 2005–2007. Beginning in 2007, tin (II) chloride (SnCl2) was added to the ground water before reaching a pre-existing air stripper leading into the A11 outfall. This process results in the formation of tin oxides, which are essentially non-toxic. Additions of SnCl2 reduce and mobilize Hg in order to volatilize the trace metal in the air stripper. These remediation efforts showed a reduction of Hg concentrations in redfin pickerel (Esox americanus) of up to 72 % in samples collected in 2010 when compared to those sampled in 2006 before the addition of SnCl2 (Looney et al. 2012).

Although Steed Pond is no longer impounded, small stream channels and beaver ponds

are still present throughout Tims Branch which flow into the Savannah River via Upper Three Runs. The Tims Branch black water second-order stream system at the time of the study contained seven beaver impounded ponds located in the northwestern region of the SRS (Fig. 1). The two ponds preceding the source for contamination (M-Area input) were labeled ponds 1A and 1 (Fig. 1). Pond 1A was considered an experimental pond as it is the new A01 outfall for wetland treated process wastewater discharges and storm water runoff. Pond 1 was considered the control for this experiment as it occurs spatially before (upgradient of) the M-Area input into Tims Branch. Pond 1 (the control) is hydrologically disconnected to pond 1A during lengthy dry periods. The five ponds downstream of the M-Area input were referred to as 2, 3, 4, , and 5B (the latter two are also known as Pond 25 in other Tims Branch studies). Biofilms, detritus, and Anuran and Anisopteran larvae were collected from all available ponds in the Tims Branch area to observe and analyze the lower trophic level status of the system.

Sample collection

To assess metals in biofilms, we provided a surface that would support their growth: 1′Å~1′ polycarbonate plates (Kröpfl et al. 2006) inserted into slots cut into untreated 2″Å~4″ Standard Yellow Pine wooden stakes. Four plates were positioned horizontally into each stake containing two on each side with a distance of three vertical inches between each plate (N=4 plates/stake). In 2010, three stakes were placed in sun lit areas of each pond enabling light to reach the surface of the biofilm plates. Biofilms were allowed to grow for 2 weeks in 2010 in the summer months before they were collected and freeze dried and a 2-week growth and collection period was repeated again in the fall. In the spring of 2011, a biofilm time scale study was initiated in ponds 1, 2, and 5B where plates were collected after 2, 4, and 8 weeks (N=12 plates per pond per time period). No time scale differences or patterns were found; therefore samples were pooled for all further analyses.

Anuran larvae were collected in the summer of 2010 from the littoral region of each pond in leaf litter and vegetated areas (1A, 1, 2, 3, 4, 5A, and 5B) using minnow traps and dip nets. . The Anuran larvae used in contaminant and/or isotopic analyses were of the Genus Rana. Collected Rana species included Rana spenocephala (leopard frog), Rana clamitans (green frog), and Rana catesbeiana (American bullfrog) tadpoles. Tadpoles were placed in deionized water for 24 h prior to processing to allow time for gut purging (Burger and Snodgrass 1998). Each individual was identified by species, weighed (in gram), measured in length (snout/vent length [in millimeter]), and assigned a Gosner stage (Gosner 1960, e.g., the measurement of development in tadpoles from fertilization to fully metamorphosed young frogs). All collected tadpoles were beyond Gosner stage 25, the larval mid-tadpole stage with independent feeding and free swimming of Anuran larvae. Digestive tracts were removed from 20 Anuran larvae per pond in ponds that had the highest abundances (1A, 1, and 2) to compare body burdens to whole tadpoles collected in the same locations. Due to low tadpole abundances in ponds 3 and 4, only ponds 1A, 1, 2, 5A, and 5B could be sampled in 2011. Dragonfly larvae, from the summer months of 2010, were collected in littoral leaf litter and vegetation from each pond using dip nets and allowed to purge for 24 h prior to weighing (in gram). Dragonfly larvae were

identified down to infraorder Anisoptera. Detritus was collected in all ponds in 2010 except for pond 1A due to drought conditions. Water samples were collected from all ponds in the summer of 2010. The fish and crayfish (Family: Cambaridae, n=27) utilized for all trophic analyses were collected in 2005–2006 from ponds 1, 2, 3, and 4 of the Tims Branch system. The fish species sampled were: mud sunfish (Acantharchus pomotis, n=60), redfin pickerel (E. americanus, n=88), golden shiner (Notemigonus crysoleucas, n=14), lake chubsucker (Erimyzon sucetta, n=66), pirate perch (Aphredoderus sayanus, n=67), dollar sunfish (Lepomis marginatus, n=59), eastern mudminnow (Umbra pygmaea, n=14), redbreast sunfish (Lepomis auritus, n=1), spotted sunfish (Lepomis punctatus, n=27), and warmouth bass (Lepomis gulosus, n=6). Due to the inability to distinguish between the effects of year versus natural variation, all years were combined for each sample matrix/taxa. All samples were collected, handled, and processed in accordance with the University of Georgia Animal Care Use Protocols.

Trace metal and stable isotope analyses

All samples were freeze-dried and ground to a consistent homogeneous matrix using a glass mortar and pestle (to maintain sample integrity and to minimize loss) in order to be processed for both stable isotope and metals analysis. Samples were prepared for trace metals analysis using microwave-assisted acid digestion based on the USEPA method 3052. All samples were analyzed for Mn, V, Cr, Ni, Cu, Zn, As, Se, Sr, Cd, Hg, Pb, andUusing a Perkin Elmer NexIONÅ~300 inductively coupled plasma-mass spectrometer (ICP-MS) operating in Kinetic Energy Discrimination mode (USEPA 6020A) at the Savannah River Ecology Laboratory, Aiken, S.C., USA (Bryan et al. 2011; Metts et al. 2012; O'Quinn 2005; Punshon et al. 2003a; Punshon et al. 2003b; Reinhart 2003). The allowable range for spike recoveries was 80–120 %. Due to the fact that a high number of Mn (above standard detection) and Cd (below standard detection) concentrations were outside of the calibration curve, they were removed from all statistical analyses. All trace metal concentrations are reported on a dryweight basis. A water sample was collected from each pond and analyzed for total and dissolved trace metals based on the USEPA method 200.8.

For stable isotope analysis, an elemental analysisisotope ratio mass spectrometer was used to measure the isotopic ratios of 15N/14N and 13C/12C. All samples were analyzed at the Stable Isotope Laboratory at the Institute of Ecology, University of Georgia, Athens, USA, using a Carlo Erba Combustion Analyzer (CHN NA1500) coupled to a Thermo-Finnigan Delta V Continuous Flow Isotope Ratio Mass Spectrometer via the Thermo-Finnigan Conflo III Interface (Atkinson et al. 2006; Gaines et al. 2002; Hunte-Brown 2006;O'Quinn 2005; Reinhart 2003; Sowder et al. 1996). Samples were compared to international standards, Pee Dee belemnite (13C/12C) and atmospheric N2 (15N/14N) to give the delta (δ) notation in per mil units (‰) for sample reporting:

$$\delta X = \left[\left(R_{sample} \Big/ R_{standard}\right) - 1\right] \times 1,000$$

Measurements were verified using in-house reference standards (bovine liver, poplar, and

DORM3) of known δ15N and δ13C values and a sample replicate was analyzed every ten samples. All standards and replicates were reproducible to better than ±0.15‰ (1σ SD) for both δ13C and δ15N.

Statistical analyses

Focal metals Ni, U, and Hg are subsequently used in the modeling portion of this study while other site-wide COPC metals are reported using summary statistics. To determine the effect of location (pond) with a simultaneous effect of trophic position (δ15N) on the COPC metals acquired by (purged) Anuran and Anisopteran larvae, biofilms, and detritus samples, analysis of covariance models were employed (ANCOVA; PROC GLM; SAS Institute 2006) for each trace metal. A multiple comparison approach was utilized to examine differences between ponds with applicable Bonferroni corrections (LSMEAN; SAS Institute 2006). To better understand the relationship of metal concentration as a function of δ15N, regression analyses were performed on each sample matrix with appropriate transformations. In this case, fish species were pooled due to low sample sizes.

A K-means analysis was utilized in order to investigate the relationship between δ15N and trace metal concentrations in Anuran larvae, the relationship between δ15N and δ13C for all sample matrices for ponds 1 and 2 (XLSTAT; Microsoft Excel 2010). The analysis is a partitioning procedure where the data are grouped into K clusters defined by the user (Fisher 1958; MacQueen 1967; Aldenderfer and Blashfield 1984; Systat 2000). K-means represents an attempt to define an optimal number of K locations where the sum of the distance from every point to each of the K centers is calculated and the empirical solution is achieved through the minimization of the distances with all points being assigned to an individual cluster. For this analysis, the class size (number of clusters)was sequentially increased (ranging from three to five) until biologically irrelevant separations were observed.

To investigate collinearity among metals within each sample type, a correlation analysis was conducted (PROC CORR; SAS Institute 2006). To further investigate this relationship, a principle component analysis (PCA) was performed on all metals for each sample matrix (PROC PRINCOMP; SAS Institute 2006). The principal components that explained 80 % or more of the variation were used as new variables for general linear models (e.g., regression) to test the effects of the principal components on δ15N (PROC GLM; SAS Institute 2006). To determine if there were differences in trace metal contribution in tadpoles based on the presence of a digestive tract, appropriate unequal and equal variance t tests were performed on whole tadpoles and tadpoles with the digestive tract removed. For all other analyses involving tadpoles, whole specimens (including the digestive tract) were used. The effects of Gosner stage on δ15N was modeled utilizing a general linear model (PROC GLM; SAS Institute 2006). To explore the effects of both Gosner stage and δ15N on metal concentrations for tadpoles, an analysis of covariance was used to explore these effects simultaneously for each pond (PROC GLM; SAS Institute 2006). For all models, type III (partial) sums of squares with their respective F statistics were used to interpret relationships.

Results

Elemental analysis of biofilms, detritus, dragonfly larvae, and anuran tadpoles confirm the presence of the three focal metals (Ni, U, and Hg) as well as all of the COPC metals analyzed (Table 1). Average Ni concentrations for each sample matrix/biota in the impacted ponds (1A, 2, 3, 4, 5A, and 5B) were higher when compared to their respective sample matrices in the control pond (1). Differences were seen between biofilm and detritus Ni concentrations with detritus being higher in upstream ponds (2–4) while biofilm showed higher concentrations in the downstream ponds (5A, 5B). Little difference was observed between Anuran and Anisopteran larvae between ponds as well as within each pond. U displayed similar trends with detritus and biofilms in impacted ponds having higher U concentrations than the control, although ponds 3 and 4 Anuran and Anisopteran larvae concentrations did not differ from the control pond (1). Hg concentrations did not follow the same trend as Ni and U. Anuran larvae contained the highest concentrations of Hg in ponds 1, 2, 5A, and 5B when compared to other sample matrices within the same pond.

ANOVA and corresponding multiple comparison tests showed that in some cases, metal concentration differed by pond with trends being biota specific [Table 2 (a–d)]. For example, metal concentrations did not differ by pond for detritus and dragonfly larvae. In contrast, biofilm and tadpole concentrations did differ between most ponds for the three focal metals (Ni, Hg, and U) but did not differ for many of the other COPC metals. To better understand how metal concentrations change within and among biota within and among ponds, δ15N was introduced as a covariate and indicated that trophic position (as expressed by δ15N) influenced metal concentration with detritus and biofilms representing baseline [Table 2(a–d); Fig. 2a–c]. Since ANCOVA results showed that pond had a large influence on the model and plots indicated that not all relationships were linear, especially for anuran tadpoles, a K-means procedure was used to find clusters of comparable two dimensional spaces/distances between δ15N and metal (in milligram per kilogram of dry weight) specifically for this biota type to identify isotopically distinct groupings (Fig. 3). The K-means analysis complemented the ANCOVA and showed distinct clustering for pond locations based on δ15N and metal values.

Since the aforementioned analyses indicated that biota were isotopically distinct, a mixing model (Phillips and Gregg 2003, Phillips et al. 2005) was attempted to construct a source-partitioning model for all sample matrices. However, because of the variation in δ15N concentration, a mixing model could not be produced using carbon source as the second variable. In contrast, the K-means procedure did yield distinct clusters that represented discrete feeding groups based on δ15N and δ13C values for all sample matrices (Fig. 4).

The PCA performed for each biota type to quantify the relationship between metals within the system and also used to convert the metal concentration data into a set of values of linearly uncorrelated variables [principal components (PC)] showed strong relationships between metals. Moreover, the first three PCs explained over 80 % of the

variation for all biota except tadpoles, in which the first five PCs explained 80 % of the variation. The ANOVA used to determine if there was a relationship between the PCs that explained most of the variation and trophic level (as expressed by δ15N) indicated that PC1 usually had the best fit [Table 3(a–c)]. However, for tadpoles, the first three principal components yielded the strongest models and for biofilms the first two principal components yielded similarly strong models [Table 3(d)].

Pond	Metal	Biofilm Mean (95 % CL)	Detritus Mean (95 % CL)	Dragonfly larvae Mean (95 % CL)	Tadpole Mean (95 % CL)	Water
1A	Ni	79.095 (7.756)	–	12.133 (0.766)	13.210 (1.115)	4.171E−6
	Hg	0.698 (0.072)	–	1.708E−4 (0)	0.168 (0.056)	7.353E−8
	U	152.821 (19.937)	–	21.296 (1.122)	27.290 (2.555)	8.110E−6
1	Ni	8.988 (5.391)	4.936 (1.353)	1.424 (0.335)	2.098 (0.462)	4.514E−7
	Hg	0.0394 (0.036)	1.683E−4 (2.09E−5)	1.309E−4 (3.91E−5)	0.428 (0.044)	7.353E−8
	U	9.024 (13.048)	2.188 (0.237)	1.170 (0.684)	5.714 (1.639)	3.678E−7
2	Ni	100.106 (6.920)	264.865 (56.753)	12.010 (1.189)	6.362 (1.555)	1.415E−7
	Hg	0.589 (0.056)	0.632 (0.210)	0.779 (0.210)	0.996 (0.088)	7.353E−8
	U	231.216 (16.687)	640.632 (53.630)	26.632 (5.521)	15.822 (3.430)	1.576E−7
3	Ni	41.072 (7.607)	50.827 (0.948)	4.092 (2.494)	7.869 (3.845)	2.159E−6
	Hg	0.056 (0.036)	1.790E−4 (0)	0.370 (0.724)	0.368 (0.050)	7.353E−8
	U	27.007 (6.324)	31.091 (9.589)	3.489 (0.931)	4.792 (2.269)	2.811E−7
4	Ni	45.597 (4.843)	95.182 (15.765)	6.461 (0.182)	–	1.241E−6
	Hg	0.046 (0.029)	0.173 (0.171)	1.110E−4 (2.06E−12)	–	7.353E−8
	U	34.478 (5.834)	51.502 (17.175)	5.292 (0.246)	–	2.794E−7
5A	Ni	147.866 (19.687)	59.328 (9.912)	12.958 (1.153)	2.413 (0.757)	1.913E−6
	Hg	0.187 (0.046)	1.683E−4 (2.09E−5)	0.317 (0.100)	1.091 (0.159)	7.353E−8
	U	331.903 (27.510)	261.397 (72.007)	331.903 (27.510)	33.268 (3.946)	9.778E−7
5B	Ni	145.506 (7.932)	80.676 (3.941)	11.968 (1.236)	15.215 (1.368)	3.001E−6
	Hg	0.093 (0.023)	0.277 (0.112)	0.093 (0.023)	0.419 (0.038)	7.353E−8
	U	194.873 (16.222)	198.711 (57.913)	24.897 (2.951)	39.860 (4.160)	2.406E−6

Table 1 Mean Ni, U, Hg (in milligram per kilogram of based on dry weights) and associated 95% confidence limits in detritus and biota sampled from seven spatially sequential impounded ponds within the Tim's Branch main tributary located on the USDOE's Savannah River Site. Pond 1 serves as a reference site for this study. Total metals for water (in milligram per liter) in each pond are shown for reference

Since trophic structure (as expressed by δ15N) very much influenced the Tims Branch system dynamics as demonstrated in the results above, a t test (assuming unequal variances unless noted) was used to determine if metal concentration (in milligram per kilogram of dry weight) in Ranidae tadpoles differed based on the removal of the digestive tract to understand metal assimilation. In all cases, Ranidae tadpoles had higher whole body burdens when the digestive tract was still in place even after 24 h of purging (Table 4). The ANCOVA models used to determine if whole tadpole growth (as expressed through Gosner stage) and trophic position affect metal concentrations showed positive relationships for U and Hg in pond 1A, Ni and U in pond 2, and U in pond 5A (Table 5). No tadpoles from other ponds yielded strong models for any of the three focal metals.

Metal	R^2	Pond		$\delta^{15}N$		Pond*$\delta^{15}N$	
		F value	Pr>*F*	*F* value	Pr>*F*	*F* value	Pr>*F*
(a) Biofilm (*n*=191)							
Ni	0.845	55.29	<0.001	0.10	0.750	2.97	0.009
Hg	0.801	51.93	<0.001	3.18	0.077	3.46	0.003
U	0.843	77.14	<0.001	0.44	0.509	3.88	0.001
(b) Detritus (*n*=17)							
Ni	0.983	2.62	0.157	0.02	0.887	1.26	0.403
Hg	0.904	0.44	0.805	0.00	0.997	0.34	0.870
U	0.994	0.95	0.523	1.49	0.277	0.65	0.673
(c) Dragonfly larvae (*n*=21)							
Ni	0.976	1.16	0.418	0.01	0.924	0.92	0.533
Hg	0.764	0.71	0.656	0.59	0.468	0.72	0.645
U	0.995	5.81	0.018	0.05	0.838	3.53	0.062
(d) Tadpole (*n*=201)							
Ni	0.636	10.99	<0.001	0.20	0.655	6.42	<0.001
Hg	0.671	8.47	<0.001	13.86	<0.001	6.13	<0.001
U	0.623	10.11	<0.001	2.18	0.142	5.55	<0.001

Table 2 (a–d) Analysis of covariance (ANCOVA) for all biota sampled in the Tim's Branch tributary to determine if metal concentration is a function of "Pond" and "trophic level (using δ15N as a proxy)" as well as interaction among main effects

In summary, the results showed that: (1) trace metals were measurable for all matrices sampled, except water; (2) some metal concentration in sample matrices were better predicted using carbon and nitrogen stable isotopes; (3) nitrogen isotope signatures differ between sequentially hydrologically linked ponds; (4) removing the digestive tracts of purged tadpoles (e.g., sediment/detritus/undigested biota) changes whole body metal concentration for COPCs in this system; (5) in some cases, a relationship exists between tadpole stage of development (Gosner stage) and trophic structure as expressed by δ15N which influences (6) trace metal concentrations.

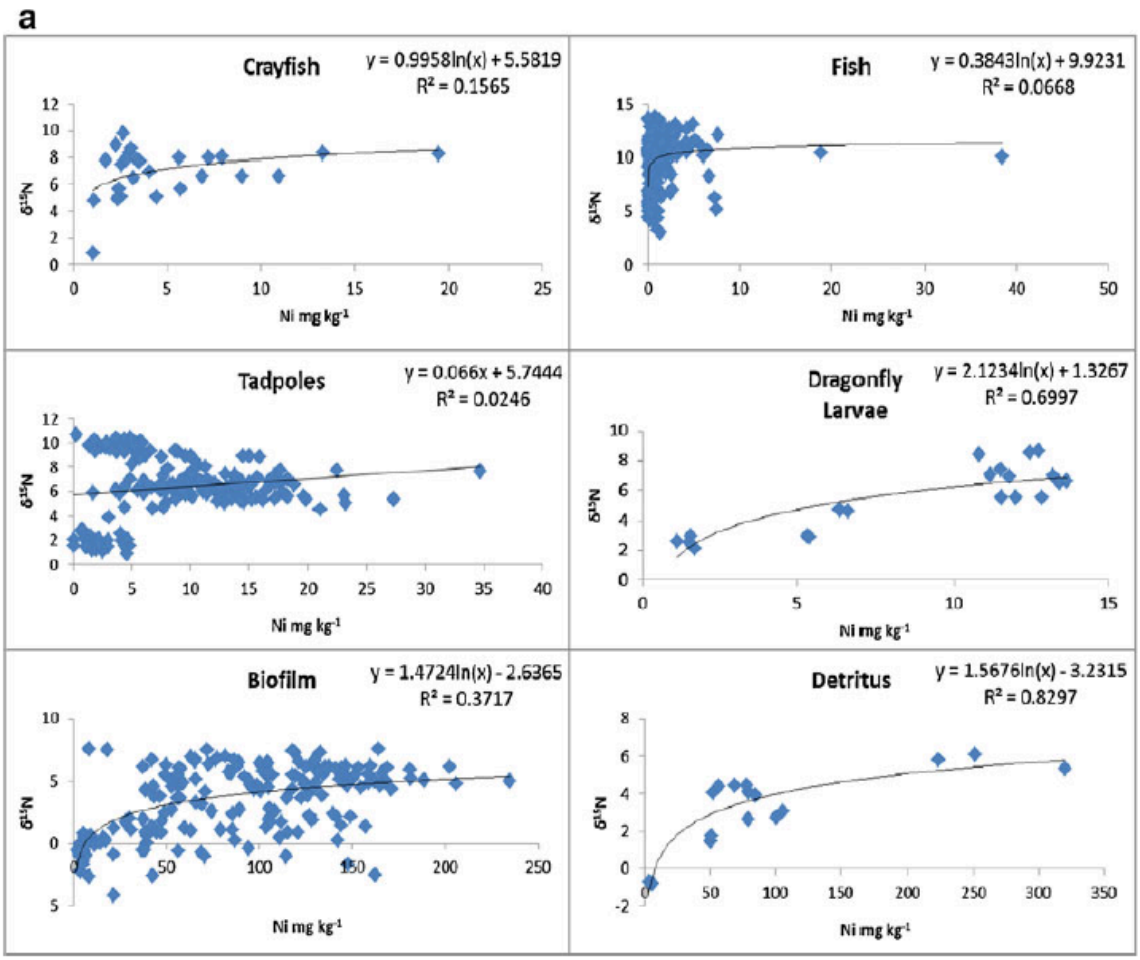
Discussion

Bioavailability

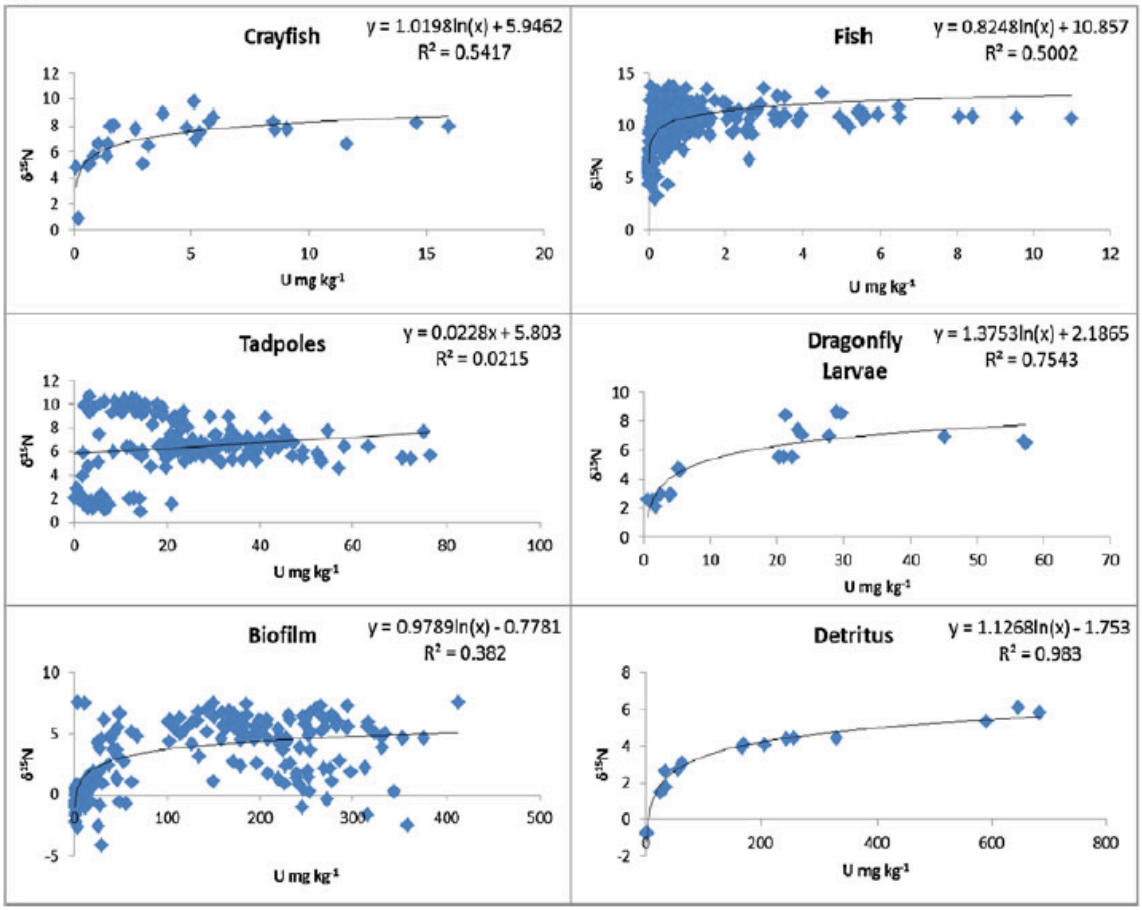
The Department of Energy performs ecological risk assessment activities under the guidelines of the USEPA, and when appropriate, prescribes even more conservative action levels. For the SRS, the focus is on prioritizing cleanup efforts to minimize detrimental effects to ecosystems and protection of public health. There has always been a question as to whether the metal contamination, especially the U contamination, in Tims Branch poses these risks. Clearly, the focal COPC metals (Ni, Hg, and U) are still in the system and can be measured in the sediment and biota. A previous terrestrial study (Punshon et al. 2003a) has shown elevated metal concentrations in these riparian soils when compared to respective stream sediments due to elevated stream flow, supporting possible transport of contaminants to the terrestrial food web through riparian vegetation uptake. Another Tims Branch study (Punshon et al. 2003b) showed a higher mobility in Ni compared to U in woody and herbaceous plants, as well as small mammal tissues with

little evidence of U bioavailability in the riparian ecosystem. Sowder et al. (1996) suggested that while U was associated with dissolved organic carbon, Ni was not and therefore was available for transport. However, another study focusing on sediments showed an increase of 1,500–2,800 % of U transported to the watershed during storm events when compared to base flow measurements indicating that U is still present in the aquatic systems even though it may not be as mobile (Batson et al. 1996; Buettner et al. 2011).

Consistent with other studies (Punshon et al. 2003a, b; O'Quinn 2005); U and Ni concentrations in biota were highly correlated while simultaneously correlating positively with other COPCs [Table 3(a–d)]. This relationship within and between metals is further exemplified by the PCA's where, for each type of biota, the first PC almost always exhibited positive loadings of similar magnitude for the metals indicating that the metals moved similarly in the system. Hg was the exception where the recent inputs were probably atmospheric and interestingly it was the only metal that negatively loaded in the first PC (for tadpoles) indicating its uptake was negatively associated with the uptake of other metals. Additionally, the correlation between Hg and the other focal metals might have been affected by the remediation activities which began in 2007. Hg contaminant contribution on the SRS has been cited from a number of sources, including site



b


Crayfish
y = 1.0198ln(x) + 5.9462
R² = 0.5417
δ15N
U mg kg-1
Fish
y = 0.8248ln(x) + 10.857
R² = 0.5002
δ15N
U mg kg-1
Tadpoles
y = 0.0228x + 5.803
R² = 0.0215
δ15N
U mg kg-1
Dragonfly Larvae
y = 1.3753ln(x) + 2.1865
R² = 0.7543
δ15N
U mg kg-1
Biofilm
y = 0.9789ln(x) - 0.7781
R² = 0.382
δ15N
U mg kg-1
Detritus
y = 1.1268ln(x) - 1.753
R² = 0.983
δ15N
U mg kg-1

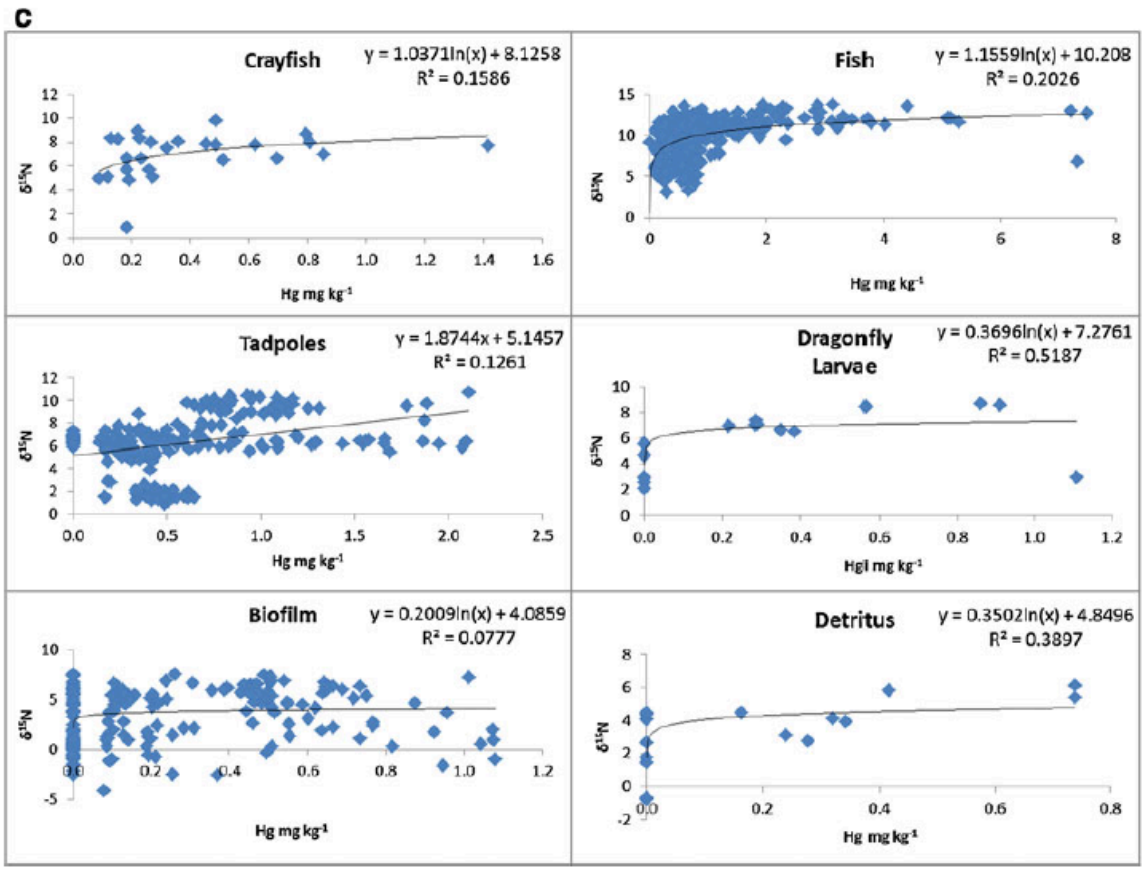


Fig. 2 a–c Ni, U, Hg (in milligram per kilogram of dry weight) in detritus and biota sampled from seven spatially sequential impounded ponds within the Tim's Branch main tributary located on the USDOE's Savannah River Site in relation to trophic position and baseline (as expressed using δ15N as a proxy). Data were pooled by pond. Note δ15N is plotted on the y-axis for display purposes (e.g., "high" vs. "low" trophic position/baseline); all statistical tests were performed using metal as the response variable

processing activities (coal combustion and waste disposal), atmospheric deposition, and most importantly with the highest contribution, contaminated water due to upstream industrial activities drawn from the Savannah River (Kvartek et al. 1994; Sugg et al. 1995). Availability of Hg to the food web, particularly in these habitat types, is associated to episodes of flooding (Rudd 1995) as Hg binds to sediments in combination with an increase in microbial processes during these periods, forming the highly bioavailable methylmercury (MeHg; Jackson 1998; Snodgrass et al. 2000). During periods of drought, sediments become exposed to an aerobic environment weakening the bonds between MeHg and the sediment resulting in possible bioavailability and uptake of the metal into the aquatic food web (Snodgrass et al. 2000). Beaver ponds and wetland habitats have also been known to increase mercury methylation (Brigham et al. 2009; Driscoll et al. 1998; Krabbenhoft et al. 1999; Mason et al. 2000; Roy et al. 2009a, b), raising concerns for Tims Branch due to the dynamic habitat types and periods of drought occurring in this system.

Previous work from Tims Branch have consistently shown that most COPC metals are not present in high levels in the upper trophic levels (Murray et al. 2010; Punshon et al. 2003b; Reinhart 2003), although some biota have shown adverse effects, most likely from low level exposures, based on specific biomarker endpoints (Murray et al. 2010). Froma risk assessment viewpoint, it is important to explain why most metals tend to diminish in concentration in the upper portions of the aquatic food web. Exposure is a concern because assimilation of even low levels of Ni and U can produce negative effects and Hg seems to be biomagnifying similarly to other aquatic systems on the SRS and throughout the southeast USA. The fact that biofilms independently differed by pond and isotopic concentration shows that it is crucial to quantify metal and isotopic baselines when examining contaminant transfer especially within a spatial context [Table 2 (a)]. The evidence suggests that each pond is starting with distinct nitrogen signatures and contaminant loads, most likely due to the relative ages of the ponds and their hydrologic state when contamination events occurred. For example, since Steed Pond is an old farm pond, the nitrogen could be associated with farming activities, such as manure input, resulting in similar trends following the rupture of the dam (Fig. 5). Despite these differences,

there are meaningful trends in δ15N, Ni, U, and Hg between ponds as these concentrations tend to increase and decrease together over the downstream spatial gradient. Our findings show that risk assessors dealing with unique COPC combinations such as this study need to pay particular attention not only to the biogeochemistry of the soil–sediment interfaces but also historical landuse to understand what may influence bioavailability.

Food webs and trophic transfer

Other than MeHg, the literature indicates that only a few metals tend to biomagnify, although a recent study suggested biomagnification of Cd had been observed throughout lower freshwater trophic levels (Croteau et al. 2005). However, Gaines et al. (2002) investigated intraspecific biomagnification in trace metals using raccoons as an indicator species and showed (via stable isotope analysis) that many of the metals on the SRS are bioavailable and in some cases biomagnify. That study was successful in controlling for interspecific variation and movement. In our study, trophic transfer is being investigated from a food web (or interspecific) approach and fish species at higher trophic levels are not confined to one pond system, making the possibility of seeing biomagnification less likely because of their movement (e.g., foraging strategy behaviors). However, certain patterns indicated that the focal metal concentrations did increase as a function of δ15N at a linear or loglinear rate (Fig. 2a–c). Again, this is possibly related to δ15N signatures associated with nitrogen inputs into Steed Pond or age and impoundment of the ponds. For example, the very high r-square value for the log-linear model associated with both U and Ni versus δ15N could be explained by high nitrogen enrichment of Steeds Pond when it was contaminated (Fig. 2a–b). Following the rupture of the dam in Steed Pond, it is likely that the contaminants and the associated δ15N flowed downstream, creating a similar pattern between the trace metals and stable isotopes. Hg does not show the same

pattern (Fig. 2c) as its input has been consistent through atmospheric deposition in addition to sporadic inputs, as is the case with the other COPCs associated with the M-Area contamination. The U and Ni contamination load in the biofilm is most likely due to sediment and detritus getting trapped in the sample matrix as U is known to be associated with dissolved organic carbon and Ni is suggested to be distributed in non-crystalline and crystalline iron oxides in Tims Branch riparian sediments (Sowder et al. 1996). One would expect low δ15Nvalues for sediment and higher δ15N values for detritus with various metal loads based on the geochemical properties of the aforementioned substrates which are what the results of this study show. Although, the history of the ponding and contamination events most likely explains why Ni and U concentrations are increasing with δ15N, physiological intraspecific biomagnification may also be playing a role. This can be seen via the relationship of both fish and crayfish in the system. Metal loads for this biota increased with δ15N for Ni, U, and Hg yet were not sampled from pond five (that presumably holds/held much of the contamination). Although the fish and crayfish were sampled prior to the SnCl2 and air stripper treatments of Hg in Tims Branch, these relationships are still present in other measured biota sampled from 2010 to 2011. Moreover, many of these fish may move throughout the stream system making their exposure heterogeneous (Fig. 3).

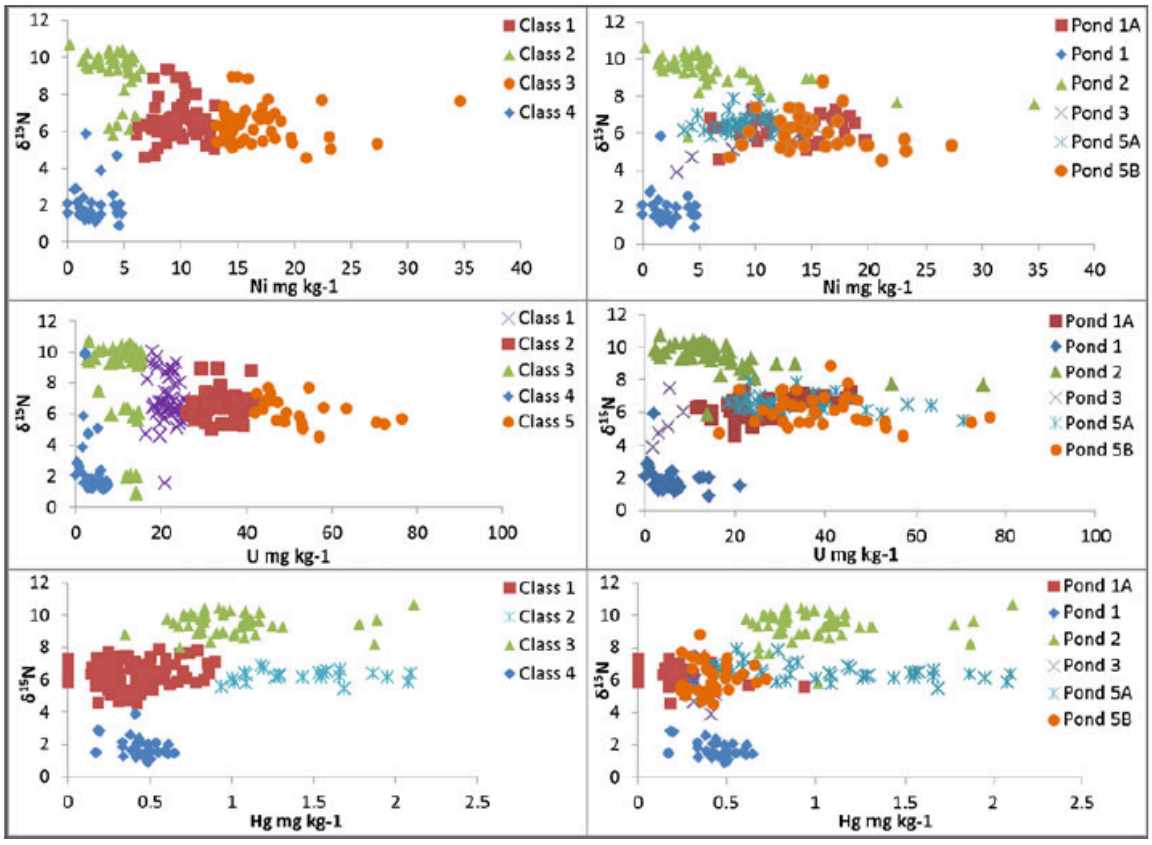


Fig. 3 K-means analysis to find clusters of comparable spatial extent represented by δ15N (trophic position/baseline) and metal (in milligram per kilogram of dry weight) to identify distinct groupings based on pond for anuran tadpoles (digestive tract intact). Graphs on the left represent the K-means clusters while the graphs on the right

represent the original data from each pond

Although many of the linear models explored in this study explained a good portion of the variation associated with metal concentration in the seven ponds, especially when using δ15N as a covariate, it was clear that many of the trophic relationships were not linear. One of the main objectives of this study was to gain insight into the movement of individual metals within the food web by utilizing carbon and nitrogen stable isotopes to better understand trophic position among biota. The K-means clustering routine provided an additional tool to investigate this process. The procedure was used to address if there are isotopically distinct separations/clusters in two-dimensional space between δ15N and metals for Anuran larvae based on pond (Fig. 3). When focusing on Ni, U, and Hg the analysis shows class separation that very much parallels the ponds, reinforcing the conclusion that each pond is isotopically distinct in terms of metal bioavailability.

This same trend can be seen when exploring δ15N and δ13C by biota. In this case, the K-means showed the trophic structure of pond 2 (the most diverse pond) in contrast to Pond 1 (control). For both ponds 1 and 2, δ15N versus δ13C plots yielded distinct classes for biofilms, tadpoles, and all other vertebrates/invertebrates again displaying classic and consistent trophic models. δ13C is commonly used to distinguish between food sources (terrestrial, freshwater, and marine) and C3 and C4 plant populations; however, C3 detritus is known to support fungal decomposing communities while C4 is known to support bacterial decomposing communities which may explain the variation observed in biofilm (Dornbush et al. 2008). In this case, δ13C was used to stabilize the trophic model due to its decreased variation. Similar spatial analyses such as the K-nearest neighbor have also been used to create trophic models based on stable isotope (δ15N versus δ13C) data (Rosing et al. 1998). In this case, the Kmean procedure was able to establish a working trophic structure model, as other procedures (e.g., mixing models: Phillips and Gregg 2003) would not converge a solution due to the fact that there were no distinct upper source terms (e.g., a mixing polygon could not be formed). Ultimately, the aforementioned analyses all consistently indicate that there still is risk to this ecosystem integrity, as the COPC metals are being assimilated and are moving into the lower trophic levels. To our knowledge, no other studies have investigated the trophic mobility of U and Ni to this extent, especially in terms of structure.

Anuran larvae and bioindicators

The effects of combinations of many trace metal contamination on Anuran larvae have been thoroughly investigated (Birdsall et al. 1986; Burger and Snodgrass 1998; Burger and Snodgrass 2000; Farag et al. 1999; Freda 1991; Gillilland et al. 2001; Lefcort et al. 1998; Rowe et al. 2009; Rowe et al. 2001; Snodgrass et al. 2004; Sparling et al. 2006), although Ni and U have not been extensively analyzed which makes this study unique. Of the existing literature, one study reports a negative correlation between Ni and the distributions of Hyla crucifer and Bufo americanus leading to possible population sinks in highly contaminated areas (Glooschenko et al. 1992). U effects associated with mining have included observed histopathological abnormalities in Rana perezi involving the liver, kidneys, spleen, lungs, and testes from a field study (Marques et al. 2009) and

decreases in snout–vent length, lower stimulus reactions, and tail deformities from a laboratory study (Marques et al. 2008).

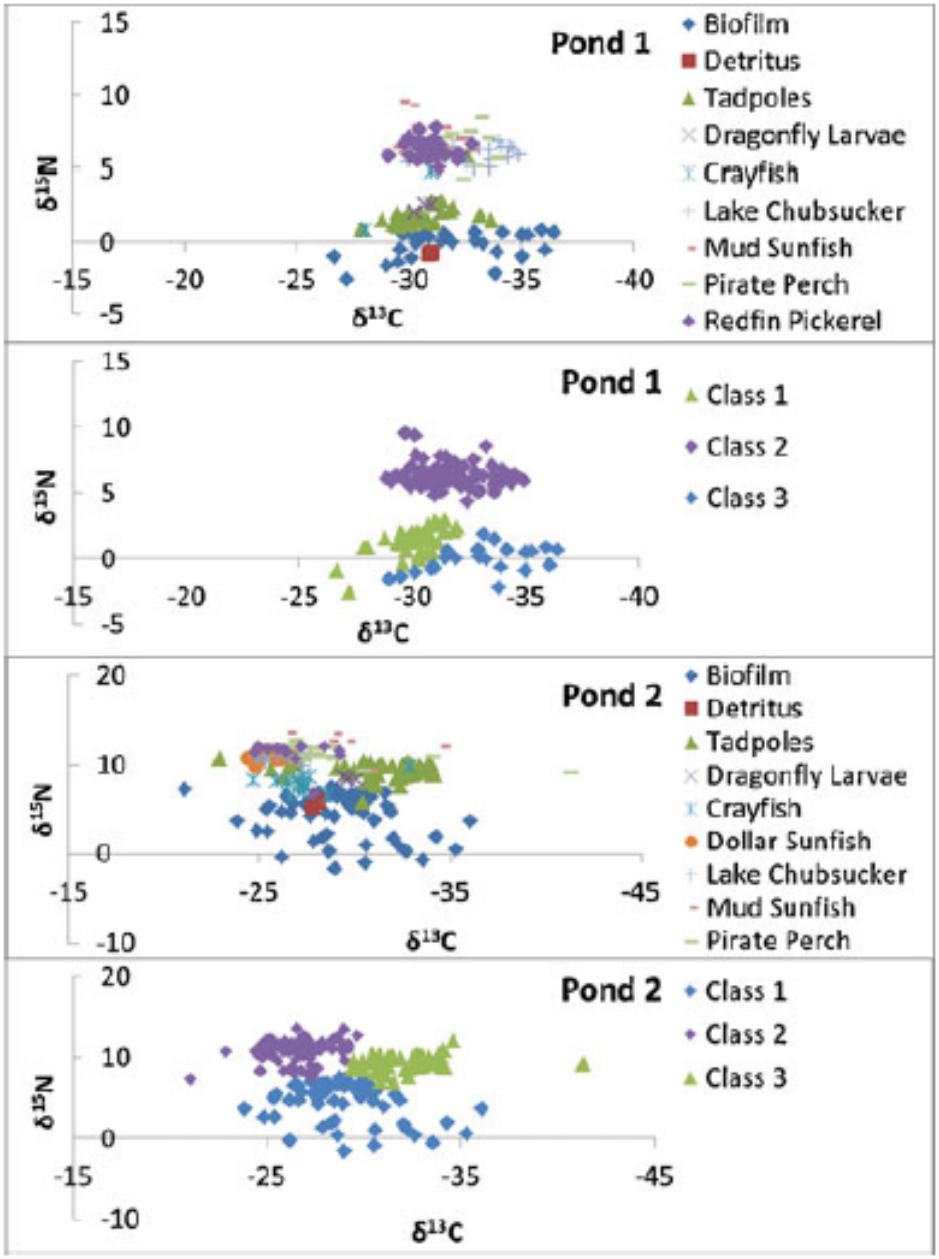


Fig. 4 K-means analysis to find clusters of comparable spatial extent represented by δ15N and δ13C to investigate the trophic structure of ponds 1 (control) and 2 (the most diverse pond). The K-means clusters are shown below the original data for each sample matrix

(a) Biofilm (n=202)

Eigenvalue for each PC with respective GLM: biofilm

PC	Eigenvalue	Proportion	Cumulative	R^2	δ^{15}N	
					F value	Pr>F
1	5.738	0.522	0.522	0.167	38.02	<0.001
2	2.105	0.191	0.713	0.124	26.86	<0.001
3	1.222	0.111	0.824	0.012	2.27	0.133

PC values: biofilm

Metal	Prin1	Prin2	Prin3
V	0.347	0.066	−0.420
Cr	0.245	−0.463	0.391
Ni	0.250	0.433	0.361
Cu	0.246	−0.493	0.314
Zn	0.358	−0.239	−0.108
As	−0.068	0.262	0.322
Se	0.316	0.312	0.266
Sr	0.295	0.151	−0.376
Hg	0.350	−0.110	−0.300
Pb	0.380	0.003	0.087
U	0.330	0.305	0.126

(b) Detritus (n=17)

Eigenvalue for each PC with respective GLM: detritus

PC	Eigenvalue	Difference	Proportion	Cumulative	R^2	δ^{15}N	
						F value	Pr>F
1	7.573	6.149	0.689	0.689	0.504	15.25	0.001
2	1.424	0.597	0.130	0.818	0.033	0.51	0.486
3	0.827	0.126	0.075	0.893	0.082	1.34	0.265

Principal component values: detritus

Metal	Prin1	Prin2	Prin3
V	0.351	0.096	−0.113
Cr	0.338	0.087	−0.306
Ni	0.359	−0.049	−0.030
Cu	0.202	0.576	0.107
Zn	0.165	−0.441	0.737
As	0.345	−0.052	0.155
Se	0.356	0.006	0.078
Sr	0.346	−0.191	−0.115
Hg	0.266	−0.009	−0.289
Pb	0.304	−0.354	−0.127
U	0.192	0.539	0.441

(c) Dragonfly larvae (n=21)*

Eigenvalue for each PC with respective GLM: dragonfly larvae

PC	Eigenvalue	Difference	Proportion	Cumulative	R^2	δ^{15}N	
						F value	Pr>F
1	5.697	3.194	0.518	0.518	0.709	46.38	<0.001
2	2.503	1.364	0.228	0.746	0.133	2.93	0.104
3	1.139	0.158	0.104	0.849	0.048	0.95	0.342

Principal component values: dragonfly larvae

Metal	Prin1	Prin2	Prin3
V	0.386	−0.006	0.247
Cr	0.228	−0.508	0.119
Ni	0.361	0.017	−0.449
Cu	0.253	−0.491	0.125
Zn	0.225	0.104	0.365
As	0.344	0.158	−0.320
Se	0.278	0.360	0.247
Sr	0.348	0.128	0.160
Hg	0.183	0.437	0.301
Pb	0.349	−0.320	0.033
U	0.285	0.159	−0.542

(d) Tadpoles (n=206)*

Eigenvalue for each PC with respective GLM: tadpoles

PC	Eigenvalue	Difference	Proportion	Cumulative	R^2	δ^{15}N	
						F value	Pr>F
1	3.955	2.148	0.360	0.360	0.088	19.23	<0.001
2	1.807	0.528	0.164	0.524	0.050	10.46	0.001
3	1.279	0.305	0.116	0.640	0.151	35.51	<0.001
4	0.974	0.116	0.089	0.729	0.008	1.50	0.221
5	0.858	0.059	0.078	0.807	0.004	0.84	0.360

Principal component values: tadpoles

Metal	Prin1	Prin2	Prin3	Prin4	Prin5
V	0.398	0.066	0.213	0.043	−0.157
Cr	0.354	−0.262	−0.188	−0.082	−0.030
Ni	0.409	0.261	−0.346	0.000	−0.055
Cu	0.144	−0.174	−0.140	0.650	0.702
Zn	0.243	−0.417	0.368	−0.112	0.092
As	0.395	0.224	0.112	−0.118	0.140
Se	0.209	0.264	0.405	−0.006	0.137
Sr	0.230	0.059	0.260	0.619	−0.550
Hg	−0.150	0.400	0.521	−0.098	0.329
Pb	0.319	−0.438	0.137	−0.342	0.089
U	0.304	0.423	−0.331	−0.181	0.117

Table 3 (a–d) PCA used to convert the raw metal concentration data into a set of linearly uncorrelated variables (PC) for detritus and biota sampled from seven spatially sequential impounded ponds within the Tim's Branch main tributary located on the USDOE's Savannah River Site. General linear models (GLM) were used to determine if the PCs that explained most of the variation were a function of trophic level (as expressed as δ15N)

The investigation to determine the best and most appropriate methodology of measuring tadpole metal concentrations showed that for this system, it is best to remove the digestive tract. Tadpoles were purged for 24 h to minimize the amount of sediment and

associated metals within the digestive tract to eliminate excess sediment to ensure a consistent comparison. One SRS study observing the effects of depuration on trace metal assimilation suggests that certain metal concentrations (Cr, As, and Pb) decreased at 24 h and again at 48 h; however, an increase was observed at 72 h (Burger and Snodgrass 1998). That study also suggested that purging tadpoles did not affect Mn, Cd, and Hg concentrations. In the current study when comparing whole purged tadpoles (24 h) to those with the digestive tract removed, Hg concentrations were also very similar (Table 4). This evidence supports the high absorption rate (>90 %) of MeHg (Berglund et al. 1971; Charbonneau et al. 1976; Miettinen 1973) suggesting that the majority of Hg consumed within the tadpoles was MeHg as opposed to inorganic Hg. In contrast, Ni and U concentrations were both approximately three times higher in whole purged tadpoles than those with the digestive tract removed, again associating these two metals with sediments and detritus located within the digestive tract (Table 4).

Metal	t value	Pr>|t|	Tadpole means	
			Whole (n=89)	DT removed (n=59)
V	12.84	<0.001	6.187	1.351
Cr	5.85	<0.001	14.522	4.861
Ni	7.13	<0.001	6.847	2.441
Cu	5.97	<0.001	30.912	12.922
Zn	5.17	<0.001	144.100	90.482
As	10.40	<0.001	1.745	0.403
Se	6.35	<0.001	2.629	0.853
Sr[a]	2.72	0.007	9.610	8.241
Hg[a]	0.92	0.359	0.544	0.475
Pb	8.76	<0.001	11.136	2.538
U	8.87	<0.001	17.081	5.318

[a] *T* test for equal variances

Table 4 T test (assuming unequal variances unless noted "a") to determine if metal concentration (in milligram per kilogram of dry weight) in Ranid tadpoles differed based on the removal of the digestive track (DT)

For whole Anuran larvae and those with the digestive tract removed, δ15N essentially remained constant as Gosner stage increased for all three ponds observed (1A, 1, and 2, Fig. 6). Not only does this present a clear separation of δ15N values between ponds but also supports that the values are stable throughout their life history. McIntyre and Flecker (2006) suggested that Rana palmipes larvae had a very slow turnover rate of δ15N with the %15N signature being essentially unchanged over time (up to 24 days). If a similar turnover rate were applied to the tadpoles in the Tims Branch system, then using this taxa with Gosner stage as a proxy for time would provide rigor to food web studies due to their ability to give a true representation of the δ15N signature in each pond.

Tadpoles in our study reflected a consistent unique δ15N signature for each pond sampled (1A, 1, and 2) showing little variation throughout Gosner stages (Fig. 6). Metal concentrations demonstrated a high variation in each pond and lacked a consistent trend as Gosner stages increased, making Gosner stage a weak predictor of trace metal concentration (Table 5). It is likely that Gosner stage is unable to predict trace metal concentration due to the effect of prolonged larval stages during periods of overwintering. Snodgrass et al. (2005) also showed that overwintering may have confounded the effect of Gosner stage on COPC trace metal concentrations in R. clamitans from the SRS. Based on these results, Anuran larvae appear to be excellent indicators of both isotopic baseline and trace metal contamination, although their life history must be considered when choosing them for environmental monitoring and assessment.

Pond	Metal	R^2	$\delta^{15}N$		Gosner stage		$\delta^{15}N$[a] Gosner	
			F value	Pr>*F*	*F* value	Pr>*F*	*F* value	Pr>*F*
1A	Ni	0.405	0.78	0.383	0.03	0.869	0.36	0.551
	U	0.439	6.71	0.014	3.41	0.073	4.47	0.041
	Hg	0.368	7.81	0.008	7.41	0.010	6.41	0.016
1 (Control)	Ni	0.379	1.05	0.314	0.26	0.616	1.02	0.3203
	U	0.261	0.17	0.685	1.65	0.207	0.11	0.747
	Hg	0.326	0.05	0.826	1.46	0.237	0.01	0.919
2	Ni	0.615	23.21	<0.0001	21.59	<0.0001	17.94	0.0001
	U	0.548	16.70	0.0002	14.28	0.0005	12.09	0.0011
	Hg	0.142	0.31	0.581	0.08	0.781	0.29	0.593
5A	Ni	0.244	0.80	0.376	1.54	0.222	1.000	0.324
	U	0.218	8.25	0.007	7.33	0.010	7.23	0.011
	Hg	0.320	0.39	0.534	0.01	0.914	0.00	0.966
5B	Ni	0.101	2.44	0.128	2.25	0.144	2.13	0.155
	U	0.087	2.06	0.162	1.83	0.186	1.77	0.193
	Hg	0.295	0.21	0.647	0.01	0.920	0.15	0.670

[a] Ponds 3 and 4 did not yield high enough sample sizes to test the hypothesis of the ANCOVA model

Table 5 ANCOVA results to test the effects of δ15N and Gosner stage on metal concentration for Anuran larvae (n=32) in the five of the seven ponds sampled in the Tim's Branch study area

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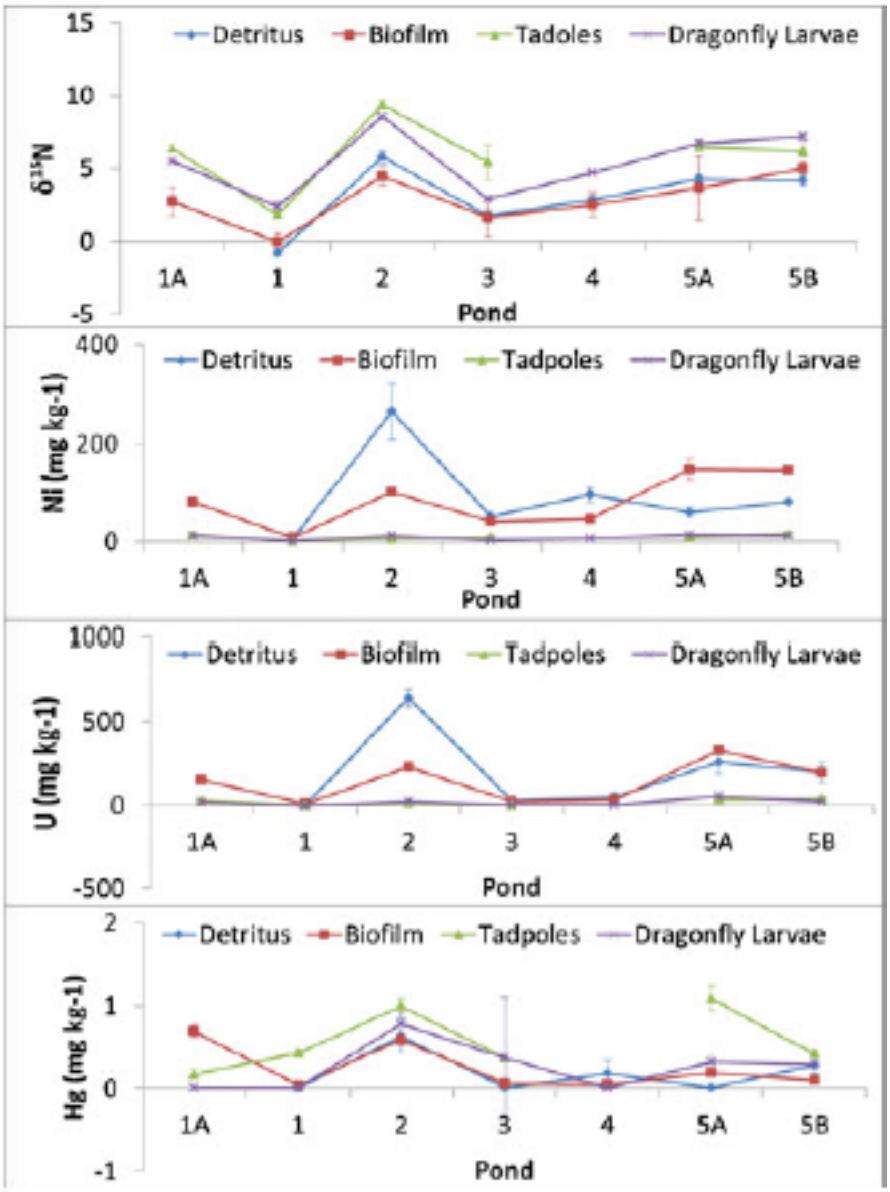

Fig. 5 Mean δ15N, Ni, U, and Hg concentrations (in milligram per kilogram of dry weight) and their corresponding 95 % confidence limits in detritus and biota sampled for each of the seven spatially sequential impounded ponds within the Tim's Branch main tributary located on the USDOE's Savannah River Site. Pond 1 serves as a control site for this study

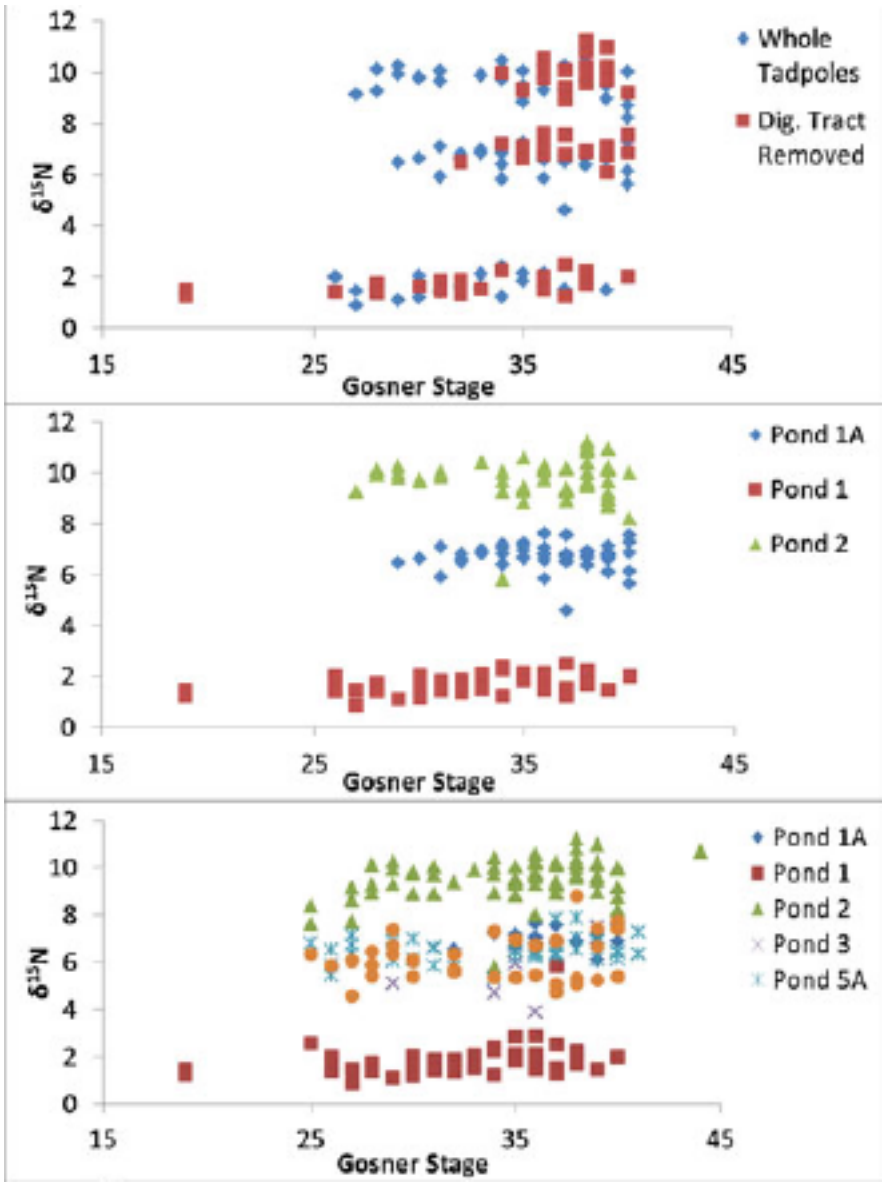

Fig. 6 δ15Nvalues as a function of Gosner stage for Ranidae larvae sampled in the Tims Branch main tributary located on the USDOE's Savannah River Site. The first graph compares whole tadpoles to digestive tract removed tadpoles for ponds 1A, 1, and 2 sampled in 2011. The second graph displays the tadpole δ15N values for each pond, 1A, 1, and 2 sampled in 2011. The last graph contains all tadpoles sampled in the Tims Branch water system separated by pond. Pond 1 serves as a control site for this study

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