Trophic Interaction vs. Direct Uptake: Pathways of Coal Combustion Pollutants into Aquatic Predators

by

Barry James Williams, Jr.

(Under the direction of Barbara Taylor)

Abstract

Coal is still used as a major source of fuel for power plants in the U. S. and worldwide. One of the many forms of the combusted waste is fly ash, a fine particulate matter that is sometimes mixed with water and pumped to settling basins. This form of waste removal is currently used at the D-Area power plant on the Savannah River Site near Aiken, SC. Many of the metal and metalloid constituents of the coal residue are known to have chronic toxic effects on biota. Definite pathways into aquatic organisms are poorly understood. The research examined the distribution of coal-associated contaminants among aquatic flora and fauna found in the D-Area impoundment, which receives coal fly-ash. Trace metal body burdens and community composition were compared at the D-Area impoundment and reference sites. Stable isotopes of carbon and nitrogen were also analyzed to determine trophic linkages and the potential for transfer of contaminants in the impoundment. A laboratory experiment was used to separate direct uptake (contact with sediments and water) from trophic interactions as a means of contaminant entry for a representative aquatic vertebrate predator. Mosquito fish were subjected to four treatments: contaminated sediment and contaminated food, contaminated sediment and uncontaminated food, uncontaminated sediment and contaminated food, and uncontaminated sediment and uncontaminated food. While microcrustaceans and worms displayed some of the highest densities of invertebrates sampled at D-Area, they along with coleopterans, molluscs, odonates and *Chaoborus* were significantly reduced in density compared to the reference site. Spatial variation of invertebrate abundances within the D-Area impoundment was associated with patterns of water flow through the impoundment and submerged aquatic vegetation. Within the D-Area impoundment, biota consistently accumulated Cd, Se and Sr; body burdens of these contaminants were associated with concentrations in sediment. Stable isotope data showed a possible food chain consisting of *Myrica*, the amphipod *Hyalella* and mosquitofish (*Gambusia holbrooki*). Contaminant levels of As, Se, Sr and Cu increased from *Myrica* to amphipods, but not from amphipods to mosquitofish. Nickel concentrations did not increase from *Myrica* to amphipods, but do increase from amphipods to mosquitofish. Our feeding trial showed that of the metals accumulated, the most important contributing factor was exposure to sediment. Selenium was the only metal that showed prey as a source of metal accumulation.

INDEX WORDS: Trophic interactions, aquatic ecology, food webs, coal combustion

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Chapter 1

Introduction

Coal is still used as a major source of fuel for power plants in the U. S. and worldwide. One of the many forms of the combusted waste is fly ash, a fine particulate matter that is sometimes mixed with water and pumped into settling basins and constitutes 62% of the solid waste of coal combustion (EPA, 1997, NRC, 2006). This form of waste removal is currently used at the D-Area power plant on the Savannah River Site near Aiken, SC. Fly ash is known to contain many trace elements such as arsenic, cadmium, lead, mercury and selenium as well as others (EPA, 1997, NRC, 2006). Many of these trace elements are known to have effects on biota that encounter the fly ash slurry. Many of the invertebrates inhabiting these polluted ecosystems suffer from reduced population densities. Studies have shown this to be the case for zooplankton (Wogram and Liess, 2001, Winner, Owen and Moore, 1990), insects (Clements et al., 1988 a,b, Diamond, Bressler and Serveiss, 2002, Specht et al., 2004) and snails (Brooks et al., 2004) to name a few examples. Additionally, vertebrates found in these ecosystems are also affected by the trace elements in fly ash. Fish exposed to fly ash have exhibited liver and kidney damage, reduced body mass, modified behavior, and fin erosion (Coughlan and Velte, 1989, Hopkins et al., 2000). There is also evidence of trophic transfer of some metals associated with fly ash (Coughlan and Velte, 1989, Ruangsomboon and Wongrat, 2006, Chan, Wang and Ni, 2003).

The focus of my research was to determine how an aquatic community responds to coal fly ash. The first component of the research compared the community structure of the impacted impoundment with a water body unaffected by coal ash. Differences between the impacted and an un-impacted impoundment could indicate a change in ecosystem functioning. The second component investigated the contaminant burdens in aquatic biota and compared them to similar data for a reference site. Stable isotopes of carbon and nitrogen were also studied from the impacted site to determine potential trophic linkages. These two types of data should elucidate the severity of impacts in the D-Area impoundment and provide insights into patterns of trophic transfer of contaminants through the food chain. The third component of the research was a laboratory experiment designed to identify the relative importance of multiple modes of contaminant uptake in an aquatic predator, *Gambusia holbrookii*. Four treatments were selected to separate direct environmental uptake from trophic uptake.

1.1 References

Brooks, B.W., J.K. Stanley, J.C. White, P.K. Turner, K.B. Wu and T.W. La Point. 2004. Laboratory and field responses to cadmium: an experimental study in effluent-dominated stream mesocosms. Environmental Toxicology and Chemistry 23: 1057-1064.

Chan, S.M., W.-X. Wang and I.-H. Ni. 2003. The uptake of cd, cr, and zn by the macroalga *Enteromorpha crinita* and subsequent transfer to the marine herbivorous rabbitfish, *Siganus canaliculatus*. Archives of Environmental Contamination and Toxicology 44: 298-306.

Clements, W.H., D.S. Cherry and J. Cairns Jr. 1988a. Impact of heavy metals on insect communities in streams: a comparison of observational and experimental results. Canadian Journal of Fisheries and Aquatic Science 45: 2017-2025.

Clements, W.H., D.S. Cherry and J. Cairns Jr. 1988b. Structural alterations in aquatic insect communities exposed to copper in laboratory streams. Environmental Toxicology and Chemistry 7: 715-722.

Coughlan, D.J. and J.S. Velte. 1989. Dietary toxicity of selenium-contaminated red shiners to striped bass. Transactions of the American Fisheries Society 118: 400-408.

Diamond, J.M., D.W. Bressler and V.B. Serveiss. 2002. Assessing relationships between human land uses and the decline of native mussels, fish and macroinvertebrates in the Clinch and Powell River watershed, USA. Envrironmental Toxicology and Chemistry 21: 1147-1155.

EPA. 1997. Profile of the fossil fuel electric power generation industry. Rep. EPA/310-R-97-007, U.S. Environmental Protection Agency, Washington D.C.

Hopkins, W.A., J.W. Snodgrass, J.H. Roe, B. Jackson, J.C. Gariboldi and J.D. Congdon. 2000. Detrimental effects associated with trace element uptake in lake chubsuckers (*Erimyzon sucetta*) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology 39: 1-7.

NRC. 2006. Managing coal combustion residues in mines. National Acadamies Press, Washington D.C.

Ruangsomboon, S. and L. Wongrat. 2006. Bioaccumulation of cadmium in an experimental aquatic food chain involving phytoplankton (*Chlorella vulgaris*), zooplankton (*Moina macrocopa*), and the predatory catfish *Clarias macrocephalus* x *C. gariepinus*. Aquatic Toxicology 78: 15-20.

Specht, W.L., D.S. Cherry, R.A. Lechleitner and J. Cairns Jr. 1984. Structural, functional and recovery responses of stream invertebrates to fly ash effluent. Canadian Journal of Fisheries and Aquatic Science 41:884-896.

Winner, R.W., H.A. Owen and M.V. Moore. 1990. Seasonal variability in sensitivity of freshwater Lentic communities to a chronic copper stress. Aquatic Toxicology 17: 75-92.

Wogram, J. and M. Liess. 2001. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. Bulletin of Environmental Contamination and Toxicology 67: 360-367.

Chapter 2

Community Profile of the D-Area Impoundment

2.1 Introduction

Many aquatic invertebrates display sensitivities to metals associated with coal-ash. The effect of heavy metals on microcrustaceans has been well studied. Wogram and Liess (2001) used *Daphnia magna* as a standard against which to measure other microcrustaceans according to metal sensitivity. They found that microcrustaceans, especially copepods and cladocerans, were the most sensitive of invertebrates. Winner, Owen and Moore (1990) found that zooplankton were more sensitive to copper during the spring season than any other season. In other experiments, it was found that the cladoceran, *Chydorus piger*, was more sensitive to cadmium than the model organism *D. magna*. *C. piger* experienced decreased longevity, lower population growth and reduced average size, which can result in higher predation by invertebrate predators (Dekker, Krips and Admiraal, 2002).

Other studies have investigated the effects of metals on insect densities. In the Clinch River, VA, insect density and richness were greatly reduced as a result of copper and zinc pollution (Clements et al., 1988a,b). The trichopteran family Hydropsychidae and the dipteran subfamily Orthocladiini dominated polluted sites while Ephemeroptera and the dipteran subfamily Tanytarsini dominated the reference sites. They also found the same results using a mesocosm experiment in which these four groups were allowed to colonize the mesocosms prior to the experiment. In another study on the Clinch River, Diamond, Bressler and Serveiss (2002) found that the EPT index (Ephemeroptera, Plecoptera, Trichoptera - a common method used as indicators of biotic stress) was lowered in areas of the river that were receiving heavy metals from mining runoff. They also found that native mussel species richness was declining due to runoff and spills from coal mines and other industrial processes. Brooks et al. (2004) found that cadmium had deleterious effects on aquatic snails. However, because common parasites of snails cannot tolerate metal pollution, these snails may thrive in polluted sites compared to pristine sites. Prat et al. (1999) found that densities of sensitive macroinvertebrates (Ephemeroptera, Trichoptera and Plecoptera) were reduced downstream of a mine waste spill containing heavy metals. Less sensitive invertebrates such as Coleoptera and Odonata experienced decreased densities but not as profound as the more sensitive invertebrates. Quigley (1981) also notes that odonates, along with dipterans, were more tolerant of metal stress. Specht et al. (1984) monitored the effects of outflow from a fly ash settling basin on stream invertebrates. They found that certain species of Ephemeroptera, Trichoptera and Plecoptera experienced decreased densities and slower recovery rates in sites downstream from the release of the fly ash compared to an upstream site. Conversely, a coleopteran species experienced increased densities in the downstream sites because of its resistance to metal toxicity and release from competition or other interactions with metal-sensitive species. Metal sensitivities in macroinvertebrates were also revealed in a study of an acid mine spill on the Guadiamar River in Spain (Solà et al., 2004). An average of 19 invertebrate families was found in a site upstream from the spill and that average was reduced to three families directly down stream and eight families 15 km further down stream. Studies of a swamp receiving fly ash runoff from settling basins on the Savannah River Site in South Carolina showed that siltation from the ash may have been the most important factor in reducing invertebrate densities, but that dipterans and odonates were the most tolerant to the stress. It was also postulated that the chronic exposure to fly ash may have been instrumental in the slow recovery rates of all aquatic life in the swamp (Cherry et al., 1979 and Cherry et al., 1984).

A reduction in invertebrate abundance or diversity can impede the ecological functions that invertebrates perform in aquatic systems. Odum (1985) reported that a loss of biodiversity in a stressed ecosystem decreases in-system nutrient cycling, resulting in nutrients leaving that system. He also stated that the loss of diversity and abundance leads to a decrease in the efficiency of resource use. Aquatic invertebrates provide trophic linkages from the microscopic producers to the vertebrate predators, thereby transferring energy from microbes to higher organisms (Covich, Palmer and Crowl, 1999, Downing, 2005). Aquatic invertebrates also function to help break down larger organic material and recycle nutrients back into the ecosystem (Covich, Palmer and Crowl, 1999, Cardinale, Palmer and Collins, 2002). The aquatic invertebrates include a wide range of taxa, from microcrustaceans such as cladocerans and copepods to macrocrustaceans such as amphipods, fairy shrimp and crayfish to insects such as dipterans, odonates and coleopterans.

Studies also show that stability of systems increases with greater biodiversity. The greater the number of species that occupy a functional group or provide a particular ecosystem service, the less effect the loss of one species has on the ecosystem as a whole (Naeem, 1998 and Walker, 1992). Lawton and Brown (1993) contend that when stressors impact ecosystems with sufficient species redundancy, it results in a change in species richness within functional groups instead of the entire loss of a functional group when one species is lost from that system. In addition to being a buffer against disturbance, Downing and Leibold (2002) found that an increased number of species can increase ecosystem productivity (decomposition rates and biomass) and system respiration. They also proposed that the loss of one or a few species may reduce this effect and indirectly have larger negative effects on ecosystem functioning.

The purpose of this study was to characterize the aquatic invertebrate community at the D-Area impoundment, a man-made swamp that receives overflow from coal-ash settling basins on the Savannah River Site (SRS) near Aiken, SC. I hypothesized that pollution-sensitive organisms, such as microcrustaceans, ephemeroptera, and odonates will have reduced densities compared to invertebrates of a similar, uncontaminated impoundment on the SRS. This invertebrate abundance data, along with data on metal burdens and a trophic transfer experiment will provide insight into the impact of coal combustion wastes on aquatic ecosystems.

2.2 Study Site

The D-Area impoundment is a 2-hectare impoundment situated near the D-Area coal-fired power plant on the SRS near Aiken, SC. The SRS is a 780-km^2 federal reserve owned by the Department of Energy and located on the Upper Coastal Plain of SC. The D-Area power plant is a 70 MW coal-powered plant situated near the Savannah River. Fly ash is mixed with water from the Savannah River to form slurry that is then pumped to a primary (15 ha) and secondary (6 ha) settling basins. Some of the water from these basins then overflows through a pipe into the impoundment. Water then leaves the impoundment via outflow pipes, which lead to Beaver Dam Creek, which empties back into the Savannah River. This configuration has been used since the late 1970s (Rowe, Hopkins and Congdon, 2002). Temperature, depth, conductivity and pH measurements were taken at each station on each sampling day. Temperature ranges of the impoundment were 12.0°C - 35.0°C; pH ranged from 6.0 - 8.3; conductivity ranged from 0.14 mS/cm - 0.77 mS/cm. Water depth at the sampling stations ranged from 9 cm to 52 cm with an average depth of 22.4 cm. Maximum depth was approximately 150 cm (personal observation).

The water coming from the overflow pipe into the impoundment carves a main channel that bisects the impoundment. Stations 1, 2, 5 and 6 are located along the edges of the channel with depths ranging from 15 to 52 cm. These collective sites stations will be referred to as the main channel. The main channel has a visible current and little vegetation other than small clumps of *Eleocharis* sp. and some *Myrica cerifera* growing on small hummocks. On either side of the main channel are backwater sections characterized by still, shallow water. Stations 3, 4 and 7-10 are located in these areas and will be referred to as backwater stations. Depth here ranges from 6 to 39 cm. These areas commonly contain *Typha latifolia*, *Juncus* sp., *Eleocharis* sp., *Chara* sp. and *Ludwigia sphaerocarpa*.

Fish found in the impoundment include mosquitofish (*Gambusia holbrookii*), largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), redbreast sunfish (*Lepomis auritus*), spotted sunfish (*Lepomis punctatus*) (Hopkins et al., 2000) and the occasional gar (*Lepisosteus* sp.). Ranid larvae are common during the breeding season. The invertebrates at the impoundment are also quite diverse, including crayfish (*Procambarus* sp.), odonates, amphipods, microcrustaceans, worms, snails and dipteran larvae. These are discussed in further detail below.

Pond 4, the reference site, is an 11-ha impoundment with a mean depth of 1.6 m and a maximum depth of 4 m. It was constructed in 1958 as part of the cooling system for a nuclear production reactor, but has not received heated effluent since 1988 (Staton et al., 2003). Water temperature ranges from 10-30 °C annually. Nearly half the pond area consists of a heavily vegetated littoral zone dominated by cattail (*Typha* spp) and floating (predominantly *Nymphaea* and *Brasenia*) and submerged (predominantly *Potamogeton*, *Myriophyllum*, *Ceratophyllum* and *Utricularia*) macrophytes (Pinder et al., 2004). Historical data on invertebrate communities was used in the comparison with data from the D-Area impoundment. The sampling of invertebrates at Pond 4 was similar to that at the D-Area impoundment of this study (Taylor et al., personal communication). Only samples collected from shallow water stations (< 2m) at Pond 4 were used in the comparisons. Invertebrate abundance and diversity data from a greater number of reference sites would be ideal for statistical comparisons, but was logistically impossible to collect during this study.

2.3 Methods

Ten sampling stations were selected randomly within the impoundment to ensure good coverage of the site and to investigate spatial heterogeneity (Figure 2.1). Benthic and planktonic organisms were sampled once per season for one year. Benthos was collected at each station using a Wildco PetitePonar grab. Each sample was rinsed through a 125-μm sieve with site water and preserved with 90% ethanol. Plankton was collected at each site with a 3.3 liter Van Dorn bottle. Plankton samples were rinsed through a 102-μm mesh sieve with tap water and preserved in a 4% formalin-sucrose mixture. Temperature was recorded using a mercury thermometer and pH was recorded using an Oakton Instruments pHTestr. Conductivity was measured using a Hanna Instruments DiST WP conductivity meter and depth was measured with a meter stick. Temperature, depth, conductivity and pH measurements were taken at each station.

All samples were brought back to the lab and processed at a later date. Invertebrates were sorted out of each sample and counted and identified using a Wild Heerbrugg Wild M5A dissecting microscope and a Zeiss Axioskop compound microscope. Samples with very high abundances of invertebrates were sub-sampled. Individuals were identified to class for ostracods and worms and to family or lower for all other organisms using Thorp and Covich (1991) and Merritt and Cummins (1984). All invertebrates were grouped into 10 categories: dipterans (DIPT), macrocrustaceans (MACR), mollusks (MOLL), odonates (ODON), worms (WORM), ephemeropterans (EPHE), microcrustaceans (MICR), coleopterans (COLE), hemipterans (HEMI), and trichopterans (TRIC). These are the same categories of invertebrates that were used in the historical data from Pond 4.

Invertebrate counts were converted to organisms$\cdot$m^{-2}. Planktonic and benthic abundances were summed to estimate total abundance at each station. Total abundances were averaged across stations to provide seasonal abundances; seasonal abundances were averaged to give annual abundances. Statistical analyses were performed using S-Plus (Insightful Corporation, Seattle).

2.4 Results

A total of 58 taxa were identified from the D-Area impoundment (Table 2.1). The taxa were combined into 10 categories based on taxonomy and body size (for crustaceans). The category DIPT excludes the family Chaoboridae, which were not found at D-Area. The majority of dipterans at D -Area were chironomids. Macrocrustaceans at D-Area consisted entirely of amphipods; no crayfish were collected in this study. The most abundant invertebrates were microcrustaceans, dipterans, and worms.

The backwater stations (stations 4 and 7-10, Figure 2.1) consistently had the highest densities of organisms for most categories. Abundances at station 3 in the main channel were also sometimes elevated. Microcrustaceans and dipterans, which were found at all stations, followed this trend most closely. Worms were found in seven of the ten stations while ephemeropterans and macrocrustaceans were found in six of the ten stations. Mollusks were found in four stations. *Corbicula* were found only in the main channel stations. Snails were only found in the backwater stations of 4 and 8. The densities of *Corbicula* were roughly twice that of gastropods (3319.5 animals$\cdot$m^{-2} and 1391.3 animals$\cdot$m^{-2}, respectively). The coleopterans were found only at two stations.

Microcrustaceans and worms showed strong seasonal trends in abundance (Figures 2.2-2.4). Both were more abundant by a factor of 10 or more in summer than in other seasons. Densities of microcrustaceans were significantly higher in the summer compared to spring ($p=0.01$) and in the summer compared to the winter ($p<0.01$). Densities of worms were significantly higher in the summer than in the spring ($p<0.01$) while densities were significantly higher in both the summer and fall compared to the winter ($p<0.01$ for both comparisons). The dipterans, in contrast, showed much less seasonal variation, although their abundances were significantly higher in both the winter and fall compared to the spring ($p=0.01$ for both comparisons). The various subfamilies and tribes that make up the DIPT category showed differing temporal trends. The Orthocladiinae had the highest density of all the chironomids during the fall season, but Tanypodinae were highest in density during the summer and winter and Chironomini had the highest density during the spring. Tanypodinae had the highest average yearly abundance. The densities of other organisms were too low or too variable to show significant temporal trends. However, abundances of odonates, mollusks, hemipterans and ephemeropterans were generally higher in summer. Macrocrustaceans, coleopterans, and trichopterans were more abundant in the fall.

The comparison of yearly abundances at Pond 4 (the reference site) and the D-Area impoundment (the study site) showed significant differences for five of the categories present in both ponds (Table 2.2). Pond 4 contained an additional category, *Chaoborus* (CHAO), a planktonic dipteran larva not found at the D area impoundment. The differences in abundances were significant for COLE (p=0.01), MICR (p<0.01), MOLL (p<0.01), ODON (p=0.01), and WORM (p<0.01), where densities were higher in the Pond 4 populations.

2.5 Discussion

The spatial distribution of invertebrates within the D-Area impoundment reflects the morphology of the site. Invertebrates were less abundant in the main channel than in in the heavily vegetated backwater stations. The main channel offers very little protection and resources for resident invertebrates because the vegetation is sparse and the current is visibly faster. It is also in this main channel area that larger fish such as sunfish and bass were observed, which in addition to current and lack of vegetation could explain the lack of some of the insects.

Comparisons with the reference site suggest that effects of the coal fly ash did not severely impair composition of the community of invertebrates at the D-Area impoundment. Among the major invertebrate groups found at Pond 4, only the *Chaoborus*, a predatory dipteran larva, was absent from D-Area. *Chaoborus* larvae typically migrate into deep water to escape predation by fish during the day; its absence from D-Area may reflect the absence of suitable refuges.

Abundances of invertebrates did differ substantially between the D-Area impoundment and the reference site. Among the most important taxa, microcrustaceans and worms were much more abundant at the reference site, although dipterans, excluding *Chaoborus*, were similar in abundance.

Many other studies have shown decreased abundances of aquatic invertebrates in the presence of coal ash pollution (Brooks et al., 2004, Clements, Cherry and Cairns, 1988a, Prat et al., 1999). However, the taxa that were depleted in the D area impoundment do not exactly correspond to taxa that were depleted in other studies. In the Clinch River, VA, Ephemeroptera populations were greatly reduced just downstream of a coal-fired power plant (Clements, Cherry and Cairns, 1988a). Elevated levels of copper and zinc were found to be the cause of this decline. D-Area does not follow this pattern. Clements, Cherry and Cairns also report that Orthocladiinae was the only dipteran sub-family whose densities were not reduced in the presence of metals. This is also not the case in this study. Orthocladiinae were abundant, but not dominant at the D-Area impoundment. They also report that Hydropsychidae, a trichopteran, was tolerant to copper and zinc. That particular family of trichopterans is mainly lotic and was not found at the D-Area impoundment. However, trichopteran densities were not significantly different between the D area impoundment and Pond 4. Also of interest is the fact that the odonates show reduced abundances in the D-Area impoundment compared with Pond 4 while in previous studies the odonates are one of the more tolerant invertebrates (Quigley, 1981 and Wogram and Liess, 2001).

The six categories of invertebrates that had significantly reduced densities in the D-Area impoundment represented three functional groups. The microcrustaceans, worms and mollusks (represented by the *Corbicula* here) are filter feeders, removing organic matter and phytoplankton from the wetland. The other half of the mollusk category, the snails, are scrapers feeding on plant material and detritus. The final two categories, the odonates and coleopterans are predators. The group consisting of *Chaoborus* that was not found in the D-Area impoundment is also a predator. The functional groups of filterers and scrapers are populated by the categories of dipterans, macrocrustaceans, ephemeropterans, and trichopterans; all of whose populations were not reduced in the D-Area impoundment. Predators were the most affected functional group with three out of the four categories of invertebrates belonging to the predator functional group (*Chaoborus*, coleopterans and odonates) showing significantly reduced densities in the D-Area impoundment compared to Pond 4. Hemipterans were the only category representing the predator functional group that did not show decreased densities in the D-Area impoundment compared to Pond 4. While the hemipterans had the highest population density of the invertebrate predators, this high abundance may cause less stability with respect to functional redundancy. However, mosquitofish (*Gambusia holbrookii*) are generalist predators and may be considered a redundant organism.

The reduced densities in five of the invertebrate categories may result in their respective functional groups being less effective in their roles in the ecosystem. This leads to the conclusion that the ash deposited in the D-Area impoundment does impact some of the organisms found to be sensitive to metals in other studies (Wogram and Liess, 2001, Brooks et al., 2004, Schmidt et al., 2002). Further investigations on the functioning of the D-Area impoundment ecosystem, such as biomass production and nutrient cycling, should be carried out to determine the extent of the impact to ecosystem functioning.

2.6 References

Brooks, B.W., J.K. Stanley, J.C. White, P.K. Turner, K.B. Wu and T.W. La Point. 2004. Laboratory and field responses to cadmium: an experimental study in effluent-dominated stream mesocosms. Environmental Toxicology and Chemistry 23: 1057-1064.

Cardinale, B.J., M.A. Palmer and S.L. Collins. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415: 426-429.

Cherry, D.S., R.K. Guthrie, E.M. Davis and R.S. Harvey. 1984. Coal ash basin effects (particulates, metals, acidic pH) upon aquatic biota: an eight- year evaluation. Water Resources Bulletin 20: 535-544.

Cherry, D.S., S.R. Larrick, R.K. Guthrie, E.M. Davis and F.F. Sherberger. 1979. Recovery of invertebrate and vertebrate populations in a coal ash stressed drainage system. Journal of the Fisheries Research Board of Canada 36: 1089-1096.

Clements, W.H., D.S. Cherry and J. Cairns Jr. 1988a. Impact of heavy metals on insect communities in streams: a comparison of observational and experimental results. Canadian Journal of Fisheries and Aquatic Science 45: 2017-2025.

Clements, W.H., D.S. Cherry and J. Cairns Jr. 1988b. Structural alterations in aquatic insect communities exposed to copper in laboratory streams. Environmental Toxicology and Chemistry 7: 715-722.

Covich, A.P., M.A. Palmer and T.A. Crowl. 1999. The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling. BioScience 49: 119-127.

Covich, A.P., M.C. Austin, F. Bärlocher, E. Chauvet, B.J. Cardinale, C.L. Biles, P. Inchausti, O. Dangles, M. Solan, M.O. Gessner, B. Statzner and B. Moss. 2004. The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. BioScience 54: 767-775.

Dekker, T., O.E. Krips and W. Admiraal. 2002. Life history changes in the benthic cladoceran *Chydorus piger* induced by low concentrations of sediment-bound cadmium. Aquatic Toxicology 56: 93-101.

Diamond, J.M., D.W. Bressler and V.B. Serveiss. 2002. Assessing relationships between human land uses and the decline of native mussels, fish and macroinvertebrates in the Clinch and Powell River watershed, USA. Envrironmental Toxicology and Chemistry 21: 1147-1155.

Downing, A.L. 2005. Relative effects of species composition and richness on ecosystem properties in ponds. Ecology 86: 701-715.

Downing, A.L. and M.A. Leibold. 2002. Ecosystem consequences of species richness and composition in pond food webs. Nature 416: 837-841.

Hopkins, W.A., J.W. Snodgrass, J.H. Roe, B. Jackson, J.C. Gariboldi and J.D. Congdon. 2000. Detrimental effects associated with trace element uptake in Lake Chubsuckers (*Erimyzon sucetta*) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology 39: 1-7.

Lawton, J.H. and V.K. Brown. 1993. Redundancy in ecosystems. Pages 255-270 in E.D. Schulze and H.A. Mooney, editors. Biodiversity and ecosystem function. Springer Verlag, New York.

Lefcort, H., D.P. Abbot, D.A. Cleary, E. Howell, N.C. Keller and M.M. Smith. 2004. Aquatic snails from mining sites have evolved to detect and avoid heavy metals. Archives of Environmental Contamination and Toxicology 46: 478-484.

Merrit, R.W. and K. W. Cummins. 1996. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Company, Dubuque, IA, USA.

Naeem, S. 1998. Species redundancy and ecosystem reliability. Conservation Biology 12: 39-45.

Odum, E.P. 1985. Trends expected in stressed ecosystems. BioScience 35: 419-422.

Prat, N., J. Toja, C. Sola, M.D. Burgos, M. Plans and M. Rieradevall. 1999. Effect of dumping and cleaning activities on the aquatic ecosystems of the Guadiamar River following a toxic flood. The Science of the Total Environment 242: 231-248.

Quigley, M.A. 1981. Freshwater macroinvertebrates. Journal of Water Pollution Control Federation 53: 1015-1027.

Rowe, C.L., W.A. Hopkins and J.D. Congdon. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: a review. Environmental Monitoring and Assessment 80: 207-276.

Solà, C., M. Burgos, Á. Plazuelo, J. Toja, M. Plans and N. Prat. 2004. Heavy metal bioaccumulation and macroinvertebrate community changes in a Mediterranean stream affected by acid mine drainage and an accidental spill (Guadiamar River, SW Spain). Science of the Total Environment 333: 109-126.

Specht, W.L., D.S. Cherry, R.A. Lechleitner and J. Cairns Jr. 1984. Structural, functional and recovery responses of stream invertebrates to fly ash effluent. Canadian Journal of Fisheries and Aquatic Science 41:884-896.

Staub, B.P. 2000. Eastern mosquitofish (*Gambusia holbrooki*) population persistence in a coal ash contaminated habitat: tolerance or adaptation? Master's Thesis, University of Georgia.

Thorp, J.H. and A.P. Covich. 1991. Ecology and Classification of North American Freshwater Invertebrates. Academic Press, Inc., New York, NY, USA.

Walker, B.H. 1992. Biodiversity and ecological redundancy. Conservation Biology 6: 18-22.

Winner, R.W., H.A. Owen and M.V. Moore. 1990. Seasonal variability in sensitivity of freshwater Lentic communities to a chronic copper stress. Aquatic Toxicology 17: 75-92.

Wogram, J. and M. Liess. 2001. Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. Bulletin of Environmental Contamination and Toxicology 67: 360-367.

Table 2.1: Categories of invertebrates at D-Area impoundment

Category	Class/Order	Family	Tribe/Genus
DIPT	order Diptera		
		family Chironomidae	
			Orthocladiinae sp.
			Podonominae sp.
			Tanytarsini sp.
			Chironomini sp.
			Tanypodinae sp.
			Diamesinae sp.
		family Ceratapogonidae	
		family Culicidae	
			Haemogogus sp.
		family Tipulidae	
		family Tanyderidae	
MACR	order Amphipoda	family Hyalellidae	
			Hyalella sp.
		family Pontoporeiidae	
			Monoporeia sp.
		family Crangonyctidae	
			Stygobromus sp.
MOLL	class Gastropoda		

Continued on next page

Category	Class/Order	Family	Tribe/Genus
		family Physidae	
	class Bivalvia		
		family Corbiculidae	
ODON	order Odonata		
		family Coenagrionidae	
WORM	class Oligochaeta		
	class Hirudinea		
EPHE	order Ephemeropta		
		family Caenidae	
		family Tricorythidae	
		family Potomanthidae	
		family Siphlonuridae	
		family Baetidae	
MICR	class Branchiopoda		
		family Bosminidae	
		family Chydoridae	
			Pleuroxus sp.
			Chydorus sp.
			Alona sp.
			Camptocercus sp.
			Alonella sp.
			Pseudochydorus sp.
			Leydigia sp.
		family Daphniidae	
			Daphnia sp.

Continued on next page

Category	Class/Order	Family	Tribe/Genus
			Simocephalus sp.
			Ceriodaphnia sp.
		family Macrothricidae	
			Ilyocryptus sp.
		family Moinidae	
		family Sididae	
			Diaphanosoma sp.
	order Copepoda		
		family Cyclopidae	
			Cyclopoidae sp.
			Macrocyclops sp.
			Paracyclops sp.
			Ectocyclops sp.
			Microcyclops sp.
			Halicyclops sp.
			Diacyclops sp.
			Thermocyclops sp.
			Cyclops sp.
			Cryptocyclops
			Mesocyclops sp.
			Tropocyclops sp.
			Eucyclops sp.
		family Temoridae	
		family Diaptomidae	
	order Ostracoda		

Continued on next page

Category	Class/Order	Family	Tribe/Genus
COLE	order Coleoptera		
		family Hydrophilidae	
HEMI	order Hemiptera		
		family Gelacostoridae	
		family Gerridae	
TRIC	order Trichoptera		
		family Polycentropodidae	
		family Hydroptilidae	

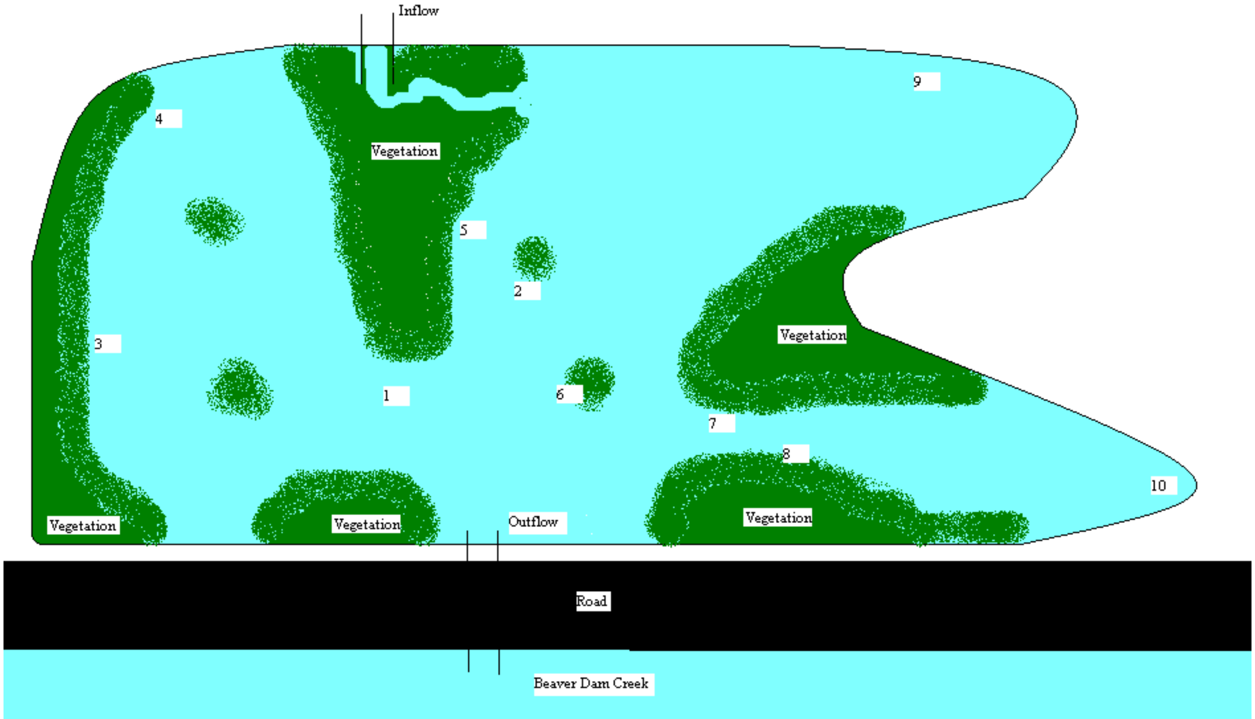

Figure 2.1: Stations within the D-Area Impoundment.
Stations are numbered, other features are labeled.

Table 2.2: Annual invertebrate densities from D-Area impoundment (study site) and Pond 4 (reference site).

	D-Area		**Pond 4**	
	mean	std	mean	std
DIPT	3.69	0.96	3.74	0.95
MICR	4.15	1.50	5.09*	0.56
MACR	0.37	0.92	0.88	1.26
MOLL	0.45	0.96	1.37*	1.16
ODON	0.13	0.60	0.62*	1.18
WORM	1.61	2.07	3.07*	1.59
EPHE	0.66	1.38	0.70	1.24
COLE	0.17	0.74	0.60*	0.92
HEMI	0.51	1.27	0.09	0.38
TRIC	0.32	0.81	0.63	1.19
CHAO	0.00	0.00	2.15*	1.21

Abundance data are $\log_{10}$ transformed. Significant differences between D-Area invertebrate densities and Pond 4 invertebrate densities are denoted by *.

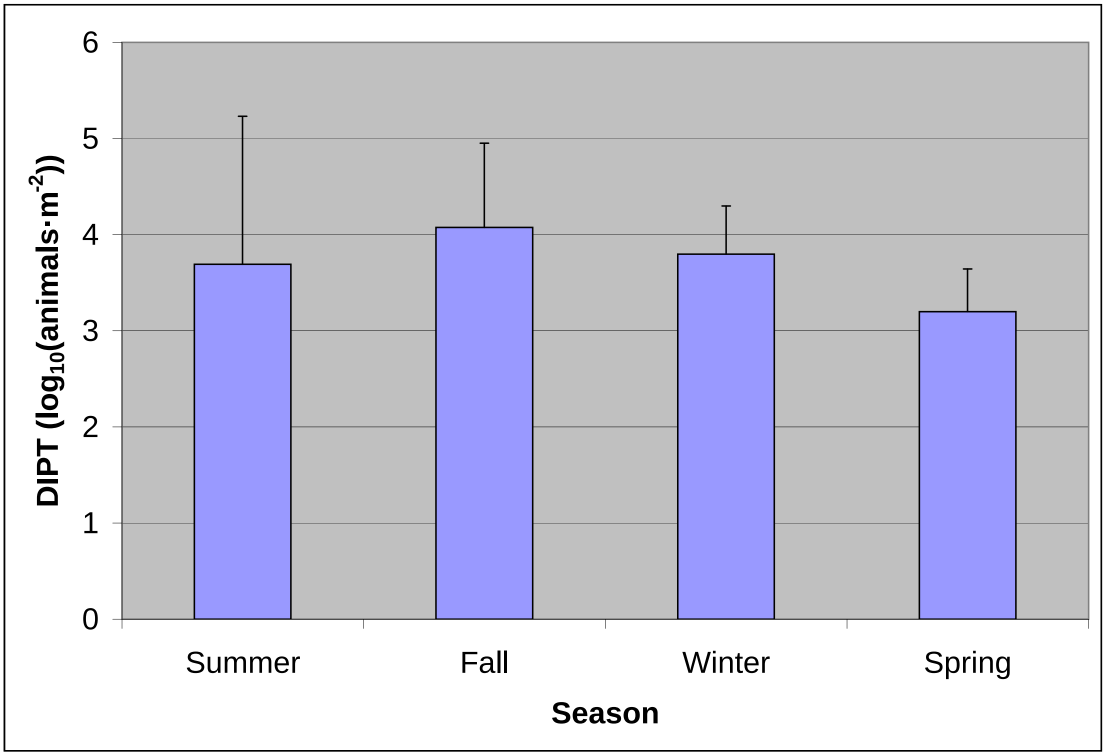

Figure 2.2: Seasonal means and ranges of dipterans (DIPT) in ASH site

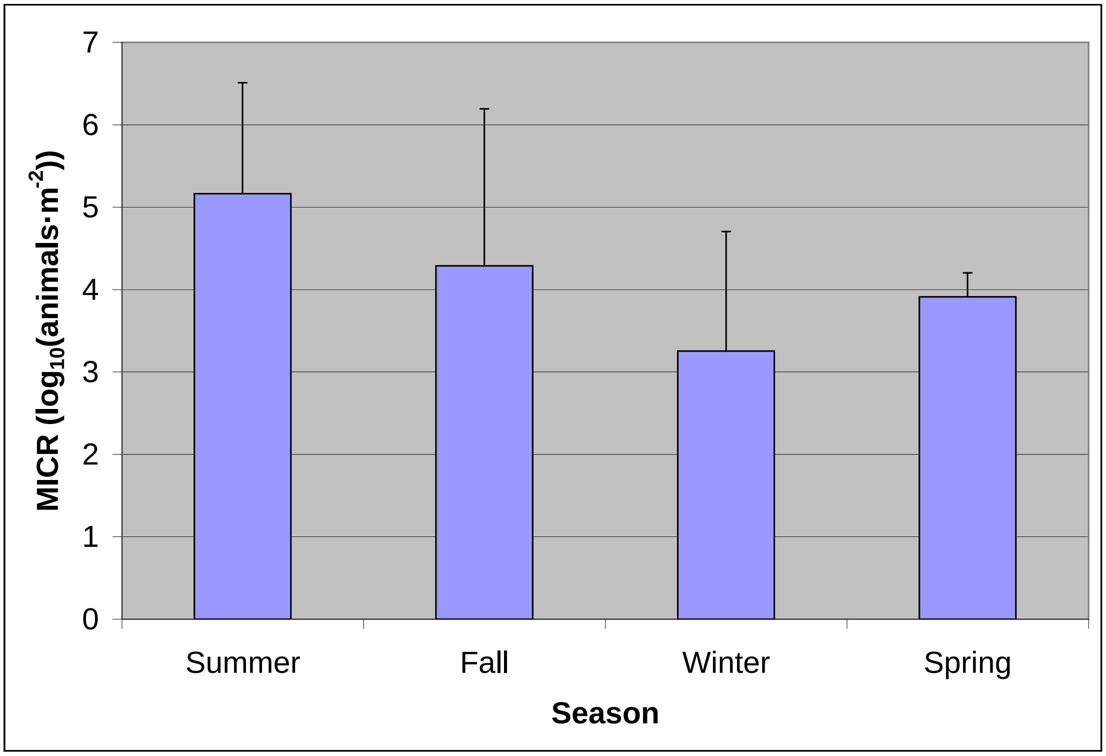

Figure 2.3: Seasonal means and ranges of microcrustaceans (MICR) in ASH site

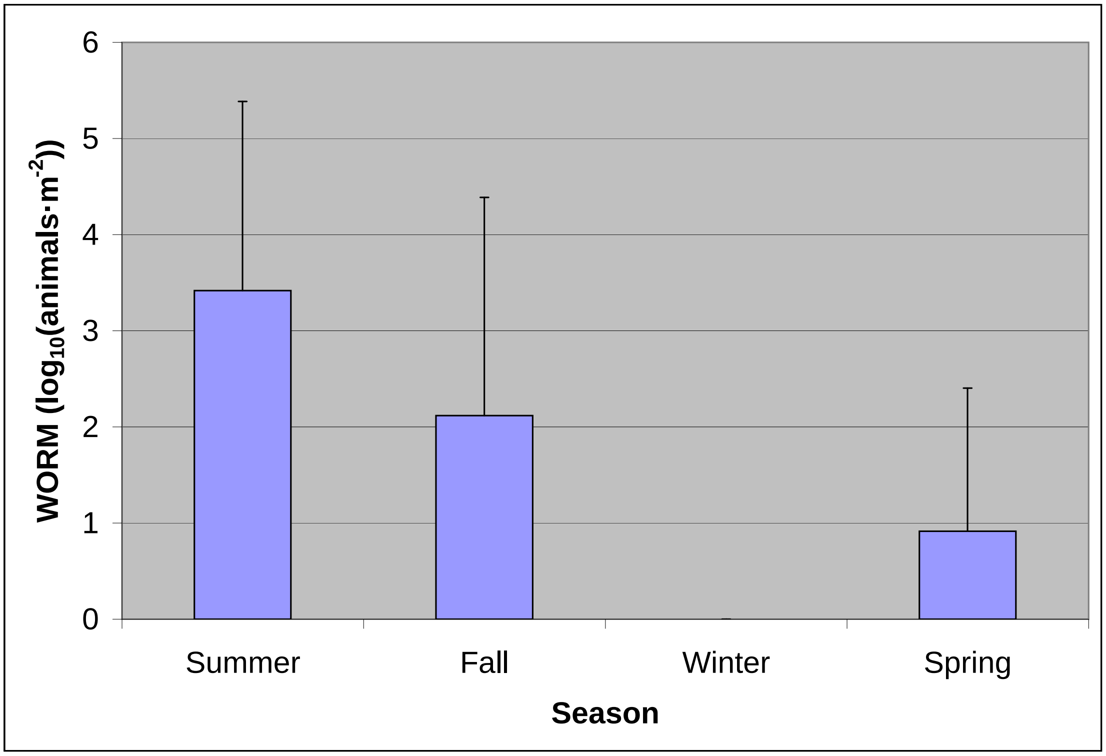

Figure 2.4: Seasonal means and ranges of worms (WORM) in ASH site

Chapter 3

Coal Combustion Related Metals in Field Collected Material and Potential for Trophic Transfer

3.1 Introduction

One of the major waste products of coal combustion for electricity is ash. The ash may be in the form of bottom ash, which is comprised of heavier, larger particles and fly ash, which is lighter and contains smaller particles of combusted coal. Management of ash typically involves placing it in landfills or in surface water retention ponds. Trace elements that are commonly associated with ash are arsenic, barium, cadmium, chromium, lead, mercury, selenium, strontium and zinc as well as other metals (EPA, 1997). The potential for colonization of retention ponds by local biota exists, raising the potential for exposure and accumulation of metals by colonizers. Some of the same metals in coal combustion wastes can be found in run-off from mining practices. Investigations of streams impacted by mining show uptake of many of the same metals as those studies done on ash impacted impoundments (Clements and Rees, 1997, Prat et al., 2004 and Solà et al., 2004). In a constructed wetland supplied with coal combustion waste leachate, iron, manganese, cobalt and nickel were removed from the water and taken up by cattail (*Typha latifolia*) and *Chara* (Ye et al. 2001). Algae and Sago pondweed (*Potamogeton pectinatus*) have been shown to accumulate selenium (Gutenmann et al., 1976). The caddisfly *Hydropsyche* showed significantly greater levels of zinc, copper and cadmium in stations downstream of an acid mine compared to upstream stations (Solà et al., 2004). Prat et al. (1999) found significant increases in zinc, copper, lead, arsenic, cadmium, antimony and thallium in plankton downstream from a flooded mine and elevated levels of the same metals, except arsenic in zooplankton. Clements and Rees

(1997) found increased levels of cadmium, copper and zinc in the gill and gut tissue of brown trout (*Salmo trutta*) that inhabited areas downstream of a mining site. Lake chubsuckers (*Erimyzon sucetta*) exposed to water, sediment and benthic invertebrates from a coal ash-contaminated swamp showed elevated levels of a suite of trace elements, including arsenic, cadmium, chromium, and selenium (Hopkins et al., 2004). Staub et al. (2004) found significantly elevated levels of selenium, arsenic, cadmium and copper in mosquitofish (*Gambusia holbrooki*) collected from a coal fly-ash settling basin system.

Naturally occurring elements may commonly exist in several forms which differ slightly in their number of neutrons. The different forms of these elements (i.e. those with different numbers of neutrons) are called isotopes. Biologically, the heavier isotopes (those with more neutrons) move more slowly through tissue than the lighter isotopes. Isotopes, mainly those of carbon and nitrogen, can be used to infer trophic linkages in an ecosystem. As one organism consumes another of a lower trophic position, the consumer accumulates the heavier nitrogen isotope, ^{15}N, at a level averaging 3 parts per thousand (per mil) greater than its food (DeNiro and Epstein, 1981, Schoeller, 1999, Minagawa and Wada, 1984), which is referred to as enrichment of the heavier isotope. The value of the measurement of the heavier isotope becomes more positive with enrichment. This is due to discrimination against the heavier isotope in metabolic processes. For example, Minagawa and Wada (1984) found the average step-wise enrichment of the heavier nitrogen isotope from phytoplankton to zooplankton to fish to be 3.1 per mil and 3.0 per mil respectively. Hansson et al. (1997) analyzed the trophic differences of ^{15}N in a marine food web consisting of particulate organic matter, zooplankton, shrimp and fish and found the average enrichment factor of ^{15}N to be 2.4 per mil. The carbon isotope of interest, ^{13}C, is used to determine the beginning of the particular food chain, as plants have different isotopic signatures depending on their method of photosynthesis (Peterson and Howarth, 1987, Odum and Barrett, 2005). The ^{13}C signature stays consistent through each increase in trophic level. In a feeding trial using carp (*Cyprinus carpio*), Focken and Becker (1998) showed that the ^{13}C values in the carp were practically the same as the feed used in the trial (-23.9 per mil for carp and -23.8 per mil for feed). Hobson and Clark (1992) further illustrate the use of the carbon isotope as an indicator of the basis of a food chain. The diets of crows and quails are shifted from wheat to corn, which have different ^{13}C signatures. Birds were sacrificed at intervals throughout the study and various tissues analyzed to determine the time until they reflected the isotopic signature of the new food source. Peterson and Howarth (1987) show one of the difficulties of using stable isotopes in food web studies. In trying to determine trophic linkages in an estuarine system, the $\delta^{13}C$ of the primary consumers did not match that of any of the proposed food sources, but lay somewhere in between. They stated that this could be the result of consumers using multiple food sources, with varying degrees of frequency or that an unknown food source may be utilized. If multiple primary producers, with differing ^{13}C values, form the base of a food chain, the resulting ^{13}C of the higher trophic levels will lie somewhere in between those of the primary producers.

The use of isotopes fills an important gap in food web studies. Before isotopes, inferences on feeding habits were formed primarily from gut content analysis. This can be problematic as some food items are digested at different rates and therefore not equally represented in the gut. Shifts in diet due to seasonal availability or changes in nutritional needs may cause further complications in determining feeding habits as prey may either be considered a consistent food source or not represented in the gut at all depending on the timing of the gut analysis.

Stable isotopes can be used to identify trophic pathways for the movement of contaminants through ecosystems. For example, Atwell, Hobson and Welch (1998) looked at mercury accumulation in an arctic marine food web. They found a linear relationship between ^{15}N and mercury levels in muscle tissue of marine animals. As ^{15}N increased so did mercury levels at a biomagnification factor of 0.2. Polar bears (*Ursus maritimus*) were the only animal that had lower mercury levels than its prey, the ringed seal (*Phoca hispida*). They explain this by the fact that polar bears preferentially feed on the skin and fat of seals which contain less mercury. Some caution should be taken when relying on isotopes to infer trophic positions. Fisk et al. (2002) used stable nitrogen isotopes, gut content analysis and organochlorine contaminants to determine the trophic status of the Greenland shark (*Somniosus microcephalus*). For comparison, they included turbot (*Reinhardtius hippoglossoides*) and ringed seal (*Phoca hispida*). Both the ringed seal and turbot are thought to be at least one trophic level below the Greenland shark and both have actually been found in the guts of Greenland sharks. Interestingly, the sharks have similar ^{15}N values as the turbot and ringed seal, suggesting a similar trophic position. Organochlorines, which biomagnify, are much higher in the Greenland shark as compared to the turbot and ringed seal, which along finding turbot and seal in the shark gut indicates that it is a trophic level higher than the turbot and seal. Fisk et al. suggest that this discrepancy is caused by the high urea concentration in elasmobranch blood that allows the shark to better osmoregulate. It is possible that the urea is isotopically light and influences the δ^{15}N of the shark making its nitrogen isotopic signature similar to that of animals a trophic level below the shark.

The first goal of this study was to determine whether body burdens of metals from coal combustion residue (CCR) in organisms in an ash impoundment were elevated relative to those of reference sites. I also used stable isotopes of carbon and nitrogen to infer potential trophic linkages among the organisms from the D-Area impoundment. The second goal of this study was to examine evidence for biotransfer of metals within the food web. For biota in which any of the CCR-related metals were elevated, we then asked whether contaminant burdens increased along plausible trophic pathways.

3.2 Study Site

The D-Area impoundment is a 2-hectare impoundment situated near the D-Area coal-fired power plant on the Savannah River Site (SRS) near Aiken, SC. The SRS is a 780-km^2 federal reserve owned by the Department of Energy and located on the Upper Coastal Plain of SC. The D-Area power plant is a 70 MW coal-powered plant situated near the Savannah River.

Fly ash is mixed with water from the Savannah River to form slurry that is then pumped to a primary (15 ha) and secondary (6 ha) settling basins. Some of the water from these basins then overflows through a pipe into the impoundment. Water then leaves the impoundment via outflow pipes, which lead to Beaver Dam Creek, which empties back into the Savannah River. This configuration has been used since the late 1970s (Rowe, Hopkins and Congdon, 2002). Temperature, depth, conductivity and pH measurements were taken at each station on each sampling day. Temperature ranges of the impoundment were 12.0°C - 35.0°C; pH ranged from 6.0 - 8.3; conductivity ranged from 0.14 mS/cm - 0.77 mS/cm. Water depth at the sampling stations ranged from 9 cm to 52 cm with an average depth of 22.4 cm. Maximum depth was approximately 150 cm (personal observation).

The water coming from the overflow pipe into the impoundment carves a main channel that bisects the impoundment. Stations 1, 2, 5 and 6 are located along the edges of the channel with depths ranging from 15 to 52 cm. These collective stations will be referred to as the main channel. The main channel has a visible current and little vegetation other than small clumps of *Eleocharis* sp. and some *Myrica cerifera* growing on small hummocks. On either side of the main channel are backwater sections characterized by still, shallow water. Stations 3, 4 and 7-10 are located in these areas and will be referred to as backwater stations. Depth here ranges from 6 to 39 cm. These areas commonly contain *Typha latifolia*, *Juncus* sp., *Eleocharis* sp., *Chara* sp. and *Ludwigia sphaerocarpa*. Fish found in the impoundment include mosquitofish (*Gambusia holbrookii*), largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), redbreast sunfish (*Lepomis auritus*), spotted sunfish (*Lepomis punctatus*) (Hopkins et al., 2000) and the occasional gar (*Lepisosteus* sp). Ranid larvae are common during the breeding season. The invertebrates at the impoundment are also quite diverse, including crayfish (*Procambarus* sp.), odonates, amphipods, microcrustaceans, worms, snails and dipteran larvae.

Reference material was collected from two small impoundments on the SRS. Both are abandoned farm ponds, built prior to 1951, and neither has received coal fly ash or other waste from industrial activities. Dick's Pond, located approximately 15 km from D-Area, is 1.0 hectares in area with maximum depth of approximately 3.6 m and has circumneutral pH. Fire Pond is located approximately 11 km from D-Area, is 3.8 hectares in area with a maximum depth of 3 m and also has circumneutral pH. A wetland with similar average depth, flow regime and biotic communities is not found anywhere on the SRS. Metal burden data from a greater number of ponds would be ideal for statistical comparisons, but was logistically impossible during this study.

3.3 Methods

Materials used in contaminant and isotope analysis were collected at the D-Area impoundment and at Fire Pond and Dick's Pond on September 4, 2003 and October 31, 2003, respectively. Sampling stations at the D-Area impoundment are described in chapter 2. Invertebrates were collected using a 102-μm Nitex net on a handheld dipnet frame. Fish were collected using a large dipnet and plants and sediments were collected by hand. Sediments from Fire Pond were collected at two distinct stations (Fire 1 and Fire 5) while biotic samples from Dick's Pond were pooled from all stations within the pond. The organisms collected at all sites were those that were easily captured and occurred in quantities that allowed for enough biomass for metal and isotope analysis. When the same species of plant collected from the D-Area imopundment could not be found in the reference sites, an ecologically similar plant (i.e. another submerged plant or another emergent plant) was used for comparison of metal burdens. This was the case for *Ludwigia*, *Typha* and *Chara*. *Ludwigia* and *Typha* were compared to *Juncus* from Fire Pond sites 1 and 5 and Dick's Pond. *Chara* was compared to *Eleocharis* from Dick's Pond. *Eleocharis* was only found at the D-Area impoundment and Dick's Pond, so there are no comparisons with Fire Pond material for this plants. *Myrica* was not found at Fire 1, so there is no comparison between that site and the D-Area impoundment. All material was brought back to the lab, rinsed in nanopure water and either freeze-dried or oven dried at 65 °C. Bulkier material was then ground using a mortar and pestle. Dried sediment was pushed through a 500-μm sieve. Dried tissue was then digested with nitric acid and hydrogen peroxide in advanced composite Teflon vessels in a MDS 2000 microwave digestion unit (CEM, Matthews, NC). Digested material was analyzed on an ICP-MS (Perkin Elmer Elan DRC Plus). Quality assurance was achieved using the standard reference materials Dolt-3 (dogfish liver), BCR-60 (aquatic plant), Apple Leaves and MESS-2 (sediment) (National Research Council Canada, Ottawa, Ontario). Percent recoveries of trace elements in certified reference material were 76.51-124.51% and blanks for all trace element analyses were below detection limits. Elements of interest were arsenic, cadmium, cobalt, chromium, copper, iron, mercury, manganese, nickel, lead, selenium, strontium, vanadium and zinc. Metal concentrations of all collected sediment and biotic material, measured in parts per million (ppm) dry mass, were compared between the D-Area impoundment and the reference sites using ANOVA (S-Plus, Insightful Corp, Seattle). All significant results are based on 95% confidence intervals. All measurements of stable isotopes were performed by the Analytical Laboratory at the Institute of Ecology, University of Georgia, Athens, GA. Tissue samples were homogenized by grinding freeze-dried samples with mortar and pestle. Approximately 2 mg sample of ground tissue were loaded into tin capsules and weighed to within 1 μg using an ultramicrobalance. Capsules were placed in the autosampler of a Carlo Erba Elemental Analyzer (NA1500) attached to a continuous flow isotope ratio mass spectrometer (Finnigan Delta+). Samples were combusted, and isotope ratios were determined by mass spectrometry on purified samples of N_2 and CO_2. Internal working standards were used for calibration to the international AIR standard (nitrogen) and PeeDee belemnite (carbon). Bovine liver and poplar were used as external working standards; samples of external standards were analyzed before and after every 10 samples. Precision of analysis for the external standards was better than +/- 0.72 per mil (2 standard deviations) for nitrogen and +/- 0.38 per mil (2 standard deviations) for carbon.

3.4 Results

Heavy metal levels in the sediments were compared for all D-Area stations and both reference sites (Tables 3.1, 3.4). Statistical comparisons between each site within the D-Area impoundment are given in Table 3.2. There was some spatial variability within the D-Area impoundment. Copper showed the most variability within the D-Area impoundment as all stations are significantly different from one another in copper levels. Station D2 had the highest levels of copper followed in descending order by D3, D1, D7, D9, and D10. Manganese, cobalt, arsenic and cadmium all showed high variability as well with each metal showing no significant difference in only one station comparison each. The highest levels of manganese were found in D1 followed in descending order by D2 and D3 (no significant difference), D7, D10, and D9. Cobalt was highest in stations D1 and D3 (no significant difference) followed in descending order by D2, D7, D9, and D10. The highest levels of arsenic were found in station D1 followed in descending order by stations D2 and D3 (no significant difference), D7, D9, and D10. Cadmium levels were highest in station D2 followed in descending order by stations D1 and D3 (no significant difference), D7, D9, and D10. While station D10 was usually lower in metal concentrations compared to the other stations, it did have concentrations of iron that were either significantly greater than other stations (D1, D3 and D9) or not significantly different from other stations (D2 and D7). Station D9 also usually had lower concentrations of metals compared to the other stations (with the exception of station D10). However, D9 showed no significant difference in concentrations of chromium and iron compared to D1 and D3; chromium and mercury compared to D7; selenium, strontium and mercury compared to D10; and lower concentrations of iron and manganese compared to D10. Iron and chromium both showed the least spatial variability with no significant difference in concentrations in 7 of the 15 inter-station comparisons. Table 3.2 shows significant differences between the D-Area impoundment station metal levels.

All D-Area stations had significantly higher levels of all metals compared to the reference sites except D10 having similar levels of Mn ($p=0.39$ compared with Fire5) and Pb ($p=0.49$

compared to Dick's Pond) to the reference sites and D9 having similar concentrations of Mn to Dick's Pond ($p=0.3$). The p-values for all other comparisons were <0.01 except manganese concentrations at D9 compared to Fire5 ($p=0.01$) and lead concentrations at D10 compared to Fire1 ($p=0.04$).

Metal values and p-values for significant differences between sites for the biotic sample material collected are given in Tables 3.5-3.16. Values from all the stations within D-Area were combined to generate the means reported in the tables. Arsenic was elevated in all biota from the D-Area impoundment when compared to material collected from the three reference sites (Fire Pond 1, Fire Pond 5 and Dick's Pond) except for *Juncus* when compared to Fire Pond stations 1 and 5 and Dick's Pond and *Typha* compared to Fire 1, where there were no significant differences. Selenium, strontium and copper were elevated in most D-Area biota. Copper was not significantly different among the *Eleocharis* samples and strontium was not significantly different among the *Myrica* and *Juncus* samples. Selenium was not significantly different among the amphipod samples, however there still exists a noticeable difference between the two groups (mean of 28.25 +/- 19.62 ppm in ASH group and mean of 3.93 +/- 0.89 ppm in REF group). Selenium was also not significantly different in *Eleocharis* from D-Area and Dick's Pond and in *Typha* samples from D-Area and Fire 1. Cadmium was elevated in the *Juncus*, *Myrica*, *Chara*, detritus, *Gambusia* and amphipod D-Area samples compared to all reference samples and in *Ludwigia* from D-Area compared to Dick's Pond. Cobalt was elevated in *Myrica*, detritus and amphipod D-Area samples compared to the reference samples. Nickel was elevated in the *Myrica*, *Chara*, detritus, *Gambusia* and amphipod D-Area samples compared to the reference samples. Zinc was elevated in the *Myrica*, *Chara* and detritus D-Area samples and vanadium was elevated in the detritus, *Chara* and *Gambusia* D-Area samples compared to the reference samples. Iron was elevated in the samples of *Myrica*, *Chara*, *Gambusia* and amphipods from the reference site compared to D-Area samples. Manganese was elevated in the *Myrica* and detritus samples from the reference site compared to D-Area and in *Ludwigia* from the D-Area impoundment compared to all reference samples. Lead was elevated in the Dick's Pond *Gambusia* when compared to D-Area, but was elevated in D-Area when compared to Fire Pond. Lead was also elevated in the reference site amphipods when compared to D-Area. Mercury was elevated in the Fire 1 samples when compared to D-Area for *Juncus* and *Ludwigia* and in the D-Area samples when compared to Fire and Dick's Ponds for *Gambusia* and in detritus when compared to Fire Pond.

The amphipod metal burdens were compared between tissue from the D-Area impoundment and combined tissue from Fire Pond and Dick's Pond as the reference site. The tissue from the D-Area impoundment was elevated in arsenic, cadmium, cobalt, copper, nickel and strontium. The reference tissue was elevated in iron, manganese and lead. There was no significant difference between the impacted site and reference sites for levels of chromium, mercury, selenium, vanadium and zinc. See Table 3.6 for statistical significances.

The *Gambusia* samples from D-Area were compared to both Dick's Pond and Fire Pond. The D-Area impoundment samples were elevated above both reference site samples for levels of arsenic, copper, nickel, selenium, strontium and vanadium. Samples from Dick's Pond were elevated above D-Area for levels of iron, mercury and lead. Fire Pond was elevated above D-Area for levels of manganese and mercury. Cadmium was elevated in *Gambusia* from D-Area compared to Dick's Pond and lead was elevated in D-Area samples compared to Fire Pond.

The stable isotopic signatures for plants are given in Table 3.17. A graph of δ^{15}N values versus δ^{13}C values for all field collected biota is given in Figure 3.1. Graphs of As, Cu, Se, Sr and Ni versus δ^{15}N for biota that constitute a plausible trophic pathway are given in Figures 3.2-3.6. δ^{15}N values for macrophytes ranged from -1.89 per mil in *Myrica* to 4.7 per mil in *Ludwigia.* Algae δ^{15}N values ranged from -0.31 per mil in *Chara* to 6.72 per mil in *Spirogyra.* Detritus had a δ^{15}N value of 3.44 per mil. Consumer δ^{15}N ranged from 4.4 per mil in *Hyalella* to 6.43 per mil in *Procambarus.* Predator δ^{15}N values ranged from 5.65 per mil in Anisoptera to 7.99 per mil in *Gambusia.* δ^{13}C values for macrophytes ranged from

-30.53 per mil in *Typha* to -27.3 per mil in *Juncus*. Algae $\delta^{13}C$ values were -23.26 per mil in *Spirogyra* and 18.79 per mil in *Chara*. Detritus had a $\delta^{13}C$ value of -27.68 per mil. $\delta^{13}C$ values in consumers were -29.15 per mil in *Hyalella* and -27.91 per mil in *Procambarus*. Predator $\delta^{13}C$ values ranged from -31.37 per mil in Zygoptera to -27.62 per mil in *Gambusia*.

3.5 Discussion

The sediment metal concentrations at D-Area were elevated in nearly all stations compared to the reference site sediments. Sediment concentrations of chromium, iron, manganese, copper, cadmium and lead are all within ranges reported in the literature for areas contaminated by fly ash while cobalt, nickel, zinc, arsenic and selenium concentrations are greater than the ranges reported for sites contaminated by coal pollution (Rowe, Hopkins and Congdon, 2002, Ye et al., 2001, Schmidt, Soucek and Cherry, 2002 and Specht et al., 1984). Within the D-Area, stations along the main channel tended to have higher sediment metal concentrations than other stations. The main channel stations, D1 and D2, are constantly receiving metal-laden water from the settling basins. It is also reasonable that stations D9 and D10 consistently have lower metal levels as these sites are along the edges of the impoundment with little flow and thus less metal replenishment.

The spatial variability in sediment concentrations results in differing risks of metal uptake by biota that inhabit various portions of the impoundment. This is best illustrated in the case of *Chara*. The spatial variability of metal levels in this alga mirrors that of sediments. The invertebrates that were sampled for heavy metals were not found in high enough abundance at all stations to make spatial comparisons among individual stations. The invertebrates were pooled from samples collected at D7, D8 and D10. It is likely that the microhabitats at D7, D8 and D10 contained more submerged vegetation that supplied cover and resources while the other stations did not have as much submerged vegetation.

Plant material collected at the D-Area impoundment had chromium, copper and lead concentrations that were all within the ranges reported in the literature for sites contaminated with coal ash while arsenic, selenium and cadmium concentrations were greater than the reported ranges in coal ash contaminated sites (Rowe et al., 2002 and Sandholm, Oksanen and Pesonen, 1973). The amphipods collected at D-Area contained concentrations of vanadium, chromium, cobalt, copper, zinc, cadmium, lead, arsenic and selenium that were within the ranges reported in the literature for coal ash contaminated sites. Strontium concentrations in amphipods were greater that those reported in the literature for coal ash contaminated sites (Rowe et al., 2002, Hopkins et al., 2004, Sandholm, Oksanen and Pesonen, 1973 and Prat et al., 1999). Copper, arsenic, chromium, cadmium, lead and selenium concentrations in mosquitofish collected in D-Area were within ranges reported in the literature while zinc concentrations were greater that those reported for coal ash contaminated sites (Rowe et al., 2002 and Staub, 2004).

While all metals were elevated in D-Area sediments over reference sediments, only three metals were consistently elevated in plant and animal tissues from D-Area. These were arsenic, selenium and strontium. Arsenic was more concentrated in submergent macrophytes than in other plant or animal tissue. Selenium and strontium had higher concentrations in the *Gambusia* and amphipods than in the plant material. Sediments had consistently higher levels of arsenic over biotic tissue, except for *Eleocharis*. Sediment selenium levels were higher than all biotic tissues except *Eleocharis*, *Gambusia* and amphipods. Concentrations of strontium are greater in *Gambusia*, amphipods, *Chara*, *Typha* and *Ludwigia* than in sediments while concentrations in *Eleocharis* are similar to sediments. The elements of iron, manganese, mercury and lead were either significantly lower or not significantly different from at least one reference site in all biotic samples except for manganese in *Ludwigia*.

Studies have been done on the ability of plants to remove and thus accumulate metals from the environment (Collins, Sharitz and Coughlin, 2005, Jackson, Kalff and Rasmussen, 1993, Su and Wong, 2003, Keskinkan et al., 2004, Peng et al., 2006 and Kamal et al., 2004). Only two of the plants collected and tested were submerged macrophytes, *Chara* (a macroalga) and *Eleocharis*. *Eleocharis* showed little ability to accumulate metals from the D-

Area impoundment. These results are peculiar since other studies have found that *Eleocharis* can accumulate metals (Jackson, Kalff and Rasmussen, 1993 and Guimarães et al., 2000). It has been found that a slightly oxic redox potential (Jackson, Kaff and Rasmussen, 1993) and low pH (Collins, Sharitz and Coughlin, 2005 and Jackson, Kalff and Rasmussen, 1993) enhance the ability of aquatic plants to accumulate metals. These soil characteristics were not measured in this study. The water pH in D-Area was circumneutral. However, other plants collected in this study did accumulate metals. Since *Chara* was not found in the reference sites, it was compared to *Eleocharis*. Given the poor ability of *Eleocharis* to accumulate metals, the use of it as a reference plant to *Chara* must be viewed cautiously. Given *Chara's* δ^{13}O signature, it is unlikely the basis of any food chain investigated in this study and further investigations into the transfer of metals from *Chara* to local fauna are nonessential for this study.

Of the three emergent macrophytes (*Typha*, *Juncus* and *Ludwigia*), *Typha* and *Juncus* show similar trends to *Eleocharis*. Neither accumulated many metals and samples of *Juncus* had elevated levels of some metals (vanadium, chromium, iron, zinc and lead) in reference site material. These results are curious and require further study. *Ludwigia* accumulated most metals (manganese, cobalt, nickel, copper, arsenic, selenium, strontium and cadmium) in levels significantly higher than reference material, but mostly only for comparisons with Dick's Pond (Table 3.13). However, *Ludwigia* was compared to reference samples of *Juncus*, so this data should be interpreted cautiously. *Ludwigia* has been shown to accumulate iron, zinc, copper and mercury (Kamal et al., 2004).

Some of the trends in the mosquitofish and amphipod data may be explained by metal interactions. However, the sample size (three) was too small to confidently determine any interactions by use of linear regression. Much work has been done on the effects of selenium accumulation (Woshner et al., 2001, Belzile et al., 2006, Levander, 1977, Lorentzen, 1998, Rudd et al., 1980, Turner and Rudd, 1983, Lindqvist, 1991 and Paulsson and Lundbergh, 1989 and 1991). One of the most studied interactions is between selenium and mercury. It has been shown that selenium can inhibit the uptake of total and methylmercury (Woshner et al., 2001, Belzile et al., 2006 and Rudd et al., 1983) and can possibly inhibit the transfer of mercury from mother to offspring (Hopkins et al., 2006). While mercury concentrations in mosquitofish and amphipods are significantly lower or not different from reference material, the species of mercury was not determined in this study. The combined effect of arsenic and selenium is that of reduced toxicity of selenium (Moxon, 1938, Howell and Hill, 1978 and Levander and Argrett, 1969). In addition, mosquitofish can be tolerant to selenium stress (Lemly, 1985 and Saiki and Ogle, 1995, Staub et al., 2004). Lorentzen (1998) reported that copper could reduce selenium accumulation by forming an insoluble complex within the gastrointestinal tract. Copper and selenium are elevated in both mosquitofish and amphipods (although not significantly for selenium), but it is unknown whether the concentrations of either would be greater in the absence of the other. Chromium is not significantly elevated in either the mosquitofish or the amphipods. This may be due to competition for binding sites with nickel as has been found by Yang and Black (1994).

The analysis of the stable isotope data shows a possible food chain with *Myrica* and *Eleocharis* at the base, amphipods as a primary consumer and *Gambusia* as a secondary consumer. Detritus had a similar ^{15}N signature as amphipods and thus could be considered a potential food of *Gambusia*, however it is unlikely that *Gambusia* are feeding on detritus. We compared metals that were consistently elevated in the D-Area impoundment (As, Se, Sr and Cu) to the ^{15}N signature of this potential food chain. There was no increase of any of these metals from amphipods to the fish. However, Chen and Folt (2000) report that planktivorous fish that were feeding on arsenic-contaminated prey accumulated arsenic, but the concentration of arsenic in fish was lower than that in the prey suggesting that it is possible for the mosquitofish to accumulate arsenic from prey, but not magnify it. Nickel is also elevated in the *Myrica*, amphipods and mosquitofish, suggesting transfer of this metal through the food chain (See Graph 3.6). The copper, selenium and strontium data agrees with our findings from a feeding trial with mosquitofish and amphipods (see next chapter).

There is an increase in Cu, Se and Sr from *Myrica* to *Hyalella*, suggesting that *Myrica*-based detritus forms the beginning of this particular food chain and thus a source of metals to higher trophic positions. It may be possible that plant-based detritus (mainly *Myrica*) is a large component of the amphipod diet and is thus the reason the total detritus analyzed in this study does not appear to be a probable link in the food chain due to its δ^15N signature. Further investigations into the metal content and isotopic signatures of other invertebrates (microcrustaceans and insects) are needed but the biomass required for those analyses was not available during this study.

In summary, biota from the D-Area impoundment did show evidence of increased burdens of metals associated with coal fly ash, however not every metal was significantly elevated in every organism sampled from the D-Area impoundment. Metal interactions may explain some of these trends while a lack of ideal reference material (e.g. *Chara* and *Ludwigia* and consistently higher lead and iron in reference samples) lends to a cautionary interpretation of the data. Isotope data revealed potential trophic linkages and evidence for an increase of metal burdens from producer to primary consumer but no further increase of metal burdens to the predator. Thus, our data suggests that prey may still be an important source of metal exposure for predators, but that biomagnification does not occur in the species assemblage I monitored.

3.6 References

Atwell, L., K.A. Hobson and H.E. Welch. 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences 55: 1114-1121.

Belzile, N., Y.-W. Chen, J.M. Gunn, J. Tong, Y. Alarie, T. Delonchamp and C.-Y. Lang. 2006. The effect of selenium on mercury assimilation by freshwater organisms. Canadian Journal of Fisheries and Aquatic Science 63: 1-10.

Chen, C.Y. and C.L. Folt. 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. Environmental Science and Technology 34: 3878-3884.

Clements, W.H. and D.E. Rees. 1997. Effects of heavy metals on prey abundance, feeding habits, and metal uptake of Brown Trout in the Arkansas River, Colorado. Transactions of the American Fisheries Society 126: 774-785.

Collins, B.S., R.R. Sharitz and D.P. Coughlin. 2005. Elemental compostition of native plants in constructed mesocosm treatment wetlands. Bioresource Technology 96: 937-948.

DeNiro, M.J. and S. Epstein. 1981. Influences of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45: 341-351.

Elifantz, H. and E. Tel-Or. 2002. Heavy metal biosorption by plant biomass of the macrophyte *Ludwigia stolonifera*. Water, Air, and Soil Pollution 141: 207-218.

EPA. 1997. Profile of the fossil fuel electric power generation industry. Rep. EPA/310- R-97-007, U.S. Environmental Protection Agency, Washington D.C.

Fisk, A. T., S.A. Tittlemier, J.L. Pranschke and R.J. Nordstrom. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland Sharks. Ecology 83: 2162-2172.

Focken, U. and K. Becker. 1998. Metabolic fractionation of stable carbon isotopes: implications of different proximate compositions for studies of the aquatic food webs using δ^{13}C data. Oecologia 115: 337-343.

Gadd, G.M. 1990. Heavy metal accumulation by bacteria and other microorganisms. Experientia 46: 834-839.

Guimarães, J.R.D., M. Meili, L.D. Hylander, E. de Castro e Silva, M. Roulet, J.B.N. Mauro and R.A. de Lemos. 2000. Mercury net methylation in five tropical flood plain regions of

Brazil: high in the root zone of flooding macrophyte mats but low in surface sediments and flooded soils. The Science of the Total Environment 261: 99-107.

Gutenmann, W.H., C.A. Bache, W.D. Youngs and D.J. Lisk. 1976. Selenium in fly ash. Science 191: 966-967.

Hansson, S., J.E. Hobbie, R. Elmgren, U. Larsson, B. Fry and S. Johansson. 1997. The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. Ecology 78: 2249-2257.

Hobson, K.A. and R.G. Clark. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. The Condor 94: 181-188.

Hopkins, W.A., S.E. DuRant, B.P. Staub, C.L. Rowe and B.P. Jackson. 2006. Reproduction, embryonic development, and maternal transfer of contaminants in the amphibia *Gastrophryne carolinensis*. Environmental Health Perspectives 114: 661-666.

Hopkins, W.A., B.P. Staub, J.W. Snodgrass, B.E. Taylor, A.E. DeBiase, J.H. Roe, B.P. Jackson and J.D. Congdon. 2004. Responses of benthic fish exposed to contaminants in outdoor microcosms-examining the ecological relevance of previous laboratory toxicity tests. Aquatic Toxicology 68: 1-12.

Howell, G.O. and C.H. Hill. 1978. Biological interaction of selenium with other trace elements in chicks. Environmental Health Perspectives 25: 147-150.

Jackson, L.J., J. Kalff and J.B. Rasmussen. 1993. Sediment pH and redox potential affect the bioavailability of Al, Cu, Fe, Mn, and Zn to rooted aquatic macrophytes. Canadian Journal of Fisheries and Aquatic Science 50: 143-148.

Kamal, M., A.E. Ghaly, N. Mahmoud and R. Côté. 2004. Phytoaccumulation of heavy metals by aquatic plants. Environment International 29: 1029-1039.

Keskinkan, O., M.Z.L. Goksu, M. Basibuyuk and C.F. Forster. 2004. Heavy metal adsorption properties of a submerged aquatic plant (*Ceratopyllum demersum*). Bioresource Technology 92 197-200.

Lemly, A.D. 1985. Toxicology of selenium in a freshwater reservior: implications for environmental hazard evaluation and safety. Ecotoxicology and Environmental Safety 10: 314-338.

Levander, O.A. 1977. Metabolic interrelationships between arsenic and selenium. Environmental Health Perspectives 19: 159-164.

Levander, O.A. and L.C. Argrett. 1969. Effects of arsenic, mercury, thallium, and lead on selenium metabolism in rats. Toxicology and Applied Pharmacology 14: 308-314.

Lindqvist, O., K. Johansson, M. Aastrup, A. Andersson, L. Bringmark, G. Hovsenius, L. Hakenson, A. Iverfeldt, M. Meili and B. Timm. 1991. Mercury in the Swedish environment - recent research on causes, consequences and corrective methods. Water, Air and Soil Pollution 55: xi-261.

Lorentzen, M., A. Maage and K. Julshamm. 1998. Supplementing copper to a fish meal based diet fed to Atlantic salmon parr affects liver copper and selenium concentrations. Aquaculture Nutrition 4: 67-72.

McNear, D.H.Jr., E. Peltier, J. Everhart, R.L. Chaney, S. Sutton, M. Newville, M. Rivers and D.L. Sparks. 2005. Application of quantitative flourescence and absorption-edge computed microtomography to image metal compartmentalization in *Alyssum murale*. Environmental Science and Technology 39: 2210-2218.

Minagawa, M. and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochimica et Cosmochimica Acta 48: 1135-1140.

Moxon, A.L. 1938. The effect of arsenic on the toxicity of seleniferous grains. Science 88: 81.

Nakajima, A. and T. Sakaguchi. 1986. Selective accumulation of heavy metals by microorganisms. Applied Microbiology and Biotechnology 24:59-64.

Paulsson, K. and K. Lundbergh. 1989. The selenium method for treatment of lakes for elevated levels of mercury in fish. Science of the Total Environment 87-88: 495-507.

Paulsson, K. and K. Lundbergh. 1991. Treatment of mercury contaminated fish by selenium addition. Water, Air and Soil Pollution 56: 833-841.

Peng, K., X. Li, C. Luo and Z. Shen. 2006. Vegetation composition and heavy metal uptake by wild plants at three contaminated sites in Xiangxi Area, China. Journal of Environmental Science and Health 40: 65-76.

Peterson, B.J. and R.W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnology and Oceanography 32: 1195-1213.

Prat, N., J. Toja, C. Sola, M.D. Burgos, M. Plans and M. Rieradevall. 1999. Effect of dumping and cleaning activities on the aquatic ecosystems of the Guadiamar River following a toxic flood. The Science of the Total Environment 242: 231-248.

Rowe, C.L., W.A. Hopkins and J.D. Congdon. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: a review. Environmental Monitoring and Assessment 80: 207-276.

Rudd, J.W.M., M.A. Turner, B.E. Townsend, A. Swick and A. Furutani. 1980. Dynamics of selenium in mercury-contaminated experimental freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Science 37: 848-857.

Saiki, M.K. and R.S. Ogle. 1995. Evidence of impaired reproduction by western mosquitofish inhabiting seleniferous agricultural drainwater. Transactions of the American Fisheries Society 124: 578-587.

Saito, M.A., J.W. Moffett, S.W. Chisholm and J.B. Waterbury. 2002. Cobalt limitation and uptake in *Prochlorococcus*. Limnology and Oceanography 47: 1629-1636.

Sandholm, M., H.E. Oksanen and L. Pesonen. 1973. Uptake of selenium by aquatic organisms. Limnology and Oceanography 18: 496-499.

Schmidt, T.S., D.J. Soucek and D.S. Cherry. 2002. Integrative assessment of benthic macroinvertebrate community impairment from metal-contaminated waters in tributaries of the upper Powell River, Virginia, USA. Environmental Toxicology and Chemistry 21: 2233-2241.

Schoeller, D.A. 1999. Isotope fractionation: why aren't we what we eat? Journal of Archaeological Science 26: 667-673.

Solà, C., M. Burgos, Á. Plazuelo, J. Toja, M. Plans and N. Prat. 2004. Heavy metal bioaccumulation and macroinvertebrate community changes in a Mediterranean stream affected by acid mine drainage and an accidental spill (Guadiamar River, SW Spain). Science of the Total Environment 333: 109-126.

Specht, W.L., D.S. Cherry, R.A. Lechleitner and J. Cairns Jr. 1984. Structural, functional and recovery responses of stream invertebrates to fly ash effluent. Canadian Journal of Fisheries and Aquatic Science 41:884-896.

Staub, B.P., W.A. Hopkins, J. Novak and J.D. Congdon. 2004. Respiratory and reproductive characteristics of eastern mosquitofish (*Gambusia holbrooki*) inhabiting a coal ash settling basin. Archives of Environmental Contamination and Toxicology 46: 96-101.

Su, D.C. and J.W.C. Wong. 2003. Chemical speciation and phytoavailability of Zn, Cu, Ni and Cd in soil amended with fly ash-stabilized sewage sludge. Environment International 29: 895-900.

Turner, M.A. and J.W.M. Rudd. 1983. The English-Wabigoon River system: III. Selenium in lake enclosures: its geochemistry, bioaccumulation, and ability to reduce mercury bioaccumulation. Canadian Journal of Fisheries and Aquatic Science 40: 2228-2240.

Wen, Y., A. Long, R. Dawson and S. Tao. 2002. Speciation and bioavailability of EDTA complexed copper in the microenvironment of fish gills. Journal of Environmental Science and Health A37: 331-342.

Woshner, V.M., T.M. O'Hara, G.R. Bratton, R.S. Suydam and V.R. Beasley. 2001. Concentrations and interactions of selected essential and non-essential elements in Bowhead and Beluga whales of arctic Alaska. Journal of Wildlife Diseases 37: 693-710.

Yang, J. and J. Black. 1994. Competitive binding of chromium, cobalt and nickel to serum proteins. Biomaterials 15: 262-268.

Ye, Z.H., S.N. Whiting, Z.Q. Lin, C.M. Lytle, J.H. Qian and N. Terry. 2001. Removal and distribution of iron, manganese, cobalt and nickel within a Pennsylvania constructed wetland treating coal combustion by-product leachate. Journal of Environmental Quality 30: 1464-1473.

Table 3.1: Metal concentrations (ppm) in sediments collected from six stations in the D-Area impoundment (n=3).

	D 1		**D 2**		**D 3**		**D 7**		**D 9**		**D 10**	
	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
V	88.03	2.70	75.83	3.30	71.70	2.11	73.75	4.45	54.18	2.62	40.47	1.53
Cr	41.14	3.54	55.82	6.31	38.67	4.42	43.97	6.10	37.28	3.80	19.57	2.20
Fe	9606.28	557.13	12222.38	861.48	8605.39	1057.84	11105.36	891.65	9051.10	695.92	11835.57	301.83
Mn	250.96	10.59	199.62	6.42	190.03	7.93	149.69	6.26	60.63	3.14	96.13	3.40
Co	52.51	0.71	42.34	1.72	54.55	1.98	27.04	1.42	21.97	1.21	11.14	0.31
Ni	108.41	1.22	118.27	4.77	109.65	3.92	95.57	4.33	75.27	4.22	33.70	1.03
Cu	190.10	5.86	222.80	5.21	211.28	1.97	132.04	4.56	106.72	6.14	38.71	0.58
Zn	237.59	19.30	302.48	12.91	245.71	14.95	249.42	8.60	175.37	9.90	106.73	1.35
As	136.13	4.75	106.86	2.64	108.92	0.29	83.92	3.82	57.45	3.45	33.75	0.91
Se	36.51	3.17	31.09	0.82	24.52	0.92	37.00	1.72	18.59	1.21	18.32	0.10
Sr	92.57	7.48	107.23	8.86	82.43	14.18	90.70	8.58	56.66	2.89	51.85	2.18
Cd	5.28	0.34	6.67	0.08	4.76	0.20	5.89	0.09	3.74	0.22	1.88	0.03
Hg	0.23	0.02	0.22	0.04	0.22	0.02	0.14	0.01	0.10	0.02	0.06	0.01
Pb	25.41	0.63	27.91	1.18	25.32	0.94	22.45	1.74	17.69	1.50	10.80	0.34

Table 3.2: Significant differences in sediments collected from six stations in the D-Area impoundment. The site with the higher metal concentration is listed next to each p-value. NSD=no significant difference.

Site Comparison	**V**	**Cr**	**Fe**	**Mn**	**Co**	**Ni**	**Cu**
D1:D2	D1 p0.01	D2 p=0.02	D2 p=0.01	D1 p<0.01	D1 p<0.01	D2 p=0.02	D2 p<0.01
D1:D9	D1 p<0.01	NSD	NSD	D1 p<0.01	D1 p<0.01	D1 p<0.01	D1 p<0.01
D1:D7	D1 p<0.01	NSD	NSD	D1 p<0.01	D1 p<0.01	D1 p<0.01	D1 p<0.01
D1:D3	D1 p<0.01	NSD	NSD	D1 p<0.01	NSD	NSD	D3 p<0.01
D1:D10	D1 p<0.01	D1 p<0.01	D10 p<0.01	D1 p<0.01	D1 p<0.01	D1 p<0.01	D1 p<0.01
D2:D9	D2 p<0.01	D2 p=0.01	D2 p<0.01	D2 p<0.01	D2 p<0.01	D2 p<0.01	D2 p<0.01
D2:D7	NSD	NSD	NSD	D2 p<0.01	D2 p<0.01	D2 p<0.01	D2 p<0.01
D2:D3	NSD	D2 p=0.01	D2 p=0.01	NSD	D3 p<0.01	NSD	D2 p=0.02
D2:D10	D2 p<0.01	D2 p<0.01	NSD	D2 p<0.01	D2 p<0.01	D2 p<0.01	D2 p<0.01
D9:D7	D7 p<0.01	NSD	D7 p=0.03	D7 p<0.01	D7 p<0.01	D7 p<0.01	D7 p<0.01
D9:D3	D3 p<0.01	NSD	NSD	D3 p<0.01	D3 p<0.01	D3 p<0.01	D3 p<0.01
D9:D10	D9 p<0.01	D9 p<0.01	D10 p<0.01	D10 p<0.01	D9 p<0.01	D9 p<0.01	D9 p<0.01
D7:D3	NSD	NSD	D3 p=0.03	D3 p<0.01	D3 p<0.01	D3 p=0.01	D3 p<0.01
D7:D10	D7 p<0.01	D7 p<0.01	NSD	D7 p<0.01	D7 p<0.01	D7 p<0.01	D7 p<0.01
D3:D10	D3 p<0.01	D3 p<0.01	D10 p<0.01	D3 p<0.01	D3 p<0.01	D3 p<0.01	D3 p<0.01

Table 3.3: Significant differences in sediments collected from six stations in the D-Area impoundment. The site with the higher metal concentration is listed next to each p-value. NSD=no significant difference.

Site Comparison	Zn	As	Se	Sr	Cd	Hg	Pb
D1:D2	D2 $p<0.01$	D1 $p<0.01$	D1 $p=0.04$	NSD	D2 $p<0.01$	NSD	D2 $p=0.03$
D1:D9	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$
D1:D7	NSD	D1 $p<0.01$	NSD	NSD	D7 $p=0.04$	D1 $p<0.01$	D1 $p=0.05$
D1:D3	NSD	D1 $p<0.01$	D1 $p<0.01$	NSD	NSD	NSD	NSD
D1:D10	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$	D1 $p<0.01$
D2:D9	D2 $p<0.01$	D2 $p<0.01$	D2 $p<0.01$	D2 $p<0.01$	D2 $p<0.01$	D2 $p=0.01$	D2 $p<0.01$
D2:D7	D2 $p<0.01$	D2 $p<0.01$	D7 $p<0.01$	NSD	D2 $p<0.01$	D2 $p=0.03$	D2 $p=0.01$
D2:D3	D2 $p<0.01$	NSD	D2 $p<0.01$	NSD	D2 $p<0.01$	NSD	D2 $p=0.04$
D2:D10	D2 $p<0.01$	D2 $p<0.01$	D2 $p<0.01$	D2 $p<0.01$	D2 $p<0.01$	D2 $p<0.01$	D2 $p<0.01$
D9:D7	D7 $p<0.01$	D7 $p<0.01$	D7 $p<0.01$	D7 $p<0.01$	D7 $p<0.01$	NSD	D7 $p=0.02$
D9:D3	D3 $p<0.01$	D3 $p<0.01$	D3 $p<0.01$	D3 $p=0.03$	D3 $p<0.01$	D3 $p<0.01$	D3 $p<0.01$
D9:D10	D9 $p<0.01$	D9 $p<0.01$	NSD	NSD	D9 $p<0.01$	NSD	D9 $p<0.01$
D7:D3	NSD	D3 $p<0.01$	D7 $p<0.01$	NSD	D7 $p<0.01$	D3 $p<0.01$	NSD
D7:D10	D7 $p<0.01$	D7 $p<0.01$	D7 $p<0.01$	D7 $p<0.01$	D7 $p<0.01$	D7 $p<0.01$	D7 $p<0.01$
D3:D10	D3 $p<0.01$	D3 $p<0.01$	D3 $p<0.01$	D3 $p=0.02$	D3 $p<0.01$	D3 $p<0.01$	D3 $p<0.01$

Table 3.4: Metal concentrations (ppm) in reference sediments (n=3).

	Dicks		**Fire 5**		**Fire 1**	
	mean	stdev	mean	stdev	mean	stdev
V	11.93	3.20	16.09	1.23	9.69	1.49
Cr	7.60^{D10}	1.73	11.68^{D10}	0.20	9.12^{D10}	2.10
Fe	1612.65	441.75	4292.73^{D3}	503.85	1696.16	236.88
Mn	51.39^{D9}	19.33	89.41^{D10}	14.75	29.67	4.89
Co	1.83	0.56	2.54	0.36	1.00	0.13
Ni	4.55	1.08	7.24	0.77	5.05	0.70
Cu	3.72	1.24	6.74	0.80	6.42	0.78
Zn	15.50	4.25	39.22	5.19	33.14	3.68
As	0.49	0.17	0.83	0.07	0.84	0.32
Se	0.62	0.24	0.81	0.11	0.67	0.10
Sr	7.80	2.86	10.94	0.77	10.41	1.87
Cd	0.02	0.01	0.05	0.01	0.03	0.00
Hg	0.02^{D10}	0.02	BDL	BDL	0.02^{D10}	0.01
Pb	9.72^{D10}	3.03	8.05^{D10}	1.04	9.35^{D10}	1.11

Superscripts indicate which stations within D-Area are not significantly different from the reference site. All other differences are significant.

Table 3.5: Metal concentrations (ppm) in *Gambusia* (n=3 per site) collected from the D-Area Impoundment (ASH) and reference sites (FIRE and DICKS).

ASH *Gambusia* pooled from the six stations within D-Area Impoundment.

	ASH *Gambusia*		**FIRE *Gambusia***			**DICKS *Gambusia***		
	mean	stdev	mean	stdev		mean	stdev	
V	0.93	0.07	0.12	0.03	$p<0.01$	0.47	0.06	$p<0.01$
Cr	2.45	0.49	2.56	0.13		2.28	0.55	
Fe	75.91	4.48	72.59	4.63		168.77	20.15	$p<0.01$
Mn	82.19	16.24	193.78	40.39	$p=0.01$	109.65	39.60	
Co	0.43	0.08	0.59	0.09		0.51	0.02	
Ni	2.64	0.37	1.77	0.20	$p<0.01$	0.14	0.02	$p<0.01$
Cu	6.61	0.45	3.15	0.33	$p<0.01$	4.04	0.41	$p<0.01$
Zn	234.94	14.99	270.64	26.52		244.44	49.43	
As	2.37	0.07	0.31	0.08	$p<0.01$	0.09	0.05	$p<0.01$
Se	15.84	1.27	1.37	0.30		4.52	2.66	$p<0.01$
Sr	335.92	59.00	155.23	23.66	$p<0.01$	93.81	13.99	$p<0.01$
Cd	0.18	0.01	0.17	0.02	$p<0.01$	0.04	0.01	$p<0.01$
Hg	0.07	0.02	0.30	0.07	$p<0.01$	2.00	0.75	
Pb	0.07	0.01	0.04	0.01	$p=0.02$	0.09	0.01	$p=0.02$

p-values for statistical comparisons between D-Area samples and reference samples are given.

Table 3.6: Metal concentrations (ppm) in amphipods (n=3).

	ASH Amphipods		**REF Amphipods**		
	mean	stdev	mean	stdev	
V	1.94	1.11	0.94	0.13	
Cr	3.63	0.96	4.40	0.19	
Fe	353.07	42.94	631.01	31.16	$p<0.01$
Mn	112.11	8.19	190.61	49.49	$p=0.05$
Co	1.72	0.35	1.08	0.09	$p=0.03$
Ni	2.15	0.47	0.60	0.26	$p<0.01$
Cu	53.30	8.91	29.28	1.57	$p=0.01$
Zn	80.38	16.89	62.66	0.15	
As	9.14	2.48	4.51	0.10	$p=0.03$
Se	28.25	19.62	3.93	0.89	
Sr	734.58	124.21	159.60	3.57	$p<0.01$
Cd	3.29	0.19	1.40	0.05	$p<0.01$
Hg	0.13	0.06	0.17	0.02	
Pb	0.55	0.04	1.13	0.26	$p=0.02$

p-values for statistical comparisons between D-Area samples and reference samples are given.

Table 3.7: Metal concentrations (ppm) in detritus (n=3).

	ASH Detritus		FIRE Detritus			DICKS Detritus		
	mean	stdev	mean	stdev		mean	stdev	
V	129.19	16.42	12.79	1.08	$p<0.01$	22.86	3.31	
Cr	54.21	8.12	9.57	0.88	$p<0.01$	16.82	2.94	$p<0.01$
Fe	20074.96	3013.79	7802.57	705.08	$p<0.01$	15171.36	2765.16	
Mn	1489.08	210.45	407.90	41.16	$p<0.01$	2148.05	339.10	$p=0.05$
Co	40.82	4.79	3.53	0.32	$p<0.01$	16.57	2.46	p¡<0.01
Ni	107.40	14.70	6.95	0.58	$p<0.01$	16.64	2.36	$p<0.01$
Cu	186.28	13.43	13.50	1.09	$p<0.01$	79.38	11.57	$p<0.01$
Zn	312.54	37.67	35.05	2.99	$p<0.01$	162.38	24.85	$p<0.01$
As	84.12	9.73	1.55	0.09	$p<0.01$	3.47	0.41	$p<0.01$
Se	30.13	3.15	0.75	0.15	$p<0.01$	1.90	0.40	$p<0.01$
Sr	195.24	27.32	9.78	0.82	$p<0.01$	30.50	4.52	$p<0.01$
Cd	7.74	1.06	0.07	0.00	$p<0.01$	0.36	0.05	$p<0.01$
Hg	0.21	0.02	0.01	0.00	$p<0.01$	0.23	0.02	
Pb	25.92	3.35	5.56	0.46	$p<0.01$	31.83	3.88	$p<0.01$

p-values for statistical comparisons between D-Area samples and reference samples are given.

Table 3.8: Metal concentrations (ppm) in *Chara* (ASH n=15), compared with *Eleocharis* (REF n=3).

	ASH *Chara*		**DICKS *Eleocharis***		
	mean	stdev	mean	stdev	
V	10.26	2.91	3.90	0.48	$p<0.01$
Cr	13.64	12.55	5.45	1.04	
Fe	775.13	272.76	8773.88	1061.32	$p<0.01$
Mn	2069.47	803.81	2006.31	346.84	
Co	15.38	5.08	12.25	1.63	
Ni	49.92	24.82	7.09	1.03	$p=0.01$
Cu	27.92	16.44	3.21	0.36	$p=0.02$
Zn	218.94	47.74	60.44	7.85	$p<0.01$
As	30.79	8.61	2.88	0.34	$p<0.01$
Se	5.11	2.26	0.35	0.12	$p<0.01$
Sr	764.00	129.56	12.69	1.81	$p<0.01$
Cd	8.02	5.20	0.14	0.03	$p=0.02$
Hg	0.10	0.09	0.01	0.03	
Pb	2.17	0.53	2.86	0.41	$p=0.05$

p-values for statistical comparisons between D-Area samples and reference samples are given.

Table 3.9: Metal concentrations (ppm) in *Juncus* (n=9).

	ASH *Juncus*		**FIRE 1 *Juncus***			**FIRE 5 *Juncus***		
	mean	stdev	mean	stdev		mean	stdev	
V	0.24	0.31	8.88	2.05	p<0.01	0.08	0.02	
Cr	0.91	1.22	5.18	0.95	p<0.01	2.10	0.38	
Fe	32.91	36.14	4709.71	258.88	p<0.01	63.56	1.05	
Mn	239.30	84.71	79.61	7.93	p=0.01	405.37	55.57	p=0.01
Co	0.58	0.27	1.53	0.34	p<0.01	0.04	0.01	p<0.01
Ni	3.48	1.36	5.30	0.84		0.47	0.17	p<0.01
Cu	8.26	3.36	8.51	1.31		2.27	0.45	p=0.01
Zn	32.96	5.42	44.96	7.36	p=0.01	75.63	11.19	p<0.01
As	2.64	2.25	1.10	0.19		0.01	0.00	
Se	1.53	0.63	0.43	0.09	p=0.01	0.03	0.02	p<0.01
Sr	12.08	9.11	12.65	2.25		3.86	0.33	
Cd	1.03	0.54	0.09	0.07	p=0.01	0.07	0.01	p=0.01
Hg	0.01	0.02	0.05	0.04	p=0.05	0.01	0.00	
Pb	0.03	0.08	7.15	1.47	p<0.01	0.08	0.03	

p-values for statistical comparisons between D-Area samples and reference samples are given. Table continued on next page.

Table 3.10: Metal concentrations (ppm) in *Juncus* (n=9) continued.

	ASH *Juncus*		**DICKS *Juncus***		
	mean	stdev	mean	stdev	
V	0.24	0.31	0.09	0.02	
Cr	0.91	1.22	1.12	0.19	
Fe	32.91	36.14	72.41	9.16	
Mn	239.30	84.71	455.79	161.02	$p=0.01$
Co	0.58	0.27	0.18	0.04	$p=0.03$
Ni	3.48	1.36	0.28	0.05	$p<0.01$
Cu	8.26	3.36	1.72	0.28	$p<0.01$
Zn	32.96	5.42	62.03	16.77	$p<0.01$
As	2.64	2.25	0.08	0.02	
Se	1.53	0.63	0.12	0.02	$p<0.01$
Sr	12.08	9.11	4.10	0.50	
Cd	1.03	0.54	0.01	0.01	$p<0.01$
Hg	0.01	0.02	0.01	0.00	
Pb	0.03	0.08	0.05	0.02	

p-values for statistical comparisons between D-Area samples and reference samples are given.

Table 3.11: Metal concentrations (ppm) in *Myrica* (n=3).

	ASH *Myrica*		**FIRE *Myrica***			**DICKS *Myrica***		
	mean	stdev	mean	stdev		mean	stdev	
V	0.24	0.02	0.23	0.01		0.19	0.03	p=0.02
Cr	0.67	0.23	0.47	0.05		0.26	0.07	p=0.04
Fe	47.15	21.47	138.30	3.73	p<0.01	219.96	31.92	p<0.01
Mn	657.33	27.37	564.12	27.20	p=0.01	899.59	112.60	p=0.02
Co	0.41	0.02	0.04	0.00	p<0.01	0.12	0.02	p<0.01
Ni	5.81	0.15	1.01	0.09	p<0.01	2.82	0.33	p<0.01
Cu	8.39	0.32	2.10	0.16	p<0.01	1.89	0.16	p<0.01
Zn	52.72	5.65	13.64	1.06	p<0.01	33.70	4.04	p<0.01
As	5.46	0.36	0.01	0.01	p<0.01	0.05	0.01	p<0.01
Se	0.53	0.05	0.04	0.01	p<0.01	0.08	0.02	p<0.01
Sr	48.30	2.40	16.03	0.97		51.27	8.40	
Cd	0.24	0.03	BDL	0.00	p<0.01	BDL	0.00	P<0.01
Hg	0.10	0.08	0.04	0.00		0.07	0.01	
Pb	0.17	0.05	0.22	0.06		0.30	0.03	

p-values for statistical comparisons between D-Area samples and reference samples are given. BDL=Below Detection Limit.

Table 3.12: Metal concentrations (ppm) in *Eleocharis* (ASH n=10, REF n=3).

	ASH *Eleocharis*		**DICKS *Eleocharis***		
	mean	stdev	mean	stdev	
V	37.48	36.19	3.90	0.48	p<0.01
Cr	25.20	20.63	5.45	1.04	
Fe	5579.16	3144.42	8773.88	1061.32	
Mn	2328.40	1516.89	2006.31	346.84	
Co	60.64	114.11	12.25	1.63	
Ni	91.35	133.11	7.09	1.03	p<0.01
Cu	45.73	55.67	3.21	0.36	
Zn	190.17	223.17	60.44	7.85	p=0.03
As	110.99	57.15	2.88	0.34	p=0.01
Se	17.01	12.43	0.35	0.12	
Sr	89.46	17.12	12.69	1.81	p<0.01
Cd	6.47	9.08	0.14	0.03	
Hg	0.23	0.32	0.01	0.03	
Pb	3.90	2.99	2.86	0.41	

p-values for statistical comparisons between D-Area samples and reference samples are given.

Table 3.13: Metal concentrations (ppm) in *Ludwigia* (ASH n=15), compared with *Juncus* (REF n=9).

	ASH *Ludwigia*		**FIRE 1 *Juncus***			**FIRE 5 *Juncus***		
	mean	stdev	mean	stdev		mean	stdev	
V	1.39	0.74	8.88	2.05	$p<0.01$	0.08	0.02	$p<0.01$
Cr	2.39	1.77	5.18	0.95	$p=0.02$	2.10	0.38	
Fe	102.85	22.31	4709.71	258.88	$p<0.01$	63.56	1.05	$p<0.01$
Mn	1067.00	281.71	79.61	7.93	$p<0.01$	405.37	55.57	$p<0.01$
Co	1.22	0.50	1.53	0.34		0.04	0.01	$p<0.01$
Ni	5.12	1.86	5.30	0.84		0.47	0.17	$p<0.01$
Cu	5.28	1.50	8.51	1.31	$p<0.01$	2.27	0.45	$p<0.01$
Zn	54.04	14.56	44.96	7.36	$p=0.01$	75.63	11.19	$p=0.03$
As	2.20	0.51	1.10	0.19	$p<0.01$	0.01	0.00	$p<0.01$
Se	1.78	0.56	0.43	0.09	$p<0.01$	0.03	0.02	$p<0.01$
Sr	204.10	40.67	12.65	2.25	$p<0.01$	3.86	0.33	$p<0.01$
Cd	0.19	0.12	0.09	0.07	$p=0.01$	0.07	0.01	
Hg	0.00	0.03	0.05	0.04	$p=0.03$	0.01	0.00	
Pb	0.22	0.17	7.15	1.47	$p<0.01$	0.08	0.03	

p-values for statistical comparisons between D-Area samples and reference samples are given. Table continued on next page.

Table 3.14: Metal concentrations (ppm) in *Ludwigia* (ASH n=15), compared with *Juncus* (REF n=9) continued.

	ASH *Ludwigia*		**DICKS *Juncus***		
	mean	stdev	mean	stdev	
V	1.39	0.74	0.09	0.02	$p<0.01$
Cr	2.39	1.77	1.12	0.19	
Fe	102.85	22.31	72.41	9.16	$p=0.03$
Mn	1067.00	281.71	455.79	161.02	$p<0.01$
Co	1.22	0.50	0.18	0.04	$p<0.01$
Ni	5.12	1.86	0.28	0.05	$p<0.01$
Cu	5.28	1.50	1.72	0.28	$p<0.01$
Zn	54.04	14.56	62.03	16.77	
As	2.20	0.51	0.08	0.02	$p<0.01$
Se	1.78	0.56	0.12	0.02	$p<0.01$
Sr	204.10	40.67	4.10	0.50	$p<0.01$
Cd	0.19	0.12	0.01	0.01	$p=0.02$
Hg	0.00	0.03	0.01	0.00	
Pb	0.22	0.17	0.05	0.02	

p-values for statistical comparisons between D-Area samples and reference samples are given.

Table 3.15: Metal concentrations (ppm) in *Typha* (ASH n=6), compared with *Juncus* (REF n=9).

	ASH *Typha*		**FIRE 1 *Juncus***			**FIRE 5 *Juncus***		
	mean	stdev	mean	stdev		mean	stdev	
V	0.22	0.12	8.88	2.05	$p<0.01$	0.08	0.02	
Cr	4.51	3.71	5.18	0.95	$p=0.02$	2.10	0.38	
Fe	74.30	20.42	4709.71	258.88	$p<0.01$	63.56	1.05	
Mn	420.27	60.27	79.61	7.93	$p<0.01$	405.37	55.57	
Co	0.43	0.20	1.53	0.34		0.04	0.01	$p=0.01$
Ni	3.29	1.88	5.30	0.84		0.47	0.17	$p=0.04$
Cu	4.12	0.41	8.51	1.31	$p<0.01$	2.27	0.45	$p<0.01$
Zn	26.67	4.64	44.96	7.36	$p=0.01$	75.63	11.19	$p<0.01$
As	1.33	0.78	1.10	0.19	$p<0.01$	0.01	0.00	$p=0.02$
Se	0.83	0.31	0.43	0.09	$p<0.01$	0.03	0.02	$p<0.01$
Sr	167.69	18.78	12.65	2.25	$p<0.01$	3.86	0.33	$p<0.01$
Cd	0.04	0.05	0.09	0.07	$p=0.01$	0.07	0.01	
Hg	0.00	0.03	0.05	0.04	$p=0.03$	0.01	0.00	
Pb	0.15	0.10	7.15	1.47	$p<0.01$	0.08	0.03	$p<0.01$

p-values for statistical comparisons between D-Area samples and reference samples are given. Table continued on next page.

Table 3.16: Metal concentrations (ppm) in *Typha* (ASH n=6), compared with *Juncus* (REF n=9) continued.

	ASH *Typha*		**DICKS *Juncus***		
	mean	stdev	mean	stdev	
V	0.22	0.12	0.09	0.02	
Cr	4.51	3.71	1.12	0.19	
Fe	74.30	20.42	72.41	9.16	
Mn	420.27	60.27	455.79	161.02	
Co	0.43	0.20	0.18	0.04	
Ni	3.29	1.88	0.28	0.05	$p=0.03$
Cu	4.12	0.41	1.72	0.28	$p<0.01$
Zn	26.67	4.64	62.03	16.77	$p<0.01$
As	1.33	0.78	0.08	0.02	$p=0.03$
Se	0.83	0.31	0.12	0.02	$p=0.01$
Sr	167.69	18.78	4.10	0.50	$p<0.01$
Cd	0.04	0.05	0.01	0.01	
Hg	0.00	0.03	0.01	0.00	
Pb	0.15	0.10	0.05	0.02	

p-values for statistical comparisons between D-Area samples and reference samples are given.

Table 3.17: Mean and standard deviation of ^{15}N and ^{13}C isotopic signatures of material collected from the D-Area Impoundment.

Material collected from multiple stations within the impoundment were pooled.

		δ^{15}**N**		δ^{13}**C**	
		mean	stdev	mean	stdev
Macrophytes	*Juncus*	4.49	0.35	-27.30	0.22
	Myrica	-1.89	0.03	-30.27	0.19
	Typha	4.43	0.09	-30.53	0.10
	Ludwigia	4.70	0.29	-28.98	0.18
	Eleocharis	-0.49	0.18	-28.55	0.16
Algae	*Chara*	-0.31	0.12	-18.79	0.12
	Spirogyra	6.72	0.08	-23.26	0.23
Detritus	Detritus	3.44	0.18	-27.68	0.16
Consumers	*Hyalella*	4.40	0.40	-29.15	1.65
	Procambarus	6.43	0.27	-27.91	0.22
Predators	Zygoptera	7.65	0.58	-31.37	0.36
	Anisoptera	5.65	0.23	-30.38	0.06
	Gambusia	7.99	0.16	-27.62	0.13

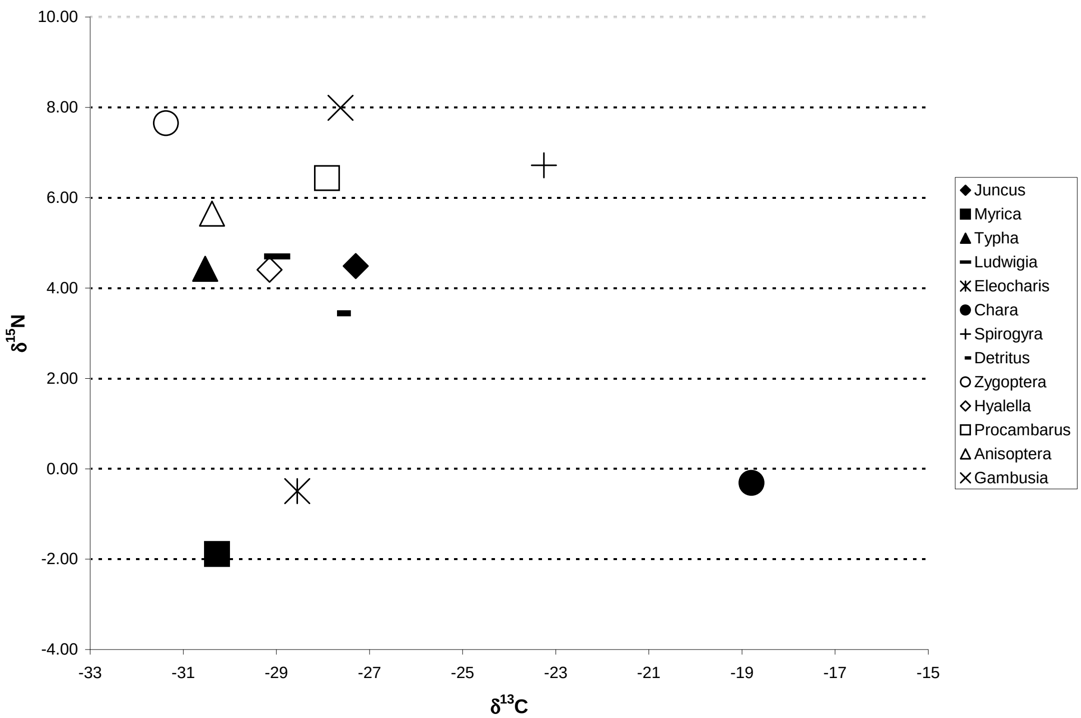

Figure 3.1: Isotopic signatures of field collected material

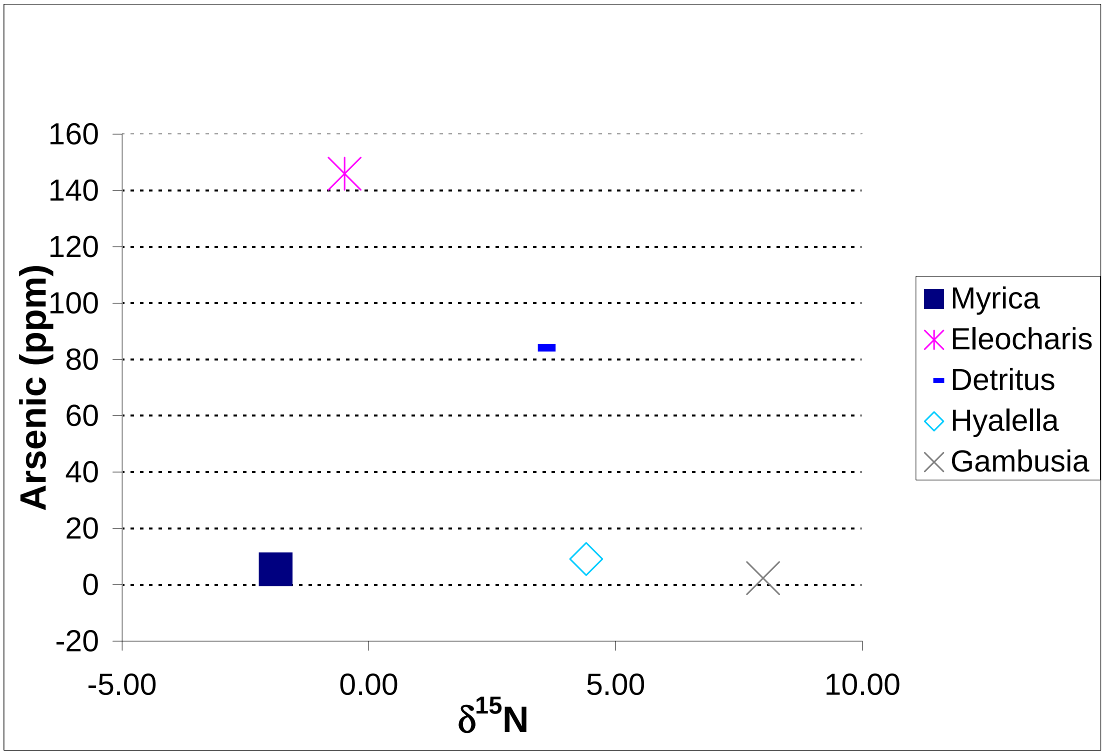

Figure 3.2: Arsenic (ppm) v. ^{15}N in potential trophic linkages

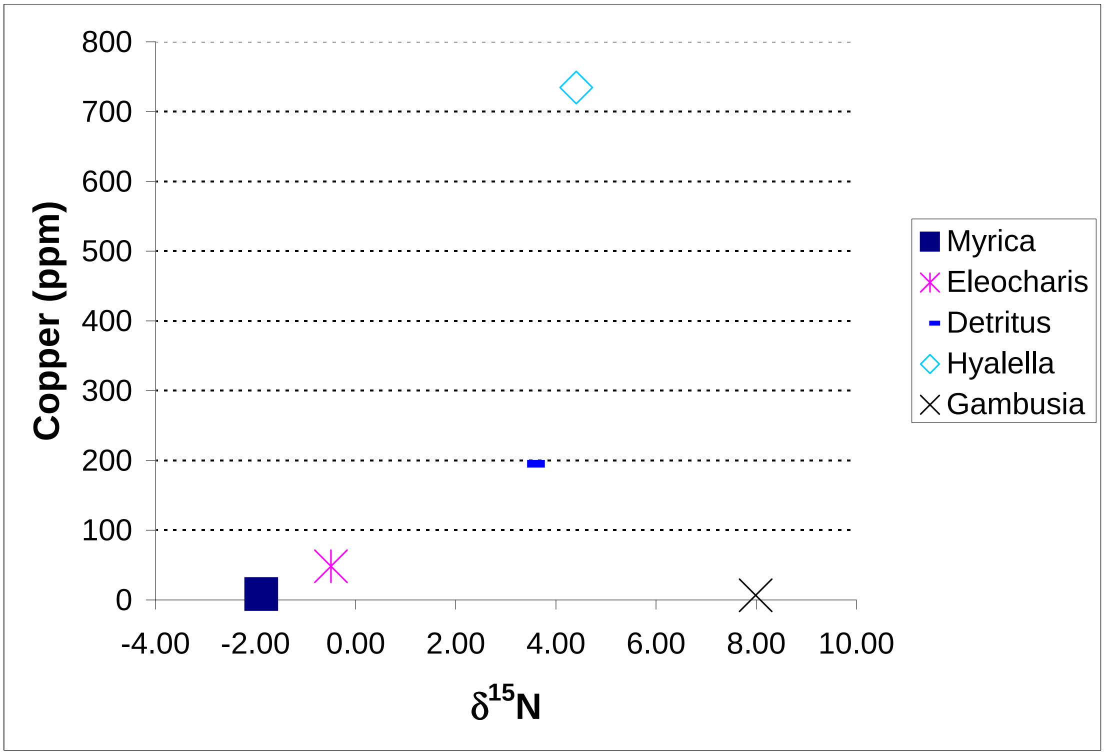

Figure 3.3: Copper (ppm) v. ^{15}N in potential trophic linkages

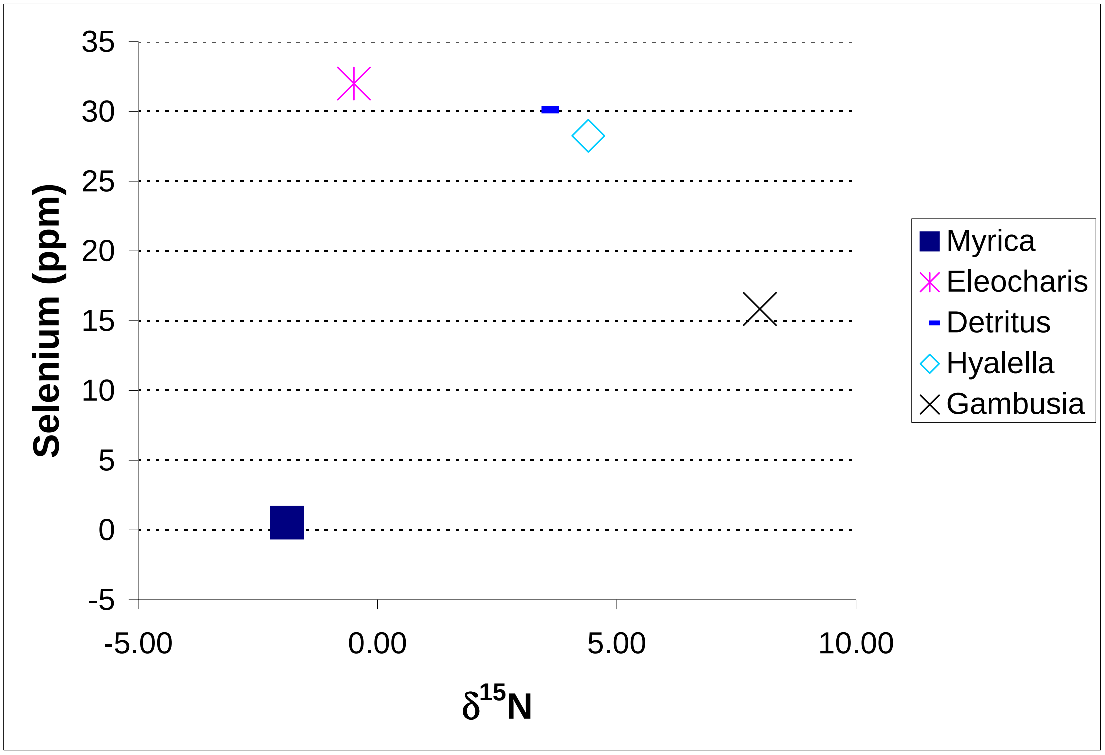

Figure 3.4: Selenium (ppm) v. ^{15}N in potential trophic linkages

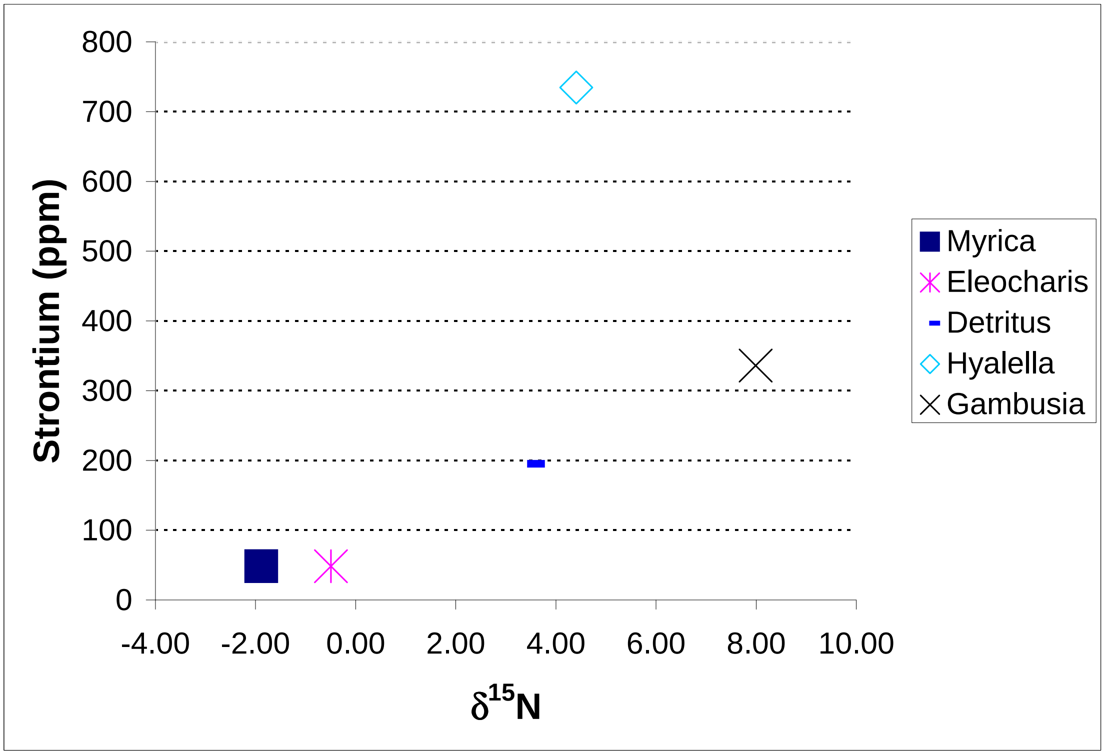

Figure 3.5: Strontium (ppm) v. ^{15}N in potential trophic linkages

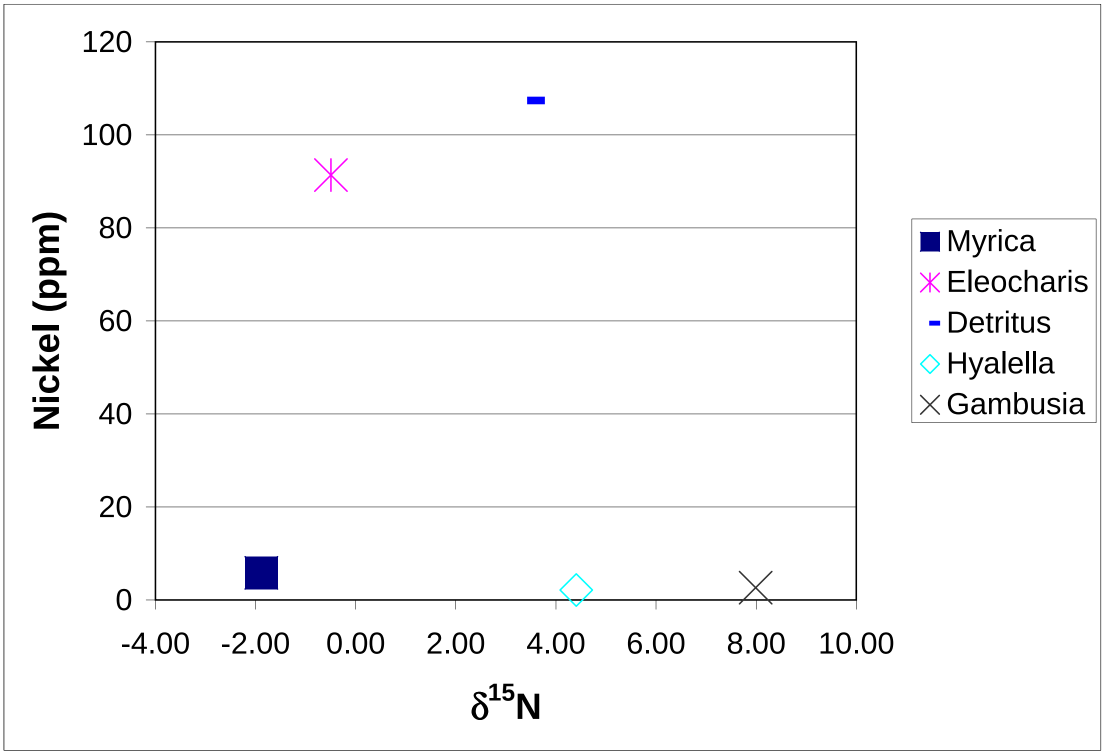

Figure 3.6: Nickel (ppm) v. ^{15}N in potential trophic linkages

Chapter 4

Route of Entry of Contaminants in a Laboratory Experiment

4.1 Introduction

To fully understand the risk associated with a contaminant in an aquatic system, it is important to understand how the contaminant moves through the biological community. Marsden and Rainbow (2004) found that amphipods accumulate contaminants via direct contact with water and trophic uptake. Amphipods have permeable body tissue and can absorb metals through the gills and other body surfaces. The rate of absorption is proportional to the concentration of metals dissolved in the water. Trophic uptake also contributes to the body burden of metals in amphipods, although which pathway contributes more has not been determined. Assimilated metals in amphipods are stored in the ventral caeca, or gut. The metals are bound to the cells lining the caeca and are not actively excreted, but leave the body when the cells that metals are bound to are naturally sloughed. To a lesser extent, metals can be stored in other tissues in the body. Metal uptake in aquatic insects has also been studied, revealing that for the larva of the megalopteran *Sialis velata* dietary exposure contributed the majority of body burdens of arsenic, cadmium, cobalt, copper, zinc and lead, while lead was also taken up in small amounts directly from water (Croisetière, Hare and Tessier, 2006). Fish are able to acquire contaminants directly from sediments and water exposure. Simon and Boudou (2001) found that the carp, *Ctenopharyngodon idella*, was able to accumulate inorganic and organic mercury directly from contaminated water. Fish are also able to acquire contaminants via contaminated food. A catfish (*Clarias macrocephalus* x *C. gariepinus*) accumulated cadmium from ingestion of the zooplankter *Moina macrocopa*, although in concentrations less than that of the zooplankter (Ruangsomboon and Wongrat, 2006). Seebaugh, Goto and Wallace (2005) found an increase in cadmium burdens in the mummichog (*Fundulus heteroclitus*) after being fed cadmium-contaminated grass shrimp (*Palaemonetes pugio*). In addition to finding evidence for direct uptake of mercury from water, Simon and Boudou (2001) revealed that mercury uptake from a combination of direct aqueous routes and trophic interaction resulted in higher body concentrations in carp than from direct uptake alone. Rabbitfish (*Siganus canaliculatus*) were able to acquire cadmium, chromium and zinc from the macroalga *Enteromorpha crinita* (Chan, Wang and Ni, 2003).

In situ studies of exposure pathways in aquatic organisms are difficult due to variables that are not easy to isolate and control. Laboratory experimentation allows manipulation and close control of specific variables, but may omit others. A combination of laboratory and field methods is a compromise that can produce ecologically relevant and repeatable results (see Hopkins et al., 2004). Generating realistically contaminated food is one of the difficulties in experimental studies of trophic pathways for heavy metals by fishes. Hopkins et al. (2004) created microcosms to study uptake of metals, comparing treatments supplemented or un-supplemented with artificial food to test hypotheses about the influence of the natural prey on exposure. The microcosms were lined with sediment and benthic resources from a control or an ash impacted site. Their results indicated that fish exposed to the ash microcosms accumulated trace metals and suggested that contaminated food resources were a key exposure pathway.

This study combined a laboratory experiment with field collections to examine possible routes of entry of heavy metals into a freshwater fish, *Gambusia holbrooki.* I examined two important exposure pathways, trophic uptake and direct uptake from water and sediment. *Gambusia* is native to the Southeastern U.S. but has been introduced worldwide as a form of mosquito control and is therefore present in a variety of habitats globally (Berra, 1981), including the coal-ash polluted impoundment used in this study (Satub et al., 2004). *Gambusia* is also omnivorous (Meffe and Snelson, 1989), allowing for a choice in model prey.

For this study we wanted prey collected from a historically contaminated site so that these prey would contain relevant levels of contamination. I chose amphipods because they are easily collected, present in the ash impoundment and reference sites, are large enough to be a substantial meal and we were able to visually determine that the fish are feeding on them. Amphipods are also used worldwide in studies ranging from bio-indicators of stressed ecosystems to trophic transfer studies. Their presence worldwide, short life cycle and relative hardiness contribute to the usefulness as a model prey organism (Marsden and Rainbow, 2004).

This study separated ash sediment exposure from ash prey exposure to determine which source of contamination contributes most to metal body burdens of freshwater fish. The metals analyzed were vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, strontium, cadmium, mercury and lead. These metals are commonly associated with coal combustion wastes, which *Gambusia* are exposed to at the D-Area impoundment. The majority of the literature reports that uptake of metals does occur through trophic interaction (Wang and Wong, 2006, Simon and Boudou, 2001, Chan, Wang and Ni, 2003, Seebaugh, Goto and Wallace, 2005), however there is little investigation into the role of direct exposure as it compares to trophic exposure. I hypothesized that exposure through contaminated prey as opposed to sediment will result in higher body burdens of the associated metals in the fish.

4.2 Methods

The D-Area impoundment is a 2-hectare impoundment situated near the D-Area coal-fired power plant on the Savannah River Site (SRS) near Aiken, SC. Fly ash is mixed with water from the Savannah River to form a slurry that is then pumped to two settling basins. Some of the water from these basins then flows into the impoundment. Water then leaves the impoundment via Beaver Dam Creek, which empties back into the Savannah River. Average temperature is 22.4 °C; average pH is 7.7; average conductivity is 0.31 mS/cm and average depth is 22.43 cm. This site was used for amphipod collections. The sediment was collected at the temporary basins since sediment collected from the impoundment created too much ammonia in the bins used to house individual fish in the feeding trial.

Fire Pond and Dick's Pond were used as the source of *Gambusia* and amphipods. Fire Pond is on the SRS and located 11-km from D-Area. It is 3.8 hectares in area, almost 3 m at maximum depth and has circumneutral pH. Dick's Pond is also on the SRS and is approximately 15 km from D-Area. Dick's Pond is 1.0 hectares in area, has a maximum depth of 3.6 m and is also circumneutral. Both are abandoned farm ponds. Playground sand was used as the reference sediment since ammonia levels in the water used in the experiment became too high using sediment from Dick's and Fire Ponds.

Gambusia were collected from Fire Pond on July 4 and July 6, 2004. Approximately 90 individuals were dip netted and placed in five-gallon buckets filled with site water and ice to cool water five degrees below ambient temperature. Fish were transported back to the lab and placed in four 10-gallon aquaria filled with aerated water from Fire Pond. Three liters of water in the tanks were replaced each day with aged tap water that was aerated with an air stone for at least three days to allow chlorine to evaporate. Fish were fed Tetramin flake food (Tetra, Blacksburg, Va) each day.

Forty six-quart plastic bins were set up in the laboratory in rows of ten bins. Half of the bins contained clean playground sand and the other half contained sediment from one of the coal ash settling basins. Adjacent bins contained alternate sediment types. All sediment was dried at 65 °C and sieved through a 500 μm sieve. Bins were filled with 2-cm sediment and 4 liters aged tap water. 20 mL filtered (at 102 μm) Fire Pond water was added to each bin to establish a natural algal flora in an attempt to reduce ammonia, and air stones were added to each bin. The bins were allowed to sit for several days before adding fish. Ammonia was checked the day prior to adding fish.

Fish were removed from the acclimation tanks and randomly assigned to one of four treatments on July 20, 2004 (n=10 fish per treatment). The four treatments were: reference sediment (sand) and reference site prey (RS/RP), reference sediment and ash impacted prey (RS/AP), ash sediment and reference prey (AS/RP) and ash sediment and ash impacted prey (AS/AP). The prey type assignments were randomly dispersed among the bins. Feeding began on July 21, 2004. The average length of each fish at the beginning of the trial was 20.8 mm and the average wet weight was 0.15 g. Weight was determined from a length to weight regression curve formulated from data from excess fish not used in the trials. Amphipods were collected daily from the D-Area impoundment, Fire Pond and Dick's Pond using a small dip net. Fish and amphipod metal burdens were not checked prior to study. Fish were given amphipods until they stopped feeding or when five minutes had passed since being offered the first amphipod. Usually, this resulted in three to ten amphipods per fish per day. One liter of water was removed every five days and replaced with enough aged tap water to bring the level up to the four liter mark (usually >1 liter). Ammonia levels were checked every 7 to 15 days for a subset of 10 bins. The ammonia assay methods followed those of the Ecosystem Center at the Marine Biological Laboratory of Woods Hole, Ma. The remaining fish not used in the feeding trial were anesthetized using MS-222 and weighed and measured for length using MorphoSys visual imaging software. This was to determine an average length to weight ratio to use to monitor the growth of fish in the feeding trial. Fish in the feeding trial were measured once using MorphoSys to detect growth. The planned end of the experiment was when the average length of the fish doubled.

At the end of the experiment, all surviving fish were allowed to void gut contents for 24 hours and then anesthetized using MS-222, weighed and measured for length. Each fish was then placed in a minus 20 °C freezer and later lyophilized. Additional sediment and amphipods were collected at both sites to determine metal content. Sediment was oven dried at 60 °C for 48 hours and amphipods were lyophilized. Dried sediment and tissue from collections and the feeding trial was then digested with nitric acid and hydrogen peroxide in advanced composite Teflon vessels in a MDS 2000 microwave digestion unit (CEM, Matthews, NC). Digested material was analyzed on an ICP-MS (PERKINS ELMER ELAN

DRC PLUS). Quality assurance was achieved using the standard reference material Dolt-3 (dogfish liver) (National Research Council Canada, Ottawa, Ontario). Percent recoveries of trace elements in certified reference material were 76.51-124.51% and blanks for all trace element analyses were below detection limits. Elements of interest were As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, V, Zn. ANOVA was used to compare treatment effects for each metal. Comparisons were made using one-way or two-way analysis of variance with the level of significance set at α=0.05. All statistical analyses were performed in S-Plus (Insightful Corp, Seattle).

4.3 Results

All elements were significantly elevated in the ASH sediment compared to the REF sediment (Table 4.1). The prey collected from ASH were elevated in Co, Ni, Cu, As, Cd and Sr while the prey collected from DICKS and FIRE were elevated in Fe and Pb (Table 4.2, significant differences are noted). The concentrations of Se did not differ significantly between prey from the D-Area and the reference sites (p=0.10). However there was a large difference in mean concentration of Se with D-Area prey having the larger concentration.

The experiment was terminated after 23 days due to high mortality. A total of 16 fish had died by that time. These fish were evenly divided among treatments. They were excluded from analysis because their exposure times were shorter than that of the other fish. The proposed endpoint was to be a doubling in mass. The average final mass of all *Gambusia* in the trials was 0.13 g; 86% of the average starting mass of 0.15 g.

Contaminant levels in the fish (Table 4.3) were analyzed using a two-way ANOVA to test the effects of prey source and sediment type and the interaction between the two. Sediment type had a significant effect for arsenic, cadmium, copper, selenium, strontium and vanadium. Prey type also had a significant effect for selenium and there was also an interaction between the two factors. For these elements, except Cd and Cu, concentrations were elevated in the

ASH sediment or prey treatments. For Cd and Cu, concentrations were elevated in the REF sediment treatments.

4.4 Discussion

This study was unique in that it investigated the importance of direct exposure and trophic exposure simultaneously for a suite of 14 contaminants associated with coal fly-ash in a common species of fish. The list of metals elevated in the fish exposed to ASH sediment is similar to the results of the previous chapter's investigation of metal burdens in field-collected fish. Vanadium, arsenic, selenium and strontium were found to be significantly higher in fish exposed to fly-ash sediment in both the field and the feeding trials. However, copper was elevated in fish from the feeding trial exposed to reference sediment as opposed to being elevated in ash exposed fish in the field. For arsenic, strontium, selenium, and vanadium, we infer that exposure to contaminated sediment contributed to the increased burdens of these metals in the fish, indicating direct uptake of the contaminant.

Selenium is the only metal that showed effects from both factors of the treatments (sediment and prey), indicating both trophic and direct uptake. This is not the only study that has found trophic and direct uptake as both contributing to the body burdens of selenium in aquatic organisms. Besser, Canfield and La Point (1993) found that cladocerans, *Daphnia magna* and bluegills, *Lepomis macrochirus*, accumulated selenium from both food sources and exposure to contaminated sediment. Hopkins et al. (2000) found results similar to this study, however the study organism, lake chubsuckers (*Erimyzon sucetta*), are benthic feeders and likely ingested sediment during the course of the experiment.

Other studies have found trophic transfer of metals to fish, but focus mainly on mercury (Simon and Boudou, 2001), cadmium and chromium (Seebaugh, Goto and Wallace, 2005, Liu, Ni and Wang, 2002, Ruangsomboon and Wongrat, 2006 and Chan, Wang and Ni, 2003). Milton and Chenery (2001) demonstrated that copper, strontium and lead could be accumulated in barramundi (*Lates calcarifer*) otoliths directly from contaminated water. While strontium is the only one of these three metals from this feeding trial that follows this trend, it should be noted that lead concentrations were significantly higher in the amphipods from the reference sites. Arsenic also accumulated directly from water into tilapia (*Oreochromis mossambicus*) through the gills (Tsai and Liao, 2006), which may be the primary route into fish used in this trial. The two treatments with ash sediment exposure (AS/RP and AS/AP) produced fish with significantly higher concentrations of arsenic while fish from the treatment that had ASH amphipods and REF sediment (RS/AP) did not have arsenic concentrations different from the control treatment (RS/RP). This may also explain the mode of entry of vanadium, strontium and selenium (in part). Dallinger et al. (1987) report that metal uptake from food may depend on the prey containing a threshold amount of trace metals. This could explain the lack of dietary uptake in our experiment, although Dallinger et al. do not report for which metals this holds true and what the threshold levels are.

Copper and cadmium results were perplexing because fish developed higher body burdens when exposed to the reference sediments. Copper and cadmium concentrations in sediment in the ASH treatments were an order of magnitude higher than in sediment in the REF treatments. Copper is only significantly elevated in the RS/RP treatment, which is also the only treatment that is significantly lowered in selenium. It is possible that the copper:selenium ratio was high enough so that there was not enough selenium to bind to copper in an insoluble complex (Lorentzen, 1998), allowing for the higher concentration of copper in mosquitofish in the RS/RP treatment. Interaction with another metal is also a probable cause of the cadmium results. Cadmium in reference sediments and reference amphipods is significantly lower than cadmium from ash sediments and amphipods, indicating that it may be out-competed for binding sites on *Gambusia* tissue by a contaminant that is elevated in one aspect of the ash treatments. Cobalt and nickel are two other elements that are significantly elevated in ASH prey and sediment, but show no differences between treatments in the feeding trials. Yang and Black (1994) found that nickel can decrease the binding ability of cobalt to tissue, which may explain the cobalt results, however they report that cobalt has no effect on the binding of nickel.

Metal speciation was not explored in this study, but it may explain some of these results. Wen et al. (2002) found that when copper is bound to an organic ligand (EDTA) it became more readily assimilated by the gills of carp (*Cyprinus carpio*) when compared to fish exposed to inorganic copper. Organic species of cadmium were also found to have higher capabilities of adsorption compared to the inorganic species (Nakajima and Sakaguchi, 1986 and Gadd, 1990). Saito et al. (2002) found that the cyanobacterium *Prochlorococcus* assimilated cobalt more quickly in conditioned growth medium (medium which had already been used to grow *Prochlorococcus*) than in fresh medium. From this they hypothesized that *Prochlorococcus* produced organic cobalt ligands which facilitated the assimilation of cobalt. Free cobalt (Co^{2+}) was only available in unconditioned media. Investigations into the abundance of the organic species of these metals in the reference sediments and ash sediments may help to explain the results.

Since four individuals died in each of the treatments during the course of the experiment, it seems unlikely that mortality was due to effects from the ash sediment or prey. It is probable the mortality observed was due to inadequate amounts of prey. The mass of amphipods fed daily to the fish was 1.3 to 4.2 mg dry mass. The cause of the high mortality was most likely due to starvation. The fish received roughly 5% of average body mass as food per day. Hopkins (2004) has shown that reduced prey intake exacerbates accumulation of metals, but the food limitation here may have been too severe. Ideally the experiment would have lasted longer and its termination may have been too early to reveal metal accumulation trends in long term exposure to contaminated prey and sediment. The experiment should be repeated and measures taken to minimize mortality. The effort involved in field collecting macroinvertebrate prey is substantial enough to suggest an alternate method of obtaining prey. Mesocosms lined with either ash or reference sediment and allowed to grow appropriate plankton and plant community would be a good alternate method while still retaining some of the integrity of the natural environs. Additionally, supplementing the fish diet with commercially available flake food would help to reduce mortality.

4.5 References

Berra, T.M. 1981. An Atlas of Distribution of the Freshwater Fish Families of the World. University of Nebraska Press, Lincoln, NE, USA.

Besser, J.M., T.J. Canfield and T.W. La Point. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. Environmental Toxicology and Chemistry 12: 57-72.

Boudou, A. and F. Ribeyre. 1981. Comparative study of the trophic transfer of two mercury compounds-$HgCl_2$ and CH_3HgCl-between *Chlorella vulgaris* and *Daphnia magna*. Influence of temperature. Bulletin of Environmental Contamination and Toxicology 27: 624-629.

Chan, S.M., W.-X. Wang and I.-H. Ni. 2003. The uptake of cd, cr, and zn by the macroalga *Enteromorpha crinita* and subsequent transfer to the marine herbivorous rabbitfish, *Siganus canaliculatus*. Archives of Environmental Contamination and Toxicology 44: 298-306.

Croisetière, L., L. Hare and A. Tessier. 2006. A field experiment to determine the relative importance of prey and water as sources of as, cd, co, cu, pb, and zn for the aquatic invertebrate *Sialis velata*. Environmental Science and Technology 40: 873-879.

Dallinger, R., F. Prosi, H. Senger and H. Back. 1987. Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia 73: 91-98.

Hopkins, W.A., J.W. Snodgrass, J.H. Roe, B. Jackson, J.C. Gariboldi and J.D. Congdon. 2000. Detrimental effects associated with trace element uptake in Lake Chubsuckers

(*Erimyzon sucetta*) exposed to polluted sediments. Archives of Environmental Contamination and Toxicology 39: 1-7.

Hopkins, W.A., J.W. Snodgrass, J.H. Roe, B.P. Staub, B.P. Jackson and J.D. Congdon. 2002. Effects of food ration on survival and sublethal responses of lake chubsuckers (*Erimyzon sucetta*) exposed to coal combustion wastes. Aquatic Toxicology 57: 191-202.

Hopkins, W.A., B.P. Staub, J.W. Snodgrass, B.E. Taylor, A.E. DeBiase, J.H. Roe, B.P. Jackson and J.D. Congdon. 2004. Responses of benthic fish exposed to contaminants in outdoor microcosms-examining the ecological relevance of previous laboratory toxicity tests. Aquatic Toxicology 68: 1-12.

Liu, X.-J., I.-H. Ni and W.-X. Wang. 2002. Trophic transfer of heavy metals from freshwater zooplankton *Daphnia magna* to zebrafish *Danio reiro*. Water Research 36: 4563-4569.

Lorentzen, M., A. Maage and K. Julshamm. 1998. Supplementing copper to a fish meal based diet fed to Atlantic salmon parr affects liver copper and selenium concentrations. Aquaculture Nutrition 4: 67-72.

Marsden, I.D. and P.S. Rainbow. 2004. Does the accumulation of trace metals in crustaceans affect their ecology-the amphipod example? Journal of Experimental Marine Biology and Ecology 300: 373-408.

Meffe, G.K. and F.F. Snelson, Jr. 1989. An ecological overview of Poecilid fishes. Pages 13-31 *in* G.K. Meffe and F.F. Snelson, Jr. (editors) Ecology and Evolution of Livebearing Fishes (Poecilidae). Prentice-Hall, Ingelwood Cliffs, NJ, USA.

Milton, D.A. and S.R. Chenery. 2001. Sources and uptake of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). Journal of Experimental Marine Biology and Ecology 264: 47-65.

Ruangsomboon, S. and L. Wongrat. 2006. Bioaccumulation of cadmium in an experimental aquatic food chain involving phytoplankton (*Chlorella vulgaris*), zooplankton (*Moina* *macrocopa*), and the predatory catfish *Clarias macrocephalus* x *C. gariepinus*. Aquatic Toxicology 78: 15-20.

Rubinstein, N.I., W.T. Gilliam and N.R. Gregory. 1984. Dietary accumulation of PCBs from a contaminated sediment source by a demersal fish (*Leiostomus xanthurus*). Aquatic Toxicology 5: 331-342.

Russell, R.W., F.A.P.C. Gobas and G.D. Haffner. 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. Environmental Toxicology and Chemistry 18: 1250-1257.

Seebaugh, D.R., D. Goto and W.G. Wallace. 2005. Bioenhancement of cadmium transfer along a multi-level food chain. Marine Environment Research 59: 473-491.

Simon, O. and A. Boudou. 2001. Direct and trophic contamination of the herbivorous carp *Ctenopharyngodon idella* by inorganic mercury and methylmercury. Ecotoxicology and Environmental Safety 50: 48-59.

Tsai, J.-W. and C.-M. Liao. 2006. A dose-based modeling approach for accumulation and toxicity of arsenic in tilapia *Oreochromis mossambicus*. Environmental Toxicology 21: 8-21.

Twiss, M.R., P.G.C. Campbell and J.C. Auclair. 1996. Regeneration, recycling and trophic transfer of trace metals by microbial food-web organisms in the pelagic surface waters of Lake Erie. Limnology and Oceanography 41: 1425-1437.

Wang, W.-X. and P. Wong. 2006. Dynamics of trace metal concentrations in an intertidal rocky shore food chain. Marine Pollution Bulletin 52: 332-356.

Xu, Y. and W.X. Wang. 2002. Exposure and potential food chain transfer factor of cd, se and zn in marine fish *Lutjanus argentimaculatus*. Marine Ecology Progress Series 238: 173-186.

Yang, J. and J. Black. 1994. Competitive binding of chromium, cobalt and nickel to serum proteins. Biomaterials 15: 262-268.

Table 4.1: Trace element content (ppm) of ash basin sediment (ASH) and playground sand (REF) used in feeding trials.

	ASH		**REF**	
	mean	stdev	mean	stdev
V	67.01	3.69	9.5	0.14
Cr	37.43	8.21	7.35	0.14
Mn	125.44	2.01	3.32	0.2
Fe	30243.05	4457.23	627.45	42.89
Co	22.11	3.32	0.44	0.02
Ni	51.15	8.91	1.77	0.08
Cu	66.96	6.1	1.28	0.06
Zn	55.69	6.95	3.69	0.07
As	108.45	1.28	0.35	0.04
Se	7.85	0.45	0.71	0.17
Sr	358.45	12.98	10.23	0.31
Cd	0.25	0.01	0.01	0
Hg	0.22	0.02	0.03	0.01
Pb	16.43	1.12	4.55	0.32

ASH sediment (n=3) from Temporary Basin and REF sediment (n=2) from playground sand. All elements differ significantly between sediment types.

Table 4.2: Whole body trace element content (ppm) of amphipods (n=3 samples of ~0.3g, approx. 1361 amphipods per sample) collected in the D-Area Impoundment (ASH) and at Fire and Dick's Ponds (REF).

	ASH Amphipods		**REF Amphipods**		
	mean	stdev	mean	stdev	
V	1.94	1.11	0.94	0.13	
Cr	3.63	0.96	4.40	0.19	
Fe	353.07	42.94	631.01	31.16	$p<0.01$
Mn	112.11	8.19	190.61	49.49	$p=0.05$
Co	1.72	0.35	1.08	0.09	$p=0.03$
Ni	2.15	0.47	0.60	0.26	$p<0.01$
Cu	53.30	8.91	29.28	1.57	$p=0.01$
Zn	80.38	16.89	62.66	0.15	
As	9.14	2.48	4.51	0.10	$p=0.03$
Se	28.25	19.62	3.93	0.89	
Sr	734.58	124.21	159.60	3.57	$p<0.01$
Cd	3.29	0.19	1.40	0.05	$p<0.01$
Hg	0.13	0.06	0.17	0.02	
Pb	0.55	0.04	1.13	0.26	$p=0.02$

p-values for significant differences are given.

Table 4.3: Whole body trace element content (ppm) of *Gambusia* (n=6 per treatment) after termination of feeding trial.

	RS/RP		**RS/AP**		**AS/RP**		**AS/AP**	
	mean	stdev	mean	stdev	mean	stdev	mean	stdev
V	0.31	0.17	0.24	0.06	0.75*	0.23	0.90*	0.31
Cr	0.58	0.28	0.54	0.12	0.51	0.17	0.58	0.26
Fe	137.63	121.01	87.44	13.92	102.53	35.75	129.14	115.49
Mn	137.33	37.47	146.98	28.77	139.15	40.26	132.35	49.88
Co	0.57	0.09	0.65	0.09	0.81	0.47	0.61	0.16
Ni	0.28	0.46	0.34	0.49	0.35	0.25	0.47	0.62
Cu	12.53*	2.24	11.70	2.60	10.48	2.58	8.37	3.80
Zn	744.93	124.29	593.82	122.80	556.88	155.45	572.38	179.24
As	0.57	0.35	0.61	0.23	1.06*	0.28	1.28*	0.41
Se	1.37	0.30	4.60*	0.83	3.40*	0.99	4.47*	0.85
Sr	162.55	8.10	180.69	17.14	213.05*	18.76	213.88*	32.61
Cd	4.21*	3.54	3.67*	2.10	0.78	0.50	0.83	0.33
Hg	0.35	0.10	0.34	0.11	0.36	0.10	0.30	0.13
Pb	0.27	0.14	0.40	0.40	0.17	0.08	0.21	0.16

Significant differences are denoted by *.

Table 4.4: p-values for contributing factors of elements that were elevated in different treatments of feeding trial

Metal	Sediment Type	Prey Source	Interaction	Trophic Exposure
V	$p<0.01$	$p=0.59$	$p=0.21$	No
Cr	$p=0.88$	$p=0.90$	$p=0.54$	No
Fe	$p=0.92$	$p=0.74$	$p=0.28$	Yes
Mn	$p=0.69$	$p=0.93$	$p=0.61$	Yes
Co	$p=0.36$	$p=0.57$	$p=0.18$	Yes
Ni	$p=0.60$	$p=0.63$	$p=0.89$	Yes
Cu	$p=0.03$	$p=0.22$	$p=0.58$	Yes
Zn	$p=0.09$	$p=0.27$	$p=0.18$	No
As	$p<0.01$	$p=0.32$	$p=0.49$	Yes
Se	$p<0.01$	$p<0.01$	$p<0.01$	Yes*
Sr	$p<0.01$	$p=0.28$	$p=0.32$	Yes
Cd	$p<0.01$	$p=0.77$	$p=0.73$	Yes
Hg	$p=0.78$	$p=0.45$	$p=0.55$	No
Pb	$p=0.14$	$p=0.37$	$p=0.63$	Yes

Trophic Exposure indicates if that particular metal was statistically elevated in amphipods.

* amphipod prey is not significantly elevated in Se, but concentrations are noticeably higher in amphipods from the D-Area impoundment.

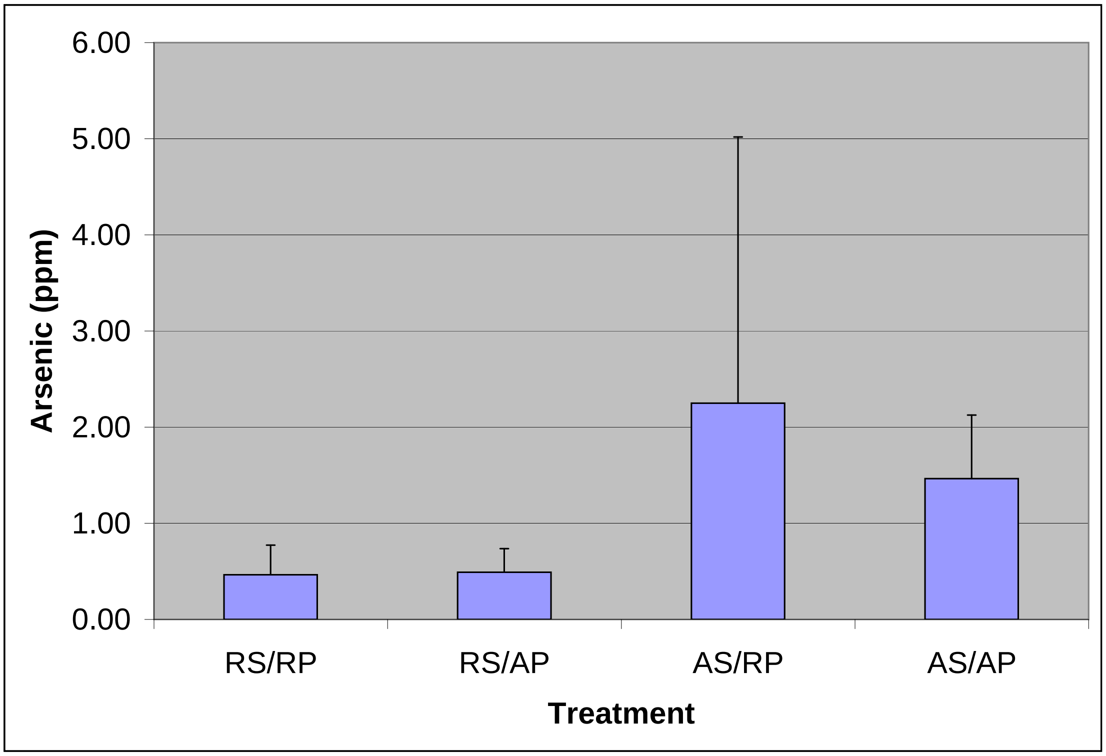

Figure 4.1: Average arsenic burdens in *Gambusia* in feeding trial treatments. Plot shows average and standard deviation; n=6 for each treatment. RS=reference sediment; AS=ash sediment; RP=reference prey; AP=ash prey.

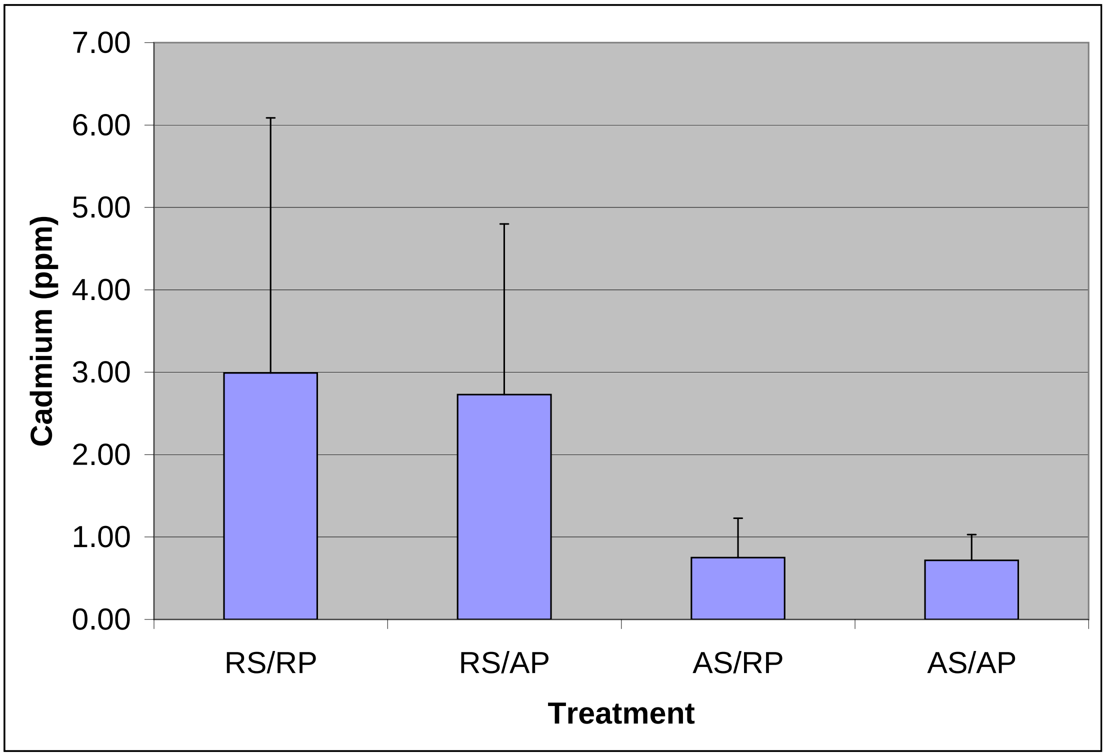

Figure 4.2: Average cadmium burdens in *Gambusia* in feeding trial treatments. Plot shows average and standard deviation; n=6 for each treatment. RS=reference sediment; AS=ash sediment; RP=reference prey; AP=ash prey.

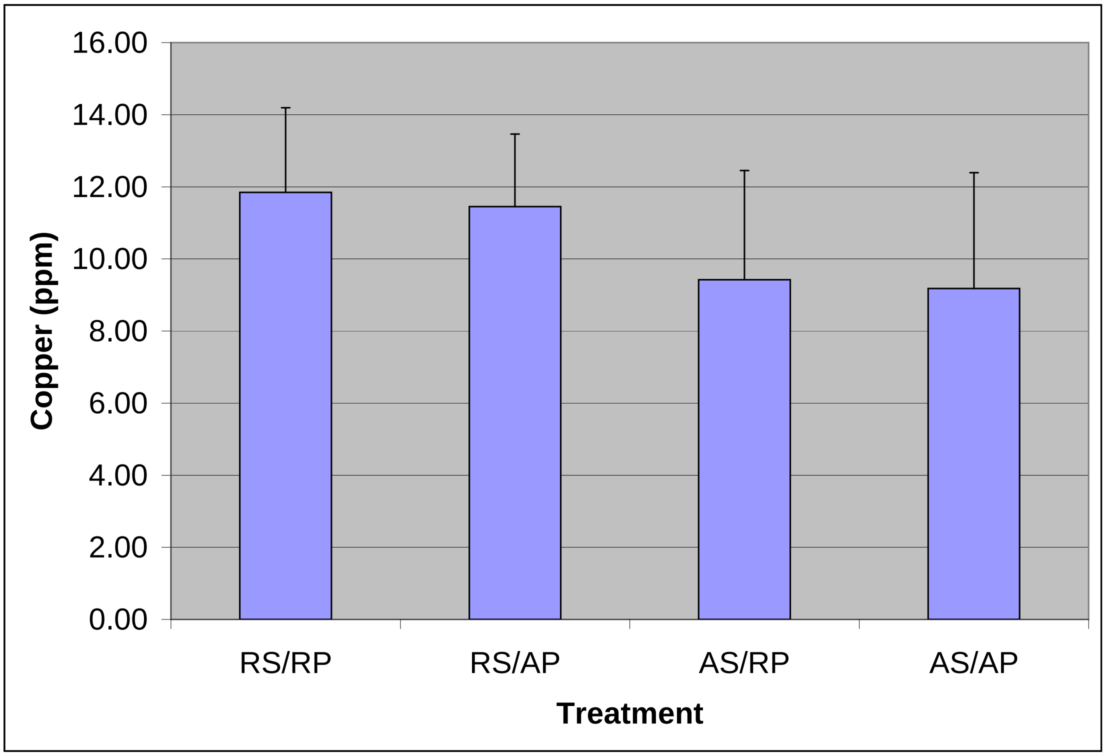

Figure 4.3: Average copper burdens in *Gambusia* in feeding trial treatments. Plot shows average and standard deviation; n=6 for each treatment. RS=reference sediment; AS=ash sediment; RP=reference prey; AP=ash prey.

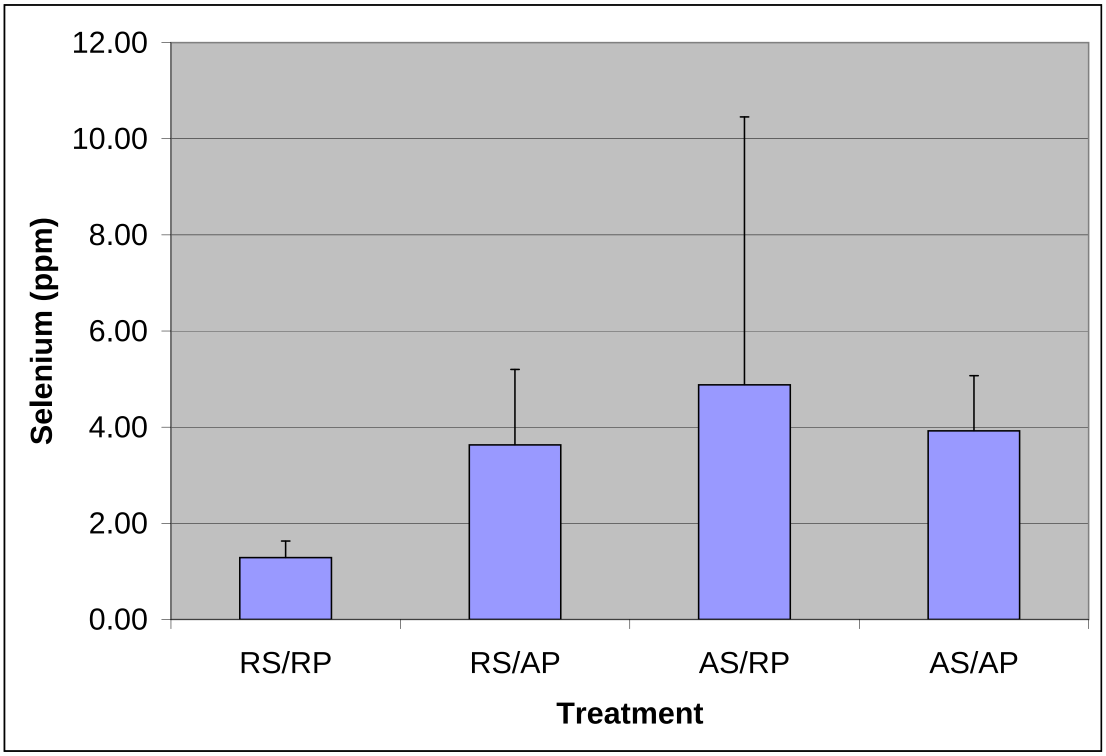

Figure 4.4: Average selenium burdens in *Gambusia* in feeding trial treatments. Plot shows average and standard deviation; n=6 for each treatment. RS=reference sediment; AS=ash sediment; RP=reference prey; AP=ash prey.

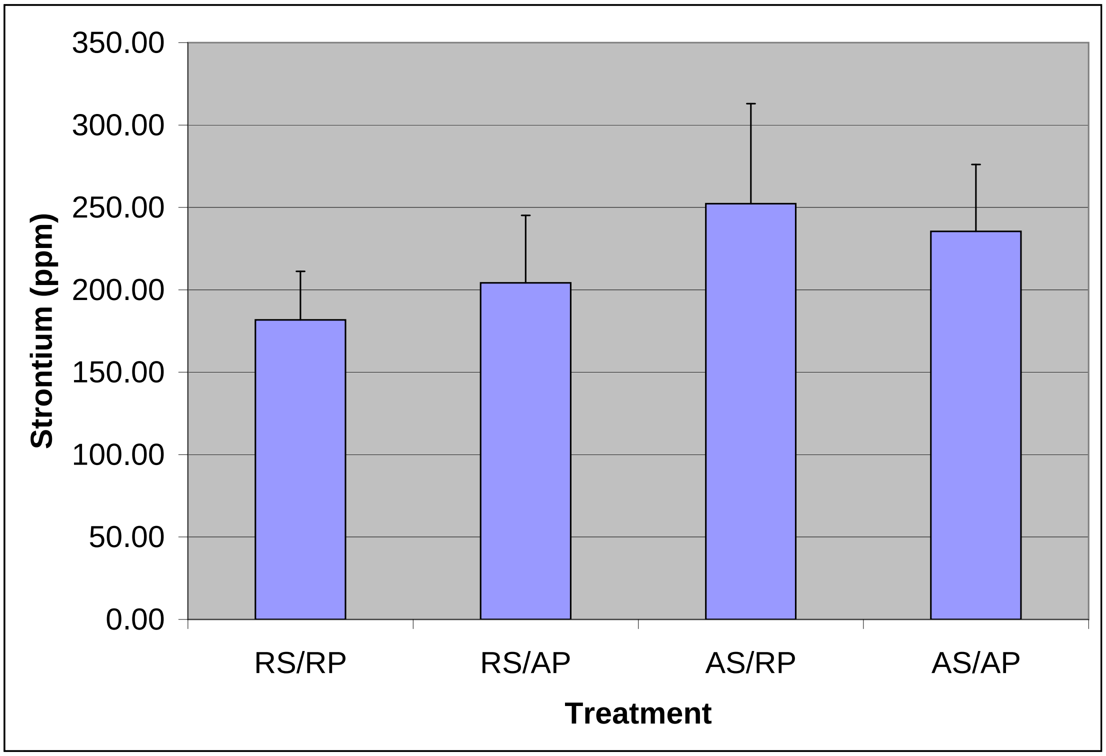

Figure 4.5: Average strontium burdens in *Gambusia* in feeding trial treatments. Plot shows average and standard deviation; n=6 for each treatment. RS=reference sediment; AS=ash sediment; RP=reference prey; AP=ash prey.

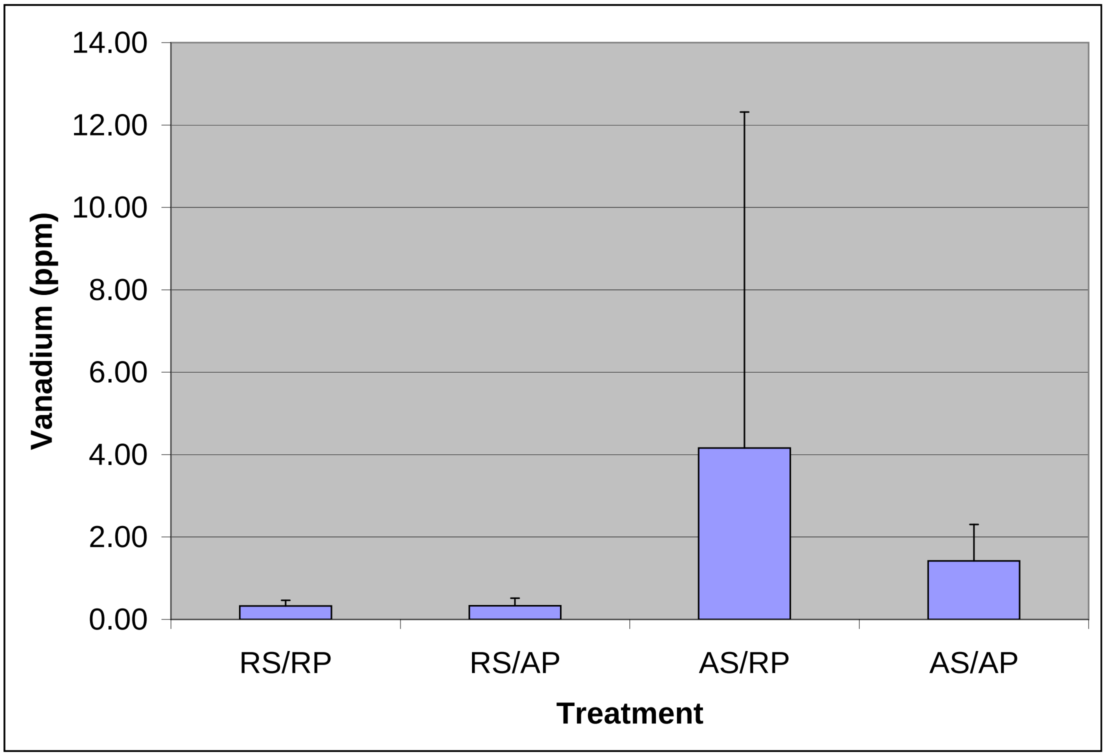

Figure 4.6: Average vanadium burdens in *Gambusia* in feeding trial treatments. Plot shows average and standard deviation; n=6 for each treatment. RS=reference sediment; AS=ash sediment; RP=reference prey; AP=ash prey.

Chapter 5

Conclusions

The aquatic invertebrate community study indicated that disposal of coal fly ash in this system was associated with a reduction of some invertebrate abundances. While no single functional group was eliminated from the system, the predators were the most impacted. Two out of four categories of predators had decreased abundances and one category was completely absent from the system. Two other functional groups, the filterers and scrapers, showed some decrease in abundances of categories but not to the extent of the predators. One of the more studied invertebrates, the ephemeropterans, has been found to be sensitive to metal pollution but did not show decreased densities in this study. It should be noted that the majority of those studies were on lotic system species while the D-Area is a lentic system. The D-Area impoundment also had a unique floral community for a Southeastern U.S. shallow impoundment. Floating-leaved aquatic plants are nearly ubiquitous in impoundments across the Savannah River Site and the surrounding area. These plants are absent from the D-Area impoundment and may have had an effect on the faunal community had they been present.

The data from the metal burden analyses showed evidence of increased metal burdens in D-Area samples compared to reference samples, however the data from plant sample comparisons is either inconsistent with the overall trends (as is the case with *Juncus*) or is based on less than ideal comparisons (*Chara*, *Typha* and *Ludwigia*). The isotopic signature data along with the metal concentration data did not show increasing metal burdens from producer to primary consumer to predator, therefore suggesting biomagnification does not occur in the proposed food chain, however there was an increase in metal concentrations from producers to primary consumers and all organisms in the food chain had elevated levels of these metals. An increase in the number of taxa sampled for isotopic signatures would provide a better understanding of the food web and potential metal transfer in the D-Area community. Inadequate biomass was prohibitive in analyzing the metal burdens and isotopic signatures of most of the invertebrates in the D-Area impoundment, but further investigations into these may offer an explanation of the lowered predator densities at D-Area.

While the results of the feeding trial were not what we postulated, some other studies do show evidence of direct uptake as being a major contribution to metal burdens of fish (Wu and Wang, 2002). While prey source was not shown to be as important a contributing factor to metal burdens of fish (except in the case of selenium - an important contaminant), it does not mean that prey from the impoundment do not pose a risk to predators (Hopkins et al., 2001). With coal continuing to be the major source of energy world-wide (Rowe, Hopkins and Congdon, 2002), it is important to continue investigating its effects on ecosystems. While resident organisms of coal combustion waste sites are the most affected, research should also include highly mobile species that may forage in an impacted site for a while and then move to a relatively pristine area, effectively increasing the reaches of the waste disposal.

5.1 References

Brooks, B.W., J.K. Stanley, J.C. White, P.K. Turner, K.B. Wu and T.W. La Point. 2004. Laboratory and field responses to cadmium: an experimental study in effluent-dominated stream mesocosms. Environmental Toxicology and Chemistry 23: 1057-1064.

Clements, W.H., D.S. Cherry and J. Cairns Jr. 1988. Impact of heavy metals on insect communities in streams: a comparison of observational and experimental results. Canadian Journal of Fisheries and Aquatic Science 45: 2017-2025.

Hopkins, W.A, J.H. Roe, J.W. Snodgrass, B.P. Jackson, D.E. Kling, C.L. Rowe and J.D. Congdon. 2001. Nondestructive indices of trace element exposure in squamate reptiles. Environmental Pollution 115: 1-7.

Prat, N., J. Toja, C. Sola, M.D. Burgos, M. Plans and M. Rieradevall. 1999. Effect of dumping and cleaning activities on the aquatic ecosystems of the Guadiamar River following a toxic flood. The Science of the Total Environment 242: 231-248.

Rowe, C.L., W.A. Hopkins and J.D. Congdon. 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: a review. Environmental Monitoring and Assessment 80: 207-276.

Xu, Y. and W.X. Wang. 2002. Exposure and potential food chain transfer factor of cd, se and zn in marine fish *Lutjanus argentimaculatus*. Marine Ecology Progress Series 238: 173-186.