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Detrimental Effects Associated with Trace Element Uptake in Lake Chubsuckers (*Erimyzon sucetta*) Exposed to Polluted Sediments

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Abstract. Lake chubsuckers (*Erimyzon sucetta*) were exposed to coal ash–polluted sediments under conservative experimental conditions (filtered artificial soft water and abundant uncontaminated food). After 4 months of exposure, fish grazing the polluted sediments had significantly elevated body burdens of Se, Sr, and V. Selenium levels were particularly elevated, reaching mean whole body concentrations of 5.6 μg/g dry mass by the end of experimental manipulations. Twenty-five percent of fish exposed to pollutants died during the study. All surviving fish exposed to ash exhibited substantial decreases in growth and severe fin erosion. Total nonpolar lipids were two times higher in fish from the control treatment, but percent lipid did not differ between treatments. Because fish were presented with the same amount of food during the study, it appears fish exposed to ash utilized more energy for daily activities and/or were less efficient at converting available energy to tissues for growth and storage. The results were particularly interesting because we were unable to detect differences in standard metabolic rate (SMR) of fish between treatments. Increased energy expenditures not detectable in estimates of maintenance based on SMR, such as costs of digestion or activity, may have contributed to decreased energetic efficiency. Our findings corroborate previous studies which have documented the toxicity of ash-derived pollutants in fish.

Over the last two decades, several studies have clearly documented the adverse effect of coal combustion wastes on aquatic communities. In the most widely cited example, 16 of 20 fish species were eliminated from Belews Lake, NC, after coal fly ash pond effluent was discharged into the reservoir (Lemly 1997). Fish inhabiting the contaminated reservoir suffered severe reproductive failure due to trophic uptake of ash-derived toxicants (most notably Se). Surviving offspring from affected females exhibited a variety of morphological abnormalities, including edema and malformations of the head, eyes, fins, and spinal column (Gillespie and Baumann 1986; Lemly 1993a). In response to ecological problems induced by pollutant disposal practices, the power plant ceased discharge of ash pond effluent into the reservoir and began landfilling their wastes (Lemly 1993a).

Despite what is known about the detrimental effects of coal ash on fish communities, approximately 20 million tons of ash continues to be discharged into aquatic systems across the United States each year (US EPA 1997). In addition to negatively impacting fish populations, such disposal procedures pose significant health risks to various wildlife species. For example, investigations near the D-area coal-fired power plant on the Savannah River Site (SRS), Aiken, SC, have revealed that numerous aquatic and semiaquatic organisms are impacted by coal combustion waste products which contain high concentrations of Se as well as at least 17 other trace elements (Hopkins *et al*. 1997). The D-area power plant discharges ash into a series of settling basins that ultimately drain into the Savannah River. Organisms utilizing the disposal basins and downstream habitats accumulate high concentrations of trace elements in their tissues (*e.g*., As, Se, Cd) and exhibit various physiological, behavioral, and developmental disruptions (Hopkins *et al*. 1997, 1998, 1999a, 1999b, 2000; Raimondo *et al*. 1998; Rowe *et al*. 1996, 1998a, 1998b; Rowe 1998).

Before draining into the Savannah River via Beaver Dam Creek, water flows from the D-area settling basins into a 2-ha drainage swamp that contains several fish species typically associated with Savannah River floodplain habitats. The primary species present in the swamp include largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), redbreast sunfish (*Lepomis auritis*), spotted sunfish (*Lepomis punctatus*), and mosquitofish (*Gambusia holbrooki*). Although American eels (*Anguilla rostrata*) also migrate from the Savannah River into the drainage swamp, other benthic fish appear to be absent from the contaminated habitat. Most notably, common benthic floodplain species, such as catfish (Ictaluridae) and suckers (Catostomidae), are either absent or in extremely low numbers within the drainage swamp. Because contaminants in coal ash–polluted sites are often concentrated

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in sediments and enter the detrital food web, some benthic species may be exposed to high dietary concentrations of contaminants by grazing surface sediments. In fact, previous investigators estimated that benthic fish in the Belews Lake system would accumulate a lethal dose of Se within 1–3 days of exposure (Cumbie 1980). Therefore, it is possible that successful colonization by some benthic species is restricted in such polluted sites due to hazardous sediment conditions.

In the current study, we sought to examine the impact of ash-contaminated sediments on a common floodplain species, the lake chubsucker (*Erimyzon sucetta*), which is absent from the D-area drainage swamp. The fact that chubsuckers are not present in the polluted swamp led to the hypothesis that this common species may be vulnerable to the contaminated sediments and therefore unlikely to successfully colonize the site. To examine the effects of contaminated sediments on chubsuckers, we monitored uptake of trace elements by fish grazing on coal ash and subsequent effects on survivorship, growth, metabolism, and lipid storage.

Materials and Methods

Animal Capture

Lake chubsuckers (mean mass 1.59 $\pm$ 0.08 g) were captured from a reference site (collection site) in minnow traps in September 1998. The collection site was a historically unpolluted Carolina bay located on the SRS. After transport to the laboratory, fish were allowed to acclimate for 5 days in artificial softwater (Rowe *et al*. 1994) at 25°C (4–5 fish/72-L tank). Fish were fed Tetramin fish flakes *ad lib* during the acclimation period. At the end of the acclimation period, all fish with visible signs of illness or weight loss were excluded from the study.

Sediment Exposure

Twenty-four 72-L tanks equipped with carbon filters, heaters, and aeration were utilized in the study. The bottom of each tank was covered with approximately 1 cm of substrate. Half of the tanks received sediment collected from the effluent outflow at the coal ash–polluted site (ash tanks), and the remaining 12 tanks received sand collected from a nearby reference site (control tanks). Sand was collected from an unpolluted terrestrial system and was hand-sifted to remove coarse debris. Tanks contained a 10 $\times$ 10 cm clay refugia, which had no bottom to ensure that fish using them maintained exposure to sediments. In the laboratory, tanks were arranged in a randomized block design and one fish was randomly assigned to each tank. Each fish was weighed to the nearest 0.001 g prior to tank assignment. Fish were fed 200 mg of Tetramin fish food every 48 h. Chubsuckers fed by grazing fish food from surface sediments after food was deposited on the bottom of tanks. Thus, fish ingest small quantities of sediment while feeding. Water temperature and dissolved oxygen were monitored at every feeding and pH was measured at the initiation and termination of the study.

Growth, Metabolic Rates, and Survivorship

Tanks were inspected 4–5 days per week for mortality and to document complete food consumption. Brightly colored food was easily detected against the contrasting and uniform background of each sediment type. On days 22, 56, 84, 108, and 124 of the experiment each fish was weighed to the nearest 0.001 g. In addition, standard metabolic rates (25°C) were determined for each fish on days 0, 84, and 124 of the experiment.

Metabolic rates were determined using a closed-circuit, computer-controlled, indirect respirometer (Micro-oxymax, Columbus Instruments, Columbus, OH). Prior to metabolic measurements, each fish was placed in a perforated 600-ml holding vessel suspended within each individual's aquarium. The vessels enabled each fish to remain within its respective tank without having access to food or sediment. After 48 hs, each postabsorptive fish was placed in a 1,100-ml respiratory chamber containing 500 ml artificial soft water (25°C) and was randomly assigned to a channel on the respirometer. In addition, one channel on the respirometer was connected to an 8.4-V battery (Procell Zinc Air Medical Battery, DA146, Duracell, Bethel, CT), which consumed a known amount of oxygen per minute. Respiratory chambers were placed in a dark environmental chamber at 25°C. Oxygen consumption of each fish was determined at 1.5-h intervals for 24 h.

Lipid Extraction and ICP-MS

After the final respiratory measurements (124 days) each fish was frozen for later lipid and trace element analysis. In addition, 13 fish captured from the collection site were frozen at the initiation of the study (September) and analyzed with experimental fish for lipids and trace elements. Prior to analysis, each fish was lyopholized and homogenized before being divided into two subsamples. One subsample of each homogenized fish was analyzed for trace element concentrations, and the other subsample was used for lipid analysis. In addition, one sediment sample from each aquarium, nine sediment samples from the collection site, and five samples of Tetramin fish food were analyzed for trace element concentrations.

All trace element analyses were performed using an inductively coupled plasma mass spectrometer (ICP-MS) (Perkin Elmer Elan 6000). Soils were digested in a microwave (CEM) with nitric acid and hydrogen peroxide in sealed PTFE (Teflon) vessels. Tissues were digested similarly to soils with the addition of hydrogen peroxide. Analysis sets of soil and fish samples included certified reference materials (MESS-2 and TORT-2, National Research Council of Canada, Ottawa, Ontario), replicates, matrix spikes, and blanks at a minimum rate of 1 per 10 samples. Replicate analyses were conducted on 10% of the samples to assess precision of analytical techniques.

The remaining subsample of each fish was used to determine total nonpolar lipid (NPL) content. Dried and homogenized subsamples were extracted with petroleum ether for 5 h using a soxhlet apparatus. Extracted samples were then dried at 60°C to a constant mass. The quantity of NPL was calculated by subtracting the dry postextraction sample mass from the dry preextraction sample mass. To ensure that a subsample of the homogenate would accurately represent whole body lipid concentration, six fish collected from the field were utilized for a validation trial. Each field-captured fish was lyopholized to a constant mass and homogenized before being divided into three validation samples. Total NPL was determined for each validation sample ($n = 3$/fish) and we concluded that less than 5% of the variability could be attributed to variance between validation samples with remaining variability attributed to among individual (fish) differences.

Data Analyses

Whole-body trace element concentrations were log-transformed to meet assumptions of normality and homoscedasticity. Trace element concentrations in fish from the collection site were compared to concentrations in fish from the experimental treatments using one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparisons. Because multiple trace elements were measured in the same fish, a sequential Bonferonni adjustment was used to adjust critical values downward. The minimum critical value was $p \leq 0.007$.

Changes in fish mass were compared between treatments using repeated measures ANOVA. Survivorship in the two treatments was compared using Chi-square analysis. We compared total quantities of NPL stored by individuals grazing the different sediment types using ANOVA. Because the amount of NPL stored is influenced by body mass, we also compared total NPL stored using a full factorial analysis of covariance (ANCOVA) with fish dry mass as a covariate. The interaction term was dropped from the final comparison because it was not statistically significant ($p = 0.098$). To confirm the results of ANCOVA, percent nonpolar lipid in experimental fish were compared using a Wilcox rank sum test.

Comparisons of SMR between treatments were made using ANCOVA with body wet mass as the covariate (Packard and Boardman 1987; Beaupre and Dunham 1995). Because standard metabolic rate is a measure of a postabsorptive animal's metabolic rate at rest, the highest 50% of oxygen consumption values were removed to minimize the effect of unobserved periods of activity on estimates of SMR (Rowe *et al*. 1998b; Hopkins *et al*. 1999b). Fish mass and mean oxygen consumption rates were log-transformed prior to analysis. We used a split plot approach to ANCOVA with time treated as plots and sediment type and fish mass treated as between and within plot effects, respectively.

Results

Mean water temperature in experimental tanks remained near 25°C in both treatments during the study (mean water temp 24.62 ± 0.26°C). Likewise, dissolved oxygen remained near 100% saturation in both treatments. Water pH remained near 7.5 in ash-treated tanks throughout the experiment; however, pH in control tanks gradually dropped from 7.5 to 4.3 during the course of the study. The decrease in pH possibly occurred due to poor buffering capacity of sand in control tanks.

Trace element concentrations in the sediments differed significantly between collection site, control tanks, and ash-treated tanks (As: $F_{2,30} = 2020.78$, $p < 0.001$; Cd: $F_{1,19} = 42.25$, $p < 0.001$; Cr: $F_{2,30} = 760.84$, $p < 0.001$; Cu: $F_{2,30} = 765.29$, $p < 0.001$; Se: $F_{1,19} = 270.60$, $p < 0.001$; Sr: $F_{2,30} = 579.70$, $p < 0.000$; V: $F_{2,30} = 1{,}370.10$, $p < 0.001$). Because Cd and Se concentrations were below detection limits in the reference tanks, we did not include Cd and Se values for the reference treatment in our analysis to avoid violations of assumptions of ANOVA. Sediment concentrations of all trace elements were significantly higher in ash-treated tanks compared to control tank sediments (Table 1). With the exception of Cd, ash-treated tanks had higher trace element concentrations than sediments from the collection site. Moreover, sediments from the collection site had significantly higher trace element concentrations compared to the control tanks (Table 1). Cadmium concentrations in sediment from the collection site were actually significantly higher than concentrations in either of the experimental treatments, probably due to the high organic content of sediment in Carolina bays compared to sand and ash. Trace element concentrations of Tetramin food are also provided in Table 1.

Mean whole-body trace element concentrations before and after experimental manipulations are provided in Figure 1. In general, ash-exposed fish tended to uptake more Se, V, As, and Sr than control fish. When compared to initial whole-body concentrations, levels of Se and Sr increased significantly in ash-exposed fish and decreased significantly in control fish (Se: $F_{2,31} = 321.18$, $p < 0.001$; Sr: $F_{2,31} = 215.18$, $p < 0.001$). Increases in As burdens were not statistically significant in ash-exposed fish, but concentrations actually decreased significantly in control fish ($F_{2,31} = 16.61$, $p < 0.001$). Although V concentrations increased significantly in both treatments, the increase was significantly higher in ash-exposed fish ($F_{2,31} = 201.36$, $p < 0.001$).

In contrast to elements that were incorporated during the study, whole-body concentrations of Cr, Cu, and Cd tended to decrease in both treatments after experimental exposure (Figure 1). Copper levels decreased to a greater extent in the control treatment than in the ash treatment ($F_{2,31} = 54.86$, $p < 0.001$). On the other hand, Cd levels actually decreased in the ash-treated fish but did not decrease significantly in control fish ($F_{2,31} = 13.12$, $p < 0.001$). Although Cr levels decreased in fish from both treatments, the decrease was not statistically significant ($F_{2,31} = 2.42$, $p = 0.106$).

All fish in the control treatment grew and survived to completion of the study whereas fish grazing the ash-contaminated sediments exhibited high mortality, poor growth rates, and severe fin erosion. By day 60 of the experiment, ash-treated fish exhibited lower body mass compared to fish in the control treatment and the difference remained significant for the duration of the study (Table 2; Figure 2). Because the feeding regime was constant throughout the study (200 mg/48 h), fish in the control treatment may have ultimately outgrown the feeding regime and become constrained by the experimental provisions. As a result, fish in the control treatment did not increase in mass for the last 16 days of the experiment. At termination of the experiment, final mean mass of control fish was 41% higher than the mass of ash-treated fish. Moreover, 0% and 25% of fish in the control and ash treatment had died, respectively ($p = 0.217$). In addition, 100% of surviving fish in the ash treatment experienced erosion of the pectoral and caudal fins. In some instances, the caudal fins eroded to the hyperel plate.

Standard metabolic rates did not differ between treatments at initiation of the study or following day 84 and day 124 of experimental exposure (Tables 3 and 4). In comparison to NPL concentrations of fish from the collection site at initiation of the study, fish in both treatments exhibited increases in NPL by the end of experimental manipulations (Table 5). Control fish stored twice as much energy in the form of NPL ($p < 0.001$), however, total NPL did not differ between treatments when fish mass was considered using ANCOVA (LS means: ash = 0.328 ± 0.021, control = 0.328 ± 0.015; $p = 0.996$; Table 5). In addition, percent NPL did not differ significantly between treatments ($p = 0.114$; Table 5).

Discussion

Trace Element Uptake

The current study examined contaminant uptake by benthic fish exposed primarily through direct contact with and ingestion of

Table 1. Trace element concentrations in sediments from the initial collection site, substrate from experimental tanks, and in Tetramin fish food

	As	Cd	Cr	Cu	Se	Sr	V
Collection site	2.70 ± 0.13	0.40 ± 0.05	16.22 ± 2.64	16.55 ± 1.24	1.97 ± 0.17	28.22 ± 2.73	30.42 ± 2.60
Control tanks	0.07 ± 0.01	BDL	0.57 ± 0.05	0.34 ± 0.06	BDL	0.88 ± 0.36	0.72 ± 0.06
Ash basin tanks	88.24 ± 2.64	0.18 ± 0.01	41.01 ± 0.75	57.52 ± 0.88	7.82 ± 0.32	380.80 ± 22.60	68.01 ± 0.67
Tetramin fish food	3.93 ± 0.02	0.16 ± 0.01	2.77 ± 0.12	9.25 ± 0.10	1.31 ± 0.09	161.66 ± 1.26	2.15 ± 0.02

All trace element concentrations are expressed as mean µg/g dry mass ± SE.
For cadmium and selenium, concentrations in control tanks were below detection limits (BDL).

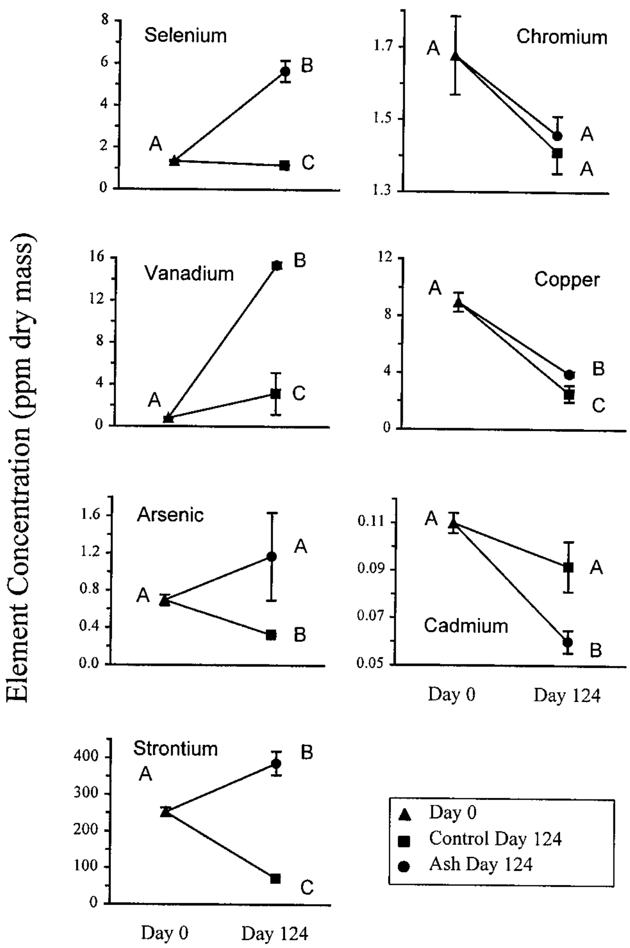

Fig. 1. Whole body concentrations (µg/g dry mass) of trace elements in lake chubsuckers (*Erimyzon sucetta*) sacrificed at the beginning and end of experimental manipulations. Fish comprising the day 0 sample were a random subsample of fish collected from the field for the experiment. Experimental fish were exposed to either coal ash–contaminated sediments (ash) or sand from a reference site (control) for 124 days. All fish were 2–3 days postabsorptive before being sacrificed for analysis. Error bars represent ± 1 SE. Common superscripts denote no significant difference ($p > 0.05$)

Table 2. Results of repeated measures ANOVA comparing changes in fish mass among treatment groups

Source	SS	df	F	p
Between subject effects				
Treatment	35.663	1	18.446	<0.001
Error (days)	36.735	19		
Within subject effects				
Days	97.799	5	344.227	<0.001
Days × treatment	13.623	5	47.948	<0.001
Error (days)	5.398	95		

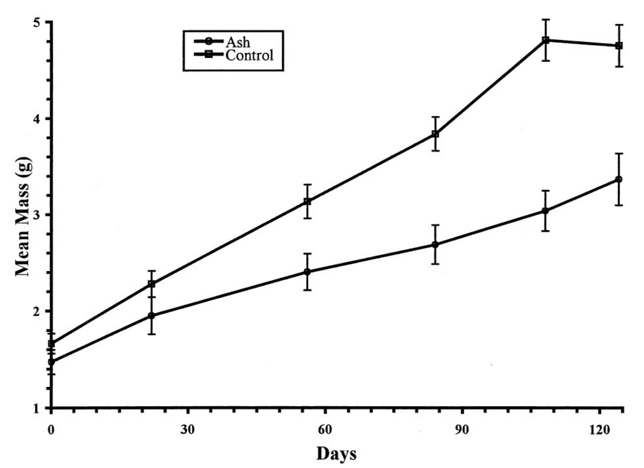

Fig. 2. Change in mean mass (g) of lake chubsuckers (*Erimyzon sucetta*) during 124-day exposure to either coal ash–contaminated sediments (ash) or sand from a reference site (control). Error bars represent ± 1 SE

coal ash–contaminated sediments. Lake chubsuckers grazing polluted sediments experienced dramatic increases in Se, Sr, and V after only 4 months of exposure. Under natural conditions, fish encounter contaminants in water, sediments, and ingested food at the ash-polluted site. Our experimental conditions were conservative; waterborne concentrations of trace elements were likely lower than those found in contaminated sites (because of continuously filtered artificial softwater), and food provided during the experiment did not contain contaminants. The rapidity of trace element accumulation, despite provision of uncontaminated food, implies severe consequences for benthic feeding fish exposed under field conditions. Such field conditions could potentially exacerbate accumulation observed in the laboratory because fish naturally exposed to polluted sediments and water would also depend on a trace element–rich food supply.

Table 3. Mean mass and standard metabolic rate (ml O_2/h ± 1 SE and ml O_2/g * h ± 1 SE) in lake chubsuckers before experimental manipulations (day 0) and after 84 and 124 days of exposure to either ash-contaminated (coal ash) or control sediments

Treatment	Mass (g)	ml O_2/h	ml O_2/g * h
Day 0			
Coal ash	1.473 ± 0.11	0.173 ± 0.01	0.118 ± 0.01
Control	1.663 ± 0.10	0.177 ± 0.01	0.108 ± 0.01
Day 84			
Coal ash	2.687 ± 0.20	0.385 ± 0.05	0.142 ± 0.01
Control	3.839 ± 0.18	0.543 ± 0.03	0.142 ± 0.01
Day 124			
Coal ash	3.000 ± 0.28	0.402 ± 0.05	0.134 ± 0.01
Control	4.952 ± 0.22	0.705 ± 0.06	0.140 ± 0.01

In contrast to fish in the ash treatment, fish in the control treatments generally exhibited decreases in whole body concentrations of trace elements compared to initial levels. Although Cr levels did not change significantly in the control fish, concentrations of five of the six remaining elements decreased significantly during the course of the experiment. Trace element levels may have decreased due to growth-influenced dilution and/or active elimination of elements. Regardless of the mechanism by which body burdens decreased, the results were expected because all trace element concentrations in sand from control tanks were significantly less than in highly organic sediments from the collection site (Table 1). However, Cd concentrations actually decreased significantly in fish exposed to ash laden sediments but did not decrease significantly in fish exposed to control sediments (Figure 1). The finding was particularly interesting given the fact that all fish species previously sampled from D-area exhibit high body burdens of Cd (Hopkins *et al.* 1999b). Fish primarily uptake Cd from water via their gills rather than direct ingestion of food or sediments, which suggests Cd concentrations in our experimental water were probably low (McCracken 1987; Hollis *et al.* 1999). Because we did not measure waterborne concentrations of contaminants in our experimental tanks it is difficult to interpret the lower Cd burdens in fish from the ash treatment.

Of the elements accumulated by chubsuckers exposed to ash, Se has received the most attention due to its well documented toxicity in fish. Previous studies in D-area indicate that resident fish have Se body burdens exceeding four times the toxic effect threshold for freshwater fish (Hopkins *et al.* 1999b; Lemly 1996, 1997). Selenium levels found in chubsuckers under our conservative experimental conditions (and short exposure time relative to resident fish species) were not nearly as high as other fish species exposed under natural conditions in D-area, but are clearly elevated enough to impair normal physiological processes. Whole-body concentrations of Se less than those found in experimentally exposed chubsuckers exhibit numerous problems, including decreased growth, histological changes, reproductive failure, and mortality (Hilton *et al.* 1980; Hodson *et al.* 1980; Ogle and Knight 1989; Hamilton *et al.* 1990; Saiki *et al.* 1992; Lemly 1996).

Biological Effects

In addition to killing 25% of the fish, coal ash exposure had a number of sublethal health consequences. Most notably, exposure to the polluted sediments had a profound effect on growth in chubsuckers. Within 60 days, control fish had attained significantly higher body masses than ash-exposed fish. By the end of the study, fish in the control treatments were 41% larger than fish exposed to ash. Differences in growth between treatments may have been conservative because fish in control treatments decreased in mass over the last 2 weeks of the study. Decreased mass was likely a result of resource limitations imposed by our feeding regime as fish became larger; fish were fed a constant amount of food throughout the study. Alternatively, because decreased pH has been associated with reduced fish growth (Faris 1986) a gradual decrease in pH in the control treatments (but not in the ash treatment) may have ultimately contributed to the final decrease in control fish growth. The fact that pH changes, and any adverse consequences associated with it, only occurred in the control treatment reinforces the likelihood that our estimates are conservative.

Because coal ash–contaminated sediments contain high concentrations of numerous potentially toxic trace elements, the current study cannot link observed fish responses to individual contaminants. In some cases, responses exhibited by chubsuckers are likely the result of interactions of contaminants in this complex anthropogenic mixture. Nevertheless, our results are consistent with a number of other investigations which document the suppressive impact of Se on fish growth (Hilton *et al.* 1980; Hilton and Hodson 1983; Hicks *et al.* 1984; Bennett *et al.* 1986; Woock *et al.* 1987). For example, fathead minnows with burdens of 5 μg/g exhibit decreased growth rates (Ogle and Knight 1989). Salmonids, which appear to be particularly susceptible to Se exposure, exhibit decreases in growth with body burdens of only 2 μg/g (Hamilton *et al.* 1990). In addition, a number of studies document decreased feeding rates in fish exposed to dietary Se (Hilton *et al.* 1980; Hilton and Hodson 1983; Finley 1985; Ogle and Knight 1989). Ogle and Knight (1989) suggest that reduced palatability of seleniferous food items may contribute to low growth rates observed in various fish species. In the current investigation, decreased growth in chubsuckers is not likely attributed to decreased feeding; food provided was not seleniferous, and fish in the ash treatment did not appear to alter feeding rates.

In addition to poor growth, all fish exposed to polluted sediments exhibited severe pectoral and caudal fin erosion. Although erosion of fin tissue is often associated with contaminant exposure (Adams *et al.* 1993; Goede and Barton 1990), the damage observed in the current study was more severe than commonly noted in field studies. Pectoral and caudal fins of 72% of fish began to erode following 22 days of exposure to ash-laden sediments. Erosion began along the ventral fin surfaces, which frequently contact the sediments, and progressed dorsally over time. By day 84 of the study, 100% of surviving chubsuckers in the ash treatment exhibited severe fin erosion. In some instances, caudal fins completely degenerated to the hyperel plate. Other benthic organisms exposed to coal fly ash exhibit deterioration of extremities and abnormalities in tissues which continuously contact the sediments. For example, 40%

Table 4. Results of ANCOVA of the effects of sediment type and fish mass on standard metabolic rate (SMR) of lake chubsuckers. SMR was measured as ml O_2 consumed/h. Mass and ml O_2 were $\log_{10}$-transformed prior to statistical analysis

Source	SS	df	MS	F	p
Sediment	0.003	1	0.003	0.830	0.414
Error (time [sediment])	0.017	4	0.004		
Log (mass)	0.634	1	0.634	73.961	0.001
Sediment × mass	0.000	1	0.000	0.021	0.891
Error (log (mass) × time [sediment])	0.034	4	0.009		

Table 5. Total nonpolar lipids (Total NPLs) and percent NPL (% NPL) in chubsuckers before experimental manipulations (collection site) and following 124 days of exposure to either ash-contaminated (coal ash) or control sediments

	Collection site	Coal Ash	Control	p Value
Total NPL (g dry mass)	0.015 ± 0.01	0.188 ± 0.03	0.407 ± 0.03	< 0.001
% NPL	4.55 ± 0.43	23.87 ± 2.80	28.84 ± 0.91	0.114

Data for total NPL and % NPL are presented as mean ± 1 SE. p value for total NPL refers to comparison of fish in experimental treatments using ANOVA. p value for %NPL refers to results of statistical comparison of fish in experimental treatments using a Wilcox-Rank sum test.

of marbled salamander larvae experimentally exposed to fly ash exhibit severe deterioration of the feet and digits (D. Scott and W. Hopkins unpublished). Bullfrog larvae that graze coal ash exhibited oral abnormalities, such as missing tooth rows and deformed labial papillae (Rowe *et al*. 1996, 1998a). Currently, it is not clear if oral abnormalities result from aberrant development or deterioration due to direct contact with sediments. Regardless of the cause, missing fins, digits, and teeth likely have severe consequences for performance in affected individuals (for example, see Rowe *et al*. 1996).

Chubsuckers from the control treatment not only increased dramatically in total body mass but also stored twice as much energy in the form of nonpolar lipids (NPLs). However, because the absolute quantity of lipid stored is influenced by body size of the organism, when proportional levels of NPLs were compared between treatments differences were not significant. Because fish in both treatments were fed the same quantity of food, it appears that chubsuckers exposed to ash were overall less efficient at converting available energy to tissues for growth and storage. Moreover, measured differences in lipid stores between treatments were probably conservative because control fish may have utilized lipids during the last 2 weeks of the experiment (the period when they lost body mass).

The apparently lower efficiency of food to tissue conversion in ash-exposed fish was not explained by differences in SMR between treatments. In addition, the lack of elevated SMR in ash-exposed fish was in contrast to results from previous studies. In general, several vertebrates and invertebrates exposed to the wastes exhibit increased SMR (an estimate of basic maintenance costs) compared to conspecifics from reference sites (Rowe *et al*. 1998b; Rowe 1998; Hopkins *et al*. 1999b). Such increases in SMR may be the result of activating energetically expensive mechanisms to deter adverse effects of pollutants (resistance costs) or utilizing mechanisms to correct damage caused by pollutants (repair costs). Organisms forced to allocate greater than normal energy to basic maintenance expenditures may experience constraints on energy available for production of tissues important for reproduction, growth, and storage.

Because SMR is the metabolic rate of a postabsorptive ectotherm at rest, increased energy expenditures occurring during digestion and activity are not included in our estimates. Increases in energy expenditures during digestion or activity could therefore explain the apparent energetic inefficiency of ash-exposed fish. For example, energy expenditures during digestion could be higher for fish simultaneously ingesting toxic sediments with their food. In addition, ingestion of toxic sediments could have negative effects on assimilation or conversion efficiency. Alternatively, fish within experimental aquaria may expend energy avoiding contact with polluted sediments. Avoidance behavior could be confounded by fin erosion; hindered swimming efficiency may result in increased activity costs.

Conclusions

The current study demonstrates that benthic feeding chubsuckers are negatively impacted when exposed to coal ash–contaminated sediments. Despite the fact that we fed chubsuckers uncontaminated food, fish grazing the polluted sediments accumulated high concentrations of Se, Sr, and V in their tissues. More important, fish that accumulated trace elements exhibited a high incidence of mortality and severe fin erosion. Even though fish in both treatments were provided with the same resources, fish from the control treatment grew more readily and stored twice as much NPL compared to ash-exposed fish. The observation that ash-exposed fish were apparently inefficient at utilizing available energy for growth and storage is particularly interesting because no differences in maintenance expenditures were detected between treatments. The negative impacts of ash-polluted sediments on chubsuckers were rapid, occurring after only several months of exposure. Because of the rapidity of the responses under conservative experimental conditions, we hypothesize that *real-world* consequences of ash exposure are potentially much more severe and could contribute to the absence of chubsuckers from the polluted habitat downstream from the D-area settling basins.

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