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Responses of benthic fish exposed to contaminants in outdoor microcosms—examining the ecological relevance of previous laboratory toxicity tests

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Abstract

Previous laboratory studies indicate that coal combustion wastes (a mixture composed of fly ash and other lower volume wastes such as bottom ash; hereafter collectively referred to as ash) adversely affect the health of benthic fish (*Erimyzon sucetta*; lake chubsucker), but fish in these studies were provided with ample uncontaminated food resources. Because aquatic disposal of ash can also adversely affect food resources for benthic fish, we hypothesized that changes in resources might exacerbate the effects of ash on fish observed in laboratory studies. We exposed juvenile *E. sucetta* in outdoor microcosms to water, sediment, and benthic resources from an ash-contaminated site or a reference site for 45 days and compared our findings to previous laboratory studies. Benthic invertebrate biomass was nearly three times greater in controls compared to ash microcosms. Total organic content of control sediment (41%) was also greater than in ash sediments (17%), suggesting that additional benthic resources may have also been limited in ash microcosms. Benthic invertebrates isolated from the ash microcosms had trace element concentrations (As, Cd, Co, Cr, Cs, Se, Sr, and V) up to 18 times higher than in weathered ash used in laboratory studies. The concentrations of trace elements accumulated by fish reflected the high dietary concentrations encountered in the ash microcosms and were associated with reduced growth (final mass = 0.07 g) and survival (25%) compared to controls (0.37 g and 67%, respectively). Accumulation of trace elements, as well as reductions in growth and survival, were more pronounced than in previous laboratory studies, suggesting that resource conditions may be important in mediating ash toxicity. Taken together, our studies suggest that ash discharge into aquatic systems is a more serious threat to the health of benthic fish than previously predicted based upon laboratory toxicity tests.
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1. Introduction

Ecotoxicologists have become increasingly aware of limitations associated with traditional laboratory

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toxicity testing and have sought methods for gaining a more holistic understanding of contaminant effects on naturally complex systems. Although reductionistic laboratory approaches in ecotoxicology are important for establishing clear cause and effect linkages between contaminants and toxicity, as well as the mechanistic basis for toxicity, such approaches usually fail to represent ecologically relevant exposure conditions. Alternatively, field studies illustrate responses of organisms to contaminants under natural environmental conditions, but such studies are often correlative, expensive, difficult to replicate, and logistically impractical. Moreover, distinguishing contaminant effects from effects of other biotic and abiotic variables (i.e., non-toxicological parameters) can be a formidable task in field studies, due to the complexity of natural systems and variability in conditions between contaminated and reference sites. Because neither laboratory nor field studies alone produce a comprehensive understanding of contaminant effects, some ecotoxicologists have adopted a pluralistic approach (e.g., Sadinski and Dunson, 1992; Rowe and Dunson, 1994), where field studies are combined with laboratory investigations to achieve a more holistic perspective. An approach that has been used less extensively by ecotoxicologists, but frequently by ecologists is construction of simulated outdoor ponds (e.g., meso- and microcosms) as a useful intermediate between field and laboratory studies (Rowe and Dunson, 1994; Boone and Semlitsch, 2001; Bridges and Boone, 2003). Use of outdoor microcosms minimizes the disadvantages associated with each independent approach (i.e., field and laboratory) by permitting control of desired variables while simultaneously maintaining some degree of natural complexity (Diamond, 1986; Rowe and Dunson, 1994; Resetarits and Fauth, 1998).

The current study was designed to examine the effects of coal combustion wastes on benthic fish exposed in outdoor microcosms. Recent laboratory studies indicate that the benthic-feeding fish, *Erimyzon sucetta*, exposed to coal ash-contaminated sediments exhibit a variety of adverse responses, and responses are most severe when food resources are reduced (Hopkins et al., 2000, 2002, 2003). Although these studies evaluated the role of food resources in mediating toxicity, they focused on manipulating uncontaminated resources (Tetramin fish food) under conservative laboratory conditions (e.g., continuously filtered uncontaminated water, constant temperature, etc.). Here, we examine the effects of aquatic coal ash disposal under more ecologically realistic conditions by exposing *E. sucetta* in outdoor microcosms containing water, sediments, and benthic invertebrates collected from a swamp downstream from a coal-burning power plant. Because aquatic disposal of solid wastes produced during coal combustion can decrease the diversity, abundance, and quality of benthic resources in downstream habitats (Cairns et al., 1970; Cherry et al., 1979a,b; Forbes and Magnuson, 1980; Forbes et al., 1981; Hatcher et al., 1992), we hypothesized that benthic fish dependent on such resources in outdoor microcosms would be affected more adversely than under laboratory conditions where such community interactions do not occur. Our approach enabled us to examine the confounding effects of altered resource conditions on the responses of benthic fish exposed to ash and to evaluate the ecological significance of our previous laboratory findings.

2. Materials and methods

2.1. Experimental design

In February 2000, 48 outdoor microcosms were established at the Aquatic Ecology Laboratory, a satellite facility of the Savannah River Ecology Laboratory near Aiken, SC, USA. We used a 2×2 fully factorial study design with two sediment types (ash or control) and two feeding regimes (unsupplemented or supplemented). Microcosms were arranged in a randomized block design (six blocks containing two replicates per sediment/feeding regime combination). The microcosms were situated under partial tree canopy and were further shaded by a 6 m × 10 m shadecloth draped 2–3 m above the microcosm array.

Microcosms were constructed of 109 l semi-transparent plastic containers (benthos dimensions = 42 cm × 73 cm). A single 21 cm × 39 cm panel in the lid of each container was removed and replaced with fiberglass insect screen. Microcosms received 14 l of sediment to produce a benthos approximately 1.5 cm deep. Sediments were collected from either a drainage swamp downstream from a coal ash-polluted site

(ash) or from a historically unpolluted reference site (control; Carolina Bay # 97) on the Savannah River Site (SRS). For detailed description of the contaminated site and studies conducted over the last 30 years at this site, see Rowe et al., 2002. Sediments were collected by removing the 2–3 cm surface layer (by shallow skimming of the surface layer with a shovel) from 16 locations distributed throughout each wetland. The 16 samples from each collection site were then placed in a single large vessel and homogenized by hand before being distributed in equal volumes to each microcosm. Microcosms also received 70 l of water collected from the site of sediment collection. Each microcosm was equipped with an airstone connected to an outdoor pump system that supplied continuous aeration throughout the study.

On 15 May 2000, juvenile *E. sucetta* (lake chubsucker) (approximate standard length = 15.0 mm) were collected from a historically unpolluted Carolina Bay (Bay # 40 on the SRS) using dipnets. Fish were allowed to acclimate to laboratory conditions for approximately 3 weeks in 72 l tanks containing artificial softwater (USEPA, 1991) at 25 °C. During the acclimation period, fish were fed Tetramin fish flakes ad libitum.

On 6 June 2000 (4 months after microcosms had been established) a single lake chubsucker was randomly assigned to each microcosm (total of 48 fish used; $N = 12$ fish per treatment). Random tank assignment was achieved by blindly drawing one fish at a time from a single large tank. Each fish was measured to the nearest 0.01 mm (standard length) prior to microcosm assignment. A dorsal image of each fish was taken with a camera, and standard length was determined using Morphosys® visual imaging analysis software according to methods described by Heulett et al., 1995. The procedure was repeated three times for each fish and the mean of the measurements was used for that fish. At initiation of the study, mean standard length of fish was 17.08 ± 0.34 mm. To avoid handling stress, initial mass of fish was not determined, but we estimated initial mass of experimental fish using linear regression comparing standard length and mass of 10 extra fish not used in the study ($R^2 = 0.88$, $P < 0.001$; estimated initial mean mass = 95.29 ± 37.34 mg). Fish assigned to unsupplemented treatments received no additional food and were reliant on resources present in the sediment within their respective microcosm. Because previous studies indicated that benthic resources can be severely reduced due to ash disposal (Cairns et al., 1970; Cherry et al., 1979a,b; Forbes and Magnuson, 1980; Forbes et al., 1981; Hatcher et al., 1992), small quantities of supplemental rations were administered to half of the experimental fish (supplemented treatment) to ensure that at least half of ash-exposed fish had adequate rations for survival (based upon rations supplied in our previous work; Hopkins et al., 2000, 2002, 2003). Fish assigned to the supplemented treatments were fed weekly rations of Tetramin equaling 16% of their initial estimated mean body mass (equivalent to 15.0 mg of Tetramin fish food per week). Finely ground Tetramin was supplied to fish in the supplementation treatments by depositing weighed rations directly on the surface sediments using a disposable plastic pipette.

2.2. Microcosm sampling

We measured water temperature, conductivity, dissolved oxygen, and pH of each microcosm prior to introducing fish. For the duration of the study, we monitored temperature of four randomly selected microcosms every 2 h using temperature-recording dataloggers (Hobologger, Onset Computer Co.).

To assess potential differences in food resource availability between ash and control sediments, we quantified invertebrate community composition in sediment samples from each microcosm. Prior to the beginning of the experiment, six sediment cores were collected from approximately the same locations within each microcosm; four of the samples were taken 2 cm from each corner and two samples were taken near the midpoint of each microcosm. Sediment samples ($V = 15$ ml) were obtained using a modified 60 ml syringe (diameter = 2.75 cm) that was inserted into the sediment as negative pressure was applied to retrieve a sediment core. The six sediment cores from each microcosm were then pooled into a single sample (producing a single composite sample per microcosm) and were preserved and stored in 95% ethanol. Because benthic fish may consume organic matter other than invertebrates from the sediments, we also collected a sediment sample (approximate dry mass 5 g) from a random subset of ash and control microcosms ($N = 5$ ash and controls) for organic content analysis.

To assess trace element concentrations (As, Cd, Co, Cr, Cs, Se, Sr, and V) in the microcosms, we collected a water sample (200 ml) and surface sediments (approximate grab sample wet mass = 25 g) from a random subset of ash and control microcosms ($N = 5$ ash and controls; see ICPMS procedures below). All samples for trace element analysis were stored frozen (−70 °C) prior to analysis.

Forty-five days after initiation of the study, fish were removed from each microcosm using modified minnow traps over a 3-day period. When a fish was not recovered using traps, we drained the microcosm to verify that the fish died during the study. Each fish was weighed (nearest 0.01 mg) and standard length of each fish (nearest 0.01 mm) was measured. Fish were then frozen at −70 °C for future trace element analysis (Permit Number SCDNR F-00-16).

2.3. Benthic sample processing

Sediment samples were stained with Rose Bengal several days before processing by accepted protocols. The sediments were rinsed on a 250 μm standard testing sieve to remove the preservatives and finer sediments. The contents were rinsed into a white, shallow plastic tray. All stained animal material was removed with a pipette or fine forceps and collected for counting. The animals were counted and identified at 12–25×. Counted samples were preserved in 4% formalin.

To compare dry mass of benthic invertebrates between treatments, counted animals were pooled in four groups of six randomly chosen microcosms from each sediment treatment (i.e., $N = 4$ composite samples per sediment type). Samples were rinsed on a 26 μm Nitex sieve and placed in a petri dish. All individuals were removed, cleaned of debris, and placed in tared plastic boats. Excess water was removed before the samples were dried for 24 h at 59 °C, and then samples were stored in a desiccator before reweighing. To provide enough biomass for trace element analysis, we pooled benthic invertebrates from all 24 microcosms in each sediment treatment (resulting in $N = 1$ pooled benthic invertebrate sample per sediment treatment).

Organic content of sediments was quantified based on loss of mass at ignition at 550 °C. Sediment samples were dried to a constant mass (5.75±0.25) at 50 °C before being placed in a muffle furnace (550 °C) for 24 h. The difference between the pre- and post-combustion masses was used to estimate organic content of each sediment type. All samples were allowed to equilibrate in a dry box (<2% humidity) prior to mass measurements.

2.4. Trace element analyses

Water, sediments, benthic invertebrates, surviving experimental fish, and three fish from the initial collection site were analyzed for trace element content using ICP-MS (Perkin–Elmer, Norwalk, CT). Water samples from each microcosm were filtered (10 ml through 0.45 μm filter) before being acidified with 0.7 ml of 70% nitric acid. Sediment samples were dried and tissue (invertebrate and fish) samples were lyophilized prior to being digested and analyzed for trace element concentrations according to the following procedures. Approximately 10–100 mg of tissue or 250 mg of sediment was digested. Nitric acid (1.0 or 5.0 ml, tissue and sediment, respectively) was added to samples before digestion in a microwave (CEM Corp., Matthews, NC) with heating steps of 60, 60, 70, and 80% microwave power for 10, 10, 15, and 20 min, respectively. Next, 0.25 or 1.0 ml of H_2O_2 (tissue and sediment, respectively) were added to the samples and microwaved at the same power and duration as the HNO_3 digestion. After digestion, samples were brought to a final volume of 5 or 25 ml (tissue and sediment, respectively) with d-d water.

Trace element analysis was performed by ICP-MS on samples diluted 1:1 with d-d water. Calibration standards covering a range of 1–500 μg/l were prepared daily by serial dilution of NIST traceable primary standards. Certified reference material (Tort 2 and Mess 2; NRC, Ottawa, Canada) and blanks were included in the digestion and analysis procedure for quality control purposes. Mean percent recoveries for trace elements in tissue reference material ranged from 83.3 to 104.3%. Coefficients of variation of percent recoveries from tissue reference material replicated among digestion sets ranged from 3.94 to 12.29%.

2.5. Statistical analyses

Trace element concentrations in water from the microcosms were not compared statistically because most elements in water from control microcosms were

below instrumentation detection limits (detection limits for As, Cd, Co, Cr, Cs, Se, Sr, and V were 2.9, 0.4, 0.2, 7.5, 0.3, 5.0, 0.2, and 6.9 μg/l, respectively). Sediment and whole body trace element concentrations were log-transformed to meet assumptions of normality and homoscedasticity. Trace element concentrations in fish from the experimental treatments were compared using MANOVA with sediment type and food treatment as main effects in the model. For exploratory purposes we also conducted a series of individual two-way ANOVAs for each element in fish tissue. Trace element concentrations in sediments were compared between the two treatments using individual ANOVAs. Because multiple trace elements were compared from the same sediment samples, a sequential Bonferonni adjustment was used to adjust critical values downward and maintain an experiment-wide error rate of $P < 0.05$. The minimum critical value for a specific test was $P \leq 0.007$.

We used a series of Fisher's exact tests to investigate the effect of sediment type and food supplementation on survival. Final mass, standard length, and condition factor of fish were compared between treatments using MANOVA, with sediment type and food supplementation treatment as main effects in the model. Final mass and standard length were log transformed prior to analysis. We calculated condition factors using the standard equation $K = [\text{fish mass (g)}/\text{fish standard length}^3\ \text{(cm)}] \times 100$ (Anderson and Gutreuter, 1984).

To examine resources available to juvenile fish in the microcosms, we compared total dry invertebrate biomass, number of benthic invertebrates, and sediment organic content among treatments using ANOVAs. Invertebrate composition was not influenced by food supplementation, because invertebrate samples were collected prior to the experiment. Therefore, food supplementation was not included in statistical comparisons of invertebrates. The total number of benthic invertebrates, total dry invertebrate biomass, and sediment organic content were compared between sediment types using one-way ANOVAs. Because oligochaetes and microcrustaceans represented the majority (96%) of biomass within the microcosms, we also compared total number of both groups among sediment treatments using one-way ANOVAs. All analyses were performed on untransformed data. Although measures of resource availability were not independent, we used individual ANOVAs because parameters in each model differed among measured resource endpoints. For all comparisons of resource abundance, we used a sequential Bonferonni adjustment to adjust critical values downward, and maintained an experiment-wide error rate of $P < 0.05$.

To compare findings from this microcosm experiment to findings from our longer (up to 124 d) laboratory exposures (Hopkins et al., 2000, 2002, 2003; Hopkins, 2001), we standardized growth and survival data from the laboratory studies to 45 days (i.e., the length of the current microcosm experiment). Specific growth rates ([ln initial mass − ln mass at 45 day]/45 day) and survival at 45 days were determined for fish in all treatments from each experiment. For each experiment, we plotted the percent reduction in specific growth rate and survival in ash-treated fish compared to the appropriate controls used in that experiment. For illustrative purposes, we also provide a graph describing two examples (i.e., Cd and Se) of trace element accumulation in fish from our series of experiments. Because whole body trace element concentrations were only determined at the end each study, we could not standardize trace element concentrations to 45 days.

3. Results

Mean conductivity and pH were higher in ash microcosms than in control microcosms (conductivity (μS/cm): 285.87 ± 6.63 versus 24.75 ± 1.41, respectively; pH: 6.42 ± 0.08 versus 5.64 ± 0.07, respectively), but temperature and dissolved oxygen were similar among all microcosms. Because microcosms were aerated, dissolved oxygen remained high (range: 7.15–8.23 mg/l). Mean initial water temperature ranged between 25.46 and 25.79 °C. Over the 45 days of the study, temperatures fluctuated between 21.78 and 30.64 °C, with overall mean temperatures remaining similar among microcosms (range of mean microcosm temperatures = 24.85–25.64 °C).

In general, trace element concentrations in water, sediment, and invertebrate samples from ash microcosms were higher than in control microcosms (Table 1). Although water concentrations were not compared statistically, concentrations of five elements were elevated in water from ash microcosms compared to control microcosms (Table 1). Sediment concentrations of seven trace elements (As, Cd, Co,

Table 1
Trace element concentrations in water, sediments, and benthic invertebrates from experimental microcosms containing water and sediments collected from either a coal ash-polluted drainage swamp or from a reference site

	Water (μg/l)		Sediments (μg/g)		Benthic invertebrates (μg/g)	
	Control	Ash	Control	Ash	Control	Ash
As	BDL	8.45 ± 2.10	0.63 ± 0.09	98.04 ± 3.70	0.93	16.69
Cd	BDL	8.09 ± 0.75	0.09 ± 0.01	4.24 ± 0.67	0.33	3.36
Co	1.24 ± 0.23	75.56 ± 6.80	2.61 ± 0.35	38.72 ± 4.48	1.97	15.16
Cr	BDL	BDL	195.50 ± 29.90	81.34 ± 3.56	5.08	29.06
Cs	BDL	0.81 ± 0.07	0.50 ± 0.08	3.03 ± 0.03	0.38	0.61
Se	BDL	BDL	0.52 ± 0.13	20.68 ± 0.39	4.56	56.94
Sr	8.16 ± 1.64	347.72 ± 12.17	13.12 ± 2.28	114.62 ± 5.25	8.84	33.33
V	BDL	BDL	6.27 ± 3.38	119.78 ± 4.63	7.16	24.72

Values for water and sediment represent the mean of samples from 5 microcosms ± 1S.E. Invertebrate concentrations are derived from a single pooled sample of all benthic invertebrates collected from each of the sediment types. Sediment and invertebrate values are presented on a dry mass basis. Water and benthic invertebrate concentrations were not compared statistically, however, sediments differed significantly ($P < 0.01$) between sediment types in concentrations of all elements. Values that were below detection limit are indicated with BDL.

Cs, Se, Sr, and V) in ash microcosms were significantly elevated above controls, and in some cases were orders of magnitude higher (Table 1; in all cases $F_{1,8} > 106.02$, $P < 0.001$). However, Cr concentrations were higher in control sediments than in ash ($F_{1,8} = 23.39$, $P = 0.001$). All trace elements were markedly higher in composite invertebrate samples from ash microcosms compared to control microcosms (Table 1).

Results of MANOVA indicated that contaminated conditions in ash microcosms significantly influenced fish whole body burdens of trace elements ($F_{8,11} = 270.80$, $P < 0.001$), but provision of supplemental food had no effect on final body concentrations (Food: $F_{8,11} = 1.65$, $P = 0.217$; Sediment X Food: $F_{8,11} = 0.71$, $P = 0.681$; Table 2). Individual ANOVAs indicated that fish exposed to ash had significantly higher whole body concentrations of all measured trace elements compared to fish from control microcosms (Table 2; in all cases $F_{1,18} > 10.33$, $P < 0.005$). Individual ANOVAs also confirmed that supplemental feeding had no effect on final trace element body concentrations (in all cases, Food Treatment: $P > 0.104$ and Sediment X Food Treatment: $P > 0.227$).

Survival of fish in microcosms was dependent upon sediment treatment. Overall, significantly ($P = 0.008$)

Table 2
Whole body trace element content of lake chubsuckers (*E. sucetta*) exposed to water, sediments, and invertebrates from either a coal ash-contaminated drainage swamp or from a reference site

	Field-captured	Control		Ash	
		Unsupplemented	Supplemented	Unsupplemented	Supplemented
As	0.36 ± 0.05	0.31 ± 0.02	0.34 ± 0.06	2.18 ± 0.19	2.43 ± 0.50
Cd	0.17 ± 0.02	0.14 ± 0.01	0.17 ± 0.03	1.30 ± 0.45	1.66 ± 0.26
Co	1.39 ± 1.14	0.54 ± 0.07	0.56 ± 0.17	2.05 ± 0.83	1.52 ± 0.23
Cr	1.26 ± 0.23	0.66 ± 0.12	0.62 ± 0.13	1.60 ± 0.69	2.49 ± 0.49
Cs	0.14 ± 0.01	0.16 ± 0.02	0.18 ± 0.02	0.32 ± 0.04	0.36 ± 0.03
Se	1.20 ± 0.06	1.50 ± 0.07	1.29 ± 0.13	70.34 ± 1.16	65.43 ± 3.24
Sr	86.94 ± 8.63	105.99 ± 7.53	122.10 ± 19.50	168.90 ± 27.80	189.70 ± 25.90
V	0.50 ± 0.09	0.35 ± 0.05	0.40 ± 0.05	1.98 ± 0.49	1.94 ± 0.17

Fish were exposed in outdoor microcosms for 45 days and were either reliant upon resources present in microcosms (unsupplemented) or were provided with supplemental uncontaminated food (supplemented). Values represent the mean of all surviving fish in each treatment ± 1S.E. Values are presented as μg/g dry mass. Multivariate analysis indicated that sediment type has significant effect ($P < 0.001$) on fish element concentrations, but that supplemental feeding did not.

fewer fish survived in the ash microcosms (25% survival) than did in the control microcosms (67%). Supplemental food had no effect on survival within either the ash or control treatments ($P = 0.640$ and 0.193, respectively). However, when fish reliant on constitutive resources were compared between sediment treatments, fish in the ash treatment had significantly lower survivorship than controls ($P = 0.003$). There was no significant difference in fish survival between the ash and control treatments when additional food was supplied ($P = 0.680$), likely because of statistically insignificant reductions in survival in control fish with supplemental food (Fig. 1).

Final standard length, mass, and condition factor of fish were adversely affected by exposure to ash sediments ($F_{3,16} = 17.43$, $P < 0.001$; Fig. 2). None of these measures was influenced by provision of supplemental food (food: $F_{3,16} = 1.77$, $P = 0.192$; sediment × food: $F_{3,16} = 1.24$, $P = 0.328$).

Benthic invertebrate composition and abundance, as well as organic content of sediments, differed between sediment treatments. Invertebrates were more abundant per sample in the ash treatment compared to controls (mean # individuals: 131 ± 15.3 versus 58 ± 5.9, respectively; $F_{1,46} = 19.65$, $P < 0.001$), due mainly to greater numbers of oligochaete worms (mean # individuals: 89 ± 9.6 versus 28 ± 4.7, respectively; $F_{1,46} = 31.81$, $P < 0.001$). The number of microcrustaceans did not differ between sediment treatments (mean # individuals: control $= 36 \pm 7.22$ versus ash $= 28 \pm 3.05$; $F_{1,46} = 1.02$, $P = 0.318$). However, because oligochaete worms were much smaller in the ash treatment than in the control treatment (estimated dry mass per individual 0.006 and 0.097 mg, respectively), total benthic invertebrate biomass was nearly three times

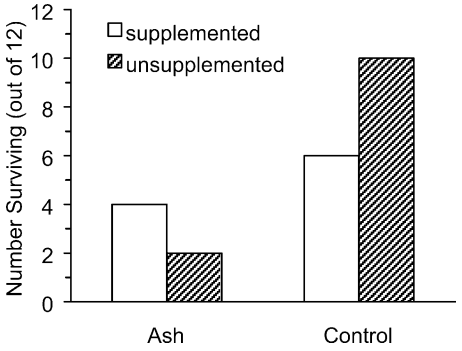

Fig. 1. Number of juvenile lake chubsuckers (*E. sucetta*) surviving following 45 days in microcosms containing water and benthos collected from a coal ash-contaminated site (ash) or from a reference site (control). Fish exposed in ash and control microcosms were either dependent upon resources naturally available in the microcosms (unsupplemented) or were provided supplemental food (supplemented).

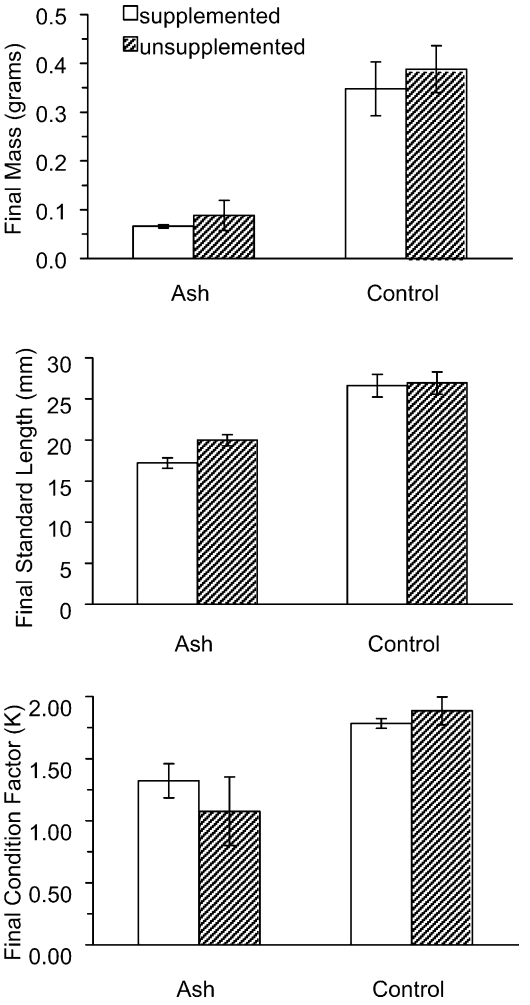

Fig. 2. Final mass (g), standard length (mm), and condition factor of juvenile lake chubsuckers (*E. sucetta*) after 45 days in microcosms containing water and benthos collected from a coal ash-contaminated site (ash) or from a reference site (control). Fish exposed in ash and control microcosms were either dependent upon resources naturally available in the microcosms (unsupplemented) or were provided supplemental food (supplemented). Data are presented as mean ± 1S.E. Multivariate analysis indicated that sediment type has significant effect ($P < 0.001$) on fish body measurements, but that supplemental food did not.

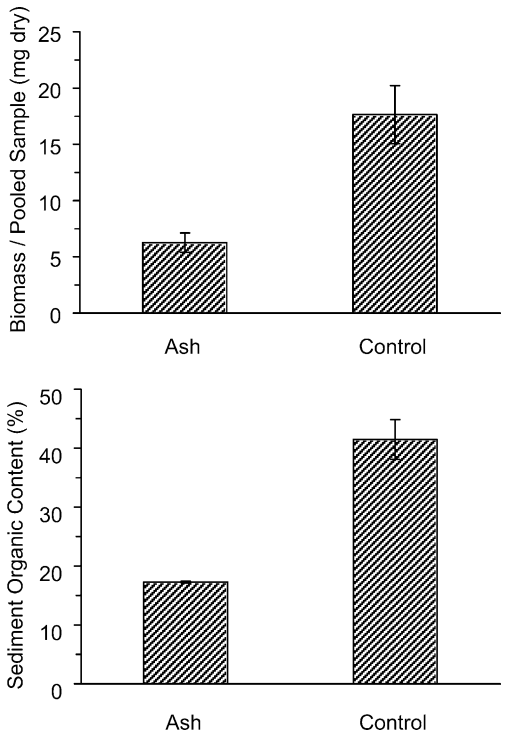

Fig. 3. Total biomass (mg dry mass) of benthic invertebrates isolated from pooled collections ($N = 4$ pooled samples/sediment treatment) and percent organic content of sediment samples from a randomly selected subset ash and control microcosms ($N = 5$ per sediment treatment). Data are presented as mean ± 1S.E. In both statistical comparisons, ash microcosms had significantly lower means than controls ($P < 0.01$).

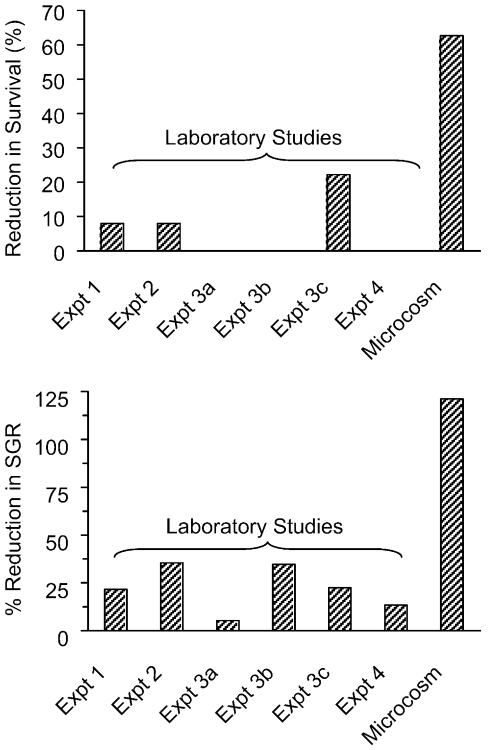

Fig. 4. Comparison of fish survival and specific growth rate (SGR) in the current study and our previous laboratory studies. Each bar represents the percent reduction in survival of fish exposed to ash compared to controls within each study through 45 days of exposure. Data for experiments 1–3 are derived from Hopkins et al., 2000, 2003, and 2002, respectively. Data from experiment 3 represents responses in fish provided with high, medium, and low food resources (Expt 3a–c, respectively). Data for experiment 4 was derived from a 45 day study examining the effects of ash on juvenile fish of equivalent body size as fish in the microcosm study (Hopkins, 2001).

greater in control microcosms than in the ash treatment ($F_{1,6} = 27.56$, $P = 0.002$; Fig. 3). In addition, percent organic content of sediments from the control microcosms was significantly higher than in the ash microcosms ($F_{1,8} = 51.23$, $P < 0.001$; Fig. 3).

The adverse responses of fish exposed to ash in outdoor microcosms were more severe than the responses of fish exposed to weathered ash in the laboratory (Fig. 4). Survival through the first 45 days of our laboratory studies only differed between ash and controls by 0–8%, except in the case where fish were placed under severe resource limitations (Fig. 4, experiment (Expt) 3c: rations equaling 4.6% of initial body mass per week); survival differences between treatments increased to 22% after 45 days when most resources were withheld (Hopkins et al., 2000, 2002, 2003; Fig. 4). In contrast, survival of fish from ash microcosms was 63% lower than in control microcosms (Fig. 4). Similarly, growth reductions were most pronounced in fish exposed to ash under outdoor microcosm conditions. In our previous laboratory work, the maximum reduction in specific growth rate of ash-exposed fish compared to controls was 35% after 45 days. In contrast, fish from ash microcosms had specific growth rates 121% lower than fish from control microcosms (Fig. 4). Trace element accumulation was generally higher in fish exposed in microcosms than in the laboratory (e.g., Fig. 5) despite the fact that microcosm exposures were much shorter than laboratory exposures.

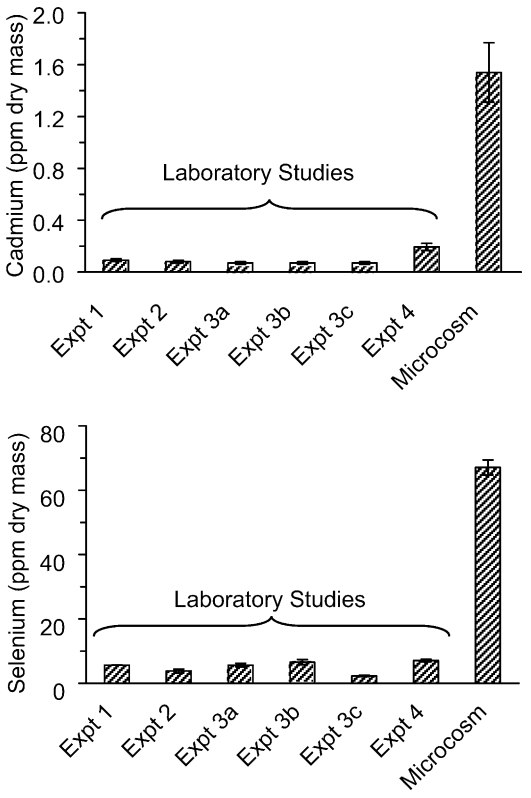

Fig. 5. Examples of differences in trace element accumulation in fish (whole body concentrations) from the current study (microcosm) compared to previous laboratory studies. For comparisons of other elements, readers should refer to the original laboratory studies (Hopkins et al., 2000, 2002, 2003). Data for experiments 1–3 are derived from Hopkins et al., 2000, 2003, and 2002, respectively. Data from experiment 3 represents accumulation in fish provided with high, medium, and low food resources (Expt 3a–c, respectively). Data for experiment 4 was derived from a 45 day study examining the effects of ash on juvenile fish of equivalent body size as fish in the microcosm study (Hopkins, 2001). Note that concentrations reported for experiments 1–3 reflect accumulation following much longer exposure (124, 100, and 78 day, respectively) than in the current microcosm study (45 day). Data are presented as mean ± 1S.E.

4. Discussion

Fish exposed to ash in outdoor microcosms accumulated contaminants and experienced a variety of adverse effects. At least eight trace elements were accumulated in fish tissues and in some cases body burdens greatly exceeded those known to adversely affect fish physiology (e.g., Se; Lemly, 1996). In addition, fish from control microcosms were five times larger (wet mass) and were in much better physical condition than surviving fish from ash microcosms. Survival was more than twice as high in controls compared to fish from ash microcosms. Provision of small weekly rations of uncontaminated food had no effect on any parameters measured.

Compared to our previous laboratory toxicity tests (Hopkins et al., 2000, 2002, 2003), the contaminated conditions experienced by fish in our outdoor microcosms more closely simulated conditions that benthic fish would naturally encounter in the contaminated field site. In our previous work, fish were exposed to weathered coal ash collected from the power plant outfall that contained little organic matter (<4%). Resources were controlled in these laboratory studies (i.e., in the form of Tetramin fish food) and uncontaminated water was continuously filtered. These laboratory tests allowed us to determine that fish accumulated contaminants directly from sediment ingestion and that accumulation was associated with reduced survival and a variety of sublethal effects (Hopkins et al., 2000, 2002, 2003). In contrast, fish in outdoor microcosms were exposed to contaminated water and sediments collected from a drainage swamp downstream from the power plant's settling basins. Unlike weathered ash from the power plant outfall, the sediments from the swamp contained contaminated food resources for benthic fish. Thus, our microcosm exposure allowed us to evaluate how fish would respond when all possible contaminant exposure routes (water, sediment, and resources) were present. Because fish responded to ash more severely in microcosms than in our previous laboratory toxicity tests (Fig. 4), our current study suggests that field conditions are more hazardous than previously inferred from laboratory studies.

The most conspicuous differences between our laboratory and microcosm studies relate to concentrations of trace elements encountered by fish in the benthos. Sediments from ash microcosms had concentrations of trace elements that in most cases (e.g., As, Cd, Cr, and Se) greatly exceeded concentrations from weathered ash used in our previous laboratory studies, probably due to organic material (e.g., invertebrates and algae) present in the microcosms. For example, benthic invertebrates, an important food source of juvenile *E. sucetta*, isolated from the ash

microcosms had trace element concentrations up to 18 times higher than concentrations found in weathered ash alone (Hopkins et al., 2000, 2002, 2003). Thus, fish ingesting resources within the microcosms were exposed to concentrations of trace elements greatly exceeding the concentrations in weathered ash encountered in our previous laboratory work.

The high body burdens of contaminants in surviving fish from the ash microcosms reflected the high dietary concentrations of trace elements encountered in the contaminated benthos. Cadmium and selenium, two elements associated with ash that are of great toxicological concern, were an order of magnitude higher in tissues of fish exposed to contaminated resources than in fish exposed to weathered ash alone (Fig. 5). In fact, elements such as Cd, Cs, and Co were elevated in fish exposed in microcosms, but were not accumulated by fish in the laboratory. Thus, exposure to microcosm conditions increased both the quantity and diversity of contaminants accumulated by fish exposed to ash. Such differences between studies are likely the result of increased trophic exposure in the microcosms, but in some cases may also relate to changes in bioavailability due to contaminant speciation. Because the ash-contaminated benthos in the microcosms contained plant and animal material that was not present in weathered ash, the microcosms likely contained higher levels of organic elemental species (e.g., seleno-amino acids and proteins) which in some cases can be more readily incorporated into fish tissues than inorganic forms (e.g., selenate or selenite).

Other factors could contribute to differences in responses of fish between our laboratory and microcosm studies. For example, because fish gills are an important site of uptake of some trace elements (Dallinger et al., 1987), differences in contaminant accumulation between studies could relate to levels of dissolved contaminants. Water used in the microcosm ash treatment was collected from the contaminated site and had elevated levels of several trace elements, whereas the laboratory studies relied on continuously filtered artificial softwater with lower concentrations of trace elements (Hopkins et al., 2000, 2002, 2003; Hopkins, 2001). Elements such as Cd can be accumulated across the gill and were elevated in the water column of the ash microcosms. However for most elements, water concentrations were so low compared to sediments that uptake from water was probably less important than exposure to the benthos (Table 1). Taken together, it is unlikely that dissolved trace element concentrations alone could explain such substantial accumulation of elements in fish from ash microcosms, particularly for elements that were not detectable in the water column.

Differences in fish responses between laboratory and microcosm studies could also relate to the quantity of resources encountered by fish while grazing surface sediments. As expected based upon previous field studies (Cairns et al., 1970; Cherry et al., 1979a,b; Forbes and Magnuson, 1980; Forbes et al., 1981; Hatcher et al., 1992), abundance of benthic invertebrates was reduced in the ash treatments compared to controls at initiation of the study. In addition, percent organic content in sediment from ash microcosms was less than half of that in control microcosms, a finding consistent with field studies that documented reduced establishment of organic material on submerged sampling plates in the coal ash-contaminated site (Newman et al., 1985; Rowe et al., 2001) and potential smothering effects of ash on benthic communities (Cherry et al., 1979a). Because juvenile *E. sucetta* consume small benthic invertebrates (Ewers and Boesel, 1935; Carlander, 1969; Shireman et al., 1978; Becke, 1983) as well as algae and detrital material associated with the benthos (Shireman et al., 1978; Becke, 1983), differences in benthic food resources may have contributed to differences in growth and survival between sediment treatments. Indeed, we previously demonstrated in the laboratory that effects of ash exposure on *E. sucetta* are exacerbated when food resources are reduced (Hopkins et al., 2002). However, supplemental food rations supplied to half of the fish in the microcosms did not attenuate the effects of ash, suggesting that enhanced trace element exposure may have been a more important factor than resource restrictions.

The experimental microcosms allowed us to capture some of the complexity of natural systems while simultaneously controlling certain environmental variables, but the results also suggest limitations of the approach. For example, mortality in the supplemented-control treatment, although not statistically different from mortality in the unsupplemented-control, would be unacceptable in laboratory bio-assays. Such mortality was not observed in our previous laboratory studies (Hopkins et al., 2000, 2002, 2003) and may be an unavoidable obstacle encountered in studies

performed under less-controlled, outdoor microcosm conditions. In situ field exposures would likely be even more problematic than microcosms because of problems associated with site-specific differences in predation rates. Covering the microcosms prevented colonization by insect predators (e.g., odonate nymphs and dytiscid beetle larvae) that might have consumed juvenile fish. However, because our microcosms were covered, potentially important prey species such as chironomids were also prevented from colonizing the microcosms. Consequently, oligochaetes and microcrustaceans comprised greater than 96% of invertebrates present in the benthos of the microcosms. Other researchers have acknowledged the benefits and limitations of using covered microcosms and have suggested combining laboratory, microcosm, and field experiments to aid in interpretation of results (Diamond, 1986; Hairston, 1989; Jaeger and Walls, 1989; Morin, 1989; Wilbur, 1989; Rowe and Dunson, 1994). Future studies using uncovered microcosms or in situ field enclosures would help determine whether benthic fish respond differently to ash in the presence of a more complex benthic community.

5. Conclusions

Our study illustrates the potential pitfalls associated with extrapolations of laboratory toxicity data to the more complex environments where organisms naturally encounter contaminants. Exposure of fish in outdoor microcosms suggested that ash is much more toxic to benthic fish than was previously postulated based upon laboratory toxicity tests, likely due to resource parameters that were not considered in the laboratory. Our results suggest that reduced food resource quality (i.e., high trace element content) appears to exacerbate the toxic effects of ash. Fish relying on contaminated resources (i.e., benthic invertebrates and other organic material) are exposed to much higher concentrations of contaminants, additional contaminants, and perhaps more bioavailable or toxic forms of contaminants than when exposed to weathered ash alone. Because resource limitations increase the toxicity of ash to benthic fish (Hopkins et al., 2002), reductions in benthic invertebrate biomass and organic content of surface sediments downstream from ash disposal facilities may be an additional factor mediating ash toxicity. Lower levels of resources could have important consequences for natural communities and competitive dynamics because fewer consumers could be supported. Taken together, our studies suggest that laboratory toxicity tests should be coupled with outdoor microcosm and/or field experiments to better understand how organisms interact with contaminants in more complex natural systems.

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References

Anderson, R.O., Gutreuter, S.J., 1984. Length, weight, and associated structural indices. In: Nielson, L.A., Johnson, D.L. (Eds.), Fisheries Techniques. American Fisheries Society, Bethesda, MD, USA.

Becke, G.C., 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison, WI, USA.

Boone, M.D., Semlitsch, R.D., 2001. Interactions of an insecticide with larval density and predation in experimental amphibian communities. Conserv. Biol. 15 (1), 228–238.

Bridges, C.M., Boone, M.D., 2003. The interactive effects of UV-B and insecticide exposure on tadpole survival, growth, and development. Biol. Conser. 113, 49–54.

Cairns Jr., J., Dickson, K.L., Crossman, J.S., 1970. The biological recovery of the Clinch River following a fly ash pond spill. In: Proceedings of the 25th Purdue Industrial Waste Conference.

Carlander, K.D., 1969. Handbook of Freshwater Fishery Biology. Iowa State University Press, Ames, Iowa, USA.

Cherry, D.S., Larrick, S.R., Guthrie, R.K., Davis, E.M., Sherberger, F.F., 1979a. Recovery of invertebrate and vertebrate populations in a coal ash-stressed drainage system. J. Fish. Res. Board. Can. 36, 1089–1096.

Cherry, D.S., Guthrie, R.K., Sherberger, F.F., Larrick, S.R., 1979b. The influence of coal ash and thermal discharges upon the distribution and bioaccumulation of aquatic invertebrates. Hydrobiologia 62, 257–267.

Dallinger, R., Prossi, F., Segner, H., Back, H., 1987. Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. 73: 91–98.

Diamond, J., 1986. Overview: laboratory experiments, field experiments, and natural experiments. In: Diamond J., Case, T. (Eds.), Community Ecology. Harper and Row Inc., New York, NY, USA.

Ewers, L.A., Boesel, M.W., 1935. The food of some Buckeye Lake fishes. Trans. Am. Fish Soc. 65, 57–70.

Forbes, A.M., Magnuson, J.J., 1980. Decomposition and microbial colonization of leaves in a stream modified by coal ash effluent. Hydrobiologia 76, 263–267.

Forbes, A.M., Magnuson, J.J., Harrell, D.M., 1981. Effects of habitat modifications from coal ash effluent on stream macroinvertebrates: a synthesis. In: Krumholz, L.A. (Ed.), Proceedings of the Warmwater Stream Symposium. Am. Fish Soc., Lawrence, KS, pp. 241–249.

Hairston, N.G., 1989. Hard choices in ecological experimentation. Herpetologica 45 (1), 119–122.

Hatcher, C.O., Ogawa, R.E., Poe, T.P., French, J.R.P., 1992. Trace elements in lake sediment, macrozoobentos, and fish near a coal ash disposal basin. J. Freshwater. Ecol. 7, 257–269.

Hopkins, W.A., 2001. Effects of coal combustion wastes on survival, physiology, and performance of the benthic feeding fish (*Erimyzon sucetta*). PhD dissertation. University of South Carolina.

Hopkins, W.A., Snodgrass, J., Roe, J.H., Jackson, B.P., Gariboldi, J.C., Congdon, J.D., 2000. Detrimental effects associated with trace element uptake in lake chubsuckers, *Erimyzon sucetta* exposed to polluted sediments. Arch. Environ. Toxicol. Chem. 39, 193–199.

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

Hopkins, W.A., Snodgrass, J.W., Staub, B.P., Jackson, B.P., Congdon, J.D., 2003. Altered swimming performance of benthic fish (*Erimyzon sucetta*) exposed to contaminated sediments. Arch. Environ. Contam. Toxicol. 44 (3), 383–389.

Jaeger, R.G., Walls, S.C., 1989. On salamander guilds and ecological methodology. Herpetologica 45 (1), 111–119.

Lemly, A.D., 1996. Selenium in aquatic organisms. In: Beyer, W.N., Heinz. G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, FL, USA, pp. 427–445.

Morin, P.J., 1989. New directions in amphibian community ecology. Herpetologica 45 (1), 124–128.

Newman, M.C., Alberts, J.J., Greenhun, V.A., 1985. Geochemical factors complicating the use of *aufwuchs* to monitor bioaccumulation of arsenic, cadmium, chromium, copper, and zinc. Water Res. 19, 1157–1165.

Resetarits, W.J., Fauth, J.E., 1998. From cattle tanks to Carolina bays: the utility of model systems for understanding natural communities. In: Resetarits, W.J., Bernardo, J. (Eds.), Experimental Ecology: Issues and Perspectives. Oxford University Press, Oxford, UK, pp. 133–151.

Rowe, C.L., Dunson, W.A., 1994. The value of simulated pond communities in mesocosms for studies of amphibian ecology and ecotoxicology. J. Herpetol. 28 (3), 346–356.

Rowe, C.L., Hopkins, W.A., Coffman, V.R., 2001. Failed recruitment of southern toads (*Bufo terrestris*) in a trace element-contaminated breeding habitat:direct and indirect effects that may lead to a local population sink. Arch. Environ. Contam. Toxicol. 40, 399–405.

Rowe, C.L., Hopkins, W.A., Congdon, J.D., 2002. Ecotoxicological implications of aquatic disposal of coal combustion residues. Environ. Monitor. Assess. 80, 207–276.

Sadinski, W.J., Dunson, W.A., 1992. A multilevel study of effects of low pH on amphibians of temporary ponds. J. Herpetol. 26 (4), 413–422.

Shireman, J.V., Stetler, R.L., Colle, D.E., 1978. Possible use of the lake chubsucker as a baitfish. Progres. Fish Cultur. 40 (1), 33–34.

Wilbur, H.M., 1989. In defense of tanks. Herpetologica 45 (1), 122–123.