

Effects of food ration on survival and sublethal responses of lake chubsuckers (*Erimyzon sucetta*) exposed to coal combustion wastes

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Abstract

Study organisms in chronic toxicological bioassays are often provided with excessive resources to remove food limitations as a confounding experimental variable. Under more ecologically realistic situations, resources are often less abundant and such restrictions may alter the responses of organisms to environmental contaminants. Here, we investigated the interaction between resource level and sediment toxicity in the lake chubsucker, *Erimyzon sucetta*. For 78 days we fed fish one of three ration levels (1X, 2X, 4X; uncontaminated food) that was grazed directly from either clean sand or coal ash-contaminated sediments. Despite provision of uncontaminated food, fish exposed to the contaminated sediments accumulated significant whole body concentrations of As, Se, Sr, and V. Food ration affected the pattern of Se accumulation, with lowest concentrations accumulated by fish supplied with the lowest rations (1X). Paradoxically, fish in the 1X-ash treatment were most adversely effected by ash-exposure, despite having Se burdens much lower than fish in the 2X- and 4X-ash treatments. Fish in the 1X-ash treatment exhibited higher mortality, lower proportional growth, and increased incidence of fin erosion compared to fish provided with higher rations. Such results may, in part, be explained by the apparent inability of fish with reduced rations to maintain positive energy balance, as evidenced by their higher standard metabolic rates compared to control fish fed similar rations. Our results underscore the importance of considering resource quantity and nutritional factors in chronic bioassays in order to draw more ecologically realistic conclusions about contaminant effects. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Coal combustion wastes; Food ration; Selenium; Growth; Metabolic rate

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1. Introduction

Environmental contaminants affect organisms not only by direct toxicity, but also via indirect effects on the organisms' environment. By altering environmental parameters such as resource availability, vegetative cover, thermal characteristics, and competitor and predator densities, contaminants may affect life history characteristics of organisms and ultimately produce population-level changes (Congdon et al., 2001). Thus, ecotoxicological studies should not be limited to examining the direct toxic action of anthropogenic substances, but should also consider how pollutant-induced changes in community structure and physical habitat affect study organisms (e.g., Boone and Semlitsch, 2001).

Resource abundance is an environmental variable frequently affected by xenobiotics, yet often unconsidered in toxicity testing. Many toxicity tests rely on feeding study organisms ad libitum during laboratory manipulations to remove food limitation as a confounding environmental factor. Clearly, rigorous standardization of conditions (e.g., resource abundance, temperature, etc.) across experimental treatments is required for direct toxic effects to be discernible from effects associated with non-toxicological variables. However, resources are often limited in natural systems and may even be reduced in contaminated habitats. Thus, excessive resource provisions in bioassays are often ecologically unrealistic and potentially overshadow the 'real world' consequences of contaminant exposure. Indeed, several laboratory toxicity tests, focussing primarily on invertebrates, suggest that nutritional or energy deficits resulting from resource limitations may increase an organism's sensitivity to pollutants (Jimenez et al., 1987; Segner, 1987; Wiederholm et al., 1987; Chandini, 1988, 1989; Bridges et al., 1997).

The current study was designed to evaluate the relationship between resource availability and responses of fish to pollutant exposure. A recent study in our laboratory indicated that benthic-feeding fish, lake chubsuckers (*Erimyzon sucetta*), supplied with ample resources exhibit a variety of sublethal effects when exposed to coal combustion

wastes (hereafter ash), but do not exhibit significant reductions in survival (Hopkins et al., 2000). Because field studies indicate that ash-discharge reduces invertebrate abundance and diversity (Cherry et al., 1979a,b; Magnuson et al., 1980; Hatcher et al., 1992), we hypothesized that under more ecologically realistic conditions (i.e., limited food resources) benthic fish exposed to ash would face more complex physiological challenges compared to our previous laboratory experiment. Here, we exposed *E. sucetta* to ash at three food ration levels and compared their survival and sublethal responses to control fish fed the same rations. We predicted that as food ration decreased, fish would exhibit a transition from sublethal to lethal responses to ash-exposure.

2. Materials and methods

2.1. Animal capture

Lake chubsuckers (mean mass = 2.76 ± 0.07 g; mean standard length = 53.56 ± 0.45 mm) were collected using minnow traps in September 1999. The collection site was a historically unpolluted Carolina bay (Bay No. 100) located on the Savannah River Site, SC. After transport to the laboratory, fish were allowed to acclimate to laboratory conditions for approx. 3 weeks. During the acclimation period, fish were held in 72 l tanks containing artificial softwater (US Environmental Protection Agency, 1991) at 25 °C and were fed Tetramin fish flakes ad libitum.

2.2. Experimental design

The bottoms of 60 glass tanks (38 l), equipped with carbon filters, heaters, and aeration, were covered with approx. 1 cm of substrate. Half of the tanks received sediment collected from the effluent outflow at the coal ash-polluted site (ash) and the remaining 30 tanks received clean sand (control; Flame sanitized sand, W.R. Bonsal Co., Charlotte, NC). Each tank also contained a $10 \times 10 \times 2$ cm clay refugia, which had no bottom so that fish using them maintained exposure to sediments.

Within each sediment treatment, fish were assigned to one of three food levels ($N = 10$ /food level; hereafter food levels referred to as 1X, 2X, and 4X). Fish assigned to the 1X food level were fed weekly rations of 4.62% of their initial mean body mass (equivalent to 42.5 mg of Tetramin fish food 3 days/week). Fish assigned to the 2X and 4X food levels received weekly rations of 9.24 and 18.48% of their initial mean mass, respectively (equivalent to 85.0 and 170.0 mg of Tetramin fish food 3 days/week). Based on our previous research where we provided abundant weekly rations to fish (44.03% of initial mean fish body mass; Hopkins et al., 2000), we consider the three low food levels chosen for the current study to represent conditions ranging from moderate to severe food limitations. Every 2 weeks, rations of Tetramin in each treatment were increased by 10% of the initial ration in order to accommodate fish growth. Because *E. sucetta* graze ground fish food from surface sediments, they ingest sediments while feeding.

In the laboratory, tanks were arranged in a randomized block design (five blocks containing two replicates of each sediment \times food treatment) and one fish was randomly assigned to each tank. Each fish was measured to the nearest 0.01 mm (standard length; see methods below) and weighed to the nearest 0.001 g prior to tank assignment. To ensure that water quality conditions remained similar among sediment and food treatments, we measured water temperature, dissolved oxygen, and pH (in a randomly selected subset of three tanks/treatment) at weekly intervals during the study.

2.3. Growth, metabolic rates, and survivorship

Tanks were inspected 4–5 days per week to document food consumption and mortality. After depositing food with a pipette directly onto the substrate, food consumption could be monitored because brightly colored food was easily detected against the contrasting and uniform background of each sediment type. On day 0, 16, 30, 44, 65, and 78 of the experiment each fish was weighed (nearest 0.001 g) and standard length of each fish (nearest 0.01 mm) was measured using Mor-

phosys[®] visual imaging analysis software according to methods described by Heulett et al. (1995). A dorsal image of each fish was taken with a camera and measured using Morphosys[®]. At every measurement interval, each fish was measured three times and the mean of the measurements was used for that fish.

Standard metabolic rates (SMR; 25 °C) were estimated for fish on day 0, 45, and 78 of the experiment. Standard metabolic rates were estimated by measuring oxygen consumption using a closed-circuit, computer-controlled, indirect respirometer (Micro-oxymax, Columbus Instruments, Columbus, OH). Prior to oxygen consumption measurements, each fish was placed in a perforated 600-ml holding vessel suspended within each individual's tank. The vessels enabled each fish to remain within its respective tank without having access to food or sediment. After 48 h, each post-absorptive fish was placed in an 1100-ml respiratory chamber containing 500 ml of artificial softwater (25 °C) and randomly assigned to a channel on the respirometer. In addition, one channel on the respirometer was connected to a 8.4 V battery (Procell Zinc Air Medical Battery, DA146, Duracell, Bethel, CT) that 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-hour intervals for 24 h. Standard metabolic rate of each fish was estimated as the mean of the lowest 50% of oxygen consumption measurements. Because SMR is the metabolic rate of a post-absorptive ectotherm at rest, removal of the highest 50% of oxygen consumption values reduces the influence of unobserved periods of activity on our estimates of SMR (Rowe et al., 1998; Hopkins et al., 1999, 2000).

2.4. Lipid extraction and ICP-MS

After the final respiratory and growth measurements (78 days) each surviving fish was frozen for lipid and trace element analysis. In addition, 10 fish from the collection site were frozen at the initiation of the study and were analyzed with experimental fish for lipids and trace elements.

Prior to analysis, each fish was lyophilized, homogenized, and 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, five sediment samples from the site of ash collection and five sand samples were lyophilized and homogenized in preparation for trace element analysis.

Sediment and fish subsamples were digested and analyzed for trace element concentrations according to the following procedures. Approx. 150 or 250 mg of sample (fish and sediment, respectively) was used for digestion. Nitric acid (2.5 or 5.0 ml, fish 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, 0.5 or 1.0 ml of H₂O₂ (fish and sediment, respectively) were added to the samples and microwaved at the same power and duration as the HNO₃ digestion. After digestion, samples were brought to a final volume of 10–25 ml (fish and sediment, respectively) with deionized water. Trace element analysis was performed by ICP–MS (Perkin Elmer, Norwalk, CT) on samples diluted 1:1 with deionized 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 certified reference materials ranged from 93.9 to 121.9%. Mean variability in percent recoveries of certified reference materials between digestion sets ranged from 2.43 to 5.54%.

Total nonpolar lipid (NPL) content of each fish subsample was determined using petroleum ether extractions. Previous validation experiments confirmed that a subsample of each homogenized fish accurately represents whole body lipid concentration (Hopkins et al., 2000). 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. All mass measurements were ac-

quired using a balance housed in a dry box at 1–2% relative humidity. The quantity of NPL was calculated by subtracting the dry post-extraction sample mass from the dry pre-extraction sample mass.

2.5. Data analyses

Ash and control sediments were not compared statistically because most elements in control sediments were below instrumentation detection limits. 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 two-way analysis of variance (ANOVA) with sediment type and food level as main effects in the model. Because multiple trace elements were measured in the same fish 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 relationships among survival, sediment type, and food levels. A series of Fisher's exact tests was also used to determine the effect of sediment and food level on frequency of fin erosion. Fish growth rates were compared between treatments using two-way ANOVA, with sediment type and food level as main effects in the model. To investigate the effects of sediment type and food levels on fish condition at the end of the study, we used a two-way analysis of covariance (ANCOVA) with standard length as a dependent variable and mass as a covariate. Mass and length were both log-transformed prior to analysis. Our comparisons using two-way ANCOVA are analogous to traditional comparisons of power functions between food treatments (Ricker, 1979). For illustrative purposes, we also 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).

A full factorial two-way ANOVA was performed on untransformed data to test for effects of sediment type, food level, and their potential

interaction on percent nonpolar lipid in fish. Mass was not included as a covariate because correlation analysis indicated that there was not a strong relationship between lipid and dry mass within treatments (in all cases $P \geq 0.09$). In addition, a Ryan Joiner test of normality indicated that no data transformation was necessary to meet assumptions of normality.

We investigated effects of sediment type and food level on SMR using repeated measures two-way ANOVA on the residuals obtained from regression analysis of mass and SMR of fish in each treatment. Because there were limits to the number of animals that could be analyzed on the respirometer at each time interval, several animals could not be analyzed during the 0 and 45 day respiratory trials (10 and 2% of fish were not analyzed, respectively). No more than one fish per sediment type-food level combination was missing from either respiratory trial. Because repeated-measures ANOVA requires no missing values, we estimated missing values as the mean of the values for that sediment type-food level combination.

3. Results

3.1. Water chemistry and trace element concentrations

Water chemistry parameters remained similar among the six treatment groups throughout the

study. Mean water temperature in experimental tanks ranged from 24.9–25.4 °C in ash tanks and 25.1–25.2 °C in reference tanks during the study. Likewise, mean water pH ranged between 6.74 and 7.47 in the six treatment groups. Dissolved oxygen remained near saturation in all treatments (range: 8.18–8.33 mg/l).

Trace element concentrations were higher in sediment from ash tanks than in sand from reference tanks. Ash tanks also had higher sediment concentrations of trace elements, with the exception of Cd, compared to sediment from the site where fish were initially collected (Table 1). Fish grazing the contaminated sediments in ash tanks accumulated significant concentrations of As, Se, Sr, and V (Table 2; $F_{1,44} = 141.24$, $P < 0.001$; $F_{1,44} = 367.01$, $P < 0.001$; $F_{1,44} = 28.90$, $P < 0.001$; $F_{1,44} = 36.36$, $P < 0.001$, respectively), but did not accumulate Cd, Cr, or Cu (in all cases $P > 0.298$). In addition, there was a significant effect of food level on whole body concentrations of Se, Sr, and V ($F_{2,44} = 22.44$, $P < 0.001$; $F_{2,44} = 35.58$, $P < 0.001$; $F_{2,44} = 5.68$, $P = 0.006$, respectively). Specifically, fish provided with more abundant resources accumulated higher concentrations of Se and V, and lower concentrations of Sr. In the case of Se there was a significant interaction between food level and sediment type ($F_{2,44} = 16.15$, $P < 0.001$). The increase in Se accumulation associated with increased food levels was much greater among fish exposed to ash-contaminated sediments compared to controls.

Table 1

Trace element concentrations in sediments from the site where fish were initially collected and from experimental tanks

	As	Cd	Cr	Cu	Se	Sr	V
Collection site ^a	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	BDL	BDL	BDL	BDL	BDL	0.67 ± 0.03	BDL
Ash tanks	58.41 ± 2.99	0.11 ± 0.02	53.96 ± 5.04	69.12 ± 2.06	6.47 ± 0.27	272.90 ± 12.90	67.27 ± 3.63
Tetramin food ^a	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

Trace element concentrations for Tetramin fish food are also provided. All trace element concentrations are expressed as mean μg dry mass \pm SE. For all elements except Sr, concentrations in reference tanks were below detection limits (BDL). Mean sediment detection limits ($\mu\text{g/g}$ dry mass) for elements in control and ash tanks (respectively) are as follows, As: 0.408, 0.651; Cd: 0.049, 0.785; Cr: 1.040, 1.659; Cu: 0.484, 0.772; Se: 0.693, 1.105; Sr: 0.024, 0.038; V: 0.968, 1.543.

^a Element concentrations for Tetramin fish food and sediment from the fish collection site were originally published in Hopkins et al. (2000).

Table 2

Whole body trace element concentrations of lake chubsuckers (*Erimyzon sucetta*) before (collection site) and after 78 days of exposure to either sand (control) or coal ash

	Collection site	Control sediment			Coal ash		
		1X	2X	4X	1X	2X	4X
As	0.24 ± 0.01	0.22 ± 0.01	0.26 ± 0.02	0.25 ± 0.01	0.44 ± 0.08	0.55 ± 0.04	0.51 ± 0.03
Cd	0.04 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Cr	1.3 ± 0.08	1.11 ± 0.11	1.38 ± 0.26	1.16 ± 0.12	1.12 ± 0.15	1.17 ± 0.12	1.11 ± 0.13
Cu	1.55 ± 0.40	2.30 ± 0.08	2.48 ± 0.16	2.41 ± 0.15	2.08 ± 0.11	2.91 ± 0.58	2.73 ± 0.21
Se	1.18 ± 0.06	1.12 ± 0.05	1.20 ± 0.03	1.21 ± 0.04	2.29 ± 0.22	6.55 ± 0.80	5.66 ± 0.54
Sr	247.00 ± 14.20	267.50 ± 12.60	171.40 ± 13.90	138.96 ± 6.87	313.50 ± 21.30	237.20 ± 13.30	195.80 ± 10.40
V	0.42 ± 0.08	0.48 ± 0.07	0.58 ± 0.09	0.83 ± 0.15	1.30 ± 0.67	2.13 ± 0.38	2.48 ± 0.40

Fish were maintained on three food levels (1X, 2X, and 4X) within their respective sediment type. All trace element concentrations are expressed as mean µg dry mass ± SE. Mean tissue detection limits (µg/g dry mass) for elements in fish are as follows, As: 0.093; Cd: 0.011; Cr: 0.236; Cu: 0.110; Se: 0.157; Sr: 0.005; V: 0.220.

3.2. Survival and sublethal effects

Effects of sediment on survival were dependent on food level (Fig. 1). Among fish exposed to ash sediments there was significant dependence ($P = 0.022$) of survival on food level; survival rates of fish were similar among the 2X- and 4X-ash treatments (90% survival), but were low (40%) at the 1X food level. In contrast, there was no significant difference in survival (90–100%) among food levels for fish exposed to control sediments ($P = 0.999$). There was also a significant dependence of survival on sediment type within the 1X food level ($P = 0.029$), but no dependence within 2X and 4X food levels was found ($P \geq 0.50$ in both cases; Fig. 1).

Patterns of change in fish mass throughout the study varied significantly among both food and sediment treatments ($F_{2,44} = 80.30$, $P < 0.001$ and $F_{1,44} = 5.69$, $P = 0.022$, respectively). The different patterns of mass change throughout the experiment are best illustrated when the effects of sediment type and food level on growth rate over the entire duration of the experiment are considered (Fig. 2). The percent difference in growth rate between fish exposed to ash and control sediments increased as food level decreased, indicating that growth differences between lower food treatments were greater than higher food treatments on a proportional basis (Fig. 2). Such a finding is not the result of a significant interaction between food

level and sediment type ($F_{2,44} = 1.00$, $P = 0.377$), but is attributed to the fact that proportional differences in growth between sediment types is most pronounced in smaller fish due to their smaller size.

Changes in fish condition during the experiment were influenced most by food levels, while the effects of ash exposure may have been obscured by differential survival among treatment combinations. Mass was significantly ($P = 0.027$) corre-

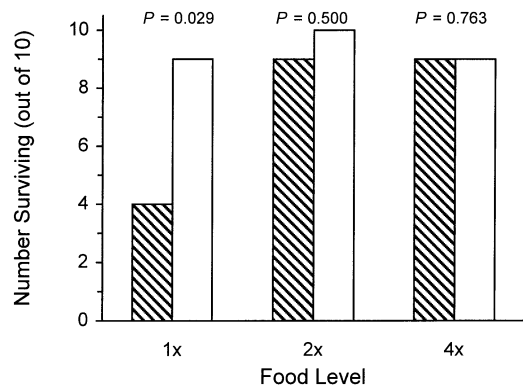


Fig. 1. Number of lake chubsuckers (*Erimyzon sucetta*) surviving until the end of 78 day exposure to either coal ash-contaminated sediments (ash; shaded bars) or sand (control; open bars) on three ration levels (1X, 2X, and 4X). Error bars represent ± 1 SE. P values refer to results of comparisons Fisher's exact tests between sediment types within each food level.

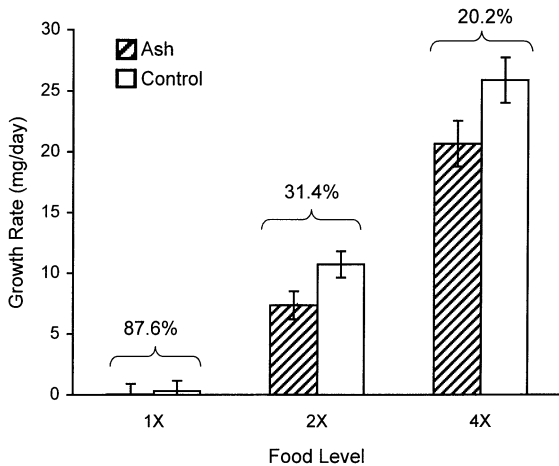


Fig. 2. Growth rate (mg/day) in lake chubsuckers (*Erimyzon sucetta*) after a 78 day exposure to either coal ash-contaminated sediments (ash) or sand (control) on three ration levels (1X, 2X, and 4X). Percentages above bracketed pairs indicate percent difference between sediment types within each food level. Error bars represent ± 1 SE.

lated with length of fish and was also significantly influenced by food level and the interaction between food level and fish standard length ($P = 0.014$ and 0.013 , respectively). Although fish in the 1X-ash treatment have higher final condition factors than 1X-control fish (Fig. 3), many fish with low condition factors in the ash treatment died while individuals with higher condition fac-

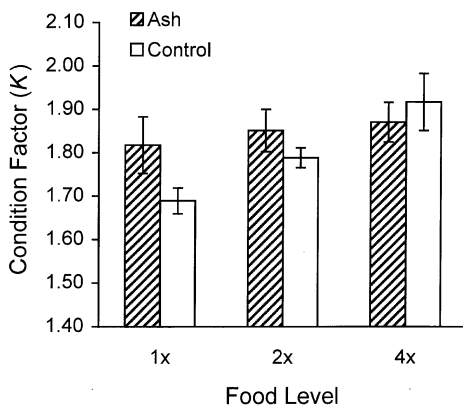


Fig. 3. Condition factor in lake chubsuckers (*Erimyzon sucetta*) after a 78 day exposure to either coal ash-contaminated sediments (ash) or sand (control) on three ration levels (1X, 2X, and 4X). Error bars represent ± 1 SE.

tors survived (Fig. 4). In contrast, fish in the 1X-control treatment had low condition factors but survived to the end of the study (Fig. 4). In addition, there was significant effect of sediment type ($P < 0.001$), but no effect of food level ($P = 0.620$), on fin erosion at the end of the study. Of the surviving fish in the 1X, 2X, and 4X ash treatments, 75, 78, and 44%, respectively, experienced erosion of the pectoral and caudal fins, whereas no reference fish experienced fin erosion. In some instances, the caudal fins of ash-exposed fish eroded to the hyperel plate.

Fish SMRs varied significantly through time among sediment type and food level treatment combinations as indicated by the significant time \times sediment \times food level interaction term in repeated measures ANOVA (Table 3). Fish SMRs were positively correlated ($R^2 = 0.39$, $P < 0.001$) with fish mass when all SMR measures were combined. Inspection of a plot of residuals against predicted values indicated a good fit of the linear model. In comparison to day 0, mass corrected SMRs remained similar or increased as the study progressed among ash-exposed fish at all three ration levels and among control fish at the 4X and 2X food levels (Fig. 5). In contrast, SMRs of control fish at the 1X food level decreased by the end of the experiment (Fig. 5).

Percent NPL was not significantly affected by sediment or the sediment by food interaction ($F_{1,44} = 0.01$, $P = 0.941$ and $F_{2,44} = 0.07$, $P = 0.933$, respectively), but was significantly influenced by food level ($F_{2,44} = 22.32$, $P < 0.001$; Fig. 6).

4. Discussion

4.1. Trace element uptake

The current study demonstrates that resource availability can affect trace element accumulation by benthic fish grazing contaminated sediments. Despite provision of uncontaminated food, fish exposed to contaminated sediments accumulated significant concentrations of As, Se, Sr, and V. In addition, three of the accumulated elements (i.e., Se, Sr, and V) were influenced by food level.

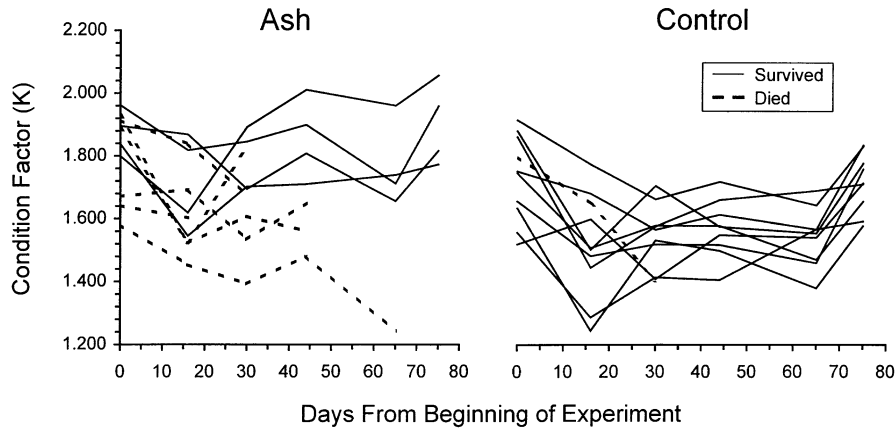


Fig. 4. Change in condition factor in lake chubsuckers (*Erimyzon sucetta*) after a 78 day exposure to either coal ash-contaminated sediments (ash) or sand (control) on the lowest food level (1X). Note that fish in the ash treatment with low condition factor tend to die during the study, whereas fish in the control treatment with low condition factor survive. Because only fish with high condition factor survive in the ash treatment, mean condition factor in ash treated fish is actually higher than control fish at termination of the study.

Regardless of sediment type, fish fed the least had the highest concentrations of Sr, implying either a growth-influenced dilution in fish provided with greater rations or discrimination of Sr in favor of Ca derived from the Tetramin fish food (Whicker and Schultz, 1982). In contrast, V concentrations in fish generally increased in both sediment treatments with resource level, indicating increased accumulation from ingested food or sediments. More importantly, the quantity of uncontaminated resources provided to fish greatly influenced Se accumulation from contaminated sediments. Fish in the 1X-ash treatment had Se burdens 60–65% lower than fish exposed at the two higher resource levels. Such a pattern of accumulation possibly reflects the manner in which Se, a sulfur analog (Lemly, 1998; O'Toole and Raisbeck, 1998), is deposited in proteinaceous tissues. Food-deprived fish may utilize available liver proteins for energy and only replace such protein reserves when resource availability increases (Collins and Anderson, 1995). Because fish in the 1X-ash treatment were clearly energy-limited and grew very little, they probably produced little Se-enriched liver or structural proteins during the study.

Of the four elements accumulated by *E. sucetta* exposed to ash, Se is of special interest due to its well-documented toxicity in fish. Selenium levels

in *E. sucetta* in the 2X- and 4X-ash treatments were comparable to concentrations previously documented in this species (Hopkins et al., 2000), and are above the chronic toxicity thresholds recommended by Lemly (1996). Whole body concentrations of Se less than those found in fish from the 2X- and 4X-ash treatments induce numerous responses in other fish species 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). Paradoxically, fish provided with the lowest rations (1X) had body burdens 50% lower than Lemly's (1996) toxicity criteria, yet this treatment group exhibited the most pronounced responses to the toxic sediments (see following Discussion). Thus, tissue burdens of Se alone, which are common criteria for assessing risk in ash-contaminated sites, may not accurately predict adverse effects under conditions of reduced resource availability.

4.2. Survival and sublethal effects

Decreased resource availability not only affected contaminant uptake, but also strongly influenced the toxicity of coal combustion wastes in

Table 3

Results of two-way repeated measures ANOVA of the effects of sediment type and food level on change in metabolic rate during the experiment

Source	DF	SS	MS	F	P
<i>Among fish</i>					
Sediment	1	0.00164	0.00164	0.06	0.808
Food level	2	0.86799	0.43399	15.84	<0.001
Sediment X food level	2	0.01141	0.00570	0.21	0.813
Error (fish within treatments)	44	1.20572	0.02740		
<i>Within fish</i>					
Time	2	0.41347	0.20674	16.33	<0.001
Time X sediment	2	0.05323	0.02662	2.10	0.128
Time X food level	4	0.31606	0.07901	6.24	< 0.001
Time X sediment X food level	4	0.12725	0.03181	2.51	0.047
Error (time)	88	1.11375	0.01266		

E. sucetta. Ninety percent of fish exposed to ash survived in the 2X and 4X ration levels, but survival decreased to 40% when resources were reduced to the 1X level. Although the interaction between food level and sediment treatment did not have a significant effect on absolute differences in growth rate, proportional differences in growth were most pronounced at lower food levels. As food ration levels decreased, the percent difference in growth rate between ash-treated and control fish increased. The percent difference in growth rate between sediment treatments was four times greater at the lowest food ration level than at the highest food level. In addition, signs of overall poor health appeared in ash-exposed fish at the lower ration levels. Fish exposed to ash at the lowest food levels showed more symptoms of disease (e.g., red sores) and higher incidence of fin erosion than fish in the 4X-ash treatment.

Resource availability may influence the toxicity of sediments via a variety of mechanisms. Decreased resource quantity or quality can lead to nutritional deficiencies altering the condition of study organisms, potentially influencing their sensitivity to contaminants (Bridges et al., 1997; Ankley and Blazer, 1988). Our results lend support to the idea that physiological condition influences sediment toxicity. Fish provided with 1X rations had lower lipid levels than fish maintained on higher rations and mortality in the 1X-ash treatment was often preceded by low condition factor. Toxicity may have increased in the lowest

food level treatment because weekly rations were inadequate to offset daily metabolic expenditures. Mass-specific metabolic rates increased slightly in most fish after initiation of the study, a response that we have previously observed when comparing

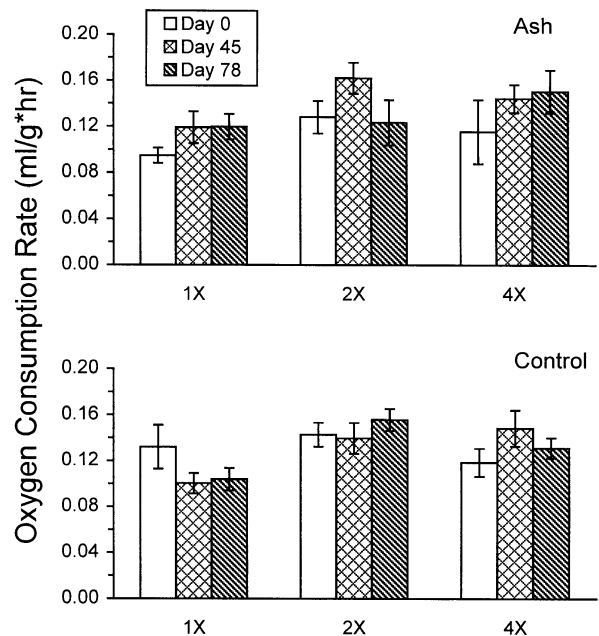


Fig. 5. Changes in standard metabolic rate (25 °C; ml oxygen/g × hour) of lake chubsuckers (*Erimyzon sucetta*) after a 78 day exposure to either coal ash-contaminated sediments (ash) or sand (control) on three ration levels (1X, 2X, and 4X). Error bars represent ± 1 SE.

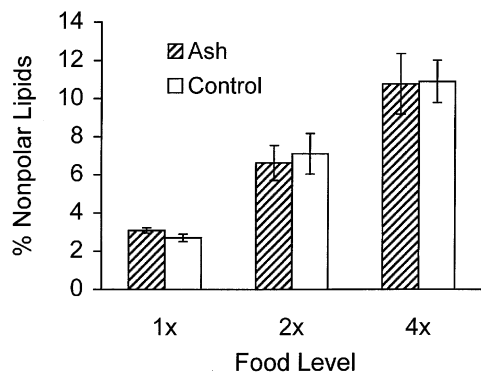


Fig. 6. Percent nonpolar lipids in lake chubsuckers (*Erimyzon sucetta*) after a 78 day exposure to either coal ash-contaminated sediments (ash) or sand (control) on three ration levels (1X, 2X, and 4X). Error bars represent ± 1 SE.

recently field-captured animals to conspecifics fed laboratory rations (e.g., Hopkins et al., 2000). In contrast to these observations, SMR of fish in the 1X-control treatment decreased as the experiment progressed. Starving organisms commonly display similar metabolic responses, presumably to conserve energy during periods of energy deficit (Newell, 1973; Jobling, 1980; Regnault, 1981). Fish in the 1X-ash treatment failed to exhibit energy-saving decreases in metabolism, suggesting that fish in this treatment group may face increased energy requirements due to costs associated with detoxification and/or pollutant-induced tissue damage. Thus, fish fed low rations may eventually succumb to ash-exposure by failing to remain in positive energy balance.

Although several studies have examined responses of invertebrates to contaminants under conditions of reduced resources (Rao and Sarma, 1986; Wiederholm et al., 1987; Chandini 1988, 1989; Kluttgen and Ratte, 1994; Bridges et al., 1997), few investigations have examined the effects of resource level on trace element uptake and toxicity in fish (Segner and Storch, 1985; Segner, 1987; Collvin, 1985). Results from invertebrate studies generally support our findings; invertebrates exhibit decreased growth and survival (as well as reproductive endpoints) when exposed to contaminants and resource restrictions simultaneously. Similarly, the few available studies on fish indicate that sensitivity to trace elements of-

ten increases as food rations decrease. Food deprivation greatly increased accumulation of Cu and Fe in roach (*Rutilus rutilus*) and guppies (*Poecilia reticulata*), respectively, and even affected the ability of fish to eliminate Cu after exposure cessation (Segner and Storch, 1985; Segner, 1987). In addition, Fe contamination was found to greatly exaggerate starvation-induced hepatic damage (Segner and Storch, 1985). In contrast to these findings, weight reduction was not different between fed and unfed perch (*Perca fluviatilis*) exposed to Cu (Collvin, 1985). Future studies examining responses of fish to contaminants under nutritional restrictions will be useful in assessing the generality of their responses under such conditions.

4.3. Conclusions

Our results, in combination with the work of others (Bridges et al., 1997; Lanno et al., 1989), underscore the importance of considering ecological variables, such as resource abundance, when evaluating the toxicity of xenobiotics. Because the nutritional status of study organisms can clearly influence their response to toxicants, results from chronic bioassays can vary considerably based solely upon the physiological condition of experimental subjects. When performing chronic bioassays, there are tradeoffs between ecological realism and factors such as assay simplification, cost effectiveness, and rapidity of performance. Obviously, all ecological variables cannot be considered while simultaneously maintaining some degree of practicality. However, as awareness increases that chronic bioassays are often more informative than acute toxicity tests, variables such as quantity and quality of resource provisions become requisite considerations.

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