Assessment and Prediction of Effects of Selenium Exposure to Larval Razorback Sucker

Final Report

Recovery Implementation Program

Project 95/CAP-6 SE

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24 November 1999

Contribution 107, Larval Fish Laboratory, Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, CO 80523, U.S.A.

Acknowledgments

We are grateful to Pat Nelson (USFWS) for administrative and technical advice. Frank Pfeifer, Mike Baker, and Roger Hamman (USFWS) provided larval razorback sucker for this investigation. Mike Baker (USBR), Steve Brinkman (Colorado Division of Wildlife), Kathy Holley (USFWS), Barb Osmundson (USFWS), and Paul Von Guerard (USGS) provided comments that improved the content of the report. Kevin R. Bestgen (Larval Fish Laboratory) clarified aspects of statistical analysis. Jay Bundy (Larval Fish Laboratory) provided assistance in the laboratory. This study was funded by the Recovery Implementation Program for the Endangered Fish Species in the Upper Colorado River basin and the National Irrigation Water Quality Program. The Recovery Program is a joint effort of the U.S. Fish and Wildlife Service, U.S. Bureau of Reclamation, Western Area Power Administration, states of Colorado, Utah, and Wyoming, Upper Basin water users, environmental organizations, and the Colorado River Energy Distributors Association.

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Razorback sucker Selenium Dietary exposure Survival Growth

Executive Summary

Selenium is a metalloid that occurs in geologic formations and surface waters in the Colorado River basin. Human activities have increased selenium concentrations in surface waters. To investigate the potential for effects on larval razorback sucker, toxicity tests were conducted to quantify the relative importance of exposure to inorganic (dissolved) and organic (dietary) selenium. Effects of exposure were evaluated by culturing a three-trophic level food chain (algae, rotifer, razorback sucker) in a series of dissolved selenium concentrations (5.35, 8.23, 11.1, 16.5, and 27.2 µg/L) and exposing the fish to gradients of dietary and dissolved selenium. Measurement endpoints included survival and growth during the 28-day exposure period.

We did not observe changes in survival or growth of larval razorback sucker in response to food-chain exposure. Other investigators have suggested that exposure to dietary selenium concentrations greater than 3 µg/g dry weight will produce adverse effects in fish. In our study, the highest concentrations of selenium in rotifer were about half of this value (1.40 µg/g). This magnitude of bioaccumulation in food organisms was less than anticipated and is the most likely explanation for lack of adverse effects on razorback sucker. Because of low dietary exposure we were not able to estimate a relationship that describes adverse effects as a function of selenium concentration in diet. Results of this study do confirm that exposure to dietary selenium concentrations below 1.4 µg/g dry weight do not adversely effect survival and growth of larval razorback sucker.

A separate study was conducted to compare the relative sensitivity of razorback sucker and fathead minnow using standard 96-hour acute toxicity tests. Exposure concentrations in acute toxicity tests were over 1000 times higher than in the dietary study. The 96-hour median lethal concentrations and 95% confidence limits (in parentheses) for razorback sucker and fathead minnow exposed to dissolved selenium were 40.8 (37.0, 44.9) and 33.3 mg/L (29.8, 37.1), respectively.

Ongoing investigations will expand this research and allow evaluation of its predictive accuracy. The new data will be combined with findings of this report to provide a description of effects over a broad range of environmental and dietary concentrations that can be used to compare predicted and observed toxicity of water collected from potential razorback sucker nursery habitats in the Colorado River basin.

Introduction

Selenium is a metalloid that occurs in geologic formations and surface waters in the Colorado River basin (Stephens and Waddell 1998). It occurs in natural waters in inorganic and organic forms and is an essential micronutrient for biological organisms. Because of its role as an essential micronutrient, selenium is readily absorbed by biological organisms. When exposure to selenium is increased due to high environmental concentrations toxic effects may result. Toxicity in fish occurs because selenium replaces sulfur in amino acids which changes structure and function of synthesized proteins (Maier and Knight 1994; Lemly 1998). Organic forms of selenium like selenoamino acids are more bioavailable than inorganic forms, thus they are more toxic and bioaccumulate rapidly. For fish, the most important exposure pathway for organic selenium is through the diet.

Human activities have increased selenium concentrations in surface waters in the Colorado River basin. Humans have increased rates at which selenium is mobilized from geologic formations, and have created conditions that allow concentration of dissolved selenium in evaporation basins. Several objectives of the Recovery Implementation Program for Endangered Fish Species in the Upper Colorado River basin relate to restoration of nursery habitats for razorback sucker. Recently it was discovered that many of these nursery habitats have elevated selenium concentrations and concern exists about potential effects on larval razorback sucker that may reside in these habitats. To investigate the potential for effects on larval razorback sucker, toxicity tests were conducted to quantify the relative importance of

exposure to inorganic (dissolved) and organic (dietary) selenium. Effects of exposure were evaluated by culturing a three-trophic level food chain (algae, rotifer, razorback sucker) and exposing the fish to gradients of dietary and dissolved selenium. Measurement endpoints included survival and growth during the exposure period. Data were analyzed using regression to describe the response of survival and growth as functions of dissolved and dietary selenium concentrations. The relationships can be used to predict potential effects of selenium concentrations in razorback sucker nursery habitats.

A separate study was conducted to compare the relative sensitivity of razorback sucker and fathead minnow to dissolved selenium. Both species were studied simultaneously under similar exposure conditions using standard 96-hour acute toxicity tests. Consequently, direct comparison of species sensitivity can be made.

Materials and Methods

Experimental animals

Algae and rotifers

Monocultures of the freshwater algae *Chlorella vulgaris* (Carolina Biological Supply Company, Burlington, North Carolina) and the rotifer *Brachionus calyciflorus* (Florida Aqua Farms, Dade City, Florida) were maintained using prescribed methods (Hoff and Snell 1987). Both organisms were maintained in a series of 20-L batch cultures with five target exposure concentrations (0.0, 2.5, 5.0, 10.0, and 20.0 µg/L dissolved selenium). Each rotifer culture was fed algae from the corresponding selenium exposure concentration (e.g., rotifers in the 20.0 µg/L treatment were fed algae from the 20.0 µg/L treatment) two or three times daily. Abundance of rotifers in batch cultures was quantified daily by subsampling.

Razorback sucker

Razorback sucker larvae were obtained from the Grand Valley Propagation Facility (U.S. Fish and Wildlife Service, Colorado River Fishery Project, Grand Junction, Colorado) and transported to laboratory culture facilities at Colorado State University (Fort Collins, Colorado). Culture-facility water temperature was 19°C. Razorback sucker larvae were reared in mass cultures until approximately 75% of fish were observed feeding on live brine shrimp nauplii or rotifers (10 days after hatching). Then, randomly-selected fish were transferred to exposure beakers for acclimation to testing conditions. Larvae were approximately 12 days old (after

hatching) at the start of the dietary exposure study and 27 days old at the start of acute toxicity tests.

Fathead minnow

Larval fathead minnows were purchased from Aquatic BioSystems Inc. (Fort Collins, Colorado). Experimental animals were acclimated to test temperature and water quality for 48 hours before toxicity tests were initiated. Larvae were approximately 3 days old (after hatching) at the start of acute toxicity tests.

Experimental design and exposure system

Exposure procedures for the water-versus-dietary exposure study (henceforth "dietary study") were based on prescribed methods for conducting early life-stage toxicity tests with fishes (ASTM 1990a). Experimental treatments were assigned to replicate exposure beakers (n=4) using a randomized, balanced 5×2 factorial design with five target exposure concentrations (0.0, 2.5, 5.0, 10.0, and 20.0 µg/L) and control or selenium-enriched rotifer diet. This experimental design was equivalent to conducting two toxicity tests simultaneously. In one test, the larvae were exposed to five dissolved selenium concentrations and control diet. In the other, larvae were exposed to five dissolved selenium concentrations and food organisms cultured in the corresponding selenium concentration. Twenty larvae were assigned to each exposure beaker (experimental unit). Larvae were transferred from mass cultures to flow-through beakers about 24 h before the toxicant metering system was activated. A continuous-flow diluter was used to generate exposure concentrations. The diluter maintained a 0.5 dilution factor and supplied a volume of 34 ml/min to exposure beakers. Beakers were polyethylene vessels having a diameter

of 12 cm and height of 15 cm. Depth of test solutions was 9.5 cm. Cool-white fluorescent lamps were the only source of illumination (530 lx), and a 12:12-h light:dark photoperiod was maintained.

Larvae were fed an average ration of 886 control or selenium-enriched rotifers daily based on their respective experimental treatment. Survival of fish in each treatment was monitored daily. Growth was quantified at the end of the 28-d exposure period by determining the average blotted wet mass and average total length (TL) of fish that survived. Average mass was measured to 0.0001 g; TL to 0.1 mm.

To compare sensitivity of razorback sucker and fathead minnow to dissolved selenium, 96-hour renewal-acute toxicity tests were simultaneously conducted with each species using methods prescribed by the American Society for Testing and Materials (1990b). Experimental treatments were assigned to replicate beakers (n=4) using a randomized, balanced design with seven target exposure concentrations (0.0, 6.38, 12.8, 25.5, 51.0, 102, and 204 mg/L selenium). Fifteen larvae were randomized to each beaker (20 larvae per beaker were inadvertently used in the razorback sucker test). Range-finding and 96-hour renewal-acute tests were conducted using 1-L polyethylene beakers containing 0.50 L of exposure solutions. Depth of test solutions was 7.3 cm. Test solutions were renewed every 24 h. Cool-white fluorescent lamps were the only source of illumination (530 lx), and a 12:12-h light:dark photoperiod was maintained.

Larvae were offered live ≤ 24-h-old brine shrimp nauplii before and during toxicity tests. During a test, brine shrimp nauplii were introduced into exposure beakers three times daily and at least 2 hours before renewal. Survival was monitored at 6, 12, 24, 48, 72, and 96 h after start of the exposure period.

Physical and chemical conditions

Dilution water for the dietary study was supplied by a well on the Colorado State University campus. Well water was treated by vigorous aeration for approximately 8 h while being heated to a test temperature of 20 ± 1°C. Alkalinity, hardness, pH, and specific conductance were measured weekly; dissolved oxygen was measured daily; water temperature was measured continuously. Dilution-water characteristics had the following ranges: dissolved oxygen, 6.8 to 7.3 mg/L; alkalinity, 233 to 251 mg/L as $CaCO_3$; hardness, 358 to 378 mg/L as $CaCO_3$; pH, 7.7 to 8.2; specific conductance, 750 to 850 µS/cm; and temperature, 19 to 22°C (see Table A3 for other dissolved constituents).

The same dilution-water source was used for acute toxicity tests. Alkalinity, hardness, pH, and specific conductance were measured at the beginning and end of the exposure period; dissolved oxygen was measured daily; water temperature was measured continuously. Dilution-water characteristics during acute tests had the following ranges: dissolved oxygen, 5.9 to 7.2 mg/L; alkalinity, 242 to 250 mg/L as $CaCO_3$; hardness, 364 to 367 mg/L as $CaCO_3$; pH, 7.8 to 8.1; specific conductance, 750 to 830 µS/cm; and temperature, 20 to 21°C.

Toxicant solutions

A selenium stock solution was prepared by dissolving sodium selenate (Na_2SeO_4; Sigma Chemical Company, St. Louis, Missouri) in dilution water. For the dietary study, concentrations were prepared by delivering the stock solution to the diluter using a peristaltic pump. In renewal-acute tests, exposure concentrations were prepared by pipetting the desired amount of a

selenium stock into beakers containing 0.50 L dilution water. Test solutions were stirred and transferred to exposure beakers within 30 minutes of preparation.

Analytical procedures

Dissolved selenium concentrations in the dietary study were measured weekly (four occasions). Concentrations in renewal-acute tests were measured on one occasion at the beginning of the exposure period. Selenium concentrations nearest the median lethal concentration were also measured 24 h after renewal to quantify any change in bioavailability of dissolved selenium. Dissolved selenium concentrations in algae and rotifer batch cultures were measured on one occasion. Preparation of batch-culture medium required pasteurization at approximately 70°C for 1 hour to prevent contamination of cultures with undesirable algae or other biological organisms. To determine if pasteurization changed dissolved selenium concentrations, the highest and lowest concentrations were measured before and after the procedure was completed.

On each sampling occasion, three 250 ml samples were collected from each exposure concentration. Samples were obtained from different exposure chambers. Samples were placed in acid-washed polyethylene bottles, and held at 4°C until analyzed by Paragon Analytics, Inc. (Fort Collins, Colorado).

Selenium concentrations in algae, rotifers, and razorback sucker larvae were also determined. Duplicate samples of algae and rotifer were collected weekly (four occasions). Razorback sucker larvae were collected at the end of the dietary study. Samples were placed in acid-washed polyethylene vials, and held at -4°C until analyzed at analytical facilities at

Colorado State University (Department of Environmental Health, Fort Collins, Colorado). All tissue concentrations are based on dry-weight determinations.

Statistical analysis

The data generated by the dietary study and acute tests represent concentration-response relationships. For both data sets, it was of interest to determine if the observed concentration responses were influenced by the experimental treatments (i.e., dietary exposure or fish species). Consequently, regression analysis was used to estimate statistical models that describe each data set, and to determine if fitted lines had similar slopes and intercepts (Oris and Bailer 1997).

Survival data were analyzed using logistic regression. PROC GENMOD (with options LINK=LOGIT, DIST=BINOMIAL, DSCALE; SAS 1993) was used to describe the response of survival as a function of the independent variables. The full regression model had the form

$$\text{logit}\,(p) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2$$

where p = survival proportion, logit (p) = natural log $[p / (1 - p)]$, β_0 = intercept, β_1, β_2 = coefficients of linear terms, X_1 = dissolved selenium concentration, X_2 = dietary selenium concentration or fish species, and β_{12} = coefficient of cross products. The coefficient of cross products tests for equal slopes of the two regression lines. A significant coefficient suggests that the regression lines are not parallel and further analyses are not required to demonstrate that the concentration-response relationships are different from each other. A nonsignificant coefficient suggests that the lines are parallel and may or may not have similar intercepts. In this case, additional analyses are required. Since the coefficient of cross products is not significant, it is omitted from the statistical model and the analysis is re-run. Interpretation of the reduced model

is straight forward: coefficient β_1 represents a test for effects due to exposure to dissolved selenium; β_2 represents a test for effects due to exposure to dietary selenium or fish species.

Analysis of fish growth during the 28-day dietary study was conducted using a similar methodology. PROC GENMOD (with options LINK=IDENTITY, DIST=NORMAL; SAS 1993) was used to describe the response of fish mass and total length as a function of dissolved and dietary selenium exposure.

Estimates of 96-hour median lethal concentrations for razorback sucker and fathead minnow in acute studies were obtained using PROC PROBIT (SAS 1990). In all cases, transformations ($\log_{10}$) were used if they improved the fit of regression models. Graphical analyses of data and residual plots were conducted to confirm that regression models were appropriate and to evaluate compliance with statistical assumptions.

Results

Toxicant solutions and analytical procedures

Exposure and tissue concentrations were adjusted for recovery of selenium in spiked samples (water: 106%, SE = 1.36; algae: 90.0%, SE=8.61; rotifer: 77.5%, SE= 5.80; fish: 87.7%, SE=11.9). Mean and standard error measured concentrations in exposure water, algae, rotifer, and fish are presented in Tables 1-3. Chemical analysis revealed that the well water used for controls contained traces of selenium at an average concentration of 5.35 (SE=0.153) μg/L. The well water was used as dilution water for all experimental treatments, consequently the selenium that it contained increased target exposure concentrations by approximately 5.35 μg/L. To account for the additional selenium, all data and analysis are presented based on measured concentrations. Previous analysis of the well water over several years did not detect selenium. Analyses conducted during this study suggest that selenium levels in the water are relatively constant and that previous analyses were either in error or did not have sufficient detection limits to quantify existing levels.

Measured selenium concentrations before and after pasteurization in the lowest and highest water exposure concentrations were 8.60 (SE=0.306) and 7.6 (SE=0.665), and 26.7 (SE=0.333) and 27.7 (SE=0.667), respectively. The average selenium concentration was about 4% higher after pasteurization. This relatively small increase was probably a result of analytical error during measurement of selenium concentrations and shows that pasteurization did not reduce toxicant concentrations.

Table 1. Summary of responses of razorback sucker exposed to dissolved and dietary selenium for 28 days. Twenty animals per replicate, four replicates per exposure concentration. Concentrations are mean (standard error) measured on four occasions ($n = 4$).

Measured selenium concentrations					
Water (µg/L)	Diet (µg/g)	Replicate	Number surviving	Average mass (g)	Average TL (mm)
5.35 (0.153)[a]	0.349 (0.0377)	a	18	.00558	11.6
5.35	0.349	b	18	.00565	11.6
5.35	0.349	c	19	.00495	11.3
5.35	0.349	d	19	.00518	11.4
5.35	0.349	a	20	.00514	11.3
5.35	0.349	b	20	.00548	11.7
5.35	0.349	c	20	.00553	11.6
5.35	0.349	d	20	.00567	11.8
8.23 (0.127)	0.349	a	16	.00639	12.0
8.23	0.349	b	18	.00537	11.3
8.23	0.349	c	20	.00514	11.3
8.23	0.349	d	20	.00542	11.7
8.23	0.474 (0.125)	a	16	.00553	11.6
8.23	0.474	b	18	.00550	11.6
8.23	0.474	c	19	.00517	11.3
8.23	0.474	d	19	.00528	11.5
11.1 (0.583)	0.349	a	16	.00693	12.2
11.1	0.349	b	18	.00568	11.7
11.1	0.349	c	19	.00532	11.4
11.1	0.349	d	19	.00632	11.8
11.1	0.627 (0.136)	a	18	.00547	11.4
11.1	0.627	b	18	.00558	11.5
11.1	0.627	c	19	.00528	11.4
11.1	0.627	d	20	.00491	11.3
16.5 (0.752)	0.349	a	18	.00552	11.7
16.5	0.349	b	18	.00555	11.8
16.5	0.349	c	18	.00566	11.6
16.5	0.349	d	19	.00596	11.8
16.5	1.19 (0.284)	a	18	.00597	11.5
16.5	1.19	b	19	.00532	11.4
16.5	1.19	c	19	.00589	11.7
16.5	1.19	d	19	.00601	11.6

Table continued on next page.

Table 1. Continued.

Measured selenium concentrations					
Water (µg/L)	Diet (µg/g)	Replicate	Number surviving	Average mass (g)	Average TL (mm)
27.2 (1.31)	0.349	a	17	.00565	11.55
27.2	0.349	b	18	.00534	11.46
27.2	0.349	c	19	.00557	11.62
27.2	0.349	d	20	.00486	11.56
27.2	1.40 (0.0852)	a	17	.00530	11.41
27.2	1.40	b	19	.00510	11.36
27.2	1.40	c	19	.00533	11.44
27.2	1.40	d	20	.00515	11.41

[a]Control.

Table 2. Summary of selenium concentrations in algae at respective water concentrations. Concentrations are mean (standard error) measured on four occasions ($n = 4$).

Water (μg/L)	Algae (μg/g)
5.35 (0.153)[a]	0.412 (0.0414)
8.23 (0.127)	0.433 (0.0468)
11.1 (0.583)	0.587 (0.0803)
16.5 (0.752)	1.16 (0.120)
27.2 (1.31)	1.46 (0.188)

[a]Control.

Table 3. Summary of mean whole-body tissue concentrations of larval razorback sucker exposed to dissolved and dietary selenium for 28 days. Whole-body concentrations are based on pooled samples from treatment replicates (n=2).

Water (µg/L)	Diet (µg/g)	Whole body (µg/g)
5.35 (0.153)[a]	0.349 (0.0377)	1.16 (0.394)
8.23 (0.127)	0.349	0.965 (0.065)
11.1 (0.583)	0.349	0.782 (0.118)
16.5 (0.752)	0.349	0.852 (0.130)
27.2 (1.31)	0.349	0.870[b]
8.23	0.474 (0.126)	0.692 (0.009)
11.1	0.627 (0.136)	0.700 (0.062)
16.5	1.19 (0.284)	0.893 (0.167)
27.2	1.40 (0.0852)	1.40 (0.250)

[a]Control.

[b]Concentration based on one sample due to missing data.

Dietary study

Changes in survival or growth of larval razorback sucker in response to selenium exposure were not detected over the concentration range studied (Table 4; Figure 1). Average survival across all exposure treatments was approximately 93%. Of the three endpoints investigated (survival, growth in mass, and growth in TL), only TL data showed a potential effect due to exposure to selenium. Statistical analysis of TL data did not detect a trend in size due to exposure ($p = 0.586$), but did suggest that fish in the dietary-exposure treatment were smaller compared to fish fed the control diet ($p = 0.0621$). This p-value is higher than the traditional value for statistical significance of $p = 0.05$, but it suggests that a subtle effect may have occurred over the concentration range investigated.

The magnitude of selenium bioaccumulation in algae and rotifer was less than expected. Concentrations in rotifer ranged from 0.0349 µg/g for the control to 1.40 µg/g for the 27.2 µg/L exposure culture (Table 3; Figure 2). Research by other investigators using similar algae and rotifer culture conditions showed that concentrations as high as about 14 µg/g could be expected in the highest dissolved selenium exposure culture (Dobbs et al. 1996).

Renewal-acute tests

Median lethal concentrations and 95% confidence limits (in parentheses) for razorback sucker and fathead minnow exposed to dissolved selenium were 40.8 (37.0, 44.9) and 33.3 mg/L (29.8, 37.1), respectively. Statistical analysis revealed that the concentration responses for the two species were parallel ($p = 0.555$; Table 5), but that intercepts of the regression lines were

Table 4. Estimates and significance probabilities for final regression models and their coefficients for razorback sucker exposed to dissolved and dietary selenium for 28 days.

Response and coefficients	Estimate	SE	*p*
	Survival (logit)		
Test of slopes			
Intercept	2.85	0.755	0.0002
Water concentration	-0.0257	0.0395	0.515
Diet concentration	-0.234	1.58	0.882
Water × diet concentration	0.0245	0.0696	0.725
Test of intercepts			
Intercept	2.61	0.333	0.0001
Water concentration	-0.0142	0.0219	0.518
Diet concentration	0.302	0.475	0.525
	Growth (g)		
Test of slopes			
Intercept	0.0053	0.0003	0.0001
Water concentration	0.0000	0.0000	0.500
Diet concentration	0.0006	0.0006	0.327
Water × diet concentration	0.0000	0.0000	0.255
Test of intercepts			
Intercept	0.0056	0.0001	0.0001
Water concentration	-0.0000	0.0000	0.667
Diet concentration	-0.0001	0.0002	0.753
	Growth (TL; mm)		
Test of slopes			
Intercept	11.6	0.146	0.0001
Water concentration	0.0035	0.0080	0.661
Diet concentration	-0.136	0.305	0.655
Water × diet concentration	0.0020	0.0133	0.881
Test of intercepts			
Intercept	11.6	0.0775	0.0001
Water concentration	0.0025	0.0046	0.586
Diet concentration	-0.179	0.0962	0.0621

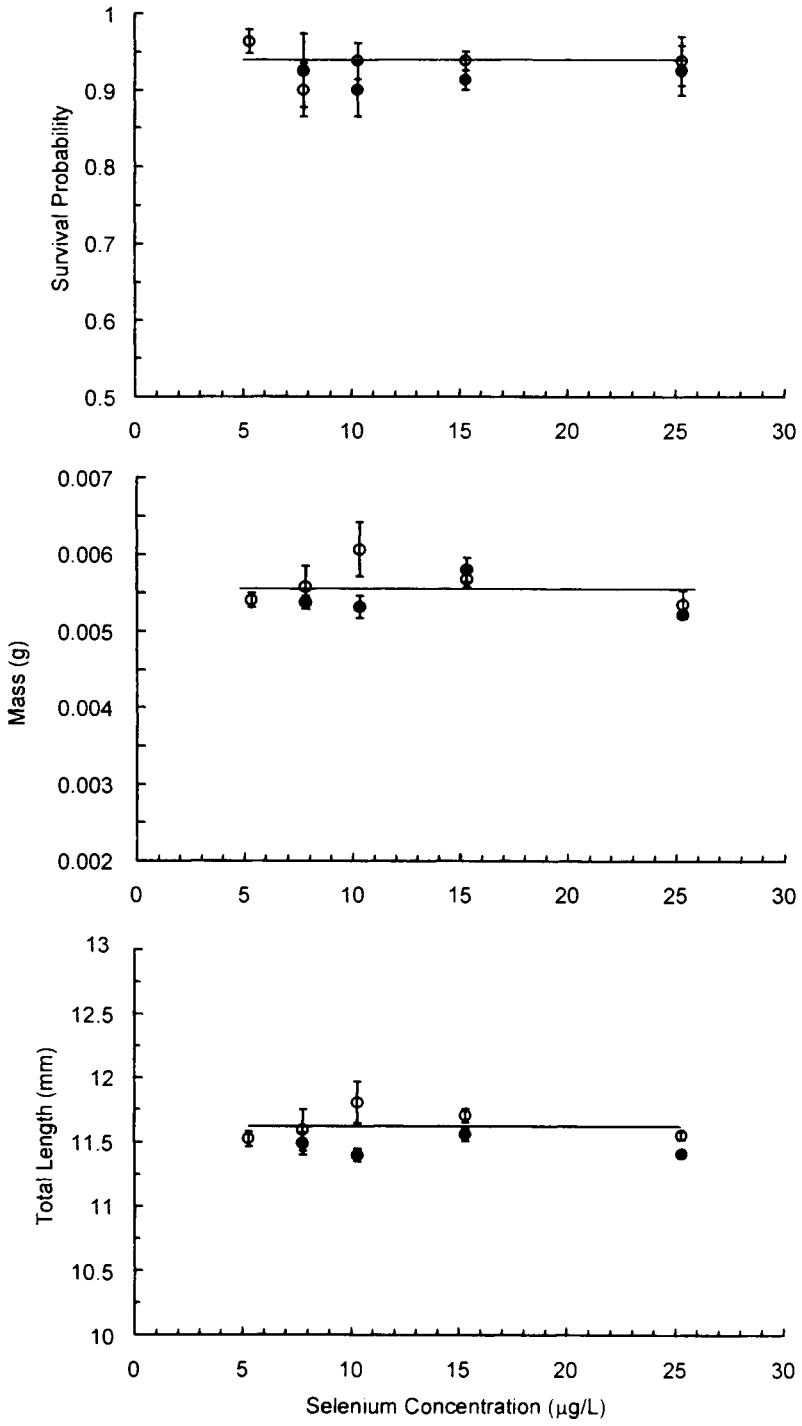

Figure 1. Plots of mean ± SE survival and growth as mass and TL as functions of dissolved selenium concentration. Open markers represent exposure to dissolved selenium and control diet, filled markers represent water and dietary exposure (n=4 for each marker). The horizontal line represents the average response of all treatments. No response as a function of selenium concentration was observed over the concentration range studied.

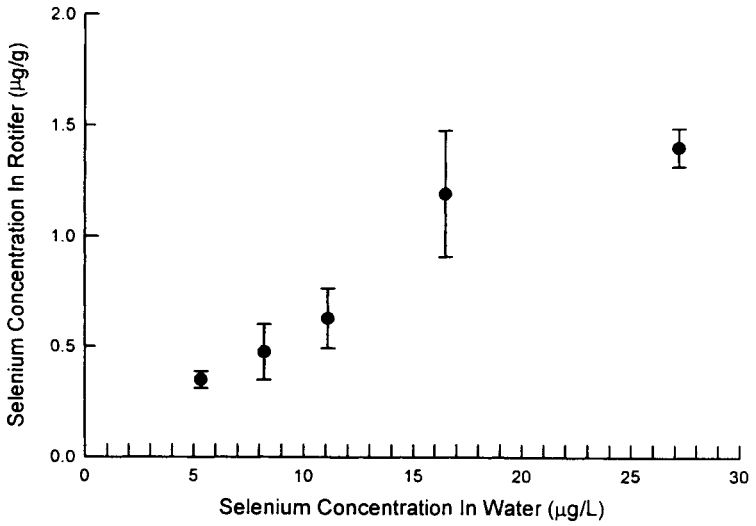

Figure 2. Plot of mean ± SE dry weight selenium concentration in rotifer as a function of dissolved selenium concentration (n=4 for each marker).

Table 5. Estimates and significance probabilities for final regression models and their coefficients for razorback sucker and fathead minnow exposed to dissolved selenium for 96 hours.

Response and coefficients	Estimate	SE	*p*
	Survival (logit)		
Test of slopes			
Intercept	-13.7	1.04	0.0001
Water concentration	8.53	0.639	0.0001
Species	-0.173	1.62	0.915
Water concentration × species	0.611	1.04	0.555
Test of intercepts			
Intercept	-14.1	0.818	0.0001
Water concentration	8.78	0.499	0.0001
Species	0.779	0.208	0.0002

different suggesting that fathead minnow were more sensitive to selenium than razorback sucker ($p = 0.0002$; Table 5). The regression equation describing probability of survival (p(survival)) of razorback sucker to dissolved selenium exposure (mg/L) has the form:

$$\text{logit} = -13.7 + 8.53 \cdot \log_{10}(\text{exposure concentration})$$

where $p(\text{survival}) = 1 - (e^{\text{logit}} / (1 + e^{\text{logit}}))$. The regression equation for fathead minnow has the form:

$$\text{logit} = -13.9 + 9.14 \cdot \log_{10}(\text{exposure concentration}).$$

These regression equations can be used to estimate the probability of survival after 96-hour exposure to any concentration of dissolved selenium (Se^{+6}).

Discussion

Dietary study

Predicted thresholds for toxic effects in fish from exposure to selenium are 2 µg/L in water and 3 µg/g in diet (Lemly 1993a; NIWQP 1998). In this study, the highest water and dietary concentrations were 27.2 µg/L and 1.40 µg/g, respectively. Adverse effects due to exposure to dissolved and dietary selenium were not observed over the concentration ranges studied in this investigation. The most likely explanation for this outcome is that the magnitude of bioaccumulation in the experimental food chain, and consequently, the level of dietary exposure was not sufficient to produce a response. The general design of our bioaccumulation food chain was based on Dobbs et al. (1996). They observed equilibrium bioconcentration factors (selenium concentration in whole organism divided by water concentration) in algae and rotifer of about 400 to 500. Bioconcentration factors in algae and rotifer in our investigation ranged from 51 to 77. This result suggests that we observed only a fraction of the potential bioaccumulation that can occur and explains why adverse effects were not observed even though dissolved selenium exposure concentrations were above the 2 µg/L threshold.

Guidelines that present threshold values for interpretation of biological effects of selenium are intended to be applied to natural systems where selenium may cycle between biological organisms and the physical environment. Cycling of selenium in a complex environment plays a major role in the potential for bioaccumulation (Lemly and Smith 1987). For example, in aquatic habitats associated with the Colorado River near Grand Junction,

Colorado, bioconcentration factors for zooplankton range from 267 to 5700 (B.C. Osmundson, pers. comm.). Our relatively simple food chain exposure system did not include many components of natural systems (e.g., sediment). However, the system was not intended to mimic all aspects of natural systems. It was intended to use a natural process to incorporate selenium into the diet of razorback sucker. The magnitude of bioaccumulation in diet of razorback sucker was less than anticipated. To account for this apparent difference in environmental cycling, we have modified exposure conditions in ongoing studies (see below) to prevent depletion of dissolved selenium in algae and rotifer cultures. However, the apparent sensitivity of bioaccumulation rates to cycling of selenium suggests that results of this and other investigations should be interpreted based on concentrations in tissues of organisms and not based on water concentrations.

Exposure to contaminants can reduce an organisms ability to obtain food, as well as increase its metabolic demand for energy (Beyers et al. 1999). Lemly (1993b) showed that selenium exposure increases metabolic stress in fish. The growth of fish in this investigation was relatively slow (from 10.6 to 11.5 mm TL in 28 days) suggesting that the quantity of food offered was smaller than the maximum ration that the fish could have consumed. Slow growth can be considered a weakness of this study, but it also suggests that fish had limited resources to offset effects of selenium exposure. Because energy for compensating effects of stress induced by selenium exposure was limited, this investigation may provide a conservative (worst-case) estimate of effects over the concentration ranges studied. Results of this study confirm that exposure to dietary selenium concentrations below 1.4 µg/g dry weight do not adversely effect survival and growth of larval razorback sucker.

Renewal-acute tests

Acute exposure to dissolved selenium showed that razorback sucker were slightly less sensitive to the contaminant than fathead minnow, but that responses of the fishes were similar. The ratio of median lethal concentrations for razorback sucker (40.8 mg/L) and fathead minnow (33.3 mg/L) is about 1.2. Ratios between 0.5 and 1.5 are considered within the range of normal experimental variation (Mayer and Ellersieck 1986).

Other investigators have compared sensitivity of razorback sucker and fathead minnow. Finger et al. (1995) conducted studies that exposed both species to water from localities in the Green River basin receiving irrigation drainage. They concluded that sensitivity of razorback sucker was similar to that of fathead minnow. Dwyer et al. (1995) exposed razorback sucker and fathead minnow to a variety of contaminants and concluded that the two species were "generally similar". These consistent conclusions produced by different investigators using different sources of selenium, different contaminants, and different techniques represent strong evidence that razorback sucker and fathead minnow are approximately equal in sensitivity to dissolved selenium.

Age of razorback sucker and fathead minnow used in renewal-acute tests were not the same (27 and 3 days after hatching, respectively). We intended to start acute toxicity tests coincident with the occasion when approximately 75% of razorback sucker began feeding. This event occurred approximately 10 days after hatching. It was chosen because it coincides with the time when larval razorback sucker probably first enter backwater or flooded bottomland habitats and exposure to relatively high selenium concentrations begins. Conflicts with simultaneously starting dietary and acute studies resulted in postponement of the latter. In general, the older fish

become, the less sensitive their response to toxicants. Consequently, the estimated median lethal concentration for razorback sucker at 27 days after hatching may be slightly higher than the value at 10 days. This test will be repeated during future investigations to assess the influence of age on sensitivity of razorback sucker.

Conclusions

Description of adverse effects as functions of dietary exposure were not possible because toxic selenium concentrations in diet were not achieved. However, results do confirm that exposure to dietary selenium below 1.4 µg/g dry weight do not adversely effect survival and growth of larval razorback sucker. The 96-hour median lethal concentrations and 95% confidence limits for razorback sucker and fathead minnow exposed to dissolved selenium were 40.8 (37.0, 44.9) and 33.3 mg/L (29.8, 37.1), respectively.

Recommendations

No recommendations can be made at this time. Useful recommendations should be provided by ongoing investigations that will extend results of research presented in this report.

Ongoing investigations

Two ongoing investigations being conducted by the authors will provide data that will expand this research and allow evaluation of its predictive accuracy. One study entitled "*Evaluation of interspecific sensitivity to dietary selenium exposure: razorback sucker versus flannelmouth sucker*" will provide data that will extend the dissolved and dietary exposure

concentration ranges to levels expected to reduce survival and growth of razorback sucker. The new data will be combined with data in this report to provide a description of effects over a broad range of environmental and tissue concentrations. Resulting descriptive relationships will be evaluated using data from the other ongoing study entitled "*Selenium effects on larval razorback sucker: field verification of laboratory results*". This study will provide data that can be used to compare predicted and observed toxicity of water collected from potential razorback sucker nursery habitats along the Colorado River.

A shortcoming of this investigation was that bioaccumulation in the experimental food chain was lower than expected. The experimental food chain was based on a design presented by Dobbs et al. (1996) and used identical species of algae, rotifer, and type of selenium. The time required for exchange of algae growth media (turnover rate) in the system described by Dobbs et al. was 2 days. In our investigation, we attempted to maximize bioaccumulation by increasing residence time of algae and growth media using a longer exchange time of approximately 10 days. The slower exchange rate may have reduced bioavailability of selenium in cultures as a result of sedimentation. Selenium may have been retained in dead algae that accumulated over time on the bottom of culture vessels. Thus, dissolved selenium may not have been available for incorporation into living algae, rotifers and fish. In future investigations, exchange rate will be increased to approximately once every 3 days. This change combined with a broader range of exposure concentrations should produce dietary exposure concentrations that exceed predicted thresholds for toxic effects in fish.

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Appendix

Table A1. Summary of mortality of razorback sucker exposed to dissolved selenium for 96 hours. Twenty animals per replicate, four replicates per exposure concentration. Concentrations are mean (standard error) measured on one occasion from replicate exposure beakers (n = 3).

Measured selenium concentration (mg/L)	Replicate	Cumulative mortality per replicate			
		24 hours	48 hours	72 hours	96 hours
210[a]	a	20			
210	b	20			
210	c	20			
210	d	20			
107(3.33)	a	6	8	18	20
107	b	4	8	17	19
107	c	3	8	18	20
107	d	6	7	18	19
53.0[a]	a	0	1	10	17
53.0	b	0	2	13	14
53.0	c	0	1	8	13
53.0	d	0	0	6	14
27.0(.333)	a	0	0	1	3
27.0	b	0	0	2	4
27.0	c	0	0	3	4
27.0	d	0	0	0	2
13.0[a]	a	0	0	0	0
13.0	b	0	0	0	0
13.0	c	0	0	0	1
13.0	d	0	0	0	1
.00530(.000142)[b]	a	0	0	0	1
.00530	b	0	0	0	0
.00530	c	0	0	0	0
.00530	d	0	0	0	0

[a]Measured concentrations were identical, consequently standard error could not be estimated.
[b]Control.

Table A2. Summary of mortality of fathead minnow exposed to dissolved selenium for 96 hours. Fifteen animals per replicate, four replicates per exposure concentration. Concentrations are mean (standard error) measured on one occasion from replicate exposure beakers (n = 3).

Measured selenium concentration (mg/L)	Replicate	Cumulative mortality per replicate			
		24 hours	48 hours	72 hours	96 hours
107(3.33)	a	4	9	12	15
107	b	5	9	12	15
107	c	3	9	12	14
107	d	8	11	14	15
53.0[a]	a	1	4	10	13
53.0	b	2	4	8	12
53.0	c	0	4	7	11
53.0	d	0	2	9	15
27.0(.333)	a	0	0	2	5
27.0	b	0	0	1	5
27.0	c	0	0	1	5
27.0	d	0	0	1	6
13.0[a]	a	0	0	0	0
13.0	b	0	0	0	0
13.0	c	0	0	0	0
13.0	d	0	0	0	0
6.53(.0333)	a	0	0	0	0
6.53	b	0	0	0	0
6.53	c	0	0	0	0
6.53	d	0	0	0	0
.00530(.000142)[b]	a	0	0	0	0
.00530	b	0	0	1	1
.00530	c	0	0	0	0
.00530	d	0	0	0	0

[a]Measured concentrations were identical, consequently standard error could not be estimated.
[b]Control.

Table A3. Average concentration of dissolved constituents in well water. All values represent mg/L.

Al	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Se	Zn
<0.1	0.16	0.10	107	<0.005	0.01	<0.01	0.03	1.2	67.0	0.01	0.07	51.2	0.01	0.10	0.00535	0.01

Well water was tested for the following contaminants since October 1995 and all were below detection: arsenic, mercury, carbaryl, malathion, alpha-BHC, beta-BHC, delta-BHC, heptachlor, aldrin, heptachlor epoxide, endosulfan I, dieldrin, 4,4'-DDE, endrin, endosulfan II, 4,4'-DDD, endosulfan sulfate, 4,4'-DDT, methoxychlor, endrin ketone, chlordane, toxaphene, endrin aldehyde, isodrin, and hexachlorocyclopentadiene.