Evaluation of Interspecific Sensitivity to Selenium Exposure:

Larval Razorback Sucker Versus Flannelmouth Sucker

Final Report to:

Recovery Implementation Program

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Razorback sucker Flannelmouth sucker Selenium Dietary exposure

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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. Elevated environmental selenium concentrations have been shown to adversely affect fish populations in other systems. The objective of this investigation was to compare the relative sensitivity of razorback sucker (*Xyrauchen texanus*) and flannelmouth sucker (*Catostomus latipinnis*) to selenium exposure using early life-stage (ELS) toxicity tests. Species comparisons were made by exposing larval fish to gradients of selenate-contaminated water (<1, 25.4, 50.6, 98.9, and 190. µg/L) and food organisms (<0.702, 1.35, 2.02, 4.63, and 8.24 µg/g). Dietary exposure was accomplished by culturing food chains (algae, rotifer, and 41-d-old razorback or 11-d-old flannelmouth sucker) in the selenium gradient. Survival, growth, and whole-body selenium concentrations of larvae were measured at the end of a 28-d exposure period.

No negative effects on survival or growth were detected. Existing guidelines suggest that exposure to dietary selenium concentrations greater than 3 µg/g dry weight produce adverse effects in fish. In our study, the highest dietary exposure concentration in rotifer was 8.24 µg/g. Results are consistent with findings of other laboratory food-chain investigations with fathead minnow. Together, results of our food-chain investigations suggest that the threshold for adverse effects from dietary exposure is above 8.24 µg/g selenium for larval razorback and flannelmouth suckers.

A separate study was conducted to compare the relative sensitivity of razorback sucker, flannelmouth sucker, and fathead minnow to dissolved selenate using standard 96-hour acute toxicity tests. Median lethal concentrations and 95% confidence limits for razorback sucker, flannelmouth sucker, and fathead minnow exposed to dissolved selenium were 35.5 (32.3, 38.8), 32.5 (28.9, 35.1), and 21.8 mg/L (18.6, 25.2), respectively.

Results of this investigation do not support the hypothesis that larval razorback sucker are more sensitive to selenium exposure than flannelmouth sucker. Both species had similar responses to dietary selenium concentrations up to 8.24 µg/g, and to acutely toxic dissolved selenium concentrations. It should be noted that larval razorback sucker were 30 and 5 days

older than flannelmouth sucker in the ELS and acute tests, respectively. It is not certain how this age difference may have influenced relative sensitivity of the two species.

Lack of detection of adverse effects from exposure does not imply that razorback sucker populations are not affected by increased selenium concentrations. There are other life stages that may be especially sensitive to exposure. Recommendations for future research are presented.

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.

Human activities have increased rates at which selenium is dissolved and mobilized from soil and geologic formations. When selenium-contaminated water collects in aquatic habitats the potential exists for elevated concentrations to produce toxic effects in resident organisms. 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 (*Xyrauchen texanus*). Razorback sucker populations are presumed to have declined in the Upper Colorado River Basin from cumulative effects of loss of physical habitat and historical temperature regime, interactions with non-native fishes, and degraded water quality (Bestgen 1990; Minckley et al. 1991; Muth et al. 1998). Recently, the impacts of changing water quality have been a special concern because it was discovered that selenium concentrations in some razorback sucker nursery habitats are above the USEPA criterion for protection of freshwater aquatic life (5 µg/L; USEPA 1987) and elevated levels of selenium were measured in

larval and adult fish (Hamilton and Waddell 1994; Waddell and May 1995; Hamilton et al. 2000). The biological significance of these findings is uncertain because other co-occurring native fishes remain relatively abundant in the Upper Colorado River Basin. The apparent paradox about why razorback sucker populations have declined while co-occurring native fishes remain abundant has led to the hypothesis that razorback sucker may be relatively more sensitive to selenium exposure. The objective of this investigation was to compare the relative sensitivity of razorback sucker and flannelmouth sucker (*Catostomus latipinnis*) to selenium exposure using early life-stage (ELS) and acute toxicity tests. Flannelmouth sucker was selected for the comparison because it is a closely related, co-occurring, native fish that has life-history characteristics in common with razorback sucker. Species comparisons were made by exposing larval fish to gradients of selenium-contaminated water and food organisms. Dietary exposure was accomplished by culturing three-trophic level food chains (algae, rotifer, and razorback or flannelmouth sucker) in selenium-contaminated water. Survival, growth, and whole-body selenium concentrations of larvae were measured at the end of a 28-d exposure period.

A separate study was conducted to compare the relative sensitivity of razorback sucker and flannelmouth sucker to dissolved selenium. Fathead minnow (*Pimephales promelas*) were also tested to provide a basis for comparing results to other investigations. All species were studied under similar exposure conditions using 96-hour renewal-acute toxicity tests. Data from the ELS and acute studies were analyzed using regression analysis to describe the responses of survival and growth as functions of selenium concentration.

Materials and Methods

Dilution water

Two sources of dilution water were used. The source of dilution water for algae cultures, rotifer cultures, and acute toxicity tests was tap water (City of Fort Collins, Fort Collins, Colorado) treated by vigorous aeration for at least 24 h while being heated to 18°C (room temperature). The treatment process reduced total residual chlorine concentration to less than the detection limit of 0.02 mg/L. Water was adjusted using procedures for preparing "very hard" water (USEPA 1991) to approximate water-quality characteristics of the Colorado River near the Colorado-Utah state line (U.S. Geological Survey records; gage 09163500). Water used for culture of algae and rotifers was pasteurized at 70°C for 1 h to prevent contamination of cultures with undesirable biological organisms. Previous investigations have demonstrated that pasteurization does not influence selenium concentration or chemical composition of water treated with this procedure (Beyers and Sodergren 1999; Hoff and Snell 1989).

Water used for dissolved exposure of razorback and flannelmouth sucker in the 28-d exposure 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. Water from this source was known to contain dissolved selenium. However, it was concluded that the benefits of flow-through exposure using this source outweighed the disadvantages associated with trace selenium concentrations. Average selenium concentration of the water was 6.16 µg/L which does not cause reduced growth or survival of fish (Beyers and Sodergren 1999).

Experimental animals

Algae and rotifers

Monocultures of the freshwater algae *Chlorella vulgaris* (Carolina Biological Supply Company, Burlington, North Carolina) were cultured using methods of Hoff and Snell (1989). Algae were maintained in a series of batch cultures with five target exposure concentrations (0.0, 25, 50, 100, and 200 µg/L dissolved selenium). Algae were cultured in 4-L polyethylene bottles containing 3 L of exposure water. From 600 to 900 ml of the algae culture were removed each day for rotifer feeding. Consequently, the replacement rate of selenium in algae cultures ranged from once every 3.3 to 5 d.

Monocultures of the rotifer *Brachionus calyciflorus* (Florida Aqua Farms, Dade City, Florida) were also cultured in a series of batch cultures with five exposure concentrations using methods of Hoff and Snell (1989). Rotifers were cultured in 20-L polyethylene bottles containing 16 L of water. Each rotifer culture was fed algae from the corresponding selenium treatment (e.g., rotifers in the 200 µg/L treatment were fed algae from the 200 µg/L treatment) two or three times daily. Abundance of rotifers in batch cultures was quantified daily by subsampling. On average, about 9% of the rotifer population in a culture was removed each day for fish feeding. Replacement rate of exposure water in rotifer cultures was once every 2 d.

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) when they were approximately 2-days old (after hatching) 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 selected for testing. Fish in mass cultures were offered ≤ 24-h-old brine shrimp nauplii twice daily. Exposures were planned to begin when 75% or more of fish were observed feeding on live brine shrimp nauplii or rotifers (10-12 days after hatching). However, exposures were delayed until razorback sucker larvae were 41-days old because flannelmouth sucker larvae were not available until that time. Consequently, razorback sucker larvae were 41-days old (after hatching; mesolarva) at the start of the ELS test, and 16-days old (protolarva) at the start of the acute test.

Flannelmouth sucker

Fertilized flannelmouth sucker eggs 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). Mean selenium concentration in fertilized eggs was 4.06 µg/g dry weight. Flannelmouth sucker were hatched in a Heath incubator at 19°C, then reared in mass cultures until selected for testing. Fish in mass cultures were offered ≤ 24-h-old brine shrimp nauplii twice daily. Larvae were 11-days old (protolarva) at the start of the acute and ELS tests.

Fathead minnow

Larval fathead minnow 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 at the start of acute tests.

Experimental design and exposure system for ELS tests

Experimental treatments were assigned to replicate exposure beakers (n=4) using a randomized, balanced 5×2 factorial design. The first factor, selenium exposure, had five levels of dissolved and corresponding dietary concentrations (control, 25, 50, 100, and 200 µg/L). The second factor, species, had two levels (razorback sucker and flannelmouth sucker).

Exposure procedures were based on prescribed methods for conducting ELS toxicity tests with fishes (ASTM 1990a). Ten larvae were assigned to each exposure beaker (experimental unit). Beakers were polyethylene vessels having a diameter of 12 cm and height of 15 cm. Depth of test solutions was 9.5 cm. 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. Larvae were transferred from mass cultures to flow-through beakers about 24 h before the toxicant metering system was activated. Cool-white fluorescent lamps were the only source of illumination (530 lx), and a 12:12-h light:dark photoperiod was maintained.

Larvae in each beaker of each experimental treatment were offered the same daily ration of living rotifers. The average daily ration was equivalent to 914 rotifers per fish. 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.

Experimental design and exposure system for acute tests

To compare sensitivity of razorback sucker and flannelmouth sucker to dissolved selenium, 96-hour renewal-acute toxicity tests were conducted using prescribed methods (ASTM 1990b; USEPA 1991). Fathead minnow were also tested to provide a basis for comparing results to other investigations. Experimental treatments were assigned to replicate beakers (n=4) using a randomized, balanced 6×2 factorial design, as described above, with six target exposure concentrations (control, 10, 20, 40, 80, and 160 mg/L selenium). Fifteen larvae were randomly assigned to each beaker. 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 once daily 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

Water temperature in the ELS test was measured continuously during the exposure period. A test temperature of 20±1 °C was maintained using a water bath. Alkalinity, hardness,

pH, and specific conductance were measured weekly; dissolved oxygen was measured daily. Dilution-water characteristics had the following means and ranges: dissolved oxygen, 7.5 (7.2-7.9) mg/L; alkalinity, 263 (253-271) mg/L as $CaCO_3$; hardness, 400 (379-411) mg/L as $CaCO_3$; pH, 8.0 (8.0-8.1); specific conductance, 887 (850-910) µS/cm.

Alkalinity, hardness, pH, and specific conductance in acute tests were measured at the beginning and end of the exposure period; dissolved oxygen was measured daily; water temperature was measured continuously. A test temperature of 20±1 °C was maintained using a water bath. Dilution-water characteristics during acute tests had the following means and ranges: dissolved oxygen, 6.5 (4.8-7.3) mg/L; alkalinity, 215 (191-231) mg/L as $CaCO_3$; hardness, 354 (348-365) mg/L as $CaCO_3$; pH, 8.5 (8.4-8.6); specific conductance, 1070 (1050-1120) µS/cm.

Characteristics of water used for algae and rotifer culture were: alkalinity, 210 (191-231) mg/L as $CaCO_3$; hardness, 349 (335-365) mg/L as $CaCO_3$; pH, 8.5 (8.4-8.6); specific conductance, 1070 (1050-1120) µS/cm. Concentrations of major cations, anions, and other general water quality characteristics were also determined (Tables A1 and A2).

Toxicant solutions

A selenium stock solution was prepared by dissolving sodium selenate (Na_2SeO_4; Sigma Chemical Company, St. Louis, Missouri) in de-ionized water. For the ELS test, concentrations were prepared by delivering the stock solution to the diluter using a peristaltic pump. In 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 ELS test were measured weekly (four occasions). Concentrations in acute tests were measured on one occasion at the beginning of the exposure period. Dissolved selenium concentrations in algae and rotifer batch cultures were measured on one occasion. On each sampling occasion, three 250-ml samples were collected. Unfiltered samples were placed in acid-washed polyethylene bottles, acidified to pH < 2 with analytical-grade nitric acid, and held at 4°C until analyzed by Paragon Analytics, Inc. (Fort Collins, Colorado). Measured concentrations were adjusted for recovery of selenium in spiked samples (99.2%, SE = 1.65).

Selenium concentrations in algae, rotifer, razorback sucker, and flannelmouth sucker were also determined for the ELS test. Duplicate samples of algae and rotifer were collected weekly (four occasions). Fish within an exposure beaker were pooled and collected at the end of the exposure period. Samples were placed in acid-washed polyethylene vials, and held at -4°C until analyzed. Algae and rotifer samples were analyzed at Colorado State University (Department of Environmental Health, Fort Collins, Colorado). Fish larvae were analyzed at North Carolina State University (Nuclear Services, Department of Nuclear Engineering, Raleigh, North Carolina). All tissue concentrations are based on dry-weight determinations. Average water content of algae, rotifer, and fish was 70.5% (SE = 1.69, n = 10), 92.3% (SE = 0.474, n = 10), and 83.0% (SE = 0.0736, n = 5), respectively. Tissue concentrations were adjusted for

recovery of selenium in spiked samples (algae and rotifer: 86.0%, SE=3.65; fish: 100.0%, SE=0.413).

Statistical analysis

The data generated by the ELS and acute tests represent concentration-response relationships. For both data sets, it was of interest to determine if the observed responses were related to the experimental treatments (i.e., selenium 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

$$\text{logit}(p) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2$$

where p = survival proportion, logit (p) = natural log $[p / (1 - p)]$, β_0 = intercept, β_1, β_2 = coefficients for linear terms of main effects, x_1 = dissolved selenium concentration, $x_2 = 0$ for razorback sucker or 1 for flannelmouth sucker, and β_3 = coefficient for interaction of main effects. A nonsignificant coefficient for interaction suggests that the concentration-response relationships for both species are parallel and may or may not have similar intercepts. A nonsignificant interaction coefficient also suggests that including the term in the statistical model increases complexity, but does not explain additional variation in the dependent variable. Consequently, when the interaction coefficient was not significant, it was omitted from the statistical model and the analysis was re-run. Interpretation of the reduced model is straight

forward: coefficient β_1 represents a test for effects due to exposure to selenium; β_2 represents a test for effects from fish species.

Analysis of fish growth in the ELS test 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 selenium concentration and fish species. The full regression model had the form

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2$$

where y = fish mass (mg) or total length (mm) and other parameters and variables are the same as described above.

Estimates of 96-hour median lethal concentrations for razorback sucker, flannelmouth sucker, and fathead minnow in acute studies were obtained using Proc Probit (SAS 1990). 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

Selenium concentrations in algae, rotifer, and fish

Selenium concentrations in algae, rotifer, and fish increased with dissolved exposure (Table 1; Fig. 1). Algae concentrations ranged from <0.183 to 5.61 µg/g. Rotifer concentrations ranged from < 0.702 to 8.24 µg/g. Razorback sucker concentrations ranged from 3.33 to 12.9 µg/g and were generally higher than flannelmouth sucker, 3.43 to 10.2 µg/g.

Fish survival in ELS tests

Reduced survival of fish in response to selenium exposure was not detected over the concentration range and duration studied (Table 2; Fig. 2). Survival ranged from 95 to 100% for razorback sucker and 82.5 to 100% for flannelmouth sucker. Statistical analysis did not detect an effect from selenium exposure ($p = 0.4028$) but did detect a difference in survival between species ($p < 0.0001$; Table 3). The species difference in survival was due to lower overall survival of flannelmouth sucker in experimental treatments and was not a result of selenium exposure.

Fish growth in ELS tests

Analysis of growth in terms of fish mass at the end of the experiment showed that there was an effect from selenium exposure (Table 2; Fig. 2). Fish mass increased as a function of

Table 1. Summary of selenium concentrations in water and organisms (dry weight). Values are mean and standard error ($n = 4$).

Water (µg/L)	Algae (µg/g)	Rotifer (µg/g)	Razorback sucker (µg/g)	Flannelmouth sucker (µg/g)
6.12 (0.140)[a]	<0.183	<0.702	3.33 (0.298)	3.43 (0.487)
25.4 (0.479)	0.907 (0.00847)	1.35 (0.146)	5.25 (0.428)	3.91 (0.129)
50.6 (1.14)	1.57 (0.0730)	2.02 (0.186)	5.92 (0.294)	4.58 (0.178)
98.9 (0.875)	2.95 (0.182)	4.63 (0.623)	8.27 (0.346)	6.81 (0.525)
190 (3.04)	5.61 (0.762)	8.24 (0.511)	12.9 (0.327)	10.2 (.522)

[a]Control.

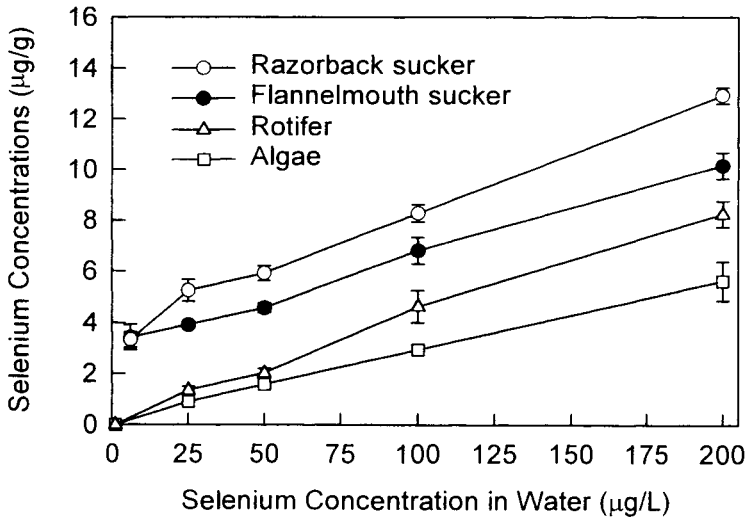

Figure 1. Selenium in algae, rotifer, larval razorback sucker, and flannelmouth sucker as functions of dissolved exposure concentration. Markers represent mean ± standard error ($n = 4$).

Table 2. Summary of responses of larval razorback sucker and flannelmouth sucker exposed to dissolved and dietary selenium for 28 days. Ten animals per replicate, four replicates per exposure concentration. Values are mean (standard error).

Selenium exposure		Survival (%)	Mass (mg)	Total length (mm)
Water (µg/L)	Diet (µg/g)			
Razorback sucker				
6.12[a]	<0.702	100.	12.0 (0.659)	15.1 (0.278)
25.4	1.35	100.	10.8 (0.410)	14.5 (0.0866)
50.6	2.02	97.5 (2.50)	12.2 (0.263)	15.0 (0.0750)
98.9	4.63	100.	12.1 (0.312)	14.9 (0.108)
190	8.24	95.0 (2.89)	12.4 (0.483)	15.0 (0.155)
Flannelmouth sucker				
6.12[a]	<0.702	97.5 (2.50)	16.6 (0.387)	17.0 (0.103)
25.4	1.35	85.0 (5.00)	15.4 (0.462)	16.9 (0.0946)
50.6	2.02	87.5 (7.50)	18.1 (0.588)	17.1 (0.180)
98.9	4.63	100.	17.7 (0.282)	17.0 (0.111)
190	8.24	82.5 (6.29)	18.4 (0.483)	17.2 (0.118)

[a]Control.

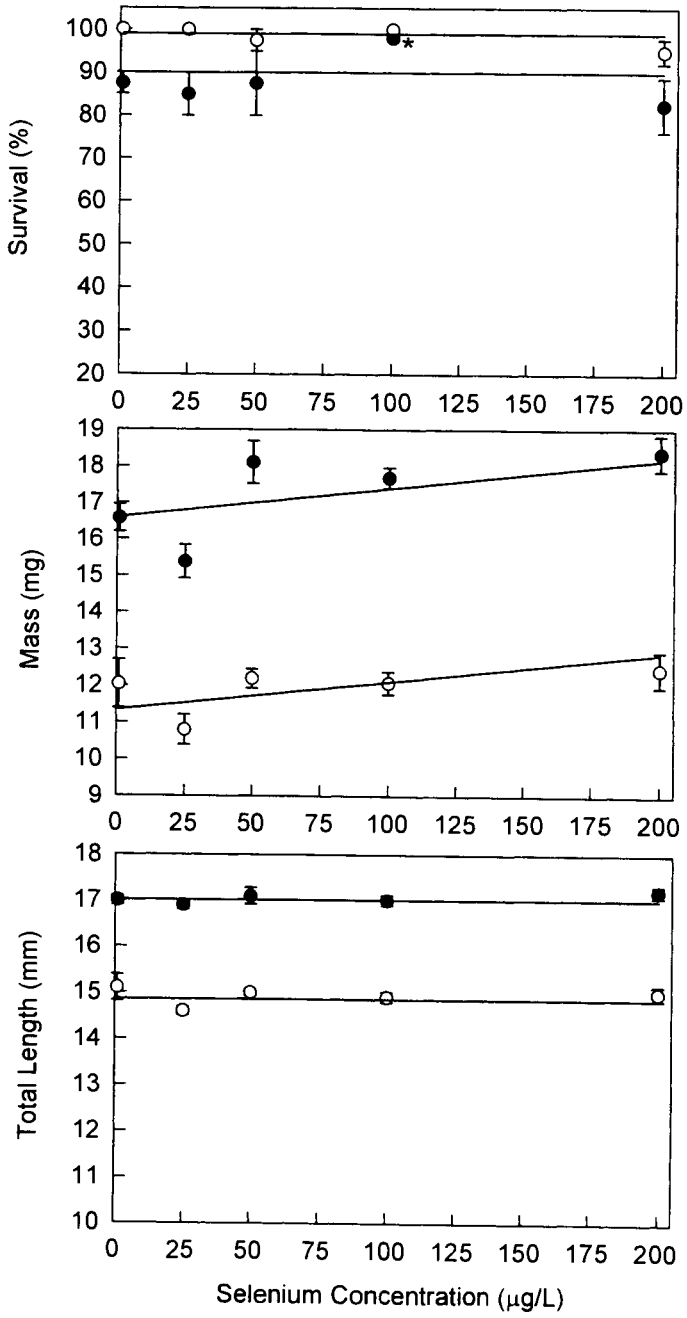

Figure 2. Survival and growth of larval razorback sucker (open markers) and flannelmouth sucker larvae (filled markers) after 28-day exposure to selenium in water and diet. Markers represent mean and standard error. Lines represent final regression models. There was no response of percent survival or total length as a function of selenium. *Marker offset to reveal overlapping data point.

Table 3. Maximum-likelihood significance probabilities for regression models describing the responses of larval razorback sucker and flannelmouth sucker exposed to dissolved and dietary selenium for 28 days.

Experimental treatment	df	Chi Square	*p*
		Survival (logit)	
Full model			
Concentration	1, 36[a]	2.78	<0.0954
Species	1, 36	13.9	<0.0002
Concentration × species	1, 36	2.24	0.1340[b]
Reduced model			
Concentration	1, 37	0.700	0.4028
Species	1, 37	17.3	<0.0001
		Growth (mg)	
Full model			
Concentration	1	10.0	0.0015
Species	1	51.5	<0.0001
Concentration × species	1	2.01	0.1560[b]
Reduced model			
Concentration	1	9.61	0.0019
Species	1	80.5	<0.0001
		Growth (TL)	
Full model			
Concentration	1	1.24	0.2644
Species	1	79.7	<0.0001
Concentration × species	1	0.359	0.5491[b]
Reduced model			
Concentration	1	1.23	0.2665
Species	1	108	<0.0001

[a]Numerator degrees of freedom, denominator degrees of freedom.

[b]No significant interaction between selenium exposure concentration and species. Consequently, this term was omitted from the statistical model and the analysis was re-run to obtain estimates for the reduced model.

selenium concentration ($p = 0.0019$; Tables 3 and 4). Statistical analysis showed that the slopes of concentration-response relationships of both species were similar (i.e., parallel; $p = 0.1560$), but razorback sucker were consistently smaller than flannelmouth sucker ($p < 0.0001$). This species effect was due to natural size differences of razorback sucker and flannelmouth sucker and was not a result of selenium exposure.

Analysis of growth in terms of total length did not detect an effect from selenium exposure ($p = 0.2665$). Statistical analysis showed that the concentration-response relationships for both species were similar ($p = 0.5491$), and that razorback sucker were consistently smaller than flannelmouth sucker ($p < 0.0001$).

Acute tests

Median lethal concentrations and 95% confidence limits (in parentheses) for razorback sucker, flannelmouth sucker, and fathead minnow exposed to dissolved selenium were 35.5 (32.3, 38.8), 32.5 (28.9, 35.1), and 21.8 mg/L (18.6, 25.2), respectively. Statistical analysis revealed that the concentration responses for razorback sucker versus flannelmouth sucker or fathead minnow were not parallel ($p = 0.0433$, $p = 0.0003$; Table5; Fig. 3). This result suggests that species responses to selenium exposure were statistically different, and that relative effects were not consistent over the concentration range. Inspection of Fig. 3 illustrates this point. The regression lines for flannelmouth sucker and fathead minnow cross the response for razorback sucker. Thus, comparing species sensitivity is complex because sensitivity rankings change with

Table 4. Parameter estimates for final regression models describing the responses of larval razorback sucker and flannelmouth sucker exposed to dissolved and dietary selenium for 28 days.

Parameter	Estimate	Standard error
	Survival (logit)	
β_0: Intercept	4.38	0.654
β_2: Species	-2.15	0.645
	Growth (mg)	
β_0: Intercept	11.3	0.292
β_1: Concentration	0.0077	0.0023
β_2: Species	5.31	0.330
	Growth (TL)	
β_0: Intercept	14.8	0.0803
β_2: Species	2.16	0.0907

Estimates for the regression equation $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2$ where x_1 = dissolved selenium concentration (µg/L) and $x_2 = 0$ for razorback sucker or $x_2 = 1$ for flannelmouth sucker.

Table 5. Maximum-likelihood significance probabilities for regression models of survival of larval razorback sucker, flannelmouth sucker, and fathead minnow exposed to dissolved selenium for 96 hours.

Experimental treatment	df	Chi Square	*p*
Razorback sucker versus flannelmouth sucker survival (logit)			
Concentration	1, 33[a]	644	<0.0001
Species	1, 33	3.43	0.0641
Concentration × species	1, 33	4.08	0.0433
Razorback sucker versus fathead minnow survival (logit)			
Concentration	1, 34	225	<0.0001
Species	1, 34	17.6	<0.0001
Concentration × species	1, 34	31.2	0.0003

[a]Numerator degrees of freedom, denominator degrees of freedom.

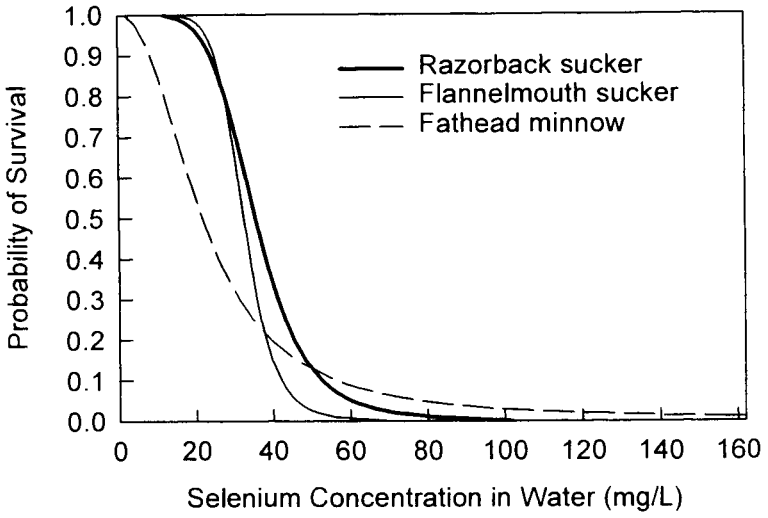

Figure 3. Probability of survival of larval razorback sucker, flannelmouth sucker, and fathead minnow exposed to dissolved selenium for 96 hours in a renewal-acute toxicity test.

concentration. Despite statistical differences, the relationships for razorback and flannelmouth sucker were similar, even overlapping over much of the concentration range. This result suggests that the observed statistical difference may not reflect a biologically important difference in sensitivity of razorback and flannelmouth sucker,

The difference between response relationships of razorback sucker and fathead minnow was larger than for flannelmouth sucker (Fig. 3). The data suggest that razorback sucker may be less sensitive than fathead minnow to dissolved selenium exposure, but a general conclusion about sensitivity cannot be made because the regression lines cross. The regression model for fathead minnow has a distinctly different shape than the other two relationships. This suggests that some other factor may have influenced the response or estimates for fathead minnow. Six of 60 fish in the control treatment for fathead minnow died during the exposure period (Table A7). Guidelines for conducting acute tests state that a test is unacceptable if there is more than 10% mortality in the control (ASTM 1990b). Our test did not violate this criterion, but the level of mortality that was observed was relatively high. In addition, the factor that was responsible for mortality in the control may have also been acting in other experimental treatments. Consequently, the fathead minnow data reported here should be used and interpreted with caution.

The regression equation describing probability of survival (p(survival)) of razorback sucker to dissolved selenium exposure (mg/L) has the form:

$$\text{logit} = -19.9 + 12.8 \cdot \log_{10}(\text{exposure concentration})$$

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

$$\text{logit} = -29.3 + 19.4 \cdot \log_{10} (\text{exposure concentration}).$$

The regression equation for fathead minnow has the form:

$$\text{logit} = -7.14 + 5.34 \cdot \log_{10} (\text{exposure concentration}).$$

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

Early life-stage test

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). These exposure thresholds were exceeded in the two highest experimental treatments. Water and dietary selenium concentrations in these treatments were 98.9 µg/L and 4.63 µg/g, and 190. µg/L and 8.24 µg/g, respectively, but corresponding reductions in survival or growth were not observed during the 28-d exposure period. Analysis of whole-body selenium concentrations confirmed that selenium was bioavailable and bioaccumulated in fish. Whole-body selenium concentrations exceeded the predicted threshold for toxic effects in fish (4 µg/g; Lemly 1993a; NIWQP 1998). Whole-body concentrations in razorback sucker ranged from 5.25 µg/g to 12.9 µg/g. Concentrations in flannelmouth sucker ranged from 4.58 µg/g to 10.2 µg/g. Selenium concentrations in flannelmouth sucker were consistently lower than razorback sucker. This was probably due to tissue dilution of whole-body concentrations because flannelmouth sucker larvae are naturally larger than razorback sucker (Snyder and Muth 1990). Tissue dilution has been observed in other selenium investigations (Bennett et al. 1986).

Results of this investigation are not consistent with predictions of established guidelines. Selenium in water and diet produced no effect or a slight positive effect. As exposure concentrations increased, both species generally attained greater mass than control fish. This effect could not have been due to different rations because rotifer abundance was quantified daily and fish in each treatment were offered an identical ration. Several explanations can be offered to account for the lack of adverse effects from exposure.

The first explanation relates to general characteristics of guidelines for toxic effects of selenium. Guidelines are generally intended to represent thresholds below which toxic effects will not be observed and may include a margin for error or safety factor. The magnitude of exposure above the threshold that is required to elicit a response will vary with environmental and biological conditions. Therefore, it is unrealistic to expect to always observe adverse effects at concentrations just above the threshold. A second characteristic of thresholds is that they are intended to protect all life stages of all fishes in all habitats. We investigated effects of exposure on one life stage, but two other important developmental stages exist (see below). These other stages may be more sensitive to selenium exposure and their responses may be more closely related to those predicted by general guidelines. In addition, cumulative effects resulting from exposure of more than one life stage may be an important consideration. Protecting the most sensitive component(s) of an organisms life cycle is critical for ensuring long-term persistence of reproducing populations. A third characteristic of thresholds is that they are dependent on type of selenium studied. The majority of laboratory investigations of selenium toxicity have used commercially refined forms of selenium like selenate, selenite, or selenomethionine. These investigations are the basis for much of the justification for predictive thresholds, but toxicity of these forms varies. Selenate, the species of selenium studied in this investigation, is generally considered to be least toxic, but it is the dominant form in natural waters of the Grand Valley, Colorado (Butler et al. 1994).

The second explanation for lack of adverse effects is related to the observed increase in fish wet mass, but not total length. This response may have been caused by selenium-induced edema in fish (Sorensen 1991). If it was caused by edema, then it represents an adverse effect.

However, Beyers and Sodergren (2000) used a similar exposure system and observed increased mass and total length in larval razorback sucker exposed to natural waters containing a mixture of selenium and other contaminants. Increased total length suggests that the observed effect was due to growth, not edema. In addition, edema is not a normal condition and there should be an energetic cost associated with its effects. Increased energetic cost from edema should cause a reduction in growth of fish. No reduction in total length was observed as selenium exposure increased. Therefore, it is unlikely that the observed effects resulted from selenium-induced edema.

A more likely explanation for the lack of adverse effects is that the thresholds for adverse effects were not achieved. A variety of data support this conclusion. The concentrations of dissolved selenium estimated to be lethal to 1% of razorback and flannelmouth sucker in 96 hours are 15.6 and 18.8 mg/L, respectively (based on equations presented in this report). These concentrations are at least 78 times higher than levels of exposure studied in the ELS test. Consequently, it is not surprising that mortality was not observed in the ELS test because dissolved concentrations were too low to induce the effect. Similarly, evidence suggest that the threshold for effects from dietary exposure are higher than the levels of exposure achieved in this investigation. Bennett et al. (1986) used a food-chain exposure system similar to the one in this investigation (algae, rotifer, fish) to investigate effects of 7-day exposure of larval fathead minnow to selenate. They observed reduced growth at 55µg/g the lowest concentration studied. Dobbs et al. (1996) also used a food-chain exposure system to investigate effects of 25-day exposure of fathead minnow to selenate. Dobbs et al. did not regulate the ration of rotifers offered to fish. Food was available to fish for the entire duration of the experiment in only two

treatments (control and 106.8 µg/L). Growth of fathead minnow was reduced in the 106.8 µg/L treatment which had corresponding dietary exposure that ranged from about 30 to 60 µg/g selenium. Ogle and Knight (1989) fed fathead minnow a prepared diet containing a mixture of sodium selenate, sodium selenite, and selenomethionine (50:25:25%). They observed reduced growth in the two highest exposure treatments, 20.3 and 29.5 µg/g. Lastly, Bertram and Brooks (1986) exposed fathead minnow to a range of dissolved and dietary selenate concentrations for 8 weeks. Growth effects were not detected in any of the dissolved and dietary exposures. The highest exposure concentrations studied were 40 µg/L in water and 7.32 µg/g selenium in the diet.

Our results are consistent with these other investigations. Together, results of all the investigations suggest that the threshold for adverse effects from dietary exposure is between the highest concentration studied in this investigation (8.24 µg/g) and the lowest observed effect concentration reported by Ogle and Knight (1989; 20.3 µg/g).

There was a difference in the age of razorback sucker and flannelmouth sucker larvae at the start of the ELS test: razorback sucker were 41-days old and flannelmouth sucker were 11-days old. This difference resulted from postponing the start of the test until flannelmouth sucker larvae were available. Postponement allowed simultaneous testing of razorback and flannelmouth sucker under identical exposure and dietary conditions. We advocate that simultaneous testing eliminated many strong sources of bias and that the resulting use of older fish is relatively unimportant. Several arguments support our contention that use of older fish was not a strong source of bias. First, it is generally believed that razorback sucker spawn in the main channel, not in nursery areas. Therefore, it is not ecologically realistic to begin an exposure

that mimics nursery-habitat conditions at the time of egg deposition or emergence from the egg. Second, dietary exposure of larvae cannot begin until the larvae start feeding. First feeding generally occurs at 10-12 days after hatching which also coincides with arrival of larvae in nursery habitats (Muth et al. 1998). Thus, the earliest that the ELS test could have started was about 10-12 days after hatching. Third, generalizations about age-related sensitivity usually are in reference to larval versus juvenile and adult life stages (Sprague 1985). Larval stages are often more sensitive than juvenile and adult stages, but sensitivity of larvae that differ in age is similar. Specific support for this contention is provided by Hamilton (1995) who reported that razorback sucker larvae ranging from 10 to 186 days old had similar sensitivity to dissolved selenium. Hamilton's results are based on acute exposures to selenite and selenate dissolved in water, therefore they do not specifically address age-related sensitivity to dietary exposure. However, we are not aware of an investigation which has demonstrated that age-related sensitivity to selenium is strongly dependent on route of exposure (i.e., dissolved versus dietary exposure). Our investigation involved both routes of exposure. Consequently, we contend that the magnitude of bias that may have been created by studying 41-day-old razorback sucker instead of 10-d-old fish is small.

Acute tests

Razorback and flannelmouth sucker had similar responses to acute exposure to dissolved selenate. Estimates of 96-hour median lethal concentrations for both species were within 10% of each other with flannelmouth sucker having the lower value (i.e., more sensitive to exposure). These data do not support the hypothesis that larval razorback sucker are more sensitive to

selenium exposure than flannelmouth sucker. Further evidence of lack of biologically meaningful differences in sensitivity of these two species is based on the ratio of median lethal concentrations (35.5 : 32.5). The ratio is about 1.09. Ratios between 0.5 and 1.5 are considered within the range of normal experimental variation (Mayer and Ellersieck 1986).

Fathead minnow were more sensitive than razorback sucker to acute exposure. The ratio of median lethal concentrations (35.5 : 21.8) is about 1.6. suggesting that fathead minnow were more sensitive to selenium exposure. In a previous investigation, Beyers and Sodergren (1999) conducted acute exposures with razorback sucker and fathead minnow and calculated a ratio of 1.2.

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 five chemicals that represent different chemical classes and toxic modes of action: carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. Results of the investigation suggested that the two species were "generally similar" (Dwyer et al. 1995). These consistent conclusions produced by different investigators using different sources of selenium, different contaminants, and different techniques represent strong evidence that razorback sucker larvae are about equal to, or less sensitive than fathead minnow to dissolved selenium. It should be noted that studies of relative sensitivity of razorback sucker and fathead minnow have been based on dissolved, not dietary, exposure concentrations. This method of estimating relative sensitivity is an accepted procedure (Mayer and Ellersieck 1986; Suter 1993). However, results

of one investigation suggest that estimates of relative sensitivity from laboratory acute toxicity tests may not accurately predict toxic effects of selenium in the field (Lemly 1985). Conflicting conclusions about the accuracy of accepted methods for assessing relative sensitivity of fishes to selenium exposure suggests that sensitivity estimates in this, and other reports, should be used and interpreted with caution.

Although razorback sucker, flannelmouth sucker, and fathead minnow had generally similar responses to selenium exposure, we did observe some variation in sensitivity. Observed variation may have been due to interspecific differences or to age and stage of development at time of exposure. There are important species differences in size at hatching: razorback sucker are 7 to10-mm long, flannelmouth sucker are 10 to 11-mm long, and fathead minnow are about 5-mm long (Snyder and Muth 1990; USEPA 1991). These differences suggest that the fishes will have different sensitivities to toxicants even if all other conditions are equal. There were also differences in age of fishes in acute exposures: razorback sucker were 16 days old, flannelmouth sucker were 11 days old, fathead minnow were 3 days old. Timing of exposure of these fishes was intended to mimic the conditions that occur in the natural environment. Arrival of razorback sucker larvae in nursery habitats is thought to coincide with first feeding (i.e., 10-12 days old; Muth et al. 1998). In contrast, fathead minnow reproduction and hatching probably takes place entirely in nursery habitats.

The broad distribution of fathead minnow, and its similar sensitivity to razorback sucker suggests that this non-native species may be a useful surrogate for evaluating toxic effects under field conditions. Surrogate species have been used for a variety of scientific purposes including: indicating anthropogenic change, indicating habitat quality for other species, and tracking

population changes of other species (Thomas 1972; O'Doherty 1999; and references cited therein). Surrogate species are not assumed to be identical to the target species; but the response of surrogate species is assumed to be correlated with the response of the target organism. Because the association between surrogate and target species is based on correlation, an evaluation study should always be conducted to confirm whether the choice of species was appropriate. If the evaluation shows that the surrogate is a reliable indicator of target species characteristics, then wider application of the method may be appropriate. Compared to razorback sucker, fathead minnow are ubiquitous in the Upper Colorado River Basin and represent about 30% of fish captured in Interagency Standardized Monitoring Program fall seine collections in the Colorado River (Miller and Snyder 2000). Because fathead minnow carry out their entire life cycle in potential razorback sucker nursery habitats, they should accumulate effects of long-term exposure and represent a worst-case biomonitor of exposure to ambient selenium concentrations. Application of the surrogate concept to fathead minnow and razorback sucker should be carefully planned. For example, it would be inappropriate to use presence or absence of adult fathead minnow in nursery habitats as an indicator of razorback sucker suitability because selenium may impair reproduction in fish. Thus, the presence of reproductively impaired adults would be a misleading indicator. In contrast, a potentially useful indicator might be evidence of recruitment of age-0 fathead minnow in a nursery habitat. Recruitment of young fish would suggest that selenium-exposed adults had successfully reproduced, and that the offspring were able to survive and grow using resources in the nursery habitat.

Other important razorback sucker life stages

The lack of detection of adverse effects from selenium exposure does not imply that razorback sucker populations may not be affected by increased environmental concentrations of the contaminant. There are two other life stages that may be especially sensitive to selenium exposure. They are young-of-year in fall, and reproductively active females. Over-winter survival of young-of-year fishes has been shown to be highly variable and is probably related to energetic reserves of fish (Oliver et al. 1979; Shuter and Post 1990; Thompson et al. 1991; Haines et al. 1998). Lemly (1993b) studied combined effects of selenium exposure and winter temperatures on bluegill. He showed that selenium exposure increased metabolic rate while winter conditions reduced food consumption. The combined effects of increased energy expenditure and reduced energy acquisition accelerated the rate of depletion of energetic reserves required for overwinter survival. Beyers et al. (1999a,b) presented an energetics-based approach that describes a mechanistic explanation for Lemly's observations. Together, the evidence that regional winter conditions are severe enough to influence overwinter survival (Thompson et al. 1991; Haines et al. 1998) and that selenium exposure during winter conditions increases mortality rates of fish (Lemly 1993b) suggests that effects on young-of-year during winter warrant further investigation.

Reproductively active female fish represent a potentially sensitive life stage because several investigators have demonstrated that selenium can be transferred from adult fish to eggs (Lemly 1998). Maternal transfer of selenium does not appear to influence reproductive behavior of adults or deposition of eggs, but survival and growth of fertilized eggs and larvae may be

affected. An investigation of reproductive effects on razorback sucker has been conducted, and a final report is in preparation (Hamilton et al. 1999). The potential for reproductive effects and need for future investigations with this life stage can be better evaluated after the final report has been made available.

Conclusions

Results of this investigation showed that razorback and flannelmouth sucker had very similar responses to selenium exposure. In 28-day early life-stage toxicity tests, survival and growth of both fishes were not negatively affected by exposure to dissolved and dietary selenium concentrations ranging up to 190 µg/L and 8.24 µg/g. The fishes also had similar responses to acute exposure to dissolved selenium. Median lethal concentrations and 95% confidence limits (in parentheses) for razorback sucker, flannelmouth sucker, and fathead minnow were 35.5 (32.3, 38.8), 32.5 (28.9, 35.1), and 21.8 mg/L (18.6, 25.2), respectively.

The intended emphasis of this investigation was to compare the sensitivity of razorback and flannelmouth sucker to dietary selenium exposure. Unfortunately neither species was adversely affected by the highest dietary selenium concentration studied (8.24 µg/g); consequently, an estimate of relative sensitivity based on dietary exposure cannot be made. However, it can be concluded that the data do not support the hypothesis that larval razorback sucker are more sensitive to selenium exposure than flannelmouth sucker over the range of exposure concentrations used in this investigation.

It should be noted that larval razorback sucker were 30 and 5 days older than flannelmouth sucker in the ELS and acute tests, respectively. It is not certain how this age difference may have influenced relative sensitivity of the two species.

Recommendations

The following are suggested topics for investigations of effects of selenium exposure on razorback sucker. These topics address important questions about effects of selenium exposure, but the merit and justification for each investigation are dependent on the direction of management activities and results of ongoing investigations. Suggested topics should be used as a basis for discussions about potentially important, unanswered questions regarding selenium exposure. Topics are not listed in order of importance.

- Conduct investigations to evaluate potential for reduced overwinter survival of young-of-year fish from selenium exposure.
- Conduct investigations to predict selenium bioaccumulation in wild adult razorback sucker and link bioaccumulation to natural movements of fish using radio telemetry.
- Conduct mesocosm-scale investigations with young-of-year fish to evaluate effects of exposure under environmentally realistic conditions of selenium cycling, physical habitat, and natural food organisms.
- Consider additional investigations on effects of selenium on reproductive success depending on conclusions of previous investigations.

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Appendix

Table A1. Concentrations of major cations of laboratory water used for culture of algae and rotifer, and well water used for early life-stage toxicity tests.

Source	Al (mg/L)	B (mg/L)	Ba (mg/L)	Ca (mg/L)	Cd (mg/L)	Cr (mg/L)	Cu (mg/L)	Fe (mg/L)	K (mg/L)	Mg (mg/L)	Mn (mg/L)	Mo (mg/L)	Na (mg/L)	Ni (mg/L)	P (mg/L)	Zn (mg/L)
Laboratory water	<0.1	0.02	0.01	31.10	<0.005	0.01	<0.01	<0.01	9.00	49.50	<0.01	<0.01	100.88	0.01	0.10	0.01
Laboratory water	<0.1	0.01	0.01	31.90	<0.005	<0.01	<0.01	0.04	9.56	50.00	<0.01	<0.01	116.60	<0.01	0.10	0.01
Mean	<0.1	0.02	0.01	31.50	<0.005	0.01	<0.01	0.04	9.28	49.75	<0.01	<0.01	108.74	0.01	0.10	0.01
Well water	<0.1	0.16	0.10	106.10	<0.005	0.02	<0.01	0.03	1.50	93.30	0.01	0.07	49.00	0.01	0.10	0.01
Well water	<0.1	0.16	0.10	109.40	<0.005	0.01	<0.01	0.03	0.80	40.60	0.01	0.07	53.40	0.01	0.10	0.01
Mean	<0.1	0.16	0.10	108.75	<0.005	0.02	<0.01	0.03	1.15	66.95	0.01	0.07	51.20	0.01	0.10	0.01

All means are based on analytical determinations that were above the limit for quantitation. Analyses were conducted by the Soil, Water and Plant Testing Laboratory, Colorado State University.

Table A2. Concentrations of major anions and water quality characteristics of laboratory water used for culture of algae and rotifer, and well water used for early life-stage toxicity tests.

Source	CO_3 (mg/L)	HCO_3 (mg/L)	Cl (mg/L)	NO_3 (mg/L)	NO_3-N (mg/L)	SO_4 (mg/L)	Alkalinity (mg/L)	Conductivity (µS/cm)	Hardness (mg/L)	TDS (mg/L)
Laboratory water	<0.1	187	13	0.8	0.2	335	153	1030	281	726
Laboratory water	<0.1	186	9	0.7	0.2	347	153	1020	285	752
Mean	<0.1	187	11	0.7	0.2	341	153	1025	283	739
Well water	<0.1	348	27	28.4	6.4	220	285	969	423	829
Well water	<0.1	341	25	26.8	6.1	226	279	969	417	820
Mean	<0.1	344	26	27.6	6.2	223	282	969	420	824

All means are based on analytical determinations that were above the limit for quantitation. Analyses were conducted by the Soil, Water and Plant Testing Laboratory, Colorado State University.

Table A3. Summary of responses of razorback sucker exposed to dissolved and dietary selenium for 28 days. Ten animals per replicate, four replicates per exposure concentration.

Selenium exposure					
Water (µg/L)	Diet (µg/g)	Replicate	Number surviving	Average mass (mg)	Average TL (mm)
6.12[a]	<0.702	a	10	13.7	15.8
6.12	<0.702	b	10	11.1	14.5
6.12	<0.702	c	10	12.5	15.3
6.12	<0.702	d	10	10.9	14.9
25.4	1.35	a	10	11.9	14.6
25.4	1.35	b	10	10.5	14.6
25.4	1.35	c	10	10.0	14.3
25.4	1.35	d	10	10.9	14.7
50.6	2.02	a	10	12.2	14.9
50.6	2.02	b	10	12.3	15.1
50.6	2.02	c	9	11.5	14.8
50.6	2.02	d	10	12.8	15.1
98.9	4.63	a	10	11.7	15
98.9	4.63	b	10	12.6	14.9
98.9	4.63	c	10	11.4	14.6
98.9	4.63	d	10	12.6	15.1
190	8.24	a	10	12.2	14.8
190	8.24	b	10	11.4	14.7
190	8.24	c	9	12.5	15
190	8.24	d	9	13.7	15.4

[a]Control.

Table A4. Summary of responses of flannelmouth sucker exposed to dissolved and dietary selenium for 28 days. Ten animals per replicate, four replicates per exposure concentration.

Selenium exposure					
Water (µg/L)	Diet (µg/g)	Replicate	Number surviving	Average mass (mg)	Average TL (mm)
6.12[a]	<0.702	a	9	15.9	16.8
6.12	<0.702	b	9	17.5	17.2
6.12	<0.702	c	9	16.0	16.9
6.12	<0.702	d	8	16.9	17.2
25.4	1.35	a	8	15.8	17.2
25.4	1.35	b	8	14.0	16.8
25.4	1.35	c	10	15.9	16.8
25.4	1.35	d	8	15.9	16.9
50.6	2.02	a	8	17.0	16.8
50.6	2.02	b	10	17.4	16.9
50.6	2.02	c	10	18.4	17.2
50.6	2.02	d	7	19.6	17.6
98.9	4.63	a	10	17.9	17.1
98.9	4.63	b	10	17.8	17.3
98.9	4.63	c	10	16.9	16.9
98.9	4.63	d	10	18.2	16.8
190	8.24	a	7	19.0	17.2
190	8.24	b	8	19.1	17.4
190	8.24	c	10	17.0	16.9
190	8.24	d	8	18.5	17.4

[a]Control.

Table A5. Summary of mortality of razorback sucker 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
160.[a]	a	1	13	15	
160.	b	5	13	15	
160.	c	1	12	15	
160.	d	2	9	13	15
80.3(0.881)	a	0	2	12	14
80.3	b	1	1	12	15
80.3	c	0	2	7	14
80.3	d	0	0	12	15
39.3(0.333)	a	0	0	5	7
39.3	b	0	0	3	13
39.3	c	0	0	3	13
39.3	d	0	0	3	8
20.0[a]	a	0	0	0	0
20.0	b	0	0	0	0
20.0	c	0	0	0	0
20.0	d	1	1	1	1
9.7(0.153)	a	0	0	0	0
9.7	b	0	0	0	0
9.7	c	0	0	0	0
9.7	d	0	0	0	0
<0.001[a]	a	0	0	0	0
<0.001	b	0	0	0	0
<0.001	c	0	0	0	0
<0.001	d	0	0	0	0

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

Table A6. Summary of mortality of flannelmouth sucker 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
160.[a]	a	0	15		
160.	b	0	15		
160.	c	0	15		
160.	d	0	14	15	
80.3(0.881)	a	0	8	15	
80.3	b	0	8	15	
80.3	c	0	6	15	
80.3	d	0	11	15	
39.3(0.333)	a	0	1	12	13
39.3	b	0	1	5	12
39.3	c	0	0	11	14
39.3	d	0	1	7	11
20.0[a]	a	0	0	0	1
20.0	b	0	0	0	0
20.0	c	0	0	0	0
20.0	d	0	0	0	0
9.7(0.153)	a	0	0	0	0
9.7	b	0	0	0	0
9.7	c	0	0	0	0
9.7	d	0	0	0	0
<0.001[a]	a	0	0	0	0
<0.001	b	0	0	0	0
<0.001	c	0	0	0	0
<0.001	d	0	0	0	0

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

Table A7. 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
160.[a]	a	12	15		
160.	b	12	15		
160.	c	0	12	13	15
160.	d	8	14	15	
80.3(0.881)	a	5	8	12	15
80.3	b	4	11	11	13
80.3	c	5	8	13	15
80.3	d	4	10	12	15
39.3(.333)	a	0	2	8	13
39.3	b	0	3	10	12
39.3	c	0	1	5	10
39.3	d	1	4	11	13
20.0[a]	a	0	0	5	12
20.0	b	0	0	1	5
20.0	c	0	0	2	5
20.0	d	0	0	1	2
9.7(.153)	a	0	0	0	1
9.7	b	0	0	0	0
9.7	c	0	0	0	3
9.7	d	0	0	0	6
<0.001[a]	a	0	0	0	0
<0.001	b	0	0	2	4
<0.001	c	0	0	0	2
<0.001	d	0	0	0	0

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