# Assessment of Exposure of Larval Razorback Sucker to Selenium in Natural Waters and Evaluation of Laboratory-Based Predictions

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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. A previous investigation conducted by the authors used a laboratory food-chain exposure system to quantify effects of selenium on razorback sucker (*Xyrauchen texanus*) larvae. The objective of this investigation was to evaluate the accuracy of predictions of the previous experiment by comparing its predictions to results observed when razorback sucker larvae are exposed to naturally occurring selenium in surface waters from three localities on the Colorado River near Grand Junction, Colorado. Assessment of predictions of the previous experiment is important because natural waters may contain different forms of selenium, as well as co-contaminants that influence the bioaccumulation and toxicity of ambient concentrations.

Razorback sucker larvae (27-days old, after hatching) were exposed for 28 d to site waters and food organisms cultured in site waters. Data were analyzed using analysis of variance to describe the response of survival and growth of fish in each site water and to describe the relative contribution of dissolved versus dietary exposure to constituents in site waters. Results were compared to predictions of the previous investigation to evaluate agreement, and the potential for adverse effects caused by selenium exposure.

Existing guidelines suggest that >3 μg/g dietary selenium, or > 4 μg/g whole-body tissue concentrations in fish will produce adverse effects. The highest dietary and whole-body concentrations achieved in this investigation were 21.8 μg/g and 42.0 μg/g, respectively. Negative effects from dietary exposure to site-water constituents were detected, but the data suggest that they were caused by co-contaminants in the diet, not selenium exposure.

Lack of detection of adverse effects from exposure does not imply that razorback sucker populations are not affected by increased selenium concentrations. There are a variety of factors which were not included in this investigation that may influence sensitivity of razorback sucker to selenium. For example, razorback sucker larvae in this investigation were not pre-exposed to

high concentrations of selenium via maternal transfer. Pre-exposure may increase effects of selenium exposure during larval development. In addition, 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 (1987) criterion for protection of freshwater aquatic life (5 μg/L) and elevated concentrations of selenium were measured in larval

and adult fish (Hamilton and Waddell 1994; Waddell and May 1995; Hamilton et al. 2000). In response to this concern, a laboratory food-chain experiment was conducted to quantify effects of selenium exposure to larval razorback sucker (Beyers and Sodergren 1999; 2000). The experiment involved exposing larval fish to gradients of selenium-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 razorback) in the selenium gradient. A conclusion of the previous investigation was that selenium exposure did not adversely affect survival or growth of razorback sucker larvae in any of the experimental treatments.

The objective of this investigation was to evaluate the accuracy of predictions of the previous experiment by repeating the food-chain experiment using water from three localities on the Colorado River near Grand Junction, Colorado. Assessment of predictions of the previous experiment is important because natural waters may contain different forms of selenium, as well as co-contaminants that influence the bioaccumulation and toxicity of ambient concentrations. Razorback sucker larvae were exposed for 28 d to site waters and food organisms cultured in site waters. Survival and growth of exposed fish were compared to responses of controls. Data were analyzed using analysis of variance to describe the response of survival and growth of fish in each site water and to describe the relative contribution of dissolved versus dietary exposure to constituents in site waters. Results were compared to predictions of the previous investigation to evaluate agreement, and the potential for adverse effects caused by selenium exposure.

## Materials and Methods

### Test waters

A total of five test waters were evaluated. The source of water for the control treatment 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. The treatment process reduced total residual chlorine concentration to less than the detection limit of 0.02 mg/L. Control 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).

Natural waters were obtained from potential and historic razorback sucker nursery habitats at three localities (Table 1). Localities were selected based on existing water quality data (Barb Osmundson, Frank Pfeifer, and Kathy Holley, U.S. Fish and Wildlife Service, personal communication) so that the investigation would include the typical range of dissolved constituents that currently occurs in fish habitats in the Grand Junction area. Localities ranged from presumed uncontaminated De Beque (DB), to moderately contaminated Orchard Mesa (OM), to highly contaminated North Pond (NP). The fifth test water was North Pond water diluted 50% with control water (NPD). This dilution provided an additional moderately contaminated test water, and also simulated potential results if North Pond contaminant concentrations were reduced by 50%.

**Table 1.** Descriptions of locations where natural waters were collected from habitats associated with the Colorado River.

| Locality name | Habitat | Location reference |
|---|---|---|
| De Beque (DB) | Backwater | Latitude N 39° 20' 18", longitude W 108° 11' 39" in Mesa County, on left bank of the Colorado River 200 m downstream of frontage road (Old Highway 6) bridge. |
| Orchard Mesa (OM) | Backwater | Latitude 39° 2' 58", longitude 108° 29' 36" in Mesa County, on left bank of Colorado River at site GF 1. |
| North Pond (NP) | Pond | Latitude 39° 6' 12", longitude 108° 38' 53" in Mesa County, Walter Walker State Wildlife Area, on right bank of Colorado River at the north side of North Pond. |

Water from each locality was collected on three occasions 28 April, 10 May, and 17 May 1999. Unfiltered water was collected from mid water column and placed in 50-L high-density polyethylene barrels. Samples were transferred on the day of collection, to laboratory facilities at Colorado State University. Samples were stored at room temperature in open collection barrels.

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 exposure of razorback sucker larvae was not pasteurized, filtered, or treated in any way.

**Experimental animals**

*Algae and rotifers*

Monocultures of the freshwater algae *Chlorella vulgaris* (Carolina Biological Supply Company, Burlington, North Carolina) were cultured in each test water using methods of Hoff and Snell (1989). Algae were cultured in 4-L polyethylene bottles containing 3 L of test water. From 600 to 900 ml of algae-containing test water were removed from each culture each day for rotifer feeding. Consequently, the replacement rate of test water 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 test waters using methods of Hoff and Snell (1989). Rotifers were cultured in 20-L polyethylene bottles containing 16 L of test water. Each rotifer culture was fed

algae from the corresponding treatment (e.g.. rotifers in the De Beque treatment were fed algae from the De Beque treatment) two or three times daily. Abundance of rotifers in batch cultures was quantified daily by subsampling. Replacement rate of test water in rotifer cultures was once every 2 d.

*Razorback sucker*

Razorback sucker larvae were obtained from the Dexter National Fish Hatchery and Technology Center (U.S. Fish and Wildlife Service, Dexter, New Mexico) 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, low abundance of rotifers in monocultures delayed start of the exposure until 27 days after hatching (mesolarva). Then, randomly-selected fish were transferred to exposure beakers containing the same water they had been reared in for 24-h acclimation to testing conditions.

**Experimental design and exposure system**

Experimental treatments were assigned to replicate exposure beakers ($n$=4) using a randomized, balanced 5×2 factorial design. The first factor, test water, had five levels or types of water (control, De Beque, Orchard Mesa, North Pond dilution, or North Pond). The second

factor, diet, had two levels (rotifers cultured in control water or site water). Thus, the experimental design partitions effects of exposure to dissolved and dietary test-water constituents.

Exposure procedures were based on prescribed methods for conducting early life-stage toxicity tests with fishes (ASTM 1990). 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. Water in each beaker was replaced daily using renewal procedures. 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 759 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.

**Physical and chemical conditions**

Water temperature 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 determined for each test water on each occasion site waters were collected, or when a new batch of control water was prepared (Table 2). Dissolved oxygen in exposure beakers was measured daily and ranged from 6.3 to 7.5 mg/L. Concentrations of major

**Table 2.** Summary of water-quality characteristics of test waters.

| Source | Mean | Standard Error | n |
|---|---|---|---|
| Control | | | |
| Alkalinity (mg/L) | 192 | 12.9 | 6 |
| Hardness (mg/L) | 319 | 26.2 | 6 |
| pH | 8.6 | 0.034 | 6 |
| Conductivity (µS/cm) | 1010 | 49.2 | 6 |
| De Beque | | | |
| Alkalinity (mg/L) | 126 | 7.8 | 3 |
| Hardness (mg/L) | 212 | 11.4 | 3 |
| pH | 8.6 | 0.249 | 3 |
| Conductivity (µS/cm) | 786 | 14.3 | 3 |
| Orchard Mesa | | | |
| Alkalinity (mg/L) | 265 | 2.7 | 3 |
| Hardness (mg/L) | 1831 | 84.4 | 3 |
| pH | 8.0 | 0.013 | 3 |
| Conductivity (µS/cm) | 3791 | 125.6 | 3 |
| North Pond 50% dilution | | | |
| Alkalinity (mg/L) | 159 | 14.2 | 3 |
| Hardness (mg/L) | 589 | 97.0 | 3 |
| pH | 8.6 | 0.088 | 3 |
| Conductivity (µS/cm) | 2573 | 388.3 | 3 |
| North Pond | | | |
| Alkalinity (mg/L) | 132 | 21.7 | 3 |
| Hardness (mg/L) | 943 | 160.5 | 3 |
| pH | 8.5 | 0.113 | 3 |
| Conductivity (µS/cm) | 3740 | 455.1 | 3 |

cations, anions, and other general water quality characteristics were also determined for each test water (Tables A1 and A2). Assessment of the influence of these other water quality elements on razorback sucker was not an emphasis of this investigation, but the measurements provide a detailed description of each test water.

**Analytical procedures**

Dissolved selenium concentration was measured in each batch of site water collected. On each sampling occasion, two 250-ml samples were collected from each site water. Samples were passed through a 0.45-μm filter, 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, rotifers, and razorback sucker larvae were also determined. Duplicate samples of algae and rotifer were collected weekly (four occasions). Razorback sucker larvae within an exposure beaker were pooled and collected at the end of the study. 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). Razorback sucker 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**

Statistical analysis of survival data was not conducted because mortalities were not observed in five of nine treatments. Lack of variation in these treatments precludes useful statistical comparisons. Consequently, data were analyzed by inspection of summary statistics and graphical plots.

Growth data were analyzed using analysis of variance. 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 each qualitatively different water-diet exposure combination. The full statistical model had the form

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

where $y$ = average fish mass (mg) or total length (mm), $\beta_0$ = intercept, $\beta_1$, $\beta_2$ = coefficients for the linear terms of main effects, $x_1$ = test water, $x_2$ = control diet or site-water diet, and $\beta_3$ = coefficient for interaction of main effects. A nonsignificant coefficient for interaction suggests that the effect of dietary exposure to site-water constituents was consistent across all treatments. 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 $\beta_1$ represents a test for effects of exposure to dissolved constituents in site waters; $\beta_2$ represents a test for effects of dietary exposure to site-water constituents.

To explore potential relationships between dissolved selenium concentrations in test waters and response variables, Pearson correlation coefficients were calculated for dissolved selenium concentrations, fish whole-body tissue concentrations, fish wet weights, and fish total lengths. The analysis was conducted using Proc Corr (SAS 1991). Correlation analysis is an exploratory technique that can identify consistent changes between two variables. Correlation analysis does not demonstrate a cause-and-effect relationship. Correlation analysis is appropriate in this case because selenium concentrations in site waters were not under investigator control. In addition, there were numerous other water-quality characteristics that were different in each site water. Thus, the observed response of razorback sucker to site waters cannot be attributed to selenium using correlation analysis, but consistent associations can be identified.

## Results

### Selenium concentrations in test waters and organisms

Mean selenium concentrations in test waters ranged from < 1 µg/L (limit for quantitation) for the control and De Beque waters to 20.3 µg/L for North Pond (Table 3; Fig. 1). In general, there was a corresponding increase in selenium concentrations in algae and rotifer in each water. The only exception to this pattern was the selenium concentration of algae in North Pond water which was not higher than the North Pond 50% dilution. Algae concentrations ranged from <0.183 to 3.74 µg/g. Rotifer concentrations ranged from < 0.702 to 21.8 µg/g.

Selenium concentrations in razorback sucker larvae increased with water and diet concentrations (Table 3; Fig. 2). The range of concentrations for fish exposed to test waters and the control diet was 2.34 to 14.4 µg/g selenium. The range of concentrations for fish exposed to test waters and the corresponding diet was 5.45 to 42.0 µg/g selenium.

### Razorback sucker survival

Reduced survival of larval razorback sucker from exposure to test waters and diets was not detected (Table 4; Fig. 3). Survival ranged from 92.5 to 100%. Survival was 100% in five of nine test-water exposure treatments. There was no consistent pattern between survival and exposure to selenium in water or diet. Survival in the North Pond exposure treatments was 100%. Mean selenium concentrations in North Pond water and diet were 20.3 µg/L and 21.8 µg/g, respectively. High survival in North Pond treatments suggests that lower survival in other test waters (including the control) was probably due to spontaneous mortality.

**Table 3.** Summary of selenium concentrations in water and organisms (dry weight). Values are mean and standard error.

| | Water (µg/L) | Algae (µg/g) | Rotifer (µg/g) | Fish (µg/g) |
|---|---|---|---|---|
| Control | | | | |
| | <1. | <0.183 | <0.702 | 2.34 (0.186) |
| De Beque | | | | |
| w | <1. | <0.183 | <0.702 | 3.04 (0.250) |
| w + d | <1. | 0.796 (0.041) | 2.10 (0.300) | 5.45 (0.341) |
| Orchard Mesa | | | | |
| w | 5.43 (0.347) | <0.183 | <0.702 | 4.35 (0.281) |
| w + d | 5.43 (0.347) | 1.55 (0.122) | 4.83 (0.238) | 11.0 (1.38) |
| North Pond 50% dilution | | | | |
| w | 10.6 (0.930) | <0.183 | <0.702 | 10.8 (1.70) |
| w + d | 10.6 (0.930) | 3.74 (0.328) | 12.0 (3.59) | 41.1 (2.74) |
| North Pond | | | | |
| w | 20.3 (1.36) | <0.183 | <0.702 | 14.4 (0.731) |
| w + d | 20.3 (1.36) | 2.30 (0.304) | 21.8 (3.76) | 42.0 (1.92) |

Estimates based on three sampling occasions for water (i.e., $n = 3$), four sampling occasions for algae and rotifer, and whole-body concentrations in fish in four replicate exposure beakers at conclusion of the 28-d exposure. W = fish were exposed to site water and rotifers cultured in control water; w + d = fish were exposed to site water and rotifers cultured in site water.

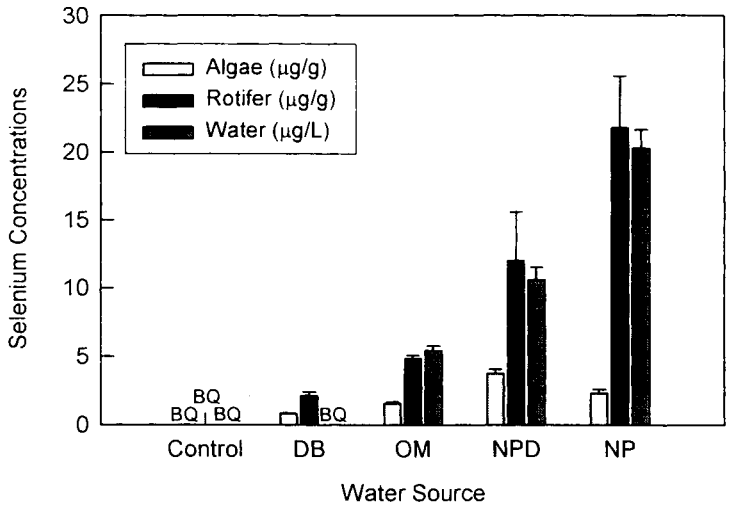


**Figure 1.** Selenium concentrations in water, algae, and rotifer in five test waters. Histograms represent mean and standard error. DB = De Beque; OM = Orchard Mesa; NPD = North Pond 50% dilution; NP = North Pond; BQ = selenium concentration below the limit for quantitation.

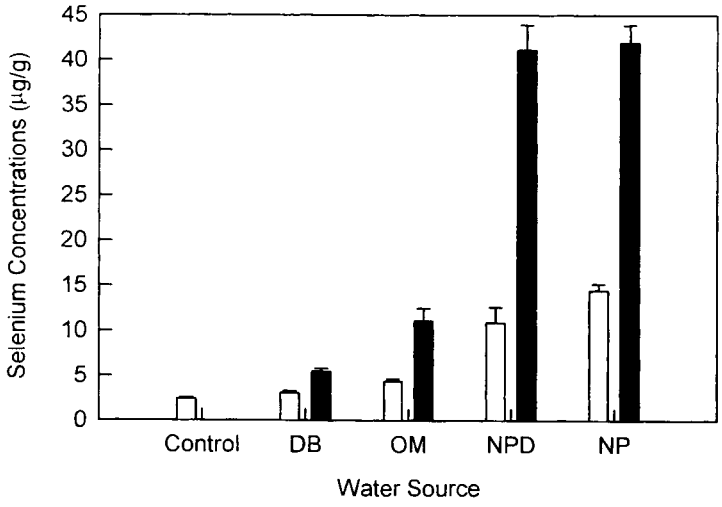


**Figure 2.** Selenium concentrations in razorback sucker larvae after exposure to test waters and control diet (open bars) or test waters and the corresponding diet (filled bars). Histograms represent mean and standard error. DB = De Beque; OM = Orchard Mesa; NPD = North Pond 50% dilution; NP = North Pond.

**Table 4.** Summary of responses of razorback sucker exposed to five test waters and diets. Ten animals per replicate, four replicates per exposure concentration. Values are mean (standard error).

| | Selenium exposure | | | | |
|---|---|---|---|---|---|
| | Water (µg/L) | Diet (µg/g) | Survival (%) | Mass (mg) | Total length (mm) |
| Control | | | | | |
| | <1. | <0.702 | 97.5 (1.64) | 10.5 (0.261) | 13.5 (0.107) |
| De Beque | | | | | |
| w | <1. | <0.702 | 97.5 (2.50) | 11.9 (0.327) | 14.0 (0.200) |
| w + d | <1. | 2.10 | 100 | 10.7 (0.427) | 13.7 (0.178) |
| Orchard Mesa | | | | | |
| w | 5.43 | <0.702 | 100 | 11.9 (0.403) | 13.8 (0.209) |
| w + d | 5.43 | 4.83 | 92.5 (0.0479) | 10.2 (0.439) | 13.5 (0.168) |
| North Pond diluted 50% | | | | | |
| w | 10.6 | <0.702 | 100 | 12.4 (0.311) | 14.2 (0.0851) |
| w + d | 10.6 | 12.0 | 97.5 (2.5) | 10.1 (0.378) | 13.5 (0.185) |
| North Pond | | | | | |
| w | 20.3 | <0.702 | 100 | 12.7 (0.528) | 14.0 (0.202) |
| w + d | 20.3 | 21.8 | 100 | 11.2 (0.339) | 13.7 (0.301) |

W = fish were exposed to site water and rotifers cultured in control water; w + d = fish were exposed to site water and rotifers cultured in site water.

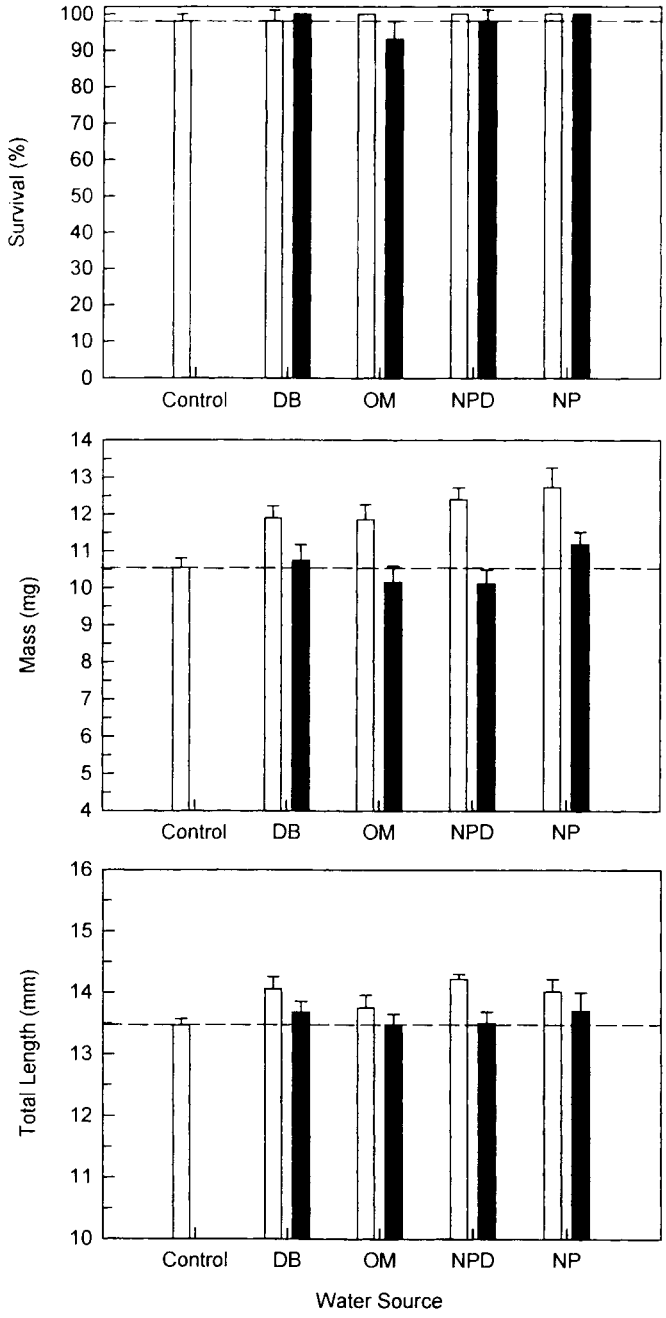


**Figure 3.** Survival and growth of razorback sucker larvae after exposure to test waters and control diet (open bars) or test waters and the corresponding diet (filled bars). Histograms represent mean and standard error. Horizontal dashed line represents control response. DB = De Beque; OM = Orchard Mesa; NPD = North Pond 50% dilution; NP = North Pond.

**Razorback sucker growth**

The growth response of razorback sucker larvae to test-water constituents was complex (Table 4; Fig. 3). Statistical analysis of growth in terms of fish mass at the end of the experiment showed that there was a positive effect from exposure to dissolved site water constituents. Fish exposed to site waters and control diet had greater mass than fish in the control treatment ($p < 0.0001$; Tables 4 and 5). A similar result was observed for total length ($p = 0.0081$) suggesting that larger size was a growth effect and not the result of contaminant-induced edema.

There was a negative effect from dietary exposure to site water constituents. Fish in dietary exposure treatments averaged 14% smaller than fish fed the control diet ($p < 0.0001$ for mass; $p = 0.0012$ for total length). The magnitude of the negative effect from dietary exposure was about the same as the positive effect from dissolved exposure. Consequently, the net effect of dissolved and dietary exposure to site-water constituents produced fish growth rates that were about equal to those observed in the controls.

**Correlation analysis**

Correlation coefficients ($r$) range from -1 to +1 (Ott 1993). Coefficients greater than 0 suggest a positive relationship; coefficients less than 0, a negative relationship between variables. A coefficient of 0 indicates no relationship.

There was a strong correlation between site-water selenium concentrations and whole-body tissue concentrations ($r = 0.699$, $p < 0.0001$; Table 6). This result is consistent with

**Table 5.** Maximum-likelihood significance probabilities for effects of dissolved and dietary exposure to constituents in five test waters.

| Experimental treatment | df | Chi Square | *p* |
|---|---|---|---|
| | | **Growth (mg)** | |
| Full model | | | |
| Test water | 4 | 27.5 | <0.0001 |
| Diet | 1 | 31.1 | <0.0001 |
| Test water × diet | 3 | 2.71 | 0.4384[a] |
| Reduced model | | | |
| Test water | 4 | 26.2 | <0.0001 |
| Diet | 1 | 29.6 | <0.0001 |
| | | **Growth (TL)** | |
| Full model | | | |
| Test water | 4 | 14.4 | 0.0061 |
| Diet | 1 | 11.0 | 0.0009 |
| Test water × diet | 3 | 2.14 | 0.5436[a] |
| Reduced model | | | |
| Test water | 4 | 13.8 | 0.0081 |
| Diet | 1 | 10.5 | 0.0012 |

[a]There was no significant interaction between test-water and diet treatments. Consequently, this term was omitted from the statistical model and the analysis was re-run to obtain estimates for the reduced model.

**Table 6.** Summary of Pearson correlation coefficients and respective probability values for the null hypothesis that correlation = 0.

| | Dissolved selenium concentration | Whole-body tissue concentration | Fish wet weight | Fish total length |
|---|---|---|---|---|
| Dissolved selenium concentration | 1 | 0.699; <0.0001 | 0.334; 0.0347 | 0.200; 0.2156 |
| Whole-body tissue concentration | 0.699; <0.0001 | 1 | -0.149; 0.3573 | -0.079; 0.6279 |

general predictions about bioaccumulation of selenium from water and diet. Whole-body concentration reflects the internal selenium dose and should be strongly related to the magnitude of adverse effects. However, whole-body selenium concentrations were only weakly correlated with fish weight ($r$ = -0.149, $p$ = 0.3573), and fish length ($r$ = - 0.079, $p$ = 0.6279). There was also a weak, positive correlation between site-water selenium concentrations and fish weight ($r$ = 0.334, $p$ = 0.0347) suggesting that exposure was associated with an increase in fish mass. Fish length was only weakly correlated with selenium exposure ($r$ = 0.200, $p$ = 0.2156). These results suggest that despite significant bioaccumulation, the negative affect on growth from exposure to site-water constituents was not strongly related to selenium.

## Discussion

### Effects of dissolved exposure to test-water constituents

Results of this investigation suggest that exposure to dissolved constituents in site waters had a positive effect. Razorback sucker exposed to site waters and control diet consistently grew to larger size 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. Consequently, the only thing that was different for fish fed the control diet was the test water. Two explanations can be offered to account for the positive influence of site waters.

First, it has been suggested that laboratory-formulated waters like the control water used in our investigation lack trace elements essential for survival and growth of biological organisms (Cowgill et al. 1986; Girling and Garforth 1989; Keating et al. 1989). Investigations with fish do not usually detect the influence of reduced availability of dissolved essential elements in laboratory waters because the elements are provided by diet. However, in our investigation, the entire food chain for the control treatment was reared in laboratory water. Consequently, for control fish, there was no alternative source for essential trace elements. In contrast, fish exposed to site waters could obtain essential elements from the water. The ultimate sources of municipal water that was used for the control treatment are rivers and reservoirs in northern Colorado. Apparently, sufficient trace elements were introduced into the water from natural sources to allow fish to survive and grow during the exposure period, but levels were inadequate to support growth rates equivalent to those observed in site waters.

A second potential explanation for faster growth of fish in site waters is related to osmotic gradient. Concentrations of many elements were higher in site waters than in the control

(Tables A1 and A2). Higher concentrations of dissolved constituents would have lowered the osmotic gradient between fish and their environment, thereby reducing the energy required for osmoregulation (Williamson et al. 1993). Energy liberated by the reduction in osmoregulation could have been re-allocated and conserved as growth. Thus, fish in site waters could have grown faster than controls because water quality reduced the energetic demands of physiological maintenance.

**Effects of dietary exposure to test-water constituents**

Experimental treatments involving exposure to site waters and control diet may have limited ecological relevance because it is unlikely that similar conditions occur in the field, but they played a critical role in uncovering the negative effect of dietary exposure in this investigation. The control-diet treatments allowed partitioning of effects of dissolved and dietary exposure, and revealed an average 14% reduction in growth of fish that were fed a diet cultured in site water. The magnitude of the negative effect was about equal to the beneficial effect of exposure to dissolved constituents. If the control-diet treatments had not been included in this experiment, only the net effect of dissolved and dietary exposure would have been observed and it is likely that it would have been concluded that exposure to site-water constituents did not influence growth of razorback sucker larvae.

**Inferred effects of selenium exposure**

A variety of factors in our experiment were beyond investigator control. Two of the most important were selenium concentrations, and presence of co-contaminants in site waters.

Because these factors could not be independently manipulated it is impossible to definitively support or refute a hypothesis that attributes observed effects to a specific contaminant. However, several lines of evidence suggest that the observed negative effects of exposure to site waters were not due to selenium.

First, selenium concentrations in site waters and diets were lowest for De Beque (<1 µg/L; 2.10 µg/g) and highest for North Pond (20.3 µg/L; 21.8 µg/g). A basic principle of toxicology is that adverse effects increase with toxic exposure; that is, there is a concentration- or dose-response relationship between the suspected cause and observed effects (McKim 1995; Rand et al. 1995). We did not observe a corresponding reduction in survival or growth of razorback sucker as selenium concentrations increased. Analysis of fish tissue concentrations showed that dissolved and dietary selenium were bioaccumulated by fish. As water and dietary concentrations increased, razorback sucker received a larger dose of selenium and whole-body concentrations increased. Thus, the lack of an exposure-response relationship despite the presence of strong selenium concentration gradients suggests that some other factor was responsible for the observed effects. This interpretation was confirmed by the correlation analysis which did not detect negative associations between selenium concentrations and fish growth although it did detect an association between exposure and whole-body concentration. Of the growth associations evaluated, the only one that showed a significant correlation with selenium concentration was fish mass, and that relationship was positive suggesting that exposure increased fish growth.

Second, we observed an effect of dietary exposure to constituents in De Beque water. Selenium concentrations in De Beque water and diet were below recommended thresholds for

toxic effects in fish. Reduced growth from dietary exposure for this locality suggests that some co-contaminant was responsible for the observed effects, and may also have been responsible for the growth reductions in other site waters.

Third, Beyers and Sodergren (2000) summarized results of several investigations that used a selenate-based food-chain system and concluded that the threshold for adverse effects from dietary exposure is between 8.24 and 20.3 µg/g selenium. The value 20.3 µg/L is the lowest concentration that affected growth of fathead minnow fed a mixture (50:25:25%) of sodium selenate, sodium selenite, and selenomethionine (Ogle and Knight 1989). The highest dietary exposure concentration in our investigation was 21.8 µg/g. This dietary concentration was greater than the lowest observed effect concentration reported by Ogle and Knight (1989), but it did not adversely influence growth or survival of razorback sucker. Selenate is generally considered to be less toxic than the other forms of selenium used by Ogle and Knight (1989). Inclusion of the other forms probably increased toxicity of their diet and may account for the disparity with our investigation. Consequently, we conclude that results of our investigation are consistent with previous research and that the observed negative effects from dietary exposure to site-water constituents were not due to selenium.

Concluding that selenium exposure did not cause adverse effects in this investigation does not imply that razorback sucker populations may not be influenced 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. Maternal transfer increases the potential for cumulative effects resulting from exposure of more than one life stage. For example, if embryos are exposed to selenium via maternal transfer, and the resulting larvae occupy nursery habitats with high selenium concentrations, then the potential exists for adverse effects from repeated, long-term exposure. 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.

**Comparison of predicted and observed responses**

Beyers and Sodergren (2000) exposed larval razorback sucker to dietary selenium concentrations up to 8.24 µg/g and achieved maximum whole-body concentrations of 12.9 µg/g in fish, but did not observe adverse effects. The highest dietary and whole-body concentrations achieved in this investigation were 21.8 µg/g and 42.0 µg/g, respectively. The concentration ranges of the two investigations do not completely overlap which complicates comparisons of predicted and observed results. For dietary or whole-body concentrations ≤ those studied by Beyers and Sodergren (2000), predictions were that survival of razorback sucker would not be affected by selenium exposure and that there would be a neutral or positive influence on growth. These predictions were consistent with the responses of fish exposed to De Beque and Orchard Mesa dietary treatments. Predictions for the North Pond site waters were not possible because of the lack of overlapping concentration ranges. Thus, results of this, and previous investigations by the authors are consistent. In contrast, results of this investigation are not consistent with established guidelines for predicting toxic effects from selenium exposure.

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 three of four site-water treatments: Orchard Mesa, North Pond-dilution, and North Pond. Water and dietary selenium exposure concentrations for these sites ranged from 5.43 µg/L and 4.83 µg/g for Orchard Mesa to 20.3 µg/L and 21.8 µg/g for North Pond, but corresponding reductions in survival or growth of razorback sucker were not observed over the 28-d exposure period. Analysis of whole-body selenium concentrations in razorback sucker confirmed that selenium was bioavailable and bioaccumulated in fish. Whole-body selenium concentrations in

all four site waters exceeded the predicted threshold for toxic effects in fish ( 4 µg/g; Lemly 1993a; NIWQP 1998). Whole-body concentrations ranged from 5.45 µg/g for De Beque to 42.0 µg/g for North Pond.

Guidelines that present thresholds for toxic effects of selenium have three characteristics that may explain the lack of agreement between predicted and observed responses. First, they 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. The 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 as described above. 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 components of an organisms life cycle is critical for ensuring long-term persistence of reproducing populations. The third characteristic of thresholds is that they are dependent on the type of selenium studied. The majority of laboratory investigations of 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 and is probably different from naturally cycling selenium. Our exposure system used a three trophic-level food chain to mimic natural processes of selenium biotransformation and bioaccumulation in algae,

rotifer, and fish. The incorporation of site waters in this investigation greatly increased the realism of exposure conditions. Naturally occurring forms of selenium from each site were introduced into our cultures and exposure system on a daily basis. However, not all aspects of natural selenium cycling were incorporated in our experiment. Sediment interactions undoubtedly play an important role in selenium cycling and may influence bioavailability in the natural environment. Despite the simplicity of laboratory food chains, we advocate that repeated collection and use of site waters incorporated many aspects of natural selenium cycling relevant to larval razorback sucker. Thus, our results should provide a good approximation of effects on larval razorback sucker under field conditions.

There was a difference in the age of razorback sucker at the beginning of this investigation (27-days old, after hatching) and the previous investigation (41-days old, after hatching; Beyers and Sodergren 1999). This difference and the inability to study younger fish resulted from logistical constraints of the two investigations. We advocate that the age of larvae is not a strong source of bias and several arguments support our contention. 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 exposures 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. 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. We are not aware of an investigation which has demonstrated that age-related sensitivity to selenium is strongly dependent on route of exposure (ie., 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 27-day-old razorback sucker instead of 10-d-old fish is small.

**Biological significance of selenium exposure**

Razorback sucker populations are presumed to have declined from cumulative effects of loss of physical habitat and historic temperature regime, interactions with non-native fishes, and degraded water quality. Quantifying the relative importance of each stressor is critical for successful management. Ranking potential stressors on the basis of sensitivity of razorback sucker response increases the likelihood that the most important limiting factors are identified and targeted for management and evaluation. Larval razorback sucker studied in this investigation were not strongly negatively affected by exposure to site waters with dissolved selenium concentrations $\leq$ 20.3 µg/L and corresponding dietary selenium concentrations $\leq$ 21.8 µg/g. Consequently, our data suggest that biologically significant effects will not occur in nursery habitats as a result of exposure of larval razorback suckers to selenium concentrations at or below these levels.

# Conclusions

This investigation used an experimental approach to evaluate the toxicity of naturally occurring forms of dissolved and dietary selenium on larval razorback sucker. Selenium concentrations in waters tested ranged from <1 to 20.3 µg/L, and corresponding dietary selenium concentrations ranged from<0.702 to 21.8 µg/g. Whole-body tissue concentrations in larval razorback sucker exposed to water and dietary selenium ranged from 2.34 to 42.0 µg/g. Despite strong concentration gradients of dissolved and dietary selenium, no adverse effects from selenium exposure were observed in this study. A weak negative effect from dietary exposure to site-water constituents was detected, but the data suggest that it was caused by co-contaminants in the diet, not selenium exposure.

Lack of detection of adverse effects from exposure does not imply that razorback sucker populations are not affected by increased selenium concentrations. There are a variety of factors which were not included in this investigation that may influence sensitivity of razorback sucker to selenium. For example, razorback sucker larvae in this investigation were not pre-exposed to high concentrations of selenium via maternal transfer. Pre-exposure may increase effects of selenium exposure during larval development. In addition, there are other life stages that may be especially sensitive to exposure.

## 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 quantify dietary exposure in nursery habitats by collecting potential prey organisms at times that correspond with habitat use by larval razorback sucker.
- 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 the 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 and on-going investigations.

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Appendix

**Table A1.** Concentrations of major cations in test waters. All values represent dissolved concentrations (mg/L).

| Site | Date | Al | B | Ba | Ca | Cr | Cu | Fe | K | Mg | Mn | Mo | Na | Ni | P | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 04/28/1999 | <0.1 | 0.02 | 0.01 | 31.10 | 0.01 | <0.01 | <0.01 | 9.00 | 49.50 | <0.01 | <0.01 | 100.88 | 0.01 | 0.10 | <0.001 | 0.01 |
| Control | 04/28/1999 | <0.1 | 0.01 | 0.01 | 31.90 | <0.01 | <0.01 | 0.04 | 9.56 | 50.00 | <0.01 | <0.01 | 116.60 | <0.01 | 0.10 | <0.001 | 0.01 |
| Mean | 04/28/1999 | <0.1 | 0.02 | 0.01 | 31.50 | 0.01 | <0.01 | 0.04 | 9.28 | 49.75 | <0.01 | <0.01 | 108.74 | 0.01 | 0.10 | <0.001 | 0.01 |
| DB | 04/28/1999 | <0.1 | 0.04 | 0.08 | 75.98 | 0.02 | <0.01 | <0.01 | 3.25 | 30.89 | 0.02 | 0.01 | 173.15 | <0.01 | 0.10 | <0.001 | <0.01 |
| DB | 04/28/1999 | <0.1 | 0.05 | 0.08 | 76.50 | 0.02 | <0.01 | 0.10 | 3.40 | 30.93 | 0.02 | 0.01 | 172.48 | <0.01 | 0.09 | <0.001 | 0.01 |
| Mean | 04/28/1999 | <0.1 | 0.05 | 0.08 | 76.24 | 0.02 | <0.01 | 0.10 | 3.33 | 30.91 | 0.02 | 0.01 | 172.82 | <0.01 | 0.10 | <0.001 | 0.01 |
| OM | 04/28/1999 | <0.1 | 0.37 | 0.03 | 418.50 | 0.10 | 0.01 | 0.09 | 4.65 | 148.59 | 0.06 | 0.04 | 329.08 | 0.03 | 0.40 | 0.005 | 0.03 |
| OM | 04/28/1999 | <0.1 | 0.36 | 0.02 | 404.50 | 0.10 | 0.01 | 0.06 | 5.02 | 143.18 | 0.01 | <0.01 | 329.07 | 0.03 | 0.40 | 0.005 | 0.03 |
| Mean | 04/28/1999 | <0.1 | 0.37 | 0.03 | 411.50 | 0.10 | 0.01 | 0.08 | 4.84 | 145.89 | 0.04 | 0.04 | 329.08 | 0.03 | 0.40 | 0.005 | 0.03 |
| NP | 04/28/1999 | <0.1 | 0.29 | 0.02 | 88.50 | 0.05 | <0.01 | 0.01 | 8.68 | 184.50 | 0.02 | 0.04 | 472.86 | 0.05 | 0.33 | 0.023 | 0.04 |
| NP | 04/28/1999 | <0.1 | 0.28 | 0.02 | 89.30 | 0.05 | <0.01 | <0.01 | 7.86 | 186.00 | 0.01 | 0.04 | 477.95 | 0.04 | 0.40 | 0.023 | 0.04 |
| Mean | 04/28/1999 | <0.1 | 0.29 | 0.02 | 88.90 | 0.05 | <0.01 | 0.01 | 8.27 | 185.25 | 0.02 | 0.04 | 475.41 | 0.05 | 0.37 | 0.023 | 0.04 |
| NPD | 04/28/1999 | <0.1 | 0.15 | 0.02 | 60.40 | 0.03 | <0.01 | <0.01 | 8.59 | 123.79 | 0.01 | 0.03 | 360.18 | 0.03 | 0.30 | 0.013 | 0.02 |
| NPD | 04/28/1999 | <0.1 | 0.15 | 0.02 | 60.20 | 0.03 | <0.01 | <0.01 | 8.90 | 122.30 | 0.01 | 0.02 | 388.50 | 0.02 | 0.30 | 0.012 | 0.02 |
| Mean | 04/28/1999 | <0.1 | 0.15 | 0.02 | 60.30 | 0.03 | <0.01 | <0.01 | 8.75 | 123.05 | 0.01 | 0.03 | 374.34 | 0.03 | 0.30 | 0.013 | 0.02 |
| DB | 05/10/1999 | <0.1 | 0.03 | 0.06 | 53.80 | 0.02 | <0.01 | <0.01 | 2.80 | 15.00 | <0.01 | <0.01 | 72.72 | <0.01 | 0.10 | <0.001 | 0.01 |
| DB | 05/10/1999 | <0.1 | 0.03 | 0.06 | 53.50 | 0.02 | <0.01 | <0.01 | 2.36 | 15.04 | <0.01 | <0.01 | 69.57 | <0.01 | 0.10 | <0.001 | 0.01 |
| Mean | 05/10/1999 | <0.1 | 0.03 | 0.06 | 53.65 | 0.02 | <0.01 | <0.01 | 2.58 | 15.02 | <0.01 | <0.01 | 71.15 | <0.01 | 0.10 | <0.001 | 0.01 |
| OM | 05/10/1999 | 0.1 | 0.37 | 0.02 | 413.22 | 0.12 | 0.02 | 0.08 | 5.10 | 142.60 | 0.05 | 0.05 | 310.50 | 0.04 | 0.30 | 0.005 | 0.04 |
| OM | 05/10/1999 | <0.1 | 0.36 | 0.02 | 420.00 | 0.10 | 0.01 | 0.10 | 4.93 | 144.20 | 0.04 | 0.05 | 312.55 | 0.03 | 0.30 | 0.006 | 0.04 |
| Mean | 05/10/1999 | 0.1 | 0.37 | 0.02 | 416.61 | 0.11 | 0.02 | 0.09 | 5.02 | 143.40 | 0.05 | 0.05 | 311.53 | 0.04 | 0.30 | 0.005 | 0.04 |
| NP | 05/10/1999 | <0.1 | 0.21 | 0.02 | 74.60 | 0.04 | <0.01 | <0.01 | 6.60 | 133.50 | 0.01 | 0.02 | 385.72 | 0.03 | 0.30 | 0.020 | 0.03 |
| NP | 05/10/1999 | <0.1 | 0.21 | 0.03 | 72.66 | 0.04 | 0.01 | <0.01 | 6.20 | 131.25 | 0.01 | 0.03 | 380.66 | 0.03 | 0.20 | 0.017 | 0.03 |
| Mean | 05/10/1999 | <0.1 | 0.21 | 0.03 | 73.63 | 0.04 | 0.01 | <0.01 | 6.40 | 132.38 | 0.01 | 0.03 | 383.19 | 0.03 | 0.25 | 0.019 | 0.03 |
| NPD | 05/10/1999 | <0.1 | 0.11 | 0.02 | 61.66 | 0.03 | <0.01 | <0.01 | 7.79 | 98.44 | 0.01 | 0.01 | 255.26 | 0.02 | 0.20 | 0.010 | 0.02 |
| NPD | 05/10/1999 | <0.1 | 0.12 | 0.02 | 61.46 | 0.03 | <0.01 | <0.01 | 7.40 | 97.32 | 0.01 | 0.01 | 278.40 | 0.02 | 0.20 | 0.010 | 0.02 |
| Mean | 05/10/1999 | <0.1 | 0.12 | 0.02 | 61.56 | 0.03 | <0.01 | <0.01 | 7.60 | 97.88 | 0.01 | 0.01 | 266.83 | 0.02 | 0.20 | 0.010 | 0.02 |

Table continued on next page.

**Table A1.** Continued.

| Site | Date | Al | B | Ba | Ca | Cr | Cu | Fe | K | Mg | Mn | Mo | Na | Ni | P | Se | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DB | 05/17/1999 | <0.1 | 0.36 | 0.06 | 56.30 | 0.02 | <0.01 | <0.01 | 2.77 | 17.10 | <0.01 | 0.01 | 80.10 | <0.01 | 0.10 | <0.001 | 0.01 |
| DB | 05/17/1999 | <0.1 | 0.04 | 0.06 | 55.00 | 0.02 | <0.01 | <0.01 | 2.80 | 16.70 | 0.01 | 0.01 | 72.77 | <0.01 | 0.10 | <0.001 | 0.01 |
| Mean | 05/17/1999 | <0.1 | 0.20 | 0.06 | 55.65 | 0.02 | <0.01 | <0.01 | 2.79 | 16.90 | 0.01 | 0.01 | 76.44 | <0.01 | 0.10 | <0.001 | 0.01 |
| OM | 05/17/1999 | 0.1 | 0.37 | 0.02 | 428.05 | 0.11 | 0.01 | <0.01 | 4.89 | 146.20 | 0.04 | 0.04 | 297.49 | 0.04 | 0.30 | 0.006 | 0.03 |
| OM | 05/17/1999 | <0.1 | 0.37 | 0.02 | 427.95 | 0.10 | 0.01 | 0.08 | 4.70 | 145.28 | 0.04 | 0.05 | 308.21 | 0.03 | 0.30 | 0.006 | 0.04 |
| Mean | 05/17/1999 | 0.1 | 0.37 | 0.02 | 428.00 | 0.10 | 0.01 | 0.08 | 4.80 | 145.74 | 0.04 | 0.05 | 302.85 | 0.04 | 0.30 | 0.006 | 0.04 |
| NP | 05/17/1999 | <0.1 | 0.23 | 0.02 | 65.98 | 0.04 | 0.01 | 0.04 | 5.69 | 138.67 | 0.01 | 0.04 | 398.72 | 0.03 | 0.30 | 0.019 | 0.03 |
| NP | 05/17/1999 | <0.1 | 0.23 | 0.02 | 66.40 | 0.04 | <0.01 | <0.01 | 6.10 | 140.25 | 0.01 | 0.04 | 398.90 | 0.02 | 0.30 | 0.020 | 0.02 |
| Mean | 05/17/1999 | <0.1 | 0.23 | 0.02 | 66.19 | 0.04 | 0.01 | 0.04 | 5.90 | 139.46 | 0.01 | 0.04 | 398.81 | 0.03 | 0.30 | 0.020 | 0.03 |
| NPD | 05/17/1999 | <0.1 | 0.12 | 0.02 | 53.70 | 0.03 | <0.01 | <0.01 | 7.56 | 94.28 | 0.01 | 0.02 | 253.24 | 0.01 | 0.20 | 0.010 | 0.02 |
| NPD | 05/17/1999 | <0.1 | 0.12 | 0.02 | 53.15 | 0.03 | <0.01 | <0.01 | 7.60 | 94.00 | 0.01 | 0.02 | 237.40 | 0.02 | 0.20 | 0.010 | 0.02 |
| Mean | 05/17/1999 | <0.1 | 0.12 | 0.02 | 53.43 | 0.03 | <0.01 | <0.01 | 7.58 | 94.14 | 0.01 | 0.02 | 245.32 | 0.02 | 0.20 | 0.010 | 0.02 |
| Means of all occasions for each site | | | | | | | | | | | | | | | | | |
| Control | | <0.1 | 0.02 | 0.01 | 31.50 | 0.01 | <0.01 | 0.04 | 9.28 | 49.75 | <0.01 | <0.01 | 108.74 | 0.01 | 0.10 | <0.001 | 0.01 |
| DB | | <0.1 | 0.09 | 0.07 | 61.85 | 0.02 | <0.01 | 0.10 | 2.90 | 20.94 | 0.02 | 0.01 | 106.80 | <0.01 | 0.10 | <0.001 | 0.01 |
| OM | | 0.1 | 0.37 | 0.02 | 418.70 | 0.10 | 0.01 | 0.08 | 4.88 | 145.01 | 0.04 | 0.05 | 314.48 | 0.03 | 0.33 | 0.005 | 0.04 |
| NP | | <0.1 | 0.24 | 0.02 | 76.24 | 0.04 | 0.01 | 0.03 | 6.86 | 152.36 | 0.01 | 0.04 | 419.14 | 0.03 | 0.31 | 0.020 | 0.03 |
| NPD | | <0.1 | 0.13 | 0.02 | 58.43 | 0.03 | <0.01 | <0.01 | 7.97 | 105.02 | 0.01 | 0.02 | 295.50 | 0.02 | 0.23 | 0.011 | 0.02 |

DB = De Beque, OM = Orchard Mesa, NP = North Pond, NPD = North Pond diluted 50%.

Cadmium concentrations were also determined, but were all below the limit for quantitation (0.005 mg/L) so were not tabulated. 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 test waters.

| Site | Date | $HCO_3$ (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) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 04/28/1999 | 187 | 13 | 0.8 | 0.2 | 335 | 153 | 1030 | 281 | 726 |
| Control | 04/28/1999 | 186 | 9 | 0.7 | 0.2 | 347 | 153 | 1020 | 285 | 752 |
| Mean | 04/28/1999 | 187 | 11 | 0.7 | 0.2 | 341 | 153 | 1025 | 283 | 739 |
| DB | 04/28/1999 | 244 | 176 | <0.1 | <0.1 | 238 | 200 | 1410 | 317 | 941 |
| DB | 04/28/1999 | 244 | 172 | 0.6 | 0.1 | 244 | 200 | 1400 | 318 | 945 |
| Mean | 04/28/1999 | 244 | 174 | 0.6 | 0.1 | 241 | 200 | 1405 | 318 | 943 |
| OM | 04/28/1999 | 300 | 303 | 5.7 | 1.3 | 1905 | 246 | 3750 | 1655 | 3416 |
| OM | 04/28/1999 | 301 | 256 | 6.2 | 1.4 | 1910 | 246 | 3760 | 1598 | 3357 |
| Mean | 04/28/1999 | 301 | 280 | 5.9 | 1.3 | 1908 | 246 | 3755 | 1627 | 3387 |
| NP | 04/28/1999 | 215 | 414 | <0.1 | <0.1 | 1463 | 176 | 4440 | 979 | 2847 |
| NP | 04/28/1999 | 215 | 456 | <0.1 | <0.1 | 1425 | 177 | 4440 | 988 | 2858 |
| Mean | 04/28/1999 | 215 | 435 | <0.1 | <0.1 | 1444 | 177 | 4440 | 984 | 2853 |
| NPD | 04/28/1999 | 204 | 213 | 0.2 | 0.1 | 1089 | 167 | 2870 | 660 | 2059 |
| NPD | 04/28/1999 | 200 | 177 | 0.6 | 0.1 | 968 | 164 | 2870 | 653 | 1926 |
| Mean | 04/28/1999 | 202 | 195 | 0.4 | 0.1 | 1029 | 166 | 2870 | 657 | 1993 |
| DB | 05/10/1999 | 161 | 85 | 1.2 | 0.3 | 80 | 132 | 793 | 196 | 473 |
| DB | 05/10/1999 | 163 | 93 | 1.8 | 0.4 | 91 | 134 | 790 | 195 | 490 |
| Mean | 05/10/1999 | 162 | 89 | 1.5 | 0.3 | 86 | 133 | 791.5 | 196 | 482 |
| OM | 05/10/1999 | 304 | 260 | 8.2 | 1.9 | 1790 | 249 | 3730 | 1617 | 3237 |
| OM | 05/10/1999 | 303 | 221 | 7.6 | 1.7 | 1771 | 249 | 3940 | 1641 | 3187 |
| Mean | 05/10/1999 | 304 | 241 | 7.9 | 1.8 | 1781 | 249 | 3835 | 1629 | 3212 |
| NP | 05/10/1999 | 169 | 272 | 1.2 | 0.3 | 1140 | 139 | 3260 | 735 | 2183 |
| NP | 05/10/1999 | 203 | 277 | 0.6 | 0.1 | 1104 | 166 | 3260 | 721 | 2176 |
| Mean | 05/10/1999 | 186 | 275 | 0.9 | 0.2 | 1122 | 153 | 3260 | 728 | 2180 |
| NPD | 05/10/1999 | 194 | 210 | 1.9 | 0.4 | 667 | 159 | 2380 | 559 | 1497 |
| NPD | 05/10/1999 | 210 | 219 | 0.3 | 0.1 | 696 | 172 | 2350 | 554 | 1570 |
| Mean | 05/10/1999 | 202 | 215 | 1.1 | 0.3 | 682 | 166 | 2365 | 557 | 1534 |
| DB | 05/17/1999 | 142 | 105 | 1.9 | 0.4 | 101 | 116 | 807 | 211 | 506 |
| DB | 05/17/1999 | 135 | 114 | 1.1 | 0.2 | 108 | 110 | 811 | 206 | 506 |
| Mean | 05/17/1999 | 139 | 110 | 1.5 | 0.3 | 105 | 113 | 809 | 209 | 506 |
| OM | 05/17/1999 | 327 | 333 | 6.6 | 1.5 | 1696 | 268 | 3370 | 1669 | 3241 |
| OM | 05/17/1999 | 315 | 301 | 6.5 | 1.5 | 1649 | 258 | 3760 | 1665 | 3159 |
| Mean | 05/17/1999 | 321 | 317 | 6.5 | 1.5 | 1673 | 263 | 3565 | 1667 | 3200 |
| NP | 05/17/1999 | 132 | 347 | 1.0 | 0.2 | 1148 | 108 | 3260 | 735 | 2238 |
| NP | 05/17/1999 | 143 | 375 | 0.5 | 0.1 | 1124 | 118 | 3280 | 742 | 2255 |
| Mean | 05/17/1999 | 138 | 361 | 0.7 | 0.2 | 1136 | 113 | 3270 | 739 | 2247 |
| NPD | 05/17/1999 | 183 | 169 | 0.5 | 0.1 | 632 | 150 | 2190 | 522 | 1393 |
| NPD | 05/17/1999 | 185 | 184 | 1.1 | 0.3 | 675 | 152 | 2190 | 519 | 1438 |
| Mean | 05/17/1999 | 184 | 177 | 0.8 | 0.2 | 654 | 151 | 2190 | 521 | 1416 |

Table continued on next page.

**Table A2.** Continued.

| Site | Date | $HCO_3$ (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) |
|---|---|---|---|---|---|---|---|---|---|---|
| Means of all occasions for each site | | | | | | | | | | |
| Control | | 187 | 11 | 0.7 | 0.2 | 341 | 153 | 1025 | 283 | 739 |
| DB | | 182 | 124 | 1.2 | 0.3 | 144 | 149 | 1002 | 241 | 644 |
| OM | | 308 | 279 | 6.8 | 1.5 | 1787 | 253 | 3718 | 1641 | 3266 |
| NP | | 180 | 357 | 0.8 | 0.2 | 1234 | 147 | 3657 | 817 | 2426 |
| NPD | | 196 | 195 | 0.8 | 0.2 | 788 | 161 | 2475 | 578 | 1647 |

DB = De Beque. OM = Orchard Mesa. NP = North Pond . NPD = North Pond diluted 50%.

Carbonate concentrations were also determined, but were all below the limit for quantitation (0.1 mg/L) so were not tabulated. 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 five test waters 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) |
| Control | | | | | | |
| | <1. | <0.702 | a | 10 | 11.2 | 13.4 |
| | <1. | <0.702 | b | 10 | 10.7 | 13.9 |
| | <1. | <0.702 | c | 10 | 11.1 | 13.4 |
| | <1. | <0.702 | d | 10 | 10.8 | 13.6 |
| | <1. | <0.702 | e | 10 | 8.8 | 12.9 |
| | <1. | <0.702 | f | 10 | 10.6 | 13.4 |
| | <1. | <0.702 | g | 9 | 10.5 | 13.6 |
| | <1. | <0.702 | h | 9 | 10.6 | 13.7 |
| De Beque | | | | | | |
| | <1. | <0.702 | a | 9 | 12.0 | 14.3 |
| | <1. | <0.702 | b | 10 | 11.5 | 13.9 |
| | <1. | <0.702 | c | 10 | 11.3 | 13.6 |
| | <1. | <0.702 | d | 10 | 12.8 | 14.5 |
| | <1. | 2.10 | a | 10 | 12.0 | 14.0 |
| | <1. | 2.10 | b | 10 | 10.6 | 14.0 |
| | <1. | 2.10 | c | 10 | 10.2 | 13.6 |
| | <1. | 2.10 | d | 10 | 10.2 | 13.2 |
| Orchard Mesa | | | | | | |
| | 5.43 | <0.702 | a | 10 | 12.7 | 13.9 |
| | 5.43 | <0.702 | b | 10 | 11.3 | 13.4 |
| | 5.43 | <0.702 | c | 10 | 11.0 | 13.5 |
| | 5.43 | <0.702 | d | 10 | 12.4 | 14.3 |
| | 5.43 | 4.83 | a | 9 | 10.8 | 13.7 |
| | 5.43 | 4.83 | b | 10 | 9.0 | 13.5 |
| | 5.43 | 4.83 | c | 10 | 9.9 | 13.0 |
| | 5.43 | 4.83 | d | 8 | 10.9 | 13.8 |

Table continued on next page.

**Table A3.** Continued.

| Selenium exposure | | | | | |
|---|---|---|---|---|---|
| Water (µg/L) | Diet (µg/g) | Number Replicate | Average surviving | Average mass (mg) | TL (mm) |
| North Pond 50% dilution | | | | | |
| 10.6 | <0.702 | a | 10 | 11.7 | 14.2 |
| 10.6 | <0.702 | b | 10 | 12.2 | 14.4 |
| 10.6 | <0.702 | c | 10 | 12.5 | 14.4 |
| 10.6 | <0.702 | d | 10 | 13.2 | 14.0 |
| 10.6 | 12.0 | a | 10 | 9.7 | 13.5 |
| 10.6 | 12.0 | b | 10 | 10.0 | 13.6 |
| 10.6 | 12.0 | c | 9 | 11.2 | 13.9 |
| 10.6 | 12.0 | d | 10 | 9.5 | 13.0 |
| North Pond | | | | | |
| 20.3 | <0.702 | a | 10 | 13.9 | 14.0 |
| 20.3 | <0.702 | b | 10 | 13.3 | 14.6 |
| 20.3 | <0.702 | c | 10 | 11.8 | 13.7 |
| 20.3 | <0.702 | d | 10 | 11.8 | 13.8 |
| 20.3 | 21.8 | a | 10 | 10.4 | 12.9 |
| 20.3 | 21.8 | b | 10 | 11.5 | 13.8 |
| 20.3 | 21.8 | c | 10 | 11.9 | 14.3 |
| 20.3 | 21.8 | d | 10 | 10.9 | 14.0 |