

Rapid Communication

# Selenium impacts on razorback sucker, Colorado: Colorado River III. Larvae

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## Abstract

Razorback sucker (*Xyrauchen texanus*) larvae from adults exposed to selenium at three sites near Grand Junction, Colorado, for 9 months were used in a 30-day waterborne and dietary selenium study. Selenium concentrations in water averaged < 1.6 µg/L from 24-Road, 0.9 µg/L from Horsethief, 5.5 µg/L from Adobe Creek, and 10.7 µg/L from the North Pond. Selenium in dietary items averaged 2.7 µg/g in brine shrimp, 5.6 µg/g in zooplankton from Horsethief east wetland, 20 µg/g in zooplankton from Adobe Creek, and 39 µg/g in zooplankton from North Pond. The lowest survival occurred in larvae fed zooplankton rather than brine shrimp. Survival of larvae at Adobe Creek and North Pond was lower in site water than in reference water. Survival of brood stock larvae was higher than Horsethief larvae even though they received the same water and dietary treatments. Arsenic concentrations in brine shrimp may have resulted in an antagonistic interaction with selenium and reduced adverse effects in larvae. Deformities in larvae from North Pond were similar to those reported for selenium-induced teratogenic deformities in other fish species. Selenium concentrations of ≥ 4.6 µg/g in food resulted in rapid mortality of larvae from Horsethief, Adobe Creek, and North Pond, and suggested that selenium toxicity in the Colorado River could limit recovery of this endangered fish.

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## 1. Introduction

The razorback sucker (*Xyrauchen texanus*) was considered common in the upper and lower Colorado River basins in historical times, but since the 1940s has become rare, with the remaining populations in the Green River and Lakes Mead and Mohave (Bestgen, 1990). The population of razorback sucker in the middle Green River basin in Utah has been estimated at about 1000 individuals in 1988 (Lanigan and Tyus, 1989) and at 300 to 600 in 1992 (Modde et al., 1996). Razorback sucker are rare in the upper Colorado River, where only 10 fish were found in the river between 1989 and 1996 (C. McAda, USFWS, personal communication).

Adult razorback suckers, some with low and some with high concentrations of selenium, have been collected at Razorback Bar, the best-known spawning site in the Green River, Utah (Stephens and Waddell, 1998). Spawning of razorback sucker has been somewhat successful because razorback sucker larvae have been collected during several years of monitoring. During the period 1992–1996, 1735 larvae were collected in the middle Green River and 440 larvae were collected in the lower Green River (Muth et al., 1997). In 1994, 11 yearlings were captured in Leota Bottoms on the Green River (Modde and Wick, 1997), and in 1993, 1 yearling was captured in the Yampa River (T. Modde, USFWS, written communication).

Elevated selenium residues have been reported in wild razorback sucker larvae collected from the middle Green River (Hamilton et al., 2000a) and it was

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speculated that these residues were sufficiently elevated to cause adverse effects in the larvae. Hamilton (1999) presented historical information on the Colorado River basin linking selenium concentrations, irrigation activities, and native fish declines. He hypothesized that historical selenium contamination from agricultural irrigation activities in the period 1890 to 1910 caused the decline of native fish inhabiting big rivers such as Colorado pikeminnow (*Ptychocheilus lucius*) and razorback sucker, and possibly others such as bonytail (*Gila elegans*), in the period 1910 to 1920 in the upper basin and in the period 1925 to 1935 in the lower basin. In this scenario, selenium exposure would not have affected adult spawning behavior, but would have adversely affected survival and recruitment of young razorback sucker to the population.

The present study was conducted at three sites near Grand Junction, Colorado, in the general area where razorback sucker have historically been observed. Adult razorback sucker were held in three sites for 9 months as part of an exposure to selenium and other inorganic elements (Hamilton et al., 2004a). After exposure, adults were spawned in late spring when water temperatures reached a level associated with naturally spawning razorback sucker and tests were conducted with eggs (Hamilton et al., 2004b). The current study was conducted with 5-day larvae from the exposed adults to determine the effects of water and dietary selenium exposure on survival and growth of larvae.

## 2. Methods and materials

Site description, methods, and results from Part I of this study (i.e., adult stocking and exposure and measurement of residues in water, sediment, aquatic invertebrates, and fish tissues) for the adult exposure were provided in Hamilton et al. (2004a). Methods and results from Part II for the egg study were provided in Hamilton et al. (2004b).

Four sources of larvae were used in tests. The sources were adults from Horsethief Canyon State Wildlife Area (HT), Adobe Creek (AC), and North Pond (WW) at Walter Walker State Wildlife Area and brood stock from HT. Larvae were separated by parental exposure and individual spawn and held at 24-Road Fish Hatchery (24-Road) for about 4 days before being transported to a mobile laboratory for the larval fish study.

For the three HT spawns (fish HT41, HT44, HT57), selenium concentrations in eggs were 6.6, 6.2, 5.8  $\mu\text{g/g}$ , egg diameters were 2.3, 2.4, 2.4 mm, and selenium concentrations in adult muscle plugs at spawning were 5.0, 3.4, 4.6  $\mu\text{g/g}$ , respectively (Hamilton et al., 2001a). For the three AC spawns (fish AC19, AC20, AC28), selenium concentrations in eggs were 43.4, 54.5, 38.0  $\mu\text{g/g}$ ,

egg diameters were 2.4, 2.3, 2.3 mm, and selenium concentrations in adult muscle plugs at spawning were 12.9, 11.7, 11.5  $\mu\text{g/g}$ , respectively (Hamilton et al., 2001a). For the three WW spawns (fish WW2, WW21, WW54), selenium concentrations in eggs were 37.2, 35.3, 34.3  $\mu\text{g/g}$ , egg diameters were 2.2, 2.2, 2.2 mm, and selenium concentrations in adult muscle plugs at spawning were 14.6, 17.3, 14.1  $\mu\text{g/g}$ , respectively (Hamilton et al., 2001a). For the three brood stock spawns, the female with passive integrated transponder (PIT) number 7F7D133914 did not have selenium measured in eggs nor egg diameters measured, but the selenium concentration in muscle plug at spawning was 13.1  $\mu\text{g/g}$ ; the female with PIT 7F7D163436 did not have the egg diameter measured, but the selenium concentration in eggs was 7.1  $\mu\text{g/g}$  and in muscle plug at spawning was 13.8  $\mu\text{g/g}$ ; and the female with PIT 7F7F365275 did not have selenium measured in eggs, but egg diameter was 2.9 mm and the selenium concentration in muscle plug at spawning was 2.6  $\mu\text{g/g}$  (Hamilton et al., 2001a).

### 2.1. Larvae test

Survival, growth, abnormal development, and whole-body residues of selenium in larvae were assessed after 30 days exposure to selenium in water and food. The study was initiated with 5-day-old larvae from three spawns from each of the four sources. Larvae from each spawn were impartially distributed two at a time to eight exposure vessels until there were 10 larvae per 2-L beaker (4 sources  $\times$  3 spawns/source  $\times$  8 vessels/spawn = 96 vessels  $\times$  10 larvae/vessel = 960 larvae). The larvae were initially held in beakers containing 800 mL of filtered (25  $\mu\text{m}$  polypropylene filter bag, Filter Specialists, Inc., Michigan City, Indiana) 24-Road water. After 4 h, an additional 800 mL of test water was added. Fifty percent of the water (800 mL) was renewed daily.

Four exposure beakers with larvae from each spawn and source were held on each of two tables in the mobile laboratory. The 48 beakers were arranged in three rows of 16 beakers on each table and their position randomly assigned. Two beakers of larvae from each spawn and source were given one of four treatments: (1) reference food (brine shrimp) and reference water (24-Road), (2) reference food and site water where adults were held, (3) site food (zooplankton) where adults were held [except larvae from HT adults and brood stock adults, which were fed zooplankton collected from a wetland located adjacent to and east of the HT ponds and whose only water source was effluent from the ponds, termed Horsethief east wetland (HTEW, Fig. 2 in Hamilton et al., 2004a)] and reference water, and (4) site food and site water. The reference water treatments were held on one table and the site water treatments were held on the other table.

Zooplankton were collected every other day from HTEW, AC, and WW using modified light traps (Espinosa and Clark, 1972). Light traps were set overnight, collected the following morning, and held in 3.8-L plastic jars filled with site water. Oxygen concentrations in the jars of zooplankton were supplemented by bubbling compressed air from an oilless air compressor through air stones.

Larvae were fed either 40 zooplankters (site food: predominately cladocerans and copepods) or 40 brine shrimp (reference food: *Artemia* sp.) nauplii per live fish once daily after water renewal. For site food, zooplankton were sieved through a US Standard Sieve #40 to separate organisms <0.425 mm in size for feeding test fish that day. The concentration of zooplankters (number of organisms per mL) in the feeding solutions was determined by counting the number of zooplankters in three separate 1-mL samples taken with a Hensen-Stemple pipette. The zooplankton were anesthetized with 1 mL of alcohol and counted using a stereoscope microscope at magnification  $\times 7$ –30 and a counting wheel (Ward's, Rochester, NY). The number of organisms in three replicate counts were averaged and the volume calculated to feed 40 organisms per live fish in each exposure beaker. A 2-mL subsample of live zooplankton was collected from each feeding solution, placed in a vial, and preserved in 70% ethyl alcohol. The zooplankton in these archived samples were categorized as cladoceran, copepod, or other invertebrate type using a stereoscopic microscope.

Brine shrimp cysts (*Artemia* sp., Aquarium Products, Glen Burnie, MD, USA; source Columbia, S.A.) were hatched in reconstituted seawater at 35 g/L salinity prepared by adding Instant Ocean seasalts to deionized water according to standard methods (ASTM, 1992). Jars containing the hatching media were aerated with air from an oilless compressor, and the jars were held in a water bath at 25 °C. One-day-old nauplii of brine shrimp were enumerated and fed at the same number of organisms per fish as described above. Aquatic invertebrates and brine shrimp not used for feeding were sieved from the media with a 153- $\mu$ m nylon screen, placed in Whirl-Pak bags, and stored frozen for selenium and inorganic element analyses as described in Hamilton et al. (2004a).

The number of live zooplankton in exposure beakers from feeding the previous day was grossly quantified daily prior to feeding fresh zooplankton. The number of live zooplankton were categorized as few (1–10 organisms), some (11–20 organisms), or many (>20 organisms).

Test waters for the study were collected every day from each site as grab samples using two 19-L carboys. Water was filtered through 25- $\mu$ m polypropylene filter bags to remove particulate matter and poured into large plastic buckets prior to use in water quality analyses and

water renewal in exposure vessels. Water was sampled weekly and analyzed for general water quality characteristics as described in Hamilton et al. (2004a). Water quality measurements included pH, conductivity, hardness, alkalinity, calcium, magnesium, chloride, ammonia, nitrate, nitrite, sulfate, total suspended solids, volatile solids, and fixed solids. One filtered (0.4  $\mu$ m) and one unfiltered water sample was collected at 3- to 7-day intervals from waters used in the renewals and analyzed for selenium concentrations, and filtered water sample was collected for analysis of inorganic elements as described in Hamilton et al. (2004a).

Water renewal in the study was accomplished by siphoning the water level down to the 800-mL calibration line using a siphon held inside a glass sleeve with the submerged end covered with a polypropylene filter cloth (285- $\mu$ m openings) attached with a latex band. The sleeve was used to prevent larvae from being siphoned out of the beaker and restrict the loss of zooplankton fed the previous day. The siphon was held stationary in the beaker to minimize disturbance of larvae and to decrease the likelihood of siphoning out small zooplankton through the filter cloth. Exposure beakers were cleaned infrequently prior to water renewal using a small brush to remove algae or other organisms on the walls. Oxygen concentrations in exposure water were supplemented by passing compressed air from an oilless air compressor through air stones.

The number of live fish in each beaker were recorded daily and all dead fish removed. Observations of fish behavior and the amount of uneaten, live zooplankton were recorded daily. Dissolved oxygen concentration and water temperature were measured daily in two replicates (one replicate with reference food and one with site food) of each water type (24-Road, HT, AC, or WW) where live fish were present. After the 30-day exposure period, the surviving fish were anesthetized with MS-222 (tricaine methanesulfonate) and measured for total length to nearest mm and weighed to the nearest 0.0001 g. Four fish from each treatment were collected as a composite sample, placed in Whirl-Pak bags, and stored frozen until analysis of selenium concentrations by neutron activation as described in Hamilton et al. (2004a). Six of the 60 samples collected contained one (one sample), two (two samples), or three (three samples) fish because there were insufficient larvae in these beakers after 30 days of exposure. Because of a rapid die-off of larvae in site food treatments, one fish from each replicate of the three spawns in a treatment were collected (composite of six larvae) at day 6 for WW larvae, day 7 for HT and AC larvae, and day 12 for brood stock larvae. Samples were placed in Whirl-Pak bags and stored frozen until selenium analysis by neutron activation.

Temperature in exposure beakers was maintained at ambient air temperature and measured daily with a

precision grade mercury thermometer. Fish were exposed under fluorescent lighting (one cool-white bulb and one wide-spectrum bulb in each light fixture) to a photoperiod that existed at the time of testing in Grand Junction and approximated 12 h light: 12 h dark.

Methods for statistical analysis were the same as those described in Hamilton et al. (2004a). In addition, the predicted time to death and 95% confidence intervals were calculated for each test group of larvae and treatment using S-Plus statistical software (Mathsoft, Seattle, WA). Daily mortality data was fitted to parametric proportional hazard regression model using a Weibull distribution. Both right and interval censoring of survival time data was accounted for in the statistical methodology. The predicted time-to-S% mortality was calculated for S = 50%, 90%, and 95%.

## 2.2. Larvae growout

Larvae that were hatched (first hatching on May 12) and held at 24-Road (first feeding May 21, 9 days old), but not used in the exposure study, were combined by adult exposure from all available spawns at HT, AC, or WW sites. Observations of these larvae allowed an evaluation of survival, growth, and selenium residues after they were held under hatchery culture conditions at 24-Road. This information was used for comparison to survival, growth, and residue measurements in the exposure study. These three sources (HT, AC, WW) were reduced to 1000 individuals per site on June 12 (larvae 31 days old), and each source was held in a separate 1.3-m-diameter tank at 24-Road. Fish were fed at 7% of body weight initially, and then the feeding rate was gradually reduced to 4%. Larvae were fed Biokyowa (250- $\mu$ m size; BioProducts, Inc, Warrenton, OR) for the first week. During the second week, fish (17 days old) were fed a mixture of Biokyowa and a larval fish food obtained from the Bozeman Fish Technology Center (USFWS, Bozeman, MT). The Bozeman diet was fed until fish were 89 days old; then it was mixed with a locally produced, *Tilapia*-based (*Tilapia* sp.) diet (Mesa Feed and Farm Supply, Grand Junction, CO) and fed for 1 week. From age 96 to 134 days, fish were fed the *Tilapia*-based diet. A sample of the Bozeman diet, but not the Biokyowa diet or *Tilapia*-based diet, was collected for selenium analysis.

At 36, 78, 108, and 134 days of age, three 20-fish subsamples were collected at random, anesthetized with MS-222, measured for total length and weight, and deformities noted. Larvae from the same three brood stock spawns used in the larval fish exposure also were measured for total length and weight at the same time as the other larvae. At 134 days of age, three composite samples of 16–20 fish were collected from HT, AC, and WW larvae, and five composites from brood stock

larvae for analysis of selenium concentrations. A composite sample of brood stock larvae was also collected at 190 days of age for analysis of selenium concentrations.

## 3. Results

### 3.1. Water quality

Water quality characteristics were consistent within water types during the study, but differed significantly among the four water types (24-Road, HT, AC, WW) (Table 1). WW water had the highest conductivity, hardness, calcium, magnesium, chloride, and sulfate, whereas AC water had the highest alkalinity, and HT water had the highest total suspended and fixed solids. Water from 24-Road had characteristics similar to those of that from HT, except for calcium, alkalinity, and total suspended, volatile, and fixed solids, all of which were higher at HT. In the exposure beakers, the mean dissolved oxygen concentrations in the four waters ranged from 7.3 to 7.4 mg/L. The mean water temperature in the exposure beakers for the four water types ranged from 20.1 to 20.4 °C.

### 3.2. Selenium and other elements in water and zooplankton

No significant difference in selenium concentration occurred between filtered and unfiltered water samples or between water samples taken from stations within a site (i.e., between HT<sub>i</sub> and HT<sub>o</sub>; among AC<sub>1</sub>, AC<sub>2</sub>, and AC<sub>3</sub>; between WW<sub>1</sub> and WW<sub>2</sub>), and consequently, the data were combined for statistical analysis. Mean selenium concentrations in water during the larvae exposure were 0.9  $\mu$ g/L for HT, 5.5  $\mu$ g/L for AC, and 10.7  $\mu$ g/L for WW, whereas concentrations were <1.6  $\mu$ g/L for 24-Road water (Table 2). Waterborne selenium concentrations at the HT, AC, and WW sites were significantly different from each other. Just prior to the beginning of the exposure, selenium concentrations in water were <1.6  $\mu$ g/L at HT, 7.4  $\mu$ g/L at AC, and 11.9  $\mu$ g/L at WW.

Waterborne concentrations of the inorganic elements that seemed to differ between the AC and WW sites included antimony, boron, calcium, potassium, lithium, magnesium, sodium, and strontium (Table 3). Concentrations of the same elements in water were also found to be significantly different between the AC and WW sites in the adult study (Hamilton et al., 2001a).

Mean selenium concentrations in zooplankton during the larvae exposure were 5.6  $\mu$ g/g at HTEW, 20  $\mu$ g/g at AC, and 39  $\mu$ g/g at WW (Table 4). Selenium concentrations in brine shrimp averaged 2.7  $\mu$ g/g (range

Table 1  
Mean (standard error in parentheses and number of samples in brackets) water quality characteristics measured in test water collected from four sites

Measure	Sites			
	HT	AC	WW	24-Road
pH	8.5b (0.1) [9]	7.9a (0.1) [9]	8.5b (0.1) [9]	7.9a (0.1) [8]
Conductivity (µmhos/cm)	424ab (13) [9]	939b (59) [9]	4,810c (250) [9]	237a (25) [8]
Hardness (mg/L as CaCO <sub>3</sub> )	156a (4) [9]	313b (18) [9]	1,300c (68) [9]	68a (2) [8]
Calcium (mg/L)	44b (1) [9]	76c (4) [9]	143d (5) [9]	22a (0) [8]
Magnesium (mg/L)	11a (0) [9]	31a (3) [9]	229b (13) [9]	3a (0) [8]
Alkalinity (mg/L as CaCO <sub>3</sub> )	102b (2) [9]	148c (14) [9]	111b (2) [9]	58a (3) [8]
Chloride (mg/L)	24ab (1) [9]	72b (6) [9]	425c (24) [9]	12a (7) [8]
Sulfate (mg/L)	87a (4) [8]	247a (15) [8]	2,080b (110) [8]	36a (1) [8]
Un-ionized ammonia (mg/L NH <sub>3</sub> -N)	<0.01 (0) [8]	<0.01 (0) [8]	<0.01 (0) [8]	<0.01 (0) [7]
Nitrate (mg/L NO <sub>3</sub> -N)	0.2a (0.1) [2]	0.1a (0.1) [2]	0.1a (0) [2]	0.1a (0.1) [2]
Nitrite (mg/L NO <sub>2</sub> -N)	0.01a (0) [3]	0.01a (0) [3]	0.01a (0.01) [3]	0.01a (0) [3]
Total suspended solids (mg/L)	14.7c (2.4) [7]	5.4ab (1.4) [7]	8.8bc (3) [7]	0.4a (0.1) [7]
Volatile solids (mg/L)	3.0b (0.2) [7]	1.5a (0.2) [7]	3.0b (0.6) [7]	0.6a (0.2) [7]
Fixed solids (mg/L)	11.7b (2.2) [7]	3.9a (1.3) [7]	5.7ab (2.4) [7]	0.1a (0.1) [7]

Note. For each measure, sites with the same letter are not significantly different.

2.5–2.8 µg/g, *n* = 6). Selenium concentrations in zooplankton at the HTEW, AC, and WW sites were significantly different from each other. Just prior to the beginning of the exposure, mean selenium concentrations in zooplankton were 6.2 µg/g at HTEW, 17 µg/g at AC, and 29 µg/g at WW. The correlation coefficient

Table 2  
Mean (standard error in parentheses, *n* = 2) selenium concentration (µg/L) in water collected from four sites near Grand Junction, Colorado

Station	Day of exposure						
	–4	3–4 <sup>a</sup>	8	11	18	24–25 <sup>a</sup>	30
24-Road	<1.6 <sup>b</sup> (–)	<1.5 (–)	<1.5 (–)	<1.5 (–)	<1.5 (–)	<1.6 (–)	<1.5 (–)
HT1	<1.6 (–)	0.7 (–)	0.8 (–)	0.8 (–)	<1.5 (–)	<1.2 (–)	<1.5 (–)
AC1	— <sup>c</sup>	2.8 (0.3)	—	—	—	4.2 (0.3)	—
AC2	—	2.5 (–)	—	—	—	6.1 (0.2)	—
AC3	7.4 (0.4)	3.9 (0.1)	3.4 (0.7)	2.6 (0.1)	2.4 (0.1)	10.4 (0.3)	10.4 (0.4)
WW1	11.9 (0.2)	13.1 (0.2)	12.0 (0.1)	11.1 (0.1)	10.8 (0.6)	9.2 (1.1)	8.1 (0.2)
WW2	—	10.7 (0.7)	—	—	—	10.6 (–)	—

Note. Waters used in the study were 24-Road, HT1, AC3, and WW1; other waters were collected to determine the variability in concentrations at the site

<sup>a</sup>Water samples at HT and AC were collected on days 4 and 25, and at WW on days 3 and 24.

<sup>b</sup><:below limit of detection.

<sup>c</sup>—: no sample.

between selenium concentrations in zooplankton and water was *r* = 0.90 (*P* < 0.0001, *n* = 68).

None of the inorganic elements measured in zooplankton were different among sites (Table 5). Copper concentrations in zooplankton were the highest at HTEW, intermediate at WW, and lowest at AC. The highest concentrations of aluminum, iron, and vanadium in zooplankton occurred at AC, whereas the highest concentrations of potassium, magnesium, manganese, and sodium in zooplankton occurred at WW. There was no consistent pattern among sites for the concentrations of these elements in zooplankton because sometimes HTEW had intermediate concentrations compared to AC and WW (aluminum, iron, potassium, vanadium), and other times AC had intermediate concentrations compared to HTEW and WW (magnesium, manganese).

### 3.3. Food utilization

Cladocerans predominated at HTEW and AC, whereas copepods predominated part of the time at WW. The ratio of cladocerans to copepods in various collections ranged from 0.14 to 72 at HTEW, 0.05 to 61 at AC, and 0.05 to 7 at WW. Algal and periphyton growth on the walls of test beakers was readily apparent, and the walls were brushed infrequently to remove the algal buildup, which also probably was a source of

Table 3  
Concentration of inorganic elements (mg/L) in water collected from AC stations on day 3 and WW stations on day 4 near Grand Junction, Colorado

Element	Station				
	AC1	AC2	AC3	WW1	WW2
Ag	0.008	0.007	<0.005 <sup>a</sup>	<0.005	<0.005
Al	<0.03	<0.03	<0.03	<0.03	<0.03
As	<0.02	<0.02	<0.02	<0.02	<0.02
B	0.061	0.059	0.055	0.290	0.290
Ba	0.133	0.093	0.111	0.074	0.072
Be	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
Bi	<0.04	<0.04	<0.04	<0.04	<0.04
Cd	85.8	74.9	67.4	175	169
Cd	<0.002	<0.002	<0.002	<0.002	<0.002
Co	0.003	0.004	0.003	<0.003	<0.003
Cr	0.009	0.008	<0.008	0.022	0.010
Cu	<0.005	<0.005	<0.005	<0.005	<0.005
Fe	0.086	0.076	0.067	0.018	0.010
K	4.4	3.2	2.4	6.4	7.0
Li	0.026	0.029	0.028	0.100	0.100
Mg	29.5	27.8	26.9	295	287
Mn	0.110	0.089	0.067	0.075	0.058
Mo	0.010	0.009	0.009	0.018	0.017
Na	76.4	75.3	71.2	846	824
Ni	0.008	<0.006	0.008	<0.006	<0.006
P	0.10	0.08	0.09	0.18	0.17
Pb	<0.007	0.042	0.032	0.010	<0.007
Sb	0.04	<0.03	<0.03	0.08	0.08
Si	1.3	0.7	0.8	0.8	0.8
Sr	0.85	0.80	0.75	1.89	1.85
Ti	<0.0009	<0.0009	<0.0009	<0.0009	<0.0009
Tl	<0.1	0.2	0.2	<0.1	<0.1
W	0.01	<0.01	<0.01	<0.01	<0.01
V	0.007	<0.005	<0.005	<0.005	<0.005
Zn	0.01	0.02	0.02	<0.01	<0.01

<sup>a</sup> <:below limit of detection.

Table 4  
Mean (standard error in parentheses and number of samples in brackets) selenium concentration (µg/g dry weight) in zooplankton collected from three sites near Grand Junction, Colorado

Site	Day of exposure <sup>a</sup>					
	–36 to –45	2–6	7–12	13–18	19–24	25–30
HTEW	6.2 (1.0) [2]	4.6 (0.2) [4]	5.2 (0.2) [3]	4.8 (0.4) [5]	5.3 (0.3) [6]	7.4 (0.2) [6]
AC	17.3 (1.2) [2]	22.6 (0.5) [4]	20.5 (0.5) [3]	19.3 (0.5) [5]	19.3 (1.5) [6]	18.4 (0.6) [6]
WW	29.4 (3.9) [2]	36.1 (2) [3]	38.6 (2.2) [3]	38.2 (2.4) [4]	41.1 (1.2) [5]	40.8 (3.1) [5]

<sup>a</sup> Zooplankton samples were arbitrarily grouped into 5–6-day intervals.

nutrition and inorganic elements, such as selenium, for the larvae. Some small food organisms such as algae, zooplankton parts, and detritus were also present in the

Table 5  
Mean (standard error in parentheses and number of samples in brackets) concentration of inorganic elements (µg/g dry weight) in zooplankton collected from three sites near Grand Junction, Colorado

Element	Site		
	HTEW	AC	WW
Ag	<1 <sup>a</sup>	1.0 (–) [1] <sup>b</sup>	<1
Al	1637 (190)ab [4]	2145 (424)b [4]	927 (153)a [4]
As	6.0 (1.0) [2]	6.5 (0.5) [2]	7.5 (1.5) [2]
B	2.4 (0.4) [4]	2.4 (0.4) [4]	2.4 (0.9) [3]
Ba	24 (4) [4]	29 (2) [4]	17 (2) [4]
Be	0.04 (0) [2]	0.07 (0.02) [3]	0.07 (–) [1]
Ca	10,312 (1625) [4]	15,000 (1773) [4]	12,260 (3407) [4]
Cd	<0.2 [1]	0.2 (–) [1]	0.4 (0.1) [2]
Co	0.5 (0.1) [3]	0.7 (0.1) [4]	0.7 (0.1) [4]
Cr	1.5 (0.3) [4]	3.5 (0.5) [2]	1.0 (–) [1]
Cu	21 (3)a [4]	11 (0)b [4]	15 (2)ab [4]
Fe	814 (95)ab [4]	1430 (256)b [4]	759 (79)a [4]
K	4075 (718)a [4]	3075 (433)a [4]	8875 (131)b [4]
Li	1.6 (0.2) [4]	1.7 (0.4) [4]	1.2 (0.2) [4]
Mg	1292 (40)a [4]	1772 (91)b [4]	1812 (55)b [4]
Mn	52 (5)a [4]	121 (11)b [4]	201 (8)c [4]
Mo	1.0 (–) [1]	1.0 (0) [4]	1.3 (0.4) [3]
Na	1222 (293)a [4]	1210 (263)a [4]	3105 (144)b [4]
Ni	1.5 (0.5) [2]	1.0 (–) [1]	1.8 (0.3) [4]
P	12,100 (471) [4]	11,750 (494) [4]	11,500 (58) [4]
Pb	<4 [2]	5.5 (0.5) [2]	<4
Si	207 (33) [4]	195 (39) [4]	119 (35) [4]
Sr	60 (9) [4]	101 (14) [4]	76 (15) [4]
Ti	26 (5) [4]	44 (8) [4]	23 (2) [4]
V	3.0 (0.4)ab [4]	5.3 (0.9)b [4]	2.3 (0.3)a [4]
Zn	92 (5) [4]	114 (12) [4]	178 (47) [4]

Note. For each element, sites with lower case letter in common are not significantly different ( $P=0.05$ ). For all sites, Bi < 5, Sb < 9, Tl < 35, W < 3.

<sup>a</sup> <:below limit of detection.

<sup>b</sup> The number of samples submitted for analysis was 4. If the number of samples shown for a site and element is less than 4; concentrations in the other samples were below the limit of detection.

site water treatments after use of the filter bags and were available to larvae.

No live brine shrimp were observed after about 3–4 h following feeding, which was probably caused by the osmotic shock of placing them in freshwater (cultured in 35 g/L salinity water). A few dead brine shrimp were observed on the bottom of some exposure beakers. Anywhere from a few (1–10 organisms) to many (>20 organisms) zooplankters were observed in the beakers prior to feeding for those treatments receiving site food collected from HTEW, AC, and WW.

### 3.4. Selenium in larvae

Concentrations of selenium measured in larvae between days 6 and 12 of exposure (Table 6) were similar to those measured in eggs (6.5 µg/g at HT, 46.5 µg/g at AC, 37.8 µg/g at WW, 6.0 µg/g for brood stock; Hamilton et al., 2004b). After 30 days of exposure in both the reference food–reference water and reference food–site water treatments, larvae from brood stock and from HT adults had less selenium than larvae from AC and WW adults. However, in both reference food treatments there was no difference in selenium concentrations between larvae from brood stock and HT adults or between larvae from AC adults and WW adults.

Table 6  
Mean (standard error in parentheses and number of samples in brackets) selenium concentration (µg/g dry weight) in razorback sucker larvae (reference [RF] or site [SF] food and either reference [RW] or site [SW] water)

Day of exposure	Site	Treatment			
		RF/RW	RF/SW	SF/RW	SF/SW
7	HT	7.3	7.9	8.8	8.6
7	AC	32.1	32.8	69	70.6
6	WW	39.7	46.9	28.3	44.5
12	BS <sup>a</sup>	7.2	6.8	8.1	9.6
30	HT	3.3aX (0.2) [6]	5.1aY (0.3) [6]	— <sup>b</sup>	—
		AC	7.7bX (0.3) [6]	12.7bY (1.2) [6]	—
	WW	9.7bX (0.9) [6]	15.2bY (0.8) [5]	—	—
	BS	3.6aX (0.2) [6]	5.2aXY (0.3) [6]	5.4XY (0.5) [6]	6.9Y (0.6) [5]

Note. For treatments RF/RW and RF/SW at 30 days of exposure (within column), values with a lower case letter in common are not significantly different ( $P = 0.05$ ). For each site at 30 days of exposure (within row), treatments with upper case letters in common are not significantly different ( $P = 0.05$ ). At days 6, 7, and 12,  $n = 1$ .

<sup>a</sup>BS: brood stock.

<sup>b</sup>No live fish.

Insufficient larvae were available in the site food treatments to measure selenium concentrations in larvae from adults held at HT, AC, and WW. However, significantly more selenium was found in larvae from adults held at HT, AC, and WW and exposed to the reference food–site water treatment than those exposed to the reference food–reference water treatment. At 30 days of exposure, significantly more selenium was found in larvae from brood stock in the site food–site water treatment than in the reference food–reference water treatment.

Larvae from AC adults and WW adults had substantially lower selenium concentrations at 30 days of exposure than at 7 days of exposure (Table 6). For example, larvae from AC adults exposed to the reference food–reference water treatment had 32.1 µg/g selenium at day 7, which decreased to 7.7 µg/g at day 30. Likewise, larvae from WW adults had 39.7 µg/g in the reference food–reference water treatment at day 6, which decreased to 9.7 µg/g at day 30.

Selenium concentrations in larvae from HT adults and brood stock were not significantly correlated with either waterborne or zooplankton concentrations of selenium. However, based on multiple regression analysis, selenium concentrations in these larvae were significantly associated with median zooplankton selenium concentrations and the mean waterborne selenium concentrations (two-variable model,  $r^2 = 0.99$ ,  $P = 0.0006$ ). For the combined four groups of larvae in the reference food–reference water and reference food–site water treatments, selenium concentrations in larvae were significantly correlated with mean waterborne selenium concentrations ( $r = 0.87$ ,  $P = 0.001$ ).

### 3.5. Growth

After 30 days of exposure in the reference food–reference water treatment, larvae from brood stock were significantly heavier and longer than larvae from HT, AC, and WW adults (Table 7). In the reference food–site water treatment, larvae from brood stock were significantly longer than larvae from the other adults, and heavier than those from HT and WW adults. Insufficient larvae were available from the site food treatments after 30 days to compare length and weight measurements.

### 3.6. Survival

Good survival occurred among razorback sucker larvae in all four groups in the reference food (brine shrimp; 2.7 µg/g)–reference water (24-Road; <1.6 µg/L) treatment (Figs. 1 and 2). In this treatment, survival of larvae from brood stock (89%) and HT adults (87%) was higher than those from AC adults (84%) or WW adults (75%) after 30 days of exposure (Fig. 1A). This

high survival of larvae suggested that the reference food and reference water treatment was adequate to maintain razorback sucker larvae including those from AC and WW adults.

Table 7  
Mean (standard error in parentheses) total length (mm) and weight (g) of razorback sucker larvae (reference [RF] or site [SF] food and either reference [RW] or site [SW] water)

Measure	Site	Treatment			
		RF/RW	RF/SW	SF/RW	SF/SW
Total length	HT	11b (0.2)	11b (0.2)	— <sup>a</sup>	—
	AC	11b (0.1)	11b (0.2)	12 <sup>b</sup>	—
	WW	11b (0.2)	11b (0.2)	—	—
	BS <sup>c</sup>	12a (0.1)	12a (0.1)	12 (0.1)	—
Weight	HT	0.0074b (0.0003)	0.0064b (0.0001)	—	—
	AC	0.0071b (0.0001)	0.0067ab (0.0004)	0.0080 <sup>b</sup>	—
	WW	0.0071b (0.0004)	0.0066b (0.0004)	—	—
	BS	0.0086a (0.0004)	0.0081a (0.0001)	0.0076 (0.0003)	—

Note. For each measure and treatment, values with a lower case letter in common are not significantly different ( $P = 0.05$ )

<sup>a</sup>No live fish.

<sup>b</sup> $n = 1$ .

<sup>c</sup>BS: brood stock.

In the reference food–site water treatment, survival of larvae from HT adults (89%) and brood stock (86%) was higher than that of larvae from AC adults (66%) or WW adults (54%) after 30 days of exposure (Fig. 1B). Selenium concentrations during the first 6 days were 3.9–7.4 µg/L in AC site water, and 11.9–13.1 µg/L in WW site water (Table 2), which may have allowed selenium bioaccumulation in algae and periphyton growing on beaker walls thus leading to a dietary exposure.

After 14 days of exposure to the site food–reference water treatment, survival of larvae from brood stock (78%) was higher than those from HT (0%), AC (2%), and WW (0%) adults (Fig. 1C). The sharp decline in survival of larvae from young adults held at the three sites started at about 5–6 days of exposure and occurred more rapidly in larvae from WW adults than in those from HT or AC adults. Selenium concentrations in zooplankton during that time period were 4.6 µg/g at HTEW, 22.6 µg/g at AC, and 36.1 µg/g at WW (Table 4). Larvae from brood stock also were fed the site food diet from the HTEW, but their survival was 62% after 30 days of exposure.

After 14 days of exposure to the site food–site water treatment, survival of larvae from brood stock (70%) was higher than those from HT (0%), AC (2%), and WW (2%) adults (Fig. 1D). The sharp decline in survival in larvae from young adults held at the three sites started at about 5–6 days of exposure, occurred more rapidly in larvae from WW adults than in those from HT or AC adults, and was similar to that observed

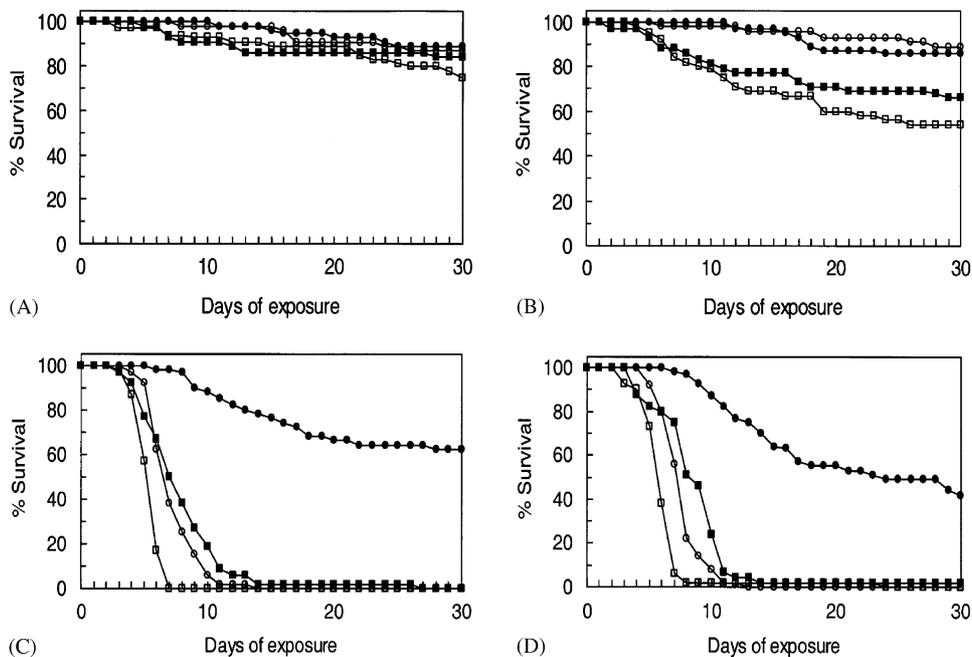


Fig. 1. Percent survival of razorback sucker larvae from four groups of adults ●: Brood stock, ○: Horseshoe Canyon State Wildlife Area, ■: Adobe Creek, and □: North Pond at Walter Walker State Wildlife Area) exposed to (A) reference food and reference water, (B) reference food and site water, (C) site food and reference water, or (D) site food and site water.

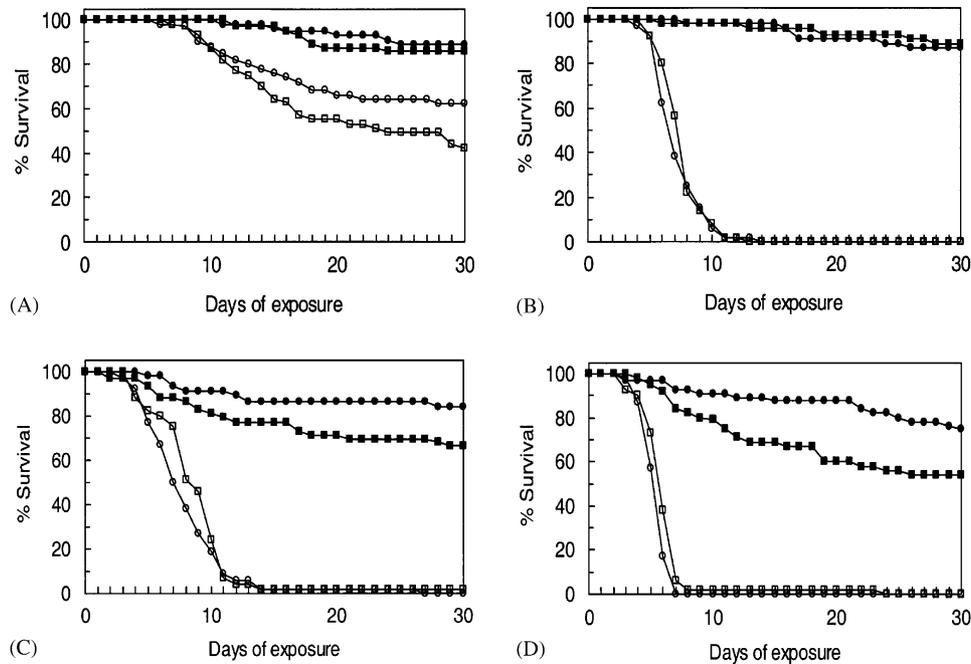


Fig. 2. Percent survival of razorback sucker larvae from four groups of adults (A) brood stock, (B) Horsethief Canyon State Wildlife Area, (C) Adobe Creek, and (D) North Pond at Walter Walker State Wildlife Area), exposed to (●) reference food and reference water, (○) site food and reference water, (■) reference food and site water, or (□) site food and site water.

in the site food–reference water treatment. After 30 days of exposure, survival of larvae from brood stock was lower in the site food–site water treatment (42%) compared to the reference food–site water treatment (62%).

The four treatments for the larvae discussed above are grouped by adult source in Fig. 2. Larvae from brood stock survived well in both reference food treatments (86–89%), but their survival was reduced in site food–reference water treatment (62%) and further reduced in the site food–site water treatment (42%) (Fig. 2A). Larvae from HT adults survived well in both reference food treatments (87–89%) but no larvae survived after 13 days in either site food treatment (Fig. 2B). Both brood stock larvae and HT larvae received the same site food, but their survival was substantially different in the site food treatments.

Larvae from AC adults survived well in the reference food–reference water treatment (84%), but their survival was reduced in reference food–site water treatment (66%). Survival of these larvae in both site food treatments decreased rapidly (50–75% at 7 days), which suggested elements in site food caused the mortality. Survival of larvae from WW adults was 75% in the reference food–reference water treatment, but was reduced to 54% in the reference food–site water treatment. Survival of WW larvae in either site food treatment decreased rapidly (0–6% at 7 days), similarly to that of larvae from HT and AC adults.

Prior to death, many of the larvae were swimming erratically or lying lethargic on the bottom of the exposure beaker. All larvae observed with scoliosis swam erratically. After death, many of the larvae were fed upon by copepods, which would recycle selenium into the food web within the beaker.

Combining the four groups of larvae, a significant negative correlation occurred between survival of larvae and selenium concentrations in larvae ( $r = -0.55$ ,  $P = 0.10$ ), which suggested a concentration response. A significant negative correlation occurred between survival of larvae and selenium in zooplankton ( $r = -0.70$ ,  $P = 0.003$ ), but not survival and selenium in water ( $r = -0.24$ ,  $P = 0.37$ ). Based on multiple regression analysis and combining the four groups of larvae, survival was significantly associated with selenium in zooplankton (one-variable model,  $r^2 = 0.46$ ,  $P = 0.03$ ) and with this same variable plus selenium concentrations in larvae (two-variable model,  $r^2 = 0.92$ ,  $P = 0.001$ ).

The predicted time to death of razorback sucker larvae for 50%, 90%, and 95% mortality are given in Table 8. At each mortality level, there were no significant differences in the predicted time to death among larvae in the reference food–reference water treatment. Nevertheless, Table 8 reveals a trend of highest survival in larvae from HT adults and brood stock, intermediate survival in larvae from AC adults, and lowest survival in larvae from WW adults. For each predicted mortality interval in the reference food–site

Table 8

Mean (95% confidence intervals in parentheses) of predicted time (days) to 50%, 90%, and 95% mortality of razorback sucker larvae (reference [RF] or site [SF] food and either reference [RW] or site [SW] water)

Predicted mortality	Treatment				
	Site	RF/RW	RF/SW	SF/RW	SF/SW
50%	HT	63.2aX (45.7–87.5)	68.2aX (48.0–96.9)	6.1bY (5.5–6.9)	6.3bYZ (5.7–7.1)
	AC	55.3aX (41.5–73.7)	37.1aYZ (30.4–45.2)	7.3bY (6.6–8.2)	7.3bY (6.6–8.2)
	WW	45.1aX (35.9–56.8)	29.6bZ (25.1–34.9)	4.4cZ (4.0–4.9)	5.6dZ (5.0–6.2)
	BS <sup>a</sup>	68.1aX (47.9–96.7)	59.1aXY (43.6–80.1)	34.0bX (28.3–40.9)	26.2bX (22.6–30.4)
90%	HT	107.0aX (77.2–148.4)	115.4aX (81.1–164.2)	10.4bY (9.3–11.6)	10.7bYZ (9.6–12.0)
	AC	93.6aX (70.1–124.8)	62.8aYZ (51.5–76.5)	12.4bY (11.1–13.9)	12.4bY (11.1–13.9)
	WW	76.4aX (60.6–96.2)	50.1bZ (42.4–59.1)	7.5cZ (6.7–8.4)	9.4dZ (8.4–10.5)
	BS	115.2aX (81.0–163.9)	100.0aXY (73.7–135.8)	57.6bX (47.9–69.2)	44.3bX (38.2–51.4)
95%	HT	120.1aX (86.6–166.6)	129.5aX (91.0–184.4)	11.7bY (10.4–13.1)	12.0bYZ (10.7–13.4)
	AC	105.0aX (78.7–140.1)	70.5aYZ (57.8–85.9)	13.9bY (12.5–15.6)	13.9bY (12.4–15.6)
	WW	85.7aX (68.0–108.1)	56.2bZ (47.6–66.4)	8.4cZ (7.5–9.4)	10.6dZ (9.5–11.8)
	BS	129.3aX (90.8–184.0)	112.3aXY (82.7–152.5)	64.6bX (53.7–77.7)	49.8bX (42.9–57.7)

Note. For each predicted mortality and site (within rows), values with a lower case letter in common are not significantly different ( $P = 0.05$ ). For each predicted mortality and treatment (within column), values with upper case letters in common are not significantly different ( $P = 0.05$ ). For the RF/RW treatment and each predicted mortality, there was no significant difference between sites ( $P = 0.05$ ).

<sup>a</sup>BS: brood stock.

water treatment, larvae from HT adults had the longest predicted time to mortality, which was similar to larvae from brood stock, whereas larvae from WW adults had the shortest predicted time to mortality (Fig. 3). For the site food–water treatments, brood stock larvae had the longest predicted time to mortality, HT and AC larvae had intermediate survival times, and WW larvae had the shortest survival time (Table 8).

A significant decrease within each predicted mortality interval occurred in predicted time to mortality between reference food treatments and site food treatments for larvae from brood stock, HT, and AC adults (Table 8). There was a significant decrease within each predicted mortality interval in predicted time to mortality among each of the four treatments for larvae from WW adults. Differences in mortality of larvae from brood stock, HT, and AC adults were associated with food source, whereas for larvae from WW adults, differences among treatments were associated with both water and food sources. Time to mortality for WW larvae fed site food was longer in the site water treatment than in the reference water treatment.

### 3.7. Larvae growout

The survival of larvae cultured at 24-Road Hatchery between 31 and 134 days of age was 93% for brood stock and HT larvae, 94% for AC larvae, and 64% for WW larvae. Larvae from adults held at HT, AC, and WW were significantly less in total length than those from brood stock at 36 days of age (Table 9). Larvae from WW also had the lowest weight at day 36. At 78 days of age, larvae from WW adults were significantly smaller in weight, and at 108 days of age significantly less in total length than larvae from the brood stock. However, by 134 days of age, there were no significant differences in weight or total length of fish among the four groups.

There were few deformities observed in fish at day 36 sampling. At day 78 sampling, deformities in the dorsal, caudal, and tail areas were recorded, as well as missing eyes (right, left, or both), and deformed opercles (Table 10). For the larvae from HT, AC, and WW adults, the total number of deformities seemed to increase over time, and seemed to differ among sites (Table 10). For example, dorsal and opercle deformities seemed most

prominent in larvae from WW adults and brood stock. Deformed snouts seemed to be unique to larvae from WW adults. Caudal, dorsal, and tail deformities seemed to be rarest in larvae from AC compared to other larvae. At 134 days of age larvae from WW adults had 2.2 times more total deformities than those from HT adults, 3.5 times more than from AC adults, and 1.8 times more than from brood stock (Table 10).

It was noted by various observers that larvae from WW adults did not school in their tank as did larvae from the other two groups or brood stock, nor did they exhibit a fright response when a person approached the tank (R. Krueger [three observations], USFWS; B.

Osmundson [one observation], USFWS; P. von Guerard [one observation], USGS; D. Butler [one observation], USGS; written communications). One observer (K. Holley) noted that larvae from AC adults had a predominance of missing eyes and darker coloration, and larvae from WW adults had blunt snouts, bent or humped backs, and bent caudal regions, whereas larvae from HT adults seemed similar in behavior to those from brood stock and both groups looked “normal.” In contrast, hatchery personnel routinely cultured the larvae on a daily basis, along with many tanks of brood stock larvae, and did not record any observational data on different schooling behavior among groups (M. Montagne and F. Pfeifer, USFWS, written communication). One observer visited the hatchery intermittently to observe the larvae in various tanks and noted that the WW fish swam at random and did not school as larvae in other tanks (K. Holley [12–15 observations], USFWS, written communication).

Mean selenium concentrations in larvae from all adult sources after 134 days of culturing at 24-Road were 0.94 µg/g (SE 0.03, *n* = 3) for HT larvae, 0.94 µg/g (SE 0.03, *n* = 3) for AC larvae, 0.98 µg/g (SE 0.02, *n* = 3) for WW larvae, and 0.98 µg/g (SE 0.03, *n* = 5) for brood stock larvae. One sample of brood stock larvae sampled after 190 days of culture at the hatchery contained 0.96 µg/g of selenium. The Bozeman diet, which was fed to larvae age 9–96 days old, contained 1.95 µg/g selenium.

4. Discussion

4.1. Water quality

Dissolved oxygen and water temperature during the test were in the acceptable range of values for tests with fish (Weber et al., 1989). Water quality characteristics

Table 9  
Mean (standard error in parentheses) total length (mm) and weight (g) of razorback sucker larvae held at 24-Road for 134 days post hatch

Measure	Site	Age (days)			
		36	78	108	134
Total length	HT	14b (0.4)	34a (0.4)	54ab (2)	64 (2)
	AC	14b (0.2)	31a (0.2)	54ab (1)	65 (2)
	WW	12b (0.1)	26b (1)	48b (1)	63 (2)
	BS <sup>a</sup>	17a (0.6)	35a (1)	56a (1)	68 (2)
Weight	HT	0.018ab (0.002)	0.45ab (0.02)	2.17 (0.15)	3.4 (0.18)
	AC	0.019ab (0.001)	0.35bc (0.01)	2.02 (0.15)	3.4 (0.36)
	WW	0.007b (0)	0.21c (0.03)	1.92 (0.1)	3.75 (0.2)
	BS	0.034a (0.004)	0.51a (0.04)	2.18 (0.17)	3.68 (0.37)

Note. For each measure and time period, values with lower case letters in common are not significantly different (*P* = 0.05).

<sup>a</sup>BS: brood stock.

Table 10  
Percent deformities in razorback sucker larvae held at 24-Road for 134 days post hatch (*n* = 60 for HT, AC, and WW; *n* = 120 for brood stock (BS))

Site	Age (days)	Caudal deformity	Dorsal deformity	Tail deformity	Total scoliosis	Eye deformity	Opercule deformity	Deformed snout	Total deformities
HT	78	3	2	2	7	5	3	0	15
	108	0	0	12	12	8	7	0	27
	134	7	0	5	12	17	18	0	43
AC	78	2	0	0	2	12	2	0	15
	108	0	2	0	2	8	5	0	13
	134	0	2	2	5	12	13	2	27
WW	78	0	15	0	17	7	8	0	30
	108	0	8	0	8	13	47	0	60
	134	10	12	20	43	8	82	53	95
BS	78	3	11	0	14	1	28	0	42
	108	0	8	0	9	0	20	0	27
	134	3	11	0	13	3	39	8	52

differed significantly among the four test waters, but those differences probably did not contribute to observed mortality of larvae, especially for waters from 24-Road, HT, and AC, which were all close to conditions in various segments of the Colorado River (Hamilton et al., 2001a). Razorback sucker are routinely spawned and cultured at Dexter National Fish Hatchery (NFH) and Technology Center, Dexter, NM, and water quality characteristics (i.e., 2350 mg/L as CaCO<sub>3</sub> hardness and 3710 µΩ/cm conductivity; R. Hamman, USFWS, personal communication) were similar to those at WW.

#### 4.2. Selenium and other elements in water

Exposure of razorback sucker larvae from AC and WW adults to site water (and reference food) had increased mortality compared to larvae exposed to reference water (and reference food). This increased mortality in the site water treatment compared to the reference water treatment suggested that some component of the site water contributed to the increased mortality. In the treatments with AC larvae exposed to the AC water, the site water had water quality characteristics (e.g., hardness, alkalinity) that were similar to those of Colorado River water, but selenium averaged 5.5 µg/L.

There was a greater difference in mortality in WW larvae between reference water and site water treatments (Fig. 2D) than observed in AC larvae exposed to AC water or reference water (Fig. 2C). There was a slight difference in water quality characteristics between WW water and AC water, but selenium averaged 10.7 µg/L and was an incremental increase of twofold higher than that in AC site water. The selenium concentration in WW water was similar to that used by Woock et al. (1987), who reported that waterborne selenium exposure to 10 µg/L caused increased mortality and deformities in fish with dietary selenium exposure (13 µg/g).

The elevated water hardness at AC and WW probably would have reduced the toxicity of some elements such as cadmium, chromium III, copper, lead, nickel, silver, and zinc (USEPA, 1998, 1999). Selenium toxicity, however, is minimally affected by changes in hardness or salinity (Hamilton and Buhl, 1990; Palawski et al., 1985; USEPA, 1998, 1999).

Selenium concentrations at AC, 4 days before study initiation and during the last 5 days, and at WW during the 30-day period, exceeded the US Environmental Protection Agency (USEPA) criterion of 5 µg/L for the protection of aquatic life (USEPA, 1987). This criterion was based on bioaccumulation of selenium through the food chain, and the resulting dietary selenium toxicity. The general consensus is that dietary exposure to selenium is more toxic to fish than waterborne exposure, and that waterborne concentrations of selenium between

2 and 5 µg/L can cause bioaccumulation of selenium in the food chain to toxic concentrations (Hamilton and Lemly, 1999; Lemly, 1996; Maier and Knight, 1994; Skorupa, 1998). However, in natural aquatic situations the two exposure routes are inseparable. The selenium concentrations in water at the three sites in the present study were elevated sufficiently to allow selenium to be bioaccumulated to concentrations above the dietary toxic threshold. However, accumulation from water to algae and periphyton growing on the exposure beaker walls probably occurred, which in turn could have been used as food and thus may have contributed to the dietary selenium exposure.

#### 4.3. Selenium and other elements in zooplankton

The selenium concentrations in zooplankton in the present study, including zooplankton collected from HTEW (4.6–7.4 µg/g), exceeded the dietary toxicity threshold of 3 µg/g proposed by Lemly (1993a, 1996) and Maier and Knight (1994) based on their review of several laboratory and field studies.

The high copper concentrations in zooplankton from HTEW match the elevated copper concentrations in sediments from HT ponds, which were the water source for the wetland (Hamilton et al., 2001a). Copper concentrations in zooplankton (11–21 µg/g) probably did not contribute to toxic effects in razorback sucker larvae because they were about 32 times lower in zooplankton than those reported by Lanno et al. (1985) to be toxic to rainbow trout (*Oncorhynchus mykiss*), and about 17 times lower than those reported by Mount et al. (1994).

Vanadium was not as elevated in zooplankton in the present study (2.3–5.3 µg/g), but was suggested as potentially contributing to the toxicity to razorback sucker larvae fed zooplankton collected from Sheppard Pond 3 (8.4 µg/g vanadium) and North Roadside Pond (9.5 µg/g vanadium) (Hamilton et al., 1996). Although no toxic threshold has been proposed for vanadium, Hilton and Bettger (1988) reported that dietary vanadium was at least as toxic, if not more toxic, as dietary selenium to juvenile rainbow trout.

The concentrations of iron, magnesium, manganese, potassium, and sodium in zooplankton in the present study were similar to concentrations reported in *Daphnia* sp. and nauplii of brine shrimp used in fish culture (Watanabe et al., 1983), and therefore probably did not cause toxic effects in razorback sucker larvae. The aluminum concentration in zooplankton in the present study was similar to background concentrations in aquatic invertebrates (Wren and Stephenson, 1991).

Selenium was the only inorganic element in zooplankton from HTEW, AC, and WW in the present study that exceeded concentrations reported to be toxic to fish. Selenium concentrations of ≥4.6 µg/g in food organisms

from HTEW adversely affected survival of razorback sucker larvae after 5–6 days of exposure. This same selenium concentration of  $\geq 4.6 \mu\text{g/g}$  also adversely affected survival of razorback sucker larvae after 6–8 days of exposure in a second razorback sucker larvae study conducted a year later (Hamilton et al., 2001b).

#### 4.4. Brine shrimp

In the present study, fish that received the reference food treatment were fed 1-day-old nauplii of brine shrimp (*Artemia* sp.) containing about  $2.7 \mu\text{g/g}$  selenium, which was close to the proposed dietary threshold of  $3 \mu\text{g/g}$ . This selenium concentration apparently was not toxic to the razorback sucker larvae because no abnormal mortality and altered growth were observed.

Although the concentrations of inorganic elements in brine shrimp were not measured in the present study, they were measured in the same lot of brine shrimp used in a second study with razorback sucker larvae (Hamilton et al., 2001b). In that study, concentrations of elements in brine shrimp were similar to concentrations of cobalt, chromium, copper, iron, manganese, molybdenum, nickel, vanadium and zinc in brine shrimp nauplii reported by Cowgill et al. (1987) (same source of cysts as in present study: Columbia, S.A.). Olney et al. (1980) and Petrucci et al. (1995) also reported somewhat similar concentrations of inorganic elements in brine shrimp nauplii from other sources such as Australia, Brazil, Italy, California, and Utah.

Of the elements measured in nauplii of brine shrimp (Hamilton et al., 2001b), arsenic and boron concentrations were elevated compared to concentrations in zooplankton, whereas concentrations of aluminum, barium, iron, manganese, silicon, strontium, titanium, and vanadium were lower than in zooplankton. The possible effects of higher concentrations of boron or lower concentrations of aluminum, barium, iron, manganese, silicon, strontium, titanium, and vanadium in brine shrimp to larvae survival compared zooplankton cannot be discounted, but the available literature strongly suggests that arsenic was a factor.

The arsenic concentration in brine shrimp nauplii in the second larvae study was  $24 \mu\text{g/g}$  (Hamilton et al., 2001b), but arsenic concentrations in brine shrimp nauplii vary widely between sources of brine shrimp cysts. Cowgill et al. (1987) reported  $9.7 \mu\text{g/g}$  in brine shrimp from Brazil,  $11.0$ – $19.5 \mu\text{g/g}$  from San Francisco, and  $15.7 \mu\text{g/g}$  from Columbia, S.A., whereas Petrucci et al. (1995) reported  $0.9$ – $3.1 \mu\text{g/g}$  from Italy.

Arsenic concentrations in brine shrimp nauplii in the second larvae study were not elevated sufficiently to cause dietary toxicity (Cockell et al., 1991), but may have ameliorated the toxic stress of dietary selenium. Arsenic compounds have been reported to protect against the toxicity of a variety of forms of selenium

including selenite, selenocystine, and selenomethionine in rats, dogs, swine, cattle, and birds (Levander, 1977). In general, arsenic exposure in water or diet protects against dietary selenium toxicity (Dubois et al., 1940; Hoffman et al., 1992; Klug et al., 1949; Moxon, 1938; Thapar et al., 1969), but combined arsenic and selenium waterborne exposure does not (Cabe et al., 1979; Frost, 1981).

Klug et al. (1950) reported that selenium residues increased in liver (28%), kidney (141%), and muscle (52%) in rats exposed to arsenic in water and selenium in the diet, compared to exposure to only selenium in the diet, and that arsenic protected against selenium-induced mortality, reduced growth, and reduced feeding. They concluded that arsenic counteracted selenium toxicity in some way other than increasing elimination. Howell and Hill (1978) also reported increased selenium residues in liver of chicks exposed to dietary selenium and either arsenic, tin, or tellurium compared to selenium exposure alone, and the interaction of these elements with selenium reduced selenium toxicity compared to selenium alone exposures. Levander and Argrett (1969) also reported that arsenic protected against selenosis in rats, yet selenium residues were increased in carcass compared to animals in the selenium alone exposure.

Selenium residues in razorback sucker larvae from HT or brood stock adults after 7–12 days of exposure to the reference food (brine shrimp) treatment were elevated at  $7.2$ – $7.3 \mu\text{g/g}$  in the reference water (24-Road) treatment and were elevated at  $6.8$ – $7.9 \mu\text{g/g}$  in the HT site water treatment. Accordingly, survival of larvae was very similar between the same food treatments (but with the different water treatments): 98–100%. Somewhat similar selenium residues in razorback sucker larvae from HT or brood stock adults were present at 30 days of exposure:  $3.3$ – $3.6 \mu\text{g/g}$  in the reference food and reference water treatment and  $5.1$ – $5.2 \mu\text{g/g}$  in the reference food and site water treatment. Accordingly, survival was very similar between the food treatments (but with the different water treatments): 86–89%. This same scenario occurred in the second larvae study with razorback sucker larvae (Hamilton et al., 2001b).

The similarity in survival of HT and brood stock larvae at 7–12 days of exposure was due to the use of the same reference food (brine shrimp). Nevertheless, the selenium concentration in brine shrimp ( $2.7 \mu\text{g/g}$ ) was near the selenium dietary toxic threshold ( $3 \mu\text{g/g}$ ; Lemly, 1996; Maier and Knight, 1994), and whole-body residues were above the whole-body adverse effect threshold ( $4$ – $5 \mu\text{g/g}$ , reviewed in Hamilton, 2002). Whole-body selenium residues in larvae were elevated, selenium concentrations in food were relatively close to the selenium dietary toxicity threshold, and arsenic concentrations ( $24 \mu\text{g/g}$ ) were elevated in brine shrimp, but survival was good. This scenario suggests that the

selenium residue in larvae fed reference food (brine shrimp) was somehow inactivated from having a toxic effect similar to the reduction of selenium toxicity by arsenic reported by others (Howell and Hill, 1978; Klug et al., 1950; Levander and Argrett, 1969).

The same reduction of selenium toxicity by arsenic probably occurred at day 30 in larvae from HT, AC, WW, and brood stock fed brine shrimp and held in 24-Road water. Survival of larvae was relatively high in the brine shrimp treatment (75–89%) compared to other food treatments (0–62%), yet whole-body residues were elevated (up to 9.7 µg/g, Table 6). Nevertheless, survival followed a concentration response: lowest survival (75%) was in WW larvae with the highest selenium residue (9.7 µg/g), intermediate (84%) in AC larvae with the intermediate selenium residue (7.7 µg/g), and highest in HT and brood stock larvae (87–89%) with the lowest selenium residues (3.3–3.6 µg/g).

A different survival pattern was present in the HTEW food treatments (6.0 µg/g arsenic in HTEW zooplankton) with either reference water (24-Road) or site water (HT) probably due to lower arsenic concentrations. Selenium residues in razorback sucker larvae from HT or brood stock adults after 7–12 days of exposure to the HTEW food treatment and reference water (24-Road) treatment were elevated at 8.1–8.8 µg/g, and in the HTEW food treatment and the HT site water treatment were elevated at 8.6–9.6 µg/g. Survival was somewhat similar between the same food treatments (but with the different water treatments) within the same adult source: 38–56% for larvae from HT adults and 82–85% for larvae from brood stock. Although residues in larvae were similar between the reference food and HTEW food treatments, survival was different. The selenium concentration in HTEW food (4.6 µg/g) was above the selenium dietary toxic threshold (3 µg/g), and whole-body residues were above the whole-body adverse effect threshold (4–5 µg/g). Considering that the whole-body residues were elevated, selenium concentrations in food were above the selenium dietary toxicity threshold, arsenic concentrations in zooplankton from HTEW were low (6.0 µg/g), and survival was lower than in the reference food treatments suggested that the selenium residue in larvae fed the site food was not inactivated due to the absence of elevated arsenic. This same scenario occurred in the second study with razorback sucker larvae (Hamilton et al., 2001b).

The relatively low arsenic concentrations compared to selenium concentrations in invertebrates in the present study was similar to collections of invertebrates from the Grand and Gunnison valleys of western Colorado (Butler et al., 1991, 1994). Consequently, in most sites in the Grand and Gunnison valleys, the amount of arsenic in food organisms is probably too low compared to selenium concentrations to alleviate selenium toxicity.

#### 4.5. Zooplankton

We collected and fed live zooplankton from the general biogeographical area where the last riverine population of razorback sucker occur, which suggests that the composition of zooplankton was probably similar to what wild larvae would have encountered. Although not counted as food, some algae and detrital material was present in the rations of zooplankton fed to razorback sucker larvae, and algae and periphyton that grew on the walls of the exposure beakers also were a food source for the larvae. Razorback sucker larvae seem to be opportunistic feeders and are capable of using zooplankton, benthic organisms, algae, and detritus as food, sometimes selectively and other times in proportion to the abundance of the item (Marsh and Langhorst, 1988; Muth, 1995; Papoulias and Minckley, 1992; Toney, 1974).

The zooplankton fed in the present study were predominantly cladocerans and copepods, whose ratio (cladoceran/copepod) varied greatly between collections. This wide variation in the ratios was similar to that reported by Grabowski and Hiebert (1989) for backwaters near Island Park, Jensen, and Ouray on the Green River.

The use of brine shrimp in the present study as a reference food organism and field-collected zooplankton seemed appropriate because the caloric content of brine shrimp and freshwater aquatic invertebrates were similar (Cummins and Wuycheck, 1971; Driver, 1981; Driver et al., 1974; Hamilton and Buhl, 1999; Schauer et al., 1980; Watanabe et al., 1983).

Larvae were fed 40 organisms/fish in the present study. Papoulias and Minckley (1990) reported in their laboratory study that 30 to 60 nauplii of brine shrimp per razorback sucker larvae per day was sufficient to maintain high survival and no reduced growth. In a feeding study where 5-day-old razorback sucker larvae were fed 20, 40, or 80 nauplii of brine shrimp per fish per day for 20 days, survival was 95% or better in all treatments (Hamilton et al., 1996).

Feeding densities in the present study (initially 400 organisms/1.6 L or 250/L) were higher than wild larvae would encounter naturally in their historic biogeographic range. Papoulias and Minckley (1992) reported good larval survival (77%, 90%, and 67%) in ponds with zooplankton densities of 43, 24, and 12 organisms/L, respectively. Cooper and Severn (1994) reported 15 organisms/L in Sheppard Bottom pond 3 on the Green River, and Grabowski and Hiebert (1989) reported 16.6 zooplankton/L in a Jensen backwater (seasonal average 4.1 zooplankton/L) and 15.1 zooplankton/L in an Ouray backwater (seasonal average 5.2 zooplankton/L) on the Green River. Papoulias and Minckley (1990, 1992) reported 25–41 zooplankton/L in Lake Mead, 83 zooplankton/L

in Lake Havasu, and 29–45 zooplankton/L in Lake Mohave.

The likelihood that razorback sucker larvae would successfully consume food was increased by using a high density of zooplankton and preselecting small sizes in the present study compared to natural occurrences. This approach also reduced the energy cost to larvae to search and capture prey. In natural wetlands fish would have unlimited access spatially to food organisms, but low food density would require larvae to forage over greater areas, which could in turn cost them more energy and subject them to greater predation pressure or competition from other species.

#### 4.6. Selenium in larvae

Most of the selenium concentrations in larvae were above the whole-body toxic threshold of  $4\ \mu\text{g/g}$  proposed by Lemly (1996) for selenium-induced adverse effects, i.e., mortality of juveniles, in a selenium-sensitive fish species. By comparison, Maier and Knight (1994) proposed  $4.5\ \mu\text{g/g}$  for the whole-body toxic threshold. These values were derived from reviews of several laboratory and field studies where selenium was the toxicant of concern. Hamilton et al. (2000a) cited three additional studies that documented adverse effects associated with whole-body selenium concentrations of  $3.6\text{--}5.5\ \mu\text{g/g}$  in juvenile fish. Information in the selenium literature linking whole-body residues of  $4\ \mu\text{g/g}$  or higher with adverse effects in fish seems sufficient to suggest that a tissue-based selenium criterion should be used for the protection of aquatic life rather than a water-based criterion (Hamilton, 2002).

At day 7 and 12, larvae from brood stock and HT adults had higher selenium residues than at day 30. The initially elevated selenium residues in HT larvae at day 7 and brood stock larvae at day 12 were probably due in part to parental transfer of selenium via eggs (HT eggs contained  $5.8\text{--}6.6\ \mu\text{g/g}$  selenium and brood stock contained  $7.1\ \mu\text{g/g}$ ). The initially high residues apparently decreased due to depuration and tissue dilution as fish grew. Investigations have reported decreases in selenium residues in fish due to tissue dilution from growth (Bennett et al., 1986) or due to depuration of initially high selenium residues in larvae while being fed food with a low selenium concentration (Birkner, 1978).

Selenium residues in razorback sucker larvae from HT after 7 days of exposure to the brine shrimp or HTEW food treatments and the 24-Road or HT water treatments were elevated at  $7.3\text{--}8.8\ \mu\text{g/g}$ , but survival was 98–100% in the brine shrimp food treatment and 38–56% in the HTEW food treatment. The difference in survival may be due in part to the selenium concentrations in food at 7 days of exposure, which were  $2.7\ \mu\text{g/g}$  in brine shrimp and  $4.6\ \mu\text{g/g}$  in zooplankton from HTEW, but more likely due to the elevated arsenic

concentrations in brine shrimp ( $24\ \mu\text{g/g}$ ) compared to those in HTEW food ( $6.0\ \mu\text{g/g}$ ). This same scenario occurred at day 30 of the current study and in the second study with razorback sucker larvae (Hamilton et al., 2001b).

The high whole-body selenium residues in razorback sucker larvae from AC ( $32\text{--}71\ \mu\text{g/g}$ ) and WW ( $28\text{--}47\ \mu\text{g/g}$ ) adults at days 6 and 7 in the present study were due in part to selenium residues in eggs because AC eggs contained  $38\text{--}54.5\ \mu\text{g/g}$  and WW eggs contained  $34.3\text{--}37.2\ \mu\text{g/g}$ . Nevertheless, others have reported similar elevated selenium residues in young fish. Bennett et al. (1986) reported whole-body selenium residues of  $52\ \mu\text{g/g}$  in 17-day-old fathead minnow (*Pimephales promelas*) larvae fed rotifers containing  $46\ \mu\text{g/g}$  selenium for 9 days, and  $61\ \mu\text{g/g}$  in 9-day-old larvae fed rotifers with  $91\ \mu\text{g/g}$  for 7 days. Dobbs et al. (1996) reported whole-body selenium residues of  $47\ \mu\text{g/g}$  in fathead minnow larvae fed rotifers containing  $40\text{--}47\ \mu\text{g/g}$  selenium for 7 days.

After 30 days of exposure in the present study, larvae from brood stock (residue  $3.6\ \mu\text{g/g}$ ) and HT (residue  $3.3\ \mu\text{g/g}$ ) adults exposed to the reference food–reference water treatment had selenium residues below the toxic threshold ( $4\ \mu\text{g/g}$ ), and concomitantly, no adverse effects on growth and survival.

Selenium residues in larvae from AC ( $7.7\ \mu\text{g/g}$ ) and WW ( $9.7\ \mu\text{g/g}$ ) adults were still elevated after 30 days in the reference food–reference water treatments, which were depurating conditions similar to those in the study conducted by Birkner (1978). Although larvae from AC and WW adults depurated selenium, there was a trend for reduced survival throughout the 30-day exposure. This trend for reduced survival suggested that even if selenium residues were depurated to  $7.7\text{--}9.7\ \mu\text{g/g}$ , adverse effects in razorback sucker larvae would continue to occur.

At 30 days of exposure, the increased selenium residues in larvae from HT ( $5.1\ \mu\text{g/g}$ ), AC ( $12.7\ \mu\text{g/g}$ ), and WW ( $15.2\ \mu\text{g/g}$ ) adults in the reference food–site water treatment compared to the reference food–reference water treatment ( $3.3$ ,  $7.7$ ,  $9.7\ \mu\text{g/g}$ , respectively) revealed that larvae were accumulating selenium from the site water exposure. Selenium uptake in fish from water exposure can occur via the gill and epithelium (Hodson et al., 1986). Selenium uptake in larvae in the site water treatments may have come from dietary uptake of algae, periphyton, and detritus growing on the walls or bottom of the exposure beakers. Selenium is readily accumulated from water into algae (Besser et al., 1993; Foe and Knight, 1986; Nassos et al., 1980; Riedel et al., 1991), periphyton (Graham et al., 1992), and detritus (Bender et al., 1991).

The lower correlation coefficient for the relation between larvae selenium and larvae survival ( $r = -0.55$ ) than for the relation between zooplankton

selenium and larvae survival ( $r = -0.70$ ) may seem counterintuitive because effects on larvae survival should be more closely linked with larvae selenium residues than with selenium residues in food. Possible explanations could be measurement error in selenium analysis of zooplankton or larvae residues or survivor bias of selenium residues, i.e., sampling live fish with selenium residues lower than in fish that died with possibly higher selenium residues. However, quality control/quality assurance measures made during selenium analyses suggest the measurements were accurate and precise. Concerning survivor bias residues, Adams (1976) and Sato et al. (1980) reported that selenium residues in live sampled fish were higher than in fish that died during selenium exposure. In contrast, Heinz et al. (1988) reported that selenium residues in live sampled birds were lower than in birds that died during selenium exposure, which suggests a survivor bias. However, Albers et al. (1996) reported similar selenium residues in birds sampled live and those that died during selenium exposure. Thus in fish, if survivor bias occurred, it would have probably resulted in higher selenium residues in live sampled fish than in dead fish and possibly skewed the relation between larvae selenium and larvae survival into a more significant correlation coefficient, which it did not relative to the relation between zooplankton selenium and larvae selenium.

#### 4.7. Growth

Growth is the culmination of many biochemical phenomena that occur in a somewhat regulated pattern, and reduced growth may occur when contaminant intoxication induces biochemical changes that interfere with normal growth processes (Mehrlé and Mayer, 1985). Some studies with selenium exposure in the water, diet, or both have reported inconsistent results: (1) reduced growth occurred in the same treatments (exposure concentration and duration) where reductions in survival occurred (Hilton et al., 1980; Klauda, 1986), (2) reduced survival occurred before reduced growth (Cleveland et al., 1993; Crane et al., 1992; Hamilton et al., 1990 [San Luis Drain diet], Hermanutz et al., 1992; Hunn et al., 1987; Woock et al., 1987), (3) reduced growth occurred before reduced survival (Hamilton et al., 1990 [selenomethionine diet]; Hilton and Hodson, 1983; Ogle and Knight, 1989), or (4) no effects occurred on growth or survival, but other pathological effects or reproductive effects occurred (Coyle et al., 1993; Hodson et al., 1980). The inconsistency between these studies was probably due to differences in species, age, exposure route and duration, selenium form, and other factors.

In the present study, the only consistent difference in growth was between brood stock larvae and larvae from the young adults held at HT, AC, and WW. This

difference was consistent with the reports that larvae from large eggs are larger at hatch and remain larger for up to 324 days post hatch than larvae from small eggs (Buss and McCreary, 1960; Gall, 1974; Kazakov, 1981; Pitman, 1979; Springate and Bromage, 1985).

#### 4.8. Survival

Razorback sucker larvae in the site food treatments probably did not die from starvation because Papoulias and Minckley (1990) reported that the point of irreversible starvation, and subsequent mortality, was between 19 and 23 days, whereas the median time to 50% mortality for unfed larvae was between 24 and 25 days. In the site food treatments of the present study, nearly complete mortality occurred in 5–12 days, which was less than the period when irreversible starvation could have influenced later mortality or the median time to 50% mortality of unfed larvae reported by Papoulias and Minckley (1990).

The process of yolk sac absorption and exposure of selenium-containing yolk (Kroll and Doroshov, 1991) probably was not associated with the observed mortality, which began at day 6 of the study. Minckley and Gustafson (1982) reported that yolk sac absorption was completed in razorback sucker at 7.5 days posthatch. The current study was started with 5-day-old fish and the mortality occurred at 6 days of exposure (total 11 days). Assuming that the larvae used in the present study had similar development to those examined by Minckley and Gustafson (1982), the larvae in the present study would have been feeding for at least 3–4 days, which is sufficient time to incur toxicity from dietary selenium in very small larval fish.

In the present study, the time frame of nearly 100% mortality in larvae receiving site food was similar to Bryson et al. (1984) who reported that juvenile bluegill (*Lepomis macrochirus*) collected from Hyco Reservoir, NC, experienced 97% mortality within a week after being fed plankton, which contained 45  $\mu\text{g/g}$  selenium. Dietary selenium concentrations close to the proposed selenium dietary toxic threshold of 3  $\mu\text{g/g}$  have been documented to cause adverse effects in young fish usually following 30–60 days of exposure. For example, Hamilton et al. (1990) reported that 5.3  $\mu\text{g/g}$  selenium in the diet reduced growth of chinook salmon (*Oncorhynchus tshawytscha*), Cleveland et al. (1993) reported that 6.5  $\mu\text{g/g}$  selenium in the diet reduced survival of bluegill, Lemly (1993b) reported that 5.1  $\mu\text{g/g}$  selenium in the diet (and 4.8  $\mu\text{g/L}$  selenium in the water) reduced survival of bluegill held under winter stress conditions, and Hamilton et al. (1996) reported rapid mortality in 5-day-old razorback sucker larvae 5–10 days after feeding zooplankton containing selenium concentrations of 3.5–25.7  $\mu\text{g/g}$ . These toxic selenium concentrations (3.5–6.5  $\mu\text{g/g}$ ) indicate that the selenium concentration

in zooplankton from HTEW (4.6 µg/g) was sufficiently elevated during the first week of exposure to reduce survival. In the second razorback sucker larvae study, selenium concentrations of 4.6 µg/g or greater were also linked with a sharp decline in survival of razorback sucker larvae at about 6–8 days of exposure (Hamilton et al., 2001a).

Waterborne exposure to site water (tested with reference food) in the present study and second larvae study contributed to the reduced survival of the larvae. The 21–28% reduction in survival of AC and WW larvae exposed to site water compared to those in reference water (Figs. 1B, 2C and D) suggested that elements in site water were toxic to larvae after 6 days of exposure when increased mortality began to occur. The site water treatments may have inadvertently contributed to dietary exposure of larvae through uptake of waterborne elements such as selenium in algae and periphyton growing on beaker walls. Other studies have also reported that combined water and dietary exposures of fish to selenium were additive (Bertram and Brooks, 1986; Woock et al., 1987). However, the magnitude of the effect of site food (tested with reference water) on mortality was much greater than that contributed by exposure to site water (tested with reference food). This prominence of adverse effects from dietary sources over waterborne sources has also been stated in review papers (Lemly, 1996; Maier and Knight, 1994; Maier et al., 1988).

In the present study, it seems reasonable to assume that dietary selenium was the primary cause of the reduced survival of larvae because (1) zooplankton types, feeding density, and energy content seemed appropriate for proper nutrition of the larvae, (2) the time frame of mortality precluded the influence of starvation, and matched those in studies of dietary selenium toxicity with fish, (3) selenium concentrations, but not other inorganic elements, were elevated in zooplankton above well-documented selenium dietary toxic thresholds, (4) the significant negative correlation between larval survival and selenium concentrations in zooplankton, and (5) selenium residues in larvae were above whole-body concentrations linked with reduced survival in young fish reported in other studies.

Likewise, it is reasonable to assume that dietary arsenic counteracted selenium toxicity because (1) brine shrimp have been documented as a suitable food organism in larval fish culture, (2) selenium concentrations in brine shrimp were near well-documented selenium dietary toxic threshold, (3) arsenic concentrations were elevated in brine shrimp (Hamilton et al., 2001b), (4) arsenic counteracts selenium toxicity and allows selenium residues to accumulate in animals above those in exposures to selenium alone but with reduced toxic effect, (5) selenium residues in HT and brood stock larvae fed brine shrimp and exposed to 24-Road water

were near whole-body concentrations linked with reduced survival in young fish reported in other studies, and (6) reduced survival did not occur in HT and brood stock larvae fed brine shrimp and exposed in 24-Road water.

#### 4.9. Delayed mortality

In the present study, a significant delay in mortality, based on the predicted time to death, occurred in razorback sucker larvae fed site food and held in site water from WW compared to larvae fed site food, but held in reference water. This delayed mortality suggested an antagonistic interaction between site food and site water that allowed the larvae to live slightly longer than those in the reference water treatment and was similar to that of razorback sucker larvae tested elsewhere (Hamilton et al., 1996). The same delayed mortality, based on the predicted time to death, occurred in razorback sucker larvae tested a year later at Grand Junction, CO, using the same waters and site food organisms as in the present study (Hamilton et al., 2001b).

The delayed mortality observed in the WW water and food treatment in the present study, as well as the two other studies (Hamilton et al., 1996, 2001b), was probably due to selenium interacting with other elements. In contrast, typical concentration–response in survival seems to have been present in the reference food–reference water treatment because there was a shorter predicted time to death for larvae from AC adults (55 days) or WW adults (45 days) than for larvae from HT adults (63 days) or brood stock adults (68 days).

#### 4.10. Growout larvae

Most of the mortality of larvae from WW adults occurred in the first 30 days of the observation period, and was greater than in larvae from AC, in spite of the similar magnitude of elevated selenium concentrations in eggs of adults held at these two sites (AC 46.5 µg/g, WW 37.8 µg/g). However, selenium concentrations in gonads were higher in fish from WW (45.5 µg/g) than in fish from AC (30.6 µg/g). Part of the reduced survival of fish, especially in the WW larvae, may have been due to the loss of weak fish through slots in the tank drain (M. Montagne and F. Pfeifer, written communication). During fish culture, feed plugged the slots in the drain pipes, and consequently, the current drains were switched to drains with slightly larger slots through which an undetermined number of weak fish probably were lost. The importance of the mortality of WW larvae associated with the change in drain slot size, and the inadvertent loss of larvae was unclear but may have

been due to their underdeveloped swimming ability due to small size.

#### 4.11. Growth in growout larvae

The greater total length and weight of larvae from brood stock at 42 days of age compared to larvae from first-time spawners was consistent with the reports that larvae from large eggs hatch into larger larvae and remain larger for a period of time after hatch than larvae from small eggs (Buss and McCreary, 1960; Gall, 1974; Kazakov, 1981; Pitman, 1979; Springate and Bromage, 1985). At 140 days of age, there were no differences in total length or weight, which was inconsistent with the reports that differences due to egg size remain in fry up to 324 days post hatch (Ojanguren et al., 1996; Pitman, 1979; Springate and Bromage, 1985).

At 84 days of age, the significant reduction in total length and weight of larvae from WW adults compared to larvae from HT and AC adults suggested a stress was transferred from the parents to the egg because larvae from all three parental exposures were hatched and cultured in the same water, and larvae were fed the same food. The disappearance of growth differences at 140 days of age among the three groups was probably due to mortality removing smaller, weak larvae from WW adults.

The consequence of reduced growth in early life stages of fish is that swimming performance is reduced and susceptibility to predation is increased (Bams, 1967; Taylor and McPhail, 1985), and feeding efficiency is reduced (Gunn and Noakes, 1987). Functions that promote fish survival also are related to body size (reviewed by Ojanguren et al., 1996). For example, sensory systems develop rapidly during early life stages and visual acuity increases with fish size, enabling large fry to better detect predators and food and resist starvation, thus allowing them access to a wider range of prey and increases the likelihood of finding appropriate food. Consequently, razorback sucker larvae with reduced growth, may be less fit to survive the rigors of competition and predation, especially in the presence of nonnative fish.

#### 4.12. Deformities in growout larvae

The incidence of deformities in larvae produced by adults from HT (43%), AC (27%), WW (95%), and brood stock (52%) were elevated compared to typical background rates of <1–3% reported in wild fish (Dahlberg, 1970; Gabriel, 1944; Gill and Fisk, 1966; Patten, 1968). The frequency of deformities in razorback sucker larvae were also higher than the range of 9–11% in other fish suggested by Bengtsson (1975) as abnormal and probably due to a “manmade” effect on wild fish.

A concentration–response relation in deformities in the present study was not expected in part due to the general inconsistency of selenium effects on growth and survival discussed above. However, Lemly (2002) compiled data from several field and lab studies and created figures showing the generalized concentration–response for percent deformed fish versus whole-body selenium and for percent teratogenic mortality versus percent deformed fish. Holm et al. (2003) also reported a concentration–response for egg selenium concentrations in rainbow trout and five deformity parameters (total deformities, craniofacial deformities, skeletal deformities, finfold deformities, and edema), but not for egg selenium concentrations in brook trout (*Salvelinus fontinalis*).

Deformities have been observed in fish larvae of other species hatched from eggs with high selenium concentrations (Coyle et al., 1993; Crane et al., 1992; Gillespie and Baumann, 1986; Hermanutz, 1992; Hermanutz et al., 1992; Pyron and Beitingner, 1989; Schultz and Hermanutz, 1990; Woock et al., 1987). Fish larvae exhibiting selenium-induced deformities were not expected to survive in natural systems, except in predator-free situations (Hermanutz, 1992; Lemly, 1993c) such as occurred in the present study. Deformities have even been documented in selenium-tolerant western mosquitofish (*Gambusia affinis*) from the San Luis Drain, CA (Saiki and Ogle, 1995). Lemly (1993c) documented selenium-induced deformities in 19 species of fish from Belews Lake, NC, and reported that selenium residues were similar in normal-appearing fish and abnormal fish. Later, Lemly (1997b) documented deformities in larval fish in Belews Lake, North Carolina, 10 years after selenium inputs to the lake were stopped, and concluded selenium in sediment was being recycled into the aquatic food chain.

Deformed snouts, i.e., pugheadedness, were observed in larvae from WW adults, but not in larvae from HT or AC adults. Pugheadedness can be caused by selenium exposure, as well as genetic anomalies causing a germinal defect during embryo development, oxygen deficiency, varying levels of temperature and oxygen, varying levels of temperature and salinity, X-irradiation, endocrine disturbances, or mixtures of environmental contaminants (Hickey, 1973; Hickey et al., 1977; Lemly, 1993c, 1997a; Slooff et al., 1986).

The lack of schooling in larvae from WW adults that was observed in the present study was also observed in razorback sucker exposed to a waterborne mixture of nine inorganic elements, including selenate and selenite, simulating Ashley Creek (Hamilton et al., 2000b). That observation was made after 21 days of exposure to the high treatment, and by 60 days of exposure, 95% mortality had occurred in larvae in that treatment. Coughlan and Velte (1989) noted nonschooling and corneal cloudiness in one or both eyes in striped bass

(*Morone saxatilis*) that were fed red shiners (*Cyprinella lutrensis*) containing selenium concentrations of 38.6 µg/g. Abnormal swimming due to opaqueness of eye lens or blindness in fish exposed to selenium has been reported (Ellis et al., 1937; Hilton et al., 1980; Woock et al., 1987). Cloudy eye lens or cornea was reported to be one of five categories of abnormalities associated with selenium exposed fish (Lemly, 1993c, 1997a, b). Cloudy eyes probably would cause partial or complete blindness, and concomitantly the loss of schooling and the appearance of abnormal swimming behavior. Abnormal schooling or swimming behavior tends to make fish more susceptible to predation (Atchison et al., 1987).

The nearly 3–6-fold higher number of eye deformities in larvae from young adults held at 24-Road for 134 days post hatch compared to larvae from brood stock may have resulted from the larvae being more susceptible to stress. If razorback sucker are a sensitive fish species to selenium exposure, then perhaps the larvae from first-time spawning adults held at HT had sufficient selenium residues from eggs to cause eye deformities.

#### 4.13. Selenium in growout larvae

At 134 days of age, razorback sucker larvae had selenium concentrations (0.94–0.98 µg/g) that were similar to background concentrations reported in control fish from laboratory studies and reference fish from field studies (<2 µg/g, Hamilton et al., 2000a). The selenium concentrations in these larvae were about a third of those in the larval fish study after 30 days of exposure to the reference food/reference water treatment (3.3 µg/g in HT larvae and 3.6 µg/g in brood stock larvae). These results reveal that the longer the larvae were fed clean food and held in clean water, the lower their selenium residues would be due to metabolic elimination of selenium. Birkner (1978) reported that fish reduced initially elevated selenium concentrations in whole body when fed food with lower selenium concentrations, whereas Bennett et al. (1986) reported that selenium concentrations in larval fish decreased as fish grew, i.e., tissue addition diluted whole-body selenium concentrations. However, selenium-induced teratogenic deformities derived from parental selenium deposited in the egg, as manifested by the deformed snout of larvae from adults held at WW, do not seem to be reversible when the selenium exposure is reduced or eliminated.

Overall, high selenium concentrations in WW larvae resulted in a unique deformity and loss of schooling not observed in other fish, and reduced their growth and survival under clean culture conditions. Selenium concentrations of ≥4.6 µg/g in food resulted in rapid mortality of larvae from HT, AC, and WW. Selenium

toxicity to razorback sucker larvae could limit the recovery of this endangered fish.

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