# EXHIBIT 14

# Impact of Selenium and Other Trace Elements on the Endangered Adult Razorback Sucker

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*Received 23 August 2001; accepted 15 March 2002*

**ABSTRACT:** A study was conducted with endangered the razorback sucker (*Xyrauchen texanus*) to determine if environmental exposure to selenium in flooded bottomland sites affected survival, growth, and egg-hatching success. Adults were stocked at three sites adjacent to the Colorado River near Grand Junction, Colorado, in July 1996: hatchery ponds at Horsethief Canyon State Wildlife Area (referred to here as Horsethief; the reference site), a diked tertiary channel at Adobe Creek, and North Pond at Walter Walker State Wildlife Area (WWSWA). Fish were collected in April 1997 and spawned. After two spawnings adults from the three sites were held at Horsethief for an 86-day selenium depuration period. Selenium concentrations at Horsethief were 1.4–3.0 μg/L in water, 0.8–0.9 μg/g in sediment, 4.5 μg/g in muscle plug, and 6.0 μg/g in eggs; at Adobe Creek, <0.7–4.5 μg/L in water, 1.2–2.5 μg/g in sediment, 16–20 μg/g in zooplankton, 9.6 μg/g in muscle plug, and 40 μg/g in eggs; and at North Pond, 3.2–17 μg/L in water, 16–94 μg/g in sediment, 32–48 μg/g in zooplankton, 14 μg/g in muscle plug, and 55 μg/g in eggs. During the depuration period, when adults from Adobe Creek and North Pond were held at Horsethief, the fish lost 7%–13% of their selenium burden in 59 days and 14%–21% in 86 days. Larvae from North Pond adults had the most deformities, followed by Adobe Creek adults, with the fewest deformities found in the Horsethief adults.  Environ Toxicol 17: 297–323, 2002; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.10064

**Keywords:** razorback sucker; selenium; endangered fish; Colorado River; eggs; inorganic elements; irrigation

## INTRODUCTION

Selenium contamination of the upper and lower Colorado River basins in water, sediment, and biota has been documented by academic studies and by U.S. Department of the Interior agency studies (reviewed in Hamilton, 1998). Historic selenium contamination of the upper and lower Colorado River basins prior to the construction of main-stem dams had been hypothesized to have contributed to the decline of native fish now listed federally as endangered (Hamilton, 1999). Other reports suggested that endangered

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fish, especially the razorback sucker (*Xyrauchen texanus*), are being adversely affected by selenium contamination in the Green, Price, Yampa, and upper Colorado rivers (Hamilton, 1998; Stephens and Waddell, 1998; Hamilton et al., 2000).

The Colorado River basin originally contained 32 native species, of which 75% were endemic because of the basin's long isolation (Minckley, 1991). The upper Colorado River provides critical habitats for four endangered fish species: Colorado pikeminnow (*Ptychocheilus lucius*), razorback sucker, humpback chub (*Gila cypha*), and bonytail (*Gila elegans*; USFWS, 1987; USDOI, 1994). A combined approach for recovery of the four endangered fish in the upper Colorado River basin was undertaken in 1987 by the Upper Colorado River Endangered Fish Recovery Program (USFWS, 1987). The goal of the 15-year program was to reestablish self-sustaining populations of the four species while allowing continued water development.

In an effort to stabilize and enhance populations of razorback sucker and other endangered fishes in the upper Colorado River, the Floodplain Habitat Restoration Program, a unit of the recovery program, undertook restoring floodplain habitats for use by razorback sucker larvae and adults. The strategy for achieving these goals was to reconnect selected floodplain habitats to the main river channel in a manner that simulated historical hydrological conditions. An important component of this program was selecting sites that after restoration would not contaminate the fish, especially with selenium.

The objective of the present study was to determine if environmental exposure of the adult razorback sucker to selenium and other inorganic elements in flooded bottomland sites affects their survival, growth, egg-hatching success, and residues.

## MATERIALS AND METHODS

This partial life-cycle chronic toxicity study was conducted by exposing adult fish to water and foods for about 9 months at three sites adjacent to the Colorado River near Grand Junction, Colorado: Horsethief Canyon State Wildlife Area (the reference site, operated by the Colorado River Fishery Project [CRFP]; hereafter referred to as Horsethief), Adobe Creek, and North Pond at Walter Walker State Wildlife Area (WWSWA; Fig. 1). All three sites are near Grand Junction, the Horsethief site about 19 km west of the city limits, the Adobe Creek site about 5 km west of the city limits, and the North Pond site about a half kilometer southwest of the city limits.

Four-year-old razorback suckers previously used in a reproductive study in Grand Junction, Colorado (Hamilton et al., 2001a) were used in this study. The fish were originally reared at the Wahweap Fish Facility, Big Water, Utah, by the Utah Division of Wildlife Resources. All the fish in the current study were progeny of a single spawn of two adult razorback suckers from the arm of the San Juan River in Lake Powell, Utah.

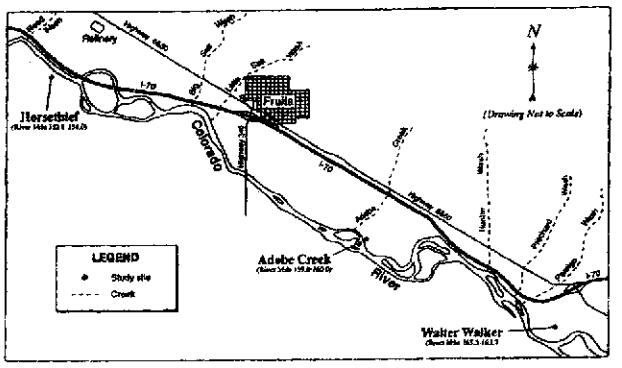


**Fig. 1.** Map of three sites in the Grand Valley near Grand Junction, Colorado, used for a reproduction study of razorback suckers.

## Site Description

The sampling stations previously established for the first study (Hamilton et al., 2001a) also were used in this one: Horsethief (HT inlet and outlet), Adobe Creek (AC1–AC7), and WWSWA (WW1–WW10). At Horsethief fish were held in earthen ponds, either pond 1 or pond 6, along with brood stock of other endangered fish. The water in the ponds was maintained by water pumped directly from the Colorado River near Fruita, Colorado, that had little selenium contamination. The Adobe Creek site was a tertiary river channel about 200 m long and 3–5 m wide; it was isolated from river flow by dikes with large gate valves at both ends, and the downstream dike had an overflow water-control structure (Fig. 2). Fish were held in the section of the channel that had sample stations AC3, AC4, and AC5. The water level at Adobe Creek was maintained with water pumped from the secondary channel (location AC2). Overflow water from an irrigation ditch (AC7) also entered the diked area. Water at the site was maintained at a depth of about 1.5 m and was believed to have relatively low levels of selenium contamination. The North Pond site was an isolated pond, about 1 ha in size with a maximum depth of 1.5 m, on a terrace about 2 m above the floodplain (Fig. 3). Water in North Pond was supplied primarily by groundwater discharge, which was believed to contain elevated selenium concentrations. The south side of North Pond had a dike and water overflow structure installed to maintain water levels and confine fish. Fish were held in North Pond at sample stations WW2 and WW3. Water levels were supplemented by inflow at WW10 from Independent Ranchman Ditch. Flooding of the Colorado River occurred in spring 1997 but did not affect water levels at Adobe Creek and North Pond.

Hatchery brood stock adults previously collected from various locations in the upper Colorado River and held at

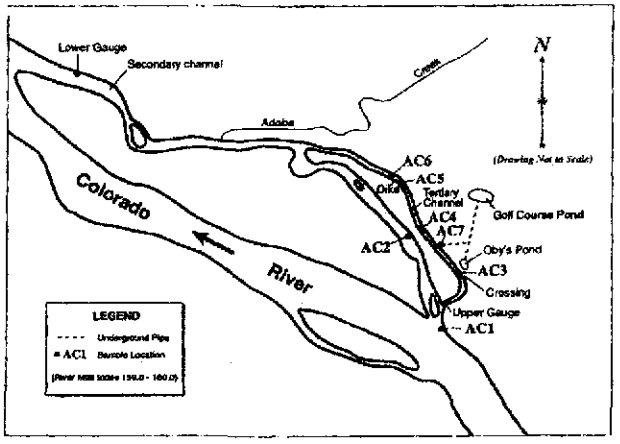


**Fig. 2.** Map of sampling stations at the Adobe Creek site, near Grand Junction, Colorado. Fish were held in the diked area, which contained sampling stations AC3, AC4, and AC5.

Horsethief were also used in the study as additional reference fish.

## Fish Stocking and Sampling

Adults were stocked at each site on July 11, 1996, as follows: Horsethief—9 females and 21 males (previously held at Horsethief for 11 months); Adobe Creek—7 females and 16 males previously held at Adobe Creek for 9 months followed by a 2-month depuration period at Horsethief, 2 females previously held at Horsethief for 11 months; North Pond—7 females and 11 males previously held at North Pond for 9 months followed by a 2-month depuration period at Horsethief, 3 females previously held at Horsethief for 11 months. On August 27, 1996 (day 47 of the exposure), an additional 10 females were stocked at each of the three sites.

Fish were collected during the third week of April 1997 for spawning. Prior to stocking, each fish (previously tagged with a passive integrated transponder [PIT]) was identified by its PIT tag, measured for length and weight, and had a muscle plug sample taken for selenium analysis from the dorsal area adjacent to the dorsal fin. Muscle plugs were collected using a 4- or 5-mm biopsy punch, placed in cryotubes, stored on ice in the field, and then stored in a freezer (−20°C) while awaiting analysis of selenium concentrations. After the muscle plug was collected, the wound was treated with full-strength Betadine solution. Fish held at Horsethief were fed the same commercial standard fish food as that fed to other stocks of razorback suckers routinely maintained there. Fish at Adobe Creek and North Pond were not fed any artificial food during the exposure and foraged for natural food items at the site.

Natural food organisms were collected from three stations where adults were held (AC3, AC5, WW2), placed in Whirl-Pak bags, stored frozen at −20°C, and analyzed for selenium concentrations. Collections were accomplished primarily with modified light traps (Espinosa and Clark, 1972) and sediment grab samplers. Light traps were set overnight, and the trapped zooplankton and other aquatic invertebrates were collected the following morning. At each sampling station the contents of all the light traps were combined and concentrated by filtering the samples through the basket of a 153-μm plankton net.

Sediment grab samples were collected in plastic jars and transported in coolers to the laboratory for separation of benthic invertebrates. Sediment samples were washed through a set of sieves and the invertebrates extracted from the debris using stainless steel forceps.

## Spawning

In late spring 1997 after the water temperature rose to 16°C and remained at that temperature for about a week (the third week in April), fish held at Adobe Creek and North Pond were captured using trap nets and electrofishing methods and transported to the fish holding building at Horsethief. Adults held in earthen ponds at Horsethief were collected using a seine. All fish were held in the holding building in 1.3-m-diameter tanks supplied with flowing Colorado River water supplemented with oxygen delivered through diffuser bars. Water temperature in the holding tanks was about 12°C. Muscle plugs were taken from fish for selenium analysis, as well as from four hatchery brood stock fish held at Horsethief.

To induce spawning, females were injected with human chorionic gonadotropin hormone at the rate of 220 international units (IU) per kilogram of body weight on each of 3 consecutive days beginning 5 days prior to the spawning date. Fish were not injected on the fourth day and were spawned on the fifth day. All males were injected at the rate of 660 IU/kg one time 5 days prior to spawning. Three females from Horsethief, four from Adobe Creek, and five

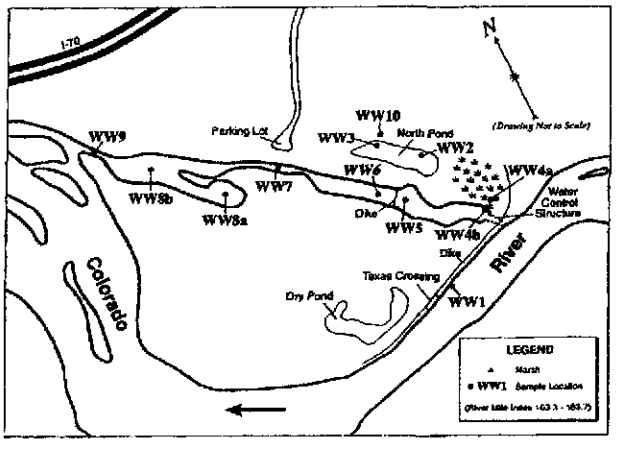


**Fig. 3.** Map of sampling stations at the Walter Walker State Wildlife Area site near Grand Junction, Colorado. Fish were held in North Pond, which contained sampling stations WW2 and WW3.

from North Pond were spawned on April 23, 1997. On April 24 two additional females from Horsethief and one from Adobe Creek were spawned. Eggs from each female were fertilized with sperm from at least one male. Fertilized eggs were washed in Colorado River water in plastic bags to reduce adhesiveness and then water-hardened in river water.

Eggs were transported to the 24-Road Fish Hatchery (hereafter referred to as 24-Road) and placed in incubation buckets with screened bottoms. Eggs from each female spawned were held in separate buckets. Buckets were held in 1.3-m-diameter tanks with recirculated 24-Road water. The water temperature at 24-Road was maintained at about 24°C for rearing activities with razorback sucker from 1996 spawns. Two samples of eggs from each spawn were collected in Whirl-Pak bags and stored frozen at −20°C until thawed for selenium analyses by atomic absorption, trace element, and major ion analyses by inductively coupled plasma (ICP) spectroscopy.

After spawning, adult fish from Adobe Creek and North Pond, along with Horsethief and brood stock fish, were held in the same earthen ponds at Horsethief for 86 days to determine the rate of selenium depuration from their muscle tissues. Fish were captured at 31, 59, and 86 days post-spawning, identified by their PIT tag, measured for length and weight, inspected for general health, and muscle plug taken for selenium analysis.

## Egg Test

For each spawn (three each for Horsethief, Adobe Creek, and North Pond and two for brood stock), eight groups of 25 eggs were placed in incubator cups for determination of survival and hatchability. Fertilized eggs that looked normal were randomly assigned to a hatching cup 5 eggs at a time until 25 eggs were placed in each cup. The egg cups were suspended in 2-L glass beakers filled with 1.2 L of filtered water (25 μm polypropylene bag filters, Filter Specialists, Inc., Michigan City, IN). Four groups (termed replicates a, b, c, and d) from each spawn were placed on each of two tables in the mobile laboratory. Eggs on one table were held in 24-Road water, and eggs on the other table were held in site water collected from the site at which the parents were exposed prior to spawning. The beakers were arranged in three rows on each table, and their positions were randomly assigned using a random numbers table.

The incubation cups were 250-mL jars with the bottom removed and a polypropylene filter cloth (with 285 μm openings) glued to the bottom using silicon adhesive. They were suspended by a latex handle in beakers containing filtered 24-Road water. After 2 h the site water treatments had 50% of the water replaced with site water. Thereafter, 50% of the water (600 mL) was renewed daily. Oxygen concentrations in exposure water were supplemented by passing compressed air from an oil-less air compressor through air stones.

Test waters for the egg test were collected as grab samples every day from each site using two 19-L carboys. Water was filtered through 25-μm 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. This water was analyzed for general water quality characteristics including pH, conductivity, hardness, alkalinity, calcium, magnesium, chloride, and ammonia. Two water samples, one filtered (0.4 μm) and one unfiltered, were collected and analyzed for selenium concentrations.

Egg cups were gently removed once daily and placed in a Petri dish with the same water as in the beaker, in which they were examined using a dissection scope to record the number of live and dead eggs and the number and types of deformities. The yolks of dead eggs appeared opaque and oddly shaped compared to live eggs. All dead eggs were removed daily. When the eggs began to hatch, the number of live and dead larvae and the number and types of deformities were recorded daily. The study was terminated at 1 day posthatch because of high mortality in all egg cups. The mortality of eggs was probably a result of a temperature acclimation problem at 24-Road that occurred prior to stocking eggs in the egg cups at the mobile laboratory. Fish were spawned and eggs were water hardened at about 12°C, then moved to the 24-Road hatchery, where the water temperature was maintained at 24°C for rearing activities with razorback suckers from 1996 spawns. During the first egg test dissolved oxygen concentrations ranged from 7.3 to 7.4 mg/L, with 82.7%–84.4% saturation, and temperature ranged from 20.0°C to 21.0°C.

On April 25, 1997, six females from Horsethief, four from Adobe Creek, and six from North Pond were spawned as described previously and a second egg test initiated. In this test eggs were used from three spawns of Horsethief, Adobe Creek, and North Pond fish, and three replicates (termed a, b, and c) were used. All other experimental conditions were as described above. After fertilization and water hardening at the spawning facility (≈12°C), the eggs were held overnight in thermal jugs that allowed slow temperature acclimation in the mobile laboratory (≈18°C), thus bypassing the holding at 24-Road. Eggs from one brood stock spawn were collected from those held at 24-Road and used in the egg test. They were stocked at 2 days postspawn. Eggs were initially held in 600 mL of Horsethief water at about 18°C, and after 2 h 300 mL of the appropriate site water or reference water was added, followed 2 h later by the addition of another 300 mL of the appropriate test water. Other procedures were as described above.

Temperature in the exposure beakers was maintained at ambient air temperature and measured daily with a precision-grade mercury thermometer. Ambient temperature in the mobile laboratory was maintained close to 20°C. Eggs and newly hatched larvae were exposed under florescent 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. Newly hatched larvae were not fed because of the short duration of the study.

Egg diameter was determined on two sets of 20 eggs from each of the three spawns from Horsethief, Adobe Creek, North Pond, and brood stock adults in the first spawning; the three spawns from Horsethief, Adobe Creek, and North Pond adults; and the brood stock spawn in the second spawning. Eggs were held in a Petri dish filled with 24-Road water so that the eggs would maintain their natural shape. The longest diameter was measured using a dissection microscope fitted with a Reichert filar micrometer eyepiece. The micrometer was calibrated by determining the mean calibration constant for a 2.0 mm distance calibrated at full-scale, 50% scale, and 20% scale calibration distances.

## Water and Sediment Sampling

Beginning in July 1996, selected water quality characteristics were measured every week *in situ*—where the fish were held at the three test sites and at 14 other sample stations on an irregular basis. In addition, site water was collected every 30 days at the three sample stations where the fish were held and analyzed for general water quality characteristics in the mobile laboratory. Water quality measurements in unfiltered water samples at the mobile laboratory included pH, conductivity, hardness, calcium, magnesium, alkalinity, and chloride. Two subsamples of each sample taken to the mobile laboratory were collected in polyethylene bottles. One sample was used for ammonia analysis and was acidified to a pH of $<2$ with concentrated sulfuric acid. The other sample was used for nitrate, nitrite, sulfate, total suspended solids, volatile solids, and fixed solids and was stored in a refrigerator at 4°C. These subsamples were shipped in a cooler with ice packs by overnight express to the Yankton FRS for analysis. All water quality characteristics were measured according to standard methods (APHA et al., 1995), except for the nitrogenous chemicals and chloride. Ammonia, nitrate, and nitrite were measured using ion-selective electrodes according to the procedures outlined by the electrode manufacturer for low-concentration measurements (Orion, 1990, 1991; ATI Orion, 1994). Chloride was measured according to a modified APHA et al. (1995) method (Hach Company, 1992).

Subsamples of water collected between December 1996 and April 1997 for water quality analyses from five sample stations (HT1, AC5, WW1, WW2, WW4a) were taken for selenium analysis and from three sample stations (HT1, AC5, WW2) for ICP analysis of inorganic elements. The samples for selenium analysis were collected monthly and those for ICP bimonthly. Filtered and unfiltered water was collected for selenium analysis. Water was filtered through a 0.4-$\mu$m polycarbonate filter using a Geotech filtration unit, and 200 mL of filtered water samples was acidified with 2 mL of ultrapure HCl and stored frozen until analysis of selenium concentrations. Two hundred milliliters of unfiltered water samples was acidified with 2 mL of ultrapure HCl and stored frozen until analysis of selenium concentrations. Samples for ICP analysis were filtered as described above and acidified with 2 mL of ultrapure $HNO_3$ and stored frozen.

Samples of sediment were collected in October 1996 and April 1997 from Horsethief, Horsethief east wetland ([HTEW] received effluent from the ponds used to hold the adult razorback suckers), Adobe Creek (AC3), and North Pond (WW2). Sediment core samples were collected by pushing a 30-cm-long, 7.6-cm-diameter PVC (polyvinyl chloride plastic) pipe (previously cut in half lengthwise) into the sediment using an apparatus that kept the sides for splitting open as the pipe was forced into the sediments. The cores were immediately frozen to maintain the longitudinal integrity of the sample and then shipped frozen. Three subsamples of each sediment core were collected by removing the end caps, splitting the pipe sides, removing the frozen sediment core, and cutting 1-cm sections from the top, middle, and bottom of each core sample. These 1-cm sections were analyzed for selenium concentrations.

## Inorganic Element Analyses

All samples collected for selenium analysis were analyzed at the Yankton FRS using a PerkinElmer model 3300 atomic absorption spectrophotometer equipped with a model MHS-10 hydride generator (AA-HG). The spectrophotometer was standardized with National Institute of Standards and Technology (NIST) standard reference material 3149 (for water). Water samples were digested using a persulfate digestion technique and total selenium determined by a modification of the method of Presser and Barnes (1984). Quality assurance and quality control measures included determination of limit of detection, procedural blanks for background equivalent concentration, percentage of relative standard deviation of triplicate sample preparation and analysis, recovery of elements from reference material, and recovery of digested-spiked sample solutions and analysis-spiked samples at the AA-HG. The mean limit of detection (LOD) was 0.7 $\mu$g/L ($SE = 0.1$, $n = 17$). For water the procedure blanks had background concentrations less than the LOD. The mean percent relative standard deviation (triplicate sample preparation and analysis) was 4.3% ($SE = 0.6$, $n = 17$). Recovery of selenium from NIST reference material 1643c water and NIST reference material 1643d was within Columbia Environmental Research Center–recommended recommended ranges. The mean percentage of recoveries of digested-spiked sample solutions was 95% ($SE = 2$, $n = 34$). Mean selenium recoveries of analysis-spiked samples analyzed for matrix suppression or enhancement was 101% ($SE = 2$, $n = 17$).

All sediment, fish egg, zooplankton, and commercial fish food samples were prepared for analyses of selenium con-

centrations by first lyophilizing the sample to a constant dry weight using a Virtis Vacu-Freezer. Fish samples were then homogenized with a food processor. Animal tissue, fish food, and sediment samples were digested using a combination nitric acid wet digestion/magnesium nitrate dry ash technique (Pettersson et al., 1986). The dry ash procedure was accomplished in a Thermolyne model FA1730 muffle furnace. Total selenium was determined by a modification of the method of Presser and Barnes (1984). Quality assurance and quality control measures were the same as for the water analyses, and the results are summarized in Table I.

Analyses of inorganic elements in water, zooplankton, and fish egg samples were performed by ICP at the Environmental Trace Substances Research Center (University of Missouri), Rolla, Missouri. The list of elements and LOD are given in Table II. For water the procedure blank had background-equivalent concentrations less than the LOD for all elements except cobalt and zinc. The mean percent relative standard deviation (duplicate sample preparation and analysis) was 9.6%, the mean spike recovery was 107%, and the recovery of trace elements from NIST reference water 1643D was within recommended ranges except for cobalt, chromium, lithium, magnesium, and zinc. For zooplankton the procedure blank had background equivalent concentrations less than the LOD for all elements, the mean percent relative standard deviation (duplicate sample preparation and analysis) was 5.9%, the mean spike recovery was 94%, and the recovery of trace elements in National Research Council of Canada (NRCC) reference material DORM2 (dogfish muscle) was within recommended ranges except for aluminum, silver, and zinc. For fish eggs the procedure blank had background equivalent concentrations less than the LOD for all elements except silicon and zinc, the mean percent relative standard deviation (duplicate sample preparation and analysis) was 8.9%, the mean spike recovery was 96%, and the recovery of trace elements in NRCC reference material DOLT2 (dogfish liver) was within recommended ranges except for arsenic, cadmium, lead, manganese, and nickel.

Muscle plugs from all the spawned fish and some of the unspawned fish were analyzed for selenium concentrations to ensure an adequate sample size for statistical analysis. Muscle plugs and larvae were prepared for analysis at the Columbia Environmental Research Center, and neutron activation analysis was performed at the University of Missouri Research Reactor (MURR), Columbia, Missouri. All sample preparations prior to neutron activation analyses and the neutron activation method were done as described in Waddell and May (1995). Samples were transported to MURR for determination of the radionuclide $^{77m}$Se (McKown and Morris, 1978). Selenium standards and quality control samples were analyzed in the same manner as animal tissues. National Institute of Standards and Technology 1577 (bovine liver) standard reference material was analyzed by MURR as quality control checks on accuracy and precision. The recovery of selenium was within the NIST-recommended range, and the percent relative standard deviation of multiple analyses ($n$ = 11) was 4.2%. Selenium values in micrograms were obtained by direct comparison of peak areas obtained for the samples with the average peak areas obtained for a set of standards. The limit of detection was 0.015 μg/g.

**TABLE I. Mean (standard error in parentheses and number of samples in brackets) quality assurance and quality control measures for selenium analysis of sediment and biological samples**

| | Matrix | | |
|---|---|---|---|
| Measure | Sediment | Fish Eggs | Aquatic Invertebrates |
| Limit of detection (μg/g) | 0.14 | 0.04 | 0.10 |
| | (0.03) | (0.01) | (0.04) |
| | [6] | [4] | [7] |
| % RSD[a] | 4.2 | 4.8 | 4.7 |
| | (0.4) | (0.5) | (1.1) |
| | [6] | [4] | [7] |
| Reference material | 0.38[b] | 1.26[c] | 1.41[c] |
| | (0.01) | (0.03) | (0.03) |
| | [6] | [4] | [7] |
| *Digested spikes*[d] | 100 | 99 | 100 |
| | (2) | (3) | (2) |
| | [12] | [8] | [14] |
| Analysis spikes[e] | 95 | 105 | 99 |
| | (5) | (7) | (4) |
| | [6] | [4] | [7] |

[a] RSD: Percent relative standard deviation for triplicate preparation and analysis.

[b] National Research Council of Canada (NRCC) reference material BCSS-1 (marine sediment; 0.43 ± 0.06 μg/g).

[c] NRCC reference material DORM-2 (dogfish muscle tissue; 1.40 ± 0.09 μg/g).

[d] Percent recovery of selenium from samples spiked with selenomethionine at the beginning of preparation for sample analysis.

[e] Percent recovery of selenium from digested samples spiked with selenite after sample preparation but before instrument analysis.

## Statistics

Data were analyzed using SAS sofware (Statistical Analysis System Institute, 1990). Analysis of variance testing was used to determine treatment effects on residues in water, sediment, zooplankton, and muscle plugs (the values logarithmically transformed) among sites and sample stations within sites. When significant differences ($p$ = 0.05) were observed, means were compared by the Bonferroni (Dunn) multiple means comparison test (Snedecor and Cochran, 1967).

Correlation analyses were used to test for relations among water quality characteristics, inorganic element concentrations, effects on fish, and tissue residue concentrations. Correlation analyses of the means with standard deviation and variance measures were conducted to determine

**TABLE II. Limit of detection of elements measured by inductively coupled argon plasma spectroscopy in water (μg/L), zooplankton (μg/g dry weight), and fish eggs (μg/g dry weight)**

| | Matrix | | |
|---|---|---|---|
| Element | Water | Zooplankton | Fish Eggs |
| Ag | 10 | 0.3 | 0.3 |
| Al | 20 | 2 | 2 |
| As | 30 | 2 | 2 |
| B | 10 | 0.5 | 0.5 |
| Ba | 0.7 | 0.05 | 0.05 |
| Be | 0.2 | 0.05 | 0.06 |
| Bi | 10 | 1 | 1 |
| Cd | 2 | 0.2 | 0.2 |
| Co | 1 | 0.3 | 0.3 |
| Cr | 9 | 1 | 1 |
| Cu | 1 | 0.2 | 0.2 |
| Fe | 7 | 0.5 | 0.5 |
| Li | 2 | 0.4 | 0.4 |
| Mg | 1 | — | — |
| Mn | 2 | 0.07 | 0.06 |
| Mo | 7 | 0.2 | 0.2 |
| Ni | 7 | 0.4 | 0.4 |
| Pb | 30 | 0.9 | 0.8 |
| Sb | 30 | 1 | 1 |
| Si | 40 | 3 | 3 |
| Sn | 30 | 1 | 1 |
| Sr | 0.2 | 0.02 | 0.02 |
| Ti | 0.5 | 0.1 | 0.09 |
| Tl | 70 | 10 | 10 |
| V | 2 | 0.2 | 0.2 |
| Zn | 1 | 0.1 | 0.09 |

if transformations were needed to meet the assumptions of normality and homogeneity of variance (M. Ellersieck, University of Missouri, personal communication). The residue data for water, sediment, zooplankton, and muscle plugs and the water quality measures in the egg test were $\log_{10}$-transformed prior to correlation analysis.

## RESULTS

### Water Quality

Water quality characteristics were significantly different at the three sites, with the water at North Pond having higher conductivity, hardness, magnesium, chloride, and sulfate than the water at Horsethief and Adobe Creek (Table III). Horsethief had a significantly higher pH than did Adobe Creek or North Pond.

Water quality, characterized primarily by conductivity, varied during the study. This variation at Horsethief and Adobe Creek was partly a result of the relatively lower conductivity values of the Colorado River during high runoff. Water quality parameters at Horsethief closely matched those of the Colorado River. Conductivity was lowest in North Pond at WW2 on October 16, 1996, which was about 3 months after the high river runoff. For Horsethief the range of values was 593–1060 μmhos/cm conductivity, 208–406 mg/L $CaCO_3$ hardness, and 106–161 mg/L $CaCO_3$ alkalinity. For Adobe Creek the range of values was 786–1260 μmhos/cm conductivity, 260–366 mg/L $CaCO_3$ hardness, and 98–150 mg/L $CaCO_3$ alkalinity. For North Pond the range of values was 1240–4630 μmhos/cm conductivity, 352–1460 mg/L $CaCO_3$ hardness, and 87–343 mg/L $CaCO_3$ alkalinity.

### Selenium and Other Elements in Water

There was no significant differences in selenium concentrations between filtered and unfiltered water at the stations within the three sites where adults were held, and the data were combined within a sample station for further statistical analysis (Table IV). Selenium concentrations in water at Horsethief and Adobe Creek were not significantly different from each other, but they were both significantly different from North Pond. Selenium concentrations at Horsethief averaged 2.2 μg/L, at Adobe Creek 2.6 μg/L, and at North Pond 7.8 μg/L. Selenium concentrations in water at Horsethief were similar to those in Colorado River samples collected at Walter Walker State Wildlife Area ([WW1] 1.6–4.4 μg/L).

The highest selenium concentrations at North Pond occurred on April 15, 1997 (17.1 μg/L), just prior to removing the adults for spawning. Most selenium in North Pond probably came from the groundwater, as demonstrated by the elevated selenium concentrations at WW4a (adjacent and east of North Pond), whose only water source was groundwater. When the Colorado River was at low flow, selenium concentrations at WW4a between December 1996 and April 1997 ranged from 82 to 152 μg/L (Table IV).

For inorganic elements in water measured by ICP, boron and lithium were significantly higher at North Pond than at Adobe Creek, strontium was significantly higher at North Pond than at Horsethief, and magnesium was significantly higher at North Pond than at Adobe Creek or Horsethief (Table V). Selenium concentrations measured by AA-HG in water from Horsethief, Adobe Creek, and North Pond were not significantly correlated on a site basis with any of the inorganic elements measured by ICP in water, except for barium. Barium was significantly correlated ($r = -0.998$, $p = 0.04$) with selenium concentrations in North Pond water. However, when the data were combined for the three sites, selenium measured by AA-HG in water was significantly correlated with five elements measured by ICP: boron ($r = 0.98$, $p = 0.0001$, $n = 8$), lithium ($r = -.99$, $p = 0.0001$, $n = 8$), magnesium ($r = 0.98$, $p = 0.0001$, $n = 8$), strontium ($r = 0.90$, $p = 0.003$, $n = 8$), and vanadium ($r = 0.99$, $p = 0.01$, $n = 4$).

**TABLE III. Mean (standard error in parentheses, $n = 9$) water quality characteristics measured in water collected at three stations near Grand Junction, Colorado**

| | Station | | |
|---|---|---|---|
| Measure | HT1 | AC5 | WW2 |
| pH | 8.2b | 7.9a | 7.9a |
| | (0.1) | (0.1) | (0.1) |
| Conductivity (μmhos/cm) | 901a | 1,000a | 2,310b |
| | (51) | (58) | (440) |
| Hardness (mg/L as $CaCO_3$) | 299a | 311a | 683b |
| | (21) | (14) | (148) |
| Calcium (mg/L) | 79a | 80a | 118a |
| | (5) | (4) | (19) |
| Magnesium (mg/L) | 25a | 27a | 94b |
| | (2) | (1) | (24) |
| Alkalinity (mg/L as $CaCO_3$) | 131a | 133a | 179a |
| | (5) | (6) | (29) |
| Chloride (mg/L) | 74a | 104a | 232b |
| | (4) | (9) | (39) |
| Sulfate (mg/L) | 233a | 239a | 830b |
| | (24) | (12) | (231) |
| Un-ionized ammonia (mg/L $NH_3$-N) | <0.01 | <0.01 | <0.01 |
| | (0) | (0) | (0) |
| Nitrate (mg/L $NO_3$-N) | 0.5a | 0.2a | 0.2a |
| | (0.1) | (0) | (0.1) |
| Nitrite (mg/L $NO_2$-N) | 0.01a | 0.01a | 0.02a |
| | (0) | (0) | (0.01) |
| Total suspended solids (mg/L) | 24.8a | 14.7a | 8.8a |
| | (6.3) | (4.9) | (3.5) |
| Volatile solids (mg/L) | 3.3a | 2.5a | 2.8a |
| | (0.8) | (0.7) | (0.9) |
| Fixed solids (mg/L) | 21.5a | 12.2a | 6.0a |
| | (5.7) | (4.3) | (2.6) |

For each measure, stations with the same letter are not significantly different ($P = 0.05$).

There was a significant positive correlation between selenium in water with several water quality characteristics at Horsethief including, from highest to lowest correlation coefficient ($r$), sulfate (0.99, $p = 0.0009$), hardness (0.99, $p = 0.001$), calcium (0.94, $p = 0.02$), conductivity (0.91, $p = 0.03$), and magnesium (0.88, $p = 0.05$), whereas there was a negative correlation for volatile solids ($r = -0.88$, $p = 0.05$). At North Pond the significant positive correlations were, from highest to lowest correlation coefficient ($r$), nitrate (0.997, $p = 0.0002$), conductivity (0.89, $p = 0.04$), and chloride (0.88, $p = 0.05$). There were no significant correlations between selenium in water and water quality characteristics at Adobe Creek.

## Selenium in Sediment

Selenium concentrations in various portions of sediment cores from Horsethief, Horsethief east wetland, Adobe Creek, and North Pond were significantly different from each other (Table VI). Selenium concentrations in the top portion of sediment cores collected on October 21, 1996, went from lowest, at Horsethief, to that at Horsethief east wetland and Adobe Creek to the highest, at North Pond. A similar pattern in selenium concentrations was observed for the top portion of sediment cores collected on April 14, 1997. In general, selenium concentrations in sediment cores tended to decrease with depth.

## Selenium in Biota

Selenium concentrations in zooplankton from Adobe Creek ranged from 15.6 to 19.6 μg/g at AC3 and was 20.0 μg/g at AC5 (Table VII). Selenium concentrations in zooplankton at North Pond ranged from 31.9 to 48.1 μg/g. Selenium concentrations in zooplankton were significantly lower at Adobe Creek than at North Pond. Selenium concentrations in zooplankton collected from the Horsethief east wetland ranged from 4.4 to 5.6 μg/g. Adult fish in the ponds did not have access to HTEW, but this site was used as a food source in other studies.

The selenium concentration in chironomids at AC5 was 48.3 μg/g for a composite sample collected on September 18 and 30, 1996. At AC3 selenium concentrations in chironomids were 33.6 μg/g for a composite sample collected on September 30 and October 5, 1996; 31.7 μg/g for a composite sample collected on October 21 and November 5, 1996; and 34.0 μg/g for a composite sample collected on April 21 and May 19, 1997. Selenium concentrations in chironomids collected from HTEW were 9.3 μg/g for a composite sample collected on September 30 and October 21, 1996; and 7.9 μg/g for a composite sample collected on April 21 and May 20, 1997. Selenium concentrations in chironomids were between 1.4 and 2.0 times higher than in zooplankton collected from the same station at about the same time. The correlation between selenium concentrations in chironomids and zooplankton was $r = 0.94$ ($p = 0.005$, $n = 6$). The Spearman correlation ($r_s$) between selenium concentrations in sediment (assuming a nonnormal distribution of selenium in sediments; Peltz and Waddell, 1991; Stephens, 1996; Zhang and Moore, 1997) and selenium concentrations in chironomids was $r_s = 0.87$ ($p = 0.05$, $n = 5$).

## Growth

There was no significant difference in length between fish stocked at the three sites on July 11, 1996, but fish at North Pond weighed significantly less (745 g) than fish stocked at Horsethief (817 g) or Adobe Creek (820 g; Table VIII). At spawning, fish were significantly shorter at Adobe Creek

**TABLE IV. Selenium concentration (μg/L) in filtered and unfiltered water at sample stations in the Horsethief Canyon State Wildlife Area (HT1), Adobe Creek (AC5), and North Pond (WW2) near Grand Junction, Colorado, where adult razorback suckers were held, and also the Colorado River (WW1) and a marsh draining into a backwater channel (WW4a)**

| Sample Type | Date | Day of Exposure | Station | | | | |
|---|---|---|---|---|---|---|---|
| | | | HT1 | AC5 | WW1 | WW2 | WW4a |
| Filtered | | | | | | | |
| | 12/09/96 | 151 | 2.8 | <0.7[a] | 3.5 | 3.9 | 84 |
| | 01/06/97 | 179 | 2.1 | 2.8 | 2.6 | 3.2 | 109 |
| | 02/10/97 | 214 | 1.9 | 2.7 | 2.1 | 6.0 | 152 |
| | 03/12/97 | 244 | 2.0 | 2.7 | 2.3 | 7.8 | 119 |
| | 04/15/97 | 278 | 1.4 | 1.2 | 1.6 | 15.6 | 98 |
| Unfiltered | | | | | | | |
| | 12/09/96 | 151 | 3.0 | 4.5 | 2.9 | 4.3 | 82 |
| | 01/06/97 | 179 | 2.6 | 3.0 | 4.4 | 3.5 | 116 |
| | 02/10/97 | 214 | 2.5 | 3.2 | 2.4 | 5.5 | 148 |
| | 03/12/97 | 244 | 1.9 | 2.9 | 2.5 | 11.0 | 125 |
| | 04/15/97 | 278 | 1.8 | 2.7 | 1.8 | 17.1 | 106 |

[a] <: Below limit of detection.

(431 mm) and weighed less (944 g) than were fish at Horsethief (443 mm, 1036 g), but there were no differences between fish at North Pond and Horsethief. Fish at Horsethief grew 4.7% in length and gained 26.8% in weight, at Adobe Creek grew 1.2% in length and gained 15.1% in weight, and at North Pond grew 2.5% in length and gained 35.0% in weight during the 286-day exposure period.

For fish stocked on August 27, 1996, there was no significant differences in fish length or weight at stocking or at spawning. The fish at Horsethief grew 8.1% in length and gained 37.8% in weight, at Adobe Creek grew 1.9% in length and gained 8.2% in weight, and at North Pond grew 3.6% in length and gained 7.0% in weight during the 239-day exposure period.

For the fish measured at spawning, 31, 59, and 86 days during the depuration phase, there were no significant differences in fish length or weight among the three sites compared with measurements made at spawning (Table VIII). However, fish from the three sites lost weight after 86 days of depuration. Horsethief fish lost 5.4% of weight (mean = 57 g), Adobe Creek fish lost 3.8% (mean = 39 g), and North Pond fish lost 2.7% (mean = 29 g).

## Selenium in Tissues

Concentrations of selenium in muscle plugs from adults held at Horsethief did not change significantly during the exposure or depuration periods (Table IX). Selenium concentrations in muscle plugs of fish held at Adobe Creek and North Pond were significantly higher than those of fish held at Horsethief at spawning (286 days of exposure) and at 31, 59, and 86 days of depuration. At spawning but not during depuration, selenium concentrations in muscle plugs of fish from North Pond were significantly higher than those from Adobe Creek. Mean selenium concentrations in muscle plugs were 1.7 times higher in fish at Adobe Creek and 1.6 times higher in fish at North Pond at spawning (April 23, 1997) compared with those at the time of stocking (July 11, 1996).

Mean selenium concentrations in muscle plugs from Adobe Creek fish at spawning in the present study were 4.2 times higher than those of fish sampled on July 6, 1995 (3.9 μg/g), prior to their initial stocking at Adobe Creek. Similarly, mean selenium concentrations in muscle plugs from North Pond fish at spawning in the present study were 5.6 times higher than those of fish sampled on July 6, 1995 (4.1 μg/g), prior to their initial stocking at North Pond. In the present study, following initial stocking at Adobe Creek and North Pond, fish were exposed for 305 days, spawned, held for 66 days of depuration at Horsethief, then restocked at North Pond for 286 days.

One fish (HT66) stocked at Adobe Creek in the present study had previously been held at Horsethief for 11 months (Hamilton et al., 2001a). At spawning in the present study, this fish had selenium residues lower than fish held at Adobe Creek for 9 months during the first study, followed by 66 days of depuration, and then exposed for 286 days in the present study. The muscle plug selenium values for this fish during the depuration phase were also lower than other fish that had previously been held at Adobe Creek. If muscle plug selenium values for this fish were omitted, the means in Table IX for the Adobe Creek would be 16.6 μg/g at 286 days, 19.0 μg/g at 316 days, 16.0 μg/g at 344 days, and 15.5 μg/g at 371 days. Likewise, two fish (HT12 and HT42) stocked at North Pond in the present study had previously been held at Horsethief for 11 months. At spawning in the present study, these fish had selenium residues lower than other fish held at North Pond for 9 months during the first study, followed by 66 days of depuration, and then 286 days in the present study. The muscle plug selenium values for

**TABLE V. Mean (standard error in parentheses and number of samples in brackets) concentration of inorganic elements (mg/L) in water collected from three stations near Grand Junction, Colorado**

| | Station | | | | Station | | |
|---|---|---|---|---|---|---|---|
| Element | HT1 | AC5 | WW2 | Element | HT1 | AC5 | WW2 |
| Ag | <0.01[a] | <0.01 | <0.01 | Mg | 23a | 26a | 90b |
| Al | 0.03 | <0.02 | 0.02 | | (3) | (1) | (33) |
| | (0) | | (—) | | [5] | [5] | [5] |
| | [3][b] | | [1] | Mn | 0.013 | 0.030 | 0.024 |
| As | <0.03 | <0.03 | 0.03 | | (0.003) | (0.008) | (0.006) |
| | | | (—) | | [5] | [5] | [5] |
| | | | [1] | Mo | <0.007 | 0.010 | 0.009 |
| B | 0.065ab | 0.049a | 0.110b | | | (0) | (0.001) |
| | (0.008) | (0.002) | (0.027) | | | [2] | [3] |
| | [5] | [5] | [5] | Ni | <0.007 | 0.007 | <0.007 |
| Ba | 0.098 | 0.115 | 0.107 | | | (0) | |
| | (0.006) | (0.009) | (0.007) | | | [3] | |
| | [5] | [5] | [5] | Pb | <0.03 | <0.03 | <0.03 |
| Be | <0.0002 | 0.0003 | <0.0002 | Sb | <0.03 | <0.03 | <0.03 |
| | | (—) | | Si | 0.74 | 0.64 | 0.45 |
| | | [1] | | | (0.16) | (0.16) | (0.11) |
| Bi | 0.02 | 0.02 | 0.03 | | [5] | [5] | [5] |
| | (0) | (0) | (0.01) | Sn | <0.03 | <0.03 | <0.03 |
| | [5] | [5] | [3] | Sr | 0.75a | 0.78ab | 1.49b |
| Cd | <0.002 | <0.002 | <0.002 | | (0.10) | (0.06) | (0.32) |
| Co | <0.001 | <0.001 | <0.001 | | [5] | [5] | [5] |
| Cr | 0.010 | <0.009 | 0.010 | Ti | 0.0011 | 0.0010 | 0.0010 |
| | (0) | | (—) | | (0.0003) | (0.0002) | (0) |
| | [4] | | [1] | | [3] | [4] | [4] |
| Cu | 0.020 | <0.001 | <0.001 | Tl | 0.07 | <0.07 | 0.09 |
| | (0.008) | | | | (—) | | (—) |
| | [5] | | | | [1] | | [1] |
| Fe | 0.023 | 0.045 | 0.028 | V | <0.002 | 0.003 | 0.004 |
| | (0.004) | (0.021) | (0.003) | | | (—) | (0.001) |
| | [5] | [5] | [5] | | | [1] | [2] |
| Li | 0.031ab | 0.027a | 0.049b | Zn | 0.005 | 0.010 | 0.009 |
| | (0.004) | (0.002) | (0.009) | | (0.001) | (0.002) | (0.002) |
| | [5] | [5] | [5] | | [4] | [5] | [5] |

For B, Li, Mg, and Sr, mean station values with letters in common are not significantly different from each other ($P = 0.05$).

[a] <: Below limit of detection.

[b] Five samples were submitted for analysis from each station. If the number of samples shown for a station and element is less than 5, concentrations in the other samples were below the limit of detection.

these fish during the depuration phase also were lower than other fish that had previously been held at North Pond. If muscle plug selenium values from these two fish were omitted, the means in Table IX for North Pond would be 25.5 μg/g at 286 days, 21.0 μg/g at 316 days, 21.7 μg/g at 344 days, and 19.0 μg/g at 371 days.

Selenium concentrations in muscle plugs of fish held at Adobe Creek for 9 months decreased about 7% after 59 days of depuration and 14% after 86 days of depuration. Of the four fish sampled after 31 days of depuration at Adobe Creek, one (fish AC18) had selenium concentrations that were 21% higher than those at spawning. Selenium concentrations in fish held at North Pond for 9 months lost 13% of their selenium load at 59 days of depuration and 21% at 86 days of depuration.

Loss of selenium during depuration from fish held at Adobe Creek and North Pond probably was not a result of tissue dilution because the fish lost weight during the depuration period. Average weight lost in fish from Adobe Creek was 3.8% and from North Pond was 2.7% at 86 days' depuration compared with measurements at spawning.

## Adult Collection and Spawning

The number of adults recaptured in April 1997 for spawning was 31 of the 35 stocked at Adobe Creek, 20 of 31 at North

**TABLE VI. Mean (standard error in parentheses, $n = 3$) selenium concentration ($\mu$g/g dry weight) in the top, middle, and bottom of sediment cores collected from four stations near Grand Junction, Colorado**

| Date | Day of Exposure | Core Section | Station | | | |
|---|---|---|---|---|---|---|
| | | | HT1 | HTEW[a] | AC3 | WW2 |
| 10/21/96 | 102 | Top | 0.42a | 0.83b | 1.21c | 94.37d |
| | | | (0.03) | (0.03) | (0.15) | (2.25) |
| | | Middle | <0.27[b] | <0.27 | <0.27 | 1.55 |
| | | | | | | (0.02) |
| | | Bottom | <0.27 | 0.30a | 0.37a | 2.91b |
| | | | | (0.02) | (0.07) | (0.07) |
| 4/14/97 | 277 | Top | —[c] | 0.91a | 2.52b | 16.00c |
| | | | | (0.02) | (0.07) | (0.38) |
| | | Middle | — | 0.33a | 0.50b | 12.67c |
| | | | | (0.01) | (0.02) | (0.05) |
| | | Bottom | — | 0.14a | 0.57b | 1.79c |
| | | | | (0.01) | (0) | (0.16) |

For each date and core section, stations with the same letter are not significantly different ($P = 0.05$).
[a] HTEW: Horsethief east wetland.
[b] <: Less than the limit of detection for that analysis.
[c] —: Not sampled.

Pond, and all 38 at Horsethief. The missing adults at Adobe Creek and North Pond were assumed to have died during the 9-month exposure period or to have escaped capture efforts. For spawning on April 23–24, 1997, 15 females from Adobe Creek were available, of which 5 were spawned; 10 from North Pond, of which 5 spawned; and 13 from Horsethief, of which 5 spawned. Several brood stock fish were spawned for other purposes, and eggs from these fish were used in egg measurements. Fish were spawned again on April 25, 1997 (6 from Horsethief and North Pond and 4 from Adobe Creek) because of nearly complete egg mortality in the first 2 days after the initial spawning.

## Selenium and Other Elements in Eggs

Mean selenium concentrations in eggs were 6.0 $\mu$g/g from Horsethief adults, 40.1 $\mu$g/g from Adobe Creek adults, and 54.7 $\mu$g/g from North Pond adults (Table X). Eggs from three brood stock fish held at Horsethief contained a mean selenium concentration of 6.9 $\mu$g/g. Selenium concentra-

**TABLE VII. Concentration of selenium ($\mu$g/g dry weight) in zooplankton and chironomids collected from four stations near Grand Junction, Colorado**

| Organism | Collection Date | Day of Exposure | Station | | | |
|---|---|---|---|---|---|---|
| | | | HTEW | AC3 | AC5 | WW2 |
| Zooplankton | 09/20/96 | 71 | 4.4 | —[a] | 20.0 | 33.9 |
| | 10/01/96 | 82 | — | 19.6 | — | — |
| | 10/03/96 | 84 | — | — | — | 31.9 |
| | 10/30/96 | 111 | — | 15.6 | — | — |
| | 10/31/96 | 112 | 5.4 | — | — | — |
| | 11/21/96 | 133 | 5.0 | 18.0 | — | 48.1 |
| | 04/08/97 | 271 | — | 17.9 | — | 42.8 |
| | 04/18/97 | 281 | — | — | — | 37.9 |
| | 04/22/97 | 285 | 5.6 | — | — | — |
| Chironomid | 09/18 & 30/96 | 69, 81 | — | — | 48.3 | — |
| | 09/30 & 10/05/96 | 81, 86 | — | 33.6 | — | — |
| | 09/30 & 10/21/96 | 81, 102 | 9.3 | — | — | — |
| | 10/21 & 11/05/96 | 102, 117 | — | 31.7 | — | — |
| | 04/21 & 05/19/97 | 284, 312 | — | 34.0 | — | — |
| | 04/21 & 05/20/97 | 284, 313 | 7.9 | — | — | — |

Chironomid samples were composited before analysis.
[a] —: Not sampled.

**TABLE VIII. Mean (standard error in parentheses and number of samples in brackets) total length (mm), and weight (g) of adult razorback suckers held at three sites near Grand Junction, Colorado**

| Measure | Day of Exposure | Date | Site | | |
|---|---|---|---|---|---|
| | | | HT | AC | WW |
| Total length | 0 | 7/11/96 | 423a | 426a | 432a |
| | | | (3) | (3) | (4) |
| | | | [29] | [25] | [21] |
| | 0[a] | 8/27/96 | 369 | 367 | 361 |
| | | | (11) | (5) | (8) |
| | | | [10] | [10] | [10] |
| | 286 | 4/23/97 | 443b | 431a | 443ab |
| | | | (3) | (3) | (4) |
| | | | [29] | [25] | [16] |
| | 239[a] | 4/23/97 | 399 | 374 | 374 |
| | | | (12) | (8) | (7) |
| | | | [6] | [6] | [4] |
| | Depuration[b] 286 | 4/23/97 | 451 | 446 | 454 |
| | | | (3) | (7) | (8) |
| | | | [6] | [4] | [5] |
| | 316 | 5/23/97 | 467 | 465 | 474 |
| | | | (4) | (8) | (10) |
| | | | [6] | [4] | [4] |
| | 344 | 6/20/97 | 456 | 459 | 467 |
| | | | (4) | (9) | (10) |
| | | | [5] | [4] | [4] |
| | 371 | 7/17/97 | 456 | 462 | 466 |
| | | | (3) | (13) | (10) |
| | | | [6] | [3] | [4] |
| Weight | 0 | 7/11/96 | 817b | 820b | 745a |
| | | | (17) | (18) | (19) |
| | | | [29] | [25] | [21] |
| | 0[a] | 8/27/96 | 547 | 512 | 511 |
| | | | (45) | (19) | (36) |
| | | | [10] | [10] | [10] |
| | 286 | 4/23/97 | 1036b | 944a | 1006ab |
| | | | (22) | (21) | (23) |
| | | | [29] | [25] | [16] |
| | 239[a] | 4/23/97 | 754 | 554 | 547 |
| | | | (80) | (45) | (51) |
| | | | [6] | [6] | [4] |
| | Depuration[b] 286 | 4/23/97 | 1052 | 1013 | 1055 |
| | | | (70) | (43) | (55) |
| | | | [6] | [4] | [5] |
| | 316 | 5/23/97 | 1056 | 1014 | 1049 |
| | | | (50) | (52) | (67) |
| | | | [6] | [4] | [4] |
| | 344 | 6/20/97 | 1055 | 997 | 1050 |
| | | | (9) | (54) | (66) |
| | | | [5] | [4] | [4] |
| | 371 | 7/17/97 | 995 | 974 | 1026 |
| | | | (41) | (65) | (80) |
| | | | [6] | [3] | [4] |

For each day of exposure and measure, sites with the same letter are not significantly different ($P = 0.05$).

[a] Measures for 10 fish stocked on August 27, 1996 (day 47 of exposure period).

[b] All fish used in the depuration phase of exposure were originally stocked on July 11, 1996: Day 286 was spawning, day 316 was 31 days' depuration, day 344 was 59 days' depuration, and day 371 was 86 days' depuration.

**TABLE IX. Mean (standard error in parentheses and number of samples in brackets) selenium concentration (μg/g dry weight) in muscle plugs of razorback suckers held at three sites near Grand Junction, Colorado**

| Day of Exposure | Date | Site | | |
|---|---|---|---|---|
| | | HT | AC | WW |
| 0[a] | 7/11/96 | 4.5a | 9.6ab | 14.2b |
| | | (0.4) | (0.6) | (2.3) |
| | | [2] | [2] | [2] |
| Depuration[b] | | | | |
| 286 | 4/24/97 | 4.6a | 16.2b | 22.8c |
| | | (0.3) | (0.8) | (1.8) |
| | | [6] | [6] | [6] |
| 316 | 5/23/97 | 5.0a | 17.5b | 20.0b |
| | | (0.3) | (2.1) | (1.5) |
| | | [4] | [4] | [4] |
| 344 | 6/20/97 | 4.9a | 15.0b | 19.8b |
| | | (0.2) | (1.2) | (2.9) |
| | | [4] | [4] | [4] |
| 371 | 7/17/97 | 5.0a | 14.0b | 18.0b |
| | | (0.5) | (1.7) | (1.2) |
| | | [4] | [3] | [4] |

For each day of exposure, sites with the same letter are not significantly different ($P = 0.05$).

[a] Day 0 data from Hamilton et al. (2001a).

[b] Day 286 was spawning, day 316 was 31 days' depuration, day 344 was 59 days' depuration, and day 371 was 86 days' depuration.

tions in eggs from adults held at Horsethief, Adobe Creek, and North Pond and from brood stock were significantly different from each other. In general, selenium concentrations tended to be lower in eggs from the second spawn (April 25) than from the first (April 23), which was especially evident in spawns from the same adult (Hamilton et al., 2001b). Eggs from the second spawn also tended to have smaller diameters, also especially evident in spawns from the same adult. Selenium concentrations in eggs were correlated with selenium concentrations in muscle plugs of adults ($r = 0.88$, $p = 0.0001$, $n = 20$).

Of the inorganic elements measured by ICP in eggs, barium, copper, iron, and manganese concentrations were significantly different among the sites. Barium concentrations in eggs from brood stock fish were significantly higher than in Horsethief fish (Table XI). Copper concentrations in eggs from fish at Horsethief were higher than in eggs from fish at Adobe Creek and North Pond, but not those from brood stock. Manganese and iron concentrations in eggs from fish at Horsethief, Adobe Creek, and North Pond were not significantly different from each other, but all were significantly higher than in eggs from brood stock. Although barium, copper, iron, and manganese concentrations in eggs had significant differences among sites, the magnitude of difference was small—with the highest concentration from 1.4 to 3.2 times greater than the lowest concentration. In contrast, in eggs there was a 14- to 15-fold magnitude of difference between the highest and lowest selenium concentrations.

## Egg Characteristics

The percentage of moisture in eggs was similar among the young adults, but the moisture in eggs from North Pond adults was significantly higher than in eggs from the brood stock (Table X). However, the difference in mean percentage of moisture between the eggs from North Pond adults and the brood stock was only 1.6% and therefore may not be biologically important. The diameters of eggs from the young adults held at the three test sites were significantly smaller (13%–17%) than those of eggs from the brood stock fish (Table X).

## Egg Test

For the first spawn the percentage of viable eggs held overnight at 24-Road was 2.7% in one spawn and ≤1% in the other eight spawns (three spawns each for Horsethief, Adobe Creek, and North Pond). Most eggs were dead, regardless of adult source, the day after the egg test was begun, even though normal-looking eggs were used in the test. Examination of the eggs revealed oil globules in 60%–95% of the eggs, that is, the lipid compounds apparently were separated from the yolk. For the second spawn the percentage of viable eggs held about 36 h at 24-Road was 5% in one spawn, 2.7% in another spawn, and ≤1% in the remaining seven. Because egg viability was essentially zero for the exposed young adults used in the study, it was not possible to link viability with other measurements such as percentage of hatch or percentage of survival.

Eggs from one brood stock (80% viability) had no measurement of selenium made because of the limited number of eggs available for use in the egg test. Egg diameter was

**TABLE X. Mean (standard error in parentheses and number of samples in brackets) selenium concentration (μg/g dry weight), percent moisture, egg diameter (mm), and percent viability of eggs from razorback suckers held at three sites near Grand Junction, Colorado, including brood stock (BS)**

| Measure | Site (Adult Designation) | | | |
|---|---|---|---|---|
| | HT | AC | WW | BS |
| Selenium | 6.0a | 40.1c | 54.7d | 6.9b |
| | (0.2) | (1.0) | (1.1) | (0.2) |
| | [6] | [6] | [6] | [3] |
| Moisture | 92.8ab | 92.4ab | 93.2b | 91.6a |
| | (0.2) | (0.3) | (0.2) | (0.7) |
| | [6] | [6] | [6] | [3] |
| Egg diameter | 2.43a | 2.57a | 2.46a | 2.94b |
| | (0.06) | (0.04) | (0.07) | (0.07) |
| | [6] | [6] | [6] | [4] |
| Viability | 0–8 | 0–6 | 0–6 | 0–80 |

**TABLE XI. Mean (standard error in parentheses and number of samples in brackets) concentration of inorganic elements (μg/g dry weight) in eggs collected from razorback suckers held at three sites near Grand Junction, Colorado, including brood stock (BS)**

| | Site (Adult Designation) | | | |
|---|---|---|---|---|
| Element | HT | AC | WW | BS |
| Ag | <0.3[a] | <0.3 | <0.3 | <0.3 |
| Al | 3 (—) [1][b] | <2 | 2 (0) [2] | 3 (—) [1] |
| As | 3 (—) [1] | <2 | 6 (4) [2] | <2 |
| B | <0.05 | <0.5 | 0.6 (—) [1] | <0.5 |
| Ba | 0.35a (0.03) | 0.37ab (0.02) | 0.40ab (0.04) | 0.54b (0.07) |
| Be | <0.06 | <0.06 | <0.06 | <0.06 |
| Bi | <1 | <1 | <1 | <1 |
| Cd | <0.2 | <0.2 | <0.2 | <0.2 |
| Co | 0.3 (—) [1] | <0.3 | <0.3 | <0.3 |
| Cr | <1 | 1 (0) [3] | <1 | <1 |
| Cu | 3.7b (0.2) [5] | 2.7a (0.1) [5] | 2.9a (0.1) [5] | 3.1ab (0.1) [3] |
| Fe | 21.2b (0.9) [5] | 20.4b (1.1) [5] | 23.2b (0.8) [5] | 15.3a (1.3) [3] |
| Li | <0.4 | <0.4 | <0.4 | <0.4 |
| Mn | 4.4b (0.8) [5] | 5.7b (0.4) [5] | 6.1b (0.4) [5] | 1.9a (0.3) [3] |
| Mo | 0.2 (0) [2] | 0.3 (—) [1] | <0.2 | <0.2 |
| Ni | 0.6 (0.1) [3] | 0.5 (—) [1] | <0.4 | 0.7 (0.2) [3] |
| Pb | 1.0 (0) [2] | 1 (—) [1] | 1 (0) [2] | 1.0 (0.1) [2] |
| Sb | 2 (0) [3] | 1 (—) [1] | <1 | 2 (0) [3] |
| Si | 7 (1) [3] | <3 | <3 | 13 (5) [3] |
| Sn | <1 | <1 | <1 | <1 |
| Sr | 2.72 (0.2) [5] | 2.67 (0.2) [5] | 3.21 (0.17) [5] | 3.11 (0.30) [3] |
| Ti | <0.09 | 0.10 (—) [1] | 0.10 (0.01) [2] | 0.29 (0.11) [3] |
| Tl | 10 (—) [1] | <10 | <10 | <10 |
| V | 0.2 (—) [1] | 0.2 (—) [1] | 0.2 (—) [1] | 0.2 (0) [2] |
| Zn | 69.4 (1.6) [5] | 67.9 (1.4) [5] | 68.6 (2.0) [5] | 62.2 (3.3) [3] |

For Ba, Cu, Fe, Mn, and Se, sites with lowercase letters in common were not significantly different ($P = 0.05$); for the other elements, there were no significant differences among sites.

[a] <: All measurements were below the limit of detection.

[b] The number of samples submitted for analysis was HT = 5, AC = 5, WW = 5, and BS = 3. If the number of samples shown for a site and element is less than the number of samples submitted, concentrations in the other samples were below the limit of detection.

3.01 mm, and the selenium concentration in muscle plugs measured on July 11, 1996, was 3.5 μg/g (no muscle plug was measured in 1997). For the three Horsethief spawns (females HT16, HT19, and HT24), selenium concentrations in the eggs were 5.0, 6.2, and 5.9 μg/g; egg diameters were 2.3, 2.4; and 2.2 mm, and selenium concentrations in the adult muscle plugs at spawning on April 23, 1997, were 4.7, 5.3, and 3.6 μg/g, respectively. For the three Adobe Creek spawns (females AC17, AC18, and AC[HT66]), selenium concentrations in the eggs were 40, 43, and 36 μg/g; egg diameters were 2.5, 2.5, and 2.5 mm; and selenium concentrations in the adult muscle plugs at spawning were 16, 19, and 14 μg/g, respectively. For the three North Pond spawns (females WW40, WW52, and WW[HT12]), selenium concentrations in the eggs were 52, 53, and 53 μg/g; egg diameters were 2.4, 2.3, 2.2 mm; and selenium concentrations in adult muscle plugs at spawning were 27, 24, and 19 μg/g, respectively.

Water quality characteristics were consistent within each water type during the 5-day egg test but differed significantly among the four types (24-Road, HT, AC, and WW; Table XII). Each water type was significantly different from the others for hardness, calcium, and magnesium. For other characteristics there were significant differences among the sites, with North Pond water generally having the highest values of the four water types. In the exposure beakers mean dissolved oxygen concentrations in the four waters ranged from 7.3 to 7.4 mg/L, percentage of saturation ranged from 83% to 84%, and mean water temperature ranged from 20.8°C to 21.3°C. Selenium concentrations ($n = 2$) measured in water were 1.2–2.2 μg/g at Horsethief, 1.4–2.4 μg/g at Adobe Creek, 12.0–13.4 μg/g at North Pond, and <1 μg/L at 24-Road.

There was no significant difference in percentage of survival or percentage of hatch in eggs among the three egg sources (Horsethief, Adobe Creek, and North Pond), whether held in 24-Road water or on-site water (Table XIII). Percentage of survival and percentage of hatch in eggs from the brood stock ($n = 1$) did not appear to differ from the other three sources of eggs (Table XIII). There was a significant correlation between percentage of hatch and percentage of survival of eggs ($r = 0.92$, $p = 0.0001$, $n = 20$), but not between survival and selenium concentration in eggs ($r = 0.42$, $p = 0.08$, $n = 18$) or in adult muscle plugs ($r = 0.19$, $p = 0.43$, $n = 20$), and not between hatch and selenium concentration in eggs ($r = 0.43$, $p = 0.08$, $n = 18$) or in adult muscle plugs ($r = 0.06$, $p = 0.79$, $n = 20$). In contrast, there was a significant correlation between egg diameter and survival ($r = 0.49$, $p = 0.003$, $n = 20$) and between egg diameter and hatch ($r = 0.64$, $p = 0.003$, $n = 20$).

## Deformities

Deformities were recorded by type rather than by individual fish, and therefore no quantification of deformities was

**TABLE XII. Mean (standard error in parentheses, $n$ = 5) water quality characteristics measured in water used in the egg study**

| | Station | | | |
|---|---|---|---|---|
| Measure | HT1 | AC5 | WW2 | 24-Road |
| pH | 8.5c | 7.8a | 8.2bc | 8.1ab |
| | (0.1) | (0) | (0.1) | (0.1) |
| Conductivity ($\mu$mhos/cm) | 483a | 786a | 4,280b | 618a |
| | (61) | (12) | (28) | (19) |
| Hardness (mg/L as $CaCO_3$) | 181b | 263c | 1,214d | 65a |
| | (5) | (5) | (7) | (0) |
| Calcium (mg/L) | 49b | 65c | 159d | 18a |
| | (1) | (1) | (1) | (0) |
| Magnesium (mg/L) | 14b | 25c | 198d | 5a |
| | (1) | (1) | (2) | (0) |
| Alkalinity (mg/L as $CaCO_3$) | 109a | 134ab | 184c | 147b |
| | (1) | (3) | (2) | (12) |
| Chloride (mg/L) | 40a | 65b | 413c | 32a |
| | (2) | (1) | (3) | (3) |
| Sulfate[a] (mg/L) | 104 | 175 | 1,710 | 33 |
| Un-ionized ammonia[a] (mg/L $NH_3$-N) | <0.01 | <0.01 | <0.01 | <0.01 |

For each measure stations with the same letter are not significantly different ($P$ = 0.05).

[a] $n$ = 1 for sulfate and un-ionized ammonia.

possible. However, from a semiquantitative (summing deformity numbers) and qualitative viewpoint, larvae from the brood stock had the most deformities recorded, followed by larvae from North Pond adults (85% of the notations for brood stock larvae), Adobe Creek adults (77% of the notations for North Pond larvae), with the least deformities in Horsethief larvae (57% of the notations for Adobe Creek larvae and 44% of the notations for North Pond larvae) (Table XIV). Deformities in the brood stock were observed earlier (day 2) than in other larvae (day 3). A variety of deformities were observed, primarily in newly hatched and developing larvae, whereas cardiac edemas were observed in developing embryos.

Deformities in brood stock larvae included edemas (abdominal and cardiac), spinal deformities (curved tail and lateral, ventral, or dorsal flexures), and constricted yolk sacs, which were observed on days 2–5 of the study. Few deformities were observed on days 2–3 of the study, with increasing numbers observed on day 4 and the most deformities observed on day 5—for larvae from Horsethief, Adobe Creek, and North Pond adults. Deformities in North Pond and Adobe Creek larvae were primarily cardiac edemas and spinal deformities. In general, there were more deformities recorded for larvae held in reference water than for those held in site water. Larvae from Horsethief adults had more abdominal edema; larvae from Adobe Creek adults had more cardiac edema and ventral, lateral, and total spinal deformities; and larvae from North Pond adults had more cardiac and abdominal edema and ventral and total spinal deformities in reference water than in site water. One larva from Adobe Creek and four larvae from North Pond had microencephaly.

Most eggs with deformed embryos either died or did not hatch. Hatched live larvae that had deformities were counted as live, even though biologically they would most likely have not lived in the natural environment.

## DISCUSSION

### Water Quality

Concentrations of cations and anions in the water at Horsethief and Adobe Creek, as characterized by conductivity, probably did not adversely affect the razorback suckers held at those sites because these concentrations were similar to those in Colorado River water. Tyus (1987) and Tyus and Karp (1990) reported that razorback suckers gathered at Ashley Creek and Stewart Lake outlet from mid-April to May prior to making a spawning migration. During

**TABLE XIII. Mean (standard error in parentheses and number of samples in brackets) percent survival and hatch of eggs held in reference water (R) or site water (S), including eggs from brood stock (BS)**

| | Site and Water Type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HT | | AC | | WW | | BS275F[a] | |
| Measure | R | S | R | S | R | S | R | S |
| Survival | 29 | 32 | 69 | 71 | 44 | 56 | 73 | 67 |
| | (15) | (16) | (17) | (13) | (6) | (9) | (—) | (—) |
| | [3] | [3] | [3] | [3] | [3] | [3] | [1] | [1] |
| Hatch | 25 | 21 | 60 | 50 | 40 | 38 | 76 | 69 |
| | (13) | (11) | (15) | (7) | (5) | (7) | (—) | (—) |
| | [3] | [3] | [3] | [3] | [3] | [3] | [1] | [1] |

Differences among sites within either water type were not significant ($P$ = 0.05).

[a] PIT 7F7F36275F.

**TABLE XIV. Number of deformities of eggs and larvae held in reference water (R) or site water (S), including brood stock (BS)**

| | Site and Water Type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HT | | AC | | WW | | BS275F[a] | |
| Measure | R | S | R | S | R | S | R | S |
| Cardiac edema | 2 | 1 | 12 | 6 | 12 | 7 | 11 | 9 |
| Abdominal edema | 5 | 2 | 2 | 1 | 8 | 2 | 7 | 3 |
| Spinal deformity | 5 | 6 | 13 | 6 | 15 | 8 | 12 | 11 |
| Constricted yolk | 1 | 1 | 0 | 0 | 0 | 0 | 6 | 2 |
| Total | 13 | 10 | 27 | 13 | 35 | 17 | 36 | 25 |

[a] PIT 7F7F36275F.

that time conductivity ranged from 1510 to 2550 μmhos/cm. as reported by Stephens et al. (1988) and Peltz and Waddell (1991). This conductivity range is relatively close to those observed in the present study at Horsethief (range = 593–1060 μmhos/cm) and at Adobe Creek (range = 786–1260 μmhos/cm).

When adults were present at North Pond, conductivity (range = 1240–4630 μmhos/cm), salinity (range = 1.0–5.0 g/L), and hardness (range = 352–1460 mg/L as $CaCO_3$) were elevated. Nelson and Flickinger (1992) reported that the acute toxicity of salinity to juvenile Colorado pikeminnow was 13.1 g/L, but for risk assessment purposes they suggested that <9.7 g/L could be tolerated. For adult razorback suckers at North Pond, a salinity of 5.0 g/L was probably not stressful. The adults held at North Pond grew in length and gained weight during the 9-month exposure, which also suggests that they were not under stress. Apparently, the adults were able to convert the energy from their diet into growth and the development of sex products, rather than having to use it all to compensate for potential stresses associated with osmoregulation and toxicants.

## Selenium and Other Elements in Water

The similarity of selenium concentrations in filtered and unfiltered water samples in the present study was consistent with investigations of selenium-contaminated flowing water systems at California's Kesterson Reservoir (Fujii, 1988; Moore et al., 1990) and at seven riverine sites associated with irrigation drainage in the San Joaquin Valley, also in California (Saiki et al., 1993). There was a potential for the selenium concentrations in filtered and unfiltered water to be different because the Adobe Creek (AC5) and North Pond (WW2) sites are semistatic, with some flow of water from irrigation supply sources or pumped river water. For pond systems at Kesterson Reservoir, Fujii (1988) and Moore et al. (1990) showed that unfiltered water samples (reported as total selenium) had higher selenium concentrations than filtered samples (reported as dissolved selenium).

The mean concentrations of selenium in river water collected at Horsethief (mean = 2.2 μg/L) and WWSWA (WW1; 4.4 μg/L) were higher than at typical reference sites in the upper Colorado River. For example, Butler et al. (1996) reported that between 1987 and 1991 selenium concentrations were <1 μg/L ($n$ = 5) in the Gunnison River downstream from the Gunnison Tunnel and between 1980 and 1992 they were ≤1 μg/L ($n$ = 22) in the Colorado River at Cameo, Colorado. However, the reaches of the Colorado and Gunnison rivers influenced by irrigation return flows from the Grand Valley and Uncompahgre Valley irrigation projects as well as by private irrigation activities have elevated concentrations of selenium and other inorganic elements, as well as general water quality characteristics, because of irrigation return flows to the rivers (Butler et al., 1989, 1991, 1994, 1996). Consequently, the elevated selenium concentrations in water from Horsethief ponds, relative to reference areas in the upper Colorado and Gunnison rivers, suggests that adults at the Horsethief reference site were exposed to slightly elevated selenium concentrations during the present study.

The elevated selenium concentrations in water at AC5 (mean = 2.6 μg/L) were due in part to inflow of irrigation return water via AC7, whose selenium concentration ranged from 12.6 to 13.2 μg/L on May 28–30, 1997. The elevated concentrations of selenium in water at WW2 (mean = 7.8 μg/L) were due in part to inflow of groundwater from the underlying cobble aquifer (Phillips, 1986). Water in the cobble aquifer sampled as part of the National Irrigation Water Quality Program (NIWQP) in 1992 at a location about 5.5 km north of WWSWA had a selenium concentration of 175 μg/L (Butler et al., 1994). Water from the cobble aquifer comes to the surface in a marsh area adjacent to WW4a, which during the present study had selenium concentrations of 82–152 μg/L.

Selenium concentrations at Adobe Creek and North Pond were typical of other surface waters in the Grand and Uncompahgre valleys that are influenced by irrigation activities. Selenium concentrations were 4–7 μg/L (median = 5 μg/L, $n$ = 11) in the Colorado River at the Colorado–Utah state line, 5–7 μg/L (median = 6 μg/L, $n$ = 11) in the Gunnison River at Whitewater, and 8–25 μg/L (median = 14 μg/L, $n$ = 20) in the Uncompahgre River (Butler et al. 1994). Selenium concentrations in water at Adobe Creek and North Pond, in addition to most waters in the irrigation-

influenced areas of the Colorado, Gunnison, and Uncompahgre rivers, were elevated compared with uncontaminated aquatic ecosystems, which typically have selenium concentrations of <1 μg/L (Maier and Knight, 1994).

The significant differences in concentrations of inorganic elements in water in the present study (boron and lithium were higher in North Pond water than in Adobe Creek water, strontium was higher in North Pond than in Horsethief, and magnesium was higher in North Pond than in Adobe Creek or Horsethief) were similar to those observed in the previous reproduction study (Hamilton et al., 2001a). In the present study the selenium concentration in North Pond water was correlated only with barium, whereas in the previous study selenium concentrations were correlated with nine elements (boron, calcium, potassium, lithium, magnesium, molybdenum, sodium, phosphorous, and strontium). Combining the three sites in the present study, selenium concentrations in water were significantly correlated with boron, lithium, magnesium, strontium, and vanadium. Finger et al. (1994) also reported a strong relation among selenium, boron, cobalt, copper, lithium, and strontium. This correlation probably depends in part on the composition of the geologic material being leached by irrigation activities (Presser et al., 1994).

The significant positive correlations between selenium concentrations in water and water quality characteristics in the present study at Horsethief (calcium, conductivity, hardness, magnesium, and sulfate), Adobe Creek (none), and North Pond (chloride, conductivity) were similar to those observed in the previous reproduction study (Hamilton et al., 2001a). The reason for the inconsistent relation between water quality measures and selenium concentrations among the three test sites is unknown. Birkner (1978) reported no relation between selenium concentrations in water (0.3–15.9 μg/L) and water quality characteristics such as sulfate, hardness, and conductivity at 6 sites in Wyoming and 11 sites in Colorado. In contrast, Finger et al. (1994) reported a high correlation between selenium concentrations in water and specific conductance ($r = 0.90$, based on a log × log plot). Stephens et al. (1992) reported that correlation coefficients ranged from 0.35 to 0.91 for selenium concentrations in water and conductivity at four drains in the Stewart Lake area of Utah, suggesting an inconsistent relation between waterborne selenium and conductivity.

## Selenium and Other Elements in Sediment

Selenium concentrations in the three core sections of sediment at Horsethief and Adobe Creek (except for one value at Adobe Creek) were near national background concentrations of <1 μg/g (Maier and Knight, 1994), considered the no-effect concentration by Stephens et al. (1997) and the no-hazard concentration by Lemly (1995). Presser et al. (1994) reported the upper limit of the expected baseline range for selenium concentrations in soils of the western United States was 1.4 μg/g. In contrast, Moore et al. (1990) used 0.5 μg/g as a reasonable selenium concentration in sediment to represent the threshold between uncontaminated, background conditions and environments with elevated selenium concentrations in sediments.

The tertiary channel at Adobe Creek was diked to hold the adult fish for the reproduction study. In June 1995 a water control structure was installed in the dike road at AC3 and an inactive beaver dam with 2–3 feet of deep water near AC5 was converted to a dike with an outflow water control structure at AC5 (D. Crabtree, U.S. Bureau of Reclamation (USBR), personal communication). The 22-month period of the two reproduction studies may have been sufficient to allow for selenium accumulation in the sediments.

Sediment concentrations at AC3 seemed to increase over time: 0.79 μg/g in May 1995, 0.95 μg/g in October 1995, 1.11 μg/g in April 1996, 1.21 μg/g in October 1996, and 2.52 μg/g in April 1997 (Hamilton et al., 2001a). The sediment collection at AC3 was more susceptible to selenium deposition than either AC4 or AC5 because of the lack of water movement at AC3 compared with AC4, which had some flow because of pumped water from AC2 and flow from AC7, and compared with AC5, the site of the water outflow structure. Consequently, selenium concentrations in sediments at Adobe Creek seemed to have increased over time until they were within Lemly's (1995) low-hazard range of 2–3 μg/g and above the no-effect category (<2 μg/g) of Stephens et al. (1997).

Selenium concentrations in sediment at North Pond probably accumulated over a number of years, perhaps more than 20. North Pond appears in aerial photos taken in 1973 and 1982 (T. Mathieson, Colorado Division of Wildlife (CDOW), personal communication). The selenium concentrations of the core samples in the present study varied widely among the core sections. The reason for this variability is unknown, but variability of selenium concentrations has been reported by Peltz and Waddell (1991), Stephens (1996), and Zhang and Moore (1997). Combining the three values (top, middle, and bottom sections) for each sample date, the average selenium concentrations for the present study were 32.9 μg/g on October 21, 1996, and 10.2 μg/g on April 14, 1997. Most selenium concentrations in sediment observed in North Pond were above the high-hazard value of >4 μg/g proposed by Lemly (1995) and above the toxic threshold guideline value, also >4 μg/g, proposed by Stephens et al. (1997).

Selenium concentrations in sediment at Adobe Creek and North Pond were typical of sediments in the Grand and Uncompahgre valleys that have been influenced by irrigation activities. Selenium concentrations in sediment during 1987–1988 in areas of the Uncompahgre Valley affected by irrigation near Delta, Colorado, were 2.2–2.3 μg/g in the Uncompahgre River, and 2.3–4.0 μg/g in the Gunnison River (Butler et al., 1991). During 1992 selenium concentration in sediment in the Uncompahgre Valley ranged from 2.0 to 6.9 μg/g in creeks and was 16 μg/g in Markley Pond

(Butler et al., 1994). Selenium concentrations observed in sediments at North Pond were higher than those concentrations but were similar to those reported for Sweitzer Lake (also known as Garnet Mesa Reservoir) in 1987–1988 (9–41 μg/g; Butler et al., 1991) and for California's Kesterson Reservoir (aggregate geometric mean = 11.8 μg/g; Moore et al., 1990). Birkner (1978) reported selenium concentrations in sediment sampled during 1976–1977 of 6.5 μg/g at Sweitzer Lake and 15.4 μg/g at Desert Reservoir, near Grand Junction, Colorado, but only 1.2 μg/g at Mac Mesa Reservoir and only 1.8 μg/g at Highline Reservoir. Barnhart (1957) reported that sediment at Sweitzer Lake had selenium concentrations of 10–40 μg/g in 1956 and that various canals and ditches in the Sweitzer Lake area had concentrations of 1–25 μg/g. Elevated selenium concentrations in sediment were reported in several creeks and washes in the Grand Valley (1.9 μg/g in Salt Creek, 6.3 μg/g in Reed Wash, 3.9 μg/g in Adobe Creek, 5.6 μg/g in Leach Creek, and 16 μg/g in Indian Wash; Butler et al., 1994, 1996).

## Selenium and Other Elements in Biota

Selenium concentrations in zooplankton at Horsethief east wetland in the present study (4.4–5.6 μg/g) were higher than those reported for the Horsethief ponds in the previous reproduction study (2.3–3.1 μg/g; Hamilton et al., 2001a). This difference may be a result of the differences in the two water bodies: the ponds were relatively devoid of zooplankton and vegetation because of the large number of large fish held in the clay-bottomed ponds, whereas the east wetland had a very dense zooplankton population, dense vegetation, and an organic-rich bottom sediment.

Selenium concentrations in zooplankton at AC3 and AC5 in the present study (15.6–20.0 μg/g) were comparable to those reported in the previous reproduction study for a similar time period (18.5–52.0 μg/g; Hamilton et al., 2001a). Selenium concentrations in chironomids at AC3 and AC5 in the present study (31.7–48.3 μg/g) also were similar to those reported in the previous reproduction study (27.9–42.1 μg/g; Hamilton et al., 2001a). Likewise, selenium concentrations in zooplankton at WW2 in the present study (31.9–48.1 μg/g) were comparable to those reported in the previous reproduction study for a similar time period (25.5–36.9 μg/g; Hamilton et al., 2001a). The significant correlation between selenium concentrations in zooplankton with chironomids in the present study ($r = 0.94$, $p = 0.005$, $n = 6$) suggests a high degree of similarity in selenium bioaccumulation in food chain organisms from different functional groups.

Selenium concentrations in zooplankton collected from Adobe Creek and North Pond were substantially above the proposed dietary toxic threshold concentration of 3 μg/g (Lemly, 1993a, 1996; Maier and Knight, 1994). This threshold concentration was derived from examination of several laboratory and field investigations on a wide variety of fish species (Maier and Knight [1994] reviewed five studies; Lemly [1993a, 1996] reviewed 11 studies and 1 additional one in two articles). Six additional studies supporting this proposed dietary toxicity threshold for fish were given in Hamilton et al. (2000). Even though selenium concentrations in water were below the current USEPA criterion of 5 μg/L (USEPA, 1987) at Adobe Creek for 12 of the 13 months selenium was monitored, selenium concentrations in food organisms during the study were 5- to 16-fold in excess of the proposed dietary toxic threshold.

The pattern of low selenium concentrations in water (<5μg/L) but elevated concentrations in benthic invertebrates (>4μg/g) in the present study was also observed in the previous reproduction study (Hamilton et al., 2001a). This is a remarkable similarity given that the selenium concentrations in chironomids collected at AC3 and AC5 in the previous study were collected from Hester–Dendy samplers suspended in the water column, whereas in this study they were collected from sediment samples. These findings are similar to those of Zhang and Moore (1996), who reported low selenium concentrations in water (0.94–1.58 μg/L) but elevated selenium concentrations in chironomids (8.12–10.4 μg/g). Others have also reported that benthic invertebrates accumulate selenium from the sediment, especially its detritus fraction (Saiki et al., 1993; Malloy et al., 1999; Peters et al., 1999).

## Growth

Between initial stocking and spawning, fish at Horsethief grew 4.7% in length and gained 26.8% in weight, similar to growth measurements in the previous study, in which it was found that fish grew 15% in length and gained 21.7% in weight (Hamilton et al., 2001a). Adults in the present study stocked at Adobe Creek had slightly greater gains in weight (15.1%) than those in the previous study (7.5%), as did adults stocked at North Pond (35.0%) compared with those in the previous study (9.2%). These data suggest that the fish previously stocked at Adobe Creek and North Pond and held for 66 days during the depuration phase in the previous reproduction study readily readapted to the Adobe Creek and North Pond sites and made substantial gains in weight. Nevertheless, the lower weight gain in fish at Adobe Creek (15.1%) compared with fish at North Pond (35.0%) might be a result of the habitat differences of the two sites. In general, the Adobe Creek site had a greater abundance of aquatic invertebrates, lower inorganic element concentrations, and better water quality than did the North Pond site.

In contrast, adults stocked at the three sites on August 27, 1996, did not do as well as they had at their previous culture site, Wahweap Fish Facility, apparently because they did not adapt as readily to the new holding conditions, especially the Adobe Creek and North Pond sites. The fish stocked on August 27, 1996, at Adobe Creek (8.2%) and at

North Pond (7.0%) showed a substantially lower weight gain than did the fish stocked on July 11, 1996, which were used in the previous reproduction study (15.1% and 35.0%, respectively). Their smaller increases in length and weight were comparable to those of the adults held at Adobe Creek and North Pond during the first reproduction study, who went through the same adaptation to the new holding conditions, and were probably from a time lag in adapting to natural foraging at Adobe Creek and North Pond.

Their relatively young age (4–5 years old) may account for the rapid growth of the previously exposed adults (5–20 mm increase/≈1 year) and the first-time exposed adults (7–30 mm increase/≈1 year) in the present study compared with the slow or negligible growth of adult razorback suckers reported by others. No growth or slow growth of adult razorback suckers has been reported by McAda and Wydoski (1980; about 2.3 mm/year over a 3.5-year period), Valdez et al. (1982), Tyus (1987; mean = 2.2 mm/year), and Roberts and Moretti (1989; mean = 1.8 mm/year). Razorback sucker populations in the Colorado River basin also have been presumed to be very old and slow growing (McCarthy and Minckley, 1987). In the present study it would not be expected to observe changes in adult growth during the relatively short exposure time period.

## Selenium in Tissues

Investigators have reported that the adult razorback sucker feeds on a variety of items including filamentous algae (Dill, 1944; Marsh, 1987), algae (Jonez and Sumner, 1954; Banks, 1964), zooplankton (Hubbs and Miller, 1953; Minckley, 1973; Allan and Roden, 1978; Marsh, 1987), diatoms (Marsh, 1987), and other aquatic invertebrates (Banks, 1964). Marsh (1987) noted that the razorback sucker had both planktivorous and benthic feeding habits. In the present study aquatic invertebrates had substantial concentrations of selenium. Researchers have concluded that selenium residues and toxicity in fish come primarily from food and secondarily from water (Sandholm et al., 1973; Birkner, 1978; Hodson and Hilton, 1983; Turner and Swick, 1983; Ogle et al., 1988). Consequently, we believe that the selenium residues in adult tissue from the present study probably came primarily from food chain organisms and secondarily from water and sediment exposure.

High selenium concentrations in the sediment at North Pond probably contributed to the high selenium concentrations in adults because other investigators have reported that the stomach contents of adults contained detritus or bottom ooze, plant debris, and benthic invertebrates, usually with associated sediment materials, such as nondescript mud, clays, or silt (Dill, 1944; Jonez and Sumner, 1954; Banks, 1964; Vanicek, 1967; Allan and Roden, 1978; Marsh, 1987). Woock (1984) reported that golden shiners (*Notemigonus cryoleucas*) maintained in enclosures that allowed access to sediments and presumably to benthic food organisms maintained high selenium concentrations in tissues even though they were exposed to clean water and clean water-column food organisms. Fish without access to sediment depurated selenium from their tissues (Woock, 1984).

Concentrations of selenium in muscle plugs in the present study increased by 73% in fish held at Adobe Creek (from 9.6 μg/g at day 0 to 16.6 μg/g at day 286, excluding fish HT66) and by 80% in fish held at North Pond (from 14.2 μg/g at day 0 to 25.5 μg/g at day 286, excluding fish HT12 and HT42). This increase over the already-elevated selenium concentrations in muscle plugs from fish measured at the end of the previous reproduction study disclosed that fish had not reached equilibrium at the end of the first 305-day exposure study. Likewise, the lower selenium residues in the one fish stocked at Adobe Creek and in the two fish stocked at North Pond (all previously held at Horsethief for 371 days) and exposed only in the present study suggested that these fish did not have sufficient time to accumulate selenium to comparable concentrations as did the fish exposed in both reproduction studies.

Most other studies have reported that selenium residues in whole-body or tissues reached an equilibrium in 60–90 days (Gissel Nielsen and Gissel-Nielsen, 1978; Sato et al., 1980; Lemly, 1982; Besser et al., 1993), but others have estimated longer periods, that is, longer than 20 weeks (Adams, 1976; Woock and Summers, 1984). Reaching equilibrium in tissue selenium concentrations depends on a variety of factors some of which are species, size, age, exposure route and concentration, and chemical form. Because the present study used adults about 100 times heavier than the fish species in the studies cited above, it would probably take longer than 19 months of exposure (with a 2-month midexposure depuration period) for tissue residues to reach an equilibrium with selenium exposure in the water and diet.

Selenium concentrations in muscle plug tissue in razorback suckers from Adobe Creek and North Pond exceeded the proposed guideline of 8 μg/g in skeletal muscle as the benchmark for probable reproductive failure (Lemly, 1996). Although not reviewed by Lemly (1996), Cumbie and Van Horn (1978) reported reproductive failure of several fish species in Belews Lake, North Carolina, at selenium concentrations of 10 μg/g or greater in skeletal muscle.

A third of the 45 wild adult razorback suckers sampled in the Green River by Waddell and May (1995) and Stephens and Waddell (1998) had selenium concentrations higher than those in the fish held at Adobe Creek (mean = 16.6 μg/g, excluding HT66), whereas 27% (12 of 45) wild razorbacks had selenium concentrations higher than North Pond (25.5 μg/g, excluding HT12 and HT42). It seems unusual that 27%–33% of wild fish had higher selenium residues because the fish in both the previous and present studies were held in elevated selenium environments for a total of 19 months and had only a 2-month midexposure opportunity to depurate selenium. The higher selenium in a

substantial portion of the wild fish reported by Waddell and May (1995) and Stephens and Waddell (1998) suggests that some wild adults choose to use habitats with high selenium in water, food organisms, or both, or are forced to do so by lack of uncontaminated habitats. It also suggests that wild razorback suckers can accumulate substantial amounts of selenium in their tissues with little apparent depuration.

## Depuration of Selenium from Tissues

Loss of selenium from muscle tissue of adult razorback sucker in the present study after 59 days of depuration were lower for fish from Adobe Creek (7%), but similar for fish from North Pond (13%), than observed in the previous reproduction study (19% and 14%, respectively, after 66 days depuration; Hamilton et al., 2001a). Loss of selenium from muscle tissue after 86 days of depuration was 14% for Adobe Creek fish and 21% for North Pond fish, which suggested a slow loss of selenium and a half life greater than 100 days. Loss of selenium from fish tissue during a depuration phase of an exposure experiment has been reported to be independent of waterborne exposure concentration (Gissel Nielsen and Gissel-Nielsen, 1978; Sato et al., 1980), but increased with dietary exposure concentration (Hilton and Hodson, 1983). Depuration of selenium from tissues depends on several factors including cleanliness of the food and water in the depurating environment, age, size, metabolic activity, season for poikilotherms, and initial selenium load of various tissues.

Researchers have reported half lives for selenium depuration of 19–30 days in various young fish (Gissel Nielsen and Gissel-Nielsen, 1978; Sato et al., 1980; Hilton et al., 1982; Bennett et al., 1986; Kleinow and Brooks, 1986; Besser et al., 1993). Half-lives of selenium reported in older or larger fish range from more than 30 days to more than 60 days (Adams, 1976; Bertram and Brooks, 1986; Lemly, 1982; Bryson et al., 1984). Consequently, the slow depuration rate of selenium from muscle tissue in adult razorback suckers in the present study seems realistic.

One fish in the present study (AC18) showed a higher selenium concentration in muscle plugs after 31 days of depuration (23 μg/g) than after spawning (19 μg/g), suggesting that selenium may have been resorbed from another tissue and deposited in muscle. This increase in selenium concentration in muscle tissue may have been the result of the fish resorbing unexpelled eggs and the selenium load being redistributed throughout its entire body. However, this fish spawned on April 23 and April 25, so most of its eggs should have been expelled, thus limiting the amount that could be resorbed. Hamman (1985) reported that of 70 hatchery-reared razorback sucker females stripped at 24-h intervals, 16 ovulated all eggs after one stripping, 51 females ovulated all eggs after two strippings, and 3 females ovulated all eggs after three strippings. Thus, some eggs may have been present in fish AC18.

## Spawning

If razorback suckers in the present study had been allowed to spawn naturally, no effects on spawning behavior would have been expected. Behaviors associated with reproductive activities of fathead minnow (*Pimephales promelas*) were not altered in fish exposed to selenium concentrations up to 30,000 μg/L for 24 h (Pyron and Beitinger, 1989). Others have also reported no effect of selenium exposure in water, diet, or both on fish spawning behavior (Bryson et al., 1984; Ogle and Knight, 1989; Hermanutz et al., 1992; Coyle et al., 1993). However, Ogle and Knight (1989) reported that no spawning occurred in adults fed a diet with a selenium concentration of 40 μg/g, a concentration similar to that in the zooplankton and chironomids in the North Pond.

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 (Stephens and Waddell, 1998). Spawning by razorback suckers in the Green River has been somewhat successful because razorback sucker larvae have been collected during several years of monitoring. During 1992–1996 larvae were collected in the Green River, 1735 in the river's middle and 440 in its lower end (Muth et al., 1997). Eleven yearlings were captured in 1994 in Leota Bottoms in the Green River (Modde and Wick, 1997) and one yearling in 1993 in the Yampa River (T. Modde, USFWS, written communication).

## Selenium and Other Elements in Eggs

Based on a review of several studies, Lemly (1996) proposed that the toxic threshold of selenium for reproductive failure was 10 μg/g in eggs or in ovaries of fish. To assess the aquatic hazard of selenium, Lemly (1995) used fish eggs as one of the five components and assigned a no-hazard rating to selenium concentrations of <3 μg/g in eggs, a minimal hazard to concentrations of 3–5 μg/g, a low hazard to concentrations of 5–10 μg/g, a moderate hazard to concentrations of 10–20 μg/g, and a high hazard to concentrations of >20 μg/g. In the present study mean selenium concentrations in eggs from razorback suckers at Adobe Creek were 40.1 μg/g and at North Pond were 54.7 μg/g, both of which were more than 2 times greater than Lemly's high-hazard concentration and 4 times greater than the proposed threshold for reproductive failure. Eggs from young adults held at Horsethief had a mean selenium concentration of 6.0 μg/g, and those from the brood stock a concentration of 6.9 μg/g, both of which fall in the low-hazard category of Lemly (1995). The results of several studies reported adverse effects on fish species at selenium concentrations in eggs or ovaries that were half the concentration of those in razorback suckers held at Adobe Creek or North Pond (Cumbie and Van Horn, 1978; Bryson et al., 1984; Gillespie and Baumann, 1986; Schultz and Hermanutz, 1990; Crane et al., 1992; Hermanutz et al., 1992; Nakamoto and Hassler, 1992), but one study reported ef-

fects at similar selenium concentrations to those in the current study (Coyle et al., 1993).

In the present study North Pond adults had higher selenium concentrations in eggs than Adobe Creek adults did, which was consistent with the higher selenium concentrations at North Pond in water (3.2–17.1 μg/L), sediment (2.9–16 μg/g), zooplankton (39 μg/g), and adult muscle plugs (25.5 μg/g) compared with selenium concentrations at Adobe Creek in water (<0.7–4.5 μg/L), sediment (1.2–2.5 μg/g), zooplankton (18 μg/g), and adult muscle plugs (16.6 μg/g).

Although three elements other than selenium (boron, lithium, strontium) were significantly elevated in water at North Pond, they were not elevated in eggs from adults held at North Pond. Boron and strontium do not bioaccumulate in fish (Nakamoto and Hassler, 1992). Limited information suggests lithium also does not accumulate to harmful concentrations in animals (Puls, 1994). In the present study concentrations of five of the inorganic elements measured in eggs (barium, copper, iron, manganese, and selenium) were significantly different among the four groups of eggs. In the previous reproduction study the concentrations of three measured inorganic elements (manganese, phosphorous, and selenium) were significantly different among the eggs in Horsethief, Adobe Creek, and North Pond (Hamilton et al., 2001a). The elements elevated in both studies were manganese and selenium. The concentrations of some elements in eggs in the present and previous reproduction studies were similar to those reported by Wooch and Cofield (1983) for arsenic, copper, and zinc in gonads of bluegill (*Lepomis macrochirus*), and to those by Bryson et al. (1984, 1985) for arsenic, copper, cadmium, and zinc in gonads of bluegill. Nakamoto and Hassler (1992) reported concentrations in gonads of bluegill similar to those found in the present study for aluminum, arsenic, barium, boron, beryllium, cadmium, chromium, copper, iron, lead, manganese, molybdenum, nickel, strontium, titanium, thallium, vanadium and zinc.

The elevated copper in eggs from young Horsethief adults may be related to the high copper concentration in sediment at Horsethief (Hamilton et al., 2001a). The elevated copper concentrations in Horsethief sediment may have resulted from the use of copper sulfate, a common aquatic herbicide and algaecide used in some fish culture activities (Hansen et al., 1983). Copper sorbs readily to pond sediments (Reinert, 1989).

Nakamoto and Hassler (1992) reported that manganese accumulated in the whole body of bluegill collected from an area dominated by irrigation return flow near Kesterson Reservoir, California, but not in gonads, which contrasts with the present study. Moller (1996) presented a hypothesis that manganese and selenium accumulate in the liver of birds because they are components in liver enzymes important in their antioxidant defense system. Yolk proteins are produced in the liver of fish (Bun Ng and Idler, 1983), and because the liver is the site of manganese and selenium accumulation, manganese may be incorporated in a way similar to selenium into yolk proteins, which in turn are incorporated into the egg. The significance of manganese in fish eggs is unknown. Overall, barium, copper, iron, and manganese concentrations in eggs among adults held at the three sites varied from 1.4-fold to 3.2-fold, compared with the 14- to 15-fold difference in selenium concentrations between eggs from Horsethief fish and eggs from Adobe Creek or North Pond fish. Thus, selenium seemed to be the only element in eggs from adults from Adobe Creek and North Pond that was elevated to concentrations of concern compared with adults from Horsethief.

## Egg Characteristics

The magnitude of difference in egg size between young adults and brood stock in the present study (13%–17%) was less than in the previous reproduction study (19%–24%; Hamilton et al., 2001a). The diameter of eggs from young adults in the present study (2.43–2.57 mm) was larger than those from adults in the previous study (2.17–2.32 mm). This difference in egg size between studies was probably a result of the older age and larger size of adults used in the present study.

The range of egg sizes measured in the present study was similar to those reported by McAda and Wydoski (1985) for flannelmouth sucker (*Catostomus latipinnis*; 2.39 mm). Toney (1974) reported egg size in wild razorback sucker (perhaps as many as 40 fish) was 2.1–3.2 mm in diameter, but fish age was unknown. Also, some eggs measured by Toney (1974) may have not been fully mature and were therefore smaller in size, as was observed in the second spawning in the present study. Muth and Ruppert (1996) reported water-hardened eggs from razorback suckers (mean total length = 575 mm) were 2.1 mm (range = 2.1–2.3 mm), which is smaller than the range of sizes in the current study even though their fish were larger than adults used in the current study.

## Egg Test

The low percentage of viable eggs from the first and second spawns probably resulted from the temperature change experienced by the eggs in the move from the Horsethief spawning facility, where water temperature was about 12°C, to the 24-Road facility, where water temperature was about 24°C. A similarly low viability occurred in brood stock spawns, with 15 spawns having less than 1% viability; 3, 20%; 1, 50%; and 3 about 80%. Blaxter (1969) and Piper et al. (1982) reported that sharp temperature changes can cause egg mortality. Egg viability was not determined on eggs transferred directly from the Horsethief spawning facility and acclimated to conditions in the mobile laboratory. Dissolved oxygen concentrations and water temperature during

the two egg tests were in the acceptable range of values for tests with eggs and embryos (Weber et al., 1989).

In the present study the percentage of hatched eggs from young adults (21%–60%) and the percentage of surviving eggs and newly hatched larvae (29%–71%) was in the range of results reported by others for the razorback sucker. Hamman (1985) reported having about 40% success in hatching and survival of larvae in their experiment. Muth and Ruppert (1996) reported that for control fish the mean percentage of eggs hatched was 26% (range 21%–35%). Inslee (1982) reported a 23% hatch of eggs collected from 71 females. In recent years at Dexter National Fish Hatchery (NFH) and Technology Center, New Mexico, the overall average percentage of razorback sucker eggs hatched has been about 50% and the survival of larvae about 35%–40% (R. Hamman, USFWS, personal communication).

No significant differences were found in percent survival and percent hatch measures among the test groups, apparently indicating no difference in effect from the selenium concentrations in the eggs and the water quality of the site waters. These results were similar to other selenium studies with fish. Hamilton et al. (2001a) also reported no significant correlations between hatch and selenium concentrations in adult razorback sucker muscle plugs or between survival and selenium concentrations in adult muscle plugs or eggs. Others also have reported no effects from selenium exposures on fertilization rate or time to first hatch of eggs (Crane et al., 1992), on fertilization or hatch (Gillespie and Baumann, 1986), on hatching success of eggs (Woock et al., 1987), on number of eggs per spawn or percent hatch (Ogle and Knight, 1989), and on number of eggs per spawn or hatchability of eggs (Coyle et al., 1993).

In contrast, Hermanutz et al. (1992) reported that percent hatch was reduced in eggs of adult fathead minnows exposed to 10 μg/L selenium in an experimental stream for 1 year. Likewise, egg exposures to high selenium concentrations in water can affect the median hatch time of rainbow trout (*Oncorhynchus mykiss*; 10,000 μg/L) and lake trout (*Salvelinus namaycush*; 20,000 μg/L; Klaverkamp et al., 1983), but concentrations up to 40,000 μg/L had no effect on the hatch of fathead minnow eggs (Halter et al., 1980). Saiki and Ogle (1995) reported that western mosquitofish (*Gambusia affinis*; whole-body selenium >100 μg/g) from the San Luis Drain, in California, which contained selenium in a concentration range of 340–390 μg/L, had a lower mean percentage of live births within broods and produced less fry than adults from a reference area.

## Deformities

Based on the number of deformity notations, larvae from adults from Adobe Creek, North Pond, and brood stock, but not Horsethief, had elevated numbers of deformities compared with the typical background deformity rates of <1%–3% (Gabriel, 1944; Gill and Fisk, 1966; Patten, 1968; Dahlberg, 1970). The number of deformities in razorback sucker larvae from Adobe Creek and North Pond and from brood stock adults seemed to be higher than the range of 9%–11% suggested by Bengtsson (1975) as abnormal and probably resulting from a "man-made" effect.

The greatest number of deformities in larvae from young adults were found in North Pond larvae, followed those in Adobe Creek larvae, with the least in Horsethief larvae. Teratogenesis is a well-documented biomarker of selenium toxicity in wild birds and fish at the embryo-larval stage (Ohlendorf et al., 1986; Hoffman and Heinz, 1988; Hoffman et al., 1988; Lemly, 1993b, 1997a, 1997b). Fish deformities from selenium exposure include lordosis, kyphosis, scoliosis, and head, mouth, gill cover, and fin deformities, in addition to edema and brain (microencephaly), heart, and eye problems. Selenium-induced deformities in fish larvae have been reported in laboratory studies (Goettl and Davies, 1977; Bryson et al., 1984; Klauda, 1986; Woock et al., 1987; Pyron and Beitinger, 1989), experimental stream studies (Schultz and Hermanutz, 1990; Hermanutz, 1992; Hermanutz et al., 1992), artificial crossing experiments (Gillespie and Baumann, 1986), and field investigations (Lemly, 1993b, 1997a, 1997b; Saiki and Ogle, 1995; Hamilton et al., 2001a). Contaminated ecosystems may require long time periods for recovery from selenium contamination because elevated incidences of deformed fry of four fish species were reported in Belews Lake, North Carolina, 10 years after selenium inputs had been stopped (Lemly, 1997b).

Lemly (1997a, page 261) stated that "in order to draw a conclusion of selenium-induced teratogenesis, the visual indicators and symptoms (deformities) must be corroborated with the presence of elevated concentrations of selenium in tissues." He went on to state that selenium concentrations of 10–20 μg/g or greater in the whole body or 6–12 μg/g in muscle would be sufficient to confirm the diagnosis. Based on selenium concentrations in eggs from Adobe Creek and North Pond adults (40.1 and 54.7 μg/g, respectively) and in muscle plugs from adults (16.6 and 25.5 μg/g, respectively) and on the teratogenesis ratings of Lemly (1997a), selenium-induced teratogenesis would have been expected at these two sites. These results were similar to a previous reproduction study in which there were deformities in 12%–26% of embryo-larvae from Adobe Creek adults and in 20%–27% of larvae from North Pond adults (Hamilton et al., 2001a).

The high number of deformities observed in larvae from brood stock and young adults in the present study may have resulted from inbreeding effects, stress from handling, or a combination of these. Dowling et al. (1996) reported that, based on mitochondrial DNA diversity, razorback suckers examined from the upper Colorado River seemed to be from a small population, closely related, and possibly derived from a limited number of females. Spawning of closely related fish can result in reduced growth rate, lower survival, reduced feed conversion, and increased numbers of

deformed fry (Kincaid, 1976a, 1976b; Piper et al., 1982). The brood stock used were generally considered old and perhaps past their prime for producing good-quality eggs. The old age of brood stock is a concern that has been expressed by some fishery workers involved in endangered-fish propagation (C. Figiel, USFWS; R. Hamman, USFWS; S. Severson, USFWS; H. Williamson, USFWS; personal communications).

Although inbreeding and old age are possibilities, it is most likely that the stress causing the deformities in brood stock larvae was the temperature change. Temperature stress can kill eggs, as demonstrated by the complete mortality of eggs from the young adults spawned at the Horsethief spawning facility, at which the water temperature was about 12°C, and from those held at 24-Road, at which the water temperature was about 24°C. Temperature stress can also cause deformities in hatched fish larvae, as can numerous other stresses (Seymour, 1959; Turner and Farley, 1971; Hickey, 1973; Sindermann, 1979).

The greatest number of deformities occurred in brood stock larvae spawned at Horsethief, then held at elevated water temperatures at 24-Road before transfer to the mobile laboratory for use in the egg test. In contrast, larvae from the young adults spawned at Horsethief were transferred to the mobile laboratory rather than the 24-Road facility and did not undergo a severe temperature stress. The egg test was conducted with eggs from one of the brood stock spawns with 80% viability. This high level of viability does not preclude the appearance of deformities from temperature stress.

Deformities have been reported in threatened or endangered fish in the Colorado River basin and in those reared in hatcheries. Fin deformities were reported by Martinez (1996) in razorback sucker larvae reared in the Colorado Division of Wildlife's Fish Research Hatchery, Bellvue, Colorado, in 1993 and again during a feeding study in 1994. She concluded that these fin deformities were a result of handling stress and not from genetic or nutritional sources because siblings that were not used in the feeding test and handled substantially less did not have the deformities. In the first reproduction study prior to the current one, the fin deformity was observed neither in the egg test nor in larvae (Hamilton et al., 2001a).

Severson et al. (1992) also reported deformities in razorback sucker larvae in a study to evaluate various feeds on larvae culture. They reported deformity rates of 0%–83% in larvae fed different diet series for 127 days posthatch. The diet that had 0% deformities in one replicate had 23% deformities in a second replicate. Spinal deformities included displaced vertebral columns, compressed spinal cords, and displaced musculature, which they speculated might have been a result of vitamin deficiency associated with the cold storage of the diets. Hickey (1973) cited three studies linking vitamin C deficiency with deformities in fish. Few deformities have been noted in razorback sucker larvae produced from wild adults spawned at Willow Beach NFH, Arizona (C. Figiel, USFWS, personal communication).

In conclusion, selenium accumulated substantially in muscle plugs and eggs of adult razorback suckers held at Adobe Creek and North Pond. This selenium accumulation in muscle plugs was lower than 27%–33% of the wild razorback suckers that were free to move about the Green River. The incidence of deformities in larvae from exposed adults followed selenium concentrations in water, food, and muscle plugs from adults. There is a growing database suggesting that selenium contamination of the Colorado River basin may be retarding the recovery of the endangered razorback sucker (Hamilton, 1998, 1999; Stephens and Waddell, 1998; Hamilton et al., 2000).

The authors thank M. Ehlers, H. Hamilton, and S. Muehlbeier of the U.S. Geological Survey (USGS); R. Dial and J. Rubalcaba of the U.S. Bureau of Reclamation (BOR), Grand Junction, Colorado; F. Pfeifer, M. Baker, C. McAda, M. Montagne, B. Scheer, R. Krueger, and B. Osmundson of the USFWS, Grand Junction, Colorado, for technical assistance; E. Callahan and M. Ellersieck for statistical assistance; K. Faerber for typing the report; and W. Beckon, A. Lemly, F. Mayer, G. Noguchi, T. Presser, and an anonymous reviewer for review comments.

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