

Rapid communication

Selenium impacts on razorback sucker, Colorado River, Colorado I. Adults

Steven J. Hamilton^{a,*}, Kathy M. Holley^b, Kevin J. Buhl^{a,*}, Fern A. Bullard^a,
L. Ken Weston^c, Susan F. McDonald^a

^aUS Geological Survey, Columbia Environmental Research Center, Field Research Station, 31247 436th Avenue, Yankton, SD 57078-6364, USA

^bUS Fish and Wildlife Service, 764 Horizon Drive, Suite 208, Grand Junction, CO 81506, USA

^cUS Bureau of Reclamation, 2765 Compass Drive, Suite 106, Grand Junction, CO 81506, USA

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Abstract

Adult razorback sucker (*Xyrauchen texanus*) were exposed to various selenium concentrations in ponds and isolated river channels of the Colorado River near Grand Junction, CO, to determine effects on their growth and residue accumulation over an 11-month period. Adults at Horsethief ponds were fed a commercial diet, whereas fish at Adobe Creek channel and North Pond foraged on natural food items. Selenium concentrations at Horsethief were 2.2 µg/L in water, 0.1–1.4 µg/g in sediment, and 2.3–3.1 µg/g in food organisms (1.1 µg/g in commercial fish food), at Adobe Creek were 3.8 µg/L in water, 0.5–2.1 µg/g in sediment, and 4–56 µg/g in food organisms, and at North Pond were 9.5 µg/L in water, 7–55 µg/g in sediment, and 20–81 µg/g in food organisms. The selenium concentrations in muscle plugs from adults at Adobe Creek (11.7 µg/g, SD=0.4, n=6) and North Pond (16.6 µg/g, SD=1.0, n=6) were greater than at Horsethief (4.5 µg/g, SD=0.2, n=6). During a depuration period adults from Adobe Creek and North Pond lost 1–2% of their selenium burden in 32 days and 14–19% in 66 days. Selenium accumulated in razorback sucker above toxic thresholds reported in other studies, yet those residues were less than those reported in muscle plugs of 40% of wild razorback sucker caught in the Green River, Utah.

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1. Introduction

The upper Colorado River provides critical habitats for four endangered fish species, Colorado pikeminnow (*Ptychocheilus lucius*), razorback sucker (*Xyrauchen texanus*), humpback chub (*Gila cypha*), and bonytail (*Gila elegans*) (USFWS, 1994). A combined approach to recovery of the four endangered fish in the upper Colorado River basin has been undertaken by the Upper Colorado River Endangered Fish Recovery Program (Recovery Program), which was initiated in 1987 (USFWS, 1987). The goal of this 15-yr program is

to reestablish self-sustaining populations of the four species while allowing continued development of water. The reasons for the decline of these species are related to a combination of factors including stream alteration (dams, irrigation withdrawals, dewatering, channelization), loss of habitat (spawning sites and backwater nursery areas), changes in flow regime, blockage of migration routes, water temperature changes, competition with and predation by introduced species, parasitism, and changes in food base (USFWS, 1987). Public Law 107–375 extended the authorization of the Recovery Program to 2008 (UCREFRP (Upper Colorado River Endangered Fish Recovery Program) and SJRBRIP (San Juan River Basin Recovery Implementation Program), 2004).

*Corresponding author. Fax: +605-665-9335.

E-mail address: kevin_buhl@usgs.gov (K.J. Buhl).

Following the discovery of selenium-contaminated irrigation return waters in the San Joaquin Valley of central California in 1982, the Department of the Interior initiated the National Irrigation Water Quality Program (NIWQP) to identify other areas in the western US that have water quality problems induced by irrigation drainage (Feltz et al., 1991). Analysis of water, bottom sediment, and biota collected since 1986 from the middle Green River, Utah, and the Grand Valley, located in western Colorado, including a portion of the Colorado, Gunnison, and Uncompahgre rivers, have confirmed the presence of inorganic elements including selenium at concentrations that could be potentially harmful to fish and wildlife (Butler et al., 1989, 1991, 1994, 1996; Peltz and Waddell, 1991; Stephens et al., 1988, 1992). Contaminant survey information in the NIWQP suggested selenium might be sufficiently elevated to be contributing to the decline of endangered fish (Hamilton, 1998, 1999).

The US Fish and Wildlife Service's (USFWS) National Contaminant Biomonitoring Program (NCBP) has documented elevated selenium concentrations in fish collected from stations located in the upper and lower Colorado River basins (Lowe et al., 1985; May and McKinney, 1981; Schmitt and Brumbaugh, 1990; Walsh et al., 1977). Selenium concentrations of 4.9–7.0 µg/g dry weight reported in the NCBP in whole-body fish from the Colorado River basin have been among the highest in the nation.

In an effort to stabilize and enhance populations of razorback sucker and other endangered fishes in the upper Colorado River, the Recovery Program has decided to restore floodplain habitats for use by razorback sucker adults and larvae. An important component of this restoration was to select sites that would not pose contaminant problems to the fish, especially from selenium. This study was conducted to derive the necessary toxicological information for assessing the suitability of selected flooded bottomlands as habitat for razorback sucker.

The present study was conducted at three sites along the Colorado River near Grand Junction, CO, in the general area where razorback sucker had historically been observed. Although wild fish move freely about the Colorado River and its tributaries, which may vary their exposure to various stresses, the adults in this study were held in specific locations as part of a exposure to selenium and other inorganic elements.

2. Materials and methods

One hundred and eight razorback sucker were acquired on July 6, 1995, as 3-yr-old adults from the Wahweap Fish Facility of the Utah Division of Wildlife Resources, Big Water, UT. Parents of these fish were

from the San Juan River arm of Lake Powell, UT, and all the fish used were progeny of a single spawn of two adult razorback sucker. Fish were treated with oxytetracycline via intraperitoneal injection before release at the three study sites to treat potential infections incurred during transport or after release at the study sites.

2.1. Site descriptions

A partial life-cycle chronic toxicity study was conducted by exposing adult fish for about 9 months to water and natural foods at three sites adjacent to the Colorado River in the Grand Valley near Grand Junction, CO: Horsethief Canyon State Wildlife Area (reference site HT), Adobe Creek (AC), and North Pond (WW) at Walter Walker State Wildlife Area (WWSWA) (Figs. 1–4). The HT site was located about 19 km west of Grand Junction, the AC site was located about 5 km west of Grand Junction, and the WW site was located about a $\frac{1}{2}$ km southwest of Grand Junction.

Sampling stations were established at each site a few months before fish were stocked. At HT, fish were held in earthen ponds with other endangered fish stock (Fig. 2). The water in the ponds was maintained by water pumped directly from the Colorado River near Fruita, CO, and contained low selenium concentrations (2.2 µg/L). The AC site was a tertiary river channel about 200 m long and 3–5 m wide that was isolated from river flow by dikes at both ends (the downstream dike had an overflow water control structure; Fig. 3). Fish were held at sample stations AC1, AC2, and AC3. The water level at AC was maintained at about 1.5 m deep with water pumped from the secondary channel, and overflow water from an irrigation ditch (AC4). Water at the site contained relatively low selenium concentrations (3.8 µg/L). The WW site was an isolated pond about 1 ha in size with a maximum depth of 1.5 m located on a terrace about 2 m above the floodplain (Fig. 4). Water in WW was supplied primarily by ground water discharge, which contained elevated selenium concentrations (9.5 µg/L). The south side of WW had a dike and water overflow structure installed to maintain water levels and confine fish. Fish were held at sample stations WW1 and WW2. Water levels were supplemented by inflow at WW3 from Independent Ranchman's Ditch. Flooding of the Colorado River in spring 1996 caused water levels at AC and WW to rise substantially for several weeks. However, to the author's knowledge, no fish escaped from these areas nor were wild adults introduced.

2.2. Fish stocking and sampling

Thirty-six adults were randomly stocked at each of three sites and maintained from July 6, 1995, to the last week of April 1996. Prior to stocking, each fish was tagged with passive integrated transponders (PIT),

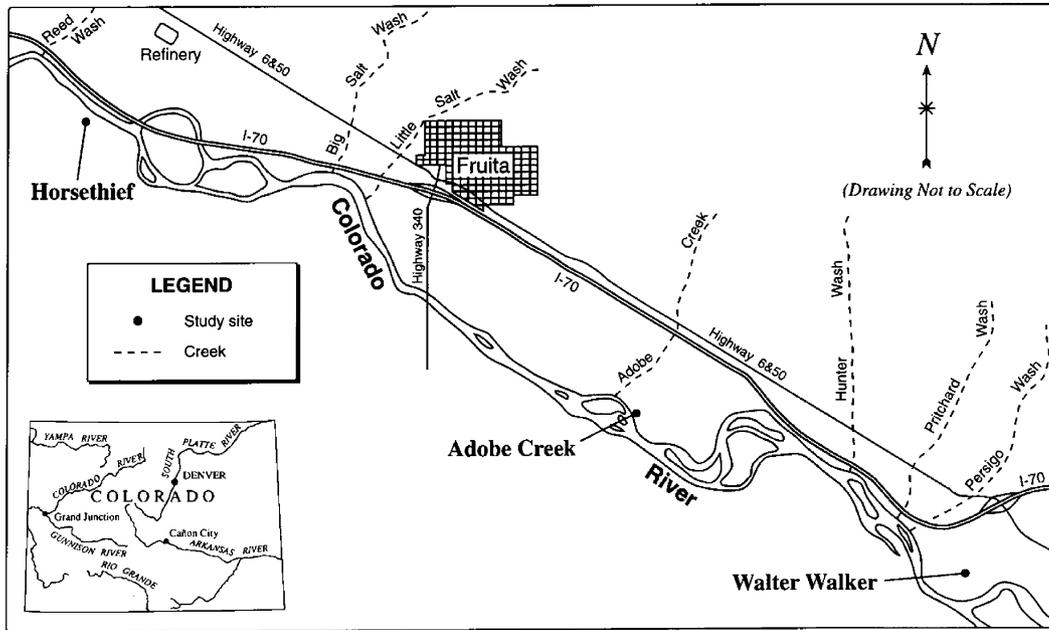


Fig. 1. Map of three sites located in the Grand Valley near Grand Junction, Colorado.

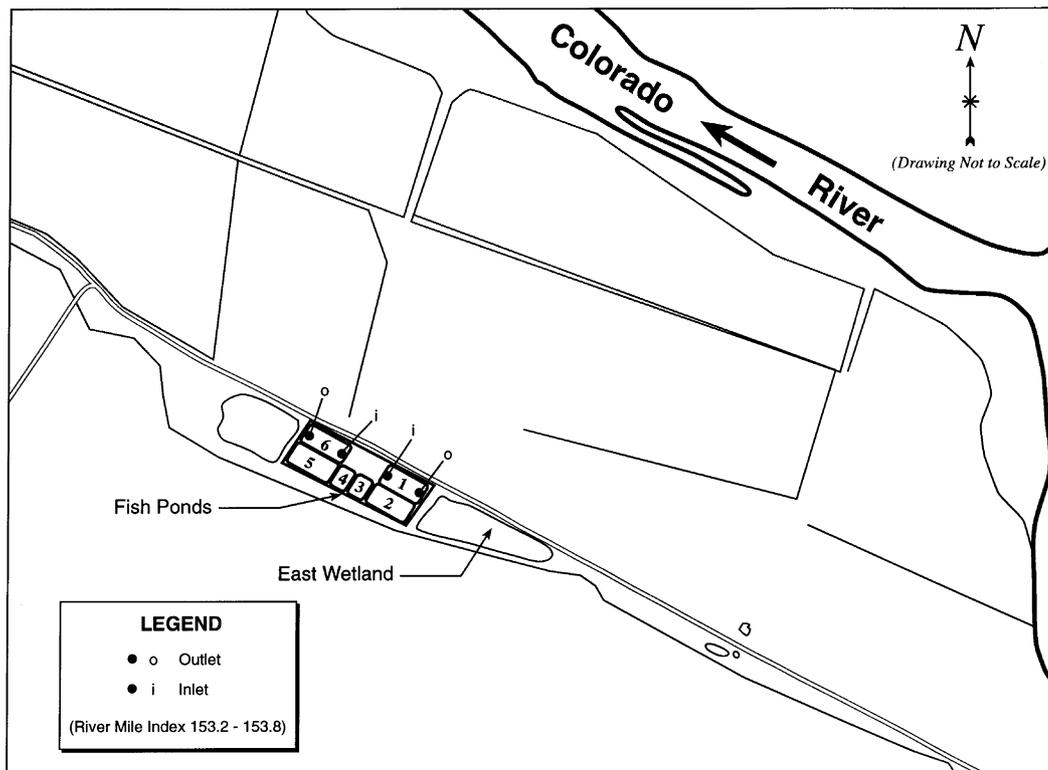


Fig. 2. Map of ponds at Horsethief Canyon State Wildlife Area, near Fruita, Colorado.

measured for total length and weight, and a muscle plug was taken from the area adjacent to the dorsal fin for use in selenium analysis. Muscle plugs were collected using a 4- or 5-mm biopsy punch, placed in cryotubes,

stored on ice in the field, stored in a freezer (-20°C) while awaiting selenium analysis, and shipped on dry ice when transported to the sample preparation laboratory. After the muscle plug was collected, the wound was

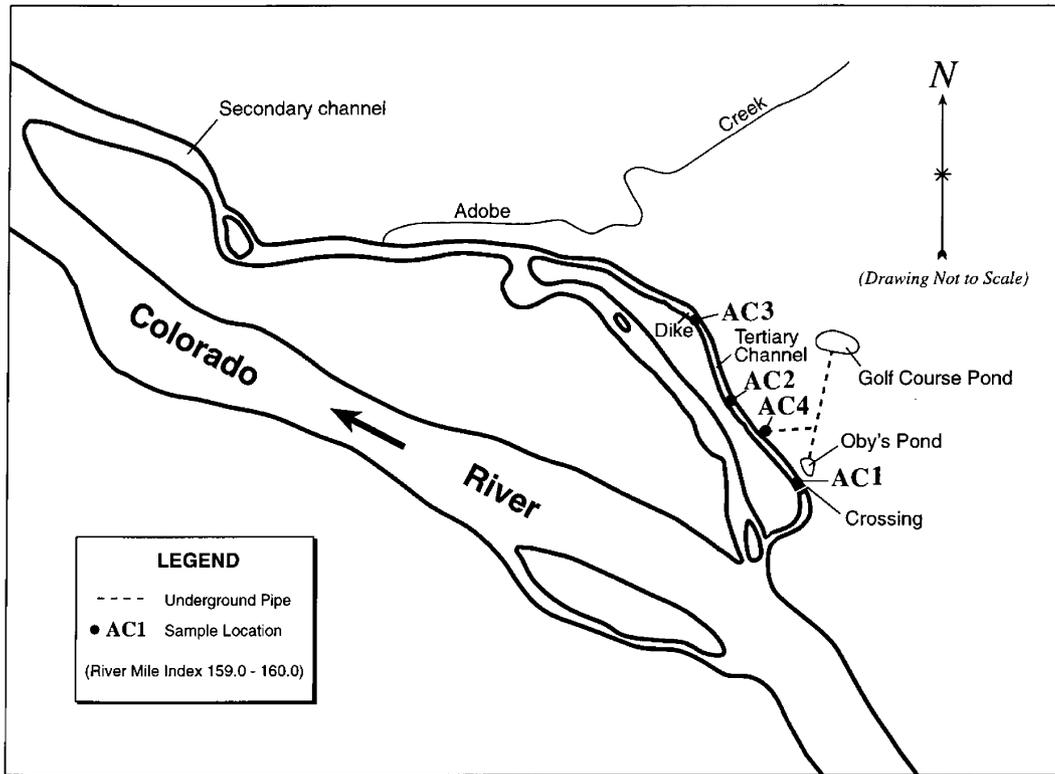


Fig. 3. Map of sample stations at Adobe Creek.

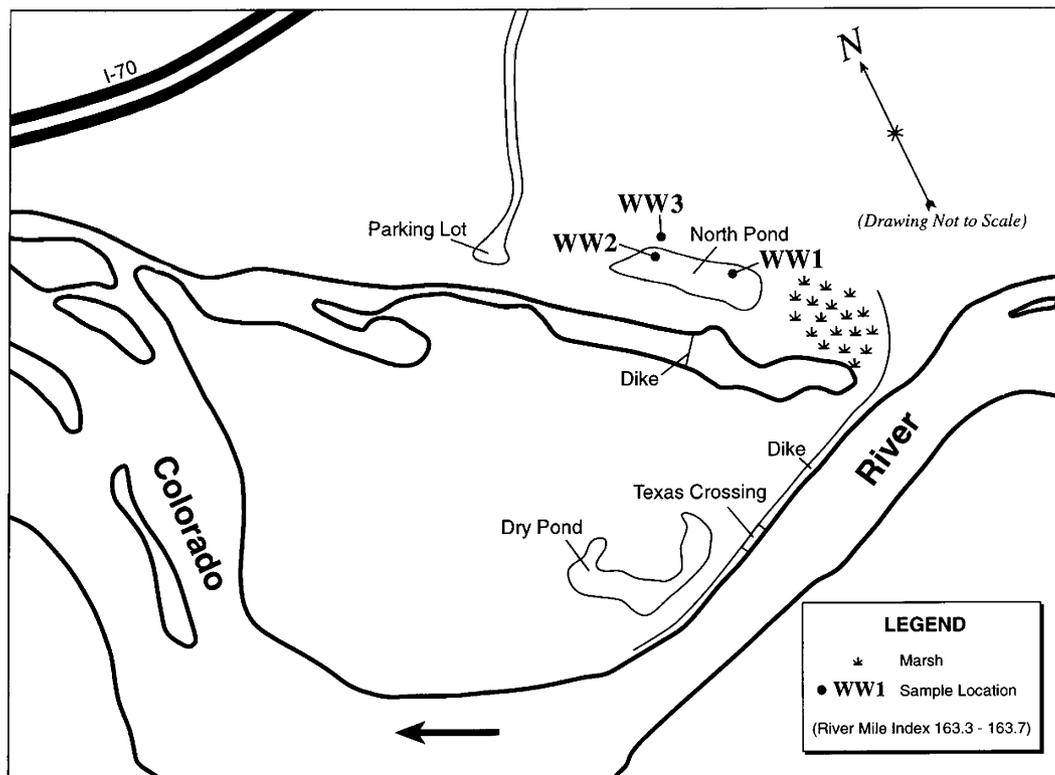


Fig. 4. Map of sample stations at North Pond, Walter Walker State Wildlife Area.

treated with full-strength Betadine solution. Fish held at HT were fed the same commercial standard fish food fed to other stocks of razorback sucker routinely maintained there. Fish at AC and WW were not fed and foraged for available food items.

Seventeen fish held at WW died at day 30 sampling because of suspected low dissolved oxygen concentrations (fish were held overnight in a hoop net and had limited access to areas of the pond with higher dissolved oxygen concentrations). WW was restocked with 17 fish from HT on August 10, 1995.

At intervals 30–60 days after stocking, fish were collected using trammel nets or boat-mounted electro-fishing equipment. Captured fish were identified by their PIT tag, measured for total length and weight, and inspected for general health, and a muscle plug was taken for selenium analysis. Not all fish stocked at AC and WW were recovered at each sampling period because of the difficulty in locating and capturing the fish.

Natural food organisms were collected monthly from the sample stations for chemical analysis. Collections were accomplished primarily using modified light traps (Espinosa and Clark, 1972) and infrequently by plankton tow net. Light traps were set overnight and the trapped zooplankton 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- μ m plankton net. The combined samples were then back-washed into a 3.8-L plastic jar filled with site water, covered, and transported to the laboratory in coolers. In the laboratory, the samples were thoroughly mixed and a subsample was collected in a glass vial, preserved in 70% ethanol, and used to make semiquantitative estimates of species composition at each station.

Generally, two sets of zooplankton samples were collected from each sample station, placed in Whirl-Pak bags, and stored frozen at -20°C . One set was analyzed for selenium concentrations and the other set was analyzed for concentrations of inorganic elements. One sample each from two different lots of the commercial diet fed to adults at the HT reference site also was collected for selenium analysis.

2.3. Depuration

At the conclusion of the study, fish were spawned and an egg test (Hamilton et al., 2004a) and larvae test (Hamilton et al., 2004b) were conducted in parts II and III of the study.

After spawning, adult fish from AC and WW, along with HT, were held in the same earthen pond at HT and fed commercial fish food for 60 days to determine the rate of selenium depuration from their tissues. Fish were captured at 30 and 60 days postspawning, identified by

their PIT tags, measured for total length and weight, and inspected for general health, and muscle plugs were taken for selenium analysis. Two fish from each group held at the three exposure sites were sacrificed after spawning, after 30 days, and after 60 days in the depuration phase of the study for analysis of selenium concentrations in various tissues. The following samples were taken from each fish: muscle tissue from the dorsal area close to where muscle plugs were removed, liver, kidney, gonad, and gall bladder. In addition, gut contents were retrieved from two fish. The samples were placed in Whirl-Pak bags and stored frozen at -20°C until selenium analysis.

2.4. Water and sediment sampling

Beginning in mid-April 1995, water quality characteristics were measured every week in situ at the sample stations and every 30 days a sample was taken for further analyses. Water quality characteristics measured weekly in situ included pH, conductivity, salinity, air temperature, water temperature, and dissolved oxygen. Water quality measurements every 30 days included pH, conductivity, hardness, calcium, magnesium, alkalinity, and chloride. Two subsamples of each 30-day sample were subjected to further analysis; one sample was used for ammonia analysis and was acidified to $\text{pH} < 2$ with concentrated sulfuric acid, and the other sample was stored in a refrigerator at 4°C and used for analysis of nitrate, nitrite, sulfate, total suspended solids, volatile solids, and fixed solids. These subsamples were shipped in a cooler with ice packs by overnight express to Yankton Field Research Station (FRS), SD, for analysis. All water quality characteristics were measured according to standard methods (APHA (American Public Health Association and American Water Works Association and Water Environment Federation), 1995), except for the nitrogenous chemicals and chloride. Ammonia, nitrate, and nitrite were measured using ion-selective electrodes and following the procedures for low concentration measurements of the electrode manufacturer (ATI (Analytical Technology Incorporated Orion), 1994; Orion Research, 1990, 1991). Chloride was measured by the mercuric nitrate titration method (Hach Company, 1992).

Subsamples of water collected between May 1995 and June 1996 for water quality analyses were also used for selenium analysis and analysis of inorganic elements. Samples for selenium analysis were collected monthly and those for inorganic elements were collected bimonthly. Filtered and unfiltered water was collected for selenium analysis. Water was filtered through a 0.4- μ 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 (but passed through a 25- μm filter bag to remove debris) was acidified with 2 mL of ultrapure HCl and stored frozen until analysis of selenium concentrations. Samples for inorganic element analysis were filtered (0.4 μm) as described above, acidified with 2 mL of ultrapure HNO₃ and stored frozen.

Sediment samples were collected in May 1995, October 1995, and April 1996 by a petit ponar grab sampler, placed in a large plastic pan and thoroughly mixed, and large pieces of debris were removed (plants, twigs, rocks, etc.). Three subsamples of sediment were collected in polyethylene bottles and stored in a freezer until analysis. One sample was analyzed for selenium, and a second sample was analyzed for inorganic elements. A second portion of each of the samples collected in October 1995 and April 1996 was analyzed for total and inorganic carbon and for total, volatile, and fixed solids, and a third portion was examined for sediment texture.

Samples for carbon analysis were oven-dried overnight at 105 °C in a Fisher Isotemp oven. Dried samples were homogenized and ground in a CRC Micro-mill. Subsamples of about 30 mg each were wrapped in aluminum foil, bagged in Whirl-pak bags, and sent to the Columbia Environmental Research Center (CERC), Columbia, MO, for analysis of total and inorganic carbon; organic carbon was determined by subtraction. Carbon analyses were accomplished with a Coulometrics Carbon Model 5020 analyzer.

Total, volatile, and fixed solids were determined by standard methods (APHA et al., 1995). Briefly, subsamples were weighed in an aluminum drying pan and air-dried prior to oven drying and muffle furnace ignition. Total solids were determined by drying the sediment overnight in a Fisher Isotemp oven at 105 °C. Constant weights were determined by loss of less than 4% or 50 mg, whichever was less. Fixed and volatile solids were determined by ignition at 550 °C for 60 min in a Thermolyne model FA1730 muffle furnace and then allowed to cool overnight in the furnace before weighing.

Particle size determination of sediments was determined by standard methods (ASTM (American Society for Testing and Materials), 1993). Samples were air-dried on fiberglass trays for 3–6 days, and large aggregates of dried sediment were crushed with a mortar and rubber-covered pestle. The dried sediment was sieved to remove particles >2.0 mm. Dried sediments were weighed and stored at 4 °C until analysis. Each sample was analyzed in duplicate. Hydrometer analyses were conducted in 1-L sedimentation cylinders or graduated cylinders using ASTM Model 152H hydrometers following standard methods (ASTM (American Society for Testing and Materials), 1990). Briefly, sediment subsamples were dispersed overnight in 40 g/

L sodium hexametaphosphate solution. A Hamilton Beach Scovill mechanical stirrer and a cup with baffles were utilized to further disperse the sample before hydrometer analysis. The results were plotted on graph paper and the percentage for the particle size of interest was interpolated from the graph. Particle sizes were classified according to the US Geological Survey classification scheme, which is based on the Wentworth grade scale: sand 0.063–2.0 mm, silt 0.004–0.062 mm, and clay <0.004 mm (Guy, 1969).

2.5. Inorganic element analyses

All samples collected for selenium analysis were analyzed at the Yankton FRS using a Perkin–Elmer 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 (water).

Water samples were digested using a persulfate digestion technique and total selenium was determined by a modification of the method of Presser and Barnes (1984). Quality assurance/quality control measures included determination of limit of detection, procedural blanks for background equivalent concentration, percent 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 limit of detection (LOD) ranged from 0.5 to 3.9 $\mu\text{g/L}$ (mean 1.4, SE 0.1, $n = 27$). The procedure blanks had background concentrations less than the LOD, which indicated no contamination from reagents or sample handling. The percent relative standard deviation (triplicate sample preparation and analysis) ranged from 0% to 8.2% (mean 4.6, SE 2.1, $n = 27$), which indicated consistent sample handling during preparation, digestion, and analysis. Recoveries of selenium from NIST reference material 1643c water and NIST reference material 1643d were within CERC recommended ranges, which indicated that the digestion and analysis procedure accurately measured selenium concentrations. The digested-spiked sample solutions had percentage recoveries ranging from 86% to 116% (mean 101, SE 8.4, $n = 27$), which indicated that the digestion procedure did not alter the amount of spiked selenium in the sample, i.e., suggested no loss of selenium in water samples during digestion procedure. Analysis of analysis-spiked samples analyzed for matrix suppression or enhancement had selenium recoveries ranging from 82% to 118% (mean 101, SE 9, $n = 27$), which indicated no interference from other water components.

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

concentrations by first lyophilizing the sample to a constant dry weight using a Virtis Vacu-Freezer. Sediment, zooplankton, and fish food samples were digested using a combination nitric acid wet digestion and 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 control/quality assurance measures were the same as for water analyses, and the results are summarized in Table 1.

Analyses of water, sediment, and zooplankton samples collected for inductively coupled argon plasma spectroscopy (ICP) analysis of inorganic elements were performed at the Environmental Trace Substances Research Center (University of Missouri), Rolla, MO.

Table 1
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

Measure	Matrix		
	Sediment	Zooplankton	Fish food
Limit of detection ($\mu\text{g/g}$)	0.19 (0.11) [4]	0.16 (0.03) [19]	0.08 (0.01) [2]
%RSD ^a	5.7 (1.0) [4]	5.6 (0.5) [19]	6.2 (1.8) [2]
Reference material	0.44 ^b (0.01) [4]	1.51 ^c (0.04) [11] 1.26 ^d (0.03) [8] 4.05 ^e (0.06) [8]	1.35 ^c (0.05) [2] 4.0 ^c (0) [2]
Digested spikes ^f	104 (3) [8]	97 (1) [38]	104 (2) [4]
Analysis spikes ^g	102 (4) [4]	100 (2) [19]	103 (2) [2]

^aRSD: Percent relative standard deviation for triplicate preparation and analysis.

^bNational Research Council of Canada (NRCC) reference material BCSS-1 (marine sediment; 0.43 ± 0.06 [SD] $\mu\text{g/g}$).

^cNRCC reference material DORM-1 (dogfish muscle tissue; 1.62 ± 0.12 [SD] $\mu\text{g/g}$).

^dNRCC reference material DORM-2 (dogfish muscle tissue; 1.40 ± 0.09 [SD] $\mu\text{g/g}$).

^eNational Bureau of Standards (NBS) 50AT (albacore tuna; 3.6 ± 0.4 [SD] $\mu\text{g/g}$).

^f% recovery of selenium from samples spiked with selenomethionine at the beginning of preparation for sample analysis.

^g% recovery of selenium from digested samples spiked with selenite after sample preparation but before instrument analysis.

The list of elements and LOD are given in Table 2. For water, the procedure blank had background equivalent concentrations less than the LOD for all elements except calcium, silicon in one blank, and antimony, calcium, and titanium in a second blank. The mean percent relative standard deviation (duplicate sample preparation and analysis) was 3.4%; the mean spike recovery was 97%; and the recovery of trace elements in Environmental Resources Associates reference water ERA9969TM was within recommended ranges except for arsenic, selenium, and thallium in one analysis and aluminum, arsenic, and thallium in a second analysis.

For sediments, the procedure blank had background equivalent concentrations less than the LOD for all elements except for silicon in one blank. The mean percent relative standard deviation (duplicate sample preparation and analysis) was 4.6%; the mean spike recovery was 97%; and the recovery of trace elements in National Bureau of Standards (NBS) reference sediment 2704 (Buffalo River sediment) was within recommended ranges except for aluminum, arsenic, barium, calcium, chromium, iron, magnesium, manganese, phosphorus,

Table 2
Limit of detection for elements measured by inductively coupled argon plasma spectroscopy in water ($\mu\text{g/L}$), sediment ($\mu\text{g/g dw}$: dry weight), and zooplankton ($\mu\text{g/g dw}$)

Element	Matrix		
	Water	Sediment	Zooplankton
Ag	5	1	1
Al	30	8	7
As	20	7	4
B	6	6	1
Ba	0.4	0.1	0.1
Be	0.2	0.2	0.04
Bi	40	4	5
Ca	20	3	1
Cd	2	0.2	0.2
Co	3	0.8	0.5
Cr	7	2	2
Cu	5	1	1
Fe	5	1	0.8
K	700	100	175
Li	3	0.7	0.5
Mg	7	0.1	0.2
Mn	1	0.2	0.1
Mo	4	0.9	0.7
Na	30	10	14
Ni	6	4	1
P	40	6	6
Pb	7	5	4
Sb	30	6	9
Si	80	3	10
Sr	0.6	0.04	0.05
Ti	0.9	0.3	0.2
Tl	100	40	35
W	10	2	3
V	5	1	1
Zn	10	0.4	0.3

potassium, silicon, sodium, titanium, vanadium, and zinc in one analysis, and the same elements plus cobalt in a second analysis.

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 6.0%, the mean spike recovery was 95%, the recovery of trace elements in NBS reference material 1566a (oyster tissue) was within recommended ranges except for aluminum, iron, phosphorus, silver, sodium, and vanadium, and the recovery of trace elements in National Research Council of Canada reference material TORT2 (lobster tomalley) was within recommended ranges except for arsenic, cobalt, lead, nickel, selenium, and vanadium.

Muscle plugs were prepared for analysis at CERC, and neutron activation analysis was performed at the University of Missouri Research Reactor (MURR), Columbia, MO. Neutron activation was used for the analysis of selenium in muscle plugs because of the small sample mass. All sample preparation prior to neutron activation analyses were 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 standard reference material 1577 (bovine liver) was analyzed by MURR as a quality control check on accuracy and precision. The recovery of selenium was within the NIST recommended range, and the percent relative standard deviation of multiple analyses ranged from 3.2% to 5.7%. Selenium values in μg were obtained by direct comparison of peak areas obtained for the samples to the average peak areas obtained for a set of standards. The limit of detection was 0.015 $\mu\text{g/g}$. Multiple muscle plugs from the same fish were not taken, so no other quality assurance measures were evaluated.

2.6. Statistics

Data were analyzed using computer programs from Statistical Analysis System Institute, Inc. (SAS (Statistical Analysis System), 1990). Concentrations below the LOD were reported as “<”; however, a value of one-half the LOD was assigned for statistical computations (Kushner, 1976; USEPA, 1996). Analysis of variance testing was used for comparisons of residues in water, sediment, zooplankton, and muscle plugs (logarithmically transformed values) among sites and sample stations within sites. When significant differences ($P = 0.05$) were observed, means were compared by the Bonferroni (Dunn) multiple mean comparison test (Snedecor and Cochran, 1967). Correlation analyses

(Pearson product–moment correlation [r], SAS, 1990) were used to test for relations among water quality characteristics and inorganic element concentrations in water, sediment, zooplankton, and tissue residues. Correlation analyses of the means with standard deviation and variance measures were conducted to determine if transformations were needed to meet the assumptions of normality and homogeneity of variance (M. Ellersieck, University of Missouri, Columbia, personal communication). The data for residues for water, sediment, zooplankton (except Ba, Ca, and Si), and muscle plugs were transformed (log 10) prior to correlation analysis. Multiple regression analyses were used to test for relations among sediment characteristics and selenium concentrations in sediment.

3. Results

3.1. Water quality

Water quality, characterized primarily by conductivity, varied over time at all the sites between May 1995 and June 1996 (Hamilton et al., 2001a). This variation was partly due to flooding of the Colorado River during high runoff, which extended from mid-June to late July in 1995 and from mid-May to late June in 1996. Water quality at HT matched closely with that in the Colorado River, but water quality changes at HT lagged behind those in the Colorado River by about 1–2 weeks. For example, the conductivity of the Colorado River on August 7, 1995, was 509 $\mu\text{S/cm}$ and increased 59% to 812 $\mu\text{S/cm}$ on August 22, whereas a similar change in conductivity at HT occurred on August 31. Water quality characteristics such as hardness and alkalinity followed changes in conductivity, being lowest during runoff and highest during low-flow periods. During the period when adults were held at HT, the range of values was 390–950 $\mu\text{S/cm}$ conductivity, 150–320 mg/L hardness as CaCO_3 , and 110–140 mg/L alkalinity as CaCO_3 . The range of other water quality constituents was 16–81 mg/L chloride, <0.1–1.2 mg/L nitrate, <0.1–0.1 mg/L nitrite, 6–40 mg/L solids, and 67–260 mg/L sulfate (Hamilton et al., 2001a).

Sample stations at AC where the adults were held received mostly ground water infiltration from the river, but also inputs of irrigation water at station AC4 and pumped river water. River water from the secondary channel was used to maintain water levels in the area where the adult fish were held, and was pumped as needed from late August to early November 1995 and from late April to early June 1996. Flows from AC4 into the adult holding area were unexpected, but were monitored when flow was observed. Uncontrolled flooding of the AC area occurred for about 1 week (~June 16–24, 1995) during high runoff flows in the

Colorado River. During the period when adults were held at AC, the range of values was 420–1110 $\mu\Omega/\text{cm}$ conductivity, 150–340 mg/L hardness as CaCO_3 , and 90–160 mg/L alkalinity as CaCO_3 . There was no consistent pattern of higher or lower water quality values between the upstream station AC1 and the downstream station AC3.

The stations at WW where adults were held received mostly upslope ground water from a cobble aquifer (Phillips, 1986) and inputs of irrigation supply water from Independent Ranchman's Ditch at WW3. Flows at WW3 were used to maintain water levels in the area where the adult fish were held and occurred as needed from late August to early November 1995 and from late April to early June 1996. Uncontrolled flooding at WW occurred for about 1 week (~June 16–24, 1995) during high runoff flows in the Colorado River. Water quality characteristics were more variable at WW1 and WW2 than at the stations at HT or AC because of the mixture of upslope ground water, irrigation flows, and uncontrolled flooding. During the period when adults were held at WW, the range of values was 1240–7140 $\mu\Omega/\text{cm}$ conductivity, 360–2160 mg/L hardness as CaCO_3 , and 110–230 mg/L alkalinity as CaCO_3 . There was a 9-fold variation in magnesium concentration (39–370 mg/L)

and 11-fold variation in sulfate concentration (340–3670 mg/L), which were greater than the 6-fold variations of conductivity and hardness. Irrigation water inflow at WW3 diluted some water quality characteristics (conductivity, hardness, magnesium, chloride, nitrate) at WW more than 2-fold between July and early August 1995 compared to September–November 1995.

3.2. Selenium in water

There was no significant difference in selenium concentrations between filtered and unfiltered water samples, and the data were combined within a sample station for further statistical analysis. Selenium concentrations in water at HT ranged from <1 to 3.9 $\mu\text{g}/\text{L}$ between July 1995 and June 1996 (Table 3). These values were similar to those in Colorado River samples collected at WWSWA (1.1–3.3 $\mu\text{g}/\text{L}$) and the secondary channel at AC (0.8–4.2 $\mu\text{g}/\text{L}$) (Hamilton et al., 2001a).

There was no significant difference in selenium concentrations at stations within a site where adults were held, i.e., no difference between HTi and HTo, among AC1, AC2, and AC3, or between WW1 and WW2, and hereafter the data were combined for further

Table 3
Concentration of selenium ($\mu\text{g}/\text{L}$) in filtered and unfiltered water samples from three sites near Grand Junction, Colorado

Sample type	Date	Day of exposure	Station								
			HTi	HTo	AC1	AC2	AC3	AC4	WW1	WW2	WW3
Filtered	05/03/95	–64	1.0	1.2	1.0	2.4	3.2	— ^a	133	115	—
	06/06/95	–30	<1.0 ^b	1.0	2.5	1.4	1.3	—	26.5	26.3	—
	07/10/95	4	<1.6	<1.6	3.7	2.0	3.2	—	6.5	5.9	—
	08/08/95	33	<2.8	<2.8	5.9	3.3	5.5	—	4.6	3.8	—
	09/12/95	68	3.0	3.0	7.5	6.4	8.1	12.2	5.3	5.8	13.8
	10/18/95	104	3.4	2.8	10.4	9.8	9.9	11.4	8.3	7.1	—
	11/14/95	131	3.7	3.3	3.4	3.4	3.4	—	4.5	5.8	—
	12/12/95	159	1.9	1.8	1.7	1.5	2.0	—	9.8	16.0	—
	01/09/96	187	—	3.3	3.4	2.1	4.3	—	6.6	8.5	—
	02/12/96	221	2.6	2.5	2.1	2.4	2.1	—	5.2	5.6	—
	03/12/96	250	2.2	2.0	2.8	1.9	1.8	—	7.2	14.0	—
	04/16/96	285	2.0	1.2	—	1.8	2.9	—	12.0	13.2	—
	04/23/96	292	<2.2	<2.2	1.9	1.9	1.8	—	16.8	19.6	—
	04/25/96	294	—	—	—	—	—	15.0	—	—	—
	05/21/96	—	<0.8	<0.8	2.5	<0.8	3.8	—	12.9	11.4	—
	06/11/96	—	<1.2	1.4	4.5	5.9	9.7	10.7	10.3	10.6	—
	Unfiltered	10/18/95	104	3.9	3.8	10.2	11.1	11.6	12.4	7.1	7.3
02/12/96		221	2.0	2.3	2.7	2.2	1.8	—	5.9	5.6	—
03/12/96		250	1.6	1.9	1.9	1.7	1.8	—	10.3	13.3	—
04/16/96		285	1.4	1.8	1.7	1.6	1.5	—	13.6	13.6	—
04/23/96		292	<1.6	<1.6	2.2	1.8	1.8	—	16.3	18.6	—
04/25/96		294	—	—	—	—	—	12.0	—	—	—
05/21/96		.	0.7	0.7	3.1	2.5	4.0	—	13.2	10.0	—
06/11/96		.	<1.6	—	3.9	6.3	10.9	12.6	8.2	—	—

^a—: No sample.

^b<: Below limit of detection.

statistical analysis. Selenium concentrations at the three sites were significantly different from each other. During the period when adults were present, mean selenium concentrations were 2.2 µg/L at HT (range <1.6–3.9 µg/L), 3.8 µg/L at AC (range 1.5–11.6 µg/L), and 9.5 µg/L at WW (range 3.8–19.6 µg/L).

The highest selenium concentrations at AC occurred 2 weeks after irrigation water containing 11.4–12.4 µg/L selenium flowed from AC4 into the channel near AC2 during September–October 1995 (Table 3). Selenium concentrations in the AC site peaked at 9.8–11.6 µg/L on October 18, 1995, which was twice as high as those in August (3.3–5.9 µg/L). A similar 2-fold increase in selenium concentrations occurred on June 11, 1996, after water containing 15 µg/L from AC4 flowed into the AC site on April 25, 1996.

The highest selenium concentrations at the WW sites occurred in May 1995 (115–133 µg/L) prior to stocking the adults and before dilution from river flooding or irrigation inflow at WW3 (Table 3). The 20-fold decrease in selenium concentrations in WW between May and July 1995 was due to flooding by the Colorado River. Some of the selenium in WW came from irrigation water containing 13.8 µg/L flowing from WW3 in September 1995.

There was a significant positive correlation between selenium in water with several water quality characteristics at HT including, from highest to lowest correlation coefficient (r), calcium ($r = 0.87$, $P = 0.0001$), hardness ($r = 0.86$, $P = 0.0001$), sulfate ($r = 0.86$, $P = 0.0001$), magnesium ($r = 0.82$, $P = 0.0001$), conductivity ($r = 0.80$, $P = 0.0001$), alkalinity ($r = 0.71$, $P = 0.0005$), and nitrate ($r = 0.63$, $P = 0.02$). At WW, the significant positive correlations were, from highest to lowest correlation coefficient (r , all were $P = 0.0001$), nitrate (0.86), hardness (0.78), magnesium (0.78), sulfate (0.76), chloride (0.75), conductivity (0.73), calcium (0.71), and nitrite (0.70). In contrast, there was a significant negative correlation between selenium in water and total dissolved solids ($r = -0.64$, $P = 0.004$) and fixed solids ($r = -0.56$, $P = 0.03$) at HT, but not at WW. There were no positive correlations between selenium in water and water quality characteristics at AC, but there was a significant negative correlation with nitrite ($r = -0.47$, $P = 0.0007$), which is the opposite of the positive correlation for WW. The reason for this lack of a correlation at AC between selenium and water quality characteristics is unknown.

3.3. Other elements in water

Eight of the inorganic elements in water (antimony, boron, calcium, lithium, magnesium, phosphorus, sodium, strontium) were significantly higher at WW than at AC, whereas one element (silicon) was higher at AC than at WW (Table 4). Selenium concentrations in water

Table 4

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

Element	Site		
	HT	AC	WW
Ag	<0.005 ^a	0.010 (—) [1] ^b	<0.005
Al	<0.03	<0.03	0.05 (0.01) [2]
As	<0.02	<0.02	<0.02
B	0.035 (—) [1]	0.049 (0.003) [15]	0.129 (0.017)* [10]
Ba	0.124 (—) [1]	0.112 (0.007) [15]	0.096 (0.005) [10]
Be	<0.0002	<0.0002	<0.0002
Bi	<0.04	<0.04	<0.04
Ca	40.1 (—) [1]	67.9 (3.3) [15]	114.9 (18.2)* [10]
Cd	<0.002	<0.002	<0.002
Co	0.011 (—) [1]	0.004 (0) [8]	<0.003
Cr	<0.007	0.008 (0) [4]	0.034 (0.015) [8]
Cu	0.034 (—) [1]	0.006 (—) [1]	<0.005
Fe	0.023 (—) [1]	0.023 (0.002) [15]	0.019 (0.006) [9]
K	2.0 (—) [1]	3.3 (0.2) [15]	4.2 (0.6) [10]
Li	0.013 (—) [1]	0.023 (0.001) [15]	0.048 (0.005)* [10]
Mg	11.0 (—) [1]	22.2 (1.3) [15]	99.7 (22.5) [10]
Mn	0.004 (—) [1]	0.032 (0.005) [15]	0.027 (0.006) [10]
Mo	0.005 (—) [1]	0.008 (0.001) [11]	0.009 (0.001) [10]
Na	20.8 (—) [1]	68.3 (5.7) [15]	294.4 (60.5)* [10]
Ni	0.007 (—) [1]	0.008 (0.001) [5]	<0.006
P	0.05 (—) [1]	0.06 (0.01) [8]	0.11 (0.02)* [9]
Pb	0.032 (—) [1]	0.026 (0.007) [7]	0.015 (0.003) [4]
Sb	<0.03	0.04 (0) [4]	0.05 (0)* [4]
Si	0.8 (—) [1]	1.3 (0.1) [15]	0.8 (0.4)* [4]
Sr	0.363 (—) [1]	0.665 (0.041) [15]	1.380 (0.258)* [10]
Ti	<0.0008	<0.0008	0.0015 (0.0005) [2]
Tl	0.20 (—) [1]	0.20 (0.06) [3]	<0.1
W	<0.01	0.01 (—) [1]	<0.01

Table 4 (continued)

Element	Site		
	HT	AC	WW
V	<0.005	0.006 (0.001) [3]	<0.005
Zn	<0.01	0.02 (0) [9]	0.02 (0) [5]

*Sites AC and WW significantly different of $P = 0.05$ level; HT not included because $n = 1$.

^a<: Below the limit of detection.

^bThe number of samples submitted for analysis was HT=1, AC=15, and WW=10. 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.

from AC were significantly correlated with zinc concentration ($r = 0.59$, $P = 0.05$) in water. For WW, selenium in water was significantly correlated with each of nine elements (boron, calcium, potassium, lithium, magnesium, molybdenum, phosphorus, sodium, strontium; range $r = 0.60$, $P = 0.05$ to $r = 0.88$, $P = 0.0001$).

3.4. Selenium in sediment

Selenium concentrations in sediment at HT and AC were not significantly different, but those at WW were significantly higher than those at the other two sites (Table 5). Selenium concentrations in sediments were not significantly different within a site; i.e., selenium concentrations were not different between HTi and HTo, among AC1, AC2, and AC3, or between WW1 and WW2. Selenium concentrations in WW sediment at WW2 collected in October 1995 were only 16–18% of those from the previous collection in May 1995 and in April 1996. The sediment samples from WW2 were reanalyzed and similar values obtained (Table 5). Station WW2 was located adjacent to station WW3 where irrigation source water and sediments entered WW (Fig. 3).

3.5. Other elements in sediment

Copper concentrations in sediment were 2–4 times higher at HT than at AC and WW (Table 6). The decrease in selenium concentrations at WW2 in October 1995, compared to the earlier and later sampling at that station, seemed to parallel low concentrations of five elements (calcium, magnesium, manganese, sodium, strontium) in sediment from the same collection period. The largest difference in concentrations of elements in sediments among HT, AC, and WW was for selenium, which was elevated at WW (Table 5).

3.6. Sediment chemistry

Total carbon and percent organic carbon in sediments were similar at the HT and AC stations, but tended to be higher at the WW stations (Table 7). Combining all stations where adults were held and the two sediment sampling periods for selenium analysis ($n = 32$), selenium sediment concentrations were positively correlated with several sediment constituents including inorganic carbon ($r = 0.70$, $P = 0.0001$), total carbon ($r = 0.80$, $P = 0.0001$), organic carbon ($r = 0.70$, $P = 0.0001$), and volatile solids ($r = 0.74$, $P = 0.0001$), and were negatively correlated with total solids ($r = -0.72$, $P = 0.0001$) and fixed solids ($r = -0.74$, $P = 0.0001$). Concentrations of total carbon, inorganic carbon, organic carbon, total solids, volatile solids, and fixed carbon in sediment were significantly correlated with each other.

Sediment particle size (clay, silt, and sand) was generally statistically different between or among stations within a site (Table 8). The statistical differences were due in part to the high variability of sediment composition, e.g., sediment from the inlet and outlet of HT ponds varied within a pond as did inlets or outlets between ponds 1 and 6 (Table 8). The only exception was the April 1996 sampling at WW1 and WW2, when there was no difference in sediment composition between the two stations in the content of clay, silt, and sand. Combining all stations where adults were held and the two sediment chemistry sampling periods ($n = 32$), selenium concentrations in sediment were positively correlated with silt ($r = 0.58$, $P = 0.0005$), negatively correlated with sand ($r = -0.39$, $P = 0.03$), but not significantly correlated with clay ($r = -0.12$, $P = 0.50$). The negative correlation between selenium concentration and sand content may explain the decrease in sediment selenium at WW1 in April 1996 in that the sand content at WW1 was increased compared to that in the October 1995 sample. Taking into account all the sediment measures, the best predictor of selenium concentration in sediment was total carbon content.

3.7. Selenium in biota

Selenium concentrations in zooplankton samples from HT were $3 \mu\text{g/g}$ or less (Table 9), which was significantly lower than at the other two sites. Selenium concentrations in zooplankton from AC and WW were not significantly different from each other.

Selenium concentrations in zooplankton at AC seemed to increase starting in late July and peaked in September 1995. At AC, selenium concentrations in zooplankton prior to stocking the adult fish ranged from 4.5 to $10.5 \mu\text{g/g}$ in May and June 1995, and after stocking, concentrations ranged from 13.7 to

Table 5

Mean (standard error in parentheses) selenium concentration ($\mu\text{g/g}$ dry weight) in sediment from three sites near Grand Junction, Colorado

Date	Day of exposure	Station									
		HTli	HTlo	HT6i	HT6o	AC1	AC2	AC3	WW1	WW2	WW2 ^a
05/03–04/95	–64 to –63	— ^b	—	0.52 (0.01)	0.56 (0)	0.79 (0.08)	1.6 (0.05)	0.75 (0.03)	31.77 (1.52)	50.59 (0.23)	55.42 (2.43)
10/17–18/95	103–104	0.12 (0.01)	1.37 (0.01)	—	—	0.95 (0.04)	1.43 (0.06)	2.1 (0.07)	30.12 (1.99)	8.16 (0.74)	7.17 (0.35)
04/24–25/96	293–294	1.19 (0.04)	1.03 (0.08)	0.16 (0.01)	0.18 (0.01)	1.11 (0.01)	0.54 (0.01)	1.08 (0.07)	11.93 (0.81)	46.07 (2.67)	45.74 (2.91)

Note. At Horsethief, sediment samples were collected from the inlet (i) and outlet (o) of ponds 1 and 6 when the fish were held in that pond ($n = 2$).

^aSamples reanalyzed.

^b—: No sample.

Table 6

Concentration of inorganic elements ($\mu\text{g/g}$ dry weight) in sediment from three sites near Grand Junction, Colorado

Element	Date	Day of exposure	Station							
			HT1	HT6	AC1	AC2	AC3	WW1	WW2	
Ag	10/17–18/95	103–104	— ^a	—	<1 ^b	2	<1	<1	<1	<1
	04/24–25/96	293–294	<1	<1	<1	<1	2	<1	<1	<1
Al	10/17–18/95	103–104	—	—	9900	9770	12,900	12,700	12,700	12,700
	04/24–25/96	293–294	10600	4910	4320	9070	8680	9860	11,000	11,000
As	10/17–18/95	103–104	—	—	<7	<7	<8	<8	<8	<8
	04/24–25/96	293–294	<7	<5	<6	<7	<7	<7	<7	<8
B	10/17–18/95	103–104	—	—	<6	<6	<6	9	7	7
	04/24–25/96	293–294	<6	<6	<6	<6	<6	6	7	7
Ba	10/17–18/95	103–104	—	—	189	255	238	226	227	227
	04/24–25/96	293–294	150	146	147	260	218	227	185	185
Be	10/17–18/95	103–104	—	—	0.6	0.6	0.8	0.6	0.7	0.7
	04/24–25/96	293–294	0.7	0.5	0.3	0.6	0.6	0.6	0.5	0.5
Bi	10/17–18/95	103–104	—	—	<4	<4	<4	<4	5	5
	04/24–25/96	293–294	5	<4	6	<4	4	<4	<4	<4
Ca	10/17–18/95	103–104	—	—	27,700	33,300	38,400	57,200	30,900	30,900
	04/24–25/96	293–294	52,600	24,400	15,500	32,200	33,000	46,300	84,300	84,300
Cd	10/17–18/95	103–104	—	—	0.6	0.8	0.8	1.2	0.8	0.8
	04/24–25/96	293–294	0.3	<0.2	<0.2	0.4	0.7	0.8	0.9	0.9
Co	10/17–18/95	103–104	—	—	6.1	6.8	8.3	7.9	6.6	6.6
	04/24–25/96	293–294	5.0	2.6	3.7	6.3	6.7	6.7	7.1	7.1
Cr	10/17–18/95	103–104	—	—	13	14	16	16	17	17
	04/24–25/96	293–294	11	4	5	11	12	14	14	14
Cu	10/17–18/95	103–104	—	—	16	17	21	20	16	16
	04/24–25/96	293–294	82	27	3	14	15	17	16	16
Fe	10/17–18/95	103–104	—	—	15,100	15,400	18,600	16,500	16,500	16,500
	04/24–25/96	293–294	11400	4680	8630	14,400	13,300	15,300	14,100	14,100
K	10/17–18/95	103–104	—	—	2000	1900	2500	3000	3100	3100
	04/24–25/96	293–294	2800	1540	590	1700	1800	2200	2500	2500
Li	10/17–18/95	103–104	—	—	14	15	19	19	19	19
	04/24–25/96	293–294	13	5	8	14	15	17	18	18
Mg	10/17–18/95	103–104	—	—	7350	8120	9780	10,600	8970	8970
	04/24–25/96	293–294	6010	2970	3420	7570	7010	9700	11,200	11,200
Mn	10/17–18/95	103–104	—	—	318	375	456	829	462	462
	04/24–25/96	293–294	312	197	191	348	341	494	1270	1270
Mo	10/17–18/95	103–104	—	—	<0.9	<0.9	<0.9	3	1	1

Table 6 (continued)

Element	Date	Day of exposure	Station						
			HT1	HT6	AC1	AC2	AC3	WW1	WW2
Mo	04/24–25/96	293–294	<0.9	<0.9	<0.9	<0.9	1	1	2
Na	10/17–18/95	103–104	—	—	1000	720	790	1570	910
	04/24–25/96	293–294	415	335	959	900	890	3340	5620
Ni	10/17–18/95	103–104	—	—	10	14	17	17	15
	04/24–25/96	293–294	10	4	6	10	10	15	14
P	10/17–18/95	103–104	—	—	580	644	685	771	700
	04/24–25/96	293–294	495	295	390	621	550	590	540
Pb	10/17–18/95	103–104	—	—	17	30	36	34	34
	04/24–25/96	293–294	22	14	17	28	29	34	29
Sb	10/17–18/95	103–104	—	—	<6	<6	<6	<6	6
	04/24–25/96	293–294	<6	<5	<6	<6	<6	6	<6
Si	10/17–18/95	103–104	—	—	2660	2820	3610	2940	2820
	04/24–25/96	293–294	3880	2580	822	2670	2460	2570	3110
Sr	10/17–18/95	103–104	—	—	109	121	145	541	164
	04/24–25/96	293–294	161	711	61	121	130	402	1080
Ti	10/17–18/95	103–104	—	—	102	94	105	133	111
	04/24–25/96	293–294	37	12	121	101	93	92	96
Tl	10/17–18/95	103–104	—	—	<40	<40	<40	<40	<40
	04/24–25/96	293–294	<40	<40	<40	<40	60	<40	<40
W	10/17–18/95	103–104	—	—	3	<2	<2	<2	<2
	04/24–25/96	293–294	<2	<2	<2	<2	<2	<2	<2
V	10/17–18/95	103–104	—	—	27	26	28	29	31
	04/24–25/96	293–294	17	6	16	22	23	26	25
Zn	10/17–18/95	103–104	—	—	70	93	115	113	92
	04/24–25/96	293–294	60	20	27	82	75	99	88

^a—: No sample.^b<: Below limit of detection.Table 7
Carbon and solids content of sediments from three sites near Grand Junction, Colorado

Date	Measure	Station								
		HTli	HTlo	HT6i	HT6o	AC1	AC2	AC3	WW1	WW2
10/17–18/95	Carbon									
	Inorganic (µg/g)	14.3	15.9	—	—	6.6	9.0	10.2	16.4	8.0
	Organic (µg/g)	11.3	12.1	—	—	8.4	16.8	22.0	37.1	23.9
	Total (µg/g)	25.6	28.0	—	—	14.9	25.7	32.2	53.5	31.9
	% Inorganic ^a	1.43	1.13	—	—	0.66	0.90	1.02	1.64	0.80
	% Organic ^a	1.59	1.20	—	—	0.84	1.68	2.20	3.71	2.39
	Solids (%)									
Total	56.4	58.7	—	—	66.2	59.0	47.2	21.5	47.2	
Volatile	3.6	4.0	—	—	1.9	4.0	5.1	11.4	5.1	
Fixed	96.4	96.0	—	—	98.1	96.0	94.9	88.6	94.9	
04/24–25/96	Carbon									
	Inorganic (µg/g)	15.3	15.0	5.3	7.5	2.6	5.7	5.6	13.6	24.5
	Organic (µg/g)	10.8	11.4	3.0	4.5	8.6	10.2	5.9	17.4	23.2
	Total (µg/g)	26.1	26.4	8.4	12.1	11.2	15.9	11.5	31.0	47.7
	% Inorganic ^a	1.53	1.50	0.53	0.75	0.26	0.57	0.56	1.36	2.45
	% Organic ^a	1.08	1.14	0.30	0.45	0.86	1.02	0.59	1.73	2.32
	Solids (%)									
Total	38.2	48.1	57.9	56.3	75.3	54.2	53.9	40.9	31.6	
Volatile	3.6	2.4	1.1	1.8	1.2	1.8	4.5	5.0	6.9	
Fixed	96.4	97.6	98.9	98.2	98.8	98.2	95.5	95.0	93.1	

^aBased on sample weight.

Table 8

Mean (standard error in parentheses) percent clay, silt, and sand in sediment from three sites near Grand Junction, Colorado

Date	Day of exposure	Texture	Station								
			HT1i	HT1o	HT6i	Ht6o	AC1	AC2	AC3	WW1	WW2
10/17–18/95	103–104	Clay	43.4a (0.5)	39.6a (0.9)	— ^a	—	9.8m (0.2)	13.8n (0.3)	21.8o (0.2)	14.6x (0.6)	18.8y (0.3)
		Silt	35.3a (1.1)	33.8a (1.4)	—	—	23.1m (0.2)	54.1n (0.3)	62.8o (0.2)	66.4y (0.8)	35.5x (0.9)
		Sand	21.1a (0.6)	26.4b (0.5)	—	—	66.7o (0.5)	31.3n (0.5)	14.9m (0.2)	18.2x (0.1)	45.4y (0.5)
04/24–25/96	293–294	Clay	36.3a (1.0)	30.8a (3.7)	7.3d (0.2)	24.4e (1.1)	2.5m (0.3)	7.1o (0.2)	4.5n (0.2)	14.8x (0.7)	16.4x (3.0)
		Silt	34.2b (1.2)	17.8a (3.2)	8.4d (0.4)	24.1e (1.7)	3.0m (0.4)	28.0o (0)	20.1n (0.7)	56.1x (1.7)	56.3x (5.5)
		Sand	29.1a (0.2)	46.2b (1.9)	84.1e (0.3)	51.1d (0.6)	94.4o (0.1)	63.9m (0.3)	75.0n (0.5)	25.7x (1.4)	25.6x (1.7)

Note. At Horsethief, sediments were collected from the inlet (i) and outlet (o) of ponds 1 and 6 when the fish were held in that pond ($n = 2$). Within a site (HT1, HT6, AC, and WW), date, and texture measure, values with the same letter in common are not significantly different ($P = 0.05$).

^a—: No sample.

Table 9

Concentration of selenium ($\mu\text{g/g}$ dry weight) in zooplankton from three sites near Grand Junction, Colorado

Date	Day of exposure	Station						
		HT6i	HT6o	AC1	AC2	AC3	WW1	WW2
05/16/95	–51	— ^a	—	6.6	6.6	9.6	43.4	66.5
06/13/95	–23	—	—	—	—	—	40.5	42.9
06/14/95	–22	—	—	4.5	—	4.9	81.3	77.7
06/15/95	–21	—	—	10.5	—	8.6	—	—
06/20/95	–16	3.0	3.0	—	—	—	—	—
07/21/95	15	—	—	—	—	—	36.6	21.2
07/28/95	22	—	—	19.1	—	13.7	—	—
08/16/95	41	—	—	18.8	19.5	25.0	20.3	22.2
08/17/95	42	—	—	—	22.2	—	25.4	25.5
09/27/95	83	—	—	—	—	—	25.2	32.5
09/28/95	84	—	—	49.6	55.6	52.0	—	—
09/29/95	85	2.3	3.1	—	—	—	25.2	32.5
10/24/95	110	—	—	—	—	—	30.5	35.2
10/25/95	111	—	—	32.4	33.7	24.9	—	—
11/05/95	122	—	—	—	—	—	29.7	21.2
12/06/95	153	—	—	20.3	21.0	20.3	—	—
04/02–04/96	271–273	—	—	18.5	16.1	—	25.5	33.3
05/19/96	318	—	—	—	—	21.8	36.9	—

Note. At Horsethief, zooplankton were collected from the inlet (i) and outlet (o) of pond 6 where fish were held.

^a—: No sample.

55.6 $\mu\text{g/g}$ between July 1995 and April 1996. There were no significant differences in selenium concentrations in zooplankton among AC1 (mean 26.4 $\mu\text{g/g}$), AC2 (mean 28.0 $\mu\text{g/g}$), and AC3 (mean 26.3 $\mu\text{g/g}$) between May 1995 and May 1996. The mean selenium concentration in zooplankton during the time period when adults were present was 28.5 $\mu\text{g/g}$ (SE 3.5 $\mu\text{g/g}$,

$n = 15$). The correlation coefficient between selenium concentrations in zooplankton and water at AC was $r = 0.58$ ($P = 0.01$, $n = 18$). This correlation analysis used water concentrations measured about 1–2 weeks before zooplankton collections to account for the delay in selenium uptake from the water by aquatic organisms.

At WW, selenium concentrations in zooplankton prior to stocking with fish ranged from 40.5 to 81.3 µg/g in May–June 1995, and after stocking, concentrations ranged from 20.3 to 36.9 µg/g between July 1995 and April 1996. Concentrations decreased due to flooding by the Colorado River and inputs of irrigation water, which lowered selenium concentrations in water from July 1995 to February 1996, after which concentrations started to rise in March 1996 (Table 3). There was no significant difference in selenium concentrations in zooplankton between WW1 (mean 28.8 µg/g) and WW2 (mean 27.3 µg/g) between May 1995 and May 1996. The mean selenium concentrations in zooplankton when adults were present was 27.1 µg/g (SE 1.6 µg/g, $n = 12$). The correlation coefficient between selenium concentrations in zooplankton and water at WW was $r = 0.60$ ($P = 0.02$, $n = 15$), using the 1–2 week lag period mentioned previously.

3.8. Growth

No significant difference was found in weight or total length among adults stocked at HT, AC, or WW in July 1995 (Table 10). At spawning, adults at HT were significantly heavier than adults from AC and WW, but there were no differences in length. Combining all fish at

a site, length generally increased about 2–4% at the three sites between stocking and spawning, whereas weight increased by 15% at HT, decreased by 2% at AC, and increased by 3% at WW. The greatest weight loss occurred at 69 days in the study and was 5.1% at HT, 9% at AC, and 5.6% at WW. However, between day 69 and spawning (day 305), weight gains were 21.7% at HT, 7.5% at AC, and 9.2% at WW.

3.9. Selenium in tissues

At the time of stocking selenium concentrations in muscle plugs in adults were 4.5 µg/g at HT, 3.9 µg/g at AC, and 4.1 µg/g at WW (Table 11). Concentrations of selenium in muscle plugs from adults held at HT did not change during the exposure or depuration periods (Table 11). However, fish held for 69 days of exposure and longer at AC (≥ 7.4 µg/g) and for 126 days and longer at WW (≥ 9.5 µg/g) had significantly higher selenium concentrations than fish held at HT. Selenium concentrations in muscle plugs at spawning (day 305) were significantly different among adults from the three sites, with fish from AC having 2.6 times and WW 3.7 times the selenium present in muscle plugs of fish from HT. Selenium concentrations in muscle plugs were 3.0 times higher in fish at AC and 4.0 times higher in fish at

Table 10

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

Site	Measure	Day of exposure and date							
		1995				1996			
		0 7/6	34 8/9	69 9/13	126 11/9	231 2/22	305 ^a 5/6	337 ^b 6/7	371 ^c 7/11
HT	Total length	409a (2) [56]	408a (3) [36]	405a (3) [30]	407a (3) [48]	411a (3) [46]	418a (3) [45]	428a (5) [6]	422a (2) [36]
	Weight	742x (14) [56]	711x (17) [36]	704x (22) [30]	750x (18) [48]	809x (19) [46]	857x (20) [45]	888 (34) [2]	812x (15) [36]
AC	Total length	409a (3) [36]	410a (4) [20]	409a (3) [23]	412ab (3) [23]	413a (3) [25]	419a (3) [32]	429 (7) [6]	427ab (3) [25]
	Weight	764x (16) [36]	739x (22) [20]	695x (15) [23]	748x (11) [23]	756xy (17) [25]	747y (15) [32]	815 (37) [2]	824x (19) [25]
WW	Total length	409a (3) [36]	410a (2) [46]	417a (5) [11]	420b (4) [15]	420a (3) [23]	426a (4) [24]	442a (11) [3]	437b (4) [20]
	Weight	748x (19) [36]	711x (15) [46]	706x (29) [11]	741x (25) [15]	733y (20) [23]	771y (23) [24]	914 (–) [1]	773x (25) [20]

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

^aSpawmed and moved fish at AC and WW to reference site HT for depuration.

^b32 days depuration.

^c66 days depuration.

Table 11

Mean (standard error in parentheses and number of samples in brackets) selenium concentration ($\mu\text{g/g}$ dry weight) in muscle plugs from razorback sucker held at three sites near Grand Junction, Colorado, and from brood stock held at Horsethief

Site	Day of exposure and date							
	1995				1996			
	0 7/6	34 8/9	69 9/13	126 11/9	231 2/22	305 ^a 5/6	337 ^b 6/7	371 ^c 7/11
HT	4.5a (0.2) [5]	4.7a (0.2) [5]	5.2a (0.4) [6]	4.7a (0.2) [9]	4.4a (0.1) [6]	4.5a (0.2) [6]	4.5a (0.2) [4]	4.5a (0.4) [2]
AC	3.9a (0.3) [8]	4.7a (0.2) [7]	7.4b (0.3) [6]	9.5b (0.5) [7]	10.6b (0.5) [5]	11.7b (0.4) [6]	11.5b (0.8) [4]	9.5ab (0.6) [2]
WW	4.1a (0.2) [7]	4.5a (0.7) [3]	6.3ab (0.3) [6]	9.5b (0.7) [7]	13.2b (1.0) [7]	16.6c (1.0) [6]	16.4b (2.3) [3]	14.2b (2.3) [2]
BS ^d	— ^e	—	—	—	—	5.1 (1.0) [14]	—	—

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

^aSpawned and moved fish at AC and WW to reference site HT for depuration.

^b32 days depuration.

^c66 days depuration.

^dBS: Brood stock.

^e—: No sample.

WW at spawning compared to those at the time of stocking. For the exposure period from day 69 to 305, selenium concentrations in muscle plugs pooled across site and sample date were correlated with the mean selenium concentration in water for the closest corresponding sample date ($r = 0.74$, $P = 0.006$, $n = 12$). For the same exposure period, selenium concentration in muscle plugs were correlated with the mean selenium concentration in zooplankton for the closest corresponding sample date ($r = 0.65$, $P = 0.04$, $n = 10$). Day 69 was selected as the beginning of the period because that was about the time when selenium concentrations in muscle plugs from fish at AC and WW were becoming significantly higher than those of reference fish at HT.

Selenium concentrations in muscle plugs of fish previously held at AC decreased about 2% after 32 days of depuration and 19% after 66 days of depuration. Of the four fish sampled after 32 days of depuration at AC, two (fish AC21 and AC28) had selenium concentrations that were 16–18% higher than those at spawning (Hamilton et al., 2001a). Selenium concentrations in fish previously held at WW lost 1% of their selenium at 32 days of depuration and 14% at 66 days of depuration. One of the three fish monitored did not spawn, yet it had a 6% loss of selenium after 32 days of depuration.

Fish from AC and WW gained weight during the depuration period. Weight gain in fish from AC sampled for muscle plugs was 1–2% at 32 days depuration and was 11–14% at 66 days depuration, whereas at WW it

was 0% at 32 days depuration and 5–22% at 66 days depuration. These magnitudes of increase in body weight were comparable to those for decreased selenium concentrations in muscle plugs.

After 305 days of exposure, selenium concentrations in muscle, liver, kidney, and gonad tissues were 2.5–4.4 times higher in fish from AC and 4.6–8.4 times higher in fish from WW than in fish from HT (Table 12). After 66 days of depuration, selenium concentrations in AC fish decreased by 20% in muscle tissue and by 67% in the gonads. In WW fish the decreases in selenium concentrations were 38% in muscle and 72% in the gonads. Selenium concentrations in muscle plugs were significantly correlated with selenium in muscle ($r = 0.92$, $P = 0.0001$, $n = 17$), liver ($r = 0.93$, $P = 0.0001$, $n = 12$), kidney ($r = 0.78$, $P = 0.0002$, $n = 17$), gonad ($r = 0.72$, $P = 0.002$, $n = 15$), and gall bladder ($r = 0.82$, $P = 0.01$, $n = 8$). Selenium concentrations measured by AA-HG in muscle tissue were 1.1 to 2.0 times higher than selenium measured by neutron activation in muscle plugs.

4. Discussion

4.1. Water quality

High concentrations of cations and anions in water, at HT and AC, as characterized by conductivity, probably did not adversely affect razorback sucker held at those

Table 12

Mean (standard error in parentheses and number of samples in brackets) selenium concentration ($\mu\text{g/g}$ dry weight) in various tissues of razorback sucker held at three sites near Grand Junction, Colorado

Site	Day of exposure ^a	Muscle	Liver	Kidney	Gonad	Gall bladder	Gut contents	
HT	305	6.3 (0.3) [4]	7.5 (1.1) [2]	4.6 (1.0) [4]	7.0 (0.4) [2]	— ^b	—	
		337	5.8 (0.1) [2]	7.9 (0.5) [2]	7.1 (0.5) [2]	5.9 (0) [2]	2.1 (–) [1]	3.9 (–) [1]
	371		6.0 (0) [2]	6.8 (0.4) [2]	6.1 (0.2) [2]	5.1 (0.7) [2]	2.0 (1) [1]	—
		AC	305	15.6 (0.8) [2]	—	14.6 (5.4) [2]	30.6 (5.8) [2]	—
	337			13.5 (1.0) [2]	17.6 (1.1) [2]	20.3 (2.6) [2]	27.5 (7.9) [2]	3.6 (–) [1]
			371	12.5 (0.7) [2]	12.2 (0.2) [2]	13.6 (0.6) [2]	10.0 (1.9) [2]	2.8 (0) [2]
WW	305	29.2 (4.2) [3]	52.1 (19.1) [2]	38.7 (15.8) [3]	45.5 (3.1) [2]	4.8 (–) [1]	—	
		337	16.2 (–) [1]	29.6 (–) [1]	37.8 (–) [1]	42.1 (–) [1]	6.2 (–) [1]	—
	371		18.0 (1.3) [2]	23.7 (–) [1]	30.1 (5.1) [2]	12.9 (1.1) [2]	4.8 (–) [1]	—

^aSpawmed at day 305 and moved fish at AC and WW to reference site HT for depuration: Day 337 = 32 days depuration, day 371 = 66 days depuration.

^b—: No sample.

sites. Tyus (1987) and Tyus and Karp (1990) reported that razorback sucker staged at Ashley Creek and Stewart Lake outlet in Utah in mid-April to May. During that time period, Stephens et al. (1988) and Peltz and Waddell (1991) reported conductivities ranged from 1510 to 2550 $\mu\Omega/\text{cm}$. These conductivities were higher than those observed in the present study when adults were present at HT (range 392–950 $\mu\Omega/\text{cm}$) and AC (range 418–1110 $\mu\Omega/\text{cm}$).

When adults were present at WW, conductivities (range 1240–7140 $\mu\Omega/\text{cm}$) and salinities (range 0.5–5.5 $\mu\text{g/L}$) were elevated. Adults held at WW gained slightly more weight than adults held at AC, which suggests that they were able to convert energy from their diet into growth and development of sex products, rather than using all their energy to compensate for potential stresses associated with osmoregulation and toxicants.

4.2. Selenium and other elements in water

The similarity of selenium concentrations in filtered and unfiltered water samples in the present study was

consistent with findings from investigations of flowing water systems at seven riverine sites in the San Joaquin Valley, California (Saiki et al., 1993). There was a potential for selenium concentrations in filtered and unfiltered water to be different during the peak algae growth in the summer because the AC sites AC1–AC3 and the WW sites WW1–WW2 were semi-static with limited flow of water except from irrigation supply sources or pumped river water. For pond systems at Kesterson Reservoir, Fujii (1988) and Moore et al. (1990) reported that unfiltered water samples (reported as total selenium) had higher selenium concentrations than filtered samples (reported as dissolved selenium). Adams (1976) reported similar findings for Lake Erie, and Seiler (1996) and Seiler et al. (2003) cautioned against assuming that total (unfiltered) and dissolved (filtered) concentrations of selenium were similar, especially in highly productive waters where there might be large amounts of algae.

The maximum concentrations of selenium in river water collected at HT (3.9 $\mu\text{g/L}$), AC (4.2 $\mu\text{g/L}$), and WWSWA (3.3 $\mu\text{g/L}$) were higher than at typical reference sites (areas above irrigation influences) in the

upper Colorado River. For example, [Butler et al. \(1996\)](#) reported that selenium concentrations were $<1 \mu\text{g/L}$ ($n = 5$) in the Gunnison River downstream from the Gunnison Tunnel near Montrose, CO, and were $\leq 1 \mu\text{g/L}$ ($n = 22$) in the Colorado River at Cameo, CO. However, the reaches of the Colorado and Gunnison rivers that are influenced by irrigation return flows have elevated concentrations of selenium and other elements and altered water quality characteristics ([Butler et al., 1989, 1991, 1994, 1996](#)). Consequently, the elevated selenium concentrations in water from HT, relative to reference areas in the upper Colorado and Gunnison rivers ([Butler et al., 1996](#)), suggested that adults at HT were exposed to somewhat elevated selenium concentrations during the study.

The elevated selenium concentrations in water at AC1–AC3 were due in part to inflow of irrigation return water at AC4, which contained $>11 \mu\text{g/L}$, on September 12 and October 18, 1995. Selenium concentrations in water at the AC site changed quickly due to the discharge of irrigation return flow at AC4 into the site. The selenium concentrations in water at AC4 exceeded the USEPA chronic criterion of $5 \mu\text{g/L}$ ([USEPA, 1987](#)) for the protection of aquatic life.

The elevated concentrations of selenium in water at WW1 and WW2 were due in part to inflow of ground water from the underlying cobble aquifer ([Phillips, 1986](#)). Water in the cobble aquifer sampled as part of the NIWQP in 1992 at a location about 5.5 km north of WWSWA had a selenium concentration of $175 \mu\text{g/L}$ ([Butler et al., 1994](#)). Water from the cobble aquifer comes to the surface in a marsh area adjacent to WW. When the Colorado River was at low flow, selenium concentrations in the marsh between September 1995 and April 1996 ranged from 54 to $138 \mu\text{g/L}$ ([Hamilton et al., 2001a](#)). The WWSWA channel and WW have been identified as a discharge area for ground water ([Butler and Osmundson, 2000](#)).

Water from Independent Ranchman's Ditch was used to maintain the water level in WW during the study. This water, sampled at WW3, had a selenium concentration of $13.8 \mu\text{g/L}$ in September 1995. Although the selenium concentration in water from WW3 exceeded the USEPA criterion of $5 \mu\text{g/L}$ for the protection of aquatic life ([USEPA, 1987](#)), this water diluted the incoming ground water, which in May 1995 was the sole source of water for WW when WW had selenium concentrations of 115– $133 \mu\text{g/L}$.

The consistently higher selenium concentrations at WW2 compared to WW1 may indicate that seepage of irrigation-derived ground water was greater on the west side of WW near WW2 (near a higher elevation, dry upland area) than on the east side near WW1 (near a lower elevation, marsh area).

Selenium concentrations in water at AC and WW were typical of other surface waters in the Grand and

Uncompahgre valleys that are influenced by irrigation activities. Selenium concentrations were $4\text{--}7 \mu\text{g/L}$ (median $5 \mu\text{g/L}$) in the Colorado River at the Colorado–Utah state line, $5\text{--}7 \mu\text{g/L}$ (median $6 \mu\text{g/L}$) in the Gunnison River at Whitewater, and $8\text{--}25 \mu\text{g/L}$ (median $14 \mu\text{g/L}$) in the Uncompahgre River at Delta, Colorado ([Butler et al., 1994](#)). Selenium concentrations in water at AC and WW, in addition to most waters in the irrigation-influenced areas of the Colorado, Gunnison, and Uncompahgre rivers, were elevated compared to uncontaminated aquatic ecosystems, which typically have $<1 \mu\text{g/L}$ in the upper Colorado River ([Butler et al., 1996](#)) and in the US ([Maier and Knight, 1994](#)).

4.3. Selenium and other elements in sediment and sediment chemistry

Selenium concentrations in sediment at HT and AC (except for one value at AC) were near national background concentrations of $<1 \mu\text{g/g}$ ([Maier and Knight, 1994](#)). The sediment selenium values observed at HT and AC were similar to those of [Stephens et al. \(1997\)](#), who listed a no-effect concentration of $<2 \mu\text{g/g}$ for effects of selenium on fish and wildlife, and [Lemly \(1995\)](#), who proposed a no-hazard rating at $<1 \mu\text{g/g}$ or a minimal hazard rating at $1\text{--}2 \mu\text{g/g}$. In contrast, [Presser et al. \(1994\)](#) and [Moore et al. \(1990\)](#) used $0.5 \mu\text{g/g}$ 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 AC was diked to hold the adult fish for the present study. In June 1995 a water control structure was installed in the dike road at AC1 and an inactive beaver dam with water 2–3 ft deep was converted to a dike with an outflow water control structure at AC3 ([D. Crabtree, USBR, personal communication](#)). The one elevated selenium concentration in sediment collected at AC3 in October 1995 ($2.1 \mu\text{g/g}$) may have come from an area influenced by the beaver dam, where selenium build up in the sediments could likely have occurred. Nevertheless, the overall low selenium concentrations at AC suggest that the 9-month duration of the present study may have been too short to allow accumulation of selenium in the sediments.

Selenium concentrations in sediment at WW probably accumulated over several years, perhaps more than 20 yr because WW appears in aerial photos taken in 1973 and 1982 ([T. Mathieson, Colorado Division of Wildlife, personal communication](#)). One interesting observation during the present study was the decrease in selenium concentrations in sediment at WW2 in October 1995 and the later increase in April 1996. The decreased sediment selenium at WW2 in October was probably due to deposition of sediment carried by water from Independent Ranchman's Ditch via an irrigation supply

canal that delivered water to WW at WW3 near WW2. Twelve discharges were recorded between August and October 1995, whereas no water was delivered between November 1995 and April 1996. Selenium concentrations in sediment also probably increased due to continued inflow of high selenium ground water (Butler and Osmundson, 2000). The lowest selenium concentration in sediment observed in WW (7.17 $\mu\text{g/g}$) was above the high hazard value of $>4 \mu\text{g/g}$ proposed by Lemly (1995) and the toxic threshold guideline value of $>4 \mu\text{g/g}$ proposed by Stephens et al. (1997).

Selenium concentrations in sediment in the present study may have underestimated concentrations available to biota because they were thoroughly mixed at the time of sampling. Several investigators have reported that selenium accumulates in the top layer of sediments, where it is more available to aquatic organisms (Cumbie, 1984; Holland, 1979; Kiffney and Knight, 1990; Oremland et al., 1990; Stephens, 1996) and can contribute to selenium uptake in the aquatic food web (Peters et al., 1999), beginning with bacterivorous and algivorous predators (protozoa) (Sanders and Gilmour, 1994).

Even though selenium concentrations in sediment at AC were near background concentrations, they were elevated compared to HT, and sediment selenium was elevated at WW compared to AC. These differences may have been due in part to elevated concentrations of organic carbon, which were highest at WW. Besser et al. (1989) reported higher selenium concentrations in sediments with high organic carbon than in sediments with lower organic carbon even though the two sediments had similar microbial activity, which is generally considered to be the mechanism of selenium entry into the sediments (Bender et al., 1991). Stephens et al. (1992) and Peltz and Waddell (1991) reported a positive relation between selenium concentrations in pond sediments at Ouray National Wildlife Refuge (NWR), Utah, and concentrations of organic material in sediments. Significant correlations between sediment selenium and the total carbon or organic content of sediment have been reported (Birkner, 1978).

The significant correlations between selenium concentrations in sediment and various carbon fractions in the present study were similar to high correlations for the same relations reported by Zhang and Moore (1996) for sediment from wetland ponds at Benton Lake NWR, Montana, MT (correlation coefficients ranged from 0.73 to 0.94). Zhang and Moore (1997) also reported high correlations between selenium concentrations in sediment and organic matter in sediment. In the present study, the negative correlation between selenium concentration in sediment and sand content was similar to that in Besser et al. (1989), who reported that more selenium was sorbed to fine-textured, highly organic sediment than to sandy sediments.

4.4. Selenium in biota

Selenium concentrations in zooplankton collected from AC and WW were substantially above the proposed dietary toxic threshold concentration of $3 \mu\text{g/g}$ (Hamilton et al., 2000; Lemly, 1993a, 1996; Maier and Knight, 1994). Even though selenium concentrations in water were below the current USEPA criterion of $5 \mu\text{g/L}$ at AC for 12 of 13 months it was monitored, selenium concentrations in food organisms during the study ($14\text{--}56 \mu\text{g/g}$) exceeded the proposed dietary toxic threshold by a factor of 5–19 fold.

Selenium concentrations in zooplankton from AC increased when waterborne selenium concentrations increased, and decreased when waterborne selenium concentrations decreased. Selenium concentrations in zooplankton at AC1–AC3 increased from $19\text{--}25 \mu\text{g/g}$ in August 1995, to $50\text{--}56 \mu\text{g/g}$ in September 1995 (6 weeks). This twofold increase of selenium in zooplankton was similar to that observed at North Roadside Pond ($17\text{--}24 \mu\text{g/g}$ on April 5, 1989 increasing to $31\text{--}40 \mu\text{g/g}$ on April 27, 1989), and at South Roadside Pond ($12\text{--}13 \mu\text{g/g}$ on April 5, 1989 increasing to $40\text{--}53 \mu\text{g/g}$ on April 27, 1989) at Ouray NWR, UT (Stephens et al., 1992). In a similar manner, selenium concentrations in zooplankton from WW decreased from $41\text{--}81 \mu\text{g/g}$ in June 1995 to $20\text{--}26 \mu\text{g/g}$ in July 1995.

The likely sources of selenium residues in zooplankton at AC and WW were water, aquatic plants such as algae, or both. Selenium in water is rapidly taken up by algae (Besser et al., 1993; Foe and Knight, 1986; Nassos et al., 1980; Riedel et al., 1991; Sandholm et al., 1973) and aquatic plants (Allen, 1991; Ornes et al., 1991). Typically, algae took up maximal concentrations in 3–24 h, whereas floating plants took about 1 week to accumulate maximal concentrations. Part of the selenium taken up by zooplankton was probably waterborne organoselenium compounds released from living algae or necrosis of dead cells (Besser et al., 1994; Cutter, 1991, 1992).

Selenium concentrations in zooplankton from AC ($25\text{--}56 \mu\text{g/g}$) and WW ($25\text{--}35 \mu\text{g/g}$) during September–October, 1995, were similar to or higher than those in aquatic invertebrates from selenium-contaminated Sweitzer Lake ($27\text{--}30 \mu\text{g/g}$; Butler et al., 1991). Birkner (1978) reported that a mixed-species sample of plankton collected in 1977 from Sweitzer Lake contained $42.5 \mu\text{g/g}$ selenium. Barnhart (1957) and Birkner (1978) suggested that the lack of reproduction of native fish and stocked game fish in Sweitzer Lake was probably due to adverse effects from selenium accumulated through the food chain.

4.5. Growth

Early weight loss in adults at the three sites was probably due to stress induced by transportation and

adaptation from the hatchery environment at Wahweap State Fish Hatchery, UT, to the hatchery ponds at HT or to natural foraging at AC and WW. Adults held at HT had a 5% weight loss in the first 69 days of the study, but apparently adapted well to the hatchery ponds and feeding regime, and concomitantly had the greatest weight gain (15% compared to -2% at AC and 3% at WW) of the three sites. The low weight gain in adults at AC compared to those held at WW might be due to the difference in habitat between the two sites.

The slow growth of adult fish in the present study was consistent with the slow or negligible growth of adult razorback sucker reported by others. Tyus (1987) reported slow growth (mean 2.2 mm/year) of 39 adults with recapture periods of 1 to 8 years. Similar slow growth of razorback sucker was reported by McAda and Wydoski (1980), Valdez et al. (1982), Minckley (1983), Roberts and Moretti (1989), and Marsh and Minckley (1991).

Effects of selenium on the growth of adult fish are not well documented, whereas two of the well-documented effects of selenium in the food chain are the elimination of fish species due to reproductive failure from aquatic ecosystems such as in Belews Lake, North Carolina (Cumbie and Van Horn, 1978; Lemly, 1985); Martin Lake, Texas (Garrett and Inman, 1984; Sorensen, 1988), and Kesterson Reservoir, California (Harris, 1986; Vencil, 1986), or the lack of reproduction at Sweitzer Lake, CO (Barnhart, 1957; Birkner, 1978). Adverse effects have been reported on survival and growth of adult fathead minnow (*Pimephales promelas*) and bluegill (*Lepomis macrochirus*) exposed to selenium in experimental streams (Hermanutz et al., 1992; Schultz and Hermanutz, 1990). However, Crane et al. (1992) reported no effects on growth of adult yellow perch (*Perca fluviatilis*) in a 288-day reproduction study where the fish were held in ponds treated with selenium concentrations up to 25 µg/L. Coyle et al. (1993) also reported no effects on adult bluegill exposed to selenium up to 10 µg/L in water and 32 µg/g in diet for 140 days.

4.6. Selenium in tissues

Selenium residues in adult razorback sucker tissue from the present study probably came primarily from food-chain organisms, and secondarily from water and sediment exposure. Investigators have reported that adult razorback sucker had both planktivorous and benthic feeding habits (Marsh, 1987), and noted sediment, sometimes referring to it as ooze, detritus, or decaying organic matter, in the gut of razorback sucker (Allan and Roden, 1978; Banks, 1964; Dill, 1944; Marsh, 1987; Vanicek, 1967).

At the time of stocking, selenium concentrations in muscle plugs from all the fish (range 3.9–4.5 µg/g) exceeded the 85th percentile (arbitrary point distinguish-

ing “high” selenium concentrations) in whole-body fish in the NCBP for the years 1971–1984 (range 2.5–3.0 µg/g; Lowe et al., 1985; May and McKinney, 1981; Schmitt and Brumbaugh, 1990; Walsh et al., 1977). Elevated selenium concentrations, exceeding the 85th percentile of the NCBP, have been reported as part of the NIWQP and USFWS contaminants investigations in the upper Colorado, Dolores, Green, Gunnison, Uncompahgre, White, and Yampa rivers and Lake Powell (reviewed in Hamilton, 1998, 1999).

Selenium concentrations in muscle plugs measured in the present study probably underestimate the concentrations in whole body of fish. In general, muscle contains less selenium than whole body due to the relatively high amounts of selenium found in spleen, liver, kidney, heart, and other tissues, especially mature ovaries (Adams, 1976; Hermanutz et al., 1992; Hilton and Hodson, 1983; Hilton et al., 1982; Kleinow and Brooks, 1986; Lemly, 1982; Lemly and Smith, 1987; Sato et al., 1980). Consequently, the estimated whole-body selenium concentrations in razorback sucker initially stocked in the present study would be about 6.5 to 7.5 µg/g (based on a conversion factor of $1.667 \times \text{muscle concentration} = \text{whole body concentration}$, Lemly and Smith, 1987). Conversion factors were 2.355 based on data from Adams (1976) for rainbow trout (*Oncorhynchus mykiss*) and 1.745 from Lemly (1982) for bluegill and largemouth bass (*Micropterus salmoides*), both of which would have increased the converted values for razorback sucker. Thus, the razorback sucker used in the present study initially had selenium residues over two times higher than the 85th percentile of the NCBP.

Concentrations of selenium in muscle plugs in the present study seemed to be increasing at day 305 in fish at AC and WW, which suggested that an equilibrium in selenium concentrations in muscle had not occurred. Most other studies have reported equilibrium in selenium residues in whole-body or tissues were reached in 60 to 90 days (Besser et al., 1993; Gissel Nielsen and Gissel-Nielsen, 1978; Lemly, 1982; Sato et al., 1980), but others have estimated longer periods, i.e., >20 weeks (Adams, 1976; Woock and Summers, 1984). Equilibrium in whole-body selenium concentrations depends on a variety of factors including species, size, age, exposure route and concentration, chemical form, and many other factors. 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 9 months for tissue residues to reach an equilibrium with selenium exposure in the water and diet. In fact, selenium concentrations increased in adults after an additional 9 months of exposure in a second reproduction study: from 9.6 µg/g in July 1996 to 16.2 µg/g in April 1997 at AC, and from 14.2 µg/g in July 1996 to 22.8 µg/g in April 1997 at WW (Hamilton et al., 2001b).

Selenium concentrations in muscle plug tissue in razorback sucker from AC and WW from 126 to 305 days of exposure (range 9.5–16.6 µg/g) exceeded the proposed guideline of Lemly (1996) of 8 µg/g in skeletal muscle as the benchmark for probable reproductive failure. Two other studies not reviewed by Lemly (1996) also support his proposed guideline of 8 µg/g for reproductive failure of fish (Crane et al., 1992; Cumbie and Van Horn, 1978).

Forty percent (18 of 45) of the wild adult razorback sucker sampled in the Green River, UT, by Waddell and May (1995) and Stephens and Waddell (1998) had selenium concentrations equal to or higher than those in the fish held at AC (mean 11.7 µg/g at spawning) and WW (16.6 µg/g at spawning). It seems unusual that 40% of wild fish had higher selenium residues because the two groups of fish in the present study were held in elevated selenium environments for 9 months and had no opportunity to move to low-selenium environments. The higher selenium in a substantial portion of the wild fish reported by Waddell and May (1995) and Stephens and Waddell (1998) suggested that some wild adults choose, or are forced, due to the lack of uncontaminated habitats, to use habitats with high selenium in water, food organisms, or both. It also suggested that wild razorback sucker can accumulate substantial amounts of selenium in their tissues without depurating selenium. Recently, Osmundson et al. (2000) reported that selenium concentrations were elevated in muscle plugs of Colorado pikeminnow sampled from the channel area at WWSWA, which has high selenium concentrations in water, sediment, food organisms, and fish (Butler et al., 1994, 1996). The maintenance of elevated selenium concentrations in some Colorado pikeminnow recaptured near WWSWA over a 3-yr period suggested that they, similarly to razorback sucker, maintain elevated selenium residues in tissue without depurating it.

In general, selenium was present in tissues, from highest to lowest, as follows: liver, kidney, ovary, whole body, muscle, and testes (Adams, 1976; Hilton et al., 1982; Lemly, 1982; Sato et al., 1980). Selenium concentrations in various tissues of razorback sucker in the present study were similar to those reported for other species, which suggested that the physiological storage of selenium in this species was similar to that in other fish species. The tissue distribution and the magnitude of difference in selenium concentrations among tissues in the present study were similar to those reported by others in several fish species (Hodson et al., 1980; Hilton and Hodson, 1983; Sager and Cofield, 1984).

Our correlation between selenium concentrations in muscle plugs and muscle tissue ($r = 0.92$) was similar to that reported by Waddell and May (1995), who examined the relation in three areas (middorsal, just behind the gill, and near the tail) of one razorback

sucker, three common carp (*Cyprinus carpio*), and three flannelmouth sucker (*Catostomus latipinnis*) and reported $r = 0.97$ (for combined species and tissue locations). In the present study, all of the fish had selenium concentrations in muscle tissue measured by AA-HG higher than those in muscle plugs measured by neutron activation, whereas Waddell and May (1995) reported that four of seven fish had slightly lower selenium concentrations in muscle tissue than in muscle plugs. Neutron activation analyses have been documented to be accurate (a measure of the degree of conformity to the assumed or accepted value) and precise (the degree of agreement of repeated measurements) for the measurement of selenium in tissue (Dermelj et al., 1996; McKown and Morris, 1978; Pillay et al., 1974).

Tissue accumulations of selenium in the current study were similar to those in a study where adult razorback sucker were held in a hoop net in WW and fed a commercial fish food diet containing 1.1 µg/g selenium for 89 days (Hamilton et al., 2001a). Selenium in muscle plugs increased from 2.1 µg/g at stocking to 6.7 µg/g after 89 days of exposure, whereas selenium in muscle plug of free-moving adults in WW was 6.3 µg/g after 69 days of exposure. Selenium concentrations were 11.4 µg/g in muscle tissue, 23.9 µg/g in liver, 27.5 µg/g in kidney, 4.5 µg/g in undeveloped gonad, and 9.1 µg/g in gall bladder. The ratio of selenium concentrations in muscle tissue measured by AA-HG to those in muscle plugs measured by neutron activation in adults held in the hoop net ranged from 1.1 to 2.3, which was similar to those from free-ranging adults from WW (ratio 1.2–2.0).

4.7. Depuration of selenium from tissues

In the present study, razorback sucker from AC and WW lost 14–19% of the selenium from their muscle tissue after 66 days of depuration, which suggested a slow loss of selenium. 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, initial selenium load of various tissues, and other factors.

Half lives for selenium depuration in various young fish were reported to be 20–30 days (Bennett et al., 1986; Besser et al., 1993; Gissel Nielsen and Gissel-Nielsen, 1978; Hilton et al., 1982; Kleinow and Brooks, 1986; Sato et al., 1980). Others have reported longer half-life depuration of 49–63 days in adult fish (Adams, 1976; Bertram and Brooks, 1986; Bryson et al., 1984; Lemly, 1982). Consequently, the slow depuration rate of selenium from muscle tissue in adult razorback sucker in the present study seemed realistic.

Two fish in the present study (AC21 and AC28) had increased selenium concentrations in muscle plugs after

30 days of depuration, which suggested that selenium may have been resorbed from another tissue and deposited in muscle. This increase in selenium concentration in muscle tissue may have been due to resorption of unexpelled eggs from the spawned fish (AC28). Likewise, the unstripped fish (AC21) may have resorbed its unspawned eggs, thus redistributing selenium to muscle tissue. It seemed unusual that the three WW fish that spawned did not depurate a greater amount of selenium. Perhaps selenium from some unexpelled eggs were resorbed and offset any selenium that was depurated during the first 32 days of depuration. Hamman (1985) reported that of 70 hatchery-reared razorback sucker females stripped at 24-h intervals, 16 ovulated all eggs after one stripping, 51 ovulated all eggs after two strippings, and 3 females ovulated all eggs after three strippings.

The concept of depuration may be misleading in the natural environment because measurements were on fish physically placed in a clean environment for the sole purpose of determining how fast their tissues can remove a contaminant. In the natural environment, fish may not be able to move to a clean environment. Depuration does not seem to be occurring in endangered fish in the Colorado River near WWSWA, Grand Junction, Colorado, because Colorado pikeminnow recaptured over a 3-year period at WWSWA seemed to be conserving selenium concentrations (9.4–16.6 µg/g) in muscle plugs from year to year (Osmundson et al., 2000). Sorensen (1988) reported that selenium tissue residues in fish from Martin Lake, Texas, were only 25% lower after a 5-yr period (1981–1986) following the drastic reduction of selenium inputs to the lake in 1978. Likewise, Lemly (1997) assessed selenium concentrations in five ecosystem components of Belews Lake, North Carolina, 10 yr after selenium inputs to the lake were stopped and found elevated selenium concentrations in sediment, benthic invertebrates, and fish that suggested a moderate hazard still existed. He also reported that teratogenic deformities in fish first observed in 1992 (Lemly, 1993b) were still presented at elevated levels in 1996.

Overall, selenium was elevated in water, food organisms, and sediments at WW and in water and food organisms at AC. This exposure resulted in elevated selenium concentrations in muscle plugs and other tissues in endangered razorback suckers above toxicity thresholds in sensitive fish species, but the thresholds in razorback sucker are unknown. Residues in muscle plugs were substantially elevated after 9 months exposure, but remained less than those measured in 40% of wild razorback suckers in the Green River. Elevated selenium concentrations in liver, kidney, ovaries, and testes of fish have been linked with adverse pathological changes in those tissues (Sorensen, 1986, 1988, 1991; Sorensen et al., 1984). Consequently,

selenium contamination of the Green and Colorado rivers should be a major concern in recovery efforts of this and other endangered fish in the Colorado River basin.

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