THE EVALUATION OF CONTAMINANT IMPACTS ON RAZORBACK SUCKER HELD IN FLOODED BOTTOMLAND SITES NEAR GRAND JUNCTION, COLORADO - 1997

Final Report

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LIST OF KEYWORDS

Razorback sucker
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Endangered fish
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Selenium
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“Thanks to science and technology, access to factual knowledge ...
is destined to become global and democratic ... what then?
The answer is clear: synthesis.
We are drowning in information, while starving for wisdom.
The world henceforth will be run by ...
people able to put together the right information at the right time,
think critically about it and make important choices wisely."

E.O. Wilson

EXECUTIVE SUMMARY

Selenium contamination of the upper and lower Colorado River basin has been documented in water, sediment, and biota in studies by U.S. Department of the Interior agencies, and academia. Concern has been raised that this selenium contamination may be adversely affecting endangered fish in the upper Colorado River basin. A previous reproduction study conducted in 1995-1996 with razorback sucker (*Xyrauchen texanus*) reported that adults readily accumulated selenium in various tissues including eggs, and that ≥4.6 µg/g selenium in food organisms caused increased mortality of larvae. The objective of the current study (conducted in 1996-1997) was to determine if environmental exposure of adult razorback sucker to selenium in flooded bottomland sites affects their survival, growth, and reproductive success.

Adult razorback sucker (4 year old) that were used in the previous reproduction study were also used in the present study. After 66 days of selenium depuration at the end of the previous reproduction study, adults were restocked at three sites on July 11, 1996: hatchery ponds at Horsethief Canyon State Wildlife Area (Horsethief; reference site, assumed to have little or no selenium contamination), a diked tertiary channel at Adobe Creek (assumed to have a low amount of selenium contamination), and North Pond (assumed to have an elevated amount of selenium contamination) at Walter Walker State Wildlife Area (WWSWA), all near Grand Junction, CO. All the young adults were from a single mating. Older brood stock (about 15 years old) held at Horsethief were used as additional adult reference fish. Adults at Horsethief were fed a commercial diet but could forage for natural food organisms in the earthen ponds, whereas fish at Adobe Creek and North Pond foraged on natural food items. Fish were collected in April 1997 for spawning and initiation of an egg study and a larval fish study. The water source at Horsethief was the Colorado River, at Adobe Creek it was Colorado River water and irrigation drainage, and at North Pond it was ground water and irrigation supply water.

Sampling stations were established at Horsethief (4 stations), Adobe Creek (7 stations), North Pond (3 stations) at WWSWA, and the channel area at WWSWA (7 stations). Water quality measurements were collected on-site weekly. Samples collected at monthly intervals (sometimes at 60-day intervals) included water samples for water quality and inorganic element analyses, and aquatic invertebrates for inorganic element analyses. Fish were collected using nets and electrofishing equipment. Sediment samples were collected twice (October 1996 and April 1997) for selenium analyses.

Fish were collected in April 1997 from the three sites after the appropriate water temperatures were reached, and moved to tanks in a building at Horsethief. Fish were induced to spawn by injection with human chorionic gonadotropin hormone. Personnel of the Colorado River Fishery Project (CRFP) office, Grand Junction, CO, used standard methods to collect fish, induce spawning, and fertilize and water harden the eggs. Samples of eggs were collected for inorganic element analyses, and the remaining eggs moved to the 24-Road Fish Hatchery (24-Road) for hatching. After two spawnings, adults from the three sites were held at Horsethief for an 86-day selenium depuration period. Muscle plugs were collected from fish at stocking, spawning, and about 30-day intervals during depuration for selenium analysis.

An egg study was conducted in a mobile laboratory housed in the garage at the CRFP office. Eggs were collected from the three spawns of adults from Horsethief, Adobe Creek, North Pond, and two spawns from brood stock fish for hatchability and survival determinations. Due to nearly complete mortality of eggs after 2 days in the egg study, a second egg study was

started with eggs from the second spawning. In this study, eggs were used from three spawns of Horsethief, Adobe Creek, and North Pond fish, and one spawn of brood stock fish. Eggs from each spawn were stocked into six egg incubator cups (each containing 25 eggs) that were suspended in 2-L exposure beakers filled with 1.2 L of either reference water (24-Road, three replicates) or site water (three replicates). Fifty percent of the water was renewed daily. Once daily, the egg cups were removed and placed in a petri dish with the same water as in the beaker, and examined using a dissection scope to record the number of live and dead eggs, and the number and type of deformities. Egg viability (i.e., number of eggs that were alive and developing embryos) in the first spawning was measured on groups of about 100 eggs from three spawns of Horsethief, Adobe Creek, and North Pond fish and two brood stock fish. Egg viability in the second spawning was measured in three spawns of Horsethief, Adobe Creek, and North Pond fish. Egg diameter in both the first and second spawnings was measured on 20 eggs from three spawns of Horsethief, Adobe Creek, and North Pond fish, and one spawn of brood stock.

A 30-day larval fish study was initiated with 5-day-old larvae and was conducted in a mobile laboratory housed in the garage at the CRFP office. No larvae were available from spawns of adults from Horsethief, Adobe Creek, or North Pond. Larvae from brood stock were used in the study. Eight groups of 10 larvae were placed in 2-L exposure beakers to assess survival, growth, abnormal development, and whole-body residues of selenium. The beakers were filled with 1.6 L of water, and fifty percent of the water was renewed daily. One set of larvae in two beakers from each source were exposed to one of 16 treatments: (1) reference food (brine shrimp) and reference water (24-Road), (2) reference food and site water from Horsethief (HT), (3) reference food and sited water from Adobe Creek (AC), (4) reference food and site water from North Pond (WW), (5) Horsethief food (zooplankton collected from Horsethief east wetland, HTEW) and reference water, (6) HTEW food and HT water, (7) HTEW food and AC water, (8) HTEW food and WW water, (9) AC food and reference water, (10) AC food and HT water, (11) AC food and AC water, (12) AC food and WW water, (13) WW food and reference water, (14) WW food and HT water, (15) WW food and AC water, and (16) WW food and WW water.

Zooplankton were collected using modified light traps, sieved to separate zooplankton <0.425 mm, and counted for feeding 40 organisms per fish in each exposure beaker. The number of live fish were counted daily, and the weight and total length of fish measured after 30 days of exposure. Larvae were collected for selenium residue analysis after 10 and 30 days of exposure.

During the adult study, selenium concentrations at Horsethief were 1.4-3.0 µg/L in water and 0.8-0.9 µg/g in the top layer of sediment, at Adobe Creek were <0.7-4.5 µg/L in water, 1.2-2.5 µg/g in the top layer of sediment, and 16-20 µg/g in zooplankton, and at North Pond were 3.2-17 µg/L in water, 16-94 µg/g in the top layer of sediment, and 32-48 µg/g in zooplankton (Summary Table 1). Selenium was considered elevated in water and food organisms at Adobe Creek, and in these same aquatic components plus sediment at North Pond. Although there were differences among the three sites in concentrations of inorganic elements in water, sediment, and biota, selenium was the only element elevated to concentrations of concern for adverse effects in fish. Selenium concentrations in fish from Horsethief were 4.5 µg/g in muscle plug and 6.0 µg/g in eggs, from Adobe Creek were 9.6 µg/g in muscle plug and 40 µg/g in eggs, and from North Pond were 14 µg/g in muscle plug and 55 µg/g in eggs. Adult razorback sucker at Adobe Creek and North Pond accumulated selenium in muscle plugs and eggs to concentrations reported in

INTRODUCTION

The Colorado River basin originally contained 32 native species, of which 75% were endemic because of the basins long isolation (Figure 1) (Minckley 1991). 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 1987, 1994). A combined approach for recovery of the four endangered fish in the upper Colorado River basin has been undertaken in 1987 by the Upper Colorado River Endangered Fish Recovery Program (USFWS 1987). The goal of the 15-year program is to reestablish self-sustaining populations of the four species while allowing continued water development.

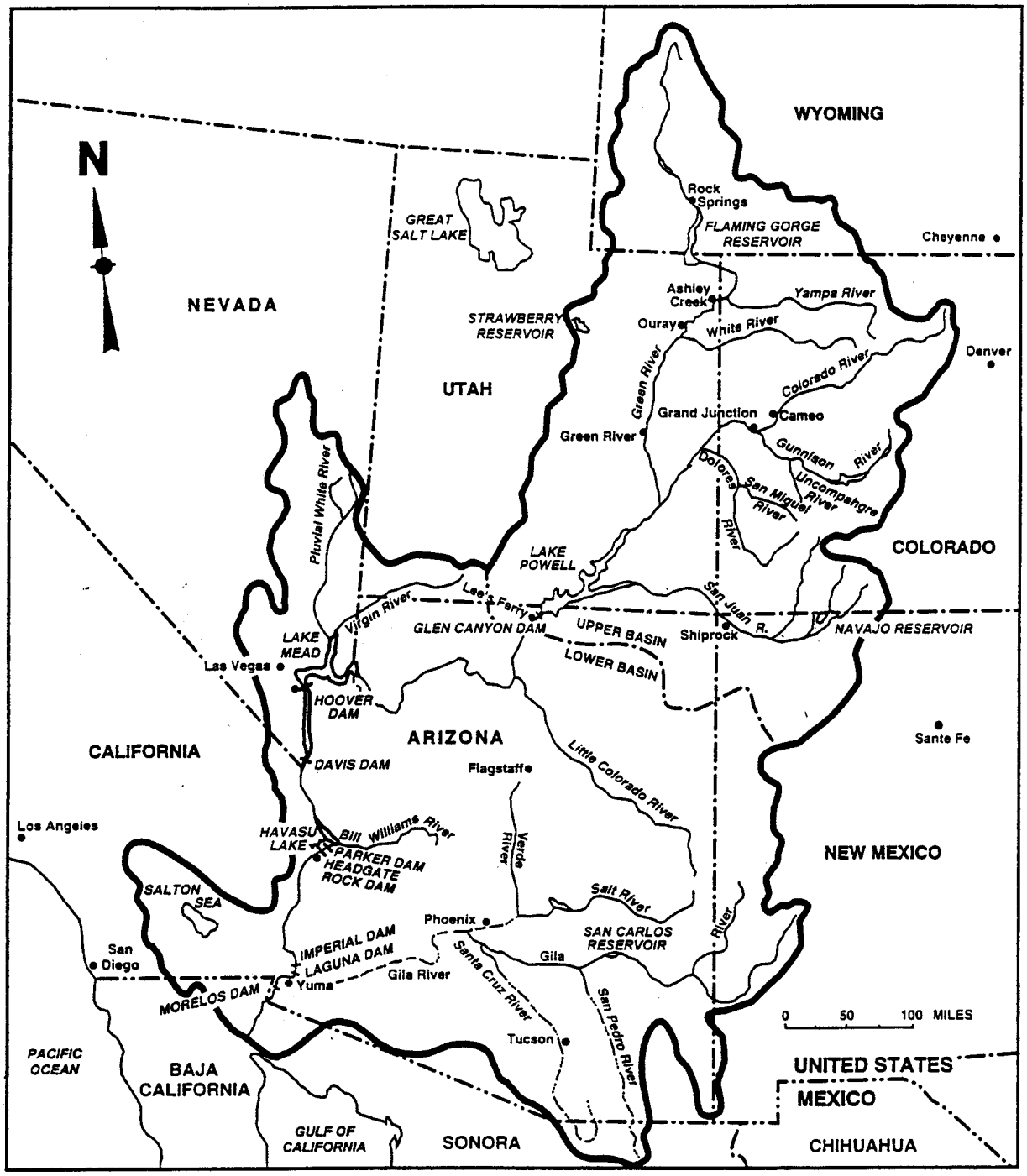
In an effort to stabilize and enhance populations of razorback sucker and other endangered fishes in the upper Colorado River, the Floodplain Habitat Restoration Program within the Recovery Program, has undertaken the task to restore floodplain habitats for use by razorback sucker larvae and adults. The proposed strategy for achieving these goals was to reconnect selected floodplain habitats to the main river channel in a manner that simulated historic hydrological conditions. An important component of this Program was to select sites, which after restoration, would not pose contaminant problems to the fish, especially from selenium.

The life history and status of the razorback sucker has been reviewed by Bestgen (1990). Briefly, the razorback sucker was considered common in the upper and lower Colorado River basins in historical times, but since the 1940's has become rare except for populations in the Green River and Lakes Mead and Mohave. Razorback suckers are generally thought to inhabit moderate to large streams and rivers and use a variety of habitats including low-velocity areas (backwaters, sloughs, oxbow lakes), near-shore runs, and shallow channels adjacent to, or over, mid-stream sandbars. The diet of razorback sucker varies depending on life stage, habitat, and food availability, and the diet of adults in rivers may be different than adults in reservoirs. Springtime congregations of razorback sucker have been found in off-channel impoundments and tributaries. Spawning behavior of razorback sucker is apparently influence by water temperature and flow (Tyus and Karp 1990), but Modde and Wick (1997) suggest that increases in discharge probably have a greater influence on initiating fish movement to spawning sites in late spring such as Razorback Bar in the Green River.

The remaining population of razorback sucker in the middle Green River basin in Utah has been estimated, using similar datasets, at about 1,000 individuals in 1988 (Lanigan and Tyus 1989) and at 300 to 600 in 1992 (Modde et al. 1996). Razorback sucker are rare in the upper Colorado River basin, where only 10 fish were found in the river between 1989 and 1996 (C. McAda, USFWS, personal communication).

Selenium contamination of the upper and lower Colorado River basins has been documented in water, sediment, and biota, in studies by the U.S. Department of the Interior agencies and academia (reviewed in Hamilton 1998). Historic selenium contamination of the upper and lower Colorado River basins prior to the construction of mainstem dams has been hypothesized to have contributed to the decline of native fish that are currently federally listed as endangered (Hamilton 1999). Other reports have suggested that endangered fish, especially razorback sucker, 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.

Figure 1. Map of Colorado River basin (modified from Carlson and Muth 1989).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UNITED STATES
MEXICO
PACIFIC OCEAN
GULF OF CALIFORNIA
GREAT SALT LAKE
STRAWBERRY RESERVOIR
FLAMING GORGE RESERVOIR
Rock Springs
Cheyenne
Denver
Ashley Creek
Yampa River
Ouray
White River
Green River
Colorado River
Grand Junction
Cameo
Gunnison River
Uncompahgre River
Dolores River
San Miguel River
Pluvial White River
LAKE POWELL
Lee's Ferry
Virgin River
GLEN CANYON DAM
UPPER BASIN
LOWER BASIN
Shiprock
San Juan R.
NAVAJO RESERVOIR
LAKE MEAD
Las Vegas
HOOVER DAM
DAVIS DAM
Flagstaff
Little Colorado River
Sante Fe
Los Angeles
HAVASU LAKE
Bill Williams River
PARKER DAM
HEADGATE ROCK DAM
Verde River
SALTON SEA
Salt River
Phoenix
SAN CARLOS RESERVOIR
San Diego
IMPERIAL DAM
LAGUNA DAM
Yuma
Gila River
Gila
Santa Cruz River
San Pedro River
MORELOS DAM
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2000).

The current study was undertaken to repeat a previous reproductive study with adult razorback sucker at the same three sites near Grand Junction, CO (Hamilton et al. 2001). Results of that study revealed unexpected early mortality in razorback sucker larvae fed zooplankton from Horsethief east wetland. That wetland was assumed to be uncontaminated by selenium because it was located on the west side of the Colorado River, up gradient from irrigated agriculture, and received only Colorado River water. However, analysis of selenium residues in zooplankton and larvae several months after the initiation of the current study disclosed selenium concentrations, but not concentrations of other inorganic elements, were elevated sufficiently in zooplankton to cause adverse effects in early life stages of fish. Selenium residues in the larvae also were elevated to the range where adverse effects have been observed in young fish (4-5 µg/g based on Hilton et al. 1980, Hamilton et al. 1986, Hunn et al. 1987, Ogle and Knight 1989, Hamilton and Wiedmeyer 1990, Hamilton et al. 1990, Cleveland et al. 1993, Lemly 1993b, Hamilton 2001). A hazard assessment of selenium concentrations at the three sites indicated a high hazard at the Adobe Creek and North Pond study sites and a moderate hazard at the Horsethief site, i.e., adverse effects on reproductive success, to razorback sucker.

The present study was conducted to derive the necessary toxicological information for assessing the suitability of selected flooded bottomlands as habitat for razorback sucker. The study was conducted at three sites near Grand Junction, CO, in the general area where razorback sucker had historically been observed, i.e., the gravel pit at what is now Walter Walker State Wildlife Area, or are currently held in hatchery ponds for propagation purposes, i.e., Horsethief Canyon State Wildlife Area. Although wild fish are free to move 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 an exposure to selenium and other inorganic elements. After exposure, adults were hand spawned in late spring when water temperatures reached a level associated with naturally spawning of razorback sucker. Following spawning, tests were conducted with eggs and larvae.

Objective

The objective of this study was to determine if environmental exposure of adult razorback sucker to selenium and other inorganic elements in flooded bottomland sites affects their survival, growth, and reproductive success.

ADULT STUDY

This section of the report will describe the methods, results, and discussion of the adult razorback sucker portion of the reproduction study.

Methods

The 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 near Grand Junction, CO: Horsethief Canyon State Wildlife Area (reference site; hereafter referred to as Horsethief), Adobe Creek, and North Pond at Walter Walker State Wildlife Area (WWSWA) (Figure 2). The Horsethief facility is operated by the Colorado River Fishery Project (CRFP), Grand Junction, CO. The Horsethief site was located about 19 km (12 miles) west of the Grand Junction city limits, the Adobe Creek site was located about 5 km (3 miles) west of the Grand Junction city limits, and the North Pond site was located about a half km to the southwest of the Grand Junction city limits.

Razorback sucker (4-year-old) previously used in a reproductive study in Grand Junction, CO, (Hamilton et al. 2001) were used in this study. The fish were originally reared at Wahweap Fish Facility, Big Water, UT, by the Utah Division of Wildlife Resources. Parents of these fish were from the San Juan River arm of Lake Powell, UT. All the fish used were progeny of a single spawn of two adult razorback sucker (female: PIT 7F7F19036C, and male: PIT 7F7D055802). An additional 36 were transferred from Wahweap to Horsethief on July 27, 1995, and an additional 26 transferred on September 8, 1995. Fish were held at Horsethief from the last week of April 1996 until July 11, 1996, prior to use in this study.

Site description

The same set of sampling stations was used in this study as previously established at each site for the first study (Hamilton et al. 2001): Horsethief (HTi [inlet] and HTo [outlet]), Adobe Creek (AC1 through AC7), and WWSWA (WW1 through WW10). At Horsethief, fish were held in earthen ponds, either pond 1 or 6, along with brood stock of other endangered fish stock (Figure 3). The water in the ponds was maintained by water pumped directly from the Colorado River near Fruita, CO, and was believed to have little or no selenium contamination. The Adobe Creek site was a tertiary river channel about 200 m long and 3 to 5 m wide and was isolated from river flow by dikes at both ends. Both dikes have large gate valves and the downstream dike also had an overflow water control structure (Figure 4). Fish were held in the section of the channel with 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 about 1.5 m deep, and was believed to have relatively low levels of selenium contamination. The North Pond site was an isolated pond about 1 ha (2.5 acre) in size with a maximum depth of 1.5 m located on a terrace about 2 m above the floodplain (Figure 5). Water in North Pond was supplied primarily by ground water 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 with sample stations WW2 and WW3.

Figure 2. Map of three sites located in the Grand Valley near Grand Junction, Colorado, used for a reproduction study with razorback sucker.

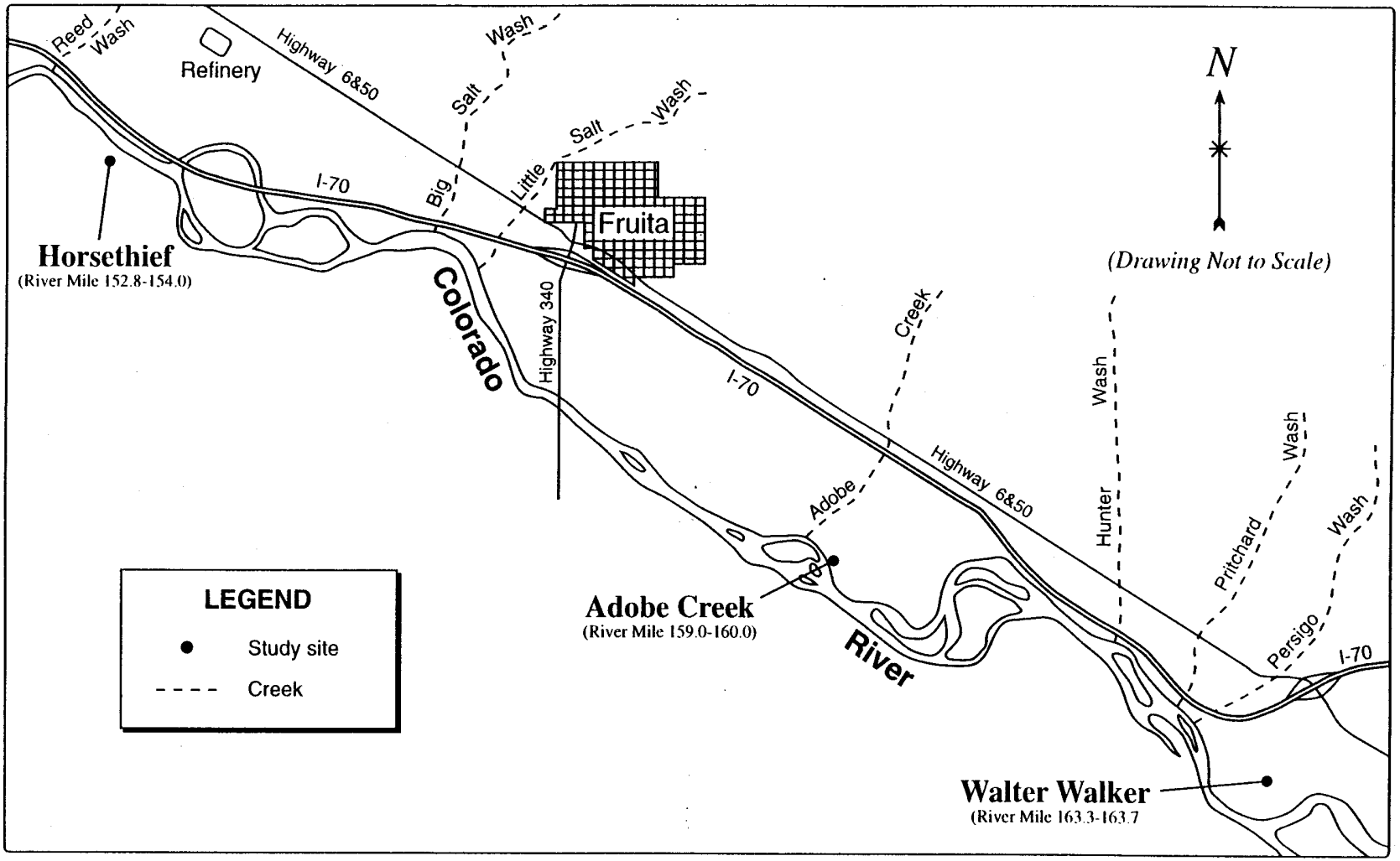
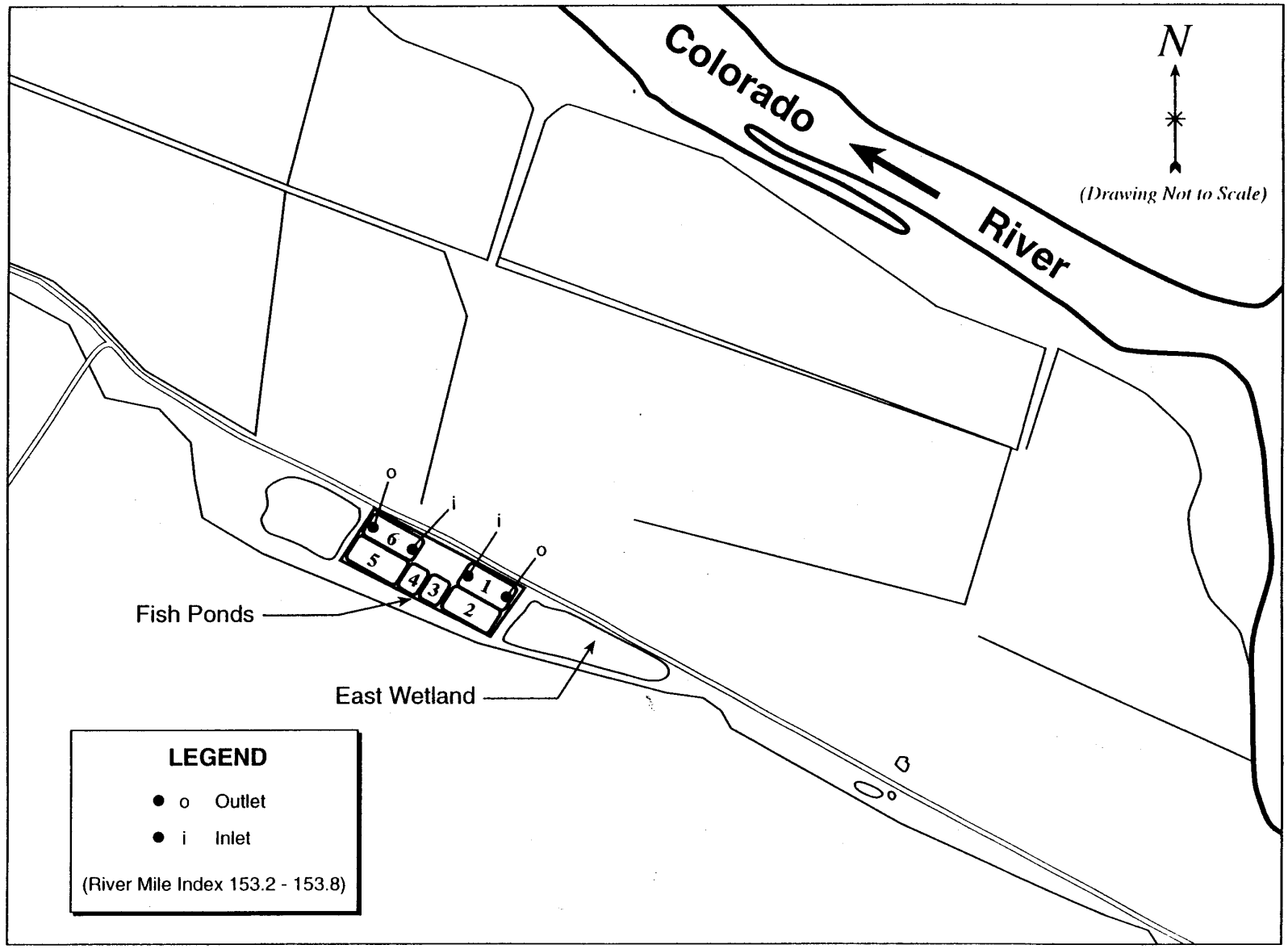

Reed Wash
Refinery
Highway 6&50
Salt Wash
Salt Wash
Big
Little
I-70
Fruita
Horsethief
(River Mile 152.8-154.0)
Colorado
Highway 340
I-70
Creek
Adobe
Highway 6&50
Wash
Hunter
Pritchard
Wash
Wash
Persigo
I-70
Adobe Creek
(River Mile 159.0-160.0)
River
Walter Walker
(River Mile 163.3-163.7
N
(Drawing Not to Scale)
LEGEND
Study site
Creek

Figure 3. Map of ponds and east wetland at Horsethief Canyon State Wildlife Area near Grand Junction, Colorado.


Colorado
River
N
(Drawing Not to Scale)
o
i
i
o
6
5
4
3
1
2
Fish Ponds
East Wetland
LEGEND
o Outlet
i Inlet
(River Mile Index 153.2 - 153.8)

9

Figure 4. Map of sampling stations at the Adobe Creek site near Grand Junction, Colorado. Fish were held in the diked area with sampling stations AC3, AC4, and AC5.

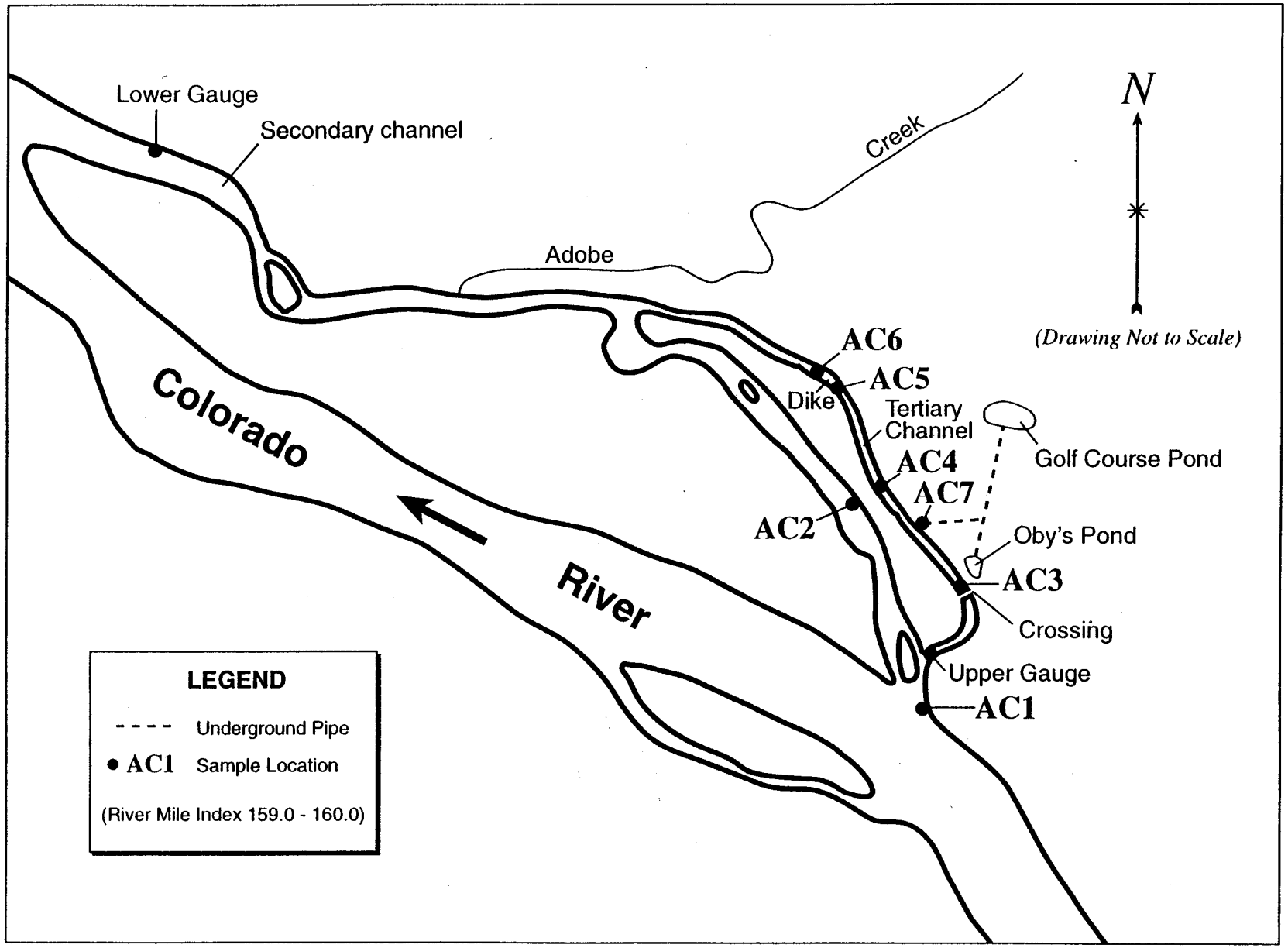
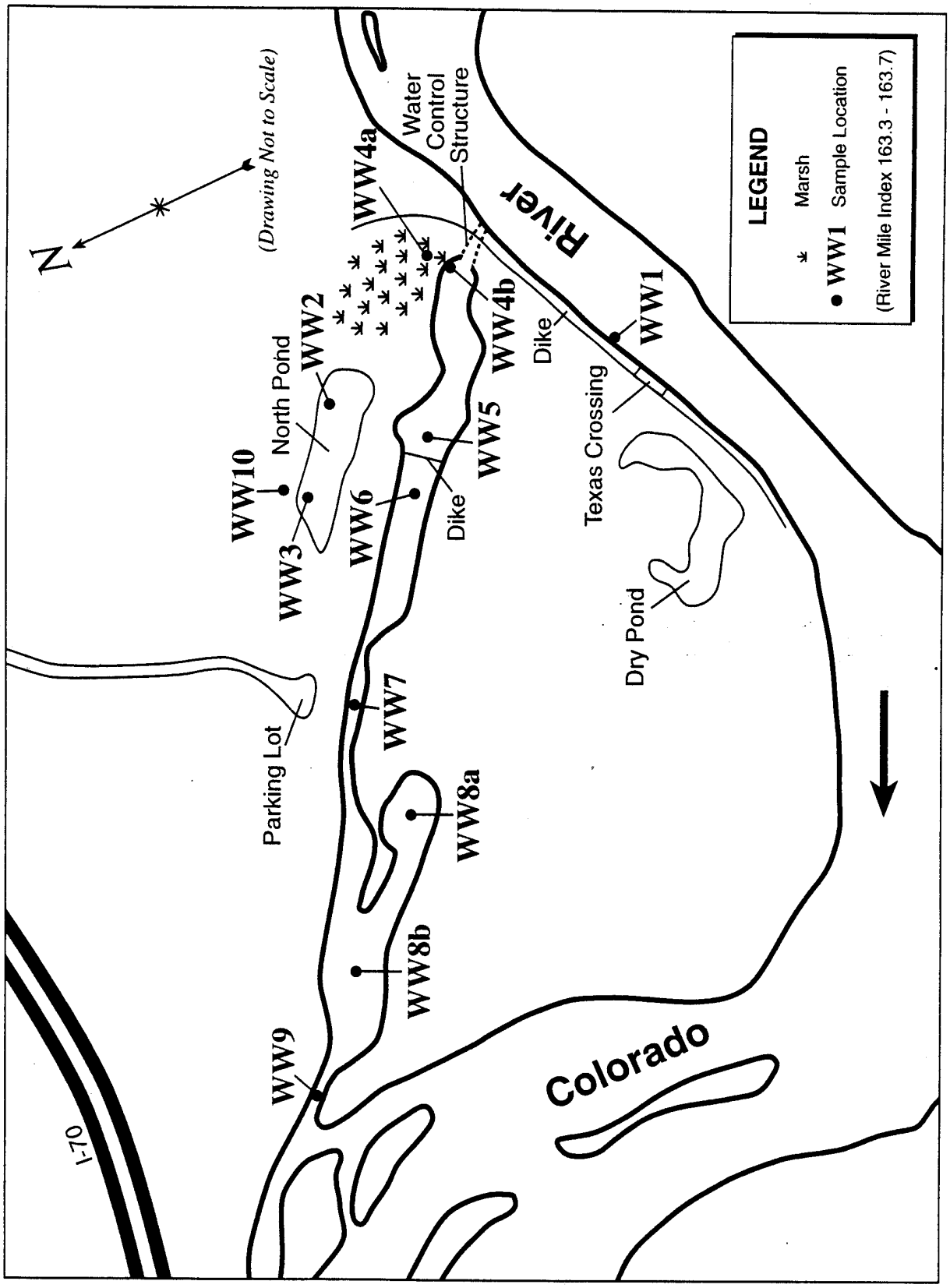

Lower Gauge
Secondary channel
Adobe
Creek
N
(Drawing Not to Scale)
AC6
AC5
Dike
Tertiary
Channel
AC4
AC7
Golf Course Pond
Oby's Pond
AC3
Crossing
Upper Gauge
AC1
AC2
Colorado
River
LEGEND
Underground Pipe
AC1 Sample Location
(River Mile Index 159.0 - 160.0)

Figure 5. Map of sampling stations at the Walter Walker State Wildlife Area site near Grand Junction, Colorado. Fish were held in North Pond with sampling stations WW2 and WW3.


N
(Drawing Not to Scale)
LEGEND
Marsh
WW1 Sample Location
(River Mile Index 163.3 - 163.7)
Water Control Structure
WW4a
WW4b
WW2
WW1
WW10
WW3
WW6
WW5
WW7
WW8a
WW8b
WW9
North Pond
Dike
Dike
Texas Crossing
Dry Pond
Parking Lot
River
Colorado
I-70

Water levels were supplemented by inflow at WW10 from Independent Ranchman's Ditch. Sampling station WW4 from the previous study was designated WW4b; a new station WW4a was established at the outfall of the marsh; WW8 from the previous study was designated WW8a; and a new station WW8b was established (Figure 4). 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 (upper Colorado River, Etter Pond adjacent to the Colorado River, the Colorado River arm of Lake Powell, and the San Juan River arm of Lake Powell) and held at Horsethief were also used in the study as additional reference fish that supplied larvae for the larval fish study.

Staff gages were installed at WW9, and near AC1 and AC6 to monitor water elevation. Gage heights were recorded daily at WW9 from June 17 to August 3, 1996, and from November 26, 1996, to June 16, 1997, at AC1 from June 17 to August 20, 1996, and at AC6 from June 17 to October 16, 1996.

Fish stocking and sampling

Adults were stocked at each site by personnel of the CRFP 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] and 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] and 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 (D. Ryden, USFWS, CRFP, Grand Junction, CO, transported the fish from Wahweap State Fish Hatchery, Big Water, UT, to Grand Junction, CO).

Fish were maintained at each site and not captured until they 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 total length and weight, and a muscle plug sample taken from the dorsal area adjacent to the dorsal fin for 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 analysis of selenium concentrations, and shipped on dry ice when transported to the sample preparation laboratory. 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 sucker routinely maintained there. Fish at Adobe Creek and North Pond were not feed 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 by atomic absorption spectrophotometry. Collections were accomplished primarily using 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. The combined samples were then backwashed 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 and preserved in 70% ethanol. This sample was used to make semi-quantitative estimates of species composition at each station.

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 of 1997 after water temperature rose to 16°C (~60°F) and remained there for about a week (third week of April), fish held at Adobe Creek and North Pond were captured by personnel of the CRFP using trap (fyke) 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 1.3 m-diameter tanks in the holding building supplied with flowing Colorado River water supplemented with oxygen delivered through diffuser bars. Water temperature in the holding tanks was about 12°C (~54°F). Muscle plugs were taken from fish for selenium analysis. Muscle plugs were also taken 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 kg of body weight on each of 3 consecutive days, beginning 5 days prior to 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 per kg one time at 5 days prior to spawning. After inspection for spawning characteristics (i.e., tubercles present and milt running in males, coloration, and plumpness of females), three females from Horsethief, four from Adobe Creek, and five 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 Colorado River water.

Eggs were transported to 24-Road Fish Hatchery (hereafter termed 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 Fish Hatchery water (hereafter termed 24-Road water). Water temperature at 24-Road was maintained at about 24°C (~75°F) 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 and trace element and major ion analyses by inductively-coupled argon 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 total length and weight, inspected for general health, and muscle plug taken for selenium analysis.

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 (Figures 3, 4, 5). In addition, site water was collected every 30 days at three sample stations where the fish were held and analyzed for general water quality characteristics in the mobile laboratory. Water quality measured in situ at each site included pH, conductivity, salinity, air temperature, water temperature, and dissolved oxygen. Water quality measurements in unfiltered water samples measured in 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 <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 then shipped in a cooler with ice packs by overnight express to the Yankton Field Research Station (FRS), SD, 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 and following the procedures for low concentration measurements of the electrode manufacturer (Orion 1990, 1991; ATI Orion 1994). Chloride was measured by a modification of 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) where adults were held for ICP analysis of inorganic elements. Samples for selenium analysis were collected monthly and those for ICP were collected bimonthly. Filtered and unfiltered water was collected for selenium analysis. Water was filtered through a 0.4 µm 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 ml 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, 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 apparatus had a removable cap that was placed on top of the pipe to hold the halves together. The removable cap had a small hole in it through which overlaying water escaped from the pipe as it was inserted in to the sediment. After pipe insertion, a rubber stopper was placed in the cap hole to create a vacuum in the pipe during removal of the pipe from the sediment so as to maintain the integrity of the sample during the removal process. After removal, the top and bottom pipe ends were capped. The cores were immediately frozen to maintain the longitudinal integrity of the sample, and 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, SD, 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 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 mean limit of detection (LOD) was 0.7 µg/L (SE 0.1, n=17). The procedure blanks had background concentrations less than the LOD, which indicated no contamination from reagents or sample handling. The mean percent relative standard deviation (triplicate sample preparation and analysis) was 4.3% (SE 0.6, n=17), 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 Columbia Environmental Research Center (CERC) recommended ranges, which indicated the digestion and analysis procedure accurately measured selenium concentrations. The mean percent recoveries of digested-spiked sample solutions was 95% (SE 2, n=34), which indicated 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. Mean selenium recoveries of analysis-spiked samples analyzed for matrix suppression or enhancement was 101% (SE 2, n=17), which indicated no interference from other water components.

All sediment, fish egg, zooplankton, brine shrimp, 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. 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 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 assurance/quality control measures were the same as for water analyses, and the results are summarized in Table 1.

Analyses of inorganic elements in water, brine shrimp, zooplankton, and fish egg samples were performed by ICP at the Environmental Trace Substances Research Center (University of Missouri), Rolla, MO. 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 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 in 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

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	Fish egg	Aquatic invertebrates and brine shrimp
Limit of detection (μg/g)	0.14 (0.03) [6]	0.04 (0.01) [4]	0.10 (0.04) [7]
% RSD[1]	4.2 (0.4) [6]	4.8 (0.5) [4]	4.7 (1.1) [7]
Reference material	0.38[2] (0.01) [6]	1.26[3] (0.03) [4]	1.41[3] (0.03) [7]
Digested spikes[4]	100 (2) [12]	99 (3) [8]	100 (2) [14]
Analysis spikes[5]	95 (5) [6]	105 (7) [4]	99 (4) [7]

[1]RSD: Percent relative standard deviation for triplicate preparation and analysis.
[2]National Research Council of Canada (NRCC) reference material BCSS-1 (marine sediment; 0.43 ± 0.06 [SD] μg/g).
[3]NRCC reference material DORM-2 (dogfish muscle tissue; 1.40 ± 0.09 [SD] μg/g).
[4]% recovery of selenium from samples spiked with selenomethionine at the beginning of preparation for sample analysis.
[5]% recovery of selenium from digested samples spiked with selenite after sample preparation but before instrument analysis.

Table 2. 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).

Element	Matrix		
	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

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 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 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 and larvae because of the small sample mass. All sample preparation prior to neutron activation analyses and the neutron activation method 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 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 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 µg/g. Multiple muscle plugs from the same fish were not taken so no other quality assurance measures were evaluated.

Statistics

Data were analyzed using computer programs from Statistical Analysis System Institute, Inc (SAS 1990). Analysis of variance testing was used to determine treatment effects on percent survival (arcsine transformed values) and growth of razorback sucker larvae, and 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 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 if transformations were needed to meet the assumptions of normality and homogeneity of variance (M. Ellersieck, University of Missouri, Columbia, personal communication). The residue data for water, sediment, zooplankton, and muscle plugs, and water quality measures in the egg and larval fish studies were log10 transformed prior to correlation analysis.

Multiple regression analyses were used to test for relations among sediment characteristics and selenium concentrations in sediment, and among selenium concentrations in larvae, water, and zooplankton, and larval survival.

The predicted time-to-death and 95% confidence intervals were calculated for each test group of larvae and treatment using S-Plus statistical software (Mathsolf, Seattle, WA). The parametric proportional hazard regression model and Weibull distribution were used in the analysis. Both right and interval censoring of survival time data was accounted for in the statistical methodology. The predicted percent time-to-death was calculated for the 50%, 90%, and 95% levels.

Results

Elevated river runoff occurred in June 23, 1996, about 2 weeks prior to fish stocking on July 11, 1996. Snow melt runoff in the Colorado River began in mid-March, 1997, increased in mid-April and increased again on May 7 (Figure 6, Appendix A). Staff gage height at WW9 increased from a low of 0.21 m prior to August 3, 1996, to 2.31 m on June 3, 1997. Limited staff gage height information at Adobe Creek revealed similar trends. No river flooding occurred at North Pond or Adobe Creek during the study. However, North Pond received water from Independent Ranchman's Ditch, and Adobe Creek received water from the secondary river channel when adults were at these sites (Appendix B).

Water quality

Water quality characteristics were significantly different at the three sites with North Pond having higher conductivity, hardness, magnesium, chloride, and sulfate than at Horsethief and Adobe Creek (Table 3). Horsethief had significantly higher pH than Adobe Creek or North Pond.

Water quality, characterized primarily by conductivity, varied over time at all the sites between July 1, 1996, and May 9, 1997 (Appendix C). This variation at Horsethief and Adobe Creek was partly due to the Colorado River during high runoff, which lowered conductivity values compared to low flow periods. Water quality parameters at Horsethief matched closely with those in the Colorado River at stations WW1 and AC1. Conductivity was lowest in North Pond at WW2 on October 16, 1996, which was about 3 months after high river runoff.

Water quality characteristics in samples measured in the mobile laboratory, such as hardness and alkalinity, followed changes in conductivity, being lowest during runoff and highest during low flow periods (Appendix D). During the period when adults were held at Horsethief, the range of values were 593-1,060 µmhos/cm conductivity, 208-406 mg/L as $CaCO_3$ hardness, and 106-161 mg/L as $CaCO_3$ alkalinity. The range of other water quality measures was also narrow, i.e., chloride, nitrate, nitrite, solids, and sulfate (Appendix D).

Sample stations at the Adobe Creek site where the adults were held received mostly ground water from the river, but also inputs of irrigation water at station AC7 and pumped river water from station AC2 (Appendix B). River water from the secondary channel (AC2) was used to maintain water levels in the area where the adult fish were held, and water was pumped from November 1996 to May 1997. Flows into the adult holding area from AC7 were unexpected, but were monitored when flow was observed. Water quality characteristics at AC5 were similar to those at HT and the Colorado River because of pumping water from AC2 and limited irrigation flows from AC7. During the period when adults were held at Adobe Creek, the range of values were 786-1,260 µmhos/cm conductivity, 260-366 mg/L as $CaCO_3$ hardness, and 98-150 mg/L as $CaCO_3$ alkalinity (Appendix D).

Figure 6. Flow (lps) of the Colorado River (Crowfoot et al. 1996, 1997) and staff gage height (m) at Walter Walker State Wildlife Area and Adobe Creek.

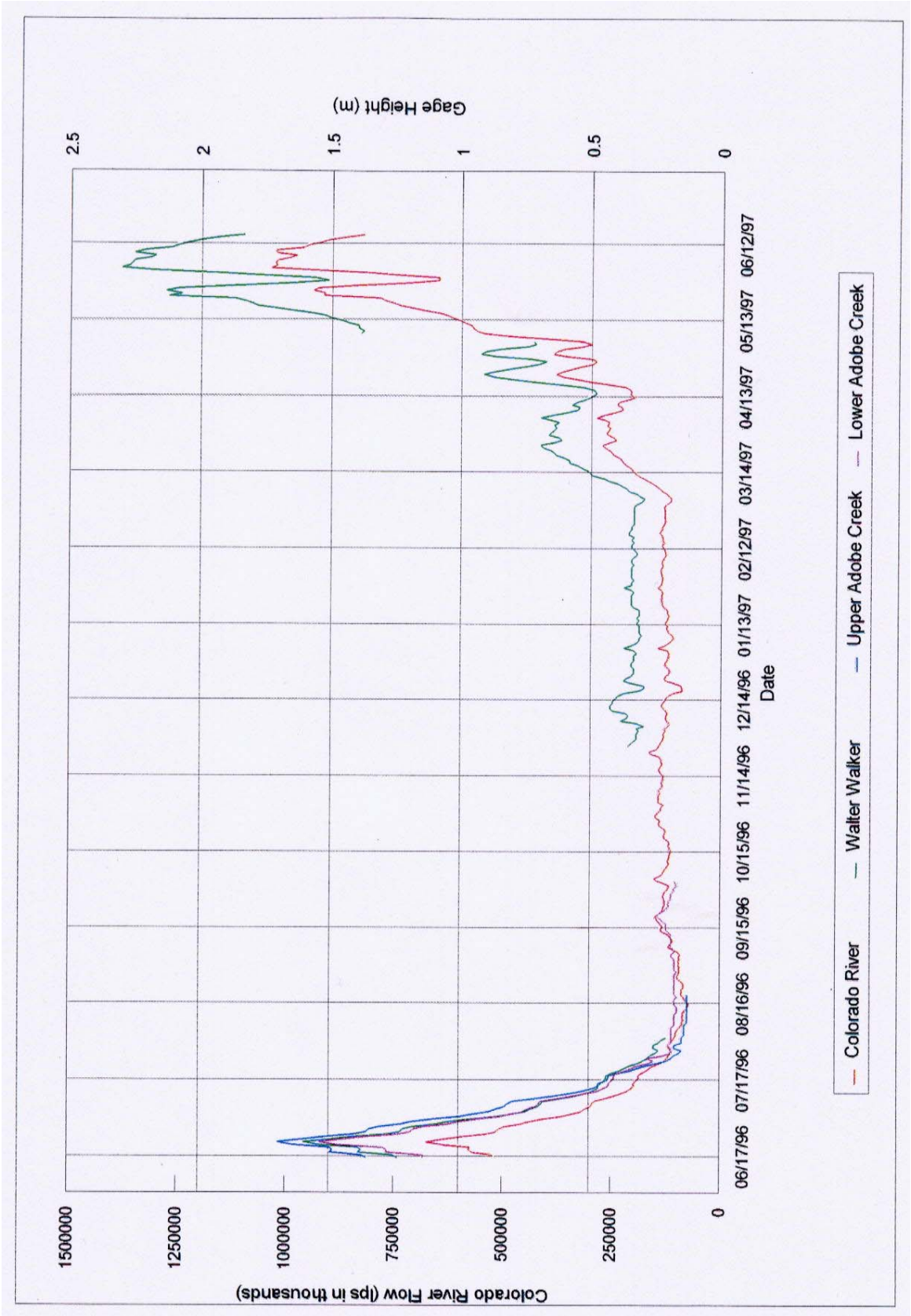

Colorado River Flow (cfs in thousands)
1500000
1250000
1000000
750000
500000
250000
0
Gage Height (m)
2.5
2
1.5
1
0.5
0
06/17/96
07/17/96
08/16/96
09/15/96
10/15/96
11/14/96
12/14/96
01/13/97
02/12/97
03/14/97
04/13/97
05/13/97
06/12/97
Date
Colorado River
Walter Walker
Upper Adobe Creek
Lower Adobe Creek

Table 3. Mean (standard error in parentheses, n=9) water quality characteristics measured in water collected at three stations near Grand Junction, Colorado. For each measure, stations with the same letter are not significantly different (*P*=0.05).

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

The stations at North Pond where adults were held (WW2 and WW3) received mostly upslope ground water from a cobble aquifer (Phillips 1986) and inputs of irrigation supply water from Independent Ranchman's Ditch via WW10. Flows from WW10 were used to maintain water levels in the area where the adult fish were held and occurred from June to October 1996 and in April 1997 (Appendix B). Water quality characteristics were more variable at WW2 than at the stations at Horsethief or Adobe Creek because of the mixture of upslope ground water, and irrigation flows (Appendix D). During the period when adults were held at North Pond, the range of values were 1,240-4,630 µmhos/cm conductivity, 352-1,460 mg/L as $CaCO_3$ hardness, and 87-343 mg/L as $CaCO_3$ alkalinity. There was a 6-fold variation in the concentrations of magnesium (37-217 mg/L) and sulfate (312-2,000 mg/L), which were greater than the 4-fold variation in values for conductivity and hardness. Surface irrigation water inflow from WW10 diluted some water quality characteristics (conductivity, hardness, magnesium, chloride, nitrate) at North Pond between August and December 1996 compared to conditions in January-April 1997.

Selenium and other elements in water

There was no significant difference 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. Selenium concentrations in water at Horsethief ranged from 1.4 to 3.0 µg/L between December 1996 and April 1997 (Table 4). These values were similar to those in Colorado River samples collected at Walter Walker State Wildlife Area (WW1; 1.6-4.4 µg/L).

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. During the period when adults were present, selenium concentrations at Horsethief averaged 2.2 µg/L (range 1.4-3.0), at Adobe Creek averaged 2.6 µg/L (range <0.7-4.5), and at North Pond averaged 7.8 µg/L (range 3.2-17.1).

The highest selenium concentrations at North Pond occurred on April 15, 1997, (15.6 µg/L) just prior to removing the adults for spawning. Most selenium in North Pond probably came from the ground water as demonstrated by the elevated selenium concentrations at WW4a (adjacent and east of North Pond), whose only water source was ground water. When the Colorado River was at low flow, selenium concentrations at WW4a between December 1996 and April 1997 ranged from 84 to 152 µg/L (Table 4).

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 5, Appendix E). Selenium concentrations measured by AA-HG in water from Horsethief, Adobe Creek, or 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 measured by AA-HG 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 4. 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 sucker 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[1]	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

[1]<: Below limit of detection.

Table 5. 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. For B, Li, Mg, and Sr, mean station values with letters in common are not significantly different from each other (*P*=0.05).

Element	Station		
	HT1	AC5	WW2
Ag	<0.01[1]	<0.01	<0.01
Al	0.03 (0) [3][2]	<0.02	0.02 (-) [1]
As	<0.03	<0.03	0.03 (-) [1]
B	0.065ab (0.008) [5]	0.049a (0.002) [5]	0.110b (0.027) [5]
Ba	0.098 (0.006) [5]	0.115 (0.009) [5]	0.107 (0.007) [5]
Be	<0.0002	0.0003 (-) [1]	<0.0002
Bi	0.02 (0) [5]	0.02 (0) [5]	0.03 (0.01) [3]
Cd	<0.002	<0.002	<0.002
Co	<0.001	<0.001	<0.001
Cr	0.010 (0) [4]	<0.009	0.010 (-) [1]

Table 5. Continued.

Element	Station		
	HT1	AC5	WW2
Cu	0.020 (0.008) [5]	<0.001	<0.001
Fe	0.023 (0.004) [5]	0.045 (0.021) [5]	0.028 (0.003) [5]
Li	0.031ab (0.004) [5]	0.027a (0.002) [5]	0.049b (0.009) [5]
Mg	23a (3) [5]	26a (1) [5]	90b (33) [5]
Mn	0.013 (0.003) [5]	0.030 (0.008) [5]	0.024 (0.006) [5]
Mo	<0.007	0.010 (0) [2]	0.009 (0.001) [3]
Ni	<0.007	0.007 (0) [3]	<0.007
Pb	<0.03	<0.03	<0.03
Sb	<0.03	<0.03	<0.03
Si	0.74 (0.16) [5]	0.64 (0.16) [5]	0.45 (0.11) [5]
Sn	<0.03	<0.03	<0.03

Table 5. Continued.

Element	Station		
	HT1	AC5	WW2
Sr	0.75a (0.10) [5]	0.78ab (0.06) [5]	1.49b (0.32) [5]
Ti	0.0011 (0.0003) [3]	0.0010 (0.0002) [4]	0.0010 (0) [4]
Tl	0.07 (-) [1]	<0.07	0.09 (-) [1]
V	<0.002	0.003 (-) [1]	0.004 (0.001) [2]
Zn	0.005 (0.001) [4]	0.010 (0.002) [5]	0.009 (0.002) [5]

[1]<: Below limit of detection.

[2]The number of samples submitted for analysis was five for each station. If the number of samples shown for a station and element is less than five, concentrations in the other samples were below the limit of detection

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 6). Selenium concentrations in the top portion of sediment cores collected on October 21, 1996, were lowest at Horsethief and highest at North Pond, with Horsethief east wetland and Adobe Creek having intermediate concentrations. A similar pattern in selenium concentrations was observed for the top portion of sediment cores collected on April 14, 1997, from Horsethief east wetland, Adobe Creek, and North Pond. In general, selenium concentrations in sediment cores tended to decrease with depth.

Selenium in biota

Selenium concentrations in zooplankton samples from Adobe Creek ranged from 15.6 to 19.6 µg/g at AC3 and was 20.0 µg/g at AC5 (Table 7). 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 North Pond. Selenium concentrations in zooplankton collected from Horsethief east wetland, which received effluent from the ponds used to hold the adult razorback sucker, ranged from 4.4 to 5.6 µg/g. However, fish in the ponds did not have access to this food source, but this site was used as a food source in the larval fish study.

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 Horsethief east wetland 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 1.4 to 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), which suggested a high degree of interconnectedness in the food web. The Spearman correlation (r_s) between selenium concentrations in sediment (assuming a non-normal 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).

Table 6. Mean (standard error in parentheses, n=3) selenium concentration (µg/g dry weight) in the top, middle, and bottom of sediment cores collected from four stations near Grand Junction, Colorado. For each date and core section, stations with the same letter are not significantly different (*P*=0.05).

Date	Day of exposure	Core section	Station			
			HT1	HTEW[1]	AC3	WW2
10/21/96	102	Top	0.42a (0.03)	0.83b (0.03)	1.21c (0.15)	94.37d (2.25)
		Middle	<0.27[2]	<0.27	<0.27	1.55 (0.02)
		Bottom	<0.27	0.30a (0.02)	0.37a (0.07)	2.91b (0.07)
4/14/97	277	Top	-[3]	0.91a (0.02)	2.52b (0.07)	16.00c (0.38)
		Middle	-	0.33a (0.01)	0.50b (0.02)	12.67c (0.05)
		Bottom	-	0.14a (0.01)	0.57b (0)	1.79c (0.16)

[1]HTEW: Horsethief east wetland.
[2]<: Less than the limit of detection for that analysis.
[3]-: Not sampled.

Table 7. Concentration of selenium (µg/g dry weight) in zooplankton and chironomids collected from four stations near Grand Junction, Colorado. Chironomid samples were composited before analysis.

			Station			
Organism	Collection date	Day of exposure	HTEW	AC3	AC5	WW2
Zooplankton	09/20/96	71	4.4	-[1]	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	-	-	-

[1]-: Not sampled.

Growth

There was no significant difference in total 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 8). At spawning, fish were significantly shorter at Adobe Creek (431 mm) and weighed less (944 g) than fish at Horsethief (443 mm, 1,036 g). Fish at Horsethief grew 4.7% in total length and gained 26.8% in weight, at Adobe Creek grew 1.2% in total length and gained 15.1% in weight, and at North Pond grew 2.5% in total 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 total length or weight at stocking or at spawning. These fish at Horsethief grew 8.1% in total length and gained 37.8% in weight, at Adobe Creek grew 1.9% in total length and gained 8.2% in weight, and at North Pond grew 3.6% in total length and gained 7.0% in weight during the 239-day exposure period.

For the fish measured at spawning, 30, 60, and 90 days during the depuration phase, there were no significant differences in fish total length or weight among the three sites compared to measurements made at spawning (Table 8). 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 9). 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 to 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. Following initial stocking at Adobe Creek, these fish were exposed for 305 days, spawned, held for 66 days of depuration at Horsethief, then restocked at Adobe Creek for 286 days in the present study. 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. Following initial stocking at North Pond, these fish were exposed for 305 days, spawned, held for 66 days of depuration at Horsethief, then restocked at North Pond for 286 days in the present study.

One fish (HT66) stocked at Adobe Creek in the present study had previously been held at Horsethief for 11 months. 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 (Appendix F). The muscle plug

Table 8. 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. For each day of exposure and measure, sites with the same letter are not significantly different (*P*=0.05).

Measure	Day of exposure	Date	Site		
			HT	AC	WW
Total length	0	7/11/96	423a (3) [29]	426a (3) [25]	432a (4) [21]
	0[1]	8/27/96	369 (11) [10]	367 (5) [10]	361 (8) [10]
	286	4/23/97	443b (3) [29]	431a (3) [25]	443ab (4) [16]
	239[1]	4/23/97	399 (12) [6]	374 (8) [6]	374 (7) [4]
	Depuration[2]				
	286	4/23/97	451 (3) [6]	446 (7) [4]	454 (8) [5]
	316	5/23/97	467 (4) [6]	465 (8) [4]	474 (10) [4]
	344	6/20/97	456 (4) [5]	459 (9) [4]	467 (10) [4]
	371	7/17/97	456 (3) [6]	462 (13) [3]	466 (10) [4]

Table 8. Continued.

Measure	Day of exposure	Date	Site HT	Site AC	Site WW
Weight	0	7/11/96	817b (17) [29]	820b (18) [25]	745a (19) [21]
	0[1]	8/27/96	547 (45) [10]	512 (19) [10]	511 (36) [10]
	286	4/23/97	1036b (22) [29]	944a (21) [25]	1006ab (23) [16]
	239[1]	4/23/97	754 (80) [6]	554 (45) [6]	547 (51) [4]
	Depuration[2]				
	286	4/23/97	1052 (70) [6]	1013 (43) [4]	1055 (55) [5]
	316	5/23/97	1056 (50) [6]	1014 (52) [4]	1049 (67) [4]
	344	6/20/97	1055 (9) [5]	997 (54) [4]	1050 (66) [4]
	371	7/17/97	995 (41) [6]	974 (65) [3]	1026 (80) [4]

[1]Measures for 10 fish stocked on August 27, 1996 (day 47 of exposure period).
[2]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 9. Mean (standard error in parentheses and number of samples in brackets) selenium concentration (µg/g dry weight) in muscle plugs of razorback sucker held at three sites near Grand Junction, Colorado. For each day of exposure, sites with the same letter are not significantly different (P = 0.05).

Day of exposure	Date	Site		
		HT	AC	WW
0[1]	7/11/96	4.5a (0.4) [2]	9.6ab (0.6) [2]	14.2b (2.3) [2]
Depuration[2] 286	4/24/97	4.6a (0.3) [6]	16.2b (0.8) [6]	22.8c (1.8) [6]
316	5/23/97	5.0a (0.3) [4]	17.5b (2.1) [4]	20.0b (1.5) [4]
344	6/20/97	4.9a (0.2) [4]	15.0b (1.2) [4]	19.8b (2.9) [4]
371	7/17/97	5.0a (0.5) [4]	14.0b (1.7) [3]	18.0b (1.2) [4]

[1]Day 0 data from Hamilton et al. (2001).
[2]Day 286 was spawning, day 316 was 31 days depuration, day 344 was 59 days depuration, and day 371 was 86 days depuration.

selenium values for this fish during the depuration phase were also the 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 9 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 (Appendix F). The muscle plug selenium value for 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 9 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 were measured in muscle plugs from four older brood stock fish held at Horsethief (Appendix F). Three of these fish had selenium measured in muscle plugs taken on both May 6, 1996 (66 days before start of present study) and April 23, 1997. On May 6, 1996, two fish (PIT 3914 and PIT 3436) had elevated selenium concentrations in muscle plugs (13.1 and 13.8 µg/g, respectively) and continued to have elevated values on April 23, 1997 (16 and 11 µg/g, respectively). The other two fish had relatively low selenium concentrations in muscle plugs (PIT 320E, 5.2 µg/g on May 6, 1996 and 4.7 µg/g on April 23, 1997; PIT D102, 3.7 µg/g on April 23, 1997).

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 (Appendix F), suggesting that eggs containing high selenium concentrations might have been resorbed, thus adding to the muscle tissue burden of selenium. 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 due to tissue dilution because the fish lost weight during the depuration period. Average weight lost in fish from Adobe Creek was 3.8% at 86 days depuration, whereas at North Pond it was 2.7% at 86 days depuration compared to measurements at spawning.

Adult collection and spawning

The number of adults recaptured in April 1997 for spawning were 31 of 35 stocked at Adobe Creek, 20 of 31 at North 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 escaped capture efforts. For spawning on April 23-24, 1997, 15 females from Adobe Creek were available and 5 were spawned; 10 females from North Pond were available and 5 were spawned; and 13 females from Horsethief were available and 5 were spawned. Several brood stock fish were spawned for other purposes, and larvae from one of these fish were used in the larval fish study. Fish were spawned again on April 25, 1997 (6 spawns from Horsethief and North Pond and 4 spawns from Adobe Creek) due to nearly complete egg mortality during the first 2 days of the egg study.

Selenium and other elements in eggs

Mean selenium concentrations in eggs were 6.0 µg/g from Horsethief adults, 40.1 µg/g from Adobe Creek adults, and 54.7 µg/g from North Pond adults (Table 10, Appendix G). Eggs from three brood stock fish held at Horsethief contained a mean selenium concentration of 6.9 µg/g. Selenium concentrations in eggs from adults held at Horsethief, Adobe Creek, North Pond, and 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 the first spawn (April 23), which was especially evident in spawns from the same adult (Appendix G). Eggs from the second spawn also tended to have smaller diameters, which also was especially evident in spawns from the same adult (Appendix G). 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 sites. Barium concentrations in eggs from brood stock fish were significantly higher than in Horsethief fish (Table 11). Copper concentrations in eggs from fish at Horsethief were higher than in eggs from fish at Adobe Creek and North Pond, but not 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 low (1.4-3.2 times difference between the lowest and highest concentrations). In contrast, the magnitude of difference between low and high selenium concentrations in eggs was 14-15 fold (measured by AA-HG).

Egg characteristics

The percent moisture in eggs was similar among the young adults in the present study, but moisture in eggs from North Pond adults was significantly higher than in eggs from brood stock (Table 10, Appendix G). However, the difference was only 1.6% between the mean percent moisture in eggs from North Pond adults compared to brood stock, and therefore may not be biologically important. The diameter 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 10, Appendix G).

Discussion

Water quality

Concentrations of cations and anions in water, as characterized by conductivity, at Horsethief and Adobe Creek probably did not adversely affect razorback sucker held at those sites because they were similar to Colorado River water. The conductivity, hardness, and alkalinity values at Horsethief and Adobe Creek were similar to those at the two Colorado River stations, AC1 (range 437-1,040 µmhos/cm) and WW1 (range 381-1,110 µmhos/cm). Conductivity, hardness, and alkalinity values in water in the present study also were similar to those measured in the previous reproductive study with razorback sucker at Horsethief (392-950 µmhos/cm conductivity, 148-324 mg/L as $CaCO_3$ hardness, 92-140 mg/L as $CaCO_3$ alkalinity)

Table 10. 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). Eggs were randomly sampled.

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

Table 11. Mean (standard error in parentheses and number of samples in brackets) concentration of inorganic elements (µg/g dry weight) in eggs collected from razorback sucker held at three sites near Grand Junction, Colorado, including brood stock (BS). For Ba, Cu, Fe, Mn and Se, sites with lower case letters in common were not significantly different (*P*=0.05); for the other elements, there were no significant differences among sites.

	Site (adult designation)			
Element	HT	AC	WW	BS
Ag	<0.3[1]	<0.3	<0.3	<0.3
Al	3 (-) [1][2]	<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) [5]	0.37ab (0.02) [5]	0.40ab (0.04) [5]	0.54b (0.07) [3]
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

Table 11. Continued.

Element	Site (adult designation)			
	HT	AC	WW	BS
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]

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

[2]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.

and Adobe Creek (418-1,110 µmhos/cm conductivity, 148-344 mg/L as $CaCO_3$ hardness, 75-189 mg/L as $CaCO_3$ alkalinity) (Hamilton et al. 2001).

Tyus (1987) and Tyus and Karp (1990) reported that razorback sucker gathered at Ashley Creek and Stewart Lake outlet in mid-April to May prior to making a spawning migration. During that time period at those two sites, Stephens et al. (1988) and Peltz and Waddell (1991) reported conductivities ranged from 1,510 to 2,550 µmhos/cm. These conductivities were relatively close to those observed in the present study when adults were present at Horsethief (range 593-1,060 µmhos/cm) and Adobe Creek (range 786-1,260 µmhos/cm).

When adults were present at North Pond, conductivity (range 1,240-4,630 µmhos/cm), salinity (range 1.0-5.0 g/L), and hardness (range 352-1,460 mg/L as $CaCO_3$) were elevated. These values were similar to those in the previous study with razorback sucker where the range of conductivity was 1,240-7,140 µmhos/cm, salinity was 0.5-5.5 g/L, and hardness was 364-2,160 mg/L as $CaCO_3$ (Hamilton et al. 2001). 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 sucker, 5.0 g/L salinity at North Pond was probably not stressful, but it is unknown what conductivity or salinity values may be stressful. This lack of stress was also suggested by the fact that adults held at North Pond gained length and weight during the 9-month exposure period. At stocking on July 11, 1996, fish averaged 432 mm total length and 745 g whereas at spawning they averaged 443 mm and 1,006 g. Adults stocked later on August 27, 1996, at North Pond also grew; stocked fish averaged 361 mm and 511 g whereas at spawning they averaged 374 mm and 547 g). Apparently, adults 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. Reduced growth is usually one of the first whole-animal indications of chronic stress in various biochemical pathways in fish (Mehrle and Mayer 1985).

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 the previous study (Hamilton et al. 2001) and with investigations of flowing water systems at Kesterson Reservoir, CA (Fujii 1988, Moore et al. 1990). Saiki et al. (1993) also reported no difference in selenium concentrations between filtered and unfiltered water samples collected from seven riverine sites associated with irrigation drainage in the San Joaquin Valley, CA, where the waters containing up to 13 µg/L selenium.

There was a potential for selenium concentrations in filtered and unfiltered water to be different because the Adobe Creek (AC5) and North Pond (WW2) sites were semi-static with little 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 attributed the higher total selenium concentrations, compared to dissolved selenium, to the sorption of selenium onto suspended solids and selenium contained in plankton. Seiler (1996) also cautioned against assuming total (unfiltered) and dissolved (filtered) concentrations of selenium were similar, especially in highly productive waters where there may be large amounts of algae.

The mean concentration of selenium in river water collected at Horsethief (3.0 µg/L), and WWSWA (WW1; 4.4 µg/L) was 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, CO. However, the reaches of the Colorado and Gunnison rivers that are influenced by irrigation return flows from the Grand Valley and Uncompahgre Valley irrigation projects as well as private irrigation activities have elevated concentrations of selenium and other inorganic elements, as well as general water quality characteristics, due to irrigation return flows to the rivers (Butler et al. 1989, 1991, 1994, 1996). Consequently, the elevated selenium concentrations in water from Horsethief ponds (mean 2.2 µg/L, range 1.4-3.0 µg/L), 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, range 1.2 to 4.5 µg/L) were due in part to inflow of irrigation return water via AC7, which contained 12.6-13.2 µg/L on May 28-30, 1997. During the previous reproduction study with razorback sucker, selenium concentrations in water at the Adobe Creek site changed quickly due to the discharge of irrigation return flow from AC7 into the site (Hamilton et al. 2001). Selenium concentrations were low between November 14, 1995, and April 23, 1996, in the tertiary channel where the razorback suckers were held (AC3, AC4, AC5). However, on April 25, 1996, shortly after irrigation drainwater (AC7) containing 15 µg/L flowed into the tertiary channel, selenium concentrations at AC5 increased from 1.8 µg/L on April 23 to 9.7 µg/L on June 11. A similar change was observed at AC3 and AC4 on June 11, 1996. The selenium concentrations in water from AC7 exceeded the USEPA chronic criterion of 5 µg/L for the protection of aquatic life (USEPA 1987), and increased selenium concentrations in the confined area where adult razorback sucker were held. In the same geographic area, water in Adobe Creek sampled near the interstate highway as part of the NIWQP in 1991-92 had a mean selenium concentration of 64 µg/L (Butler et al. 1994). Butler et al. (1989) noted that virtually all of the flow in Adobe Creek (not the same as the test site used in the present study) was irrigation return flow and drainage water.

The elevated concentrations of selenium in water at WW2 (mean 7.8 µg/L, range 3.2 to 17.1 µg/L) 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 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. The WWSWA channel and North Pond have been identified by the U.S. Geological Survey as a discharge area for ground water (D. Butler, USGS, personal communication).

Water from Independent Ranchman's Ditch was used to maintain the water level in North Pond during the study. Although water samples were not collected from WW10 during the present study, water sampled at WW10 on September 11, 1995, had a selenium concentration of 13.8 µg/L (Hamilton et al. 2001). Although the selenium concentration in water from WW10 exceeded the USEPA criterion of 5 µg/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

North Pond. Prior to stocking fish in the first reproduction study with razorback sucker, North Pond water had selenium concentrations of 115-133 µg/L (Hamilton et al. 2001). The relatively low selenium concentration in North Pond during the time when adult razorback sucker were present in this study, compared to concentrations in May 1995, were probably due to inflow of water from WW10 or flooding from the Colorado River in 1996.

Selenium concentrations at Adobe Creek and North Pond observed in the present study 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 CO-UT 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). However, 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 to uncontaminated aquatic ecosystems, which typically have <1 µg/L (Maier and Knight 1994).

Some people have suggested that native fish in the Colorado River basin may have evolved in a selenium-rich environment because of the presence of high selenium soils derived from Mancos Shale (e.g., A. Archuleta, USFWS, written communication). To address the issue of background waterborne selenium concentrations in streams and water bodies in Mancos Shale areas in the Grand Uncompahgre valleys with no irrigation activity (some areas had grazing activity that disturbed the soils), David Butler of the U.S. Geological Survey searched the area extensively and located seven areas for sampling (Butler and Osmundson 1999). At Wells Gulch at Fools Hill at Highway 50, west of Delta in the Gunnison River basin in an area with some grazing activity, selenium concentrations in water were <1 µg/L. At Cheney Reservoir in the Uncompahgre River basin in an area with no grazing or irrigation, selenium concentrations in water were <1-1 µg/L, 2-2.4 µg/g in sediments composed almost exclusively from Mancos Shale, and 4.2 µg/g in aquatic invertebrates. At Little Salt Wash above Government Highline Canal in the Grand Valley in an area with no irrigation and minimal grazing activity, selenium concentrations in water during runoff events on February 20, 1996, (1.5 cfs) were 3 µg/L and on March 24, 1997, (0.99 cfs) were 1 µg/L. At Big Salt Wash above Government Highline Canal in the Grand Valley in an area with no irrigation activity, four selenium measurements in water ranged from 2 to 3 µg/L, but perennial flows were low, ranging from 0.08 to 0.14 cfs. At east Salt Creek downstream from Camp Gulch in the Grand Valley in an area with little irrigation activity, selenium concentrations in water were <1 µg/L during runoff events on December 4, 1995 (2.2 cfs) and February 16, 1996 (5.4 cfs). At West Salt Creek downstream below Prairie Canyon in the Grand Valley in an area with no irrigation and some grazing, selenium concentrations in water <1 µg/L during two runoff events on December 4, 1995 (0.99 cfs) and March 13, 1997 (5.3 cfs). In only one area with low flows (0.16-0.24 cfs) at West Salt Creek near S Road in the Grand Valley, selenium concentrations in water were elevated (9-10 µg/L) due to the presence of salt crusts. Because selenium concentrations in water draining high selenium soils were relatively low, i.e., ~1 µg/L, the hypothesis of aquatic environments with elevated selenium, i.e., selenium enriched, enhancing the possibility of selenium adaptation by native fish seems unlikely. These waterborne selenium concentrations are also lower than those in streams and rivers below areas influenced by irrigation activities.

The significant differences in concentrations of inorganic elements in water in the present study (boron and lithium higher in North Pond water than in Adobe Creek water, strontium

higher in North Pond than in Horsethief, and magnesium higher in North Pond than in Adobe Creek or Horsethief) were similar to those observed in the previous reproduction study (Hamilton et al. 2001). In the previous study, nine elements (antimony, boron, calcium, lithium, magnesium, phosphorous, sodium, strontium) were significantly higher in North Pond water than in Adobe Creek water.

In the present study, selenium concentrations in North Pond water measured by AA-HG was correlated only with barium measured by ICP, whereas in the previous study selenium concentrations were correlated with nine elements (boron, calcium, potassium, lithium, magnesium, molybdenum, sodium, phosphorous, and strontium) measured by ICP. Combining the three sites in the present study, selenium concentrations in water measured by AA-HG were significantly correlated with boron, lithium, magnesium, strontium, and vanadium. Finger et al. (1994) also reported a strong relation between 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, i.e., elevated elements in soil will generally leach out in proportion to their concentration in soil depending on absorption coefficients. Reviews of the relation between geologic sources of selenium and their movement and potential consequences have been described in Presser and Ohlendorf (1987), Presser et al. (1994), and Presser and Piper (1998).

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) and North Pond (chloride, conductivity) were similar to those observed in the previous reproduction study (Hamilton et al. 2001). In that study, the significant positive correlations between selenium concentrations in water and water quality characteristics at Horsethief were with alkalinity, calcium, conductivity, hardness, magnesium, nitrate, and sulfate and at North Pond with calcium, chloride, conductivity, hardness, magnesium, nitrate, nitrite, and sulfate. In both studies there were no correlations between waterborne selenium and water quality characteristics at Adobe Creek.

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 17 sites: 6 in Wyoming and 11 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 log x 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, UT, which suggested 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; top portion of core collected on April 14, 1997, 2.52 µg/g) were near national background concentrations of <1 µg/g (Maier and Knight 1994). The sediment selenium values observed at Horsethief and Adobe Creek also were similar to those of Stephens et al. (1997) who listed a no effect concentration of <2 µg/g for effects of selenium on fish and wildlife, and Lemly's (1995) no hazard concentration of <1 µg/g or a

minimal hazard concentration of 1-2 µg/g. Presser et al. (1994) reported the upper limit of the expected baseline range for selenium concentrations in soils of the western U.S. 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. Neither Lemly (1993a, 1995, 1996b) nor Maier and Knight (1994) proposed a toxic threshold for selenium concentrations in sediment.

The tertiary channel at Adobe Creek was diked to hold the adult fish for the two reproduction studies. In June 1995, a water control structure was installed in the dike road at AC3 and an inactive beaver dam with 2-3 foot deep water near AC5 was converted to a dike with an outflow water control structure at AC5 (D. Crabtree, USBR, personal communication). The 22-month period of the two reproduction studies may have been sufficient to allow for selenium accumulation in the sediments. Selenium concentrations in sediment from AC3 in the present study (1.21-2.52 µg/g in top section of core) were higher than those reported in the previous reproduction study (0.79 to 1.11 µg/g; Hamilton et al. 2001). However, in the previous study, the sediment samples were mixed during sample collection, whereas in the present study the sediment was collected as a core sample, immediately frozen, and the top, middle, and bottom sections removed for analysis. Nevertheless, it is interesting to note that the 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. Site AC3 was more susceptible to selenium deposition than either AC4 or AC5 because of the lack of water movement at AC3 compared to AC4, which had some flow due to pumped water from AC2 and flow from AC7, and compared to AC5 where the water outflow structure was located. No flooding occurred at Adobe Creek during the present study, thus no flushing flows disturbed selenium deposition in the sediments at AC3. Consequently, selenium concentrations in sediments at Adobe Creek seemed to have increased over time to the point where they were within the low hazard range of Lemly (1995; 2-3 µg/g) and above the “no effect” category of Stephens et al. (1997).

Selenium concentrations in sediment at North Pond probably accumulated over several years, perhaps more than 20 years. North Pond appears in aerial photos taken in 1973 and 1982 (T. Mathieson, CDOW, personal communication). The core samples in the present study had a wide variation in selenium concentrations among the core sections. The reason for this variability is unknown. Combining the three values (top, middle, and bottom section) for each sample date, the average selenium concentrations for the present study are 32.9 µg/g on October 21, 1996, and 10.2 µg/g on April 14, 1997, which are similar to selenium concentrations in sediment for well-mixed samples previously reported for WW2 (11.9-31.8 µg/g; Hamilton et al. 2001). Most of the selenium concentrations in sediment observed in North Pond were above the high hazard value of >4 µg/g proposed by Lemly (1995) and the toxic threshold guideline value of >4 µg/g proposed by Stephens et al. (1997).

Accumulation of selenium in the top layer of sediments is generally the result of deposition of dead organic material from the water column and incorporation in the detrital food chain (Weres et al. 1989, Bender et al. 1991, Graham et al. 1992). Graham et al. (1992) reported that in a pond study, selenium rapidly disappeared from the water column and correspondingly increased in sediments and biota, especially periphtyton. One component of the sediments is the detrital layer, which is partly composed of bacteria. Bender et al. (1991) reported selenium was rapidly removed from the water column by bacteria and cyanobacteria and incorporated into a

detrital-like mat composed of anaerobically processed grass clippings. In their experiment initial selenium concentrations of 40 mg/L were undetectable after 27 days of microbial activity.

Ponds at Kesterson NWR, CA, had selenium concentrations in sediment of 5-10 µg/g, but surficial detrital material (decomposing organic matter) contained 40-130 µg/g (Presser et al. 1994). Bottom sediment from the main collective drain at Kesterson NWR, which was described as rich in organics, contained 92 µg/g in sediments, but 308 µg/g in detritus (Presser et al. 1994). Others have reported similar results (Saiki 1986a, Saiki et al. 1993).

Others have also reported that selenium accumulates in the top layer of sediments (Holland 1979, Cumbie 1984, 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). However, Zhang and Moore (1996) reported that selenium concentrations in sediments can be high in the surficial sediment or at lower depths and were associated with changes in organic carbon content in the sediment.

Selenium concentrations in sediment at Adobe Creek and North Pond are typical of sediments in the Grand and Uncompahgre valleys that are influenced by irrigation activities. Selenium concentrations in sediment during 1987-1988 in areas of the Uncompahgre Valley affected by irrigation near Delta, CO, 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 concentrations in sediment in the Uncompahgre Valley ranged from 2.0 to 6.9 µg/g in creeks and 16 µg/g in Markley Pond (Butler et al. 1994). Selenium concentrations observed in sediments at North Pond (12-55 µg/g) were higher than those concentrations, but were similar to those reported for Sweitzer Lake (aka Garnet Mesa Reservoir) in 1987-1988 (9-41 µg/g; Butler et al. 1991), and Kesterson Reservoir, CA (aggregate geometric mean of 11.8 µg/g; Moore et al. 1990). Birkner (1978) reported selenium concentrations in sediment sampled during 1976-1977 were 6.5 µg/g at Sweitzer Lake and 15.4 µg/g at Desert Reservoir, near Grand Junction, CO, but only 1.2 µg/g at Mac Mesa Reservoir and 1.8 µg/g at Highline Reservoir. Barnhart (1957) reported that sediments at Sweitzer Lake had 10-40 µg/g selenium in 1956, but various canals and ditches in the Sweitzer Lake area had 1 to 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). In a toxicity test with these five sediments, embryo-larvae of fathead minnow (*Pimphales promelas*) died within 24 hours of exposure (Butler et al. 1996). There was significant mortality of the amphipod *Hyallela azteca* in a toxicity test with sediments from Reed Wash and Adobe Creek, but not from Salt Creek, Leach Creek, or Indian Wash (Butler et al. 1996). In both toxicity tests, the cause of mortalities was unknown. Selenium concentrations in a variety of fish species collected from these washes and creeks (common carp *Cyprinus carpio*, roundtail chub *Gila robusta*, fathead minnow, speckled dace *Rhinichthys osculus*, white sucker *Catostomas commersoni*, bluehead sucker *Catostomus discobolus*, flannelmouth sucker *Catostomus latipinnis*) averaged 13.4 µg/g in Salt Creek, 11.4 µg/g in Reed Wash, 10.3 µg/g in Adobe Creek, 7.1 µg/g in Leach Creek, and 9.3 µg/g in Indian Wash (Butler et al. 1994).

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 from Horsethief ponds in the previous reproduction study (2.3-3.1 µg/g; Hamilton et al. 2001). This difference may be due to 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. 2001). 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. 2001). 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. 2001). The significant correlation between selenium concentrations in zooplankton with chironomids in the present study ($r=0.94$, $P=0.005$, $n=6$) suggested 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, 1996b, 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 5 studies, Lemly 1993a reviewed 11 studies, Lemly 1996b reviewed 1 additional study). 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 at Adobe Creek for 12 of 13 months it was monitored, selenium concentrations in food organisms during the study (15.6-48.3 µg/g) exceeded the proposed dietary toxic threshold (3 µg/g) by a factor of 5 to 16 fold.

Zooplankton can rapidly take up selenium from the water and accumulate it with no adverse effects on reproduction except at very high selenium concentrations, i.e., >400 µg/L (Halter et al. 1980, Nassos et al. 1980, Reading and Buikema 1983, Salki et al. 1985, Foe and Knight 1986, Ingersoll et al. 1990). Similarly, Salki et al. (1985) found no effects on seasonal abundance of zooplankton exposed to selenium concentrations as high as 100 µg/L in large enclosures. However, two studies have reported adverse effects on invertebrates. Boyum and Brooks (1988) found that waterborne and dietary selenium exposure of daphnids reduced survival and greater reductions occurred in the waterborne exposure than in the combined water and dietary exposure. Dobbs et al. (1996) reported that rotifers biomass was reduced by exposure to 108 µg/L selenium in their three-trophic food chain experiments.

The likely sources of selenium residues in zooplankton at Adobe Creek and North Pond were water, aquatic plants such as algae, bacteria, and particulate matter. Selenium in water is rapidly taken up by algae (Sandholm et al. 1973, Nassos et al. 1980, Foe and Knight 1986, Riedel et al. 1991, Besser et al. 1993), aquatic plants (Allen 1991, Ornes et al. 1991), and bacteria (Bender et al. 1991). Typically, algae took up maximal concentrations in 3-24 hours, whereas floating plants took about 1 week to accumulate maximal concentrations. In those

studies, neither algae nor aquatic plants exhibited adverse effects from selenium exposure except at high concentrations, i.e, ≥100 µg/L for algae (Foe and Knight 1986) or 1,250 µg/L for floating plants (Ornes et al. 1991). Part of the selenium taken up by zooplankton was probably waterborne organoselenium compounds released from living algae or necrosis of dead cells (Cutter 1986, 1989, 1991, 1992, Besser et al. 1994).

Even though waterborne selenium concentrations at Adobe Creek (<0.7-2.8 µg/L) and North Pond (3.2-15.6 µg/L) were lower than at Sweitzer Lake (10-170 µg/L; Butler et al. 1991), selenium concentrations in zooplankton and chironomids from Adobe Creek (15.6-48.3 µg/g) at AC3-AC5 and in zooplankton from North Pond (31.9-48.1 µg/g) were similar to or higher than those in aquatic invertebrates from selenium-contaminated Sweitzer Lake (27-30 µg/g; Butler et al. 1991). Birkner (1978) reported that a mixed-species sample of plankton collected in 1977 from Sweitzer Lake contained 42.5 µg/g selenium. Barnhart (1957) and Birkner (1978) suggested that the lack of reproduction of native fish such as flannelmouth sucker and stocked game fish such as channel catfish (*Ictalurus punctatus*) in Sweitzer Lake was probably due to adverse effects from selenium accumulated through the food chain.

Selenium concentrations in aquatic invertebrates from Horsethief east wetland, Adobe Creek, and North Pond were also higher than those from other aquatic environments in the upper Colorado River, which may reflect differences between lentic and lotic environments, although the three test sites were not entirely lentic, i.e., non-flowing. Aquatic invertebrates in the Uncompahgre River at Delta, CO, contained selenium concentrations of 4.1 µg/g, and in the Gunnison River at Delta, CO, were 5.6-6.8 µg/g (Butler et al. 1991), which exceed the proposed dietary toxicity threshold of 3 µg/g (Maier and Knight 1994, Lemly 1996b).

Butler et al. (1994) reported two examples of low selenium concentrations in water, yet elevated concentrations in aquatic invertebrates from the Uncompahgre Valley: Horsefly Creek (<1 µg/L, 6.1 µg/g, respectively) and South Fork (<1 µg/L, 4.8 µg/g, respectively). Stephens et al. (1992) reported four examples from the Green River valley: Sheppard Bottom pond 5 (3-4 µg/L, 4.4-8.9 µg/g, respectively), Desilting Basin (3-5 µg/L, 3-9 µg/g, respectively), Big Island Pond (2-5 µg/L, 5-6 µg/g, respectively), and Felters, Shoveler, and Pintail ponds (1-5 µg/L in adjacent waters, 6-11 µg/g, respectively). Birkner (1978) reported two examples from the Grand Valley: Mac Mesa Reservoir (2.2 µg/L, 7.7 µg/g, respectively), and Highline Reservoir (4.2 µg/L, 7.7 µg/g, respectively), as well as four other locations in Colorado and two in Wyoming. Hamilton et al. (2001) also reported examples from sites AC3, AC4, and AC5 in the previous reproduction study with razorback sucker: between May and July 1995 water had selenium concentrations of 1.0-3.7 µg/L and zooplankton had 4.5-10.5 µg/g, and between November 1995 and May 1996 water had 1.7-4.3 µg/L and zooplankton had 16.1-21.8 µg/g. These examples from locations in the Uncompahgre, Grand, and Green River valleys were similar to the selenium loading study of Maier et al. (1998), which revealed that aquatic invertebrates exposed to low waterborne selenium concentrations can accumulate selenium residues that reach or exceed the proposed dietary toxicity threshold of 3 µg/g. Other examples of low waterborne selenium concentrations (<5 µg/L) associated with elevated selenium concentrations in food organisms above the proposed dietary toxic threshold (3 µg/g) have been reported by Holland (1979), Schroeder et al. (1988), Peltz and Waddell (1991), Hamilton et al. (1996), and Lemly (1997b).

In aquatic ecosystems with elevated selenium concentrations in water, the amount accumulated in aquatic invertebrates may be variable due in part to the productivity of the water body. Butler et al. (1994) and Stephens et al. (1992) reported examples of moderate to high

selenium concentrations in water and high selenium concentrations in aquatic invertebrates. In the Uncompahgre Valley, examples include: Markley Pond (5 µg/L, 32 µg/g, respectively) and Loutsenhizer Arroyo (130 µg/L, 26 µg/g, respectively). In the Green River valley, examples include: Winter Storage Pond (8-9 µg/L, 37-42 µg/g, respectively), Drain J3 (58 µg/L, 37 µg/g, respectively), Stewart Lake (4-11 µg/L, 10-16 µg/g, respectively), North Roadside Pond (10-85 µg/L, 12-40 µg/g, respectively), and South Roadside Pond (39 µg/L, 12-53 µg/g, respectively). Birkner (1978) reported that Desert Reservoir in the Grand Valley had selenium concentrations of 12.5 µg/L in water and 31.3 µg/g in a mixed-species plankton sample. These elevated concentrations of selenium in aquatic invertebrates were comparable to those at Adobe Creek and North Pond.

The same pattern of low selenium concentrations in water, but elevated concentrations in benthic invertebrates has been observed in the present study as well as in the previous reproduction study. In the present study water from Horsethief had selenium concentrations of 1.4-3.0 µg/L and chironomids from Horsethief east wetland had 7.9-9.3 µg/g, and similarly, water from Adobe Creek had <0.7-4.5 µg/L and chironomids had 31.7-34.0 µg/g. In the previous reproduction study, selenium concentrations in water from AC3-AC5 between November 1995 and April 1996 were 1.7-4.3 µg/L and in chironomids were 34.0-40.2 µg/g. (Hamilton et al. 2001). It is remarkable that the selenium concentrations in chironomids collected at AC3, AC4, and AC5 are similar between the two studies because in the previous study chironomids were collected from Hester-Dendy samplers suspended in the water column whereas in the present study the chironomids were collected from sediment samples.

Both of these findings are similar to those reported by Zhang and Moore (1996). They found low selenium concentrations in water (0.94-1.58 µg/L), but elevated selenium concentrations in chironomids (8.12-10.4 µg/g). The high correlation coefficient between selenium concentrations in chironomids and sediment (r_s=0.87, P=0.05, n=5) in the present study supports the general consensus that benthic invertebrates accumulate selenium from the sediment, especially the detritus faction of the sediment (Saiki et al. 1993, Malloy et al. 1999, Peters et al. 1999). Saiki et al. (1993) reported selenium concentrations in sediment, detritus, and chironomids from several locations in the San Joaquin Valley, and the Spearman correlation coefficient (r_s) was 0.56 (P=0.03, n=14) for sediment and detritus, 0.48 (P=0.08, n=14) for sediment and chironomids, and 0.91 (P=0.0001, n=14) for detritus and chironomids. Malloy et al. (1999) reported the correlation coefficient for sediment and chironomids was 0.87 (P=0.001, n=10).

Growth

Adults stocked at Horsethief in the previous reproduction study and restocked at Horsethief on July 11, 1996, for the present study had generally similar gains in total length and in weight compared to the previous study. In the present study between initial stocking and spawning, fish at Horsethief grew 4.7% in total length and gained 26.8% in weight compared to the previous study where fish grew 15% in total length and gained 21.7% in weight. However, adults in the present study stocked at Adobe Creek grew 1.2% in total length and gained 15.1% in weight compared to the previous study where fish grew 9% in total length and gained 7.5% in weight. Likewise, adults stocked at North Pond grew 2.5% in total length and gained 35.0% in weight compared to the previous study where fish grew 5.6% in total length and gained 9.2% in

weight. This 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 moderate gains in total length and substantial gains in weight. Nevertheless, the lower weight gain in fish at Adobe Creek (15.1%) compared to fish at North Pond (35.0%) might be due to differences in habitat between 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 the North Pond site.

In contrast, adults stocked at the three sites on August 27, 1996, apparently did not readily adapt to the new holding conditions, especially for the Adobe Creek and North Pond sites, compared to their previous culture site, Wahweap Fish Facility. The weight gain for fish stocked on August 27, 1996, was substantially less at Adobe Creek (8.2%) and North Pond (7.0%) than for the fish stocked on July 11, 1996, and used in the previous reproduction study (15.1% and 35.0%, respectively). Their smaller increases in total length and weight are 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 was probably due to a time lag in adapting to natural foraging at Adobe Creek and North Pond.

The relatively rapid growth of the previously-exposed adults (5-20 mm increase/~1 year) and the first-time use adults (7-30 mm increase/~1 year) in the present study may be due to their relatively young age (4-5 years old) compared to the slow or negligible growth of adult razorback sucker reported by others. McAda and Wydoski (1980) reported that one adult razorback sucker had not grown after 1.5 years between captures, and a second fish had grown about 2.3 mm/year over a 3.5 year period. Valdez et al. (1982) reported little or no change in length of five razorback sucker recaptured up to 1 year later. All of the razorback sucker collected by McAda and Valdez were considered to be old fish; no juveniles or sub-adults were collected (C. McAda and R. Valdez, personal communication). Other razorback sucker populations in the Colorado River basin also are presumed to be very old (McCarthy and Minckley 1987). Tyus (1987) reported slow growth (mean 2.2 mm/year) of 39 adults with recapture periods of 1 to 8 years. Roberts and Moretti (1989) found slow growth (mean 1.8 mm/year) in six adults recaptured after 1 year in the San Juan Arm of Lake Powell. Although not based on recapture of the same adults, the total length of adults in Lake Mohave increased by 5 mm/year up to 1964 (Minckley 1983), but thereafter (1965-1980s), there was no change (Marsh and Minckley 1991).

In the present study, it would not be expected to observe changes in adult growth during the relatively short exposure time period because razorback sucker are a slow growing, long-lived fish (McAda and Wydoski 1980, Valdez et al. 1982, Tyus 1987, Roberts and Moretti 1989, 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 is the elimination of fish species from aquatic ecosystems such as in Belews Lake, NC (Cumbie and Van Horn 1978, Lemly 1985), Martin Lake, TX (Garrett and Inman 1984, Sorensen 1988), Kesterson Reservoir, CA (Harris 1986, Vencil 1986), or the lack of reproduction as documented at Sweitzer Lake, CO (Barnhart 1957, Birkner 1978). Adverse effects have been reported on survival and growth of adult fathead minnow and bluegill (*Lepomis macrochirus*) exposed to a selenium concentration of 10 µg/L in experimental streams conducted by the USEPA at Monticello, MN, (Hermanutz et al. 1992, Schultz and Hermanutz 1990). However, Coyle et al. (1993) reported no effects on growth in adult bluegill exposed to selenium up to 10 µg/L in water

and 32 µg/g in diet for 140 days. Crane et al. (1992) also 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 of either 0, 2, 10 or 25 µg/L.

Selenium in tissues

Selenium residues in adult 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 feed 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 razorback sucker had both plankivorous and benthic feeding habits. Several investigators noted sediment, sometimes referring to it as ooze, detritus, or decaying organic matter, in the gut of razorback sucker (Dill 1944, Banks 1964, Vanicek 1967, Allan and Roden 1978, Marsh 1987).

In the present study, selenium concentrations in muscle plugs from all the fish at the time of stocking (4.5 µg/g in Horsethief adults, 9.6 µg/g in Adobe Creek adults, 14.2 µg/g in North Pond adults) exceeded the 85th percentile (arbitrary point distinguishing "high" concentrations) in the National Contaminant Biomonitoring Program (NCBP) for the years 1971-1984 (Walsh et al. 1977, May and McKinney 1981, Lowe et al. 1985, Schmitt and Brumbaugh 1990). The NCBP has documented temporal and geographic trends in concentrations of persistent environmental contaminants, including selenium, in whole-body of fish that may threaten fish and wildlife. The 85th percentile concentrations of selenium in samples from the NCBP were 2.9 µg/g (reported as wet weight, converted to dry weight assuming 73% moisture [average of percent moisture in 1978-1981 and 1984 samples]) in 1972-1973, 3.0 µg/g (reported as wet weight, converted to dry weight assuming 73% moisture) in 1976-1977, 2.5 µg/g (reported as wet weight, converted to dry weight based on a mean moisture of 72% for 591 samples in the 1978-1981 collection) in 1978-1981, and 2.8 µg/g (reported as wet weight, converted to dry weight based on a mean moisture of 74% for 315 samples in the 1984 collection) in 1984, the last year of the program (Walsh et al. 1977, May and McKinney 1981, Lowe et al. 1985, Schmitt and Brumbaugh 1990).

Selenium concentrations in muscle plugs measured in the present study probably underestimate the concentrations in whole-body of fish. One report stated that fillets (i.e., muscle) had more selenium than whole body in bluegill and largemouth bass (*Micropterus salmoides*) collected at a variety of sites in central California associated with irrigation drainage (Saiki et al. 1991), which was the opposite of the majority of the literature. 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, Sato et al. 1980, Lemly 1982, Hilton et al. 1982, Hilton and Hodson 1983, Kleinow and Brooks 1986, Lemly and Smith 1987, Hermanutz et al. 1992). Consequently, the estimated whole-body selenium concentrations in razorback sucker initially stocked in the present study would be about 7.5 µg/g for Horsethief, 16.0 µg/g for Adobe Creek, and 23.7 µg/g for North Pond (based on a conversion factor of 1.667 x muscle concentration=whole body concentration, Lemly and Smith 1987). Other conversion factors are 2.355 based on data from Adams (1976) for rainbow trout (*Oncorhynchus mykiss*), and 1.745 from Lemly (1982) for bluegill and largemouth bass, both of

these factors would have increased the estimated whole-body selenium concentrations in razorback sucker. Thus, the razorback suckers used in the present study initially had selenium residues over 2 times higher for Horsethief fish, 5 times higher for Adobe Creek fish, and 8 times higher for North Pond fish than the 85th percentile of the NCBP.

Concentrations of selenium in muscle plugs in the present study increased by 73% in fish held at Adobe Creek (9.6 µg/g at day 0 and 16.6 µg/g at day 286, excluding fish HT66), and 80% in fish held at North Pond (14.2 µg/g at day 0 and 25.5 µg/g, 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 study, which was suggested in Hamilton et al. (2001). Likewise, the lower selenium residues in the one fish stocked at Adobe Creek (previously held at Horsethief for 371 days) and in the two fish stocked at North Pond (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 in fish exposed in both reproduction studies.

The elevated selenium concentrations in muscle plugs from two older brood stock (PIT 3914 and 3436) on both May 6, 1996, and April 23, 1997, suggested that these two fish are using a microhabitat in the hatchery ponds where selenium has apparently accumulated in the food chain, i.e., detrital or benthic. Chironomids collected from the Horsethief ponds in the previous reproduction study had selenium concentrations of 8.0 and 13.6 µg/g (Hamilton et al. 2001).

Most other studies have reported that selenium residues in whole-body or tissues reached an equilibrium in 60 to 90 days (Gissel Nielsen and Gissel-Nielsen 1978, Sato et al. 1980, Lemly 1982, Besser et al. 1993), but others have estimated longer periods, i.e., >20 weeks (Adams 1976, Woock and Summers 1984). Reaching 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 19 months of exposure (305 days in the previous study, followed by 66 days depuration, then 286 days in the present study) for tissue residues to reach an equilibrium with selenium exposure in the water and diet.

The fish in the present study may not have reached an equilibrium between selenium concentrations in muscle tissue and those in food chain organisms because selenium concentrations in fish generally continue to increase until they reach the concentrations in their food (Table 4 in Hamilton et al. 2000). In the present study, selenium concentrations were 17.9-20.0 µg/g in zooplankton and 31.7-48.3 µg/g in chironomids at Adobe Creek and 31.0-48.1 µg/g in zooplankton at North Pond. The estimated whole-body selenium concentration in adults from Adobe Creek was 27.7 µg/g (16.6 µg/g in muscle plug [excluding fish HT66] x 1.667 conversion factor) and from North Pond was 42.5 µg/g (25.5 µg/g [excluding fish HT12 and HT42] x 1.667 conversion factor). These values are similar to the selenium concentrations in food organisms (i.e., bioaccumulation factor of one or less). In most laboratory studies with dietary exposure to selenium, selenium accumulations to concentrations in whole-body fish similar to those in the diet (Bennett et al. 1986, Hamilton et al. 1990, Crane et al. 1992, Lemly 1993b), however, in field studies where fish have had time to equilibrate with environmental conditions, fish often accumulate selenium concentrations from 1.4 to 2.6 times selenium concentrations in their food (Barnhart 1957, Birkner 1978, Woock 1984, Saiki 1986). It is possible that if the adult fish were

held at Adobe Creek and North Pond longer than 19 months the selenium concentrations in their muscle plugs would have continued to increase.

Selenium concentrations in muscle plug tissue in razorback sucker from Adobe Creek (16.6 µg/g excluding fish HT66) and North Pond (25.5 µg/g excluding fish HT12 and HT42) after 286 days of exposure exceeded the proposed guideline of 8 µg/g in skeletal muscle as the bench mark for probable reproductive failure (Lemly 1996b). This value was based on a review of 17 laboratory and field studies of selenium effects on various fish species. One other study not reviewed by Lemly (1996b) also support his proposed guideline of 8 µg/g for reproductive failure of fish. Cumbie and Van Horn (1978) reported reproductive failure of several fish species in Belews Lake, NC, at selenium concentrations of 10 µg/g or greater in skeletal muscle. In addition, Crane et al. (1992) reported no hatch of eggs from adult yellow perch exposed for 288 days to 25 µg/L in a pond system; fish contained 23 µg/g in muscle and 28 µg/g in gonads (reported as wet weight, converted to dry weight assuming 75% moisture).

Thirty-three percent (15 of 45) of the wild adult razorback sucker 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 to 33% of wild fish had higher selenium residues because the two groups of fish in the present study were held in elevated selenium environments for a total of 19 months (9 months in the present study and 10 months in the previous study) and had only a 2-month opportunity to move to low selenium environments, i.e., 66-day depuration period. 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 with little depuration. Modde (1993) stated that "Based on the distribution of razorback sucker, it is likely that a significant portion of the remaining razorback sucker population in the middle Green River have been exposed to selenium contamination." Modde's statement was supported by information in Waddell and May (1995) and Stephens and Waddell (1998). 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-year period suggested that they, similar to razorback sucker, maintain elevated accumulated selenium residues in muscle tissue without depurating it.

Selenium concentrations in whole-body fish in the Colorado River basin, measured as part of the NCBP, have been among the highest in the nation (Walsh et al. 1977, Lowe et al. 1985, Schmitt and Brumbaugh 1990). They exceeded the 85th percentile in whole-body fish collected in 1972-1973 at 5 of 6 Colorado River basin stations (Green River at Vernal, UT [only upper basin station], and Colorado River at Imperial Reservoir, Lake Havasu, Lake Mead, Lake Powell, all AZ). Selenium concentrations in whole-body fish also exceeded the 85th percentile in 1978-1981 and 1984 at 6 of 7 stations (same five as above) plus Colorado River at Yuma, AZ.

Elevated selenium concentrations, exceeding the 85th percentile of the NCBP, in other fish species have been reported as part of the NIWQP and USFWS contaminants investigations

in the upper Colorado, Dolores, Green, Gunnison, Uncompahgre, White, and Yampa rivers (Butler et al. 1991, 1994, 1996, Krueger 1988, 1991, Krueger et al. 1991, Stephens et al. 1988, 1992, Osmundson 1989, 1992, Peltz and Waddell 1991, Rose 1992, Waddell and Wiens 1992, 1994a, 1994b, Wiens and Waddell 1996). Elevated selenium concentrations have also been found in fish (Bussey et al. 1976, Kidd and Potter 1978, Potter and Drake 1989, Waddell and Wiens 1993) from Lake Powell and also in net plankton (Kidd and Potter 1978). These elevated concentrations in Lake Powell prompted Stanford and Ward (1991) to caution that "Relatively high [selenium] concentrations were observed in the larger fishes of Lake Powell (Potter and Drake 1989) and warrant further work, as selenium may inhibit egg production in squawfish [Colorado pikeminnow] and other native fishes of the Colorado River."

High selenium concentrations in 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 (i.e., larvae of mayfly, caddis fly, midge), 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 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 sediments depurated selenium from their tissues (Woock 1984).

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. 2001). The lower amount of selenium loss in fish from Adobe Creek seems unusual and may reflect variability in depuration rates. Loss of selenium from their muscle tissue after 86 days of depuration was 14-21%, which suggested a slow loss of selenium. 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). Loss of selenium also is faster in small, younger fish (Bennett et al. 1986) than in larger, older fish (Bertram and Brooks 1986). 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.

Besser et al. (1993) reported a 20-day half life of selenium residues in juvenile bluegill, although the authors thought the fish had not reached an equilibrium of selenium uptake before depuration was started, which could have lengthened the half life period. Kleinow and Brooks (1986) reported a 19-day half life for selenate and selenite, and a 27-day half life for selenomethionine in whole body of adult fathead minnow. They also reported that the half lives were longer in muscle tissue (33 days for selenate, 41 days for selenite, and 42 days for selenomethionine) than in whole body. The longer half life for selenomethionine was probably due to its incorporation into protein and tissue, which would require more metabolic work to

eliminate. Others have reported half lives for selenium depuration of 28-30 days in various young fish (Gissel Nielsen and Gissel-Nielsen 1978, Sato et al. 1980, Hilton et al. 1982, Bennett et al. 1986), but Adams (1976) estimated a half life of 63 days in whole body of adult fathead minnows and muscle of rainbow trout, whereas Bertram and Brooks (1986) graphically showed a half life of about 49 days for adult fathead minnows exposed to selenium in the diet. In sub-adult bluegill and largemouth bass with elevated selenium concentrations in tissues, Lemly (1982) noted no significant decrease in selenium concentrations in muscle, liver, kidney, and spleen after 30 days depuration, which suggested a half life greater than 30 days. Similarly, Bryson et al. (1984) reported a half life of selenium residues in adult bluegill of about 60 days. Consequently, the slow depuration rate, i.e., >86 days, of selenium from muscle tissue in adult razorback sucker in the present study seemed realistic.

One fish in the present study (AC18) had increased selenium concentrations in muscle plugs after 31 days of depuration (23 µg/g) compared to spawning (19 µg/g), 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 fish and the selenium load redistributed to the whole body. However, this fish was spawned on April 23 and 25 so most of the eggs should have been expelled, thus limiting the amount that might be resorbed. Hamman (1985) reported that of 70 hatchery-reared razorback sucker females stripped at 24-hour 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.

The concept of depuration may be misleading in the natural environment because those 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. Sorensen (1988) reported that selenium tissue residues in fish from Martin Lake, TX, were only 25% lower after a 5-year period (1981-1986) following the drastic reduction of selenium inputs to the lake in 1978. In 1983, 4 years after selenium inputs to Martin Lake, TX, were stopped, selenium concentrations in ovary of redear sunfish were 20-24 µg/g (reported as wet weight, converted to dry weight assuming 75% moisture) (Sorensen and Bauer 1984), which are two times higher than the toxicity threshold of 10 µg/g in ovary or eggs. Likewise, Lemly (1997b) assessed selenium concentrations in five ecosystem components of Belews Lake, NC, 10 years 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 teratogenic deformities that were first observed in 1992 (Lemly 1993c) were still presented at elevated levels in 1996.

Depuration does not seem to be occurring in endangered fish in the Colorado River near WWSWA, Grand Junction, CO, because Colorado pikeminnow recaptured over a 2 or 3-year period seemed to be conserving selenium concentrations in muscle plugs from year to year, i.e., if the selenium concentrations were elevated 1 year, they were elevated in subsequent years (Osmundson et al. 2000). Waddell and May (1995) and Stephens and Waddell (1998) did not collect muscle plugs from any adult razorback sucker that were recaptured, thus it is unknown if any changes in selenium concentrations have occurred over time (B. Waddell, USFWS, personal communication).

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 (approach behavior, leading behavior, lateral display, tail-beating, vibrating, butting) were not altered in fish exposed to selenium concentrations up to 30,000 µg/L for 24 hours (Pyron and Beitinger 1989). Ogle and Knight (1989) reported no effects on number of spawns in fathead minnow exposed up to 30 µg/g selenium in the diet for 98 days. Hermanutz et al. (1992) reported no effect on the number of active nests for bluegill exposed to 10 µg/L selenium in experimental streams for 40 weeks. Others have also reported no effect of selenium exposure in water, diet, or both on fish spawning behavior (Coyle et al. 1993, Bryson et al. 1984). However, Ogle and Knight (1989) reported no spawning occurred in adults fed 40 µg/g selenium in the diet, which was similar to the concentration of selenium in zooplankton and aquatic invertebrates from North Pond.

We cannot state whether natural spawning would have been successful or not in the present study because fish were induced to spawn by injection of hormones followed by hand stripping of eggs and milt. However, negative effects of selenium on fish reproduction in the upper Colorado River basin have been observed at Sweitzer Lake where few native or nonnative fish have been found to reproduce (Barnhart 1957, Birkner 1978). At Sweitzer Lake, schools of young fish were observed in the fall following earlier observations of spawning by fathead minnow, red shiner (*Cyrinella lutrensis*), and plains killifish (*Fundulus zebrinus*) (Barnhart 1957). Although flannelmouth sucker were abundant in the lake, no young fish were observed (Barnhart 1957).

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 of razorback sucker in the Green River has been somewhat successful because razorback sucker larvae have been collected during several years of monitoring. During 1992-1996 1,735 larvae were collected in the middle Green River and 440 larvae collected in the lower Green River (Muth et al. 1997). In 1994, 11 yearlings were captured in Leota Bottoms on the Green River (Modde and Wick 1997), and in 1993, one yearling was captured in the Yampa River (T. Modde, USFWS, written communication).

Selenium and other elements in eggs

Lemly (1996b) proposed a selenium toxic threshold of 10 µg/g in eggs or ovary of fish for reproductive failure based on a review of several studies. 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, minimal hazard at 3-5 µg/g, low at 5-10 µg/g, moderate at 10-20 µg/g, and high at >20 µg/g. In the present study, mean selenium concentrations in eggs from razorback sucker at Adobe Creek were 40.1 µg/g and at North Pond were 54.7 µg/g, which were two times greater than Lemly's high hazard and four times higher 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 eggs from brood stock had 6.9 µg/g, which falls in the low hazard category of Lemly (1995).

In the previous reproduction study with razorback sucker, there seemed to be an

incongruity in selenium concentrations in fish eggs from Adobe Creek (46.5 µg/g) and North Pond (37.8 µg/g) because North Pond had higher selenium concentrations in water (4-20 µg/g), sediment (7-55 µg/g), and fish muscle plugs (16.6 µg/g) than did Adobe Creek (water 1.5-12 µg/g, sediment 0.5-2.1 µg/g, and fish muscle plug 11.7 µg/g) (Hamilton et al. 2001). However, North Pond had similar selenium concentrations in zooplankton (27.1 µg/g), and chironomids (11-45 µg/g) compared to Adobe Creek (zooplankton 28.5 µg/g, and chironomids 28-45 µg/g). The higher selenium concentrations in eggs of Adobe Creek fish may have been due to the substantially higher selenium concentrations in zooplankton at Adobe Creek during September 1995 (49.6-55.6 µg/g) compared to North Pond 25.2-32.5 µg/g). If razorback sucker begin deposition of egg yolks in the fall prior to spawning, the fall time period could be a critical time for deposition of selenium in eggs.

In the present study, North Pond adults had higher selenium concentrations in eggs (54.7 µg/g) than Adobe Creek adults (40.1 µg/g). This relation in egg selenium between sites 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 fish muscle plugs (25.5 µg/g) compared to 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 fish muscle plugs (16.6 µg/g). If September were a critical period for selenium deposition in eggs as hypothesized above, selenium concentrations in eggs in the present study followed the same pattern in zooplankton during September 1996: 20.0 µg/g at Adobe Creek and 33.9 µg/g at North Pond.

If razorback sucker have a similar three-part reproductive cycle as rainbow trout (i.e., spawn in December, previtellogenesis in December-March, endogenous vitellogenesis in May-July, and exogenous vitellogenesis in July-December; Bun Ng and Idler 1983), then the cycle for razorback sucker near Grand Junction, CO, would be spawn in May, previtellogenesis in May-August, endogenous vitellogenesis in August-December, and exogenous vitellogenesis in December-May. The vitellogenic phase of oocyte growth and protein incorporation can last 9 or more months (Tyler and Sumpter 1996). Vitellogenesis is the phase when selenoproteins are incorporated into oocytes in fish (Kroll and Doroshov 1991). Selenium in fish eggs is carried as part of the yolk precursor proteins, lipovitellin and phosvitin, and is incorporated into egg immunoglobulin and vitellogenin (Kroll and Doroshov 1991). These proteins are produced in the liver of fish (Bun Ng and Idler 1983), and the liver of fish accumulates more selenium than most other tissues types in fish (Adams 1976, Lemly 1982, Sager and Cofield 1984).

Bryson et al. (1985) studied the uptake of selenium in the gonads of bluegill and reported that long-term exposure of fish was less important than exposure during the maturation of the ovaries. Navas et al. (1997) also reported that a critical time for the development of high quality eggs was during vitellogenesis. They found that European sea bass (*Dicentrarchus labrax*) had higher quality eggs if females were fed a lipid-enriched diet during vitellogensis than if females were fed during previtellogenesis or prior to spawning. In an analogous manner, a critical exposure time of female razorback sucker in September 1995 (i.e., during endogenous vitellogenesis) could have occurred when selenium concentrations were higher in zooplankton from Adobe Creek (49.6-55.6 µg/g) than at North Pond (25.2-32.5 µg/g), and resulted in higher selenium concentrations in eggs in the previous reproduction study (Hamilton et al. 2001).

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 sucker 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), whereas one study reported effects at similar selenium concentrations (Coyle et al. 1993).

Selenium concentrations in eggs of razorback sucker have also been reported to be elevated in the Green River. Hamilton and Waddell (1994) reported that selenium concentrations were 3.7, 4.7, and 10.6 µg/g in eggs (4.4, 7.1, and 32.0 µg/g, respectively, in muscle plugs) from three wild adult razorback sucker collected in 1992 from Razorback Bar in the Green River. These selenium concentrations were similar to those reported in eggs of razorback sucker near Razorback Bar in 1988 (4.9 µg/g, Peltz and Waddell 1991), but lower than those in 1992 (28 µg/g, Waddell and Wiens 1992). This latter selenium concentration of 28 µg/g was comparable to those in fish from Adobe Creek (40.1 µg/g) and North Pond (54.7 µg/g). Hamilton and Waddell (1994) concluded that the selenium concentrations in eggs of two razorback suckers (10.6 and 28 µg/g) were sufficiently elevated, i.e., exceeded the proposed toxic threshold of 10 µg/g (Lemly 1996b), to suspect reproductive problems may be contributing to the decline of razorback sucker in the upper Colorado River basin. The only other report of selenium concentrations in gonads from an endangered fish in the upper Colorado River basin was from a Colorado pikeminnow collected in 1982, which had 6.5 µg/g (Peltz and Waddell 1991). Kroll and Doroshov (1991) reported elevated and variable selenium concentrations in eggs (3.1-29.3 µg/g) of wild white sturgeon (*Acipenser transmontanus*) caught in the Sacramento River and San Francisco Bay, CA, which were similar to the wide variation reported for razorback sucker (3.7-28 µg/g) (Peltz and Waddell 1991, Waddell and Wiens 1992, Hamilton and Waddell 1994).

In the present study, the mean selenium concentration in eggs from fish at Horsethief was 6.0 µg/g (4.6 µg/g in muscle plug), whereas eggs from fish at Adobe Creek contained 40.1 µg/g (16.6 µg/g in muscle plug) and at North Pond contained 54.7 µg/g (25.5 µg/g in muscle plug). Because muscle plugs from the captive-held adults in the present study contained lower selenium concentrations than in 27-33% of the wild adults collected from the Green River (Waddell and May 1995, Stephens and Waddell 1998), and the selenium concentrations in eggs were higher than in muscle plugs of the captive adults, it seems reasonable to assume that wild adults with elevated selenium concentrations in muscle plugs would probably contain elevated selenium concentrations in eggs, which would probably result in elevated selenium residues in larvae and in their reduced survival.

Although three elements other than selenium (boron, lithium, strontium) were significantly elevated in water at North Pond in the present study, 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, 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, three inorganic elements measured in eggs (manganese, phosphorous, and selenium) were significantly different among the Horsethief, Adobe Creek, and North Pond (Hamilton et al. 2001). The same 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, and by Bryson et al. (1984, 1985) for arsenic,

copper, cadmium, and zinc in gonads of bluegill. Nakamoto and Hassler (1992) reported similar concentrations of aluminum, arsenic, barium, boron, beryllium, cadmium, chromium, copper, iron, lead, manganese, molybdenum, nickel, strontium, titanium, thallium, vanadium and zinc in gonads of bluegill as in the present study. However, in the previous study (Hamilton et al. 2001), concentrations of aluminum, barium, iron, titanium, and thallium were substantially higher than those in the present study or reported in Nakamoto and Hassler (1992).

The elevated copper in eggs from young Horsetheif adults may be related to the high copper concentration in sediments at Horsethief reported in the previous reproduction study (Hamilton et al. 2001). The elevated copper concentrations in Horsethief sediments may have been 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 whole-body of bluegill collected from an area dominated by irrigation return flow near Kesterson Reservoir, CA, but not in gonads, which contrasts with the present study. Moller (1996) has 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 (Bun Ng and Idler 1983), and because the liver is the site of manganese and selenium accumulation, manganese may be incorporated into the yolk proteins similar to selenium, which in turn is incorporated into the egg. The significance of manganese in fish eggs is unknown. Overall, the magnitude of difference in barium, copper, iron, and manganese concentrations in eggs among adults held at the three sites was low (1.4-3.2) compared to the 14-15 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 to 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. 2001). The diameter of eggs from young adults in the present study (2.43-2.57 mm) was larger than from these adults in the previous study (2.17-2.32 mm). Although the difference in egg size seemed to be due to adult age in the present study, McFarlane and Franzin (1978) have reported that egg size, egg and larval survival, and adult longevity were reduced in white sucker from a lake with cadmium, copper, and zinc contamination compared to white sucker from a physically and chemically similar lake located nearby.

The difference in egg size between older brood stock and the young adults used in the present study suggested potential differences in fitness of the resulting larvae. Based on studies with salmonids, older females (which typically are also larger) generally produce larger eggs and fry compared to younger females (Buss and McCreary 1960, Gall 1974, Pitman 1979, Kazakov 1981, Springate and Bromage 1985). Moreover, for females of the same age, larger eggs produced larger fry that had better swimming stamina than those from smaller eggs (Ojanguren et al. 1996). Larger fry hatched from larger eggs tend to have a faster growth rate than those from smaller eggs (Gall 1974, Pitman 1979), although one investigator reported no difference in growth rate between fry from larger or smaller eggs (Springate and Bromage 1985). Larger eggs

produce fry with higher survival than those from smaller eggs (Bagenal 1969, Marsh 1986, Hutchings 1991), whereas others have reported no difference in survival (Springate and Bromage 1985), or decreased survival (Fowler 1972, Beacham and Murray 1985) due possibly to dissolved oxygen problems because large eggs have a lower surface to volume ratio for oxygen exchange. Still others have reported that larger eggs produced larger fry that had better burst swimming and predator avoidance (Taylor and McPhail 1985), and increased fitness (Hutchings 1991). Similar findings have been reported for non-salmonids (Baynes and Howell 1996, Marsh 1986). The range of egg sizes measured in the present study (2.43-2.57 mm for young adults; 2.94 mm for older brood stock) was similar to those reported by McAda and Wydoski (1985) for flannelmouth sucker (2.39 mm). Toney (1974) reported egg size in wild razorback sucker (perhaps as many as 40 fish) was 2.1 to 3.2 mm in diameter, but fish age was unknown. Also, some of the eggs measured by Toney (1974) may have not been fully mature, and 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 sucker (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.

The only significant difference in percent moisture in eggs in the present study was between North Pond fish (93.2%) and brood stock (91.6%). In the previous reproduction study there was 20-22% higher percent moisture in eggs from young adults compared to eggs from brood stock (Hamilton et al. 2001). The influence of the percent moisture in eggs on the fitness of the egg and resulting fry is unclear. Ojanguren et al. (1996) reported that percent moisture in eggs from brown trout (*Salmo trutta*) ranged from 55.3 to 61.4%, but this small range of variation (6%) was unrelated to female size. In the present study, the variation in moisture content of eggs between brood stock and young adults held at North Pond was relatively small (1.6%) and probably was not biologically significant.

The adults used in the present study were second-time spawners, which may have produced eggs and resulting fry of higher quality than those produced by first time spawners in the previous reproduction study. Brooks et al. (1997) reviewed the literature and reported that females produce better quality eggs in their second season, survival of eyed eggs was better for second time spawners than first timers, and survival to hatch increased in successive spawning seasons.

EGG STUDY

This section of the report will describe the methods, results, and discussion of the egg study portion of the reproduction study.

Methods

Adult razorback sucker previously held for 9 months at either Horsethief, Adobe Creek, or North Pond and brood stock held at Horsethief were spawned on April 23, 1997. Eggs used in the present study came from three spawns of Horsetheif adults, Adobe Creek adults, and North Pond adults, and two spawns of brood stock. A detailed description of the spawning procedure is given in the Adult Study.

After the eggs water hardened and held overnight at 24-Road, a group of eggs from each spawn was transferred to the mobile laboratory for the egg study. The laboratory was housed at the CRFP garage in Grand Junction, CO. For each spawn (three each for Horsethief, Adobe Creek, and North Pond, and two for brood stock [PIT 7F7B020404 and PIT 7F7B1B706D]),

eight groups of 25 eggs were placed in incubator cups for survival and hatchability determinations. Fertilized eggs that looked normal were randomly assigned to a hatching cup five 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 (25 µm polypropylene bag filter, Filter Specialists, Inc., Michigan City, IN) water. 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 where the parents were exposed prior to spawning. The beakers were arranged in three rows on each table and their position was randomly assigned using a random numbers table.

Incubator cups and 2-L beakers were acid-cleaned before use and calibrated at the 600-ml and 1.2-L levels. The incubation cups were 250-ml (pint) jars with the bottom removed and a polypropylene filter cloth (285 µm openings) glued with silicon adhesive to the bottom. They were suspended by a latex handle in beakers containing 1.2 L of filtered 24-Road water. After 2 hours, 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 study were collected every day from each site as grab samples using two 19-L carboys. Water was prefiltered 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 sampled five times and analyzed for general water quality characteristics. During the egg study, water quality measurements include pH, conductivity, hardness, alkalinity, calcium, magnesium, chloride, and ammonia. One filtered (0.4 µm) and one unfiltered water sample 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, and examined using a dissection scope to record the number of live and dead eggs, and the number and type of deformities. All dead eggs were removed daily. When the eggs began to hatch, the number of live and dead larvae, and the number and type of deformities were recorded daily and all dead larvae removed. The study was terminated at 1 day posthatch due to high mortality in all egg cups. The mortality of eggs was probably due to a temperature acclimation problem at 24-Road that occurred prior to stocking eggs in the egg cups. During the first egg study, dissolved oxygen concentrations ranged from 7.3 to 7.4 mg/L with 82.7 to 84.4% saturation, and temperature ranged from 20.0 to 21.0 ºC.

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 study initiated. In this study, 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, the eggs were held overnight in the mobile laboratory before being counting into the egg cups for the egg study. Eggs from one brood stock spawn (PIT 7F7F36275F) were collected from those held at 24-Road and used in the egg study. They were stocked at 2 days post spawn. Eggs were initially held in 600 ml of Horsethief water, and after 2 hours, 300 ml of the appropriate site water or reference water was added, followed 2 hours later by the addition of another 300 ml of the appropriate test water. Other procedures were as described above.

A checklist was maintained at the mobile laboratory during the egg study and each item

on the checklist (i.e., lights, aeration, etc.) was checked daily to document test conditions. Temperature in 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 as close to 20°C as possible. Eggs and larvae were exposed under flourescent 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 hours light:12 hours dark. Newly hatched larvae were not fed because of the short duration of the study.

Viability of eggs (i.e., defined as alive and developing) was measured on a group of about 100 eggs from the first spawning on April 23, 1997 (three spawns each from adults held at Horsethief, Adobe Creek, and North Pond). Eggs were maintained at 24-Road for 1 day before checking viability. A sample of eggs was removed from the incubation bucket and placed in a small petri dish marked into eight sections on the outside bottom. One person counted the number of live and dead embryos in the dish using dissection microscope set at 7x magnification. Viability was measured in eggs from the second spawning on April 25 (three spawns each from adults held at Horsethief, Adobe Creek, and North Pond) as described above. Eggs were maintained at 24-Road for about 36 hours before checking percent viability.

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, and three spawns from Horsethief, Adobe Creek, North Pond adults, and one 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 the full scale, 50%, and 20% scale calibration distances.

Methods for water quality analysis, selenium analysis, and statistic analysis of data were the same as those described in the Adult Study.

Results

For the first spawn, percent viability of 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, on the day after starting the egg test, even though normal-looking eggs were used in the test. Examination of eggs revealed oil globules in 60 to 95% of the eggs, i.e., lipid compounds apparently separated from the yolk. For the second spawn, percent viability of eggs held about 36 hours at 24-Road was 5% in one spawn, 2.7% in a second spawn, and ≤1% in the other seven spawns. The low viability of eggs in the first and second spawns was probably due to a temperature acclimation problem at 24-Road that occurred prior to accessing egg viability. Similar low viability occurred in brood stock spawns where 15 spawns had less than 1% viability, three had 20%, one had 50%, and three had about 80%. 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 percent hatch or survival.

In the egg study, eggs from brood stock PIT 7F7F36275F (spawn number 97-16, 80% viability) had no measurement of selenium made due to the limited number of eggs available for use in the egg study. Egg diameter was 3.01 mm, and the selenium concentration in muscle plug

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

Water quality characteristics were consistent within each water type during the 5-day egg study (Appendix H), but differed significantly among the four types (24-Road, HT, AC, WW) (Table 12). Each water type was significantly different from each other 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 and percent saturation ranged from 83 to 84%, and mean water temperature ranged from 20.8 to 21.3°C. Selenium concentrations (n=2) measured in water were 1.2-2.2 µg/L at Horsethief, 1.4-2.4 µg/L at Adobe Creek, 12.0-13.4 µg/L at North Pond, and <1 µg/L at 24-Road.

There was no significant difference in percent survival or percent hatch of eggs among the three egg sources (Horsethief, Adobe Creek, and North Pond) held in 24-Road water or in site water (Table 13). Percent survival or percent hatch of eggs from brood stock (n=1) did not seem to be different from the other three sources of eggs (Table 13).

The lack of significant differences in egg survival and hatch among the three egg sources may have been due to the variation in survival and hatch among the three replicates for the nine sets of eggs tested (Appendices I, J, K, L). There was a significant correlation between percent hatch and percent survival of eggs (r=0.92, P=0.0001, n=20), but not between survival and selenium concentrations in eggs (r=0.42, P=0.08, n=18) or in adult muscle plug (r=0.19, P=0.43, n=20) or between hatch and selenium concentrations in eggs (r=0.43, P=0.08, n=18) or in adult muscle plug (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), which suggested that larger eggs had better survival and hatch.

Deformities were recorded by type rather than by individual fish, and therefore, no quantitation of deformities was possible. However, from a semi-quantitative and qualitative viewpoint, larvae from brood stock had the most deformities recorded, followed by larvae from North Pond adults (about two-thirds of the notations for broodstock larvae), Adobe Creek adults (half the notations as for North Pond larvae), and the least deformities in Horsethief larvae (a third of the notations as for Adobe Creek larvae or a sixth of the notations as for North Pond larvae). Deformities in brood stock were observed at about the same time of development concerning they were stocked in the test at 2 days posthatch and eggs from the young adults were stocked at 1 day posthatch. 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) and spinal deformities (curved tail, and lateral, ventral, or dorsal flexures) that were observed on days 2

Table 12. Mean (standard error in parentheses, n=5) water quality characteristics measured in water used in the egg study. For each measure, stations with the same letter are not significantly different (*P*=0.05).

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

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

Table 13. Mean (standard error in parentheses and number of samples in brackets) percent survival and hatch of eggs in the egg study. Eggs that looked "normal" were selected for the test and were held in either reference water (R: 24-Road) or site water (S) during the 5-day test, and included eggs from brood stock (BS). Differences among sites within either water type were not significant (*P*=0.05).

	Site and water type							
	HT		AC		WW		BS275F[1]	
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]

[1]PIT 7F7F36275F.

through 5 of the egg 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. For larvae from Horsethief, Adobe Creek, and North Pond adults, there were more deformities recorded for larvae held in reference water than those held in site water, whereas for brood stock there were similar numbers of deformities in the two water types. Larvae from Horsethief adults had more abdominal edema, larvae from Adobe Creek adults had more cardiac edema, 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

The low percent viability of eggs from the first and second spawns was probably due to the temperature change experienced by the eggs in the move from the Horsethief spawning facility where water temperature was about 12ºC and the 24-Road facility where the water temperature was about 24ºC. Blaxter (1969) and Piper et al. (1982) reported that sharp temperature changes can cause mortality of eggs. ASTM (1992a) recommends that water temperature acclimation should be changed at a rate not to exceed 3ºC within 72 hours. For the second spawn, ice was added to one tank at 24-Road to lower the water temperature closer to that at the Horsethief spawning facility, but the 24ºC room temperature exceeded the capacity to cool the water. Egg viability was not determined on eggs transferred directly from the Horsethief spawning facility and acclimated to conditions in the mobile laboratory.

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

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). Although selenium concentrations in eggs and water quality of the site waters differed significantly, neither apparently affected percent survival or percent hatch of eggs because there were no significant differences in those measures among the test groups. These results were similar to other selenium studies with fish. Hamilton et al. (2001) also reported no significant correlations between hatch and selenium concentrations in adult muscle plugs or between survival and selenium concentrations in adult muscle plugs or eggs. Crane et al. (1992) reported no effects on fertilization rate or time to first hatch in eggs from yellow perch exposed to 2 or 10 µg/L selenium in ponds for 288 days. However, they reported that no eggs hatched from the 25 µg/L treatment where selenium concentrations in adults were 23 µg/g in muscle and 28 µg/g in gonads

(both reported as wet weight, converted to dry weight assuming 75% moisture). Gillespie and Baumann (1986) reported no effects on fertilization or hatch in bluegill eggs containing 27-32 µg/g selenium (reported as wet weight, converted to dry weight assuming 75% moisture). Woock et al. (1987) observed no effect on hatching success of eggs from bluegill exposed to selenium concentrations up to 30 µg/g in diet and 30 µg/L in water for 260 days. Ogle and Knight (1989) reported no effects on the number of eggs per spawn or percent hatch in fathead minnows exposed to selenium concentrations up to 30 µg/g in diet for 98 days. Coyle et al. (1993) reported no effects on the number of eggs per spawn or hatchability of eggs from adult bluegill exposed to selenium concentrations up to 10 µg/L in water and up to 33 µg/g in diet for 60 days.

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 (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 hatch of fathead minnow eggs (Halter et al. 1980). Hodson et al. (1980) reported that exposure of fertilized rainbow trout eggs to 28 µg/L selenium in water caused a small (3.5%), but significant decrease in hatch. Saiki and Ogle (1995) reported that western mosquitofish (*Gambusia affinis*) (whole-body selenium >100 µg/g) from the San Luis Drain, CA, which contained 340-390 µg/L selenium, had a lower mean percentage of live births within broods and produced less fry than adults from a reference area with selenium concentrations of 0.4 µg/L in water and <1.6 µg/g in fish.

Deformities in eggs and larvae

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 to typical background deformities rates of <1-3% (Gabriel 1944, Gill and Fisk 1966, Patten 1968, Dahlberg 1970). The amounts of deformities in razorback sucker larvae from Adobe Creek, North Pond, and brood stock adults seemed to be higher than the range of 9 to 11% suggested by Bengtsson (1975) as abnormal and probably due to a “man-made” effect.

Physiological abnormalities in fish can take many forms including scoliosis, vertebral deformities, cranial deformities such as pugheadedness and deformed operculae, fin deformities including fin erosion, and genetic anomalies (Hickey 1973). Anomalies resulting in deformities can occur at several points during development including mutation or recombination of genes, during embryonic or larval development, or during the adult life stage. Most deformities develop during the sensitive early life stages of animals. Causes of deformities in fish can include incubation at inappropriate water temperature, low dissolved oxygen concentration, elevated salinity (for freshwater fish), exposure to X-irradiation or ultraviolet irradiation, diet deficiencies of vitamins such as vitamin C, mineral deficiencies, hormone-induced ovulation, and toxic substances such as pesticides (DDT, 2,4-dinitrophenol, oxadiazon, fenitrothion, toxaphene, Kepone, trifluralin, malathion, parathion, other organophosphate compounds), inorganic elements (cadmium, lead, selenium, zinc) (Bengtsson 1975, Hickey 1973, Sindermann 1979, Hiraoka and Okuda 1983, Lemly 1997a).

The deformities in larvae from the young adults were highest in North Pond larvae,

intermediate in Adobe Creek larvae, and lowest 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 1993c, 1997a, 1997b). Fish deformities include lordosis (concave curvature of lumbar and caudal regions of spine), kyphosis (convex curvature of thoracic region of the spine), scoliosis (lateral curvature of the spine), 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 1993c, 1997a, 1997b, Saiki and Ogle 1995, Hamilton et al. 2001). 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, NC, 10 years after the selenium inputs were stopped (Lemly 1997b).

Lemly (1997a) 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 goes on to state that selenium concentrations of 10-20 µg/g or greater in whole-body or 6-12 µg/g in muscle, would be sufficient to confirm the diagnosis. In the present study, the most deformities in embryos and larvae from young adults were from fish held at North Pond, with lesser occurrences in larvae from fish held at Adobe Creek (half the deformity notations as for North Pond larvae), and the least deformities in larvae from fish held at Horsethief (a third of the notations as for Adobe Creek). 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 the teratogenesis ratings of Lemly (1997a), selenium-induced teratogenesis would have been expected from selenium exposure. These results were similar to the previous reproduction study where there were 12-26% deformities in embryo-larvae from Adobe Creek adults and 20-27% in larvae from North Pond adults. An adverse impact on the reproductive success would be expected from selenium exposure in the previous reproduction study based on the teratogenesis rating of Lemly (1997a) because of the elevated selenium concentrations in eggs (46 and 38 µg/g, respectively), muscle plugs (12 and 17 µg/g, respectively), and muscle tissue (16 and 29 µg/g, respectively) of adults (Hamilton et al. 2001).

The high number of deformities observed in larvae from brood stock or young adults in the present study may have been due to inbreeding effects, stress from handling, or a combination. Dowling et al. (1996) reported that, based on mitochondrial DNA diversity, razorback sucker examined from the upper Colorado River seemed to be from a small population, were 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 to be old and perhaps past their prime in terms of producing good quality eggs. Old age of brood stock is a concern expressed by some fishery workers involved in endangered fish propagation (C. Figiel, USFWS; R. Hamman, USFWS; S. Severson, USFWS; H. Williamson, USFWS; personal communications). As an example, R. Hamman (USFWS, Dexter NFH and Technology Center, Dexter, NM) has noted that the 1974

year class brood stock of Colorado pikeminnow produced good quality eggs and larvae at 10-20 years of age, but after age 20, egg viability has decreased.

Although inbreeding and old age are possibilities, the most likely stress causing the deformities was the temperature change. Temperature stress can kill eggs as demonstrated by the complete mortality of eggs from the young adults spawned in the present study at the Horsethief spawning facility where water temperature was about 12ºC and held at 24-Road where the water temperature was about 24ºC. Temperature stress can also cause deformities in hatched fish larvae as can numerous other stresses including genetics, low dissolved oxygen concentrations, dietary deficiencies, ultraviolet irradiation, salinity, and organic and inorganic contaminants (Seymour 1959, Turner and Farley 1971, Hickey 1973, Sindermann 1979).

The greatest number of deformities occurred in brood stock larvae that were spawned at Horsethief, then held at elevated water temperatures at 24-Road before transfer to the mobile laboratory for use in the egg study compared to the young adults spawned at Horsethief then transferred to the mobile laboratory. Of 22 spawns of brood stock held at 24-Road in 1997, three had about 80% viability, one had 50%, three had 20%, and the remaining 15 had less than 1%. The egg study was conducted with eggs from one of the spawns with 80% viability. This high level of viability does not preclude the appearance of deformities from temperature stress.

It should be noted that eggs from various sources used in the egg study were hand selected using "normal" appearance as the criterion. Criteria for use of fish and fish eggs in laboratory studies is that they are free of disease, uniform in size and age, have good nutrition, and holding conditions (temperature, water quality, photoperiod, uncrowded, minimal handling) (ASTM 1989). The guidelines further state that organisms should be free of signs of stress, physical damage, mortality, and external parasites, and that abnormal or injured individuals not be used in tests, nor should organisms that exhibit abnormal behaviors such as not eating, flashing, flipping, swimming erratically, gasping at the surface, hyperventilating, hemorrhaging, producing excessive mucus, or showing abnormal color. These types of guidelines were developed for use with routinely tested organisms such as rainbow trout and fathead minnow with well-known culture procedures, past successful use in testing, and ease of handling. Consequently, toxicant concentrations that cause effects in razorback sucker eggs hand-selected according to standard testing guidelines (ASTM 1989) could tend to underestimate toxicant impact in the environment where a variety of environmental and organismal conditions would occur.

Deformities have been reported in threatened or endangered fish in the Colorado River basin or reared in hatcheries. Fin deformities were reported in razorback sucker larvae reared at the Colorado Division of Wildlife's Fish Research Hatchery, Bellvue, CO, in 1993, and again during a feeding study in 1994 (Martinez 1996). She concluded that the 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. The fin deformity was not observed in larvae in the egg study, nor in the fish from the first reproduction study (Hamilton et al. 2001).

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 between 0 and 83% in larvae fed different diet series for 127 days posthatch. The diet that had 0% deformities in one replicate also 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 due to vitamin deficiency associated with cold storage of the diets.

Deformities in razorback sucker larvae produced and reared at Dexter NFH and Technology Center, Dexter, NM, are a problem that needs to be addressed (H. Williamson, USFWS, personal communication), and may be related to nutritional deficiencies (R. Barrows, USFWS, personal communication to H. Williamson). On the other hand, few deformities have been noted in razorback sucker larvae produced from wild adults spawned at Willow Beach NFH, AZ (C. Figiel, USFWS, personal communication).

Inbreeding

The young razorback sucker adults used in the reproduction studies in 1997 (present study) and in 1996 (Hamilton et al. 2001) came from a single mating in 1992, and thus the spawnings in 1996 and 1997 were of brother and sister. Progeny could have exhibited inbreeding effects such as reduced growth rate, lower survival, reduced feed conversion, and reduced fertility (Moav and Wohlfarth 1966, Ryman 1970, Kincaid 1976a, 1976b, Piper et al. 1982).

In the present egg study, the percent hatch and percent survival of embryos were not significantly different between embryos from the young adults held at the three sites and embryos from brood stock. Lack of differences in hatch and survival suggests that inbreeding, if present, probably did not influence these measurements. Based on semi-quantitative and qualative measures of deformities, larvae from brood stock had the most deformities, followed by larvae from North Pond, Adobe Creek, and the least from Horsethief. For larvae from the young adults, this pattern suggests an effect from parental exposure. The larvae from brood stock may have had the most deformities due to the water temperature problem at 24-Road.

In the first reproduction study (Hamilton et al. 2001), the percent viability, percent hatch, and mortality of deformed embryos were not significantly different between embryos from the young adults held at the three sites and embryos from brood stock. The percent deformities of embryos and newly hatched larvae was not significantly different among the three groups of young adults, except there were more deformed embryos from Adobe Creek adults held in site water than in reference water, thus suggesting an effect from site water exposure for Adobe Creek larvae. The range of percent hatch and percent survival in the present study and in the first reproduction study (Hamilton et al. 2001) were similar to those reported for razorback sucker embryos and newly hatched larvae (Inslee 1982, Hamman 1985, Muth and Ruppert 1996).

LARVAL FISH STUDY

This section of the report will describe the methods, results, and discussion of the larval fish portion of the reproduction study.

Methods

No larvae were available from Horsethief, Adobe Creek, or North Pond adults. Larvae from one brood stock female (PIT 7F7D163436; 605 mm total length, 2.6 kg) spawned on April 23, 1997, were used in the study. Larvae were cultured at 24-Road for about 4 days before being transported to the mobile laboratory for the larval fish study. Brood stock adults were held at Horsethief before spawning.

A detailed description of the spawning procedure is given in the Adult Study.

The study was conducted for 30 days to assess survival, growth, development, and whole-body residues of selenium in brood stock larvae. The study was initiated with 5-day-old larvae (11 mm total length, 0.004 g, n=20). After the egg study was completed, the 2-L beakers were acid washed and recalibrated at the 800-ml and 1.6-L levels for use in the larval fish study. Larvae were impartially distributed two at a time to eight exposure vessels until there were 10 larvae per beaker. The larvae were initially held in beakers containing 800 ml of filtered (25 µm polypropylene filter bag) 24-Road water. After 4 hours, an additional 800 ml of test water was added. Fifty percent of the water (800 ml) was renewed daily.

Exposure beakers with larvae were held on each of two tables in the mobile laboratory. The beakers were arranged in three rows on each table and their position randomly assigned based on a random numbers table. One set (three beakers termed replicate a, b, and c) of larvae were held on each table and were exposed to one of 16 treatments in a four by four factorial experiment (four water treatments and four food treatments) as follows:

1. reference food (brine shrimp: BSH) and reference water from 24-Road,
2. reference food and site water from Horsethief (HT; i.e, Colorado River water),
3. reference food and site water from Adobe Creek (AC),
4. reference food and site water from North Pond (WW),
5. Horsethief food (collected from Horsethief east wetland, HTEW) and reference water from 24-Road,
6. HTEW food and site water from HT,
7. HTEW food and site water from AC,
8. HTEW food and site water from WW,
9. AC food and reference water from 24-Road,
10. AC food and site water from HT,
11. AC food and site water from AC,
12. AC food and site water from WW,
13. WW food and reference water from 24-Road,
14. WW food and site water from HT,
15. WW food and site water from AC,
16. WW food and site water from WW.

The various treatment numbers are shown in a matrix as follows:

		Food treatment			
		BSH	HTEW	AC	WW
Water treatment	24-Road	1	5	9	13
	HT	2	6	10	14
	AC	3	7	11	15
	WW	4	8	12	16

Although the environmentally realistic treatments were considered numbers 6, 11, and 16, the purpose of the experiment was to determine the toxicity of the various combinations of foodborne and waterborne selenium.

Zooplankton were collected every other day from Horsethief east wetland (HTEW), Adobe Creek (AC3), and North Pond (WW2) using modified light traps (Espinosa and Clark

1972). Light traps were set overnight and the trapped zooplankton were collected the following morning. The zooplankton were transported to and stored in the mobile laboratory in 3.8-L plastic jars filled with site water. Oxygen concentrations in the jars of zooplankton held in the laboratory were supplemented by bubbling compressed air from an oil-less air compressor through air stones.

Larvae were fed either 40 zooplankters or 40 brine shrimp (*Artemia* sp.) nauplii per live fish once daily after water renewal. For the site food, about half of the zooplankton sample was sieved through a U.S. Standard Sieve #40 to separate zooplankton <0.425 mm in size for feeding test fish that day. The concentration of zooplankters (number of organisms per ml) in the feeding solutions was determined by counting the number of zooplankters in three separate 1-ml samples taken with a Hensen-Stemple pipette. The zooplankton were anesthetized with 1 ml of alcohol and counted using a stereoscope microscope at 7-30x magnification and a counting wheel (Ward's, Rochester, NY). The numbers of organisms in three replicate counts were averaged and the volume calculated to feed 40 organisms per live fish in each exposure beaker.

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

A 2-ml subsample of live zooplankton was collected from each feeding solution, placed in a small vial, and preserved in 70% ethyl alcohol. The zooplankton in these archived samples were later categorized as cladoceran, copepod, or other invertebrate type using a stereoscope microscope.

Survival of zooplankton from the three sites and brine shrimp was tested in the four test waters (24-Road, HT, AC, and WW) using the same exposure beakers as used in the fish test but without fish present (total 16 beakers: 4 foods x 4 waters). Each beaker had 1600 ml of test water and 400 organisms placed in them. Zooplankton survival was monitored after 24 and 48 hours. No water renewals or zooplankton renewals were done. The zooplankton survival test was repeated three times.

Test waters for the larval fish study were collected every day from each site as grab samples using two 19-L carboys. Water was prefiltered 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 sampled weekly and analyzed for general water quality characteristics. During the larval fish study, water quality measurements included pH, conductivity, hardness, alkalinity, calcium, magnesium, chloride, ammonia, nitrate, nitrite, sulfate, total suspended solids, volatile solids, and fixed solids. One filtered (0.4 µm) and one unfiltered water sample was collected at 3- to 4-day intervals from waters used in the renewals and analyzed for selenium concentrations.

Water renewal in the larvae study was accomplished by siphoning the water level down to the 800-ml calibration line using a siphon held inside a glass sleeve with the submerged end covered with a polypropylene filter cloth (285 µm openings) attached with a latex band. The

sleeve was used to prevent larvae from being siphoned out of the beaker and restrict the loss of zooplankton fed the previous day. The siphon was held stationary in the beaker to minimize disturbance of larvae and to decrease the likelihood of siphoning out small-sized zooplankton through the filter cloth. Exposure beakers were cleaned infrequently to remove any build-up of algae or other organisms on the walls using the glass sleeve prior to water renewal. Oxygen concentrations in exposure water were supplemented by passing compressed air from an oil-less air compressor through air stones.

The number of live fish in each beaker was recorded daily and all dead fish removed. Observations of fish behavior and the amount of uneaten live zooplankton were recorded daily starting at day 7 of the exposure. Dissolved oxygen concentration, conductivity, and water temperature were measured daily in one replicate of the 16 treatments held on each table.

Total length (TL) of individual fish and composite weight of 20 fish were measured at day 0 (mean 11mm TL, 0.004 g). Total length and weight of one fish from each replicate beaker of the 16 treatments held on both tables was measured at day 10. After the 30-day exposure period, the surviving fish in all treatments were measured for total length and weight. All fish were anesthetized with MS-222 (tricaine methanesulfonate) before growth measurements were made. All total length measurements were to the nearest mm using a measuring board, and weight was to the nearest 0.0001 g using a 4-place analytical balance.

After 10 days of exposure, a composite sample was formed by collecting one fish from each of six exposure beakers (replicates a, b, and c on each of two tables) for analysis of selenium concentrations by neutron activation. After 30 days of exposure, a composite sample was formed by collecting all remaining fish in the three replicates on one table (two composite samples per treatment). Composite samples contained from 2 to 23 fish depending on the number of live fish after 30 days of exposure. Fish were placed in Whirl-Pak bags and stored frozen until analysis of selenium concentrations.

A checklist was maintained at the mobile laboratory during the larval fish study and each item on the checklist (i.e., lights, aeration, etc.) was checked daily to document test conditions. Temperature in 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 as close to 20°C as possible. Fish were exposed under fluorescent lighting (one cool-white bulb and one wide-spectrum bulb in each light fixture) to a photoperiod that existed at the time of testing in Grand Junction, which approximated 12 hours light: 12 hours dark.

Methods for water quality analysis, selenium analysis, ICP analysis, and statistical analysis of data were the same as those described in the Adult Study.

Results

Water quality characteristics were consistent within a water type during the 30-day larval fish study (Appendix M), but differed significantly among the four water types (24-Road, HT, AC, WW) (Table 14). North Pond water had the highest pH, conductivity, hardness, calcium, magnesium, chloride, and sulfate, whereas Adobe Creek water had the highest alkalinity. Water from 24-Road had similar characteristics to that from Horsethief, except for pH, hardness, calcium, and fixed solids, which were lower in 24-Road water. In the exposure beakers, the mean dissolved oxygen concentrations in the four waters ranged from 7.0 to 7.2 mg/L. The mean water temperature in the exposure beakers for the four water types ranged from 21.7 to 22.2°C.

Table 14. Mean (standard error in parentheses and number of samples in brackets) water quality characteristics measured in water used in the razorback sucker larvae study. For each measure, stations with the same letter are not significantly different (*P*=0.05).

	Station			
Measure	HT1	AC5	WW2	24-Road
pH	8.6b (0.1) [27]	7.9a (0) [27]	8.7b (0.1) [27]	7.9a (0) [27]
Conductivity (µmhos/cm)	417a (9) [27]	957b (30) [27]	5,230c (75) [27]	454a (11) [27]
Hardness (mg/L as $CaCO_3$)	162b (2) [27]	323c (7) [27]	1,474d (36) [27]	68a (1) [27]
Calcium (mg/L)	45b (0) [27]	77c (1) [27]	155d (3) [27]	20a (0) [27]
Magnesium (mg/L)	12a (0) [27]	32b (1) [27]	264c (7) [27]	4a (0) [27]
Alkalinity (mg/L as $CaCO_3$)	113ab (1) [27]	181c (4) [27]	109a (5) [27]	123b (3) [27]
Chloride (mg/L)	29a (1) [14]	89a (5) [14]	590b (60) [14]	36a (2) [14]
Sulfate (mg/L)	83a (2) [13]	231a (17) [13]	2,336b (153) [13]	32a (1) [13]
Un-ionized ammonia (mg/L NH_3-N)	<0.01 (0) [12]	<0.01 (0) [12]	<0.01 (0) [12]	<0.01 (0) [12]

Table 14. Continued.

Measure	Station			
	HT1	AC5	WW2	24-Road
Nitrate (mg/L NO_3-N)	0.1a (0) [4]	0.2a (0.1) [4]	0.3a (0.1) [4]	12.6b (3.2) [4]
Nitrite (mg/L NO_2-N)	0.01a (0) [4]	0.03a (0.02) [4]	0.03a (0.01) [4]	0.09a (0.04) [4]
Total suspended solids (mg/L)	25.8a (4.7) [4]	8.5a (0.6) [4]	25.0a (10.7) [7]	<2 (-) [4]
Volatile solids (mg/L)	9.3a (2.1) [4]	3.6a (0.6) [4]	13.9a (6.1) [4]	<2 (-) [4]
Fixed solids (mg/L)	16.4b (2.8) [4]	4.8ab (0.4) [4]	11.0ab (6.6) [4]	0a (0) [4]

Larvae used in the study were from brood stock PIT 7F7D163436 (spawn number 97-05, 80% viability). Measurement of selenium or diameter in eggs from this female were not made due to the late selection of larvae from this spawn for use in the study. The selenium concentration in muscle plug from the female on April 23, 1997, was 11 µg/g. The selenium concentrations in this fish the previous year (July 11, 1996) were 7.1 µg/g in eggs and 13.8 µg/g in muscle plug (Hamilton et al. 2001).

Selenium in water

There was no significant difference in selenium concentrations between filtered and unfiltered water samples from HTi, AC3, or WW2. Mean selenium concentrations in water during the larval fish exposure were <1 µg/L for 24-Road, 1.6 µg/L (range 0.9-2.8) for Horsethief, 3.4 µg/L (range 2.1-5.8) for Adobe Creek, and 13.3 µg/L (range 4.3-19.4) for North Pond (Table 15). Waterborne selenium concentrations at the three sites were significantly different from each other when the data were combined for each site for the entire study. However, for each measurement day, HT and AC waters tended to have similar selenium concentrations except at day 6 and 28, whereas WW water was different from the other waters except at days 3 and 6 (Table 15). Just prior to the beginning of the exposure, selenium concentrations in water were 2.2 µg/L at Horsethief, 2.8 µg/L at Adobe Creek, and 13.4 µg/L at North Pond. On days 26, 27, and 28, water flowing into Adobe Creek from AC7 had selenium concentrations of 13.0, 12.6, and 13.2 µg/L, respectively.

Selenium and other elements in zooplankton and brine shrimp

Zooplankton from the Horsethief site were collected from the east wetland, which received effluent from the culture ponds. Mean selenium concentrations in zooplankton during the 30-day larval fish exposure were 6.0 µg/g (range 4.2-8.8 µg/g, n=17) at Horsethief east wetland, 32.4 µg/g (range 19.6-41.9 µg/g, n=17) at Adobe Creek, and 52.5 µg/g (range 36.2-60.2 µg/g, n=12) at North Pond (Table 16). Selenium concentrations in brine shrimp averaged 3.2 µg/g (range 3.0-3.6 µg/g, n=7). Zooplankton concentrations at the three sites were significantly different from each other and from brine shrimp during the larval fish exposure. Selenium concentrations in zooplankton at Horsethief east wetland prior to the beginning of the exposure were 4.4 µg/g on September 20, 5.4 µg/g on October 31, 5.0 µg/g on November 21, 1996, and 5.6 µg/g on April 22, 1997. Selenium concentrations in zooplankton at AC3 prior to the beginning of the exposure were 17.9 µg/g on April 8, 1997, and at WW2 they were 42.8 µg/g on April 8 and 37.9 µg/g on April 18, 1997. When the zooplankton data were pooled across the sites, the correlation coefficient between selenium concentrations in zooplankton and water was $r=0.78$ ($P<0.0001$, n=43).

Selenium concentrations in chironomids collected from Horsethief east wetland were 9.3 µg/g in a composite sample collected on September 30 and October 21, 1996, and 7.9 µg/g in a composite sample collected on April 21 and May 20, 1997. Selenium concentrations in chironomids from AC5 were 48.3 µg/g in a composite sample collected on September 18 and 30, 1996, and from AC3 were 33.6 µg/g in a composite sample collected on September 30 and October 5, 1996, 31.7 µg/g in a composite sample collected on October 21, and November 5, 1996, and 34.0 µg/g in a composite sample collected on April 21 and May 19, 1997. Selenium concentrations in chironomids were 1.4 to 2.0 times higher than in zooplankton collected from the same station at about the same time.

Table 15. Mean (standard error in parentheses, n=2) selenium concentration (µg/L) measured by AA in water used in the razorback sucker larvae study. Waters used in the study were sites HT1, AC3, WW2, and 24-Road (<1 µg/L in all samples). For each row, values with a letter in common are not significantly different (*P*=0.05).

Day of exposure	Station		
	HT1	AC3	WW2
3	2.2^{a} (0.6)	3.4^{ab} (0.6)	13.7^{b} (0)
6	1.4^{a} (0.2)	3.7^{b} (0)	4.6^{b} (0.3)
10	1.4^{a} (0.2)	3.4^{a} (0.6)	13.6^{b} (0.3)
14	1.5^{a} (0.4)	3.4^{a} (0.6)	14.0^{b} (1.5)
18	1.2^{a} (0.4)	3.0^{a} (0.2)	14.8^{b} (0.6)
21	1.3^{a} (0.1)	2.6^{a} (0.2)	16.9^{b} (2.5)
25	2.4^{a} (0)	2.3^{a} (0.2)	12.6^{b} (2.0)
28	1.8^{a} (0.2)	5.2^{b} (0.6)	16.0^{c} (0.8)

Table 16. Mean (standard error in parentheses and number of samples in brackets) selenium concentration (µg/g dry weight) in zooplankton collected from three stations near Grand Junction, Colorado.

Station	Day of exposure[1]				
	-10 to -24	1-7	8-14	15-21	22-29
HTEW	5.6 (0.1) [2]	4.6 (0.1) [5]	5.6 (0.2) [4]	6.1 (0.6) [4]	8.1 (0.4) [4]
AC3	17.9 (-) [1]	23.8 (1.7) [5]	36.9 (0.6) [4]	35.2 (2.2) [4]	35.8 (2.7) [4]
WW2	40.4 (2.4) [2]	41.9 (3.0) [4]	58.5 (1.1) [3]	57.8 (1.0) [2]	57.3 (1.3) [3]

[1]: Zooplankton samples were arbitrarily grouped into 7-day intervals for this table.

Eleven of the inorganic elements measured by ICP in zooplankton had significant differences among locations (Table 17). Brine shrimp had significantly lower concentrations of aluminum, barium, manganese, silicon, strontium, titanium, and vanadium than zooplankton from the three stations. In contrast, brine shrimp had significantly higher arsenic concentrations than the three stations. In general, zooplankton from Horsethief, AC3, and WW2 had similar trace elements concentrations except that zooplankton from Horsethief east wetland had significantly more arsenic and less molybdenum than zooplankton from AC3 or WW2.

Types of zooplankton

The types of zooplankton collected from the three sites and fed to razorback sucker larvae were predominantly cladocerans and copepods (Table 18). Cladocerans dominated at Horsethief east wetland and Adobe Creek, whereas copepods dominated at North Pond. The mean ratio of cladocerans to copepods was 31 (range from 1.1-195) at Horsethief east wetland, 27 (range 2.5-200) at Adobe Creek, and 0.2 (range 0-0.5) at North Pond. Although not quantified, larvae also received some algae, zooplankton parts, and detritus during feeding. Algal and periphyton growth on the walls of test beakers was readily apparent, and the walls were brushed infrequently to remove the algal buildup, which also probably was a source of nutrition and inorganic elements, such as selenium, for the larvae. Some small food organisms and particles were also present in the site water treatments, which passed through the 25 µm filter bags.

The number of live zooplankton in exposure beakers from feeding on the previous day was grossly quantified daily prior to feeding fresh zooplankton. Few live brine shrimp were observed after about 3-4 hours following feeding. Nauplii of brine shrimp were cultured in 35 g/L salinity water, and placing them in freshwater (used as the test media) probably caused an osmotic shock, which lead to their death if not consumed within a few hours after being placed in the beakers. A few dead brine shrimp were observed on the bottom of some exposure beakers. Anywhere from a few (<20 organisms) to many (>100 organisms) zooplankters were observed in the beakers prior to feeding for those treatments receiving zooplankton collected from the Horsethief east wetland, Adobe Creek, and North Pond. However, the number of zooplankters tended to decrease as the total food ration decreased, which was based on the number of live fish in the exposure beaker.

Survival of zooplankton from the three sites held in the four test waters (24-Road, HT, AC, WW) was good after 24 and 48 hours in beakers with the test water. In most beakers, >100 zooplankters were observed after 24 hours and >20 to <100 were observed after 48 hours. For brine shrimp (BSH), <20 were observed after 24 hours, and none were observed after 48 hours.

Selenium in larvae

At 10 days of exposure, concentrations of selenium measured in larvae from 24-Road or HT water treatments combined with either the brine shrimp or HTEW food treatments (6.1-7.0 µg/g; Table 19) were similar to those measured in brood stock eggs (6.5-7.1 µg/g; Appendix G), which were the source of larvae in this study. In general, selenium concentrations in larvae after 10 days of exposure tended to increase in larvae as dietary selenium concentration increased (from lowest to highest: brine shrimp, HTEW, AC, WW) within each of the water treatments. Likewise, selenium concentrations in larvae tended to increase in larvae as waterborne selenium

Table 17. Mean (standard error in parentheses and number of samples in brackets) concentration of inorganic elements (µg/g dry weight) in brine shrimp and in zooplankton collected from three stations near Grand Junction, Colorado.

Element	Brine shrimp	Zooplankton station		
		HTEW	AC3	WW2
Ag	0.4 (-) [1]	<0.3[2]	0.3 (0) [3]	0.4 (0) [3]
Al	32 (20)a [2]	2,790 (1,080)b [4]	1,890 (270)b [4]	1,210 (520)b [3]
As	24 (0)c [2]	6.5 (-)b [1]	3.0 (0)a [2]	3.0 (-)a [1]
B	14.5 (1.5)b [2]	3.4 (1.1)ab [4]	2.2 (0.4)a [4]	3.7 (1.0)ab [3]
Ba	0.4 (0)a [2]	27 (5)b [4]	31 (2)b [4]	22 (4)b [3]
Be	<0.05	0.14 (0.04) [2]	0.05 (-) [1]	<0.05
Bi	<1	2.5 (0.5) [2]	2.5 (0.5) [2]	2.0 (-) [1]
Cd	<0.2	0.2 (1) [1]	-	0.3 (0.1) [3]
Co	<0.3	0.8 (0.2) [3]	0.5 (0.1) [4]	0.7 (0.2) [3]
Cr	<1	3.2 (0.7) [3]	2.3 (0.3) [3]	2.7 (0.4) [3]
Cu	20 (2) [2]	27 (6) [4]	13 (2) [4]	21 (7) [3]
Fe	410 (30) [2]	1,360 (500) [4]	1,320 (160) [4]	1,190 (380) [3]
Li	<0.4	2.8 (0.9) [3]	1.5 (0.1) [4]	2.0 (-) [1]

Table 17. Continued.

Element	Brine shrimp	Zooplankton station		
		HTEW	AC3	WW2
Mn	9 (1)a [2]	42 (7)b [4]	82 (13)bc [4]	162 (45)c [3]
Mo	0.4 (0)a [2]	0.4 (0.1)a [4]	1.0 (0.2)b [4]	1.0 (0.1)b [3]
Ni	0.7 (-) [1]	1.7 (0.6) [3]	1.0 (0.2) [4]	2.0 (0.6) [3]
Pb	1.0 (-) [1]	2.0 (1.0) [2]	2.1 (0.7) [4]	2.8 (1.3) [3]
Sb	2 (0) [2]	<1	<1	<1
Si	6 (-)a [1]	594 (178)b [4]	444 (84)b [4]	297 (110)b [3]
Sn	<1	<1	<1	<1
Sr	12 (0)a [2]	69 (4)b [4]	85 (16)b [4]	55 (9)b [3]
Ti	1 (0)a [2]	46 (16)b [4]	40 (5)b [4]	41 (15)b [3]
Tl	<10	<10	<10	<10
V	0.3 (0)a [2]	5.2 (1.8)b [4]	4.8 (0.4)b [4]	4.3 (1.4)b [3]
Zn	123 (14) [2]	110 (13) [4]	280 (129) [4]	143 (36) [3]

[1]The number of samples submitted for analysis was 2 for brine shrimp, 4 for HTEW and AC3, and 3 for WW2. If the number of samples given for a station and element is less than the number submitted, concentrations in the other samples were below the limit of detection.
[2]<: Below the limit of detection.

Table 18. Mean (standard error in parentheses and number of samples in brackets) number of cladocerans and copepods in archive samples of aquatic invertebrates collected from three stations near Grand Junction, Colorado.

Station	Zooplankton type	Day of exposure[1]			
		1-7	8-14	15-21	22-29
HTEW	Cladoceran	469 (242) [7]	243 (28) [7]	142 (22) [6]	146 (32) [8]
	Copepod	168 (105) [7]	25 (7) [7]	5 (2) [6]	1 (1) [8]
AC3	Cladoceran	209 (45) [7]	257 (39) [7]	128 (20) [6]	145 (40) [8]
	Copepod	11 (3) [7]	11 (1) [7]	9 (3) [6]	12 (3) [8]
WW2	Cladoceran	27 (9) [7]	32 (8) [7]	4 (1) [6]	4 (1) [8]
	Copepod	128 (37) [7]	123 (22) [7]	125 (16) [6]	57 (4) [8]

[1]: Zooplankton samples were arbitrarily grouped into 7-day intervals for this table.

Table 19. Mean (standard error in parentheses, n=1 at day 10, n=2 at day 30) selenium concentration (µg/g dry weight) in razorback sucker larvae. Larvae were exposed for 10 days and 30 days to either reference food (brine shrimp [BSH]) or site food (zooplankton from Horsethief east wetland [HTEW], Adobe Creek [AC], or North Pond [WW]) and either reference water (24-Road [24-R]) or site water (Horsethief [HT], Adobe Creek [AC], or North Pond [WW]). For food treatments at day 30 (within a column), values with a lower case letter in common are not significantly different (*P*=0.05). For water treatments at day 30 (within a row), values with upper case letters in common are not significantly different (*P*=0.05). There was a significant interaction between food and water treatments.

Day of exposure	Water treatment	Food treatment			
		BSH	HTEW	AC	WW
10	24-R	6.3	7.0	11	16
	HT	6.7	6.1	9.1	6.7[1]
	AC	8.8	11	17	28
	WW	11	13	14	23
30	24-R	5.2aA (0.3)	-[2]	26aB (1)	41aC (1)
	HT	5.2aA (0)	8.2aB (1.8)	24aC (2)	52bD (4)
	AC	12bA (0)	26bB (0)	32bC (2)	52bD (2)
	WW	16cA (0)	26bB (0)	41cC (0)	64cD (0)

[1]Two smaller than normal fish in composite.
[2]No live fish.

concentration increased (from lowest to highest: 24-Road, HT, AC, WW) within each of the food treatments.

At 30 days of exposure, the trends suggested in the selenium concentrations in larvae at 10 days of exposure were confirmed (Table 19). Selenium concentrations in larvae were significantly increased in larvae as dietary selenium concentration increased (from lowest to highest: brine shrimp, HTEW, AC, WW) within each of the water treatments. Likewise, selenium concentrations in larvae were significantly increased in larvae as waterborne selenium increased for the AC and WW waters within the brine shrimp, HTEW, and AC food treatments, and for the HT, AC, and WW waters within the WW food group compared to 24-Road water. The effects from food treatments were more prominent than for the water treatments.

At day 10 of the exposure, selenium residues in larvae from all treatments combined were significantly correlated with dietary selenium concentrations (r=0.81, P=0.0002, n=15), but not with waterborne selenium concentrations (r=0.30, P=0.27, n=15). Similarly, at day 30 of the exposure, selenium residues in larvae from all treatments combined were significantly correlated with dietary selenium concentrations (r=0.89, P=0.0001, n=15), but not with waterborne selenium concentrations (r=0.29, P=0.29, n=15). Based on multiple regression analysis using a two-variable model, selenium concentrations in larvae from all treatments combined at day 10 were significantly associated with median dietary selenium concentrations and the mean waterborne selenium concentrations (r^2=0.75, P=0.0003). Selenium concentrations in larvae from all treatments combined at day 30 also were significantly associated with mean dietary selenium concentrations and median waterborne selenium concentrations (two-variable model, r^2=0.91, P=0.0001).

Growth

At 10 days of exposure, there was no significant difference in total length or weight of larvae due to water treatments or to an interaction of water and food treatments (Table 20). However, there were significant differences in total length and weight of larvae due to food treatments. Larvae fed brine shrimp were significantly longer and weighed more than larvae fed HTEW, AC, or WW foods.

At 30 days of exposure, there was no significant difference in total length and weight of larvae due to food treatments or an interaction between water and food treatments (Table 21). For the HT water treatment, larvae fed the HTEW food treatment were significantly longer than larvae fed the brine shrimp food treatment, but were not longer than larvae fed the AC and WW food treatments. The total length and weight of fish that died during the last 8 days of the 30-day exposure period tended to be smaller than those that lived to the day 30 measurement period (Table 22). The magnitude of difference in larvae growth between live and dead fish fed the brine shrimp treatment tended to be less than in the HTEW, AC, and WW food treatments.

Survival

There was good survival among razorback sucker larvae fed the reference food (brine shrimp) and held in water from 24-Road (83%) or Horsethief (81%) (Table 23, Appendix N-Q, Figure 7). Survival of larvae in these treatments (81-83%) was significantly higher than those fed brine shrimp and held in North Pond water (52%) after 30 days of exposure (Table 23, Figure

Table 20. Mean (standard error in parentheses) total length (mm) and weight (g) of razorback sucker larvae after 10 days of exposure. Larvae were exposed to either reference food (brine shrimp [BSH]) or site food (zooplankton from Horsethief east wetland [HTEW], Adobe Creek [AC], or North Pond [WW] and either reference water (24-Road [24-R]) or site water (Horsethief [HT], Adobe Creek [AC], or North Pond [WW]). For both total length and weight, the BSH treatment was significantly different from the HT, AC, and WW treatments, which were not significantly different from each other (P=0.05). No significant effect was determined due to water treatment, and no significant interaction between food treatment and water treatment.

Measure	Water treatment	Food treatment			
		BSH	HTEW	AC	WW
Total length	24-R	11 (0.3)	10 (0.2)	10 (0.2)	10 (0.2)
	HT	11 (0.2)	10 (0.3)	10 (0)	10 (0.2)
	AC	10 (0.4)	10 (0.2)	10 (0.3)	11 (0.3)
	WW	11 (0.3)	10 (0.2)	10 (0.2)	10 (0.2)
Weight	24-R	0.004 (0.001)	0.003 (0)	0.003 (0.001)	0.003 (0)
	HT	0.005 (0)	0.003 (0.001)	0.002 (0)	0.004 (0)
	AC	0.005 (0.001)	0.003 (0)	0.004 (0.001)	0.004 (0.001)
	WW	0.006 (0.001)	0.004 (0)	0.003 (0)	0.003 (0.001)

Table 21. Mean (standard error in parentheses) total length (mm) and weight (g) of razorback sucker larvae after 30 days of exposure. Larvae were exposed to either reference food (brine shrimp [BSH]) or site food (zooplankton from Horsethief east wetland [HTEW], Adobe Creek [AC], or North Pond [WW]) and either reference water (24-Road [24-R]) or site water (Horsethief [HT], Adobe Creek [AC], or North Pond [WW]). For the HT water treatment (within a row), measurement values with lower case letters in common are not significantly different (*P*=0.05). There was no significant differences due to food treatments or interaction between food treatments and water treatments for total length or weight.

Measure	Water treatment	Food treatment			
		BSH	HTEW	AC	WW
Total length	24-R	14 (0.2)	-[1]	13 (0.5)	13 (0.5)
	HT	13a (0.3)	15b (0.9)	13ab (0.6)	13ab (0.5)
	AC	13 (0.3)	11 (0.2)	12 (0.5)	12 (0.5)
	WW	13 (0.3)	12 (0.5)	13 (0.5)	13 (0.5)
Weight	24-R	0.011 (0.001)	-	0.010 (0.001)	0.012 (0.002)
	HT	0.009 (0.001)	0.013 (0.003)	0.009 (0.002)	0.010 (0.001)
	AC	0.009 (0.001)	0.006 (0.001)	0.007 (0.001)	0.007 (0.001)
	WW	0.011 (0.001)	0.008 (0.001)	0.010 (0.001)	0.010 (0.002)

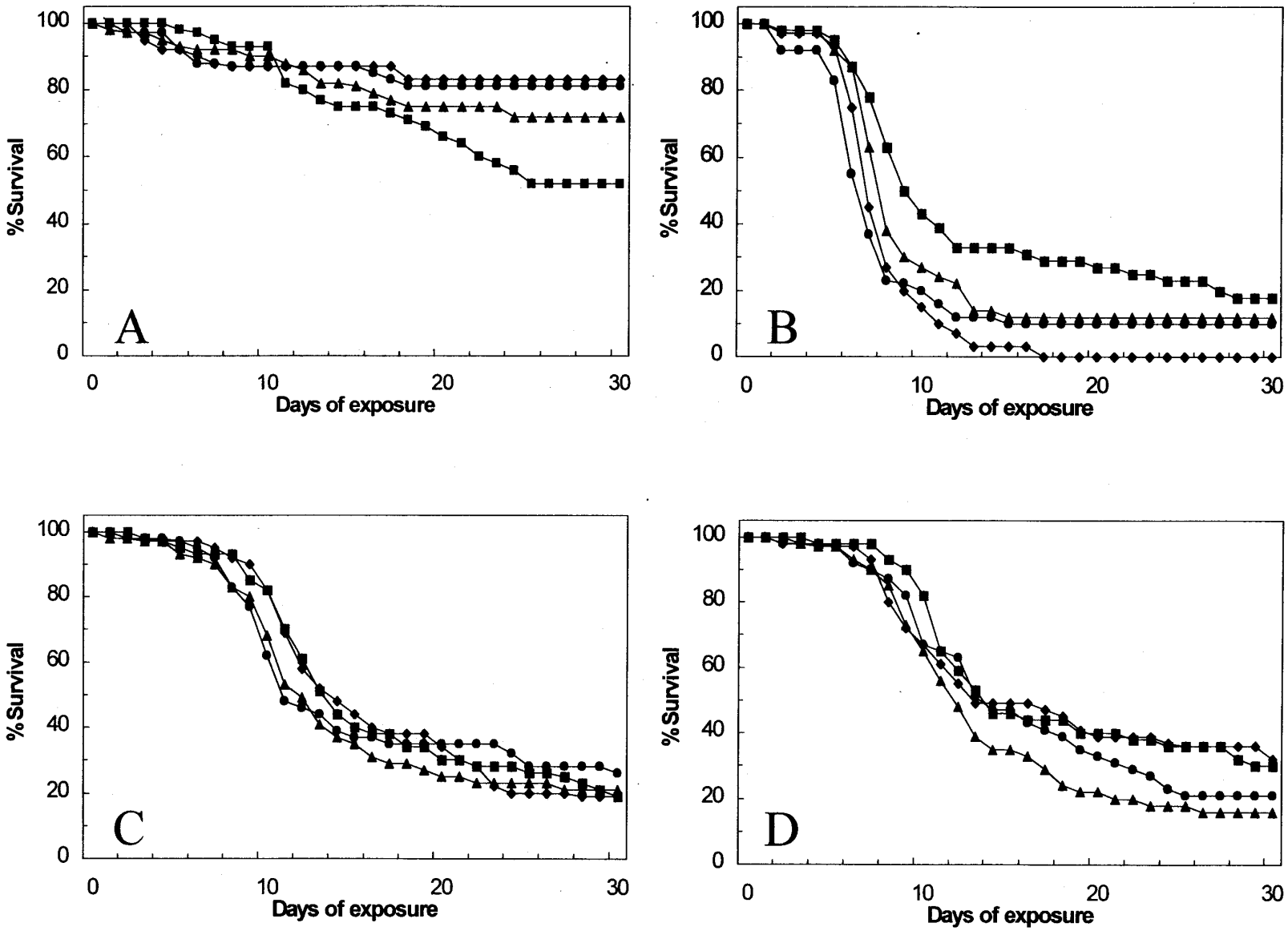
[1]-: No live fish.

Table 22. Mean (standard error in parentheses) total length (mm) and weight (g) of live and dead (died within 8 or less days of day 30 sampling) razorback sucker larvae. Larvae were exposed for 30 days to either reference food (brine shrimp [BSH]) or site food (zooplankton from Horsethief east wetland [HTEW], Adobe Creek [AC], or North Pond [WW]) and either reference water (24-Road [24-R]) or site water (Horsethief [HT], Adobe Creek [AC], or North Pond [WW]).

		Food treatment							
	Water	BSH		HTEW		AC		WW	
Measure	treatment	Live	Dead	Live	Dead	Live	Dead	Live	Dead
Total length	24-R	14 (0.2)	12 (2.5)	-[1]	-	13 (0.5)	10 (0.2)	13 (0.5)	10 (0.3)
	HT	13 (0.3)	-	15 (0.9)	-	13 (0.6)	10 (0.1)	13 (0.5)	10 (0.2)
	AC	13 (0.3)	11 (0.8)	11 (0.2)	-	12 (0.5)	9 (1.2)	12 (0.5)	10 (-)
	WW	13 (0.3)	10 (0.6)	12 (0.5)	9 (0.1)	13 (0.5)	9 (0.3)	13 (0.5)	9 (0.5)
Weight	24-R	0.011 (0.001)	0.008 (0.002)	-	-	0.010 (0.001)	0.002 (0.001)	0.012 (0.002)	0.004 (0.001)
	HT	0.009 (0.001)	-	0.013 (0.003)	-	0.009 (0.002)	0.003 (0.001)	0.010 (0.001)	0.003 (0.001)
	AC	0.009 (0.001)	0.006 (0.001)	0.006 (0.001)	-	0.007 (0.001)	0.004 (0.001)	0.007 (0.001)	0.003 (-)
	WW	0.011 (0.001)	0.004 (0.001)	0.008 (0.001)	0.002 (0)	0.010 (0.001)	0.003 (0)	0.010 (0.002)	0.001 (0)

[1]-: No live fish.

Figure 7. Mean (n=3) percent survival of razorback sucker larvae fed either brine shrimp (A), or zooplankton from Horsethief east wetland (B), Adobe Creek (C), or North Pond (D) and exposed in either 24-Road Fish Hatchery water (♦), Horsethief water (●), Adobe Creek water (▲), or North Pond water (■).



90

Table 23. Mean (n=3) percent survival of razorback sucker larvae exposed to either reference food (brine shrimp [BSH]) or site food (zooplankton from Horsethief east wetland [HTEW], Adobe Creek [AC], or North Pond [WW]) and either reference water (24-Road [24-R]) or site water (Horsethief [HT], Adobe Creek [AC], or North Pond [WW]). For food treatments (four water treatments per food treatment) at day 10, 20, and 30, survival values with lower case letter in common are not significantly different (*P*=0.05). There was no significant differences due to interaction of food treatments and water treatments at day 10, but there were significant interactions at days 20 and 30.

	Food and water treatment															
	BSH				HTEW				AC				WW			
Day	24-R	HT	AC	WW	24-R	HT	AC	WW	24-R	HT	AC	WW	24-R	HT	AC	WW
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	98	100	100	100	100	100	100	100	98	100	100	100	100	100
2	100	97	97	100	97	92	98	98	98	98	98	100	98	98	100	100
3	95	97	97	100	97	92	98	98	98	98	97	98	98	98	98	100
4	92	97	95	100	97	92	98	98	97	98	97	97	98	98	97	98
5	92	92	93	98	93	83	92	95	97	97	93	95	97	97	97	98
6	90	88	92	97	75	55	87	87	97	95	92	93	97	92	93	98
7	88	88	92	95	45	37	63	78	95	92	90	93	93	90	90	98
8	87	87	92	93	27	23	38	63	92	83	83	93	80	87	85	93
9	87	87	90	93	20	22	30	50	90	77	80	85	72	82	73	90
10	87a	87a	90a	93a	15a	20a	27ab	43b	82b	62a	68ab	82b	67a	67a	65a	82a
11	87	87	88	82	10	16	24	39	69	48	53	70	61	65	56	65
12	87	87	86	80	7	12	22	33	58	46	49	61	55	63	48	59
13	87	87	82	77	3	12	14	33	52	44	41	51	49	51	39	53
14	87	87	82	75	3	12	14	33	48	39	37	44	49	47	35	46
15	87	87	81	75	3	10	12	33	44	37	35	40	49	47	35	46
16	87	85	79	75	3	10	12	31	40	37	31	38	49	43	33	44
17	87	83	77	73	0	10	12	29	38	35	29	38	47	41	29	44
18	83	81	75	71	0	10	12	29	38	35	29	34	45	39	24	44

Table 23. Continued.

	Food and water treatment															
	BSH				HTEW				AC				WW			
Day	24-R	HT	AC	WW	24-R	HT	AC	WW	24-R	HT	AC	WW	24-R	HT	AC	WW
19	83	81	75	69	0	10	12	29	38	35	27	34	41	35	22	40
20	83b	81b	75ab	66b	0a	10b	12b	27c	34a	35a	25a	30a	39a	33a	22a	40a
21	83	81	75	64	0	10	12	27	30	35	25	30	39	31	20	40
22	83	81	75	60	0	10	12	25	28	35	23	28	39	29	20	38
23	83	81	75	58	0	10	12	25	22	35	23	28	39	27	18	38
24	83	81	72	56	0	10	12	23	20	32	23	28	37	23	18	36
25	83	81	72	52	0	10	12	23	20	28	23	26	36	21	18	36
26	83	81	72	52	0	10	12	23	20	28	23	26	36	21	16	36
27	83	81	72	52	0	10	12	20	20	28	21	25	36	21	16	36
28	83	81	72	52	0	10	12	18	19	28	21	23	36	21	16	32
29	83	81	72	52	0	10	12	18	19	28	21	21	36	21	16	30
30	83b	81b	72ab	52a	0a	10b	12b	18b	19a	26a	21a	19a	32a	21a	16a	30a

7A). This high survival of larvae suggested that the reference food and reference water treatments were adequate to maintain razorback sucker larvae.

Overall, there were significant differences in larvae survival due to food treatments between day 5 and 30 of the exposure, and due to water treatments between days 6 and 10 of the exposure. There was a consistent interaction between food and water treatments between days 19 and 30 of the exposure. The sharp decline in survival of larvae started at about 6-8 days of exposure in the Horsethief east wetland food treatments and at 8-10 days in the Adobe Creek and North Pond food treatments (Figure 7). The decline occurred more rapidly in larvae fed the Horsethief east wetland food treatment than those fed the Adobe Creek or North Pond food treatments. Selenium concentrations in zooplankton during that time period were 4.6 µg/g in zooplankton from Horsethief east wetland, 23.8 µg/g at Adobe Creek, and 41.9 µg/g at North Pond (Table 16).

For the brine shrimp food treatment, there were no significant differences in larvae survival in the four water treatments after 10 days of exposure, but there were significant differences after 20 and 30 days of exposure (Table 23, Figure 7A). At 20 and 30 days of exposure, survival of larvae in WW water was lower than those in 24-Road and HT waters. For the HTEW food treatment, larvae survival was significantly higher in WW water than in 24-Road and HT waters after 10 days of exposure, and higher than those in 24-Road water after 20 and 30 days of exposure (Table 23, Figure 7B). For the AC food treatment, larvae survival was significantly lower in HT and AC waters compared to those in 24-Road and WW waters after 10 days of exposure (Table 23, Figure 7C). However, there were no significant differences in survival among the four water treatments after 20 and 30 days of exposure for the AC food treatment. For the WW food treatments, there were no significant differences in survival among the four water treatments after 10, 20, or 30 days of exposure (Table 23, Figure 7D).

For the 24-Road water treatment, larvae survival after 10 days of exposure was significantly less in the HT food treatment than in the other food treatments, and less in the WW food treatment than in the brine shrimp and AC food treatments (Table 24, Figure 8A). For the HT, AC, and WW water treatments, larvae survival after 10 days of exposure was significantly lowest in the HTEW food treatment, intermediate in the AC and WW food treatments, and highest in the brine shrimp food treatment (Table 24, Figures 8B, C, D). At 20 and 30 days of exposure, the pattern of high survival in brine food treatments, intermediate in the AC and WW food treatments, and lowest in HT food treatments continued and was most prominent in the 24-Road, HT, and AC water treatments, but not in the WW water treatment (Table 24).

Figure 7 is grouped to show the effects of the four water treatments on the four food treatments. For the brine shrimp food treatments, Figure 7A shows the best larvae survival occurred in the 24-Road and HT water treatment after 30 days of exposure, intermediate survival in the AC water treatment, and the worst in the WW water treatment. For the HTEW food treatments, Figure 7B shows the least survival in 24-Road water and the highest survival in WW water, which is the opposite of Figure 7A and suggests an antagonistic interaction between HTEW food and WW water. Water from WW had the highest hardness, calcium, magnesium, chloride, sulfate, nitrate, boron, lithium, and strontium compared to other test waters. These or other water quality characteristics or trace elements may have contributed to the suggested antagonistic interaction.

For the AC food treatments, Figure 7C shows AC food caused a further reduction in survival in the WW water and increased survival in 24-Road water after 30 days of exposure

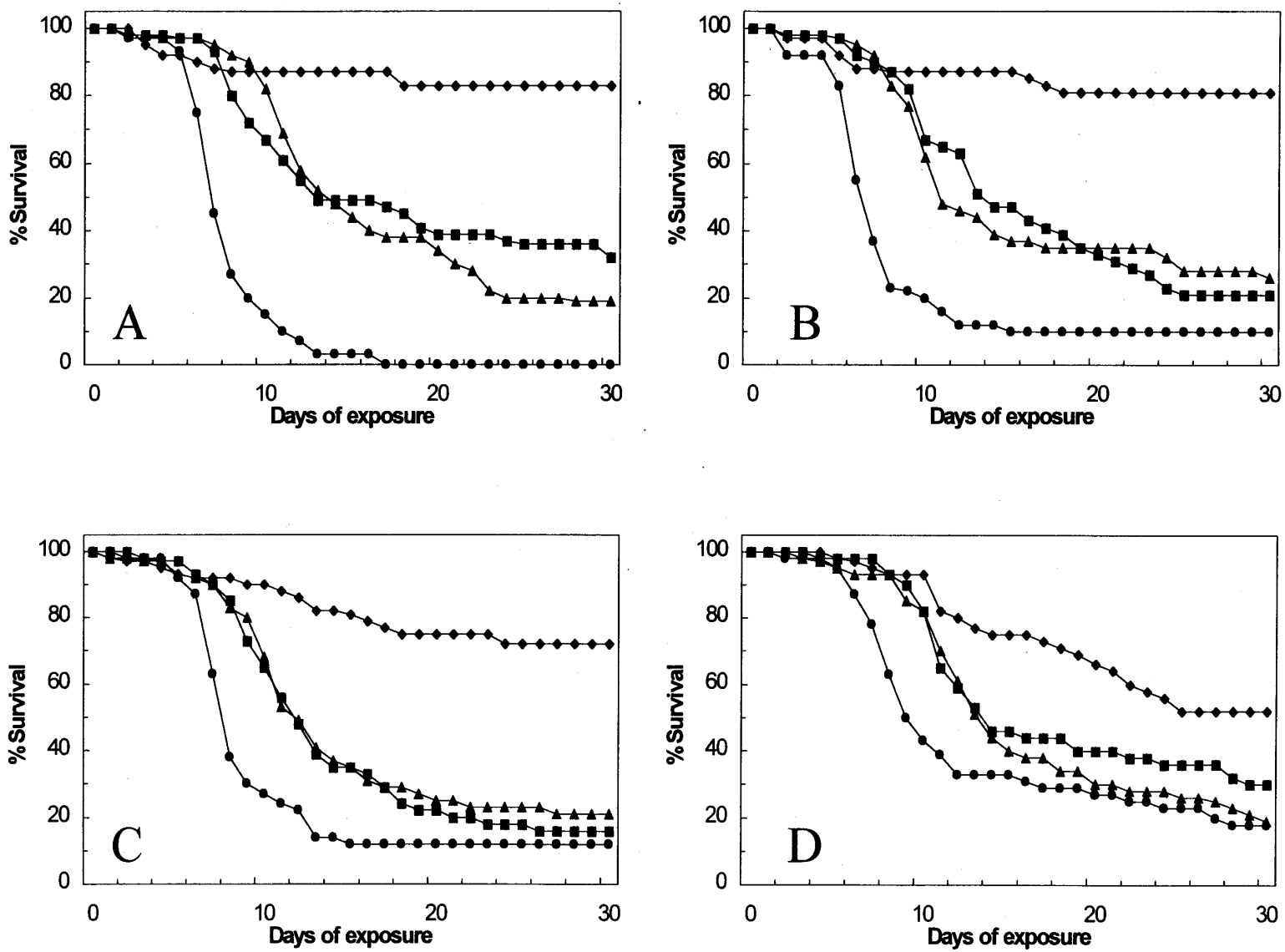
Table 24. Mean (n=3) percent survival of razorback sucker larvae exposed to either reference water (24-Road [24 -R]) or site water (Horsethief [HT], Adobe Creek [AC], or North Pond [WW]) and either reference food (brine shrimp [BSH]) or site food zooplankton from Horsethief east wetland [HTEW], Adobe Creek [AC], or North Pond [WW]). For water treatments (four food treatments per water treatment) at day 10, survival values with lower case letter in common are not significantly different (*P*=0.05). At days 20 and 30, there were no significant differences in survival due to water treatment. There were no significant differences due to interaction of food treatments and water treatments at day 10, there were significant interactions at days 20 and 30.

	Water and food treatment															
	24-R				HT				AC				WW			
Day	BSH	HTEW	AC	WW	BSH	HTEW	AC	WW	BSH	HTEW	AC	WW	BSH	HTEW	AC	WW
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	100	100	100	100	100	100	98	100	98	100	100	100	100	100
2	100	97	98	98	97	92	98	98	97	98	98	100	100	98	100	100
3	95	97	98	98	97	92	98	98	97	98	97	98	100	98	98	100
4	92	97	97	98	97	92	98	98	95	98	97	97	100	98	97	98
5	92	93	97	97	92	83	97	97	93	92	93	97	98	95	95	98
6	90	75	97	97	88	55	95	92	92	87	92	93	97	87	93	98
7	88	45	95	93	88	37	92	90	92	63	90	90	95	78	93	98
8	87	27	92	80	87	23	83	87	92	38	83	85	93	63	93	93
9	87	20	90	72	87	22	77	82	90	30	80	73	93	50	85	90
10	87c	15a	82c	67b	87c	20a	62b	67b	90c	27a	68b	65b	93a	43c	82b	82b
11	87	10	69	61	87	16	48	65	88	24	53	56	82	39	70	65
12	87	7	58	55	87	12	46	63	86	22	49	48	80	33	61	59
13	87	3	52	49	87	12	44	51	82	14	41	39	77	33	51	53
14	87	3	48	49	87	12	39	47	82	14	37	35	75	33	44	46
15	87	3	44	49	87	10	37	47	81	12	35	35	75	33	40	46
16	87	3	40	49	85	10	37	43	79	12	31	33	75	31	38	44
17	87	0	38	47	83	10	35	41	77	12	29	29	73	29	38	44
18	83	0	38	45	81	10	35	39	75	12	29	24	71	29	34	44

Table 24. Continued.

	Water and food treatment															
	24-R				HT				AC				WW			
Day	BSH	HTEW	AC	WW	BSH	HTEW	AC	WW	BSH	HTEW	AC	WW	BSH	HTEW	AC	WW
19	83	0	38	41	81	10	35	35	75	12	27	22	69	29	34	40
20	83	0	34	39	81	10	35	33	75	12	25	22	66	27	30	40
21	83	0	30	39	81	10	35	31	75	12	25	20	64	27	30	40
22	83	0	28	39	81	10	35	29	75	12	23	20	60	25	28	38
23	83	0	22	39	81	10	35	27	75	12	23	18	58	25	28	38
24	83	0	20	37	81	10	32	23	72	12	23	18	56	23	28	36
25	83	0	20	36	81	10	28	21	72	12	23	18	52	23	26	36
26	83	0	20	36	81	10	28	21	72	12	23	16	52	23	26	36
27	83	0	20	36	81	10	28	21	72	12	21	16	52	20	25	36
28	83	0	19	36	81	10	28	21	72	12	21	16	52	18	23	32
29	83	0	19	36	81	10	28	21	72	12	21	16	52	18	21	30
30	83	0	19	32	81	10	26	21	72	12	21	16	52	18	19	30

Figure 8. Mean (n=3) percent survival of razorback sucker larvae exposed to either 24-Road Fish Hatchery water (A), Horsethief water (B), Adobe Creek water (C), or North Pond water (D), and fed either brine shrimp (♦), or zooplankton from Horsethief east wetland (●), Adobe Creek (▲), or North Pond (■).



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than shown in Figure 7B. The closeness of the survival curves in Figure 7C suggests a strong influence of the AC food treatment seemingly independent of the four water treatments. For the WW food treatment, Figure 7D shows slightly higher survival in the 24-Road and WW waters than shown in Figure 7C, suggesting a further increase in an antagonistic interaction.

Figure 8 is grouped to show the effects of the four food treatments on the four water treatments. For the 24-Road water treatment, Figure 8A shows that the highest survival after 30 days exposure was in the brine shrimp treatment, intermediate in the AC and WW food treatments, and lowest in the HTEW food treatment. For the HT water treatment, Figure 8B shows slightly lower survival in the brine shrimp, AC, and WW food treatments than in Figure 8A, suggesting a toxic effect of HT water. In contrast, the HTEW food treatment shows higher survival than in Figure 8A, suggesting an antagonistic interaction that reduced the toxic effect of the HTEW food. For the AC water treatment, Figure 8C shows a further decrease in survival in the brine shrimp, AC, and WW food treatments, suggesting a greater toxic effect of AC water compared to HT water (Figure 8B). The HTEW food treatment shows greater survival in Figure 8C than in Figures 8A or 8B. For the WW water treatment, Figure 8D shows survival was further reduced in the brine shrimp treatment and increased in the HTEW food treatment compared to Figures 8A, 8B, and 8C. The change in survival in the HTEW food treatment in Figure 8D suggested a greater antagonistic interaction than shown in Figures 8B and 8C.

Prior to death, most of the larvae in the treatments fed zooplankton from Horsethief east wetland, Adobe Creek, or North Pond were lethargic or immobilized on the bottom of the exposure beaker. All larvae observed with scoliosis swam erratically. As mentioned earlier, live zooplankton were observed in beakers prior to daily feeding, which suggests that sufficient zooplankton were present to sustain the larvae.

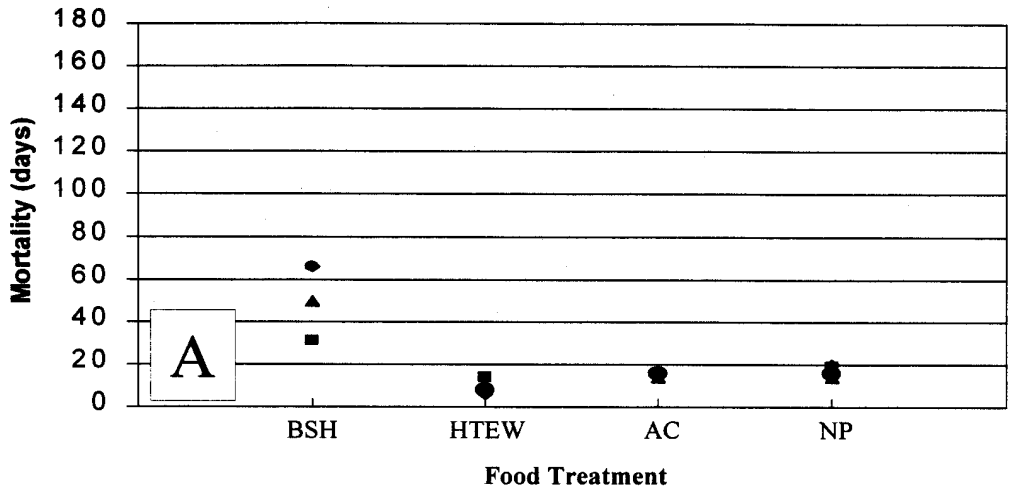
At day 10 of the exposure, survival of larvae from all treatments combined was not significantly correlated with waterborne concentrations of selenium (r=0.23, P=0.39, n=16), zooplankton selenium concentrations (r=0.22, P=0.42, n=16), or larval selenium residues (r=0.17, P=0.54, n=15), probably due to the early stage of the exposure. At day 30 of the exposure, survival of larvae from all treatments combined was not significantly correlated with waterborne concentrations of selenium (r= -0.06, P=0.83, n=16) or zooplankton selenium concentrations (r= -0.36, P=0.17, n=16), but there was a significant negative correlation with larval selenium residues (r= -0.55, P=0.03, n=15). Combining day 10 and 30 data, there was a significant negative correlation between larvae survival and selenium concentrations in larvae (r= -0.51, P=0.004, n=30). Based on multiple regression analysis, survival of larvae was significantly associated with selenium concentrations in larvae (one-variable model, r^2=0.27, P=0.004, n=30). Adding mean selenium concentrations in zooplankton to the model (two-variable model, r^2=0.34, P=0.004, n=30), and median selenium concentrations in water (three-variable model, r^2=0.41, P=0.003, n=30) improved the model slightly.

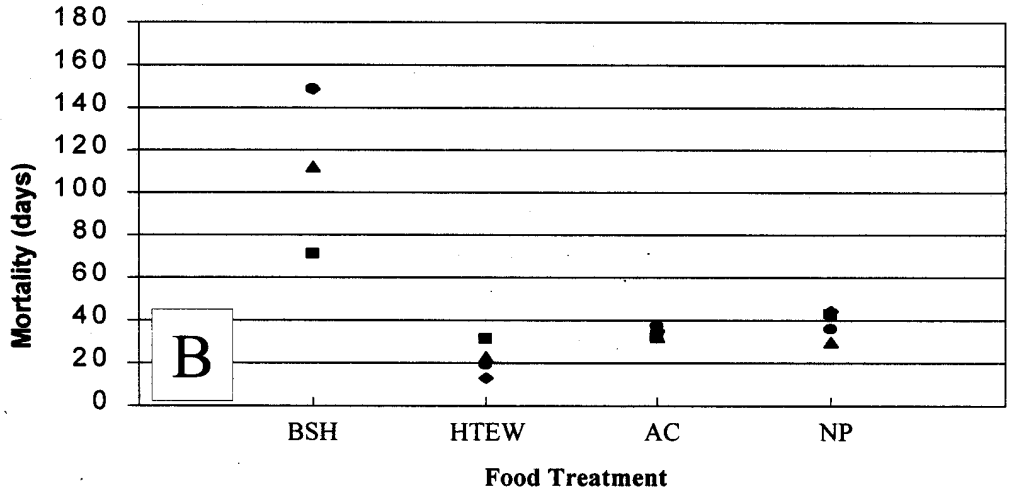
The predicted time-to-death of razorback sucker larvae for 50, 90, and 95% mortality are given in Table 25 and shown in Figures 9 and 10. The predicted time-to-death among larvae was longest in larvae fed brine shrimp and held in 24-Road or Horsethief water, intermediate in larvae held in Adobe Creek water, and shortest in larvae held in North Pond water. For each predicted mortality level, predicted time-to-death of larvae fed zooplankton from Horsethief east wetland, Adobe Creek, and North Pond was shorter than for brine shrimp. For the HT, AC, and WW food treatments, predicted time-to-death tended to be shortest for HT food, intermediate for AC food, and longest for WW food regardless of the water treatment. Specific to the HT food

Table 25. Mean (95% confidence intervals in parentheses) predicted time (days) to 50, 90, and 95% mortality of razorback sucker larvae. Larvae were exposed for 30 days to either brine shrimp (BSH) or zooplankton from Horsethief east wetland, Adobe Creek, or North Pond and water from 24-Road, Horsethief, Adobe Creek, or North Pond.

Predicted mortality	Water treatment	Food treatment			
		BSH	HTEW	AC	WW
50%	24-R	66 (44-99)	6 (5-7)	15 (13-19)	20 (16-24)
	HT	66 (44)	8 (7-10)	16 (14-20)	16 (13-19)
	AC	50 (35-69)	10 (8-12)	14 (12-17)	14 (11-16)
	WW	31 (24-40)	14 (11-17)	16 (13-19)	19 (16-23)
90%	24-R	149 (99-224)	13 (11-16)	35 (29-42)	44 (36-54)
	HT	149 (99-224)	19 (16-23)	37 (31-45)	36 (30-44)
	AC	112 (80-157)	23 (19-28)	32 (27-39)	30 (25-37)
	WW	71 (55-91)	31 (26-38)	35 (29-43)	43 (35-53)
95%	24-R	179 (119-269)	16 (14-19)	42 (34-50)	53 (43-65)
	HT	178 (118-268)	23 (19-27)	44 (36-54)	43 (35-52)
	AC	133 (95-188)	27 (23-33)	39 (32-47)	36 (30-44)
	WW	85 (65-109)	37 (31-45)	42 (35-51)	51 (42-63)

Figure 9. Mean predicted time (days) to 50% (A), 90% (B), and 95% (C) mortality of razorback sucker larvae fed either brine shrimp (BSH), or zooplankton from Horsethief east wetland (HTEW), Adobe Creek (AC), or North Pond (NP), and exposed in either 24-Road Fish Hatchery water (♦), Horsethief water (●), Adobe Creek water (▲), or North Pond water (■).


180
160
140
120
100
80
60
40
20
0
Mortality (days)
A
BSH
HTEW
AC
NP
Food Treatment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Mortality (days)
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BSH
HTEW
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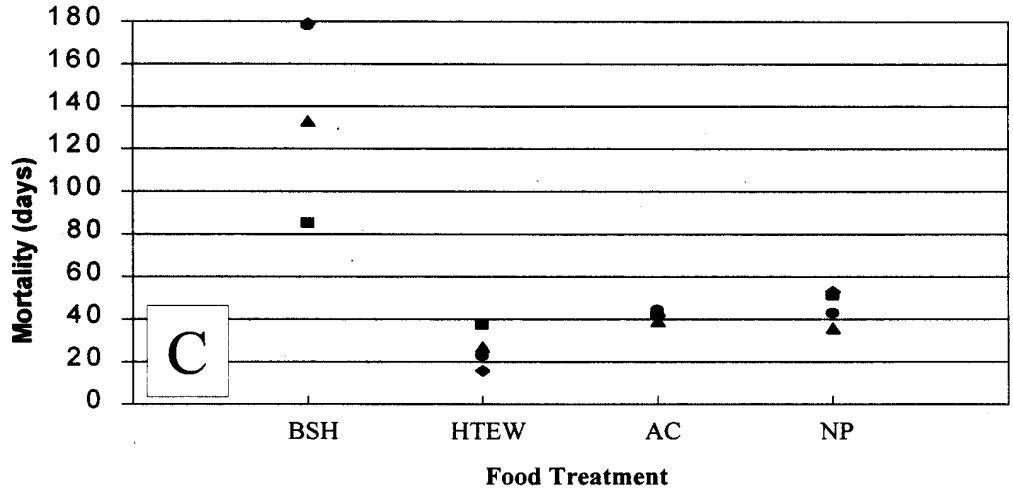
Food Treatment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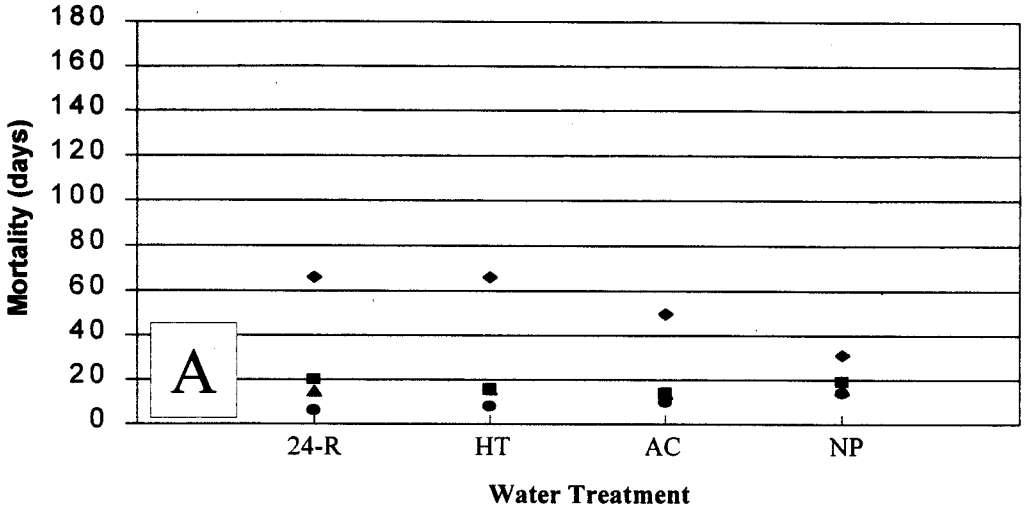
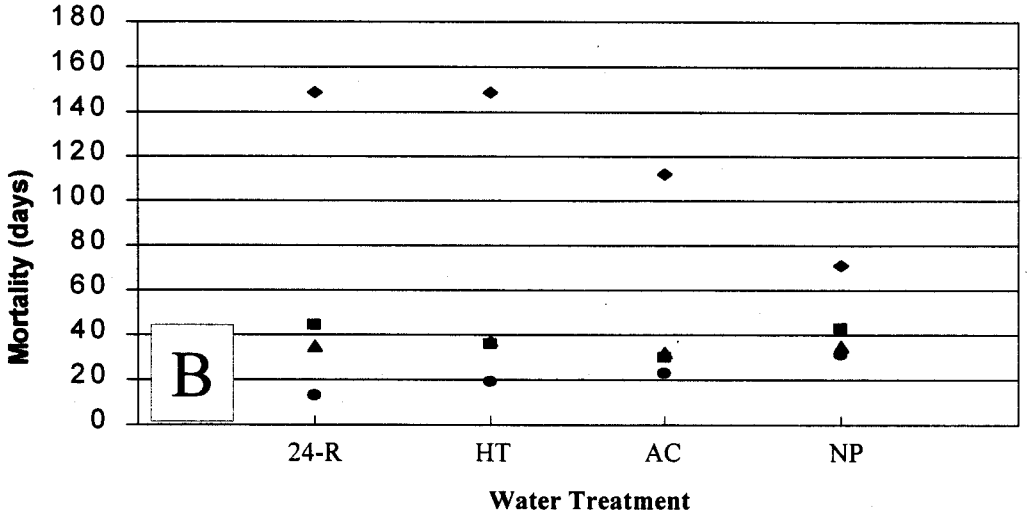
20
0
Mortality (days)
C
BSH
HTEW
AC
NP
Food Treatment

Figure 10. Mean predicted time (days) to 50% (A), 90% (B), and 95% (C) mortality of razorback sucker larvae exposed to either 24-Road Fish Hatchery water (24-R), Horsethief water (HT), Adobe Creek water (AC), or North Pond water (NP), and fed either brine shrimp (♦) or zooplankton from Horsethief east wetland (●), Adobe Creek (▲), or North Pond (■).


180
160
140
120
100
80
60
40
20
0
Mortality (days)
A
24-R
HT
AC
NP
Water Treatment


180
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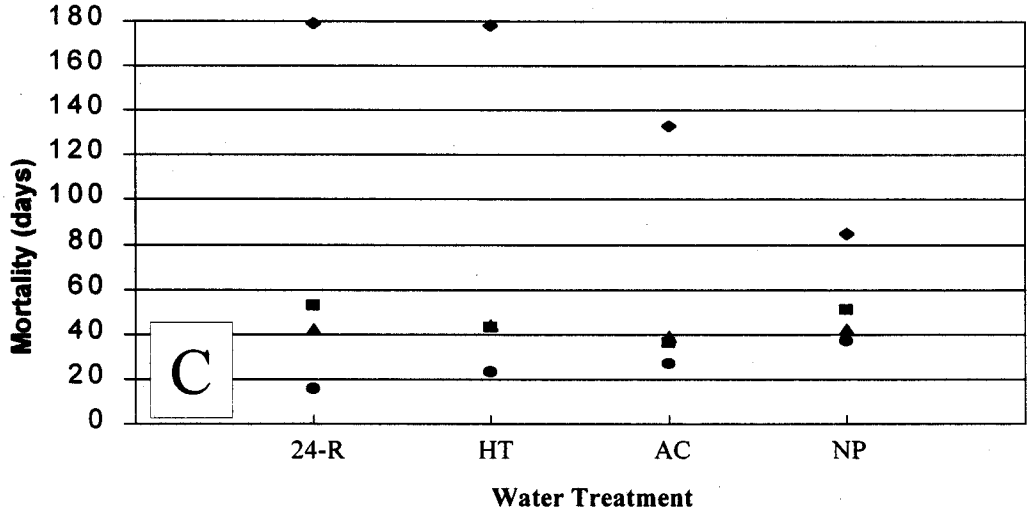
40
20
0
Mortality (days)
B
24-R
HT
AC
NP
Water Treatment


180
160
140
120
100
80
60
40
20
0
Mortality (days)
C
24-R
HT
AC
NP
Water Treatment

treatment, predicted time-to-death tended to be shortest in HT water, intermediate in AC water, and longest in WW water.

Discussion

Razorback sucker larvae used in the present study were 5-day-old at the start of the exposure, which seemed to be the appropriate age for initiating a dietary and waterborne test. Tyus and Severson (1990) started their feeding studies with razorback sucker larvae at 5-days posthatch, and Papoulias and Minckley (1992) started their feeding study with razorback sucker larvae at 7-days posthatch. Papoulias and Minckley (1992) reported that within 1 day of stocking (age 8 days posthatch) larvae had phytoplankton, diatoms, and detritus in their stomachs, and that the following day (age 9 days posthatch) these same food items plus rotifers, nauplii, cladocerans, invertebrate eggs, and chironomids were found in larvae stomachs. Minckley and Gustafson (1982) reported that 9 day-old razorback sucker larvae fed on ground aquarium fish food (Tetramin). Toney (1974) reported that razorback sucker larvae 6 days posthatch swam to the surface and fed on baby food, i.e., strained beef liver. In the studies by Papoulias and Minckley (1992) and Minckley and Gustafson (1982) it is possible that razorback sucker larvae would have started feeding earlier if the studies had been started at an earlier age or food was presented to the larvae at an earlier age.

Water quality

Dissolved oxygen and water temperature during the test were in the acceptable range of values for tests with fish (Weber et al. 1989). Water quality characteristics differed significantly among the four test waters, but those differences probably did not contribute to observed mortality of larval fish in the study, especially for waters from 24-Road, Horsethief, and Adobe Creek, which were all close to conditions in the Colorado River (i.e., Horsethief water). Razorback sucker are routinely spawned and cultured at Dexter NFH and Technology Center, Dexter, NM, and water quality characteristics there were 2,350 mg/L as $CaCO_3$ hardness, 3,710 µmhos/cm conductivity, and 3,570 mg/L total dissolved solids (R. Hamman, USFWS, personal communication). Pimentel and Bulkley (1983) reported that the total dissolved solids preference (measured as conductivity) of Colorado pikeminnow, bonytail, and humpback chub ranged from 3,610 to 5,106 µmhos/cm. Considering the culture activities at Dexter NFH, and assuming razorback sucker have a similar preference to conductivity as the three endangered fish tested by Pimentel and Bulkley (1983), then the water quality from Horsethief and Adobe Creek probably would not adversely affect larvae in the present study. The information in Pimentel and Bulkley (1983) was taken from Bulkley et al. (1981), who used razorback sucker juveniles in other tests (temperature preference, swimming tests, and blood analysis), but there was no mention of why razorback sucker were not used in the total dissolved solids testing.

Water quality characteristics were elevated in North Pond water but probably were not a stress to the larval fish. Nelson and Flickinger (1992) reported that the 96-hour LC50 for salinity to Colorado pikeminnow ranged from 11.8 to 14.7 g/L. In the present study with razorback sucker larvae, the maximum conductivity of North Pond water was 6,030 µmhos/cm, which probably contained 4 g/L salinity based on the salinity/conductivity measurement at WW9 on August 1, 1996 (6,030 µmhos/cm and 4.0 g/L salinity; Appendix C). If razorback sucker have a

similar sensitivity to salinity as Colorado pikeminnow, then stress due to elevated conductivity in the treatments with water from North Pond would not be expected.

Selenium in water

Waterborne concentrations of inorganic elements at Horsethief were not elevated sufficiently to have caused direct toxicity from waterborne exposure based on the results of studies with other fish. Waterborne selenium concentrations that cause direct toxic effects generally have to be in the 47 to >100 µg/L range (Goettl and Davies 1977, Hodson et al. 1980, Hamilton et al. 1986, Hunn et al. 1987). However, Woock et al. (1987) has reported that low (relative to chronic waterborne toxicity thresholds) waterborne selenium exposure to 10 µg/L in addition to dietary selenium exposure (13 µg/g) can increase mortality and the incidence of deformities in fish above that present in dietary only exposures.

This scenario may have occurred in the present study. Exposure of razorback sucker larvae to the North Pond water and brine shrimp treatment caused decreased survival compared to the 24-Road or Horsethief water and brine shrimp treatments. This decreased survival in the North Pond water treatment suggested that some component of the North Pond water, either water quality characteristics or inorganic elements contributed to the increased mortality. However, North Pond water had water quality characteristics (i.e., hardness, conductivity) that were lower than characteristics reported to cause adverse effects in Colorado pikeminnow (Nelson and Flickinger 1992). Consequently, water quality characteristics of North Pond water were probably not harmful to the larvae. The only inorganic element in the North Pond water that was elevated to a concentration of concern was selenium, which averaged 13.3 µg/L.

For the Adobe Creek water and brine shrimp treatment, there was a trend for lower survival compared to the 24-Road and Horsethief water treatments, but higher survival than the North Pond water treatment (Figure 7A), which suggests there was in incremental increase in a stressor. It seems unlikely that the change in water quality would have caused the incremental increase in mortality because there was no substantial difference in water quality characteristics between Adobe Creek and Horsethief or 24-Road water. Furthermore, the elevated hardness at Adobe Creek probably would have reduced the toxicity of some elements known to be affected by hardness such as cadmium, chromium III, copper, lead, nickel, silver, and zinc (USEPA 1998, 1999). Selenium toxicity, however, is minimally affected by changes in hardness or salinity (Palawski et al. 1985, Hamilton and Buhl 1990, USEPA 1998, 1999). The only inorganic element in Adobe Creek water that was elevated to a concentration of concern was selenium, which averaged 3.4 µg/L (range 2.1-5.8 µg/L) – a small incremental increase of over that in Horsethief (1.2-2.4 µg/L) or 24-Road (<1 µg/L) water. The selenium concentration in Adobe Creek water was less than that used by Woock et al. (1987), who reported that waterborne selenium of 10 µg/L caused increased mortality and deformities in larval bluegill over that from dietary selenium exposure alone.

Selenium concentrations in Adobe Creek water during the last days of exposure and in North Pond water during the 30-day period (except for one small time period), exceeded the USEPA criterion of 5 µg/L for the protection of aquatic life (USEPA 1987). This criterion was based on bioaccumulation of selenium through the food chain, and the resulting dietary selenium toxicity. The general consensus is that dietary exposure to selenium is more toxic to fish than waterborne exposure, and that waterborne concentrations of selenium between 2 and 5 µg/L can

cause bioaccumulation of selenium in the food chain to toxic concentrations (Maier and Knight 1994, Lemly 1996b, Skorupa 1998, Hamilton and Lemly 1999). However, in natural aquatic situations the two exposure routes are inseparable. The selenium concentrations in water from Adobe Creek and North Pond in the present study were elevated sufficiently to allow selenium to be bioaccumulated to concentrations above the dietary toxic threshold. This accumulation from water to algae and periphyton growing on the exposure beaker walls probably occurred, which in turn may have contributed to the dietary selenium exposure. This scenario could lead to the possibly incorrect impression that low waterborne selenium exposure (3.4 µg/L in Adobe Creek water and 13.3 µg/L in North Pond water) contributed directly to the increased mortality observed in the site water treatments compared to the Horsethief and 24-Road water treatments.

Selenium and other elements in zooplankton

The selenium concentrations in zooplankton in the present study, including zooplankton collected from Horsethief east wetland (4.2 to 8.8 µg/g), exceeded the dietary toxicity threshold of 3 µg/g proposed by Lemly (1993a, 1996b) and Maier and Knight (1994) based their review of several laboratory and field studies. This threshold for selenium-sensitive fish species was similar to the dietary toxic threshold for birds (3-8 µg/g based on reproductive impairment) and mammals (3 µg/g based on reduced longevity) (USDOI 1998). As early as 1980, Hilton et al. (1980) speculated that selenium in the diet in excess of 3 µg/g might be toxic to rainbow trout. They also reported that rainbow trout needed dietary selenium concentrations between 0.15 and 0.38 µg/g to maintain maximal plasma glutathione peroxidase activity (enzyme important in the oxidative stress defense system of animals), and homeostasis was maintained up to 1.25 µg/g in the diet. Sharma and Singh (1984) described selenium as a paradox because it is essential in the diet of animals at about 0.1 µg/g, but toxic at >1 µg/g. Lakin (1973) reported that dietary selenium in animals must meet a minimal concentration of 0.04 µg/g, was beneficial up to 0.1 µg/g, but was toxic above 4 µg/g. These three reports suggest that the difference between essential concentrations and toxic concentrations can be as little as 10 fold.

The high copper concentrations in zooplankton from Horsethief east wetland match the elevated copper concentration in sediments from Horsethief ponds reported previously (Hamilton et al. 2001), which were the water source for the wetland. In the present study, copper concentrations in zooplankton (13-27 µg/g) probably did not contribute to toxic effects in razorback sucker larvae because they were about 25 times lower than those reported by Lanno et al. (1985) to be toxic to 12-g rainbow trout exposed to a copper-supplemented commercial diet containing 665 µg/g copper, or about 13 times lower than those reported by Mount et al. (1994) to be toxic to swim-up rainbow trout fed copper-exposed brine shrimp containing >350 µg/g copper.

Vanadium was not as elevated in zooplankton in the present study (4.3-5.2 µg/g) as in a study at Ouray NWR, UT, where vanadium was suggested as potentially contributing to the toxicity to razorback sucker larvae fed zooplankton collected from Sheppard Pond 3 (8.4 µg/g vanadium) and North Roadside Pond (9.5 µg/g vanadium) (Hamilton et al. 1996). No toxic threshold has been proposed for vanadium, but Hilton and Bettger (1988) reported that vanadium toxicity to juvenile rainbow trout was between 1.2 µg/g in their control treatment and 10 µg/g, which was their next higher treatment concentration.

The concentrations of iron, magnesium, manganese, potassium, and sodium in

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

Selenium was the only inorganic element in zooplankton from Horsethief east wetland, Adobe Creek, and North Pond in the present study that exceeded concentrations documented to be toxic to fish. Selenium in brine shrimp (3.2 µg/g) also exceeded the proposed selenium dietary toxic threshold (3 µg/g) for selenium-sensitive fish species and is discussed in the following section. Selenium concentrations of ≥4.6 µg/g in food organisms from Horsethief east wetland adversely affected survival of razorback sucker larvae after 6-8 days of exposure. This same selenium concentration of ≥4.6 µg/g also adversely affected survival of razorback sucker larvae after 5-6 days of exposure in the first reproduction study conducted a year earlier (Hamilton et al. 2001).

Brine shrimp

In the present study, fish that received the reference food treatment were fed 1-day-old nauplii of brine shrimp (*Artemia* sp.). A systematic survey of eight strains of *Artemia* by the International Study on *Artemia* (ISA) reported that the source of the *Artemia* can have a major influence on the suitability for fish culture (Leger et al. 1986). They concluded that the reasons for the difference between a poor-quality and a variable-quality *Artemia* strain were complex, and due in part to a lack of knowledge of the nutritional requirements of the test fish species being cultured in the ISA study. The analysis of the ISA *Artemia* strains for amino acids, inorganic elements, pesticides, caloric content, and carotenoids yielded no data that could be related to the growth and survival of test fish during the culture testing. Leger et al. (1986) acknowledged that *Artemia* could not be used as a complete substitute for natural zooplankton or artificial diets, which have meet with limited success.

Brine shrimp used in the present study contained selenium concentrations of 3.2 µg/g (range 3.0 – 3.6 µg/g), which was close the proposed dietary threshold of 3 µg/g. This selenium concentration apparently was not toxic to the razorback sucker larvae because no abnormal mortality and altered growth were observed. However, that does not necessarily mean that long-term feeding of fish at that selenium concentration is safe.

It might be possible that long-term feeding of brine shrimp with 3.2 µg/g of selenium could be toxic. Most laboratory studies are too short in duration to determine the adverse effects of long-term (>12 months) exposures on fish. We are not aware of any studies conducted for a long-term period (>1 year) where fish were exposed to dietary selenium at low concentrations (2-4 µg/g). However, in the previous reproduction study, razorback sucker larvae fed zooplankton containing selenium concentrations of 4.6 µg/g or higher died within 10 days, which suggests that lower concentrations might be lethal over a longer period of exposure (Hamilton et al. 2001).

Selenium concentrations in brine shrimp used in the present study seemed to be elevated compared to other studies. Analysis of brine shrimp (Aquarium Products, Glen Burnie, MD, source Columbia, S.A.) used in culture at the Yankton FRS had selenium concentrations of 3.0 µg/g in 1992 (reported as wet weight, converted to dry weight assuming 88.4% moisture

measured in 1996), 2.4 µg/g in 1993 (reported as wet weight, converted to dry weight assuming 88.4% moisture), and 2.7 µg/g in 1996 (Hamilton et al. 2001). Coyle et al. (1993) also used brine shrimp from Aquarium Products and reported selenium concentrations of 2.7 µg/g. Olney et al. (1980) reported selenium concentrations of 1.0 µg/g in nauplii from Italy, 2.1 µg/g from Utah, and 0.8 µg/g from San Pablo Bay, CA, and below detection from Brazil. Petrucci et al. (1995) reported that adult brine shrimp from Italy contained selenium concentrations of 0.4-1.7 µg/g. Cowgill et al. (1987) reported that cysts contained 0.6 µg/g from Brazil, South America, 1.7 µg/g from Columbia, South America, and 0.2-2.0 µg/g from San Francisco Bay, CA. Interestingly, cysts in six cans from the same lot of brine shrimp from San Francisco Bay had a 10-fold variation in selenium concentration (0.19, 0.43, 0.48, 0.90, 1.20, 1.96 µg/g), which the authors concluded indicated that the actual geographic location of the collections were different. We do not know why brine shrimp from Columbia, South America, seem to have higher selenium concentrations than brine shrimp from other locations.

The concentrations of inorganic elements in brine shrimp was similar to concentrations of cobalt, chromium, copper, iron, manganese, molybdenum, nickel, vanadium and zinc in brine shrimp nauplii reported by Cowgill et al. (1987) (same source of cysts as in present study: Columbia, S.A.). Olney et al. (1980) and Petrucci et al (1995) also reported somewhat similar concentrations of inorganic elements in brine shrimp nauplii from other sources such as Australia, Brazil, Italy, California, and Utah.

Selenium interacts with several inorganic elements in fish, birds, and mammals (Diplock 1976, Whanger 1981, Marier and Jaworski 1983, Sorensen 1991). These interactions can be additive, antagonistic, or synergistic, and in some cases the interaction is reversed, i.e., antagonistic changed to synergism. In general, selenium toxicity is alleviated by antimony, arsenic, bismuth, cadmium, copper, germanium, lead, mercury, silver, tellurium, tin, and tungsten (Diplock 1976, Levander 1977, Howell and Hill 1978, Whanger 1981, Marier and Jaworski 1983), whereas chromium, cobalt, fluorine, molybdenum, nickel, tellurium, uranium, vanadium, and zinc apparently have no effect on selenium toxicity (Hill 1975, Ewan 1978).

Of the inorganic elements measured by ICP in nauplii of brine shrimp, arsenic and boron concentrations were elevated compared to concentrations in zooplankton, whereas concentrations of aluminum, barium, iron, manganese, silicon, strontium, titanium, and vanadium were lower than in zooplankton. However, only arsenic seemed elevated to concentrations of concern. The possible effects of higher concentrations of boron or lower concentrations of aluminum, barium, iron, manganese, silicon, strontium, titanium, and vanadium in brine shrimp compared zooplankton cannot be discounted, but the available literature strongly suggests that arsenic was a factor.

The arsenic concentration in brine shrimp nauplii in the present study was 24 µg/g. Arsenic concentrations in brine shrimp nauplii can vary widely between sources of brine shrimp cysts. Cowgill et al. (1987) reported arsenic concentrations of 9.7 µg/g in Brazilian brine shrimp, 11.0-19.5 µg/g in San Franciscan brine shrimp, and 15.7 µg/g in Columbian brine shrimp, whereas Petrucci et al. (1995) reported arsenic concentrations of 0.9-3.1 µg/g in Italian brine shrimp.

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

(Levander 1977). The protective effect of arsenic against selenium toxicity has been observed in rats, dogs, swine, cattle, and birds (Levander 1977). In general, arsenic exposure in water or diet protects against dietary selenium toxicity (Moxon 1938, Dubois et al. 1940, Klug et al. 1949, 1950, Levander and Argrett 1969, Thapar et al. 1969, Howell and Hill 1978, Hoffman et al. 1992), but combined arsenic and selenium waterborne exposure does not (Cabe et al. 1979, Frost 1981).

Dubois et al. (1940) and Klug et al. (1949) reported that the toxicity of selenite, selenomethionine, selenocystine, and seleniferous grain was reduced in rats by exposure to arsenic as either arsenite or arsenate, but not as arsenic sulfides. Klug et al. (1950) reported that selenium residues increased by 28% in liver, 141% in kidney, and 52% in muscle in rats exposed for 12 weeks to arsenic in water and selenium in the diet, compared to exposure to only selenium in the diet. Klug et al. (1950) also reported that arsenic protected against selenium-induced mortality, reduced growth, and reduced feeding and concluded that arsenic counteracts selenium toxicity in some way other than increasing elimination. Howell and Hill (1978) also reported increased selenium residues in liver of chicks exposed to dietary selenium and either arsenic, lead, tin, or tellurium compared to selenium exposure alone, and the interaction of these elements with selenium reduced the toxicity of selenium compared to selenium alone exposures. Levander and Argrett (1969) also reported that arsenic protected against selenosis in rats, and that selenium residues were increased in carcass compared to animals in the selenium alone exposure. Others have concluded that arsenic exposure increases the elimination of selenium in bile in short-term (1-10 hours) injection experiments (Diplock 1976, Levander 1977, Whanger 1981, Marier and Jaworski 1983). In a reciprocal manner, selenium has been reported to reduce arsenic-induced teratogenic deformities in hamsters (Holmberg and Ferm 1969).

Selenium residues in razorback sucker larvae after 10 days of exposure to the brine shrimp treatment and the 24-Road or Horsethief water treatments were elevated at 6.3-6.7 µg/g. Similarly, selenium residues in razorback sucker larvae after 10 days of exposure in the HTEW food treatment and the 24-Road or Horsethief water treatments residues were 6.1-7.0 µg/g. However, survival was very different between the two food treatments (with the same water treatments): 87% in the brine shrimp treatment, but 15-20% in the HTEW food treatment. The difference in survival of larvae may be due in part to the selenium concentrations in food at 10 days of exposure: 3.2 µg/g in brine shrimp verse 5.0 µg/g in zooplankton from HTEW. Nevertheless, selenium concentrations in brine shrimp were above the selenium dietary toxic threshold (3 µg/g; Maier and Knight 1994, Lemly 1996b), and whole-body residues were above the whole-body adverse effect threshold (4-5 µg/g based on Hilton et al. 1980, Hamilton et al. 1986, Hunn et al. 1987, Ogle and Knight 1989, Hamilton and Wiedmeyer 1990, Hamilton et al. 1990, Cleveland et al. 1993, Lemly 1993b, Hamilton 2001). Considering that the whole-body residues were similar, and selenium concentrations in food were relatively close, but survival was very different suggests that the selenium residue in larvae fed the brine shrimp diet (containing elevated arsenic concentrations of 24 µg/g, whereas HTEW food contained 6.5 µg/g) was somehow inactivated from having a toxic effect similar to the counteraction of selenium toxicity by arsenic reported by Klug et al. (1950) and Levander and Argrett (1969). This same scenario probably occurred in the previous study with razorback sucker larvae based on the whole-body residue and survival pattern (Hamilton et al. 2001).

The same counteraction of selenium toxicity by arsenic probably occurred at day 30 in larvae exposed to the brine shrimp food treatment and 24-Road or Horsethief water treatments

where whole-body residues were 5.2 µg/g. Survival of larvae fed brine shrimp was relatively high (52-83%) compared to the other food treatments (0-32%). Nevertheless, survival followed a concentration response: lowest survival (52%) in larvae exposed to North Pond water and with whole-body residues of 16.0 µg/g, intermediate survival (72%) in larvae exposed to Adobe Creek water and with whole-body residues of 12.0 µg/g, and highest survival (81-83%) in larvae exposed to 24-Road or Horsethief water and with whole-body residues of 5.2 µg/g.

In the absence of the protective effect of elevated arsenic (6.5 µg/g in HTEW zooplankton), a different survival pattern was present in the HTEW food treatment with either 24-Road or HT water treatments. The whole-body selenium concentration in razorback sucker larvae after 10 days of exposure to the HTEW food treatment and 24-Road water treatment was elevated at 7.0 µg/g. Similarly, selenium residues in razorback sucker larvae after 10 days exposure to HTEW food and HT water treatment was elevated at 6.1 µg/g. Accordingly, survival was very similar between the same food treatments (but with the different water treatments): 15-20%. The similarity in survival within the same food treatment, but different water treatments, was due to the use of the HTEW site food. The selenium concentration in HTEW food at 10 days of exposure (5.0 µg/g) was above the selenium dietary toxic threshold (3 µg/g; Maier and Knight 1994, Lemly 1996b), and whole-body residues were above the whole-body adverse effect threshold (4-5 µg/g based on Hilton et al. 1980, Hamilton et al. 1986, Hunn et al. 1987, Ogle and Knight 1989, Hamilton and Wiedmeyer 1990, Hamilton et al. 1990, Cleveland et al. 1993, Lemly 1993b, Hamilton 2001). Considering that the whole-body residues were elevated, selenium concentrations in HTEW zooplankton were above the selenium dietary toxicity threshold, arsenic concentrations in zooplankton from HTEW were low (6.5 µg/g), and survival was lower than in the brine shrimp food treatments (which had elevated arsenic concentrations) suggests that the selenium residues in larvae fed the HTEW food were not ameliorated in the absence of arsenic, which is the opposite of the counteraction of selenium toxicity by arsenic reported by Klug et al. (1950) and Levander and Argrett (1969). This same scenario probably occurred in the previous study with razorback sucker (Hamilton et al. 2001). Arsenic concentrations in the brine shrimp diet may have also reduced selenium toxicity in the present study in the AC and WW water treatments where indirect toxicity from waterborne selenium accumulation in periphyton and detritus may have occurred unintentionally through the diet exposure route. One reviewer of the report of the previous reproduction study (Hamilton et al. 2001) also suggested that the brine shrimp diet may have "saved" the razorback sucker larvae from the toxic effects of selenium (G. Noguchi, USFWS, personal communication).

The relatively low arsenic concentrations in invertebrates in the present study compared to selenium concentrations was similar to collections of invertebrates from the Grand and Gunnison valleys. Butler et al. (1994) reported low arsenic concentrations compared to selenium concentrations in aquatic invertebrates collected from several locations in the upper Colorado River basin: 2.4 µg/g arsenic & 6.1 µg/g selenium at Horsefly Creek, 0.9 µg/g arsenic & 26 µg/g selenium at Loutsenhizer Arroyo, 1.5 µg/g arsenic & 4.8 µg/g selenium at Smith Fork, 0.5 µg/g arsenic & 2.0 µg/g selenium at North Fork Gunnison River at Paonia, 3.5 µg/g arsenic & 11 µg/g selenium at Indian Wash, 1.4 µg/g arsenic & 32 µg/g selenium at Markley Pond. Butler et al. (1991) also reported similar results for five other locations in the upper Colorado River basin. In only one composite sample of aquatic invertebrates from Happy Canyon, Spring, and Dry creeks in the Uncompahgre River basin the arsenic concentration was substantially greater (9.1 µg/g)

than the selenium concentration (2.8 µg/g) (Butler et al. 1991). In most locations in the Grand and Gunnison valleys, the amount of arsenic in food organisms seemed to be sufficiently low compared to selenium concentrations to not alleviate selenium toxicity.

Zooplankton

Dabrowski (1984) reviewed the literature on feeding of fish larvae and stated that zooplankton were the natural food of fish larvae, and that "zooplankton can be used as a model for the formulation of artificial feed." He went on to discuss problems in the culture of fish larvae associated with formulating nutritionally adequate, artificial dry diets, and rearing intensive mono-cultures of live invertebrates for feeding fish. In the present study, we collected and fed live zooplankton from the general biogeographical area where the last riverine population of razorback sucker occur, which suggests that the composition of zooplankton was probably similar to what wild larvae would have encountered. In addition, although not counted as food, some algae and detrital material was present in the rations of zooplankton fed to razorback sucker larvae, and algae and periphyton that grew on the walls of the exposure beakers also were a food source for the larvae.

The zooplankton fed in the present study were predominantly cladocerans and copepods, whose ratio (cladoceran/copepod) varied greatly between collections, i.e., Horsethief east wetland 1.1 to 195, Adobe Creek 2.5 to 200, and North Pond 0 to 0.5. In the previous study, the ratio of cladocerans to copepods was 0.9 to 72 at Horsethief east wetland, 0.05 to 61 at Adobe Creek, and 0.05 to 7 at North Pond (Hamilton et al. 2001). This wide variation in the ratios of the cladocerans to copepods was similar to that reported by Grabowski and Hiebert (1989) for backwaters near Island Park, Jensen, and Ouray on the Green River. The wide range in these ratios may reflect changes in water temperature, light intensity, time of placement or retrieval of light traps, seasonal community structure, water flow, or other unknown factors.

Razorback sucker larvae seem to be opportunistic feeders and are capable of using zooplankton, benthic organisms, algae, and detritus as food, sometimes selectively and other times in proportion to the abundance of the item. Marsh and Langhorst (1988) found that stomachs of razorback sucker larvae in Arizona Bay backwater in Lake Mohave contained *Bosmina* (Cladoceran) 55%, rotifers 53%, and copepoda 45% of the time. They also reported larvae exhibited a positive selection for cladocerans, especially *Bosmina*, and strong negative selection for rotifers. In a more detailed report of their research with razorback sucker larvae, Langhorst and Marsh (1986) reported that larvae used all available habitats (limnetic, benthic, and macrophytic) as evidenced by the presence in larvae stomachs of *Macrothrix*, a cladoceran associated with vegetation. Gut analysis of razorback sucker larvae stocked into, and collected 1 week later, from a backwater in the Salt River, AZ, indicated that chironomid larvae were the dominant food (J. Brooks, USFWS, personal communication, cited in Bestgen 1990). Papoulias and Minckley (1992) reported that razorback sucker larvae (7 days posthatch) were eating sessile diatoms, phytoplankton, and detritus the first day after stocking in ponds, were eating rotifers, nauplii, cladocerans, insect eggs, and chironomids by 8 days posthatch. Muth (1995) examined gut contents of razorback sucker larvae collected in low velocity habitats of the middle and lower Green River, and reported similar items were consumed including rotifers, chironomid larvae, filamentous and colonial algae, diatoms, and organic debris. From a fish culture standpoint, Toney (1974) reported that razorback sucker larvae actively fed on small amounts of baby food

and strained beef liver for the first 2 weeks after hatch, and after placement in an outside raceway, they "gorged themselves" on Cladocera and blackfly larvae.

From a caloric standpoint, aquatic invertebrates, including cladocerans and copepods, have similar energy content as nauplii of brine shrimp (5.32 kcal/g, Hamilton and Buhl 1999; source was Columbia, S.A.), which were used as reference food in the present study. Driver et al. (1974) reported that the caloric values (kilocalories/gram:kcal/g) measured by calorimeter for 33 species of aquatic invertebrates ranged from 3.70 to 6.97 kcal/g (the calculated mean using the mean value for the reported ranges was 5.52 kcal/g SE ±1.01). Driver (1981) later reported that the caloric values for 24 species of aquatic invertebrates ranged from 3.68 to 6.27 kcal/g dry weight with a mean of 5.22 kcal/g. Cummins and Wuycheck (1971) reported that the caloric content was 4.30-6.28 kcal/g for cladocerans, 5.33-7.38 kcal/g for copepods, and 5.47 kcal/g (grand mean) for all aquatic invertebrates in their study.

Watanabe et al. (1983) assessed the nutritional values of live organisms used for mass propagation of fish and reported that the essential fatty acid (EFA) contents, but not mineral content, of live food organisms determined the chief nutritional value for fish larvae. They reported that the EFA content in two cladocerans, *Moina* and *Daphnia*, made them suitable as food organisms for fish larvae. From a calculated energy content standpoint, the energy content of *Moina* (5.1 kcal/g) and *Daphnia* (4.3 kcal/g) was similar to that of other common live culture foods such as brine shrimp (4.4 kcal/g from San Francisco Bay, 4.7 kcal/g from South America, 4.2 kcal/g from Canada), a rotifer (*Brachionus plicatilis*; 4.7-5.4 kcal/g), and two marine copepods (4.1-5.2 kcal/g). Schauer et al. (1980) used a wet oxidation technique to measure energy content of five geographic strains of brine shrimp (Australia, Brazil, Italy, San Francisco Bay, and Utah) and reported values ranging from 5.4 to 6.0 kcal/g with a mean of 5.6 kcal/g (reported as J/g; J x 0.239=cal). Because the caloric content of brine shrimp and freshwater aquatic invertebrates were similar, the use of brine shrimp in the present study as a reference food organism and field-collected zooplankton seemed appropriate. However, some investigators have reported that natural food organisms either alone or in combination with brine shrimp nauplii were better for culturing fish than brine shrimp nauplii alone (Colesante 1989, Mason et al. 1992, Ronnestad et al. 1998, Shields et al. 1999). Brine shrimp also have been used as substitutes for natural food organisms in a dietary toxicity study with rainbow trout (Mount et al. 1994).

In the present study, larvae were fed 40 organisms/fish (250 organisms/L or 25 organisms per L per fish for 10 fish). Papoulias and Minckley (1990) reported in their laboratory study that 30 to 60 nauplii of brine shrimp per fish per day was sufficient to maintain high survival and no reduced growth, whereas 12 or less nauplii per fish per day resulted in substantial mortality and reduced growth. Papoulias and Minckley (1992) reported high larval survival (77, 90, and 67%) in ponds with zooplankton densities of 43, 24, and 12 organisms/L, respectively. In a feeding study where 5-day-old razorback sucker larvae were fed 20, 40, or 80 nauplii of brine shrimp per fish per day for 20 days, survival was 95% or better in all treatments (Hamilton et al. 1996). They also tested 24-day-old razorback sucker and confirmed that as little as 20 nauplii per fish per day would maintain a high survival rate (97%).

Likewise, Marsh and Langhorst (1988) concluded that nutritional factors such as type, number, or size of available foods did not affect survival or growth of razorback sucker larvae in Lake Mohave, which had 1.55 zooplankton/L (reported as 1,554 zooplankton/m^3) or in Arizona Bay backwater, which had 0.38 zooplankton/L (reported as 377 zooplankton/m^3). In contrast,

Papoulias and Minckley (1990, 1992) suggested that at those low zooplankton densities, larval survival might be reduced in Lake Mohave, but not at the more common densities reported for Lake Mead (25-41 zooplankton/L), Lake Havasu (83 zooplankton/L), or Lake Mohave (29-45 zooplankton/L) (Papoulias and Minckley 1992). However, Papoulias and Minckley (1990, 1992) failed to acknowledge that Marsh and Langhorst (1988) reported that razorback sucker larvae were abundant and growing (>20 mm total length) in the Arizona Bay backwater site, which had 0.38 zooplankton/L. Larvae at that site only disappeared after the berm isolating the backwater from the main lake was breached allowing predators to enter, after which all larvae disappeared in 30 days. The organism densities in the field studies by Papoulias and Minckley (1992) and Marsh and Langhorst (1988) encompassed all possible sizes, whereas the zooplankton used in the present study were preselected to be <0.425 mm, which would have represented only a portion of the various sizes present in a field situation and presumably available to larval fish.

Feeding densities in the present study were higher than wild larvae would encounter naturally. Razorback sucker larvae in the present study were initially fed either 250 organisms/L, which was 17 times greater than the 15 organisms/L, which included all possible sizes, reported by Cooper and Severn (1994) for Sheppard Bottom pond 3 at Ouray NWR, UT. Grabowski and Hiebert (1989) reported higher zooplankton concentrations in backwaters (seasonal average 0.4-1.5 zooplankton/L) than in river sections (0.04-1.0 zooplankton /L) near Island Park, Jensen, and Ouray on the Green River in 1987-1988. They reported high concentrations of 16.6 zooplankton/L in a Jensen backwater (seasonal average 4.1 zooplankton/L) and 15.1 zooplankton/L in an Ouray backwater (seasonal average 5.2 zooplankton/L).

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

The size of zooplankton fed to larvae in the present study (<0.425 mm) seemed to be of the appropriate size range. Langhorst and Marsh (1986) reported that razorback sucker larvae 11-14 mm total length selected smaller plankton to feed on compared to all sizes available in the plankton population at large. They found size selection was especially notable for *Daphnia*, which averaged 0.66 to 0.86 mm in length, in larvae stomachs. The selected *Daphnia* were about half the average size (1.42 to 1.60 mm) in their plankton samples. Papoulias and Minckley (1992) found that razorback sucker larvae exhibited prey-size selection, based on body width, and consumed prey from 0.1 to 0.4 mm in width.

Good survival of zooplankton from the three sites held in the four test waters (24-Road, HT, AC, WW), but without fish present, suggests that the zooplankters were not adversely affected by short-term exposure to the test waters. Even if zooplankters died after placement in the exposure beakers, they would have fallen to the bottom and been available to feeding larvae through the detrital food chain.

By selecting small-sized zooplankton for feeding in the present study, we may have inadvertently selected zooplankton with elevated inorganic residues compared to larger-sized invertebrates. For example, van Hattum et al. (1991, 1996) reported that invertebrate size, rather

than species or sex, explained most of the variation in inorganic element accumulation in aquatic invertebrates. Timmermans et al. (1989) and Farag et al. (1998) also reported that small-sized aquatic invertebrates had higher concentrations of inorganic elements than larger-sized invertebrates. Consequently, Farag et al. (1998) concluded that early life stages of fish may be exposed to larger doses of inorganic elements that larger older life stages. In the present study, the small-sized zooplankton fed to razorback sucker larvae were the appropriate size due to morphological considerations, but small-sized invertebrates may have presented a greater dose of inorganic elements, including selenium, to the razorback sucker larvae.

Selenium in larvae

The whole-body toxic threshold for selenium-induced adverse effects, i.e., mortality of juveniles and reproductive failure, in a selenium-sensitive fish proposed by Lemly (1996b) was 4 µg/g, whereas Maier and Knight (1994) proposed 4.5 µg/g as the concentration above which adverse affects occur in fish and wildlife. These values were derived from a review of several laboratory and field studies where selenium was the toxicant of concern. Hamilton et al. (2000) cited three additional studies that documented whole-body selenium concentrations of 3.6 to 5.5 µg/g in juvenile fish were associated with adverse effects. Information in the selenium literature linking whole-body residues of 4 µg/g or higher with adverse effects in fish seems sufficient to suggest that a tissue-based selenium criterion should be used for the protection of aquatic life rather than a water-based criterion (Hamilton 2001).

At day 10, larvae fed brine shrimp and held in 24-Road or Horsethief water had higher selenium residues than at day 30. The initially high residues apparently decreased due to depuration and tissue dilution as fish grew. Investigations have reported decreases in selenium residues in fish due to tissue dilution from growth (Bennett et al. 1986) or due to depuration of initially high selenium residues in larvae while being fed food with a low selenium concentration (Birkner 1978). An interesting example is given in Birkner (1978) who conducted a 90-day study with juvenile fathead minnow that initially had a whole-body selenium concentration of 13.9 µg/g. After 90 days of exposure, fish fed zooplankton with selenium concentrations of 1.2 µg/g had whole-body residues of 5.0-5.7 µg/g, those fed zooplankton with 5.7 µg/g had a whole-body residue of 5.2-7.0 µg/g, and those fed zooplankton with 11.8 µg/g had a whole-body residue of 10.3-11.0 µg/g. Thus, fish depurated selenium from their initial whole-body residue to close to the concentration in their food.

Selenium residues in razorback sucker larvae after 10 days of exposure to the brine shrimp or HTEW food treatments and the 24-Road or Horsethief water treatments were elevated at 6.1-7.0 µg/g. However, survival was very different between the two food treatments within the same water treatment: 87% in the brine shrimp food treatment, but 15-20% in the HTEW food treatment. The elevated whole-body residues in larvae fed the brine shrimp food treatment (6.3-6.7 µg/g), but lack of adverse effects on survival of larvae suggested that something counteracted the toxic effects that should have been manifested by the selenium tissue residues. Whole-body residues above 4-5 µg/g have been associated with adverse effects (Hilton et al. 1980, Hamilton et al. 1986, Hunn et al. 1987, Ogle and Knight 1989, Hamilton and Wiedmeyer 1990, Hamilton et al. 1990, Cleveland et al. 1993, Lemly 1993b, Hamilton 2001). The difference may be due in part to the selenium concentrations in food at 10 days of exposure, which were 3.2 µg/g in brine shrimp and 5.0 µg/g in zooplankton from HTEW. More likely, the

elevated arsenic concentrations (24 µg/g) in brine shrimp inactivated the toxic effect of the elevated selenium residues in larvae much like the counteraction of selenium toxicity by arsenic reported by Klug et al. (1950) and Levander and Argrett (1969) as discussed above. The same scenario probably occurred at day 30 in larvae in exposed to the brine shrimp treatment and 24-Road or Horsethief water treatments where whole-body residues were 5.2 µg/g. Those selenium residues were higher than those typically reported in control fish from laboratory studies or in reference fish from field studies, which were ≤2 µg/g (Hamilton et al. 2000).

The high whole-body selenium residues in razorback sucker larvae fed zooplankton from Adobe Creek or North Pond and exposed in Adobe Creek or North Pond waters for 10 days (14-28 µg/g) were similar to those reported by others. Bennett et al. (1986) reported whole-body selenium residues of 52 µg/g in 17-day-old fathead minnow larvae fed rotifers containing 46 µg/g selenium for 9 days, and 61 µg/g in 9-day-old larvae fed rotifers with 91 µg/g for 7 days. Dobbs et al. (1996) reported whole-body selenium residues of 47 µg/g in fathead minnow larvae fed rotifers containing 40-47 µg/g selenium for 7 days.

At 30 days of exposure, the increased selenium residues in larvae fed brine shrimp and exposed in water from Adobe Creek or North Pond compared to the 24-Road or Horsethief water treatments revealed that larvae were accumulating selenium. There was a trend at 10 days of exposure for increased selenium residues in larvae fed brine shrimp and exposed in the Adobe Creek or North Pond waters. This selenium uptake may have come from either waterborne or dietary exposure. Selenium uptake in fish from water exposure can occur via the gill and epithelium (Hodson et al. 1986). Selenium uptake in larvae in the Adobe Creek and North Pond water treatments may have come from dietary uptake of algae, periphyton, and detritus growing on the walls or bottom of the exposure beakers. Selenium is readily accumulated from water into algae (Nassos et al. 1980, Foe and Knight 1986, Riedel et al. 1991, Besser et al. 1993), periphyton (Graham et al. 1992), and detritus (Bender et al. 1991). Algae, periphyton, and detritus are consumed by razorback sucker larvae (Papoulias and Minckley 1992). In the present study, larvae probably accumulated selenium residues indirectly via a dietary exposure route in the Adobe Creek and North Pond water treatments.

Growth

In the present study, there was no consistent difference in growth of larvae in food or water treatments. Some studies with selenium exposure in the water, diet, or both have reported inconsistent results: (1) reduced growth occurred in the same treatments (exposure concentration and duration) where reductions in survival occur (Hilton et al. 1980, Klauda 1986), (2) reduced survival occurred before reduced growth (Hunn et al. 1987, Woock et al. 1987, Hamilton et al. 1990 [San Luis Drain diet], Crane et al. 1992, Hermanutz et al. 1992, Cleveland et al. 1993), (3) reduced growth occurred before reduced survival (Hilton and Hodson 1983, Ogle and Knight 1989, Hamilton et al. 1990 [selenomethionine diet]), or (4) no effects occurred on growth or survival, but other pathological or reproductive effects occurred (Hodson et al. 1980, Coyle et al. 1993). The inconsistency between these studies was probably due to differences in species, age, exposure route and duration, selenium form, and other factors.

The smaller total length and weight of fish that died in the last 8 days of the 30-day study compared to fish that were measured alive at the end of the study, suggested that these fish were stressed and metabolic energy was shifted from growth functions to combating the stresses.

Growth is the culmination of many biochemical phenomena that occur in a somewhat regulated pattern, and reduced growth may occur when contaminant intoxication induces biochemical changes that interfere with normal growth processes (Mehrle and Mayer 1985). If these smaller fish had not died, the consequence of reduced growth at these early life stages is that swimming performance would be reduced and susceptibility to predation would be increased (Bams 1967, Taylor and McPhail 1985), and feeding efficiency would be reduced (Gunn and Noakes 1987). Functions that promote fish survival are also related to body size (Ojanguren et al. 1996). For example, sensory systems develop rapidly during early life stages and visual acuity increases with fish size, enabling large fry to better detect predators and food and resist starvation, thus allowing them access to a wider range of prey, which increases the likelihood of finding appropriate food. Consequently, razorback sucker larvae with reduced growth, may be less fit to survive the rigors of competition and predation, especially with nonnative fish. Predation on larval and juvenile razorback sucker by invertebrates and fish has been reported (Marsh and Langhorst 1988, Marsh and Brooks 1989, Horn et al. 1994, Modde and Wick 1997).

Survival

Razorback sucker larvae in the present study probably did not die from starvation because Papoulias and Minckley (1990) reported that the point of irreversible starvation, and subsequent mortality, was between 19 and 23 days, whereas the median time to 50% mortality for unfed larvae was between 24 and 25 days. In the HTEW, AC, and WW food treatments in the present study, nearly complete mortality occurred in 10 to 14 days, which is less than the period when irreversible starvation could have influenced later mortality or the median time to 50% mortality of unfed larvae reported by Papoulias and Minckley (1990).

The process of yolk sac absorption probably was not associated with the observed mortality beginning at day 6 of the study. Minckley and Gustafson (1982) reported that hatching in razorback sucker occurred at 131 hours of development, and yolk sac absorption occurs at 311 hours of development – a difference of 180 hours = 7.5 days posthatch. The current study was started with 5-day-old fish and the mortality occurred at 6 days of exposure [total 11 days]. Assuming that the larvae used in the present study had similar development to those examined by Minckley and Gustafson (1982), the larvae in the present study would have been feeding for at least 3-4 days (11-7.5=3.5 days), which is probably sufficient time to incur toxicity from dietary selenium in very small larval fish. The actual amount of time of feeding in the present study was probably longer because Minckley and Gustafson (1982) conducted their study in 14ºC water, whereas in the present study the holding temperature at 24-Road for egg development and first 4 days posthatch was about 24ºC and the first 6 days in the mobile laboratory was about 21ºC. At the lower temperatures of Minckley and Gustafson (1982), development would have taken longer than in the present study, whereas at the higher temperatures in the present study, development time would have been shorter and the time at first feeding earlier than suggested by Minckley and Gustafson (1982). Consequently, yolk sac absorption probably did not have an influence on larval mortality.

In the present study, the time frame of nearly 100% mortality in exposures receiving HTEW food was similar to Bryson et al. (1984) who reported that juvenile bluegill experienced 97% mortality within a week after being fed plankton, which contained 45 µg/g selenium and were collected from Hyco Reservoir, NC. Selenium concentrations in the diet at concentrations

close to the proposed selenium dietary toxic threshold of 3 µg/g have been documented to cause adverse effects in young fish usually following 30-60 days of exposure. For example, Hamilton et al. (1990) reported that 5.3 µg/g selenium in the diet reduced growth of chinook salmon (*Oncorhynchus tshawytscha*), Cleveland et al. (1993) reported that 6.5 µg/g selenium in the diet reduced survival of bluegill, and Lemly (1993b) reported that 5.1 µg/g selenium in the diet (and 4.8 µg/L selenium in the water) reduced survival of bluegill held under winter stress conditions. In the previous reproduction study, razorback sucker larvae had reduced survival within the first 10 days of exposure when fed zooplankton with selenium concentrations of 4.6 µg/g (Hamilton et al. 2001), which is similar to the results of the present study

The time frame of mortality in the present study was also similar to another study with razorback sucker larvae where larvae were fed zooplankton with different concentrations of selenium collected from sites at Ouray NWR, UT (Hamilton et al. 1996). In one test initiated with 5-day-old larvae, rapid mortality in larvae began 5-10 days after feeding zooplankton containing selenium concentrations of 3.5 to 25.7 µg/g. These selenium concentrations, as well as those reported by Hamilton et al. (1990; 5.3 µg/g), Cleveland et al. (1993; 6.5 µg/g), and Lemly (1993b; 5.1 µg/g) indicate that the selenium concentration in zooplankton from Horsethief east wetland (average 4.6 µg/g for days 1-7; Table 16) was sufficiently elevated to cause the reduced survival, which started at day 6. In the first reproduction study with razorback sucker, selenium concentrations of 4.6 µg/g or greater were also linked with a sharp decline in survival of razorback sucker larvae at about 5-6 days of exposure (Hamilton et al. 2001).

In addition, waterborne exposure to AC or WW water (tested with brine shrimp) contributed to the reduced survival of the larvae (Figure 7A). Other studies have also reported that combined water and dietary exposures of fish to selenium was additive (Bertram and Brooks 1986, Woock et al. 1987). However, the magnitude of the effect of HTEW, AC, or WW food (tested with 24-Road water) on mortality (Figure 8A) was much greater than that contributed by exposure to AC or WW water (tested with brine shrimp) (Figure 7A). This prominence of adverse effects from dietary sources over waterborne sources has also been stated in review papers (Maier et al. 1988, Maier and Knight 1994, Lemly 1996b). Arsenic concentrations in the brine shrimp diet may have also reduced selenium toxicity in the AC and WW water treatments where indirect toxicity from waterborne selenium accumulation in periphyton and detritus may have occurred unintentionally through the diet exposure route.

In the present study, because (1) zooplankton types, feeding density, and energy content seemed appropriate for proper nutrition of the larvae, (2) the time frame of mortality precluded the influence of starvation or yolk absorption, and matched those in studies of dietary selenium toxicity with fish, (3) selenium concentrations, but not other inorganic elements, were elevated in zooplankton above well-documented selenium dietary toxic thresholds, (4) the significant negative correlation between larval survival at day 30 and selenium concentrations in zooplankton, and (5) selenium residues in larvae were above whole-body concentrations linked with reduced survival in young fish reported in other studies, it seems reasonable to assume that a diagnostic pattern has been formed that leads to the conclusion that dietary selenium was the primary cause of the reduced survival of larvae (discussed further in the section on hazard assessment).

Likewise, because (1) brine shrimp have been documented as a suitable food organism in larval fish culture, (2) selenium concentrations in brine shrimp were near well-documented selenium dietary toxic threshold, (3) arsenic concentrations were elevated in brine shrimp, (4)

arsenic counteracts selenium toxicity and allows selenium residues to accumulate in animals above those of selenium alone exposures without having a toxic effect, (5) selenium residues in larvae fed brine shrimp were above whole-body concentrations linked with reduced survival in young fish reported in other studies, and (6) reduced survival did not occur in larvae fed brine shrimp, it seems reasonable to assume a diagnostic pattern has been formed that leads to the conclusion that dietary arsenic counteracted selenium toxicity thus allowing good survival of larvae fed brine shrimp.

Delayed mortality

In the present study, a significant delay in mortality, based on the predicted time-to-death, occurred in razorback sucker larvae fed HTEW food and exposed in HT, AC, or WW waters compared to the 24-Road water treatment, and in larvae fed AC and WW food compared to the HTEW food treatment. The same delayed mortality, based on the predicted time-to-death, occurred in razorback sucker larvae tested in 1996 using the same sites for collection of waters and food organisms as in the present study (Hamilton et al. 2001). The delayed mortality observed in the present study also was similar to that of razorback sucker larvae held in water from Sheppard Bottom pond 1 and fed food organisms from either North Roadside Pond or South Roadside Pond compared to larvae fed food organisms from Sheppard Bottom pond 1 at the Ouray NWR, UT (Hamilton et al. 1996).

For the HT, AC, and WW food treatments, predicted time-to-death tended to be shortest for HT food, intermediate for AC food, and longest for WW food, which suggested an antagonistic interaction, i.e., higher inorganic element concentrations in AC and WW foods compared to HT food resulted in a delayed predicted time-to-death regardless of the water treatment. As discussed earlier in the brine shrimp section, selenium toxicity is alleviated by antimony, arsenic, bismuth, cadmium, copper, germanium, lead, mercury, silver, tellurium, tin, and tungsten (Diplock 1976, Levander 1977, Howell and Hill 1978, Whanger 1981, Marier and Jaworski 1983). It seems reasonable to assume that inorganic components in AC and WW foods interacted with selenium in AC and WW foods, regardless of the water exposure, to delay the predicted time-to-death because the effect occurred in all four water tested.

Specific to the HT food treatment, predicted time-to-death tended to be shortest in HT water, intermediate in AC water, and longest in WW water, which suggested an antagonistic interaction, i.e., higher inorganic element concentrations in AC and WW waters compared to HT water interacted with inorganic elements in HT food resulting in a delayed predicted time-to-death. It seems reasonable to assume that inorganic components in AC and WW waters interacted with some element(s) in HT food (selenium was the only element elevated to concentrations of concern) to cause the delay in predicted time-to-death.

If a typical concentration-response relation was present in the current study, larvae fed AC (32.4 µg/g) or WW (52.5 µg/g) food, regardless of water exposure, should have died sooner than larvae fed HTEW food (6.0 µg/g). However, the difference in the predicted time-to-death between the HTEW food and WW food (at the 50% level: 14 days in 24-Road water, 8 days in HT water, 4 days in AC water, and 5 days in WW water) suggested that selenium toxicity from HTEW food was exhibited in the 24-Road water treatment without alleviation by other inorganic elements, but interactions with other elements in HT, AC, and WW waters alleviated some of the selenium toxicity. Nevertheless, the delayed mortality in the present study (4-14 days) was

probably not significant from a biological standpoint.

It has been suggested that the delayed mortality of larvae fed food from either Adobe Creek or North Pond compared to larvae fed food from Horsethief argues against a concentration response related to selenium concentrations in food (Recovery Program reviewer, written communication). They suggested that perhaps the food organisms at Horsethief had a more toxic species of selenium, and therefore, was more toxic to larvae than the species of selenium in food organisms at Adobe Creek or North Pond. Investigators have reported that algae and bacteria contain several seleno–analogues of sulphur amino acids incorporated mostly into protein, but also in amino acid fractions of extracts (Wrench 1978, Foda et al. 1983, Bottino et al. 1984). The forms reported include selenocysteic acid, selenomethionine, selenocystine, selenocysteine, Se-methyl selenocysteine, selenoxide form and selenone form of Se-methyl selenocysteine, Se-methyl selenomethionine, and hydrogen selenide. In spite of several organic selenium components being identified in plants and bacteria, only two organic forms of selenium have been tested with fish to determine their toxicity: selenomethionine (Sandholm et al. 1973, Woock et al. 1987, Ogle and Knight 1989, Hamilton et al. 1990, Besser et al. 1993, Cleveland et al. 1993, Coyle et al. 1993) and selenocystine (Bryson et al. 1985). In one test comparing selenomethionine and selenocystine, the investigators found that accumulation of selenomethionine in fish was similar to accumulation from selenium-laden natural zooplankton, and both had higher accumulation than from selenocystine (Bryson et al. 1985). The greater effect from exposure to selenomethionine compared to selenocystine was probably due to the greater ability of fish to digest selenomethionine than selenocystine (Bell and Cowey 1989). The limited toxicological information on the toxicity of various forms of organic seleno-compounds does not seem to support a suggestion of differential seleno-compounds in Horsethief zooplankton versus zooplankton from Adobe Creek and North Pond.

The more rapid mortality of larvae fed the HTEW diet compared to the AC and NP diets suggested a concentration response was not present. Similar to the results of the present study, nine other studies have reported no concentration-response in exposures of fish to dietary selenium (Hilton et al. 1980, Bryson et al. 1984, Bennett et al. 1986, Woock et al. 1987, Coughlan and Velte 1989, Ogle and Knight 1989, Hamilton et al. 1990, Cleveland et al. 1993, Coyle et al. 1993). In contrast, only two studies have reported a concentration response (Goettl and Davies 1977, Hamilton et al. 1990), and three studies have reported a partial concentration response limited in part by the experimental design (Geottl and Davies 1978, Hilton et al. 1980, Lemly 1993b). There is a narrow range of selenium concentrations between nutritionally optimal (i.e., 0.3 µg/g) and potentially toxic dietary exposures (i.e., 2 µg/g) to vertebrate animals, about a 7-to 10-fold range (Wilber 1980, NRC 1989). This small range of selenium concentrations between homeostasis and toxicity suggests that the range between sublethal effects and lethal effects also would be rather small, possibly over a range of selenium concentrations spanning 2-3 µg/g. Thus, a readily apparent concentration-response would not be expected unless the exposure concentrations were, for example, 2, 3, 4, 5, 6, 7, and 8 µg/g, and there were sufficient replicates to identify small statistical differences. Consequently, the majority of dietary selenium studies with fish do not report a concentration response, which is similar to the present study and the first reproduction study (Hamilton et al. 2001).

The three treatment combinations representing environmental conditions were the HT water and HTEW food treatment, the AC water and AC food treatment, and the WW water and WW food treatment. The delayed mortality of larvae in the WW water and WW food treatment,

along with the greater survival (30%) after 30 days of exposure, compared to the HT water/HTEW food treatment (10% survival) or AC water /AC food treatment (21% survival) does not imply that North Pond is a better site for rearing razorback sucker larvae than Horsethief east wetland or Adobe Creek. The conditions at Horsethief east wetland although relatively low in selenium concentrations (1.6 µg/g in water, 4.6 µg/g in zooplankton, 0.9 µg/g in sediment top layer) combined with the low concentrations of other inorganic elements and water quality characteristics (i.e., probably no antagonistic interactions) resulted in elevated selenium concentrations in larvae (8 µg/g) and low survival (10%). Based on reports of the NIWQP, the conditions at North Pond (13 µg/g in water, 42 µg/g in zooplankton, 16 µg/g in sediment top layer) or Adobe Creek (3.4 µg/g in water, 24 µg/g in zooplankton, 2.5 µg/g in sediment top layer) are less common in the upper Colorado River than are those at Horsethief east wetland (Bulter et al.1991, 1994, 1996). Even though survival of larvae was higher in the AC water /AC food treatment and in the WW water/WW food treatment, and selenium residues in were 32 µg/g in the AC treatments and 64 µg/g in the WW treatments, it is unlikely that those larvae could have carried these residue concentrations without adverse effects occurring as they moved to cleaner environments and metabolized the residue load.

It should be noted that larvae used in the larval fish study were from unexposed brood stock and larvae were hand selected using the criterion in ASTM (1989). Criteria for use of fish in laboratory studies is that they are free of disease, uniform in size and age, have good nutrition, and holding conditions (temperature, water quality, photoperiod, uncrowded, minimal handling) (ASTM 1989). The guidelines further state that organisms should be free of signs of stress, physical damage, mortality, and external parasites, and that abnormal or injured individuals not be used in tests, nor should organisms exhibit abnormal behaviors such as not eating, flashing, flipping, swimming erratically, emaciated, gasping at the surface, hyperventilating, hemorrhaging, producing excessive mucus, or showing abnormal color. These types of guidelines were developed primarily for use with routinely tested organisms such as rainbow trout and fathead minnow with well-known culture procedures, past successful use in testing, and ease of handling. Consequently, toxicant concentrations that cause effects in razorback sucker larvae hand-selected according to standard testing guidelines (ASTM 1989) could tend to underestimate toxicant impact in the environment where a variety of environmental and organismal conditions would occur.

HAZARD ASSESSMENT

Lemly (1995) presented a protocol for aquatic hazard assessment of selenium, which was formulated primarily in terms of the potential for food-chain bioaccumulation and reproductive impairment in fish and aquatic birds. The protocol incorporated five ecosystem components including water, sediment, benthic invertebrates, fish eggs, and bird eggs. Each component was given a numeric score based on the degree of hazard: 1, no identifiable hazard; 2, minimal hazard; 3, low hazard; 4, moderate hazard; 5, high hazard. The final hazard characterization was determined by adding the individual scores and comparing the total to the following evaluation criteria: 5, no hazard; 6-8, minimal hazard; 9-11, low hazard; 12-15, moderate hazard; 16-25, high hazard. Lemly (1996a) modified his protocol for use with four ecosystem components due to the difficulty in collecting residue information for all five components in an assessment. He adjusted the final ecosystem-level hazard assessment to the following four-component evaluation

criteria: 4, no hazard; 5-7, minimal hazard; 8-10, low hazard; 11-14, moderate hazard; 15-20, high hazard.

Lemly (1995) defined five categories of hazards as follows: (1) high hazard denotes an imminent, persistent toxic threat sufficient to cause complete reproductive failure in most species of fish and aquatic birds; (2) moderate hazard indicates a persistent toxic threat of sufficient magnitude to substantially impair, but not eliminate reproductive success; some species will be severely affected whereas others will be relatively unaffected; (3) low hazard denotes a periodic or ephemeral toxic threat that could marginally affect the reproductive success of some sensitive species, but most species will be unaffected; (4) minimal hazard indicates that no toxic threat identified but concentrations of selenium are slightly elevated in one or more ecosystem components (water, sediment, invertebrates, fish eggs, bird eggs) compared to uncontaminated reference sites; (5) no hazard denotes that no toxic threat is identified and selenium concentrations are not elevated in any ecosystem component. The following hazard rating table is based on Lemly (1996a):

		Hazard				
		None	Minimal	Low	Moderate	High
Ecosystem component	Water (µg/L)	<1	1-2	2-3	3-5	>5
	Sediment (µg/g)	<1	1-2	2-3	3-4	>4
	Benthic invertebrate (µg/g)	<2	2-3	3-4	4-5	>5
	Fish eggs (µg/g)	<3	3-5	5-10	10-20	>20

These protocols have been used to assess the selenium hazard to aquatic ecosystems at Ouray NWR, UT, the Animas, LaPlata, and Mancos rivers in the San Juan River basin, and three Wildlife Management Areas in Nevada (Lemly 1995, 1996a, 1997c). Although the original protocol was published in 1995, apparently no critiques have been published pointing out any deficiencies in the protocol (D. Lemly, USFS, personal communication).

Present study

The information from the present study was used with the four-component hazard assessment (Table 26). The final hazards were moderate at Horsethief and high at Adobe Creek and North Pond. It is interesting to note that even though sediments at Adobe Creek were ranked as a minimal hazard because selenium concentrations were low, selenium concentrations in water, invertebrates, and fish eggs ranked as high hazards. The outcome of the hazard assessment protocol coincides with the observations in the razorback sucker larvae study with larvae fed zooplankton from Adobe Creek and North Pond, but not from Horsethief. The sharp increase in mortality during the first week of exposure of razorback sucker larvae fed zooplankton containing 4.6 µg/g selenium from Horsethief east wetland suggested that the protocol may be conservative. This selenium concentration in zooplankton from Horsethief east

Table 26. Hazard assessment of selenium in the razorback sucker reproduction study.

Site and environmental component	Selenium concentration[1]	Evaluation by component		Totals for the site	
		Hazard	Score	Score	Hazard
Horsethief					
Water	<1.4-3.0	Moderate	4		
Sediment (HTEW)	0.8-0.9	Minimal	2	14	Moderate
Invertebrate (HTEW)	7.9-9.3	High	5		
Fish egg	6.0	Low	3		
Adobe Creek					
Water	<0.7-4.5	Moderate	4		
Sediment	1.2-2.5	Low	3	17	High
Invertebrate	32-48	High	5		
Fish egg	40.1	High	5		
Walter Walker					
Water	3.2-17.1	High	5		
Sediment	2.9-16.0	High	5	20	High
Invertebrate[2]	11-45	High	5		
Fish egg	54.7	High	5		

[1]Selenium concentrations in µg/L for water, µg/g for sediment, benthic invertebrate, and fish egg.
[2]Data from Hamilton et al. (2001).

wetland (4.6 µg/g) was also the same as what was linked with a sharp decline in survival of razorback sucker larvae in the previous reproduction study (Hamilton et al. 2001).

It should be noted that the selenium values used in the protocol for water and fish eggs came from the hatchery ponds at Horsethief (sediment and chironomids were from HTEW), whereas the zooplankton used in the larval study came from Horsethief east wetland, which received effluent only from the hatchery ponds and had elevated selenium concentrations in chironomids. The sediments in Horsethief east wetland were also probably rich in organic material, and thus probably contain a large reservoir of selenium available to the wetland ecosystem (even though the top layer of sediment had 0.8 to 0.9 µg/g selenium). These incongruities, (i.e, (1) the use of zooplankton in the lab test and benthic invertebrates in the protocol, and (2) use of water, sediment, chironomid, and fish egg values from the hatchery pond source) may have caused the hazard estimate from the protocol to be lower than the actual outcome of the toxicity test with razorback sucker larvae.

The protocol specifies use of selenium concentrations in benthic invertebrates, but not water-column invertebrates such as zooplankton, as the dietary component of the protocol. The proposed dietary threshold for adverse effects from selenium in sensitive aquatic organisms was 3 µg/g (Maier and Knight 1994, Lemly 1993a, 1996b), but the dietary source or type, i.e., water-column organisms, benthic organisms, plant material, detritus, or prepared diet, was not specified. It seems reasonable that if the dietary toxic threshold is 3 µg/g, then in the protocol, the high hazard should be associated with a value closer to 3 µg/g rather than >5 µg/g.

In the present study, several inorganic elements were measured in water, zooplankton, and fish eggs, but only selenium was elevated to concentrations reported to cause adverse effects in fish. In the previous study, several inorganic elements were measured in water, zooplankton, sediment, and fish eggs, and only selenium was elevated to concentrations reported to cause adverse effects in fish (Hamilton et al. 2001). This scenario has occurred in other contaminant investigations. For example, Sorensen (1988) stated that "Fish kills [at Belews Lake, NC, and Martin Lake, TX] were considered a direct result of selenium release into the main basin of the lakes because several hundred analyses for metals, metalloids, physiochemical parameters, and pesticides provided essentially negative results except for sufficiently high levels of selenium in the water (about 5 µg/L) to warrant concern." Lemly (1985) reviewed information in 10 studies of potential causes for the cause of fishery problems at Belews Lake (16 species eliminated, 2 species present as adults only, 1 species recolonized, and 1 species unaffected), and of the 16 inorganic elements of concern, only selenium was present at elevated concentrations in water and fish. Saiki and Lowe (1987) measured several inorganic and organic chemicals in water and biota collected from Kesterson Reservoir area, CA, and concluded only selenium was elevated sufficiently to be of concern to fisheries resources. Nakamoto and Hassler (1992) measured 20 trace elements in fish from the Merced River and Salt Slough, San Joaquin Valley, CA, which was primarily irrigation return flows, and concluded only selenium was present at toxic concentrations. Gillespie and Baumann (1986) concluded that selenium was the element causing the deformities and reduced survival of bluegill larvae and not other elements (arsenic, cadmium, copper, lead, mercury, zinc) present in females from Hyco Reservoir, NC. Bryson et al. (1984) concluded that selenium was the only element elevated sufficiently in zooplankton collected from Hyco Reservoir, NC, and not other elements (arsenic, cadmium, copper, mercury, or zinc), to cause the 97% mortality of juvenile bluegill after 1 week of dietary exposure. Montgomery Watson (1998) concluded that selenium was the major element of concern associated with phosphate mining activities in the Blackfoot River watershed of southeastern Idaho and not other

elements (cadmium, manganese, nickel, vanadium, and zinc). Skorupa (1998) reviewed 12 environmental case studies of clearly confirmed or highly probable selenium poisoning in nature, even though other inorganic elements were present.

Comparison with environmental conditions

The validity of extrapolating laboratory results, such as the egg study and larval fish study in the present investigation, to field situations had been questioned because of the lack of environmental realism with single-species tests (Cairns 1983, Kimball and Levin 1985). Yet, most investigators acknowledge that ecosystem studies require more subjective scientific judgments than do laboratory tests, are prohibitively expensive to conduct, lack theoretical basis to explain and relate the confounding morass of data collected, and lack of standardized protocols for conducting ecosystem studies. In contrast, several investigators have independently reviewed numerous laboratory and field studies of a wide variety of chemical contaminants and concluded that laboratory-derived information is a good predictor of ecosystem effects (Slooff et al. 1986, Arthur 1988, Emans et al. 1993, Sprague 1997). Sprague (1997) also reviewed several reports and concluded that ambient toxicity tests were good indicators of field effects.

Using the species of concern, similar conditions such as water quality, temperature, photoperiod, and relevant biological endpoints that can be linked to population level effects enhances achieving comparability between laboratory and field studies (Kenaga 1982). The present study incorporated these concerns in the experimental design by evaluating the effects on adults, eggs, and larvae of endangered razorback sucker exposed to water, sediment, and food organisms at three sites in aquatic locations in their current range in the upper Colorado River.

Extrapolating the results of the present study to the potential hazard in the field is a practical need in implementing the actions necessary to the recovery of endangered razorback sucker. Although single species testing has been questioned when used to evaluate the hazard to a community or ecosystem (Cairns 1983), this approach is appropriate when assessing the hazard of selenium in its various forms and combined with other inorganic elements from a source (i.e., irrigation activities) to specific organisms of concern (i.e., federally-listed endangered fishes). Razorback sucker were tested because they were endangered and indigenous to the upper Colorado River basin, not because they were considered the “most sensitive” species, which is the goal of most toxicity testing (Cairns 1986).

Comparing the biological effects concentration (BEC) with the expected (or measured) environmental concentrations (EEC) is a basic principle in evaluating the hazard of toxicants to aquatic life (Kimerle et al. 1979). The BEC/EEC quotient has been referred to as the margin of uncertainty. In the early stages of hazard evaluation (i.e., acute toxicity tests) neither the BEC nor EEC is a precise value, but rather are values with wide confidence intervals due to variability associated with the BEC and EEC (Mount 1981). For example, the BEC will vary with the species and life stage tested, organism health, genetic constitution, route of exposure, water quality, and acclimation. The EEC will vary depending on water quality of the receiving water, mixing characteristics (flow rate, input rate, etc.), degradation (photo, biological, or chemical), and seasonal changes. Many of these concerns were specifically addressed in the experimental design of the present study, thus removing a substantial amount of uncertainty in the BEC and EEC values.

To evaluate the hazard of selenium in the present study, the margin of uncertainty was

calculated by comparing the BEC (4.6 µg/g dietary selenium concentration causing adverse effects on razorback sucker larvae) with the EEC measured in potential food organisms of razorback sucker larvae collected from various aquatic locations in the upper Colorado River basin including the Green River. Margins of uncertainty of one [BEC/EEC=1] using field study-derived data indicate a high potential for environmental hazard, whereas higher margins (i.e., >1) indicate low potential (OECD 1989). Other examples of uncertainty factors include 10 for good quality chronic toxicity data, 100 for limited or poor chronic data, and 1000 for no chronic data (OECD 1989). The U.S. Environmental Protection Agency recommends similar safety factors (USEPA 1984), and there is international, national, and federal support of uncertainty (safety) factors in hazard assessments (Dourson and Stara 1983).

Several studies have reported selenium concentrations >4.6 µg/g in aquatic invertebrates in aquatic habitats in the upper Colorado River basin (Barnhart 1957, Birkner 1978, Butler et al. 1991, 1994, 1996, Peltz and Waddell 1991, Stephens et al. 1992, Waddell and Wiens 1994a, Hamilton et al. 1996, Wiens and Waddell 1996), and other reports have reported selenium concentrations in aquatic invertebrates greater than the dietary selenium threshold of 3 µg/g (Osmundson 1989, 1992). Consequently, the BEC/EEC ratio would be less than one for several locations in the upper Colorado River basin, which suggests that adverse effects on razorback sucker recruitment is probably occurring.

Assessing the hazard of selenium to razorback sucker using either the selenium hazard assessment protocol suggested by Lemly (1995, 1996a) or the BEC/EEC ratio (USEPA 1984, OECD 1989) suggests that selenium is probably adversely affecting the survival of razorback sucker larvae. Data in the previous reproduction study with razorback sucker also indicated selenium was adversely affecting the survival of larvae (Hamilton et al. 2001). Other reports have suggested adverse effects from selenium were occurring in aquatic organisms in the Colorado River basin (reviewed in Hamilton 1998, 1999). Based on selenium concentrations measured in water, sediment, aquatic invertebrates, and fish given in published NIWQP reports of studies in the Green River and upper Colorado River basins, and biological effects measured in the present study and the previous study (Hamilton et al. 2001), selenium contamination is adversely affecting razorback sucker. Recovery of razorback sucker should include addressing selenium contamination issues in the upper Colorado River basin, in addition to on-going efforts to address other factors contributing to the decline of endangered fish in the upper Colorado River basin such as 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 and clarity changes, competition with and predation by introduced species, parasitism, and changes in food base.

Selenium is a concern in the Colorado River basin

Following the discovery of selenium-contaminated irrigation return waters in the San Joaquin Valley of central California in 1982, the Department of the Interior (DOI) initiated the National Irrigation Water Quality Program (NIWQP) to identify other areas in the western U.S. that have water quality problems induced by irrigation drainage (Feltz et al. 1991). The NIWQP investigations focused on irrigation drainage facilities constructed by the DOI where the drainwater was to a national wildlife refuge, or had the potential to impact migratory birds or endangered species. The upper Colorado River basin including the middle Green River basin of Utah and the upper Colorado, Gunnison, and Uncompahgre rivers in northwestern Colorado

were identified as areas needing further study. Analysis of water, bottom sediment, and biota collected since 1986 from the middle Green River basin and the Grand Valley, located in western Colorado and includes 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; Stephens et al. 1988, 1992; Peltz and Waddell 1991).

The NIWQP studies provided a basic foundation of survey information on the occurrence of selenium in fish collected from a variety of aquatic ecosystem components that suggested selenium and possibly other contaminants might be sufficiently elevated to be contributing to the decline of endangered fish. Selenium in soils of the western states is derived from weathering of outcrops of Cretaceous marine rocks in the Rocky Mountain and Great Plains regions, which comprises an area of approximately 300,000 square miles (Presser et al. 1994).

The U.S. Fish and Wildlife Services' National Contaminant Biomonitoring Program (NCBP) has documented elevated selenium concentrations in fish collected from stations located in the upper and lower Colorado River basins. The NCBP monitors temporal and spatial trends in concentrations of persistent environmental contaminants, including selenium, that may threaten fish and wildlife, and uses the 85th percentile as an arbitrary point distinguishing "high" concentrations (Walsh et al. 1977, May and McKinney 1981, Lowe et al. 1985, Schmitt and Brumbaugh 1990). Selenium concentrations in whole-body fish from the Colorado River basin have been among the highest in the nation (Walsh et al. 1977, Lowe et al. 1985, Schmitt and Brumbaugh 1990). In samples collected in 1972-1973, selenium concentrations exceeded the 85th percentile in fish at five of six stations in the Colorado River basin: Green River at Vernal, UT (the only upper basin station) and Colorado River at four sites in Arizona (Imperial Reservoir, Lake Havasu, Lake Mead, and Lake Powell). In 1978-1981 and 1984, selenium concentrations exceeded the 85th percentile at six of seven stations; the five above plus the Colorado River at Yuma, AZ. The only station at which selenium concentrations in fish have not exceeded the 85th percentile was on the Gila River (San Carlos Reservoir, AZ).

Prior to the NIWQP and NCBP studies, studies in the 1930s by the U.S. Department of Agriculture reported elevated selenium concentrations in water in the upper and lower Colorado, Gunnison, and San Juan rivers due to irrigation activities (Anderson et al. 1961). Elevated selenium concentrations in water 48 and 112 km southeast of the mouth of the Colorado River in the Gulf of California were also reported. The long-term contamination of the lower Colorado

River basin may have been one of the factors contributing to the disappearance of endangered fish in the early 1930s as reported by Dill (1944). More recently, elevated selenium concentrations in water, sediment, and biota in the lower Colorado River basin documented in a NIWQP study were identified as coming from the upper basin (Radtke et al. 1988, Radtke and Kepner 1990).

Widespread selenium contamination of the Colorado River basin has been reported. In the upper basin, Stephens and Waddell (1998) reviewed several NIWQP investigations, data in the National Water Data Storage and Retrieval System of the USGS, DOI Floodplain Habitat Restoration Program, and contaminant assessment reports of the USFWS, and reported that selenium was present at concentrations harmful to fish and wildlife at several locations in the Green River basin including Ashley Creek, Anderson Bottom in the Canyonlands area, Desert Lake Waterfowl Management Area, Escalante Ranch, Sheppard Bottom in Ouray NWR, Stewart Lake, Pariette Wetlands, and the Price and Yampa rivers. Bussey et al. (1976) measured 10 inorganic elements in various fish tissues collected from Lake Powell and concluded that only

selenium was elevated to concentrations of concern from a human consumption standpoint. In muscle tissue, selenium concentrations were 12.2 µg/g in largemouth bass and 16.8 µg/g in black crappie (*Pomoxis nigromaculatus*), which were higher than the proposed toxic threshold (8 µg/g) for adverse effects in fish (Lemly 1996b), whereas they were 6.4 µg/g in walleye (*Stizostedion vitreum*).

Hamilton (1998) reviewed similar sources plus university studies primarily from the lower Colorado River basin and reported selenium contamination throughout the upper and lower Colorado River basin. He concluded that selenium concentrations were sufficiently elevated to be causing reproductive problems in endangered fish such as the razorback sucker. In a follow-up paper, he reviewed historical data on selenium concentrations in the upper and lower basins, along with historical records and reviews of the occurrence of native, later endangered fish, and presented a hypothesis that suggested selenium contamination from irrigated agriculture in the 1890-1910 period caused the decline of native fish in the upper basin in the 1910-1920 period and in the lower basin in the 1925-1935 period (Hamilton 1999).

UNCERTAINTIES

As with any field study, there are a variety of uncertainties that are present because of the inherent variability's associated with field studies compared to lab studies, which are generally conducted under highly controlled conditions (USEPA 1992). In field studies site characteristics can vary between sites and between locations within a site during the course of a study including physical environment (e.g., atmospheric and water temperature, hydrologic conditions, water quality, habitat variables such as sediments, plant, invertebrate, and fish communities), organisms behavior, exposure to stressors such as exposure concentration and duration, mixtures of organic and inorganic chemicals, interaction of physical and chemical stressors, and interactions with other ecological components (USEPA 1992). The variation in these kinds of site differences combined with the lack of standard methods for field studies results in uncertainties in the interpretation of results from field studies. However, USEPA (1992) acknowledges that professional judgment based on scientific expertise is needed for the interpretation of scientific data, particularly from field studies, which is frequently difficult and complex, conflicting or ambiguous, or incomplete.

Some uncertainties in the present study that were suggested during a meeting to review the study reports with the Biology Committee included (1) identifying potentially confounding factors, (2) potential effects from inbreeding, (3) whether deformities are an accurate measure of effect, (4) genetic adaptation of fish to selenium in specific areas, (5) selenium dietary threshold of 4.6 µg/g, (6) unexplained interaction/synergistic effects, (7) variation in study results between years, (8) no consistent quantitative response of fish to selenium concentrations, (9) application of "worst-case" situations at Adobe Creek and North Pond to the wild, (10) variability of zooplankton concentration and composition among years and sites, and (11) use of brine shrimp nauplii as a reference treatment for zooplankton treatments. Below is a brief discussion of uncertainties associated with the above 11 items.

1. Identifying potentially confounding factors. The major confounding factor was the use of inbred fish for the study [discussed below]. Other factors of lesser importance are (1) possible interactions between inorganic elements in water, diet, and water and diet [discussed below], (2) differences in the three sites such as water quality, habitat, and

chemical, physical, and biological composition [inherent in all field studies], (3) use of brine shrimp as a reference treatment for field collected zooplankton used as selenium treatments [discussed in "Brine shrimp" section in the Larval Fish study discussion and below], and (4) differences in zooplankton composition within sites, among sites, and between years [discussed below].

2. Influence of inbreeding on the results of the Egg study. All the fish used were progeny of a single spawn of two adult razorback sucker (female: PIT 7F7F19036C, and male: PIT 7F7D055802). The young razorback sucker adults used in the reproduction studies in 1996 (Hamilton et al. 2001) and 1997 (this study) came from a single mating in 1992, and thus the spawnings in 1996 and 1997 were of brother and sister. Progeny could have exhibited inbreeding effects such as reduced growth rate, lower survival, reduced feed conversion, reduced fertility, and increased numbers of deformed fry (Moav and Wohlfarth 1966, Ryman 1970, Kincaid 1976a, 1976b, Piper et al. 1982). The potential effects from inbreeding were discussed previously in sections titled "Inbreeding" in the Egg study discussion and in the 1996 study report (Hamilton et al. 2001). Briefly, the lack of significant differences in percent viability, percent hatch, and mortality of embryos between embryos from young adults and brood stock, and the similarity of percent hatch and percent survival reported by other investigators for razorback sucker, suggest that inbreeding was not a major factor in the Egg study. In contrast to the first reproduction study, which was the first spawning of the young adults, the second spawning a year later produced similar sized eggs as brood stock that had been spawned multiple times. The uncertainty associated with the influence of inbreeding on the results of the Egg study seems low to moderate.
3. Use of deformities as an accurate measure of selenium effects. Several factors were mentioned in the discussion section of the Egg study that causes deformities. Several papers were cited in the "Deformities in eggs and larvae" section of the Egg study to show that selenium could be responsible for the observed deformities. Deformities by themselves do not indicate that selenium or other specific stresses caused the deformities. However, the observed deformities in the present study coincided with elevated selenium residues in eggs, adult muscle plugs, and adult muscle tissue thus corroborating the likely possibility that selenium caused the deformities as suggested by Lemly (1997a). The uncertainty associated with the use of deformities as an accurate measure of selenium effects would be high in the absence of corroborating data, but low in the present study because of the substantial amount of corroborating data.
4. Possibility of genetic adaptation of fish to selenium in specific areas, i.e., the Colorado River basin. This aspect of the study was discussed in the "Selenium and other elements in water" section of the Adult study discussion. Although it may be possible for short-lived, rapid reproducing fish to evolve to a chronic selenium stress, no evidence was found in the literature that any animal, i.e., invertebrate, reptile, amphibian, fish, bird, or mammal, has adapted to chronic selenium exposure. The uncertainty associated with a possible genetic adaptation by fish in the Colorado River basin to long-tern selenium stress, while a popular supposition, seems low.
5. Selenium dietary threshold of 4.6 µg/g. This value was associated with reduced larval survival in both the first study using larvae from young adults (Hamilton et al. 2001) and in the second reproduction study with larvae from brood stock conducted a year later (this study). The occurrence of similar reduced survival in two different studies with different

sources of razorback sucker larvae conducted a year a part suggests that there is a consistent sensitivity in razorback sucker larvae to dietary selenium. This value also coincides with several published papers, which were discussed in the "Selenium and other elements in zooplankton" section in the Larval Fish study discussion. The general consensus is that the threshold for dietary effects is about 3 µg/g (Lemly 1993a, 1996b, Maier and Knight 1994). The uncertainty associated with adverse effects from dietary selenium occurring at 4.6 µg/g in food organisms seems low.

6. Unexplained interactions/synergistic effects. In any field study, a wide variety of interactions are possible between stressors and ecological components (USEPA 1992). Although many inorganic elements were measured in water, sediment, and invertebrates in the present study, it is possible that other unmeasured elements may have contributed to interactions that affected the results. Likewise, the toxicological literature is incomplete concerning interactions between inorganic elements, thus interactions may have occurred between measured elements that have not yet been documented in the literature. Many water quality and sediment characteristics were measured, but the measurements were not exhaustive, and the literature is incomplete concerning interactions between water quality and sediment characteristics and chemical stressors such as inorganic elements, thus interactions between these variables may have influenced the results. No measurements were made of potential organic stressors, nor habitat differences, invertebrate community differences, and other ecological components, which may have aided in the interpretation of the results of the study. In spite of the possible shortage of information on chemical, physical, or biological stressors, ecosystem components, and possible interactions, the available information from the study seems consistent with the available literature, especially concerning selenium interactions and effects. One example of the literature aiding in explaining a seemingly odd result was the discussion of the interaction of arsenic and selenium in the "Brine shrimp" section of the Larval Fish study discussion. The uncertainty associated with interactions/synergistic effects seems low to moderate.
7. Variation in study results between years. Several study results that were consistent between years including (1) adults accumulated selenium in muscle plugs and eggs relative to the amount in food organisms at the location where they were held [the one exception in 1996 was Adobe Creek adults, but the possibility of a three-part reproduction cycle was discussed as a likely explanation in the "Selenium and other elements in eggs" section of the Adult study discussion], (2) eggs with elevated selenium had no effects on percent hatch or percent survival after hatch, (3) 4.6 µg/g selenium in zooplankton caused reduced survival of razorback sucker larvae from young adults in 1996 and from brood stock in 1997, (4) rapid mortality of larvae in 4-6 days after exposure to Horsethief water and zooplankton from Horsethief east wetland in 1996 and 1997 [consistent with results in the Ouray study conducted in 1994], (5) delayed mortality of larvae receiving the North Pond water and food treatments in both 1996 and 1997 [consistent with the Ouray study; suggested a synergistic interaction], and (6) similar hazard assessment ranking in 1996 and 1997. Overall, there was a consistent response of adults and larvae to selenium exposure in 1996 and 1997. The uncertainty associated with variation in study results between years seems low.
8. Lack of a consistent quantitative response of fish to selenium concentrations in water, diet, or both. A few study results showed a concentration response including (1)

selenium accumulation in razorback sucker larvae after 30 days of exposure in 1996 [larvae starting with elevated selenium from parental transfer through egg yolk from Adobe Creek or North Pond adults depurated selenium to concentrations in their food for the Adobe Creek and North Pond treatments], (2) a significant negative correlation occurred between survival of larvae and selenium concentrations in larvae (r= -0.55, P=0.10) in 1996 [survival followed a concentration response: lowest survival (75%) was in North Pond larvae with the highest selenium residue (9.7 µg/g), intermediate (84%) in Adobe Creek larvae with the intermediate selenium residue (7.7 µg/g), and highest in Horsethief and brood stock larvae (87-89%) with the lowest selenium residues (3.3-3.6 µg/g)], and (3) in 1997 survival followed a concentration response [lowest survival (52%) in larvae fed North Pond zooplankton and with whole-body residues of 16.0 µg/g, intermediate survival (72%) in larvae fed Adobe Creek zooplankton and with whole-body residues of 12.0 µg/g, and highest survival (81-83%) in larvae fed brine shrimp or zooplankton from HTEW and with whole-body residues of 5.2 µg/g]. The lack of a concentration response in the majority of published selenium studies was discussed in the "Delayed mortality" section of the Larval Fish study discussion (9 studies had no concentration response, 3 had a partial response and 2 had a concentration response). Thus, results from the 1996 study and the 1997 study place them in the partial response category. The uncertainty associated with the presence of limited concentration responses in the studies seems low.

9. Application of "worst-case" situations at Adobe Creek and North Pond to the wild. One objective of the study experimental design was to exposure adult razorback sucker to environmental concentrations of selenium that would cause accumulation of elevated selenium in adult tissue, which was achieved for adults held at Adobe Creek and North Pond. For the 1996 study, selenium concentrations in muscle plugs from adults held at Adobe Creek and North Pond for 9 months were lower than 40% of the wild razorback sucker adults sampled from the Green River (Waddell and May 1995, Stephens and Waddell 1998). For the 1997 (present) study, selenium concentrations in muscle plugs from adults held at Adobe Creek and North Pond for 9 months in 1996, followed by 60 days depuration and a second 9-month exposure, were lower than 33% of the wild razorback sucker adults sampled from the Green River (Waddell and May 1995, Stephens and Waddell 1998). Although holding fish for one 9-month exposure in the 1996 study and a second 9-month exposure in the 1997 study may seem like a worst-case situation, the exposure failed to cause the adults to accumulate selenium comparable to those in wild fish, which have opportunities to move about the Green River. One uncertainty present is the length of time the wild adults accumulated selenium compared to the relatively rapid accumulation by adults held at Adobe Creek and North Pond. There are no studies differentiating between the chronic effects of relatively rapid (i.e., 9 months) verse slow (i.e., several years) accumulation of selenium in fish. The selenium accumulation in muscle plugs from adults held at Adobe Creek and North Pond apparently was not a worst-case situation because 33-40% of wild fish have higher selenium residues in muscle plugs. The uncertainty associated with the comparison of the worst-case situation in the present study with possible effects in wild fish seems low.
10. Variability of zooplankton concentration and composition among years and sites. The ratio of cladocerans to copepods was different between sites within a year, different at the same site between years, and different within a site over a 30-day period. This type of

variation in zooplankton composition was similar to that reported by Grabowski and Hiebert (1989) for backwaters near Island Park, Jensen, and Ouray on the Green River. The zooplankton used in the Larval Fish studies were native to the area where razorback sucker have been historically found in the upper Colorado River, and therefore, were probably appropriate to use in the study. Natural variation in invertebrate communities would be expected spatially and temporally in aquatic ecosystems. Razorback sucker are opportunistic feeders using whatever food source is presence, sometimes selecting certain organisms and other times selectively avoiding certain organisms, as discussed in the "Zooplankton" section in the Larval Fish study discussion. The uncertainty associated with the variability of zooplankton concentration and composition within sites, among sites, and among years seems low.

11. Use of brine shrimp nauplii as a reference for zooplankton treatments. We assumed that zooplankton from Horsetheif east wetland would be the reference treatment because (1) the wetland was located on the south side of the Colorado River and away from the majority of irrigation operations, which occur on the north side of the river, (2) some irrigation occurred near the wetland, but it was down gradient from the wetland, (3) the up-gradient side of the wetland was undisturbed land, and (4) the water source of the wetland was the Colorado River, and it was the same water used at the Horsethief Fish Facility where adult endangered fish were held. The brine shrimp nauplii treatment was added as an additional reference treatment for comparison to the HTEW diet treatment. Subsequent chemical analysis revealed elevated selenium in zooplankton from HTEW and high mortality in larvae receiving the diet, whereas slightly elevated selenium in brine shrimp, but low mortality probably due to antagonistic interactions between selenium and elevated arsenic in brine shrimp. Other potential differences between brine shrimp and zooplankton were addressed in the discussion section of Larval Fish study such as caloric content, food size, and food density, but no differences were found in the literature to suspect a substantial difference between the two diet types. The uncertainty associated with the use of brine shrimp as a reference treatment for zooplankton treatment is low.

Overall, the most prominent uncertainties in the study were the possible effects of inbreeding on the results of the Egg study and the use of brine shrimp nauplii as the reference treatment for the zooplankton treatments. The discussion section of Egg study addresses inbreeding concerns and concludes that the effects observed were more likely related to exposure than inbreeding. The discussion sections also address concerns about the nutritional comparability of the two diet types and concludes that the two diets were probably similar. Although the results of the field study may seem difficult and complex, conflicting or ambiguous, or incomplete to some readers, it is the professional judgment of the first author of this report, based on 26 years of research experience including 17 years of selenium research experience, that selenium was the primary stressor in the study and that the effects observed are consistent with the selenium literature.

One additional uncertainty is the comparison of information in the present study with information presented in three reports investigating the effects of selenium and other elements on razorback sucker larvae, flannelmouth sucker, and fathead minnow by Beyers and Sodergren (1999, 2001a, 2001b). Those reports seem incomplete and less than scientifically rigorous. Some of the problems in the reports include: (1) inadequate information or citations of analytical

chemistry methods precludes drawing conclusions about residues measured in various types of samples, (2) there were little or no quality assurance/quality control information given for chemical analyses, (3) comparisons were made between larvae of different fish species tested at different ages (up to 30 days difference in age), (4) many exceptions to cited test methods (ASTM 1992a, 1992b) were made, but not noted, and seriously conflicted with the methods, (5) control water contained >5 µg/L selenium, which exceeded the USEPA criterion (USEPA 1987), (6)growth of razorback sucker was substantially lower than reported by others for similar aged larvae (Papoulias and Minckley 1990, 1992, Marsh and Langhorst 1988) thus casing doubt on statements about growth effects, (7) inappropriate storage of site exposure water in Beyers and Sodergren (2001a) probably allowed the loss of selenium during storage (USEPA guidelines for use of site waters in toxicity tests is given in Lewis et al. 1994 and Weber 1993), and (8) little discussion of how the results of the studies relate to the selenium literature. In Beyers and Sodergren (2001a) the statement is made that "Larval razorback sucker studied in this investigation were not strongly negatively affected by exposure to site waters with dissolved selenium concentrations ≤20.3 µg/L and corresponding dietary selenium concentrations ≤21.8 µg/g," which is substantially different than the selenium literature (Lemly 1986, Maier et al. 1987, 1988, Maier and Knight 1994, Lemly 1996b, Skorupa et al. 1996, Skorupa 1998, Hamilton and Lemly 1999, Lemly 1999), yet the conclusion is not discussed. These concerns preclude discussing the results of Beyers and Sodergren (1999, 2001a, 2001b) relative to those in the present study.

CONCLUSIONS

1. Selenium was elevated in water, food-chain organisms, and sediments at North Pond, and elevated in adult tissues including eggs. Selenium was sufficiently elevated in food organisms collected from to cause reduced survival of razorback sucker larvae.
2. Selenium was elevated in water and food-chain organisms at Adobe Creek, and elevated in adult tissues including eggs. Selenium was sufficiently elevated in food organisms collected from Adobe Creek to cause reduced survival of razorback sucker larvae.
3. Although selenium was substantially elevated in eggs from adults held at Adobe Creek and North Pond, percent hatch and percent survival of eggs and newly hatched larvae was not different than that of reference eggs and newly hatched larvae.
4. Selenium was elevated sufficiently in food-chain organisms collected from Horsethief east wetland, Adobe Creek, and North Pond to adversely affect the survival of razorback sucker larvae from brood stock.

RECOMMENDATIONS

Selenium concentrations in water, food organisms, fish, and fish eggs should be taken into consideration in making management decisions.

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Appendix A. Average gage height (m) at one station at Walter Walker State Wildlife Area (WW9) and two stations at Adobe Creek (AC1 and AC6), and average flow (lps) of the Colorado River at the Colorado-Utah state line.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
06/17/96	1.24	1.36	1.14	518,200
06/18/96	1.26	1.38	1.14	521,000
06/19/96	1.39	1.50	1.28	572,000
06/20/96	1.38	1.49	1.28	577,700
06/21/96	1.39	1.50	1.30	574,800
06/22/96	1.54	1.64	1.47	637,100
06/23/96	1.60	1.70	1.54	676,800
06/24/96	1.50	1.61	1.46	642,800
06/25/96	1.41	1.52	1.36	594,700
06/26/96	1.28	1.41	1.24	540,900
06/27/96	1.23	1.36	1.19	515,400
06/28/96	1.22	1.35	1.18	509,700
06/29/96	1.18	1.31	1.14	498,400
06/30/96	1.09	1.23	1.05	461,600
07/01/96	1.05	1.19	1.01	441,700
07/02/96	0.95	1.10	0.93	407,800
07/03/96	0.86	1.00	0.85	368,100
07/04/96	0.81	0.95	0.81	345,500
07/05/96	0.75	0.89	0.76	320,000
07/06/96	0.73	0.86	0.74	311,500
07/07/96	0.70	0.83	0.71	297,300
07/08/96	0.69	0.82	0.70	294,500
07/09/96	0.68	0.80	0.69	288,800
07/10/96	0.62	0.72	0.63	268,700
07/11/96	0.56	0.64	0.57	245,200
07/12/96	0.52	0.58	0.52	228,800
07/13/96	0.49	0.53	0.47	213,500
07/14/96	0.47	0.49	0.45	203,900
07/15/96	0.47	0.47	0.43	197,900

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
07/16/96	0.46	0.47	0.43	200,200
07/17/96	0.45	0.44	0.42	195,100
07/18/96	0.44	0.43	0.41	188,300
07/19/96	0.44	0.43	0.41	189,400
07/20/96	0.42	0.40	0.40	183,800
07/21/96	0.39	0.35	0.37	172,500
07/22/96	0.37	0.32	0.34	164,000
07/23/96	0.35	0.29	0.32	153,800
07/24/96	0.32	0.25	0.28	141,000
07/25/96	0.30	0.21	0.26	129,400
07/26/96	0.28	0.19	0.28	122,300
07/27/96	0.27	0.18	0.22	119,500
07/28/96	0.25	0.16	0.19	114,400
07/29/96	0.24	0.15	0.19	113,600
07/30/96	0.26	0.18	0.19	119,200
07/31/96	0.26	0.18	0.20	121,200
08/01/96	0.24	0.15	0.19	113,800
08/02/96	0.23	0.15	0.19	109,900
08/03/96	0.21	0.15	0.19	105,900
08/04/96	-	0.14	0.19	102,200
08/05/96	-	0.14	0.19	102,500
08/06/96	-	0.14	0.19	100,000
08/07/96	-	0.14	0.19	94,300
08/08/96	-	0.14	0.18	88,600
08/09/96	-	0.13	0.18	87,500
08/10/96	-	0.13	0.18	86,400
08/11/96	-	0.13	0.18	87,200
08/12/96	-	0.13	0.18	86,650
08/13/96	-	0.13	0.17	86,400
08/14/96	-	0.13	0.17	81,600
08/15/96	-	0.13	0.17	75,900

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
08/16/96	-	0.13	0.18	73,100
08/17/96	-	0.13	0.18	77,000
08/18/96	-	0.13	0.18	81,800
08/19/96	-	0.13	0.17	84,700
08/20/96	-	0.13	0.18	90,600
08/21/96	-	-	0.18	88,600
08/22/96	-	-	0.18	88,600
08/23/96	-	-	0.18	85,200
08/24/96	-	-	0.18	85,000
08/25/96	-	-	0.18	88,300
08/26/96	-	-	0.18	96,300
08/27/96	-	-	0.18	101,100
08/28/96	-	-	0.18	97,700
08/29/96	-	-	0.17	94,600
08/30/96	-	-	0.18	94,900
08/31/96	-	-	0.18	95,100
09/01/96	-	-	0.18	96,300
09/02/96	-	-	0.18	95,700
09/03/96	-	-	0.18	94,900
09/04/96	-	-	0.19	95,100
09/05/96	-	-	0.19	93,400
09/06/96	-	-	0.18	100,000
09/07/96	-	-	0.18	112,100
09/08/96	-	-	0.19	122,300
09/09/96	-	-	0.19	116,400
09/10/96	-	-	0.19	109,900
09/11/96	-	-	0.19	111,300
09/12/96	-	-	0.19	117,200
09/13/96	-	-	0.19	122,000
09/14/96	-	-	0.20	137,900
09/15/96	-	-	0.21	137,900

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
09/16/96	-	-	0.21	136,800
09/17/96	-	-	0.21	138,200
09/18/96	-	-	0.22	145,300
09/19/96	-	-	0.23	148,700
09/20/96	-	-	0.24	152,300
09/21/96	-	-	0.24	142,400
09/22/96	-	-	0.22	136,200
09/23/96	-	-	0.21	134,200
09/24/96	-	-	0.20	132,800
09/25/96	-	-	0.20	130,500
09/26/96	-	-	0.20	130,300
09/27/96	-	-	0.19	132,500
09/28/96	-	-	0.19	133,900
09/29/96	-	-	0.19	132,500
09/30/96	-	-	0.18	127,400
10/01/96	-	-	0.18	122,600
10/02/96	-	-	0.17	120,100
10/03/96	-	-	0.17	130,800
10/04/96	-	-	0.18	148,900
10/05/96	-	-	-	154,000
10/06/96	-	-	-	140,500
10/07/96	-	-	-	134,200
10/08/96	-	-	-	130,500
10/09/96	-	-	-	127,100
10/10/96	-	-	-	122,000
10/11/96	-	-	-	120,300
10/12/96	-	-	-	122,000
10/13/96	-	-	-	119,200
10/14/96	-	-	-	118,600
10/15/96	-	-	-	116,900
10/16/96	-	-	-	116,700

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
10/17/96	-	-	-	118,900
10/18/96	-	-	-	121,800
10/19/96	-	-	-	118,900
10/20/96	-	-	-	123,700
10/21/96	-	-	-	131,100
10/22/96	-	-	-	131,700
10/23/96	-	-	-	129,400
10/24/96	-	-	-	128,300
10/25/96	-	-	-	134,800
10/26/96	-	-	-	143,000
10/27/96	-	-	-	143,000
10/28/96	-	-	-	140,500
10/29/96	-	-	-	150,400
10/30/96	-	-	-	151,200
10/31/96	-	-	-	145,800
11/01/96	-	-	-	140,200
11/02/96	-	-	-	138,200
11/03/96	-	-	-	132,800
11/04/96	-	-	-	145,300
11/05/96	-	-	-	144,100
11/06/96	-	-	-	144,700
11/07/96	-	-	-	144,700
11/08/96	-	-	-	140,500
11/09/96	-	-	-	144,400
11/10/96	-	-	-	136,500
11/11/96	-	-	-	136,200
11/12/96	-	-	-	137,300
11/13/96	-	-	-	133,900
11/14/96	-	-	-	133,700
11/15/96	-	-	-	135,100
11/16/96	-	-	-	142,200

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
11/17/96	-	-	-	144,700
11/18/96	-	-	-	139,300
11/19/96	-	-	-	136,800
11/20/96	-	-	-	137,900
11/21/96	-	-	-	138,500
11/22/96	-	-	-	143,600
11/23/96	-	-	-	161,700
11/24/96	-	-	-	166,800
11/25/96	-	-	-	149,500
11/26/96	0.36	-	-	144,100
11/27/96	0.35	-	-	141,600
11/28/96	0.34	-	-	138,800
11/29/96	0.33	-	-	133,700
11/30/96	0.33	-	-	133,100
12/01/96	0.33	-	-	134,200
12/02/96	0.32	-	-	129,400
12/03/96	0.33	-	-	131,400
12/04/96	0.30	-	-	123,700
12/05/96	0.33	-	-	118,900
12/06/96	0.39	-	-	124,900
12/07/96	0.37	-	-	128,300
12/08/96	0.36	-	-	128,000
12/09/96	0.37	-	-	124,900
12/10/96	0.42	-	-	127,100
12/11/96	0.43	-	-	132,800
12/12/96	0.43	-	-	138,200
12/13/96	0.42	-	-	136,200
12/14/96	0.42	-	-	131,700
12/15/96	0.41	-	-	128,600
12/16/96	0.40	-	-	123,200
12/17/96	0.38	-	-	115,000

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
12/18/96	0.32	-	-	90,900
12/19/96	0.30	-	-	91,200
12/20/96	0.30	-	-	93,200
12/21/96	0.35	-	-	109,600
12/22/96	0.38	-	-	128,300
12/23/96	0.37	-	-	133,100
12/24/96	0.35	-	-	128,600
12/25/96	0.34	-	-	121,800
12/26/96	0.34	-	-	120,100
12/27/96	0.34	-	-	123,700
12/28/96	0.35	-	-	129,400
12/29/96	0.35	-	-	130,500
12/30/96	0.34	-	-	126,900
12/31/96	0.34	-	-	123,200
01/01/97	0.34	-	-	122,000
01/02/97	0.33	-	-	120,600
01/03/97	0.34	-	-	124,600
01/04/97	0.38	-	-	148,700
01/05/97	0.35	-	-	132,800
01/06/97	0.33	-	-	122,900
01/07/97	0.32	-	-	115,500
01/08/97	0.32	-	-	111,300
01/09/97	0.31	-	-	112,700
01/10/97	0.32	-	-	120,600
01/11/97	0.32	-	-	124,300
01/12/97	0.32	-	-	126,600
01/13/97	0.32	-	-	126,000
01/14/97	0.33	-	-	130,000
01/15/97	0.32	-	-	125,200
01/16/97	0.33	-	-	124,600
01/17/97	0.32	-	-	120,600

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
01/18/97	0.32	-	-	125,700
01/19/97	0.32	-	-	126,300
01/20/97	0.34	-	-	132,500
01/21/97	0.35	-	-	137,300
01/22/97	0.35	-	-	137,600
01/23/97	0.35	-	-	140,200
01/24/97	0.35	-	-	138,500
01/25/97	0.35	-	-	137,900
01/26/97	0.34	-	-	136,500
01/27/97	0.35	-	-	139,600
01/28/97	0.38	-	-	148,400
01/29/97	0.35	-	-	141,000
01/30/97	0.35	-	-	138,500
01/31/97	0.34	-	-	137,300
02/01/97	0.35	-	-	136,800
02/02/97	0.35	-	-	138,800
02/03/97	0.35	-	-	139,900
02/04/97	0.35	-	-	140,500
02/05/97	0.35	-	-	137,600
02/06/97	0.34	-	-	136,200
02/07/97	0.35	-	-	138,500
02/08/97	0.35	-	-	136,200
02/09/97	0.34	-	-	132,200
02/10/97	0.33	-	-	129,400
02/11/97	0.33	-	-	130,000
02/12/97	0.34	-	-	132,800
02/13/97	0.35	-	-	136,800
02/14/97	0.35	-	-	138,800
02/15/97	0.34	-	-	134,200
02/16/97	0.35	-	-	136,500
02/17/97	0.34	-	-	136,200

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW-9	~AC-1	~AC-6	
02/18/97	0.36	-	-	140,200
02/19/97	0.36	-	-	143,600
02/20/97	0.35	-	-	139,600
02/21/97	0.35	-	-	137,300
02/22/97	0.34	-	-	134,500
02/23/97	0.34	-	-	131,700
02/24/97	0.34	-	-	131,400
02/25/97	0.34	-	-	133,400
02/26/97	0.34	-	-	132,800
02/27/97	0.34	-	-	132,200
02/28/97	0.34	-	-	133,400
03/01/97	0.34	-	-	133,700
03/02/97	0.33	-	-	129,400
03/03/97	0.31	-	-	119,800
03/04/97	0.30	-	-	116,100
03/05/97	0.32	-	-	122,900
03/06/97	0.33	-	-	127,400
03/07/97	0.36	-	-	136,800
03/08/97	0.37	-	-	144,700
03/09/97	0.39	-	-	152,300
03/10/97	0.41	-	-	161,100
03/11/97	0.44	-	-	170,200
03/12/97	0.46	-	-	180,400
03/13/97	0.50	-	-	194,800
03/14/97	0.51	-	-	199,600
03/15/97	0.52	-	-	204,200
03/16/97	0.54	-	-	210,700
03/17/97	0.56	-	-	220,000
03/18/97	0.59	-	-	228,500
03/19/97	0.59	-	-	232,500
03/20/97	0.61	-	-	238,100

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
03/21/97	0.64	-	-	249,800
03/22/97	0.65	-	-	254,900
03/23/97	0.66	-	-	258,800
03/24/97	0.69	-	-	270,400
03/25/97	0.70	-	-	277,500
03/26/97	0.65	-	-	261,600
03/27/97	0.62	-	-	244,700
03/28/97	0.63	-	-	249,500
03/29/97	0.67	-	-	264,200
03/30/97	0.66	-	-	267,000
03/31/97	0.65	-	-	260,800
04/01/97	0.64	-	-	259,100
04/02/97	0.65	-	-	265,300
04/03/97	0.63	-	-	260,800
04/04/97	0.66	-	-	269,900
04/05/97	0.70	-	-	291,700
04/06/97	0.65	-	-	273,300
04/07/97	0.60	-	-	250,000
04/08/97	0.56	-	-	231,900
04/09/97	0.55	-	-	231,600
04/10/97	0.58	-	-	242,100
04/11/97	0.56	-	-	240,100
04/12/97	0.53	-	-	218,900
04/13/97	0.51	-	-	203,900
04/14/97	0.49	-	-	213,200
04/15/97	0.49	-	-	210,700
04/16/97	0.50	-	-	212,400
04/17/97	0.54	-	-	222,900
04/18/97	0.64	-	-	264,500
04/19/97	0.73	-	-	303,000
04/20/97	0.81	-	-	334,100

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
04/21/97	0.88	-	-	365,300
04/22/97	0.92	-	-	385,100
04/23/97	0.88	-	-	371,000
04/24/97	0.83	-	-	351,100
04/25/97	0.76	-	-	325,600
04/26/97	0.71	-	-	300,200
04/27/97	0.68	-	-	291,700
04/28/97	0.74	-	-	300,200
04/29/97	0.87	-	-	356,800
04/30/97	0.93	-	-	390,800
05/01/97	0.91	-	-	382,300
05/02/97	0.85	-	-	365,300
05/03/97	0.75	-	-	322,800
05/04/97	0.72	-	-	303,000
05/05/97	0.73	-	-	325,600
05/06/97	-	-	-	393,600
05/07/97	-	-	-	481,400
05/08/97	1.39	-	-	549,400
05/09/97	1.38	-	-	566,300
05/10/97	1.40	-	-	574,800
05/11/97	1.40	-	-	577,700
05/12/97	1.44	-	-	591,800
05/13/97	1.47	-	-	606,000
05/14/97	1.51	-	-	623,000
05/15/97	1.53	-	-	634,300
05/16/97	1.58	-	-	651,300
05/17/97	1.66	-	-	690,900
05/18/97	1.72	-	-	716,400
05/19/97	1.79	-	-	744,700
05/20/97	1.81	-	-	758,900
05/21/97	1.84	-	-	781,500

Appendix A. Continued.

Date	Average gage height (m)			Colorado River near CO-UT State Line
	WW9	~AC1	~AC6	
05/22/97	1.88	-	-	790,000
05/23/97	2.13	-	-	920,300
05/24/97	2.08	-	-	917,500
05/25/97	2.14	-	-	945,800
05/26/97	2.09	-	-	934,500
05/27/97	1.86	-	-	832,500
05/28/97	1.65	-	-	722,100
05/29/97	1.52	-	-	654,100
05/30/97	1.57	-	-	654,100
05/31/97	1.75	-	-	739,100
06/01/97	1.92	-	-	809,900
06/02/97	2.19	-	-	943,000
06/03/97	2.31	-	-	1,042,100
06/04/97	2.28	-	-	1,027,900
06/05/97	2.27	-	-	1,030,700
06/06/97	2.26	-	-	1,027,900
06/07/97	2.20	-	-	999,600
06/08/97	2.18	-	-	982,600
06/09/97	2.26	-	-	1,027,900
06/10/97	2.22	-	-	1,030,700
06/11/97	2.12	-	-	968,400
06/12/97	2.09	-	-	954,300
06/13/97	2.05	-	-	926,000
06/14/97	2.01	-	-	911,800
06/15/97	1.93	-	-	866,500
06/16/97	1.84	-	-	826,900

[1]-: No measurement.

Appendix B. Water flow from Independent Ranchman's Ditch via WW10 to North Pond at Walter Walker State Wildlife Area, and from secondary channel (AC2) of Colorado River to diked teritary channel at Adobe Creek.

North Pond		Adobe Creek	
Date water on	Date water off	Date water on	Date water off
06/10/96	06/12/96	11/13/96	11/14/96
06/24/96	06/26/96	11/20/96	11/21/96
07/08/96	07/11/96	11/27/96	11/28/96
07/15/96	07/18/96	12/04/96	12/05/96
07/29/96	07/31/96	12/11/96	12/12/96
08/07/96	08/08/96	12/18/96	12/19/96
08/12/96	08/14/96	12/23/96	12/24/96
08/20/96	08/22/96	12/29/96	12/30/96
09/02/96	09/04/96	12/31/96	01/04/97
09/10/96	09/11/96	01/09/97	01/10/97
09/23/96	09/29/96	01/17/97	01/19/97
10/01/96	10/04/96	01/25/97	01/27/97
10/07/96	10/09/96	02/03/97	02/04/97
10/23/96	10/27/96	02/10/97	02/11/97
04/14/97	04/15/97	02/17/97	02/18/97
		02/24/97	02/25/97
		03/03/97	03/04/97
		03/10/97	03/11/97
		03/17/97	03/19/97
		03/24/97	03/27/97
		04/07/97	04/08/97
		04/14/97	04/15/97
		05/07/97	05/07/97[1]

[1]15 hours.

Appendix C. Water quality characteristics measured on-site near the inlet (i) and outlet (o) of ponds when fish were held in that pond at Horsethief Canyon State Wildlife Area, Adobe Creek, and North Pond near Grand Junction, Colorado.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
HT6i	07/01/96	-10	32.0	23.0	8.60	395	<0.5	7.6
	07/09/96	-02	31.0	24.4	8.65	445	<0.5	7.2
HT1i	07/25/96	14	35.0	25.8	8.60	788	<0.5	8.9
	08/01/96	21	35.0	25.1	8.59	898	1.0	10.4
	08/13/96	33	32.5	22.8	8.38	1,040	1.0	11.9
HT6i	09/17/96	68	13.0	16.9	8.15	1,060	0.5	8.1
	09/26/96	77	20.0	16.3	8.15	1,080	0.5	10.0
	10/04/96	85	18.0	15.7	8.38	1,030	1.0	9.5
	10/11/96	92	13.5	16.3	8.00	1,080	1.0	8.6
	10/16/96	97	14.5	14.7	8.15	1,000	1.0	8.1
	11/01/96	113	16.0	8.5	7.90	1,020	1.0	12.2
	11/12/96	124	10.0	6.8	7.92	1,000	1.0	11.5
	11/22/96	134	11.5	7.6	7.64	974	1.0	10.8
	12/04/96	146	5.0	3.3	7.76	939	0.5	14.0
	12/09/96	151	12.0	4.0	9.20	918	1.0	11.7
	12/18/96	160	-1.0	1.2	8.69	917	1.0	13.2
	12/23/96	165	4.0	1.4	8.35	910	1.0	12.9
	12/31/96	173	5.0	2.5	8.38	877	0.5	12.2
	01/06/97	179	0	1.4	8.32	910	1.0	11.8
	01/17/97	190	2.0	2.2	8.19	886	1.0	13.2
	01/24/97	197	5.0	2.7	8.24	871	1.0	NS[3]
	01/31/97	204	7.0	4.7	8.41	817	1.0	12.6
	02/07/97	211	6.0	4.1	8.42	832	1.0	12.7
	02/10/97	214	5.0	3.3	8.46	854	1.0	12.1
	02/19/97	223	11.0	6.1	8.47	782	1.0	11.7

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
HT6i	03/05/97	237	9.0	6.4	8.32	776	1.0	11.9
	03/12/97	244	10.5	8.0	8.06	740	0.5	10.5
	03/21/97	253	23.5	13.4	8.18	707	0.5	10.2
	04/04/97	267	13.0	11.6	7.61	605	0.5	10.0
	04/11/97	274	7.0	8.8	8.74	579	0.5	12.2
	04/15/97	278	16.0	12.4	8.00	593	0.5	10.2
	04/25/97	288	18.5	18.9	8.95	453	0.5	11.4
	05/09/97	302	26.5	19.2	8.62	472	1.0	7.1
HT6o	07/01/96	-10	25.0	23.8	8.60	404	<0.5	7.7
	07/09/96	-02	32.0	24.8	8.56	442	<0.5	7.4
HT1o	07/25/96	14	38.0	24.8	8.84	753	<0.5	9.4
	08/01/96	21	39.0	25.5	8.59	891	0.5	10.9
AC1	07/01/96	-10	21.0	17.0	8.03	437	<0.5	8.3
	07/09/96	-02	26.0	19.4	8.12	537	<0.5	8.2
	07/25/96	14	33.0	21.8	8.43	852	0.5	8.7
	08/01/96	21	31.0	23.0	8.26	1,040	1.0	8.5
AC2	07/01/96	-10	23.0	17.0	8.12	431	<0.5	8.2
	07/09/96	-02	28.0	19.0	8.21	531	<0.5	8.3
	07/25/96	14	30.0	22.1	8.48	847	0.5	9.3
	08/01/96	21	29.5	23.1	8.23	1,040	0.5	8.2
AC3	07/01/96	-10	22.5	21.2	7.59	970	1.0	3.8
	07/09/96	-02	26.0	25.3	7.80	895	1.0	3.4
	07/25/96	14	29.0	24.0	8.15	714	0.5	3.1
	08/01/96	21	30.5	24.7	8.05	905	1.0	2.7
	04/25/97	288	21.5	20.5	8.05	766	0.5	6.2
	05/09/97	302	23.0	19.0	7.79	1,020	1.0	6.9

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
AC4	07/01/96	-10	23.0	21.5	7.99	643	<0.5	4.3
	07/09/96	-02	28.0	24.5	8.26	606	<0.5	4.8
	07/25/96	14	29.5	24.3	8.61	709	<0.5	6.0
	08/01/96	21	30.0	25.1	8.47	798	0.5	6.0
AC5	07/01/96	-10	24.5	22.3	8.74	580	0.5	8.5
	07/09/96	-02	30.5	27.8	9.25	570	0.5	9.5
	07/25/96	14	32.0	25.9	8.72	688	<0.5	7.8
	08/01/96	21	31.5	26.0	8.40	736	0.5	6.4
	08/13/96	33	24.0	22.9	8.03	938	0.5	6.6
	09/17/96	68	12.0	17.7	7.71	1,160	0.5	5.6
	09/26/96	77	17.0	16.2	7.58	1,140	1.0	7.5
	10/04/96	85	15.5	15.0	7.35	1,110	1.0	7.3
	10/11/96	92	15.0	15.0	7.42	1,110	1.0	7.4
	10/16/96	97	14.5	13.8	7.75	1,140	1.0	6.5
	11/01/96	113	14.0	8.4	7.79	1,170	1.0	10.2
	11/12/96	124	10.0	6.0	7.52	1,260	1.0	13.9
	11/22/96	134	13.5	7.1	7.62	1,220	1.0	12.6
	12/04/96	146	2.5	4.0	7.39	1,250	1.0	14.3
	12/09/96	151	6.0	4.8	8.09	1,140	1.0	11.2
	12/18/96	160	-4.0	1.9	8.35	1,070	1.0	15.1
	12/23/96	165	3.0	2.4	7.96	1,230	1.0	17.4
	12/31/96	173	6.5	2.1	8.11	978	1.0	12.8
	01/06/97	179	0.0	1.9	8.34	895	1.0	12.5
	01/17/97	190	NS	NS	NS	NS	NS	NS
	01/24/97	197	5.0	4.1	8.33	999	1.0	NS
	01/31/97	204	11.0	4.7	8.30	817	1.0	14.2

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
AC5	02/07/97	211	10.0	5.9	8.25	787	1.0	13.7
	02/10/97	214	2.0	4.1	8.34	832	1.0	15.0
	02/19/97	223	12.0	7.6	8.21	824	0.5	12.0
	03/05/97	237	11.0	6.7	8.16	922	1.0	10.3
	03/12/97	244	10.5	9.5	8.13	852	0.5	9.9
	03/21/97	253	28.5	13.4	7.77	899	1.0	8.0
	04/04/97	267	18.0	11.8	7.62	802	0.5	8.8
	04/11/97	274	7.5	9.2	8.61	788	0.5	11.2
	04/15/97	278	14.5	12.6	8.16	786	0.5	10.0
AC6	07/01/96	-10	24.5	22.1	8.34	688	0.5	7.8
	07/09/96	-02	30.0	27.3	8.12	1,050	1.0	9.7
	07/25/96	14	32.0	26.5	8.26	1,750	1.0	9.5
	08/01/96	21	32.0	27.0	8.12	2,020	1.5	8.7
AC7	07/01/96	-10	26.5	22.9	9.11	521	<0.5	10.3
	07/09/96	-02	NS	NS	NS	NS	NS	NS
	07/25/96	14	NS	NS	NS	NS	NS	NS
	08/01/96	21	NS	NS	NS	NS	NS	NS
WW1	07/01/96	-10	30.5	18.6	8.04	393	<0.5	8.4
	07/09/96	-02	26.0	18.7	8.25	495	<0.5	8.0
	07/16/96	5	26.5	20.1	8.19	607	0.5	7.8
	07/25/96	14	27.0	21.0	8.25	812	0.5	7.5
	08/01/96	21	27.5	21.5	7.92	964	0.5	7.2
	08/13/96	33	24.5	20.5	7.97	1,090	1.0	7.0
	09/17/96	68	11.0	15.3	8.22	1,040	1.5	8.1

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW1	09/27/96	78	15.0	12.8	7.87	1,040	1.0	9.7
	10/04/96	85	12.5	13.5	7.92	897	1.0	8.6
	10/11/96	92	22.5	14.6	8.45	998	0.5	9.5
	10/16/96	97	11.0	12.5	7.84	1,050	1.0	9.5
	11/01/96	113	9.0	6.6	8.26	1,000	1.0	10.8
	11/12/96	124	7.0	5.3	7.18	962	1.0	12.0
	11/21/96	133	17.0	7.9	8.03	1,040	0.5	11.4
	12/04/96	146	-2.0	1.0	10.29	923	1.0	12.6
	12/09/96	151	1.0	1.7	9.70	991	1.0	13.1
	12/18/96	160	-7.0	0.9	8.86	1,020	1.0	13.4
	12/23/96	165	3.0	0.9	8.44	1,110	1.0	12.5
	12/31/96	173	5.0	1.9	8.55	895	0.5	12.1
	01/06/97	179	-3.0	1.1	8.26	920	1.0	12.0
	01/17/97	190	4.0	1.2	8.78	917	1.0	13.9
	01/24/97	197	6.0	3.1	8.21	1,030	1.0	NS
	01/31/97	204	5.0	3.1	7.10	1,030	1.0	11.9
	02/07/97	211	6.0	2.2	7.51	886	1.0	11.6
	02/10/97	214	1.0	2.8	7.53	868	1.0	11.8
	02/19/97	223	8.5	4.7	7.57	817	0.5	11.0
	03/05/97	237	5.0	5.6	7.01	953	1.0	12.0
	03/12/97	244	11.5	6.5	7.69	773	0.5	11.2
	03/21/97	253	17.0	9.3	6.83	714	0.5	9.9
	04/04/97	267	14.0	9.3	8.01	571	0.5	10.4
	04/11/97	274	0.5	5.6	8.56	715	1.0	12.5
	04/15/97	278	14.5	8.3	7.58	646	0.5	10.2
	04/25/97	288	20.0	11.1	8.36	545	0.5	10.0

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW1	05/09/97	302	19.0	12.5	8.12	381	1.0	9.3
WW2	07/01/96	-10	32.5	28.9	8.52	3,490	2.5	8.7
	07/09/96	-02	28.5	25.9	8.39	3,740	2.5	3.6
	07/25/96	14	29.0	26.4	8.75	2,090	1.5	7.5
	08/01/96	21	30.0	26.4	8.34	1,750	1.0	7.3
	08/13/96	33	25.5	24.4	8.21	1,670	1.5	7.4
	09/17/96	68	10.5	16.0	8.30	1,570	1.0	4.7
	09/26/96	77	19.0	17.9	7.95	1,390	1.0	9.7
	10/04/96	85	13.0	15.0	7.72	1,300	1.0	8.6
	10/11/96	92	25.0	17.9	8.08	1,270	1.0	9.3
	10/16/96	97	10.0	15.0	7.54	1,240	1.0	7.5
	11/01/96	113	11.0	7.6	7.58	1,270	1.0	10.9
	11/12/96	124	9.0	6.6	7.66	1,310	1.0	12.8
	11/22/96	134	13.5	8.7	7.82	1,310	1.0	10.1
	12/04/96	146	0	2.8	8.28	1,390	1.0	13.1
	12/09/96	151	4.0	4.0	8.60	1,340	1.0	12.6
	12/18/96	160	-3.0	1.4	8.34	1,640	1.0	11.4
	12/23/96	165	5.0	0.5	8.06	1,690	1.0	9.1
	12/31/96	173	6.0	3.6	7.90	1,610	1.0	8.5
	01/06/97	179	-2.0	1.4	7.99	1,820	1.0	4.1
	01/17/97	190	5.0	4.9	7.90	1,950	1.0	8.8
	01/24/97	197	7.0	4.9	8.02	1,950	1.0	NS
	01/31/97	204	8.0	3.9	7.79	2,510	2.0	6.9
	02/07/97	211	5.0	2.9	7.83	2,770	2.0	7.3
	02/10/97	214	2.0	3.0	8.11	2,930	2.0	7.9
	02/19/97	223	11.0	7.3	8.00	3,480	2.0	6.8

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW2	03/05/97	237	7.0	6.6	8.01	4,320	3.0	8.3
	03/12/97	244	15.0	9.7	7.97	4,240	3.0	8.0
	03/21/97	253	21.0	14.4	7.37	6,270	5.0	4.8
	04/04/97	267	12.5	13.5	7.89	6,410	3.5	9.5
	04/11/97	274	0.0	6.0	8.51	6,280	4.0	11.9
	04/15/97	278	15.0	11.1	7.93	4,630	3.0	11.4
	04/25/97	288	21.0	17.5	8.38	4,670	3.0	11.0
	05/09/97	302	21.5	20.3	8.45	4,610	4.0	9.3
WW3	07/01/96	-10	33.5	28.4	8.63	3,470	2.5	11.3
	07/09/96	-02	28.5	23.6	8.21	2,060	1.0	5.3
	07/25/96	14	29.0	25.0	8.70	1,700	1.0	7.9
	08/01/96	21	29.0	25.5	8.35	1,580	1.0	7.7
WW4a	07/01/96	-10	30.5	28.6	7.48	3,930	4.0	5.4
	07/09/96	-02	26.0	22.7	7.51	3,760	2.5	2.4
	07/16/96	5	23.0	21.0	7.50	7,580	5.0	2.7
	07/25/96	14	28.0	22.2	7.66	8,980	6.0	6.0
	08/01/96	21	24.5	19.9	7.34	8,310	5.0	3.1
	08/13/96	33	20.5	17.7	7.14	12,780	9.5	2.7
	09/17/96	68	10.0	13.6	7.42	12,780	13.0	4.7
	09/27/96	78	17.0	14.5	7.64	12,510	8.0	8.2
	10/04/96	85	11.5	11.2	7.89	8,830	7.0	6.7
	10/11/96	92	25.0	15.3	8.01	9,210	5.5	8.2
	10/16/96	97	7.0	10.0	8.20	11,210	8.0	8.1
	11/01/96	113	14.5	6.4	7.83	8,530	7.0	8.5
	11/12/96	124	4.0	3.6	7.42	11,840	9.0	9.3
	11/22/96	134	14.0	9.3	6.95	12,140	8.0	2.6

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW4a	12/04/96	146	-3.0	0.4	8.94	10,380	7.0	11.0
	12/09/96	151	1.0	1.9	8.45	11,630	6.0	12.0
	12/18/96	160	-1.0	-0.5	8.04	13,650	7.5	14.4
	12/23/96	165	4.0	1.1	7.64	12,880	8.0	10.4
	12/31/96	173	7.0	1.7	7.68	10,810	6.0	10.4
	01/06/97	179	-3.0	1.1	7.65	11,040	6.0	12.6
	01/17/97	190	3.0	2.3	7.58	14,120	9.0	10.9
	01/24/97	197	5.0	4.7	7.61	11,430	8.0	NS
	01/31/97	204	6.0	1.9	7.62	12,530	8.0	7.3
	02/07/97	211	8.0	0.9	7.58	12,970	8.0	11.7
	02/10/97	214	2.0	0.6	7.57	11,240	7.0	12.1
	02/19/97	223	9.5	3.4	7.68	11,920	6.5	7.7
	03/05/97	237	7.0	2.6	7.53	12,230	7.0	10.2
	03/12/97	244	9.0	5.9	7.69	11,020	7.0	6.2
	03/21/97	253	15.5	10.2	7.32	11,150	7.0	4.3
	04/04/97	267	13.5	12.3	7.73	9,240	6.0	4.9
	04/11/97	274	0.0	3.2	8.40	10,280	6.0	10.0
	04/15/97	278	13.5	10.1	7.55	11,180	7.0	5.6
	04/25/97	288	22.5	17.5	8.09	8,170	6.0	12.3
	05/09/97	302	19.0	15.7	7.85	3,040	2.0	4.8
WW4b	12/09/96	151	1.0	1.5	8.61	1,270	1.0	12.0
	12/18/96	160	0.0	-0.3	7.98	11,610	7.0	13.4
	12/23/96	165	4.0	0.4	7.97	3,210	2.0	12.4
	12/31/96	173	6.0	2.1	8.36	1,160	1.0	11.6
	01/06/97	179	-3.0	0.8	7.41	2,040	1.0	10.9
	01/17/97	190	3.0	1.3	8.36	1,460	1.0	13.7

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW4b	01/24/97	197	6.0	3.5	8.01	2,040	1.0	NS
	01/31/97	204	6.0	3.0	7.28	2,590	2.0	11.6
	02/07/97	211	8.0	2.1	7.43	1,600	1.0	8.2
	02/10/97	214	2.0	1.3	7.99	1,830	1.0	11.5
	02/19/97	223	11.0	5.2	7.91	1,040	0.5	10.6
	03/05/97	237	6.0	4.2	7.51	1,160	1.0	11.3
	03/12/97	244	8.5	6.5	8.07	928	1.0	10.5
	03/21/97	253	15.0	9.8	7.23	705	0.5	9.2
	04/04/97	267	13.5	10.6	7.93	2,760	3.0	9.6
	04/11/97	274	0.0	5.1	8.67	1,610	1.0	12.2
	04/15/97	278	14.0	8.9	7.95	1,010	1.0	9.8
	04/25/97	288	22.5	15.7	8.36	2,680	2.0	11.8
	05/09/97	302	18.5	12.6	8.14	400	0.5	8.8
WW5	07/01/96	-10	32.0	29.8	8.36	2,020	1.5	10.5
	07/09/96	-02	27.0	25.7	8.23	3,160	2.0	6.9
	07/16/96	5	27.0	24.8	8.11	3,920	3.0	4.3
	07/25/96	14	27.0	25.0	8.12	6,000	3.5	4.8
	08/01/96	21	28.0	24.8	7.85	6,520	4.0	4.0
	08/13/96	33	25.0	22.9	7.73	7,810	5.5	5.4
WW6	07/01/96	-10	31.5	28.7	8.23	1,780	1.5	9.6
	07/09/96	-02	29.5	24.4	8.10	2,830	2.0	6.5
	07/16/96	5	26.0	24.3	8.00	3,950	2.5	7.0
	07/25/96	14	27.5	24.7	8.06	5,530	3.5	9.0
	08/01/96	21	27.5	24.5	7.87	5,550	3.5	9.1
	08/13/96	33	25.0	19.9	7.77	4,320	4.0	6.2
	09/17/96	68	11.0	11.8	8.07	3,610	2.0	5.4

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW6	09/27/96	78	13.5	17.8	8.02	4,230	3.0	12.7
	10/04/96	85	12.5	13.8	7.66	4,710	3.0	5.4
	10/11/96	92	24.0	17.0	7.90	5,370	4.0	8.8
	10/16/96	97	9.5	13.2	7.72	4,780	3.5	4.8
	11/01/96	113	10.5	6.5	8.20	5,880	4.5	9.2
	11/12/96	124	9.0	8.0	7.55	4,960	3.5	8.6
	11/22/96	134	14.0	10.7	8.05	5,500	4.0	10.4
	12/04/96	146	2.0	5.9	8.02	5,670	3.5	14.0
	12/09/96	151	4.0	1.9	8.60	1,070	1.0	12.3
	12/18/96	160	-3.0	0.7	8.60	1,120	1.0	13.8
	12/23/96	165	5.0	1.0	8.40	1,110	1.0	12.4
	12/31/96	173	10.0	2.1	8.53	889	1.0	11.8
	01/06/97	179	-2.0	0.9	8.39	926	1.0	12.5
	01/17/97	190	3.0	1.7	8.38	1,080	1.0	13.3
	01/24/97	197	7.0	3.8	8.14	1,010	1.0	NS
	01/31/97	204	9.0	4.2	7.98	995	1.0	12.2
	02/07/97	211	6.0	2.5	8.05	1,050	1.0	11.9
	02/10/97	214	2.0	1.5	8.41	907	<0.5	11.6
	02/19/97	223	9.0	5.2	8.23	965	0.5	10.9
	03/05/97	237	6.0	4.4	8.21	989	1.0	11.1
	03/12/97	244	9.0	6.7	8.37	769	0.5	10.6
	03/21/97	253	20.0	10.0	7.79	701	0.5	9.7
	04/04/97	267	11.5	10.0	7.91	841	0.5	9.9
	04/11/97	274	0.0	4.6	8.94	819	0.5	12.1
	04/15/97	278	14.5	9.1	8.16	718	0.5	9.6
	04/25/97	288	25.0	15.9	8.38	666	0.5	10.2

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW6	05/09/97	302	21.5	13.4	8.05	424	0.5	8.6
WW7	07/01/96	-10	20.5	19.5	7.58	782	1.0	6.4
	07/09/96	-02	26.0	22.7	7.61	2,670	2.5	4.4
	07/16/96	5	23.5	21.8	7.83	4,050	2.5	6.5
	07/25/96	14	28.0	22.4	7.59	6,840	6.0	6.5
	08/01/96	21	25.0	20.5	7.46	6,560	4.0	4.0
	08/13/96	33	19.5	16.7	7.47	4,990	3.5	4.9
WW8a	07/01/96	-10	NS	NS	NS	NS	NS	NS
	07/09/96	-02	26.0	24.6	7.93	1,310	1.0	6.7
	07/16/96	5	24.0	23.2	8.05	2,800	2.0	6.5
	07/25/96	14	26.0	24.2	8.46	4,370	3.0	11.3
	08/01/96	21	26.0	22.9	7.73	6,250	6.5	6.0
	08/13/96	33	20.0	20.5	7.97	7,660	5.5	4.7
	09/17/96	68	9.0	14.0	8.67	6,270	5.0	4.7
	09/27/96	78	15.5	15.5	8.59	5,800	4.0	9.6
	10/04/96	85	13.5	13.4	8.14	5,270	4.0	7.2
	10/11/96	92	19.0	14.3	8.08	5,470	3.5	8.4
	10/16/96	97	13.5	11.7	8.11	5,900	3.5	8.5
	11/01/96	113	14.0	7.2	7.82	5,910	4.0	9.9
	11/12/96	124	11.0	6.5	7.53	6,190	4.0	11.3
	11/21/96	133	18.0	11.2	7.75	7,470	4.0	18.5
	12/04/96	146	6.0	3.2	8.02	6,680	4.0	>20.0
	12/09/96	151	3.0	2.5	8.24	1,230	1.0	12.4
	12/18/96	160	NS	NS	NS	NS	NS	NS
	12/23/96	165	NS	NS	NS	NS	NS	NS
	12/31/96	173	6.5	1.4	8.26	1,000	1.0	11.5

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW8a	01/06/97	179	-1.0	0.7	8.20	1,120	<0.5	12.5
	01/17/97	190	NS	NS	NS	NS	NS	NS
	01/24/97	197	6.0	0.9	8.31	1,110	1.0	NS
	01/31/97	204	10.0	2.3	8.16	1,240	1.0	14.2
	02/07/97	211	8.0	2.6	8.14	1,400	1.0	14.0
	02/10/97	214	2.0	1.1	8.39	920	1.0	11.9
	02/19/97	223	7.0	3.3	7.55	940	0.5	10.3
	03/05/97	237	10.0	5.4	8.07	1,120	1.0	9.9
	03/12/97	244	6.5	7.6	7.43	900	1.0	9.0
	03/21/97	253	12.5	10.8	6.72	823	1.0	6.7
	04/04/97	267	14.0	12.4	8.32	922	1.0	8.8
	04/11/97	274	3.0	5.4	8.90	1,120	0.5	12.3
	04/15/97	278	10.5	9.6	7.75	779	0.5	8.9
	04/25/97	288	NS	NS	NS	NS	NS	NS
	05/09/97	302	19.0	13.8	7.80	509	1.0	5.8
WW8b	09/17/96	68	7.0	11.8	8.26	5,880	4.0	3.9
	09/27/96	78	16.0	17.6	8.55	5,820	4.0	9.5
	10/04/96	85	15.0	11.6	8.47	3,560	2.0	8.8
	10/11/96	92	23.0	15.3	7.84	5,950	4.5	5.9
	10/16/96	97	12.0	9.0	7.70	6,190	4.0	6.0
	11/01/96	113	14.0	9.3	8.02	6,070	4.0	8.9
	11/12/96	124	13.0	4.8	7.45	6,020	3.5	11.0
	11/21/96	133	17.0	11.9	8.05	7,340	4.0	14.8
	12/04/96	146	2.0	4.0	7.49	6,500	4.5	>20.0
	12/09/96	151	5.0	2.3	8.41	1,060	1.0	13.2
	12/18/96	160	-1.0	0.7	8.41	1,310	1.0	13.4

Appendix C. Continued.

Station	Date	Day of exposure	Air temp. (EC)	Water temp. (EC)	pH	Cond.[1] (µmhos/cm)	Salinity (g/L)	D.O.[2] (mg/L)
WW8b	12/23/96	165	NS	NS	NS	NS	NS	NS
	12/31/96	173	10.0	3.7	8.45	1,010	0.5	11.4
	01/06/97	179	-1.0	0.7	8.44	1,120	1.0	12.1
	01/17/97	190	5.0	3.7	8.33	1,180	1.0	14.3
	01/24/97	197	8.0	4.6	8.37	1,310	1.0	NS
	01/31/97	204	12.0	4.6	8.13	1,640	1.0	11.6
	02/07/97	211	8.0	4.3	7.86	1,320	1.0	11.4
	02/10/97	214	1.0	1.6	8.52	904	1.0	12.0
	02/19/97	223	7.0	4.7	7.84	980	0.5	11.0
	03/05/97	237	10.0	7.0	8.39	1,070	1.0	10.7
	03/12/97	244	7.5	6.1	8.03	939	0.5	10.5
	03/21/97	253	13.0	10.3	7.23	834	0.5	10.2
	04/04/97	267	13.0	11.5	8.34	943	1.0	9.5
	04/11/97	274	4.5	5.7	8.82	792	0.5	13.2
	04/15/97	278	10.0	8.4	7.59	732	0.5	9.9
	04/25/97	288	20.5	17.8	8.24	812	0.5	8.8
	05/09/97	302	11.5	13.0	7.91	519	1.0	7.4
WW9	07/01/96	-10	20.5	20.2	7.71	661	0.5	6.8
	07/09/96	-02	22.5	22.3	7.34	1,260	1.0	4.6
	07/16/96	5	21.5	20.0	7.66	1,160	1.0	5.7
	07/25/96	14	28.0	21.7	8.15	4,480	3.0	12.7
	08/01/96	21	23.0	20.4	8.22	6,030	4.0	10.2
	08/13/96	33	17.0	15.3	8.15	9,210	8.5	7.8
WW10	07/09/96	-02	28.0	21.5	8.12	697	<0.5	6.2
	07/25/96	14	29.0	21.6	8.26	856	1.0	5.5
	08/01/96	21	29.0	22.0	8.25	1,010	1.0	5.5

Appendix C. Continued.

[1]Cond. = conductivity (µmhos/cm) corrected to 25°C.
[2]D.O. = dissolved oxygen.
[3]NS: No sample collected due to no water flow, flooding, ice cover, or instrument or probe problem.

Appendix D. Water quality characteristics in water collected from Horsethief Canyon State Wildlife Area (HT1), Adobe Creek (AC5), and North Pond (WW2). Characteristics were measured in a mobile laboratory at Grand Junction, Colorado (pH, conductivity, hardness, calcium, magnesium, alkalinity, and chloride) or at Yankton, South Dakota (sulfate, un-ionized ammonia, nitrate, nitrite, total suspended solids, volatile solids, and fixed solids). All un-ionized ammonia concentrations (mg/L NH_3-N) were <0.01 mg/L.

Measure	Date	Day of exposure	Station		
			HT1	AC5	WW2
pH					
	08/13/96	33	8.5	7.7	8.1
	09/17/96	68	8.3	7.7	8.0
	10/16/96	97	8.3	8.0	7.9
	11/12/96	124	8.1	7.8	7.8
	12/09/96	151	7.9	7.6	7.6
	01/06/97	179	8.3	8.2	7.7
	02/10/97	214	8.3	8.2	7.9
	03/12/97	244	8.0	8.0	8.0
	04/15/97	278	8.2	8.1	8.1
Conductivity[1] (µmhos/cm)					
	08/13/96	33	1,040	938	1,670
	09/17/96	68	1,060	1,160	1,570
	10/16/96	97	996	1,140	1,240
	11/12/96	124	996	1,260	1,310
	12/09/96	151	918	1,140	1,340
	01/06/97	179	910	895	1,820
	02/10/97	214	854	832	2,930
	03/12/97	244	740	852	4,240

Appendix D. Continued.

Measure	Date	Day of exposure	Station		
			HT1	AC5	WW2
Hardness (mg/L as $CaCO_3$)					
	04/15/97	278	593	786	4,630
	08/13/96	33	336	280	428
	09/17/96	68	406	340	384
	10/16/96	97	350	344	352
	11/12/96	124	330	366	352
	12/09/96	151	300	362	374
	01/06/97	179	276	306	558
	02/10/97	214	248	262	890
	03/12/97	244	236	278	1,460
	04/15/97	278	208	260	1,350
Calcium (mg/L)					
	08/13/96	33	86	71	73
	09/17/96	68	110	89	66
	10/16/96	97	90	90	78
	11/12/96	124	86	95	79
	12/09/96	151	78	93	81
	01/06/97	179	71	78	111
	02/10/97	214	64	66	162
	03/12/97	244	68	73	227
	04/15/97	278	56	67	182

Appendix D. Continued.

Measure	Date	Day of exposure	Station		
			HT1	AC5	WW2
Magnesium (mg/L)					
	08/13/96	33	30	25	60
	09/17/96	68	32	29	53
	10/16/96	97	31	29	38
	11/12/96	124	28	31	37
	12/09/96	151	25	32	42
	01/06/97	179	24	27	68
	02/10/97	214	21	23	118
	03/12/97	244	16	23	216
	04/15/97	278	17	22	217
Alkalinity (mg/L as $CaCO_3$)					
	08/13/96	33	106	98	97
	09/17/96	68	161	138	87
	10/16/96	97	130	140	119
	11/12/96	124	141	150	128
	12/09/96	151	135	150	135
	01/06/97	179	132	140	201
	02/10/97	214	120	115	270
	03/12/97	244	128	127	343
	04/15/97	278	122	138	227

Appendix D. Continued.

Measure	Date	Day of exposure	Station		
			HT1	AC5	WW2
Chloride (mg/L)					
	08/13/96	33	78	96	158
	09/17/96	68	75	138	178
	10/16/96	97	77	136	153
	11/12/96	124	79	133	149
	12/09/96	151	84	109	152
	01/06/97	179	87	92	184
	02/10/97	214	75	80	254
	03/12/97	244	69	86	424
	04/15/97	278	43	70	432
Sulfate (mg/L)					
	08/13/96	33	311	236	522
	09/17/96	68	336	262	463
	10/17/96	98	296	259	317
	11/12/96	124	261	278	312
	12/09/96	151	222	292	345
	01/06/97	179	195	221	524
	02/10/97	214	175	190	996
	03/12/97	244	170	222	1,990
	04/15/97	278	135	190	2,000

Appendix D. Continued.

Measure	Date	Day of exposure	Station		
			HT1	AC5	WW2
Nitrate (mg/L NO_3-N)					
	08/13/96	33	0.2	<0.1	0.1
	09/17/96	68	1.1	0.2	0.2
	10/17/96	98	0.2	0.4	<0.1
	11/12/96	124	0.4	0.4	<0.1
	12/09/96	151	0.4	0.3	<0.1
	01/06/97	179	0.4	0.4	<0.1
	02/10/97	214	1.1	0.2	0.2
	03/12/97	244	0.2	<0.1	0.4
	04/15/97	278	0.3	<0.1	0.8
Nitrite (mg/L NO_2-N)					
	08/13/96	33	<0.01	<0.01	<0.01
	09/17/96	68	0.01	<0.01	<0.01
	10/17/96	98	<0.01	<0.01	<0.01
	11/12/96	124	<0.01	<0.01	<0.01
	12/09/96	151	0.01	0.01	<0.01
	01/06/97	179	<0.01	0.01	<0.01
	02/10/97	214	<0.01	<0.01	0.04
	03/12/97	244	<0.01	<0.01	0.04
	04/15/97	278	0.01	0.01	0.05

Appendix D. Continued.

Measure	Date	Day of exposure	Station		
			HT1	AC5	WW2
Total suspended solids (mg/L)					
	08/13/96	33	14.2	51.7	3.7
	09/17/96	68	45.3	11.4	3.4
	10/17/96	98	12.9	14.4	3.0
	11/12/96	124	9.8	3.1	2.1
	12/09/96	151	3.3	4.3	2.1
	01/06/97	179	34.3	7.3	35.0
	02/10/97	214	10.0	15.2	12.0
	03/12/97	244	57.0	15.5	7.7
	04/15/97	278	36.7	9.5	10.0
Volatile solids (mg/L)					
	08/13/96	33	4.6	6.8	1.3
	09/17/96	68	4.7	1.2	1.2
	10/17/96	98	1.3	1.3	1.4
	11/12/96	124	1.1	0.7	0.8
	12/09/96	151	0.4	0.8	0.7
	01/06/97	179	3.3	1.7	9.5
	02/10/97	214	1.5	3.0	4.0
	03/12/97	244	6.0	2.7	3.3
	04/15/97	278	7.0	4.5	3.0

Appendix D. Continued.

Measure	Date	Day of exposure	Station		
			HT1	AC5	WW2
Fixed solids (mg/L)					
	08/13/96	33	9.6	44.8	2.3
	09/17/96	68	40.7	10.2	2.2
	10/17/96	98	11.6	13.1	1.6
	11/12/96	124	8.6	2.4	1.4
	12/09/96	151	2.9	3.5	1.4
	01/06/97	179	31.0	5.7	25.5
	02/10/97	214	8.5	12.2	8.0
	03/12/97	244	51.0	12.8	4.3
	04/15/97	278	29.7	5.0	7.0

[1]Conductivity (µmhos/cm) corrected to 25°C.

Appendix E. Concentration of inorganic elements (mg/L) in water collected from various sample stations near Grand Junction, Colorado.

Element	Date	Day of exposure	Station		
			HT1	AC5	WW2
Ag	08/13/96	33	<0.01	<0.01	<0.01
	10/16/96	97	<0.01	<0.01	<0.01
	12/19/96	151	<0.01	<0.01	<0.01
	02/10/97	214	<0.01	<0.01	<0.01
	04/15/97	278	<0.01	<0.01	<0.01
Al	08/13/96	33	<0.02	<0.02	<0.02
	10/16/96	97	0.03	<0.02	0.02
	12/19/96	151	0.03	<0.02	<0.02
	02/10/97	214	<0.02	<0.02	<0.02
	04/15/97	278	0.03	<0.02	<0.02
As	08/13/96	33	<0.03	<0.03	<0.03
	10/16/96	97	<0.03	<0.03	0.03
	12/19/96	151	<0.03	<0.03	<0.03
	02/10/97	214	<0.03	<0.03	<0.03
	04/15/97	278	<0.03	<0.03	<0.03
B	08/13/96	33	0.082	0.051	0.088
	10/16/96	97	0.082	0.052	0.066
	12/19/96	151	0.065	0.052	0.065
	02/10/97	214	0.055	0.042	0.120
	04/15/97	278	0.043	0.047	0.210

Appendix E. Continued.

Element	Date	Day of exposure	Station		
			HT1	AC5	WW2
Ba	08/13/96	33	0.113	0.144	0.094
	10/16/96	97	0.085	0.098	0.102
	12/19/96	151	0.085	0.149	0.126
	02/10/97	214	0.113	0.105	0.122
	04/15/97	278	0.094	0.107	0.091
Be	08/13/96	33	<0.0002	<0.0002	<0.0002
	10/16/96	97	<0.0002	<0.0002	<0.0002
	12/19/96	151	<0.0002	<0.0002	<0.0002
	02/10/97	214	<0.0002	0.0003	<0.0002
	04/15/97	278	<0.0002	<0.0002	<0.0002
Bi	08/13/96	33	0.02	0.01	0.03
	10/16/96	97	0.02	0.02	0.04
	12/19/96	151	0.03	0.03	<0.01
	02/10/97	214	0.02	0.03	0.02
	04/15/97	278	0.03	0.02	<0.01
Cd	08/13/96	33	<0.002	<0.002	<0.002
	10/16/96	97	<0.002	<0.002	<0.002
	12/19/96	151	<0.002	<0.002	<0.002
	02/10/97	214	<0.002	<0.002	<0.002
	04/15/97	278	<0.002	<0.002	<0.002

Appendix E. Continued.

Element	Date	Day of exposure	Station		
			HT1	AC5	WW2
Co	08/13/96	33	<0.001	<0.001	<0.001
	10/16/96	97	<0.001	<0.001	<0.001
	12/19/96	151	<0.001	<0.001	<0.001
	02/10/97	214	<0.001	<0.001	<0.001
	04/15/97	278	<0.001	<0.001	<0.001
Cr	08/13/96	33	<0.009	<0.009	<0.009
	10/16/96	97	0.010	<0.009	<0.009
	12/19/96	151	0.010	<0.009	<0.009
	02/10/97	214	0.010	<0.009	<0.009
	04/15/97	278	0.010	<0.009	0.010
Cu	08/13/96	33	0.017	<0.001	<0.001
	10/16/96	97	0.050	<0.001	<0.001
	12/19/96	151	0.005	<0.001	<0.001
	02/10/97	214	0.004	<0.001	<0.001
	04/15/97	278	0.025	<0.001	<0.001
Fe	08/13/96	33	0.010	0.029	0.027
	10/16/96	97	0.036	0.020	0.023
	12/19/96	151	0.027	0.028	0.034
	02/10/97	214	0.021	0.020	0.038
	04/15/97	278	0.020	0.130	0.020

Appendix E. Continued.

Element	Date	Day of exposure	Station		
			HT1	AC5	WW2
Li	08/13/96	33	0.038	0.029	0.037
	10/16/96	97	0.040	0.031	0.036
	12/19/96	151	0.031	0.031	0.035
	02/10/97	214	0.027	0.025	0.051
	04/15/97	278	0.020	0.021	0.084
Mg	08/13/96	33	29	25	55
	10/16/96	97	29	27	37
	12/19/96	151	24	31	39
	02/10/97	214	21	22	110
	04/15/97	278	15	24	210
Mn	08/13/96	33	0.024	0.018	0.006
	10/16/96	97	0.012	0.018	0.029
	12/19/96	151	0.009	0.034	0.043
	02/10/97	214	0.010	0.021	0.018
	04/15/97	278	0.008	0.058	0.024
Mo	08/13/96	33	<0.007	0.010	0.008
	10/16/96	97	<0.007	<0.007	<0.007
	12/19/96	151	<0.007	<0.007	<0.007
	02/10/97	214	<0.007	<0.007	0.008
	04/15/97	278	<0.007	0.010	0.010

Appendix E. Continued.

Element	Date	Day of exposure	Station		
			HT	AC5	WW2
Ni	08/13/96	33	<0.007	0.007	<0.007
	10/16/96	97	<0.007	0.007	<0.007
	12/19/96	151	<0.007	<0.007	<0.007
	02/10/97	214	<0.007	<0.007	<0.007
	04/15/97	278	<0.007	0.008	<0.007
Pb	08/13/96	33	<0.03	<0.03	<0.03
	10/16/96	97	<0.03	<0.03	<0.03
	12/19/96	151	<0.03	<0.03	<0.03
	02/10/97	214	<0.03	<0.03	<0.03
	04/15/97	278	<0.03	<0.03	<0.03
Sb	08/13/96	33	<0.03	<0.03	<0.03
	10/16/96	97	<0.03	<0.03	<0.03
	12/19/96	151	<0.03	<0.03	<0.03
	02/10/97	214	<0.03	<0.03	<0.03
	04/15/97	278	<0.03	<0.03	<0.04
Si	08/13/96	33	0.91	1.10	0.57
	10/16/96	97	0.52	0.46	0.44
	12/19/96	151	0.49	0.20	0.56
	02/10/97	214	1.30	0.60	0.04
	04/15/97	278	0.50	0.86	0.66

Appendix E. Continued.

Element	Date	Day of exposure	Station		
			HT1	AC5	WW2
Sn	08/13/96	33	<0.03	<0.03	<0.03
	10/16/96	97	<0.03	<0.03	<0.03
	12/19/96	151	<0.03	<0.03	<0.03
	02/10/97	214	<0.03	<0.03	<0.03
	04/15/97	278	<0.03	<0.03	<0.03
Sr	08/13/96	33	0.95	0.77	0.81
	10/16/96	97	0.98	0.93	1.05
	12/19/96	151	0.76	0.92	1.09
	02/10/97	214	0.61	0.63	2.07
	04/15/97	278	0.46	0.65	2.41
Ti	08/13/96	33	0.0006	0.0007	<0.0005
	10/16/96	97	0.0010	0.0010	0.0010
	12/19/96	151	<0.0005	0.0016	0.0010
	02/10/97	214	<0.0005	<0.0005	0.0009
	04/15/97	278	0.0016	0.0007	0.0010
Tl	08/13/96	33	<0.07	<0.07	<0.07
	10/16/96	97	0.07	<0.07	0.09
	12/19/96	151	<0.07	<0.07	<0.07
	02/10/97	214	<0.07	<0.07	<0.07
	04/15/97	278	<0.07	<0.07	<0.07

Appendix E. Continued.

Element	Date	Day of exposure	Station		
			HT1	AC5	WW2
V	08/13/96	33	0.002	0.003	<0.002
	10/16/96	97	0.002	<0.002	<0.002
	12/19/96	151	0.003	<0.002	0.003
	02/10/97	214	<0.002	<0.002	<0.002
	04/15/97	278	0.003	<0.002	0.005
Zn	08/13/96	33	<0.001	0.009	0.005
	10/16/96	97	0.004	0.013	0.016
	12/19/96	151	0.006	0.016	0.008
	02/10/97	214	0.006	0.006	0.010
	04/15/97	278	0.003	0.007	0.005

[1]<: Below limit of detection.
[2]-: No sample.

Appendix F. Concentration of selenium (µg/g dry weight) in muscle plugs from razorback sucker held at three sites near Grand Junction, Colorado, and from brood stock held at Horsethief Canyon State Wildlife Area.

			Day of exposure and date				
			0	286[1]	316[2]	344[3]	371[4]
Site	Fish no.	Sex	7/11/96	4/23/97	5/23/97	6/20/97	7/17/97
HT	16	F	-[5]	4.7	5.4	5.1	4.9
	17	F	4.9	-	-	-	-
	19	F	-	5.3	5.6	5.4	5.9
	24	F	-	3.6	4.3	4.3	3.7
	29	F	-	5.3	4.8	4.8	5.6
	50	F	-	4.1	-	-	-
	74	F	4.1	-	-	-	-
	77	F	-	4.9	-	-	-
AC	13	F	-	16	-	-	-
	14	F	-	18	18	17	-
	17	F	-	16	16	14	14
	18	F	-	19	23	17	17
	21	F	10	-	-	-	-
	23	F	8.9	-	-	-	-
	37	F	-	14	-	-	-
	HT66	F	-	14	13	12	11
WW	1	F	17	-	-	-	-
	12	F	12	-	-	-	-
	27	F	-	24	20	18	18
	40	F	-	27	-	-	-
	52	F	-	24	19	19	18
	53	F	-	27	24	28	21
	HT12	F	-	19	17	14	15
	HT42	F	-	16	-	-	-

Appendix F. Continued.

			Day of exposure and date				
			-66	286[1]	316[2]	344[3]	371[4]
Site	Fish no.	Sex	5/06/96	4/23/97	5/23/97	6/20/97	7/17/97
BS	1 (0404)[6]	F	3.6	-	-	-	-
	2 (320E)	M	5.2	4.7	-	-	-
	3 (3914)	F	13.1	16	-	-	-
	4 (3436)	F	13.8	11	-	-	-
	5 (OC6B)	F	6.3	-	-	-	-
	6 (253C)	F	-	-	-	-	-
	7 (4F7E)	M	2.7	-	-	-	-
	8 (706D)	F	4.3	-	-	-	-
	9 (081D)	F	2.5	-	-	-	-
	10 (275F)	F	3.5	-	-	-	-
	11 (5275)	F	2.6	-	-	-	-
	12 (5964)	M	3.5	-	-	-	-
	13 (607A)	M	3.2	-	-	-	-
	14 (6363)	M	3.8	-	-	-	-
	15 (6D7B)	M	3.3	-	-	-	-
	16 (D102)	F	-	3.7	-	-	-

[1]Spawned and moved fish at AC and WW to reference site HT for depuration.
[2]31 days depuration.
[3]59 days depuration.
[4]86 days depuration.
[5]-: No sample.
[6]The last four PIT numbers are given for brood stock.

Appendix G. Mean (standard error in parentheses, n=2) selenium concentration (µg/g dry weight), precent moisture, diameter (mm), and percent viability of eggs from razorback sucker held at three sites near Grand Junction, Colorado. Fish were spawned on 4/23/97 and 4/25/97; date in table is measurement date.

Site	Fish[1] number	Selenium		Moisture (%)		Diameter		Viable (%)	
		4/23	4/26	4/23	4/26	4/23	4/26	4/25	4/27
HT	16	-[2]	5.0 (0)	-	93.1 (0.4)	-	2.29 (0.06)	-	8
	19	6.2 (0)	-	92.2 (0)	-	2.52 (0.01)	2.40 (0.04)	~1	0
	24	5.9 (0.1)	-	92.4 (0)	-	-	2.24 (0)	-	0
	29	6.6 (0.2)	6.4 (-)	93.4 (0.2)	92.8 (-)	2.62 (0.01)	-	~1	-
	50	6.7 (0)	6.0 (0.1)	92.8 (0.1)	92.4 (0.2)	2.50 (0.04)	-	~0.5	-
	77	6.1 (0.2)	-	93.0 (0)	-	-	-	-	-
AC	13	40.1 (1.1)	-	92.4 (0.2)	-	-	-	2.7	-
	14	-	38.4 (-)	-	91.3 (-)	-	-	-	-
	17	40.2 (0.3)	-	92.4 (0.2)	-	2.59 (0)	2.49 (0)	0	5.7
	18	43.1 (0.2)	-	92.4 (0.1)	-	2.61 (0.02)	2.49 (0.04)	-	1.8
	37	41.9 (0.5)	-	93.2 (0.2)	-	2.75 (0.01)	-	0	-
	HT66	36.2 (0.8)	-	92.8 (0)	-	-	2.49 (0.01)	-	0

Appendix G. Continued.

Site	Fish[1] number	Selenium		Moisture (%)		Diameter		Viable (%)	
		4/23	4/26	4/23	4/26	4/23	4/26	4/25	4/27
WW	27	59.0 (1.9)	54.0 (1.8)	93.3 (0)	91.8 (0.7)	2.52 (0)	-	0	-
	40	51.8 (0.3)	-	93.0 (0.2)	-	2.60 (0.01)	2.41 (0)	0	0
	52	-	52.6 (-)	-	93.6 (-)	-	2.32 (0.01)	-	1.2
	53	55.0 (0.6)	55.2 (0.2)	93.3 (0.2)	93.2 (0)	2.66 (0.02)	-	~5	-
	HT12	53.0 (2.0)	-	93.8 (0.2)	-	-	2.23 (0.02)	-	5.9
	HT42	60.0 (2.0)	57.0 (-)	92.9 (0.1)	93.6 (-)	-	-	-	-
BS	0404	6.5 (0.2)	-	90.4 (0.1)	-	2.85 (0.02)	-	0	0
	275F	-	-	-	-	-	3.01 (0.02)	-	80
	3914	7.1 (0.2)	-	91.8 (0.2)	-	3.10 (0.02)	-	-	0
	706D	7.0 (0.1)	-	92.6 (0.2)	-	2.79 (0.01)	-	1	1

[1]The last four PIT numbers are given for brood stock (BS): 0404 = 7F7B020404; 275F = 7F7F36275F; 3914 = 7F7D133914; 706D = 7F7B1B706D.
[2]-: No sample.

Appendix H. Water quality characteristics measured in test water used in the egg study.

Measure	Date	Day of exposure	Station			
			HT1	AC5	WW2	24-Road
pH						
	04/25/97	-1	8.5	7.8	8.4	8.3
	04/27/97	1	8.4	7.8	8.1	7.7
	04/28/97	2	8.3	7.8	8.1	8.0
	04/29/97	3	8.6	8.0	8.3	8.1
	04/30/97	4	8.8	7.7	8.1	8.2
Conductivity[1] (µmhos/cm)						
	04/25/97	-1	254	799	4,270	676
	04/27/97	1	509	743	4,240	583
	04/28/97	2	520	796	4,290	636
	04/29/97	3	624	780	4,220	624
	04/30/97	4	509	812	4,380	572
Hardness (mg/L as $CaCO_3$)						
	04/25/97	-1	172	280	1,200	65
	04/27/97	1	176	252	1,200	65
	04/28/97	2	176	264	1,220	65
	04/29/97	3	202	252	1,210	64
	04/30/97	4	178	266	1,240	64

Appendix H. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC5	WW2	24-Road
Calcium (mg/L)						
	04/25/97	-1	47	63	162	18
	04/27/97	1	47	65	160	19
	04/28/97	2	48	66	160	18
	04/29/97	3	53	63	155	18
	04/30/97	4	48	67	158	18
Magnesium (mg/L)						
	04/25/97	-1	13	30	193	5
	04/27/97	1	14	22	194	4
	04/28/97	2	14	24	199	5
	04/29/97	3	17	23	200	5
	04/30/97	4	14	24	205	5
Alkalinity (mg/L as $CaCO_3$)						
	04/25/97	-1	107	130	193	181
	04/27/97	1	110	133	182	161
	04/28/97	2	110	135	185	151
	04/29/97	3	109	127	184	124
	04/30/97	4	111	147	178	116

Appendix H. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC5	WW2	24-Road
Chloride (mg/L)						
	04/25/97	-1	35	65	402	26
	04/27/97	1	40	64	416	28
	04/28/97	2	40	66	416	28
	04/29/97	3	46	64	410	38
	04/30/97	4	40	68	422	42
Sulfate (mg/L)						
	04/25/97	-1	-	-	-	-
	04/27/97	1	-	-	-	-
	04/28/97	2	104	175	1,710	33
	04/29/97	3	-	-	-	-
	04/30/97	4	-	-	-	-
Un-ionized ammonia (mg/L NH_3-N)						
	04/25/97	-1	-	-	-	-
	04/27/97	1	-	-	-	-
	04/28/97	2	<0.01	<0.01	<0.01	<0.01
	04/29/97	3	-	-	-	-
	04/30/97	4	-	-	-	-

[1]Conductivity (µmhos/cm) corrected to 25°C.

Appendix I. Percent survival of razorback sucker eggs and newly hatched larvae from young adults and brood stock held at Horsethief Canyon State Wildlife Area near Grand Junction, Colorado. Eggs and newly hatched larvae were held in either reference water (24-Road) or site water in the 5-day egg study. The test was initiated with "normal" looking eggs.

Day of exposure	Date	Fish number and replicate											
		HT16			HT19			HT24			BS275F[1]		
		a	b	c	a	B	c	a	b	c	a	b	c
Reference water													
0	4/26	100	100	100	100	100	100	100	100	100	100	100	100
1	4/27	72	64	64	76	56	80	68	36	40	72	80	80
2	4/28	44	32	52	68	44	64	0	0	0	68	80	80
3	4/29	36	28	48	68	44	64	0	0	0	68	80	80
4	4/30	36	28	44	64	36	64	0	0	0	68	80	80
5	5/01	36	24	44	56	36	64	0	0	0	68	80	72
Site water													
0	4/26	100	100	100	100	100	100	100	100	100	100	100	100
1	4/27	68	68	64	64	76	76	56	52	32	84	88	84
2	4/28	44	48	40	56	52	64	0	0	0	80	88	68
3	4/29	44	48	40	56	52	56	0	0	0	80	80	64
4	4/30	44	44	40	56	52	56	0	0	0	72	80	56
5	5/01	44	36	40	56	52	56	0	0	0	68	76	56

[1]Brood stock (BS) eggs stocked at 2 days post spawn; PIT 7F7F36275F.

Appendix J. Percent survival of razorback sucker eggs and newly hatched larvae from adults held at Adobe Creek and Walter Walker near Grand Junction, Colorado. Eggs and newly hatched larvae were held in either reference water (24-Road) or site water in the 5-day egg study. The test was initiated with "normal" looking eggs.

		Fish number and replicate																	
Day of		AC17			AC18			AC (HT66)			WW40			WW52			WW (HT12)		
exposure	Date	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Reference water																			
0	4/26	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	4/27	84	88	88	68	88	88	100	96	100	68	48	72	72	80	84	76	72	88
2	4/28	76	80	80	32	36	64	100	96	100	64	40	60	36	32	44	52	52	84
3	4/29	76	80	76	32	36	64	100	96	100	56	40	56	36	28	44	52	44	80
4	4/30	76	80	76	32	36	64	100	92	100	56	36	56	36	28	44	48	40	72
5	5/1	72	72	76	32	24	60	96	92	100	56	36	48	36	24	40	48	40	72
Site water																			
0	4/26	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	4/27	84	92	88	76	76	92	100	100	100	76	88	76	72	72	88	88	84	80
2	4/28	72	84	76	52	52	68	96	100	100	76	68	64	36	36	64	72	64	76
3	4/29	68	84	76	52	52	60	96	100	100	68	64	56	32	36	64	68	64	76
4	4/30	68	80	72	52	52	56	96	92	100	68	60	56	28	32	60	64	64	76
5	5/01	64	72	68	52	44	56	96	88	100	68	60	56	24	32	60	64	64	76

Appendix K. Percent hatch of razorback sucker eggs from young adults and brood stock held at Horsethief Canyon State Wildlife Area near Grand Junction, Colorado. Eggs were held in either reference water (24-Road) or site water in the 5-day egg study. The test was initiated with "normal" looking eggs.

Day of exposure	Date	Fish number and replicate											
		HT16			HT19			HT24			BS275F[1]		
		a	b	c	a	b	c	a	b	c	a	b	c
Reference water													
0	4/26	0	0	0	0	0	0	0	0	0	0	0	0
1	4/27	0	0	0	0	0	0	0	0	0	0	0	0
2	4/28	0	0	0	0	0	0	0	0	0	60	72	64
3	4/29	0	4	0	0	0	0	0	0	0	68	80	80
4	4/30	4	16	8	8	0	12	0	0	0	68	80	80
5	5/01	32	28	40	56	36	36	0	0	0	68	80	80
Site water													
0	4/26	0	0	0	0	0	0	0	0	0	0	0	0
1	4/27	0	0	0	0	0	0	0	0	0	0	0	0
2	4/28	0	0	0	0	0	0	0	0	0	52	60	36
3	4/29	0	0	0	0	0	0	0	0	0	68	84	56
4	4/30	0	0	4	0	8	0	0	0	0	68	84	56
5	5/01	24	36	40	32	20	36	0	0	0	68	84	56

[1]Brood stock (BS) eggs stocked at 2 days post spawn; PIT 7F7F36275F.

Appendix L. Percent hatch of razorback sucker eggs from adults held at Adobe Creek and Walter Walker near Grand Junction, Colorado. Eggs were held in either reference water (24-Road) or site water in the 5-day egg study. The test was initiated with "normal" looking eggs.

		Fish number and replicate																	
Day of		AC17			AC18			AC (HT66)			WW40			WW52			WW (HT12)		
exposure	Date	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Reference water																			
0	4/26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	4/27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	4/28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	4/29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	4/30	12	24	28	4	0	4	8	12	12	0	0	0	28	24	12	16	16	56
5	5/01	56	60	64	24	24	52	80	84	92	40	28	44	32	28	36	44	32	72
Site water																			
0	4/26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	4/27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	4/28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	4/29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0
4	4/30	8	0	12	12	4	4	0	4	0	4	0	0	4	16	4	4	16	4
5	5/01	28	60	48	40	44	40	60	72	56	60	32	32	12	20	44	24	56	64

Appendix M. Water quality characteristics measured in test water used in the razorback sucker larvae study.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
pH						
	05/03/97	1	8.9	8.1	8.5	8.2
	05/06/97	4	8.3	8.1	8.5	8.1
	05/07/97	5	8.6	7.8	8.1	8.2
	05/08/97	6	8.8	8.0	8.7	8.1
	05/09/97	7	8.4	7.9	8.0	8.1
	05/10/97	8	8.2	7.9	8.3	8.1
	05/11/97	9	8.0	8.0	8.1	8.1
	05/12/97	10	8.1	8.1	8.5	8.0
	05/13/97	11	8.0	8.1	8.1	8.1
	05/14/97	12	8.2	7.8	8.1	8.0
	05/15/97	13	8.6	7.9	8.8	8.0
	05/16/97	14	8.7	8.0	9.0	8.1
	05/17/97	15	8.4	7.9	8.4	8.1
	05/18/97	16	8.4	8.0	9.2	8.1
	05/19/97	17	8.4	7.8	8.7	8.0
	05/20/97	18	8.7	8.0	9.3	8.1
	05/21/97	19	8.6	7.8	8.7	8.1
	05/22/97	20	8.8	7.9	9.2	8.0
	05/23/97	21	8.7	7.8	8.6	7.6
	05/24/97	22	9.0	7.8	9.6	7.7
	05/25/97	23	8.9	7.8	9.0	7.5
	05/26/97	24	8.9	7.9	9.2	7.6
	05/27/97	25	8.8	7.7	8.9	7.5
	05/28/97	26	9.0	8.0	9.1	7.6
	05/29/97	27	8.8	7.8	9.3	7.6

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Conductivity[1] (µmhos/cm)						
	05/30/97	28	8.8	7.9	9.3	7.8
	05/31/97	29	8.7	7.9	8.9	7.7
	05/03/97	1	484	875	4,470	572
	05/06/97	4	536	964	4,610	583
	05/07/97	5	400	950	4,700	450
	05/08/97	6	520	1,010	4,800	541
	05/09/97	7	525	1,520	5,250	525
	05/10/97	8	468	936	4,930	520
	05/11/97	9	408	918	4,890	459
	05/12/97	10	408	927	5,100	408
	05/13/97	11	404	824	4,690	404
	05/14/97	12	418	1,020	5,100	479
	05/15/97	13	408	1,010	5,050	459
	05/16/97	14	408	1,010	5,100	438
	05/17/97	15	380	1,020	5,000	420
	05/18/97	16	360	1,020	5,200	430
	05/19/97	17	360	1,080	5,350	463
	05/20/97	18	361	1,050	5,410	435
	05/21/97	19	385	1,060	6,030	416
	05/22/97	20	411	875	5,530	433
	05/23/97	21	422	1,090	5,410	438
	05/24/97	22	420	1,010	5,640	409
	05/25/97	23	416	985	5,520	433
	05/26/97	24	358	829	5,250	409
	05/27/97	25	431	884	5,530	453
	05/28/97	26	379	747	5,520	332
	05/29/97	27	390	750	5,500	460
	05/30/97	28	400	735	5,720	469

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Hardness (mg/L as $CaCO_3$)						
	05/31/97	29	410	753	5,830	426
	05/03/97	1	174	292	1,270	64
	05/06/97	4	166	298	1,280	64
	05/07/97	5	172	320	1,280	64
	05/08/97	6	168	310	1,320	63
	05/09/97	7	169	438	810	63
	05/10/97	8	170	306	1,350	62
	05/11/97	9	170	318	1,360	63
	05/12/97	10	172	324	1,475	63
	05/13/97	11	174	340	1,425	62
	05/14/97	12	172	340	1,415	62
	05/15/97	13	166	340	1,430	62
	05/16/97	14	164	342	1,460	68
	05/17/97	15	156	346	1,500	68
	05/18/97	16	140	344	1,500	69
	05/19/97	17	146	356	1,570	68
	05/20/97	18	156	352	1,650	74
	05/21/97	19	158	356	1,660	72
	05/22/97	20	158	352	1,600	72
	05/23/97	21	160	356	1,610	72
	05/24/97	22	160	336	1,640	76
	05/25/97	23	164	324	1,660	78
	05/26/97	24	158	284	1,540	74
	05/27/97	25	162	288	1,530	74
	05/28/97	26	164	256	1,630	74
	05/29/97	27	150	268	1,570	74
	05/30/97	28	152	264	1,600	68
	05/31/97	29	166	280	1,670	70

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Calcium (mg/L)						
	05/03/97	1	47	72	160	18
	05/06/97	4	45	73	152	18
	05/07/97	5	46	80	152	19
	05/08/97	6	46	74	145	18
	05/09/97	7	45	86	110	18
	05/10/97	8	46	75	144	18
	05/11/97	9	46	78	144	18
	05/12/97	10	47	77	157	18
	05/13/97	11	47	80	147	18
	05/14/97	12	46	82	141	18
	05/15/97	13	45	80	139	18
	05/16/97	14	45	79	144	20
	05/17/97	15	43	84	149	19
	05/18/97	16	42	83	157	20
	05/19/97	17	40	85	154	20
	05/20/97	18	46	83	168	20
	05/21/97	19	44	86	168	21
	05/22/97	20	44	83	170	22
	05/23/97	21	45	85	168	22
	05/24/97	22	45	82	158	22
	05/25/97	23	46	82	171	23
	05/26/97	24	45	70	157	23
	05/27/97	25	44	69	163	22
	05/28/97	26	45	66	170	22
	05/29/97	27	42	66	158	22
	05/30/97	28	42	66	163	20
	05/31/97	29	45	62	170	21

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Magnesium (mg/L)						
	05/03/97	1	14	27	211	5
	05/06/97	4	13	28	219	5
	05/07/97	5	14	29	219	4
	05/08/97	6	13	30	233	4
	05/09/97	7	14	54	130	4
	05/10/97	8	13	29	241	4
	05/11/97	9	13	30	243	4
	05/12/97	10	13	32	263	4
	05/13/97	11	14	34	257	4
	05/14/97	12	14	33	258	4
	05/15/97	13	13	34	263	4
	05/16/97	14	13	35	267	4
	05/17/97	15	12	33	274	5
	05/18/97	16	8	33	269	5
	05/19/97	17	11	35	288	4
	05/20/97	18	10	35	299	6
	05/21/97	19	12	34	301	5
	05/22/97	20	12	35	286	4
	05/23/97	21	12	35	289	4
	05/24/97	22	12	32	303	5
	05/25/97	23	12	29	300	5
	05/26/97	24	11	26	279	4
	05/27/97	25	13	28	273	5
	05/28/97	26	12	22	293	5
	05/29/97	27	11	25	286	5
	05/30/97	28	11	24	290	4
	05/31/97	29	13	30	303	4

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Alkalinity (mg/L as $CaCO_3$)						
	05/03/97	1	104	171	171	112
	05/06/97	4	108	169	147	107
	05/07/97	5	110	179	146	107
	05/08/97	6	109	168	126	110
	05/09/97	7	111	175	177	107
	05/10/97	8	113	175	125	103
	05/11/97	9	114	180	118	105
	05/12/97	10	117	180	116	104
	05/13/97	11	119	190	113	102
	05/14/97	12	118	194	103	100
	05/15/97	13	115	193	90	109
	05/16/97	14	114	191	87	118
	05/17/97	15	110	199	93	115
	05/18/97	16	106	194	90	129
	05/19/97	17	103	203	90	135
	05/20/97	18	111	196	83	133
	05/21/97	19	112	201	98	145
	05/22/97	20	115	202	88	143
	05/23/97	21	115	208	101	139
	05/24/97	22	116	194	92	139
	05/25/97	23	117	191	101	139
	05/26/97	24	117	169	105	136
	05/27/97	25	117	168	113	136
	05/28/97	26	117	145	98	139
	05/29/97	27	110	149	85	136
	05/30/97	28	113	147	87	136
	05/31/97	29	119	149	95	128

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Chloride (mg/L)						
	05/03/97	1	35	75	442	49
	05/06/97	4	35	82	442	45
	05/07/97	5	33	84	448	41
	05/08/97	6	30	82	456	38
	05/09/97	7	34	132	1,340	42
	05/10/97	8	-	-	-	-
	05/11/97	9	32	93	515	42
	05/12/97	10	-	-	-	-
	05/13/97	11	33	94	510	38
	05/14/97	12	-	-	-	-
	05/15/97	13	-	-	-	-
	05/16/97	14	-	-	-	-
	05/17/97	15	28	103	575	31
	05/18/97	16	-	-	-	-
	05/19/97	17	-	-	-	-
	05/20/97	18	23	96	564	26
	05/21/97	19	-	-	-	-
	05/22/97	20	26	102	620	25
	05/23/97	21	-	-	-	-
	05/24/97	22	27	96	615	28
	05/25/97	23	-	-	-	-
	05/26/97	24	-	-	-	-
	05/27/97	25	27	76	540	26
	05/28/97	26	-	-	-	-
	05/29/97	27	22	60	564	28
	05/30/97	28	-	-	-	-
	05/31/97	29	26	65	625	44

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Sulfate (mg/L)						
	05/03/97	1	-	-	-	-
	05/06/97	4	93	204	2,050	30
	05/07/97	5	93	224	2,080	30
	05/08/97	6	-	-	-	-
	05/09/97	7	90	407	706	29
	05/10/97	8	-	-	-	-
	05/11/97	9	90	219	2,160	29
	05/12/97	10	-	-	-	-
	05/13/97	11	88	230	2,300	30
	05/14/97	12	-	-	-	-
	05/15/97	13	83	236	2,430	30
	05/16/97	14	-	-	-	-
	05/17/97	15	83	238	2,450	33
	05/18/97	16	-	-	-	-
	05/19/97	17	-	-	-	-
	05/20/97	18	79	247	2,660	37
	05/21/97	19	-	-	-	-
	05/22/97	20	74	238	2,650	33
	05/23/97	21	-	-	-	-
	05/24/97	22	78	237	2,700	33
	05/25/97	23	-	-	-	-
	05/26/97	24	-	-	-	-
	05/27/97	25	-	-	-	-
	05/28/97	26	78	168	2,650	31
	05/29/97	27	76	170	2,690	33
	05/30/97	28	-	-	-	-
	05/31/97	29	75	180	2,840	32

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Un-ionized ammonia (mg/L NH_3-N)						
	05/03/97	1	-	-	-	-
	05/06/97	4	<0.01	<0.01	<0.01	<0.01
	05/07/97	5	-	-	-	-
	05/08/97	6	-	-	-	-
	05/09/97	7	<0.01	<0.01	<0.01	<0.01
	05/10/97	8	-	-	-	-
	05/11/97	9	<0.01	<0.01	<0.01	<0.01
	05/12/97	10	-	-	-	-
	05/13/97	11	<0.01	<0.01	<0.01	<0.01
	05/14/97	12	-	-	-	-
	05/15/97	13	<0.01	<0.01	<0.01	<0.01
	05/16/97	14	-	-	-	-
	05/17/97	15	<0.01	<0.01	<0.01	<0.01
	05/18/97	16	-	-	-	-
	05/19/97	17	-	-	-	-
	05/20/97	18	<0.01	<0.01	0.01	<0.01
	05/21/97	19	-	-	-	-
	05/22/97	20	<0.01	<0.01	<0.01	<0.01
	05/23/97	21	-	-	-	-
	05/24/97	22	<0.01	<0.01	0.01	<0.01
	05/25/97	23	-	-	-	-
	05/26/97	24	-	-	-	-
	05/27/97	25	<0.01	<0.01	0.01	0.01
	05/28/97	26	-	-	-	-
	05/29/97	27	<0.01	0.01	<0.01	0.01
	05/30/97	28	-	-	-	-
	05/31/97	29	<0.01	<0.01	<0.01	<0.01

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Nitrate (mg/L NO_3-N)						
	05/03/97	1	-	-	-	-
	05/06/97	4	<0.1	<0.1	0.2	17.8
	05/07/97	5	-	-	-	-
	05/08/97	6	-	-	-	-
	05/09/97	7	-	-	-	-
	05/10/97	8	-	-	-	-
	05/11/97	9	-	-	-	-
	05/12/97	10	-	-	-	-
	05/13/97	11	<0.1	<0.1	0.2	16.8
	05/14/97	12	-	-	-	-
	05/15/97	13	-	-	-	-
	05/16/97	14	-	-	-	-
	05/17/97	15	-	-	-	-
	05/18/97	16	-	-	-	-
	05/19/97	17	-	-	-	-
	05/20/97	18	<0.1	0.2	0.3	11.9
	05/21/97	19	-	-	-	-
	05/22/97	20	-	-	-	-
	05/23/97	21	-	-	-	-
	05/24/97	22	-	-	-	-
	05/25/97	23	-	-	-	-
	05/26/97	24	-	-	-	-
	05/27/97	25	-	-	-	-
	05/28/97	26	0.2	0.5	0.5	4.0
	05/29/97	27	-	-	-	-
	05/30/97	28	-	-	-	-
	05/31/97	29	-	-	-	-

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Nitrite (mg/L NO_2-N)						
	05/03/97	1	-	-	-	-
	05/06/97	4	<0.01	<0.01	0.01	0.07
	05/07/97	5	-	-	-	-
	05/08/97	6	-	-	-	-
	05/09/97	7	-	-	-	-
	05/10/97	8	-	-	-	-
	05/11/97	9	-	-	-	-
	05/12/97	10	-	-	-	-
	05/13/97	11	0.02	<0.01	0.02	0.06
	05/14/97	12	-	-	-	-
	05/15/97	13	-	-	-	-
	05/16/97	14	-	-	-	-
	05/17/97	15	-	-	-	-
	05/18/97	16	-	-	-	-
	05/19/97	17	-	-	-	-
	05/20/97	18	0.01	0.08	0.04	0.19
	05/21/97	19	-	-	-	-
	05/22/97	20	-	-	-	-
	05/23/97	21	-	-	-	-
	05/24/97	22	-	-	-	-
	05/25/97	23	-	-	-	-
	05/26/97	24	-	-	-	-
	05/27/97	25	-	-	-	-
	05/28/97	26	0.011	0.081	0.045	0.190
	05/29/97	27	-	-	-	-
	05/30/97	28	-	-	-	-
	05/31/97	29	-	-	-	-

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Total suspended solids (mg/L)						
	05/03/97	1	-	-	-	-
	05/06/97	4	31.6	8.6	7.2	0.4
	05/07/97	5	-	-	-	-
	05/08/97	6	-	-	-	-
	05/09/97	7	-	-	-	-
	05/10/97	8	-	-	-	-
	05/11/97	9	-	-	-	-
	05/12/97	10	-	-	-	-
	05/13/97	11	20.8	9.2	6.4	0.7
	05/14/97	12	-	-	-	-
	05/15/97	13	-	-	-	-
	05/16/97	14	-	-	-	-
	05/17/97	15	-	-	-	-
	05/18/97	16	-	-	-	-
	05/19/97	17	-	-	-	-
	05/20/97	18	15.2	9.4	48.3	0.4
	05/21/97	19	-	-	-	-
	05/22/97	20	-	-	-	-
	05/23/97	21	-	-	-	-
	05/24/97	22	-	-	-	-
	05/25/97	23	-	-	-	-
	05/26/97	24	-	-	-	-
	05/27/97	25	-	-	-	-
	05/28/97	26	35.5	6.7	38.0	1.6
	05/29/97	27	-	-	-	-
	05/30/97	28	-	-	-	-
	05/31/97	29	-	-	-	-

Appendix M. Continued.

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Volatile solids (mg/L)						
	05/03/97	1	-	-	-	-
	05/06/97	4	10.8	2.8	4.8	0.9
	05/07/97	5	-	-	-	-
	05/08/97	6	-	-	-	-
	05/09/97	7	-	-	-	-
	05/10/97	8	-	-	-	-
	05/11/97	9	-	-	-	-
	05/12/97	10	-	-	-	-
	05/13/97	11	6.0	4.0	3.4	1.0
	05/14/97	12	-	-	-	-
	05/15/97	13	-	-	-	-
	05/16/97	14	-	-	-	-
	05/17/97	15	-	-	-	-
	05/18/97	16	-	-	-	-
	05/19/97	17	-	-	-	-
	05/20/97	18	6.0	5.2	18.0	0.9
	05/21/97	19	-	-	-	-
	05/22/97	20	-	-	-	-
	05/23/97	21	-	-	-	-
	05/24/97	22	-	-	-	-
	05/25/97	23	-	-	-	-
	05/26/97	24	-	-	-	-
	05/27/97	25	-	-	-	-
	05/28/97	26	14.5	2.6	29.5	2.4
	05/29/97	27	-	-	-	-
	05/30/97	28	-	-	-	-
	05/31/97	29	-	-	-	-

Appendix M. Continued

Measure	Date	Day of exposure	Station			
			HT1	AC3	WW2	24-Road
Fixed solids (mg/L)						
	05/03/97	1	-	-	-	-
	05/06/97	4	20.8	5.8	2.4	0
	05/07/97	5	-	-	-	-
	05/08/97	6	-	-	-	-
	05/09/97	7	-	-	-	-
	05/10/97	8	-	-	-	-
	05/11/97	9	-	-	-	-
	05/12/97	10	-	-	-	-
	05/13/97	11	14.8	5.2	3.0	0
	05/14/97	12	-	-	-	-
	05/15/97	13	-	-	-	-
	05/16/97	14	-	-	-	-
	05/17/97	15	-	-	-	-
	05/18/97	16	-	-	-	-
	05/19/97	17	-	-	-	-
	05/20/97	18	9.2	4.2	30.3	0
	05/21/97	19	-	-	-	-
	05/22/97	20	-	-	-	-
	05/23/97	21	-	-	-	-
	05/24/97	22	-	-	-	-
	05/25/97	23	-	-	-	-
	05/26/97	24	-	-	-	-
	05/27/97	25	-	-	-	-
	05/28/97	26	21.0	4.1	8.5	0
	05/29/97	27	-	-	-	-
	05/30/97	28	-	-	-	-
	05/31/97	29	-	-	-	-

Appendix M. Continued.

[1]Conductivity (µmhos/cm) corrected to 25°C.
[2]-: No data.

Appendix N. Percent survival of razorback sucker larvae exposed to reference water (24-Road) and fed food from four sites in a 30-day study.

	Food treatment,[1] side, and replicate																							
	BSH						HTEW						AC						WW					
	Left			Right			Left			Right			Left			Right			Left			Right		
Day	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2	100	100	100	100	100	100	100	90	100	90	100	100	100	100	90	100	100	100	100	90	100	100	100	100
3	90	90	90	100	100	100	100	90	100	90	100	100	100	100	90	100	100	100	100	90	100	100	100	100
4	80	80	90	100	100	100	100	90	100	90	100	100	100	90	90	100	100	100	100	90	100	100	100	100
5	80	80	90	100	100	100	90	90	90	90	100	100	100	90	90	100	100	100	90	90	100	100	100	100
6	80	80	90	100	100	90	70	60	60	90	90	80	100	90	90	100	100	100	90	90	100	100	100	100
7	80	80	90	90	100	90	40	60	40	60	50	20	100	90	90	90	100	100	90	80	100	90	100	100
8	80	80	80	90	100	90	30	30	30	30	20	20	100	80	80	90	100	100	70	80	90	80	80	80
9	80	80	80	90	100	90	20	20	20	30	20	10	100	80	70	90	100	100	70	60	70	70	80	80
10	80	80	80	90	100	90	20	20	10	20	10	10	100	40	60	90	100	100	70	60	60	60	70	80
11	80	80	80	90	100	90	20	20	0	20	0	0	78	40	60	68	89	78	70	48	48	60	58	80
12	80	80	80	90	100	90	20	20	0	0	0	0	67	40	60	56	67	56	58	48	48	60	58	57
13	80	80	80	90	100	90	20	0	0	0	0	0	56	27	60	56	56	56	58	48	36	48	58	46
14	80	80	80	90	100	90	20	0	0	0	0	0	44	27	60	56	44	56	58	48	36	48	58	46
15	80	80	80	90	100	90	20	0	0	0	0	0	33	27	60	45	44	56	58	48	36	48	58	46
16	80	80	80	90	100	90	20	0	0	0	0	0	33	13	48	45	44	56	58	48	36	48	58	46

Appendix N. Continued.

	Food treatment,[1] side, and replicate																							
	BSH						HTEW						AC						WW					
	Left			Right			Left			Right			Left			Right			Left			Right		
Day	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
17	80	80	80	90	100	90	0	0	0	0	0	0	33	13	48	45	33	56	58	48	36	48	47	46
18	80	80	69	90	100	79	0	0	0	0	0	0	33	13	48	45	33	56	58	48	36	48	47	34
19	80	80	69	90	100	79	0	0	0	0	0	0	33	13	48	45	33	56	47	48	24	48	47	34
20	80	80	69	90	100	79	0	0	0	0	0	0	33	13	48	34	22	56	47	48	12	48	47	34
21	80	80	69	90	100	79	0	0	0	0	0	0	22	13	36	34	22	56	47	48	12	48	47	34
22	80	80	69	90	100	79	0	0	0	0	0	0	22	0	36	34	22	56	47	48	12	48	47	34
23	80	80	69	90	100	79	0	0	0	0	0	0	22	0	24	34	22	33	47	48	12	48	47	34
24	80	80	69	90	100	79	0	0	0	0	0	0	22	0	24	22	22	33	47	48	12	48	35	34
25	80	80	69	90	100	79	0	0	0	0	0	0	22	0	24	22	22	33	47	48	12	48	35	23
26	80	80	69	90	100	79	0	0	0	0	0	0	22	0	24	22	22	33	47	48	12	48	35	23
27	80	80	69	90	100	79	0	0	0	0	0	0	22	0	24	22	22	33	47	48	12	48	35	23
28	80	80	69	90	100	79	0	0	0	0	0	0	11	0	24	22	22	33	47	48	12	48	35	23
29	80	80	69	90	100	79	0	0	0	0	0	0	11	0	24	22	22	33	47	48	12	48	35	23
30	80	80	69	90	100	79	0	0	0	0	0	0	11	0	24	22	22	33	47	48	12	24	35	23

[1]BSH: Brine shrimp; HTEW: Horsethief east wetland; AC: Adobe Creek; WW: North Pond at Walter Walker.

Appendix O. Percent survival of razorback sucker larvae exposed to site water (Horsethief Canyon State Wildlife Area) and fed food from four sites in a 30-day study.

	Food treatment,[1] side, and replicate																							
	BSH						HTEW						AC						WW					
	Left			Right			Left			Right			Left			Right			Left			Right		
Day	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2	80	100	100	100	100	100	80	90	100	100	80	100	100	90	100	100	100	100	100	100	90	100	100	100
3	80	100	100	100	100	100	80	90	100	100	80	100	100	90	100	100	100	100	100	100	90	100	100	100
4	80	100	100	100	100	100	80	90	100	100	80	100	100	90	100	100	100	100	100	100	90	100	100	100
5	60	100	100	90	100	100	60	90	100	100	60	90	100	90	100	90	100	100	100	100	90	100	100	90
6	60	100	100	90	90	90	20	50	70	80	30	80	100	90	100	90	100	90	90	90	90	90	100	90
7	60	100	100	90	90	90	0	30	50	80	10	50	90	90	90	90	100	90	90	90	90	80	100	90
8	60	100	100	80	90	90	0	20	20	70	0	30	90	80	70	90	100	70	80	90	80	80	100	90
9	60	100	100	80	90	90	0	20	20	70	0	20	90	70	70	90	80	60	80	90	80	70	90	80
10	60	100	100	80	90	90	0	20	20	70	0	10	60	50	60	80	80	40	70	90	50	40	70	80
11	60	100	100	80	90	90	0	20	20	58	0	0	48	50	36	69	69	13	70	90	38	40	70	80
12	60	100	100	80	90	90	0	20	20	35	0	0	48	50	36	57	69	13	70	79	38	40	70	80
13	60	100	100	80	90	90	0	20	20	35	0	0	48	38	36	57	69	13	47	68	38	27	70	57
14	60	100	100	80	90	90	0	20	20	35	0	0	48	25	36	57	57	13	47	68	38	13	58	57
15	60	100	100	80	90	90	0	20	20	23	0	0	48	25	24	57	57	13	47	68	38	13	58	57
16	60	100	100	69	90	90	0	20	20	23	0	0	48	25	24	57	57	13	47	56	38	13	47	57

Appendix O. Continued.

	Food treatment,[1] side, and replicate																							
	BSH						HTEW						AC						WW					
	Left			Right			Left			Right			Left			Right			Left			Right		
Day	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
17	60	100	100	69	79	90	0	20	20	23	0	0	36	25	24	57	57	13	47	45	38	13	47	57
18	60	100	100	57	79	90	0	20	20	23	0	0	36	25	24	57	57	13	47	45	38	13	47	46
19	60	100	100	57	79	90	0	20	20	23	0	0	36	25	24	57	57	13	47	45	38	13	35	34
20	60	100	100	57	79	90	0	20	20	23	0	0	36	25	24	57	57	13	35	45	38	13	35	34
21	60	100	100	57	79	90	0	20	20	23	0	0	36	25	24	57	57	13	35	45	38	13	23	34
22	60	100	100	57	79	90	0	20	20	23	0	0	36	25	24	57	57	13	35	45	25	13	23	34
23	60	100	100	57	79	90	0	20	20	23	0	0	36	25	24	57	57	13	35	34	25	13	23	34
24	60	100	100	57	79	90	0	20	20	23	0	0	24	25	24	46	57	13	35	34	12	13	23	23
25	60	100	100	57	79	90	0	20	20	23	0	0	24	25	24	46	34	13	35	22	12	13	23	23
26	60	100	100	57	79	90	0	20	20	23	0	0	24	25	24	46	34	13	35	22	12	13	23	23
27	60	100	100	57	79	90	0	20	20	23	0	0	24	25	24	46	34	13	35	22	12	13	23	23
28	60	100	100	57	79	90	0	20	20	23	0	0	24	25	24	46	34	13	35	22	12	13	23	23
29	60	100	100	57	79	90	0	20	20	23	0	0	24	25	24	46	34	13	35	22	12	13	23	23
30	60	100	100	57	79	90	0	20	20	23	0	0	24	25	24	34	34	13	35	22	12	13	23	23

[1]BSH: Brine shrimp; HTEW: Horsethief east wetland; AC: Adobe Creek; WW: North Pond at Walter Walker.

Appendix P. Percent survival of razorback sucker larvae exposed to site water (Adobe Creek) and fed food from four sites in a 30-day study.

	Food treatment,[1] side, and replicate																							
	BSH						HTEW						AC						WW					
	Left			Right			Left			Right			Left			Right			Left			Right		
Day	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	90	100	100	100	100	100	100	100	100	100	100	100	90	100	100	100	100	100	100	100	100	100	100	100
2	90	100	90	100	100	100	100	90	100	100	100	100	90	100	100	100	100	100	100	100	100	100	100	100
3	90	100	90	100	100	100	100	90	100	100	100	100	80	100	100	100	100	100	100	100	90	100	100	100
4	90	100	80	100	100	100	100	90	100	100	100	100	80	100	100	100	100	100	100	90	90	100	100	100
5	90	100	80	100	90	100	100	80	90	80	100	100	70	100	90	100	100	100	100	90	90	100	100	100
6	90	100	80	90	90	100	100	60	80	80	100	100	70	100	90	90	100	100	100	80	90	100	90	100
7	90	100	80	90	90	100	70	50	60	50	90	60	70	100	90	90	100	90	100	70	90	100	90	90
8	90	100	80	90	90	100	40	20	50	50	30	40	70	100	80	80	90	80	100	50	80	100	90	90
9	90	100	80	90	80	100	30	20	40	40	20	30	70	100	80	70	80	80	100	50	50	80	80	80
10	90	100	80	90	80	100	30	20	30	30	20	30	70	90	70	60	60	60	90	50	30	80	80	60
11	90	100	69	90	80	100	15	20	30	30	20	30	58	68	70	36	48	36	79	50	30	69	57	48
12	90	100	69	79	80	100	0	20	30	30	20	30	58	56	58	36	48	36	68	50	30	69	34	36
13	90	100	69	56	80	100	0	20	15	0	20	30	35	56	58	24	36	36	56	38	15	57	34	36
14	90	100	69	56	80	100	0	20	15	0	20	30	35	56	47	24	36	24	56	25	15	57	23	36
15	90	100	69	45	80	100	0	20	15	0	20	15	35	45	47	24	36	24	56	25	15	57	23	36
16	90	100	69	45	80	89	0	20	15	0	20	15	23	45	35	24	36	24	56	25	15	57	23	24

Appendix P. Continued.

	Food treatment,[1] side, and replicate																							
	BSH						HTEW						AC						WW					
	Left			Right			Left			Right			Left			Right			Left			Right		
Day	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
17	90	89	69	45	80	89	0	20	15	0	20	15	23	45	35	24	24	24	56	12	15	57	23	12
18	90	89	69	45	80	78	0	20	15	0	20	15	23	45	35	24	24	24	45	12	15	34	23	12
19	90	89	69	45	80	78	0	20	15	0	20	15	23	45	23	24	24	24	34	12	15	34	23	12
20	90	89	69	45	80	78	0	20	15	0	20	15	23	34	23	24	24	24	34	12	15	34	23	12
21	90	89	69	45	80	78	0	20	15	0	20	15	23	34	23	24	24	24	34	12	15	23	23	12
22	90	89	69	45	80	78	0	20	15	0	20	15	23	22	23	24	24	24	34	12	15	23	23	12
23	90	89	69	45	80	78	0	20	15	0	20	15	23	22	23	24	24	24	34	12	15	23	11	12
24	90	78	69	45	69	78	0	20	15	0	20	15	23	22	23	24	24	24	34	12	15	23	11	12
25	90	78	69	45	69	78	0	20	15	0	20	15	23	22	23	24	24	24	34	12	15	23	11	12
26	90	78	69	45	69	78	0	20	15	0	20	15	23	22	23	24	24	24	22	12	15	23	11	12
27	90	78	69	45	69	78	0	20	15	0	20	15	23	22	23	24	24	12	22	12	15	23	11	12
28	90	78	69	45	69	78	0	20	15	0	20	15	23	22	23	24	24	12	22	12	15	23	11	12
29	90	78	69	45	69	78	0	20	15	0	20	15	23	22	23	24	24	12	22	12	15	23	11	12
30	90	78	69	45	69	78	0	20	15	0	20	15	23	22	23	24	24	12	22	12	15	23	11	12

[1]BSH: Brine shrimp; HTEW: Horsethief east wetland; AC: Adobe Creek; WW: North Pond at Walter Walker.

Appendix Q. Percent survival of razorback sucker larvae exposed to site water (North Pond at Walter Walker State Wildlife Area) and fed food from four sites in a 30-day study.

	Food treatment,[1] side, and replicate																							
	BSH						HTEW						AC						WW					
	Left			Right			Left			Right			Left			Right			Left			Right		
Day	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2	100	100	100	100	100	100	100	100	90	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100	90	100	100	100	100	100	90	100	100	100	100	100	100	100	100	100
4	100	100	100	100	100	100	100	100	90	100	100	100	100	100	90	100	100	90	100	90	100	100	100	100
5	100	90	100	100	100	100	90	100	80	100	100	100	90	100	90	100	100	90	100	90	100	100	100	100
6	100	90	100	100	90	100	90	100	80	90	70	90	90	100	90	90	100	90	100	90	100	100	100	100
7	100	90	100	100	80	100	80	100	60	90	60	80	90	100	90	90	100	90	100	90	100	100	100	100
8	100	90	100	100	70	100	70	70	40	80	60	60	90	100	90	90	100	90	90	90	100	100	90	90
9	100	90	100	100	70	100	70	50	40	60	60	20	90	80	90	90	80	80	90	90	100	90	80	90
10	100	90	100	100	70	100	60	40	40	60	40	20	90	80	80	90	70	80	80	80	90	90	70	80
11	78	90	100	78	70	78	60	13	40	60	40	20	68	69	80	90	70	46	80	69	68	56	35	80
12	78	90	100	67	70	78	48	13	40	36	40	20	56	69	46	79	70	46	69	69	56	56	35	69
13	78	90	100	56	70	67	48	13	40	36	40	20	56	34	34	68	70	46	57	57	56	45	35	69
14	78	90	100	56	70	56	48	13	40	36	40	20	56	34	23	56	47	46	57	57	56	34	23	46
15	78	90	100	56	70	56	48	13	40	36	40	20	45	34	11	56	47	46	57	57	56	34	23	46
16	78	90	100	56	70	56	36	13	40	36	40	20	45	34	11	45	47	46	57	57	56	34	12	46

Appendix Q. Continued.

	Food treatment,[1] side, and replicate																							
	BSH						HTEW						AC						WW					
	Left			Right			Left			Right			Left			Right			Left			Right		
Day	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
17	67	90	100	56	70	56	24	13	40	36	40	20	45	34	11	45	47	46	57	57	56	34	12	46
18	67	90	100	56	58	56	24	13	40	36	40	20	45	34	11	45	47	23	57	57	56	34	12	46
19	67	90	89	56	58	56	24	13	40	36	40	20	45	34	11	45	47	23	46	46	56	34	12	46
20	67	68	89	56	58	56	24	13	40	24	40	20	34	34	11	45	35	23	46	46	56	34	12	46
21	67	68	78	56	58	56	24	13	40	24	40	20	34	34	11	45	35	23	46	46	56	34	12	46
22	67	68	78	44	47	56	24	13	40	24	27	20	34	34	11	45	23	23	34	46	56	34	12	46
23	67	56	78	44	47	56	24	13	40	24	27	20	34	34	11	45	23	23	34	46	56	34	12	46
24	67	56	67	44	47	56	12	13	40	24	27	20	34	34	11	45	23	23	34	46	56	34	0	46
25	67	56	67	33	47	44	12	13	40	24	27	20	34	23	11	45	23	23	34	46	56	34	0	46
26	67	56	67	33	47	44	12	13	40	24	27	20	34	23	11	45	23	23	34	46	56	34	0	46
27	67	56	67	33	47	44	12	13	27	24	27	20	34	23	11	34	23	23	34	46	56	34	0	46
28	67	56	67	33	47	44	12	13	27	12	27	20	22	23	11	34	23	23	34	46	45	34	0	34
29	67	56	67	33	47	44	12	13	27	12	27	20	22	23	11	34	12	23	34	46	34	34	0	34
30	67	56	67	33	47	44	12	13	27	12	27	20	11	23	11	34	12	23	34	46	34	34	0	34

[1]BSH: Brine shrimp; HTEW: Horsethief east wetland; AC: Adobe Creek; WW: North Pond at Walter Walker.