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Rapid communication

Reduced growth and survival of larval razorback sucker fed selenium-laden zooplankton

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Abstract

Four groups of larval razorback sucker, an endangered fish, were exposed to selenium-laden zooplankton and survival, growth, and whole-body residues were measured. Studies were conducted with 5, 10, 24, and 28-day-old larvae fed zooplankton collected from six sites adjacent to the Green River, Utah. Water where zooplankton were collected had selenium concentrations ranging from <0.4 to 78 μg/L, and concentrations in zooplankton ranged from 2.3 to 91 μg/g dry weight. Static renewal tests were conducted for 20 to 25 days using reference water with selenium concentrations of <1.1 μg/L. In all studies, 80–100% mortality occurred in 15–20 days. In the 28-day-old larvae, fish weight was significantly reduced 25% in larvae fed zooplankton containing 12 μg/g selenium. Whole-body concentrations of selenium ranged from 3.7 to 14.3 μg/g in fish fed zooplankton from the reference site (Sheppard Bottom pond 1) up to 94 μg/g in fish fed zooplankton from North Roadside Pond. Limited information prior to the studies suggested that the Sheppard pond 1 site was relatively clean and suitable as a reference treatment; however, the nearly complete mortality of larvae and elevated concentrations of selenium in larvae and selenium and other elements in zooplankton indicated that this site was contaminated with selenium and other elements. Selenium concentrations in whole-body larvae and in zooplankton from all sites were close to or greater than toxic thresholds where adverse effects occur in fish. Delayed mortality occurred in larvae fed the two highest selenium concentrations in zooplankton and was thought due to an interaction with other elements.

Keywords: Selenium; Razorback sucker; Green River; Diet exposure

1. Introduction

Discovery of contaminated irrigation return waters in the San Joaquin Valley of central California in 1982 (Ohlendorf et al., 1986; Saiki, 1986) prompted the Department of the Interior (DOI) to initiate a program to identify other areas in the western United States that have water quality problems induced by irrigation drainage (Feltz et al., 1991; Seiler et al., 2003). These investigations focused on irrigation drainage facilities constructed by DOI, where the receiving water was a national wildlife refuge (NWR), or had the potential to adversely impact migratory birds or endangered species.

The middle Green River basin, located in northeastern Utah, was identified as one area needing further study because it provides sensitive habitats for four endangered fish species: Colorado pikeminnow (*Ptychocheilus lucius*), razorback sucker (*Xyrauchen texanus*), humpback chub (*Gila cypha*), and bonytail (*Gila elegans*). The middle Green River is defined as the drainage area consisting of the Green River and its tributaries between Flaming Gorge Dam and the city of Green River, Utah (Fig. 1).

Several researchers have documented reproduction, but low or no recruitment in razorback sucker in the upper Colorado River basin (Lanigan and Tyus, 1989; Tyus, 1987; Wick et al., 1982). However, young-of-year razorback sucker were positively identified in a collection from the lower Green River near Hell Roaring

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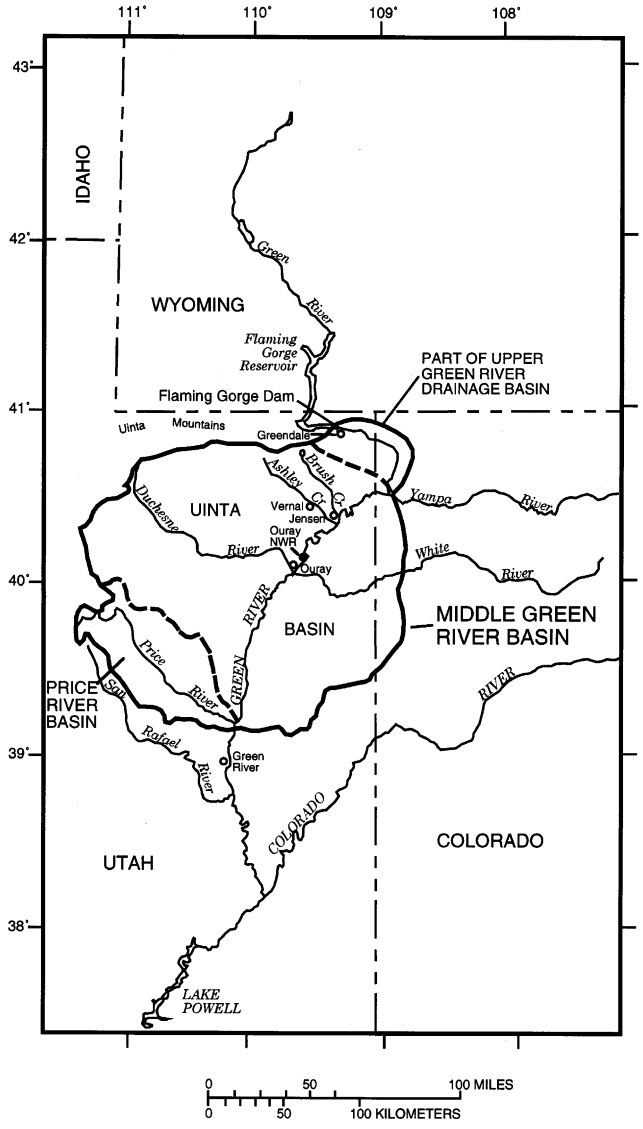

Fig. 1. The Green River and major tributaries, and locations of NWRs within the middle Green River basin.

Canyon in 1991 (Gutermuth et al., 1994), and 28 juvenile razorback sucker (74–125 mm) were collected in a wetland on the Ouray National Wildlife Refuge (NWR) in 1995 (Modde, 1996).

Major causes suggested for the decline of endangered fishes in the upper Colorado River basin include physical changes in river habitat and water quality problems due to dams, which reduce water temperature, decrease turbidity, alter seasonal and annual flow patterns, and reduce habitat for adult and larval fish such as spawning bars and flooded bottomlands (USFWS, 1987). In addition, numerous introduced fish species have increased predation and competition (reviewed by Tyus and Saunders, 2000). In certain situations, predation by macroinvertebrates on larval razorback sucker also may be adversely affecting their survival (Horn et al., 1994).

Analysis of water, bottom sediments, and biota collected from the middle Green River basin since 1986 has confirmed the presence of boron, selenium, and zinc at concentrations that could be potentially harmful to fish and wildlife (Stephens et al., 1988, 1992; Peltz and Waddell, 1991; Waddell and Stanger, 1992). Based on the DOI studies in the middle Green River, selenium is the element of principle concern because of its propensity for food-chain bioaccumulation (Besser et al., 1993; Saiki, 1986; Sandholm et al., 1973) and its dietary toxicity at low concentrations (Hamilton et al., 1990; Hilton et al., 1980; Hodson and Hilton, 1983). Low waterborne selenium concentrations have been documented to adversely affect reproduction in fish in experimental field studies (Hermanutz et al., 1992; Schultz and Hermanutz, 1990) and in water bodies in Colorado (Barnhart, 1957), North Carolina (Cumbie and Van Horn, 1978), and Texas (Garrett and Inman, 1984; Sorensen, 1988).

The objective of the study was to evaluate effects of selenium on the survival, growth, and whole-body residues in larval razorback sucker exposed to water and fed zooplankton collected at Ouray NWR. Wetlands in floodplains such as those found at Ouray are believed to be important nursery habitats for larval razorback sucker (Modde et al., 1995; Tyus and Karp, 1990) because of their high invertebrate production (Mabey, 1993).

2. Methods

An on-site toxicity investigation was conducted at the Ouray NWR from May 21 to June 26, 1994, using a mobile laboratory. Two lots of larval razorback sucker were used in four studies. Study 1 was initiated with 5-day-old larvae, Study 2 with 10-day-old larvae, Study 3 with 24-day-old larvae, and Study 4 with 28-day-old larvae. One lot of larvae was from one female from Etter Pond near DeBeque, Colorado, that was spawned at Grand Junction, Colorado. Fertilized eggs were transported to Ouray Native Fish Facility (NFF) where they were held before testing. Following unexpected mortality in Studies 1 and 2, Studies 3 and 4 were initiated using a second lot of larvae from one female from the Colorado River arm of Lake Powell that was spawned at Ouray NFF.

Larvae were cultured at Ouray NFF in filtered river water (hardness 488 mg/L as $CaCO_3$, alkalinity 191 mg/L as $CaCO_3$, pH 8.0, $<1\,\mu g/L$ selenium). Larvae from the first lot were fed live nauplii of brine shrimp (*Artemia* sp.; Aquarium Products, Glen Burnie, MD) prior to testing in Studies 1 and 2. Larvae from the second lot were fed a commercial diet (Biokyowa B-250

diet, Biodiet Products, Warrenton, OR) ad libitum prior to testing in Studies 3 and 4.

Ten fish were placed in each of four replicate 2000-mL beakers containing 400 mL of filtered river water and 400 mL of Sheppard Bottom pond 1 (S1) water to initiate acclimation. After 4 h, an additional 800 mL of S1 water was added. Thereafter, 50% of the water was removed and replaced daily with S1 water. All S1 water was filtered through a 153-μm mesh plankton net to remove debris and plankton. Water in exposure beakers was aerated continuously with compressed air from an oil-less air pump. Temperature in the mobile laboratory was maintained at $20 \pm 3\,^{\circ}C$. The photoperiod was maintained at about 12 light:12 dark.

Live zooplankton were collected every other day either by plankton tow net or modified light trap (Espinosa and Clark, 1972) from six sites at Ouray NWR where various amounts of selenium in water and biota were measured: North Roadside Pond (beaver pond about 400 yards upstream; NR) and South Roadside Pond (SR) had high concentrations, Sheppard Bottom pond 5 (S5) moderate concentrations, and Sheppard Bottom ponds 1 (S1), 3 (S3), and 4 (S4) low concentrations (Stephens et al., 1988, 1992; Fig. 2). Zooplankton from each site were concentrated by filtering water through the collection bucket of a plankton tow net and then held in 3 L of site water. All six sites were used in Studies 1 and 2, whereas Study 3 included S1, NR, and SR, and Study 4 included S3, S4, and S5. Zooplankton were separated by size using a standard No. 40 stainless-steel sieve. Plankton passing through the sieve were <0.425 mm and were used in the feeding studies because this size is equivalent to 24-h-old nauplii of brine shrimp, which are readily consumed by larval razorback sucker. After sieving and prior to counting and feeding, zooplankton were held in S1 water.

Zooplankton in a 2-mL sample were counted by pipetting with a 2-mL Hensen–Stempel pipette and using a stereoscope microscope at $0.7\times$ magnification and a Wards zooplankton counting wheel. Some detritus and algae were present in samples, but were not counted as food particles. The number of zooplankton in three replicate counts were averaged and the volume was calculated to feed 20 or 40 zooplankton per fish in each exposure vessel. Based on 1600 mL test water volume and 10 fish per vessel, the 20 zooplankton per fish feeding rate was equivalent to 125 zooplankton/L and the 40 zooplankton rate to 250 zooplankton/L.

Each larval fish was fed 20 zooplankton in Study 1 (Days 1–10) and in Study 2 (Days 1–4). Thereafter, the feeding rate was 40 zooplankton per fish in Study 1 (Days 11–25), Study 2 (Days 5–25), and Studies 3 and 4 (Days 1–20). Fish were fed zooplankton once daily after water renewal. Exposure beakers were brushed twice during the study to dislodge algal and periphyton

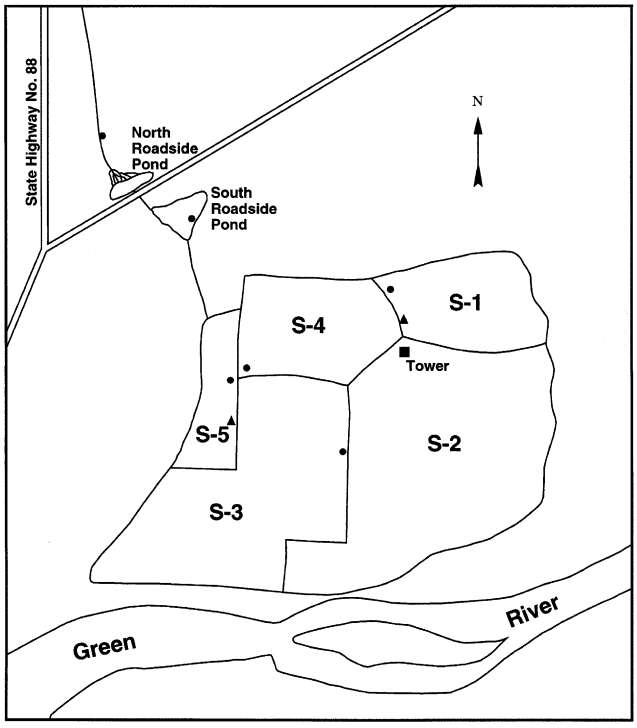

Fig. 2. Six collection sites (●) for zooplankton and water. New sites in S1 and S5 are shown with a triangle (▲).

growth on beaker walls, and the material was allowed to settle and then removed by siphoning at water renewal. This algae and periphyton probably provided some food to the larval razorback sucker. The S1 water used in renewals was collected daily and measured for water quality characteristics (Table 1). Water temperature and dissolved oxygen concentrations were measured daily in one replicate of each treatment in each study. Water quality characteristics were measured weekly in samples collected from the six sites where zooplankton were collected (Table 2). Water characteristics were measured using standard methods (APHA et al., 1989).

Mortality of fish was recorded daily and dead fish were removed. At the termination of Studies 3 and 4, all live fish were measured for total length and weight and Fulton-type condition factor was calculated as $K = (W/L^3)Z$, where W is weight (g), L is total length (mm), and Z is an arbitrary scaling factor (100,000) (Nielsen and Johnson, 1983). No measurements of fish size were made in Studies 1 and 2.

2.1. Supplemental feeding study

A study was conducted at Yankton, South Dakota, to determine the influence of feeding density of live brine shrimp on survival and growth of larval razorback sucker. The feeding study was conducted because there was a concern about the appropriate number of food

Table 1
Mean and standard error (in parentheses) of water quality characteristics measured in reference water from two sites at Sheppard Bottom pond 1 at Ouray NWR

Characteristic	Study no.			
	1	2	3	4
Site 1 (May 22–June 1)				
pH	7.9 (0)	7.9 (0)		
Conductivity (μmhos/cm)[a]	930 (34)	920 (36)	—	—
Hardness (mg/L as $CaCO_3$)	392 (12)	378 (16)	—	—
Calcium (mg/L)	79 (3)	75 (3)	—	—
Magnesium (mg/L)	48 (1)	46 (1.8)	—	—
Alkalinity (mg/L as $CaCO_3$)	320 (9)	315 (13)	—	—
Chloride (mg/L)	41 (1)	39 (2)	—	—
Sulfate (mg/L)	175 (9)	171 (13)	—	—
n	11	7	—	—
Site 2 (June 2–26)				
pH	7.6 (0)	7.6 (0)	7.6 (0)	7.6 (0)
Conductivity (μmhos/cm)[a]	530 (8)	530 (6)	530 (6)	530 (6)
Hardness (mg/L as $CaCO_3$)	210 (2)	211 (2)	212 (2)	214 (2)
Calcium (mg/L)	52 (0)	53 (0)	53 (0)	54 (1)
Magnesium (mg/L)	19 (1)	19 (0)	19 (0)	19 (0)
Alkalinity (mg/L as $CaCO_3$)	172 (3)	176 (2)	178 (3)	182 (3)
Chloride (mg/L)	14 (0)	14 (0)	13 (0)	13 (0)
Sulfate (mg/L)	116 (8)	113 (6)	112 (6)	102 (3)
n	14	19	21	19

[a]Conductivity μmhos/cm at 25 °C.

organisms per fish that were essential to maintaining good survival and growth of larval razorback suckers. One lot of fish was used in three tests. Eyed eggs were received from Dexter National Fish Hatchery, New Mexico, and were from brood stock from the Yampa River, Colorado. Eggs and larvae were cultured in Yankton well water (hardness 292 mg/L as $CaCO_3$, alkalinity 175 mg/L as $CaCO_3$, pH 8.2). Larvae were fed live nauplii of brine shrimp (*Artemia* sp.) prior to testing.

Three tests were conducted; one with 5-day-old larvae, a second with 10-day-old larvae, and a third with 24-day-old larvae. Ten fish were placed in each of four replicate 2000-mL beakers containing 1600 mL of well water at room temperature. Water in exposure beakers was aerated continuously. Beakers were held on a table at room temperature. The photoperiod was maintained at about 12 light:12 dark.

Brine shrimp (24 h old) were collected daily from a culture jar and diluted about 10-fold. Organisms were sampled with a 2-mL Hensen–Stempel pipette and counted with a stereoscope microscope at 0.7 × magnification and a Wards zooplankton counting wheel. The number of organisms in three replicate counts was averaged and the volume calculated to feed 20, 40, or 80 organisms per fish for the number of live fish in each exposure vessel. Fish were fed once daily after water renewal and were fed for 20 days.

Water quality characteristics were measured weekly in test water using standard methods (APHA et al., 1989). Test water had corrected conductivity 824 μmhos/cm, hardness 278 mg/L as $CaCO_3$, alkalinity 169 mg/L as $CaCO_3$, calcium 81 mg/L, magnesium 18 mg/L, chloride 12 mg/L, and sulfate 242 mg/L. Dissolved oxygen concentrations were greater than 8.4 mg/L in test vessels. Water temperature was measured daily in test vessels and averaged 17.4, 17.6, and 16.6 °C in tests with larvae at 5, 10, and 24 days old, respectively.

Mortality was measured daily and dead fish were removed. At the termination of each feeding study, all live fish were measured for total length and a pooled weight was determined in the tests with 5- and 10-day-old larvae (fish were too small for measurement of individual weight), but weighed individually in the test with 24-day-old larvae.

2.2. Elemental analysis

Water samples were collected weekly at the six sites for residue analysis of selenium and other elements. Water was filtered through a 0.45-μm polycarbonate filter and 200 mL was preserved with 2 mL ultrapure HCl and stored frozen. Concentrations of selenium in water samples were determined with a Perkin-Elmer Model 2280 atomic absorption spectrophotometer equipped with a Model MHS-10 hydride generator

Table 2
Mean and standard error (in parentheses) of water quality characteristics of water collected at sites at Ouray NWR where zooplankton were collected for feeding razorback sucker

Characteristic	Site					
	S1	S3	S4	S5	SR	NR
Study 1 [n = 4]						
pH	7.8 (0.1)	7.6 (0.1)	8.1 (0)	7.5 (0.1)	8.3 (0.2)	7.9 (0.2)
Conductivity (μmhos/cm)[a]	660 (140)	680 (26)	1100 (62)	1480 (110)	2040 (30)	1950 (32)
Hardness (mg/L as $CaCO_3$)	302 (53)	254 (3)	405 (19)	469 (29)	353 (25)	430 (10)
Calcium (mg/L)	66 (8)	59 (0)	85 (8)	108 (7)	76 (10)	104 (2)
Magnesium (mg/L)	33 (8)	26 (1)	47 (1)	48 (3)	40 (1)	41 (4)
Alkalinity (mg/L as $CaCO_3$)	245 (42)	166 (4)	230 (10)	262 (22)	244 (25)	299 (3)
Chloride (mg/L)	27 (7)	32 (2)	77 (3)	107 (12)	198 (3)	192 (18)
Sulfate (mg/L)	147 (20)	193 (15)	251 (34)	441 (153)	316 (101)	339 (98)
Study 2 [n = 3]						
pH	7.7 (0.1)	7.8 (0.2)	8.2 (0.1)	7.4 (0.1)	8.3 (0.2)	7.7 (0)
Conductivity (μmhos/cm)[a]	630 (100)	700 (28)	1050 (66)	1590 (130)	2010 (38)	1950 (22)
Hardness (mg/L as $CaCO_3$)	256 (45)	254 (3)	374 (29)	478 (36)	325 (24)	421 (1)
Calcium (mg/L)	60 (7)	58 (1)	76 (10)	109 (8)	65 (10)	106 (1)
Magnesium (mg/L)	26 (7)	26 (2)	44 (2)	50 (4)	39 (1)	38 (1)
Alkalinity (mg/L as $CaCO_3$)	212 (38)	166 (4)	212 (17)	278 (31)	218 (24)	305 (5)
Chloride (mg/L)	20 (6)	35 (2)	71 (4)	124 (12)	195 (4)	189 (19)
Sulfate (mg/L)	124 (16)	211 (15)	224 (14)	368 (90)	429 (104)	396 (90)
Study 3 [n = 3]						
pH	7.7 (0.1)	—	—	—	8.5 (0.2)	7.6 (0)
Conductivity (μmhos/cm)[a]	530 (12)	-	—	—	1990 (50)	1930 (16)
Hardness (mg/L as $CaCO_3$)	211 (4)	—	—	—	313 (29)	421 (1)
Calcium (mg/L)	53 (1)	—	—	—	59 (11)	106 (1)
Magnesium (mg/L)	19 (1)	—	—	—	40 (0)	38 (1)
Alkalinity (mg/L as $CaCO_3$)	175 (4)	—	—	—	203 (26)	309 (4)
Chloride (mg/L)	14 (1)	—	—	—	196 (6)	170 (3)
Sulfate (mg/L)	110 (9)	—	—	—	499 (110)	447 (106)
Study 4 [n = 3]						
pH	—	8.1 (0.2)	8.2 (0.1)	7.3 (0)	—	—
Conductivity (μmhos/cm)[a]	—	760 (20)	940 (20)	1950 (150)	—	—
Hardness (mg/L as $CaCO_3$)	—	253 (7)	316 (17)	560 (30)	—	—
Calcium (mg/L)	—	52 (4)	56 (4)	126 (5)	—	—
Magnesium (mg/L)	—	30 (1)	43 (2)	60 (4)	—	—
Alkalinity (mg/L as $CaCO_3$)	—	165 (8)	173 (14)	345 (24)	—	—
Chloride (mg/L)	—	39 (1)	64 (3)	157 (13)	—	—
Sulfate (mg/L)	—	229 (22)	268 (23)	561 (57)	—	—

[a]Conductivity μmhos/cm at 25 °C.

(AA-HG). The spectrophotometer was standardized with National Institute of Standards and Technology (NIST) standard reference material 3149 (water). Samples were digested using a persulfate digestion technique and total selenium was determined by a modification of the method of Presser and Barnes (1984). The limit of detection (LOD) ranged from 0.4 to 2.4 μg/L, the procedure blanks had background equivalent concentrations less than the LOD, the percent relative standard deviation for triplicate sample preparation and analysis was 5.4%, and recovery of selenium was within recommended ranges in National Bureau of Standards (NBS) reference material 1643b water (8.2–11.2 μg/L) and NIST standard reference material 3149 (8.99–11.01 μg/L). The digested sample spike solutions had a mean percentage recovery of 98%. Field spiked samples had a mean percentage recovery of 90%. Spiked samples analyzed for matrix suppression or enhancement had mean selenium recoveries of 99%.

A second set of 200-mL water samples was filtered and preserved with 2 mL ultrapure HNO_3 and stored frozen. These samples were analyzed by inductively coupled argon plasma (ICP) analysis for 31 elements at the Environmental Trace Substances Research Center, Columbia, Missouri. The procedure blank had background equivalent concentrations less than the LOD for all elements except Ca and Tl, the mean percentage relative standard deviation (duplicate sample

Table 3
Mean and standard error (in parentheses, $n = 24$) of estimated limit of detection for inorganic elements in water (mg/L) and tissue (μg/g)

Element	Water	Tissue
Ag	0.009 (0.002)	0.4 (0.1)
Al	0.024 (0.001)	2.0 (0.3)
As	0.030 (0.003)	4.2 (0.5)
B	0.009 (0.002)	0.6 (0.1)
Ba	0.001 (0)	0.06 (0.01)
Be	0.0002 (0)	0.03 (0)
Bi	0.020 (0)	4.5 (0.5)
Ca	0.056 (0.006)	1.3 (0.2)
Cd	0.002 (0)	0.3 (0)
Co	0.004 (0)	0.6 (0.1)
Cr	0.007 (0)	1.8 (0.2)
Cu	0.004 (0)	0.3 (0)
Fe	0.007 (0)	0.6 (0.1)
K	0.273 (0.018)	31 (3)
Li	0.001 (0)	0.2 (0)
Mg	0.014 (0.001)	0.3 (0)
Mn	0.001 (0)	0.08 (0.01)
Mo	0.006 (0.001)	0.5 (0.1)
Na	0.079 (0.003)	2.2 (0.3)
Ni	0.008 (0)	1.7 (0.2)
P	0.062 (0.005)	7 (1)
Pb	0.027 (0.002)	3 (0)
Sb	0.037 (0.002)	3 (0)
Si	0.036 (0.001)	14 (2)
Sn	0.030 (0.003)	2 (0)
Sr	0.001 (0)	0.05 (0.01)
Ti	0.001 (0)	0.1 (0)
Tl	0.076 (0.001)	7 (1)
V	0.002 (0)	0.4 (0.1)
Zn	0.002 (0)	0.1 (0)
Zr	0.003 (0)	—

preparation and analysis) was 1.3%, the mean spike recovery was 97%, and the recovery of elements in Environmental Resources Associates reference water ERA9947TM was within recommended ranges. The estimated limits of detection are given in Table 3.

Aquatic macroinvertebrates collected from the six sites and retained on the standard No. 40 stainless-steel sieve (≥0.425 mm) were separated by order. These samples and a portion of the zooplankton that passed through the sieve (<0.425 mm), along with some tadpoles collected in light traps, were stored frozen and analyzed for selenium concentration by AA-HG. Prior to analysis, samples were freeze-dried and the analysis results reported on a dry weight basis. The LOD was 0.15 μg/g, the procedure blanks had background equivalent concentrations less than the LOD, the percent relative standard deviation (triplicate sample preparation and analysis) was 3.7%, and recovery of selenium was within recommended ranges in NIST standard reference material 3149 (8.99–11.01 μg/L) and National Research Council of Canada (NRCC) reference material DORM-1 (dogfish muscle) (1.35–1.91 μg/g). The digested sample spike solutions had a mean percentage recovery of 100%, and spiked samples analyzed for matrix suppression or enhancement had mean selenium recoveries of 98%.

A portion of the zooplankton that passed through the sieve (<0.425 mm) was collected, stored frozen, and analyzed for 30 elements by ICP. Prior to analysis, samples were freeze-dried and the analysis results reported on a dry weight basis. The procedure blank had background equivalent concentrations less than the LOD for all elements except Ag, Ca, and Zn in one blank and Ca, Co, Fe, Mg, Sr, and Zn in a second blank (concentrations of these elements exceeded their LOD by very little), the mean percentage relative standard deviation (duplicate sample preparation and analysis) was 5.3%, the mean spike recovery was 90%, and the recovery of elements in NBS reference material 1566A (oyster tissue) was within the recommended range. The estimated limits of detection are given in Table 3.

Live fish at the end of Studies 2, 3, and 4 were collected for analysis of selenium concentrations by neutron activation at the University of Missouri, Columbia, Missouri. No fish were available from Study 1. Prior to analysis, samples were freeze-dried and the results reported on a dry weight basis. The LOD was 15 ng/g, the percentage relative standard deviation (triplicate sample preparation and analysis) was 3.1%, and recovery of selenium in NIST standard reference material 1577 (bovine liver) was within the recommended range (3.5 ± 0.4 μg/g).

2.3. Statistical analyses

Toxicant effects on percentage survival (arcsine-transformed values), growth, and whole-body concentrations of elements in fish (logarithmically transformed values) were evaluated by one-way analysis of variance. Food density effects on percentage survival (arcsine-transformed values) and growth in fish in the supplemental feeding study were evaluated by one-way analysis of variance. Treatment means were compared with the least-significant-difference multiple-means comparison test (Snedecor and Cochran, 1967). Pearson correlation analysis was used to test the relation between selenium concentrations in zooplankton and larval razorback sucker. Concentrations below the LOD were reported as "<"; however, a value of one-half the LOD was assigned for statistical computations (Kushner, 1976; USEPA, 1996). For each study, differences between survival curves and the estimated median time to death values from survival curves were analyzed using the accelerated failure time model outlined in Dixon and Newman (1991) and Lawless (1982) based on a Weibull distribution to model the data. Treatment curves were compared by the Bonferroni method to determine significant differences.

3. Results

3.1. Water quality

Prior to initiation of the studies, personnel at Ouray NWR used water from the Green River to flood Sheppard Bottom ponds. Water flowed into S1, then S2 and S4 simultaneously, and from S2 into S3, and from S3 to S5. In January 1994, the water level in S1 was full due to gravity water flow from the Green River, and S2, S3, and S5 were dry; S4 had some water (Dan Schaad, USFWS, personal communication). Gravity flow water was intermittently diverted to S2 and S3 from January 5 until February 18. No river water was available from February 4 until late April due to low river elevation. Pelican Lake water (208 acre feet) was delivered to Sheppard Bottom through a new pipeline from April 10 through May 16. Gravity flow water from the Green River was diverted to Sheppard Bottom from April 26 through June 12. These waters were comingled and diverted to S1, S2, S3, and S4. Some water entered S5 via the connecting water control structure with S3. The collection site for aquatic invertebrates at S5 was moved on May 27 (Day 6 of Study 1; Day 1 of Study 2) from near the connecting water control structure for S3 to a location 100 m south because the S5 site was being substantially diluted by S3 water (Fig. 2).

The water quality at the S1 reference site was relatively stable for the first 7 days of use, but the water level started to drop due to evaporation and by Day 11 zooplankton abundance had decreased substantially. Consequently, the collection site for reference water used in renewals and zooplankton was moved about 100 m south on June 2 (Day 12 of Study 1; Day 7 of Study 2) (Fig. 2). The new site was about 40 m from the supply canal and water control structures for S2 and S4. The water quality differed between S1 sites and was closer to Green River water during the latter half of Study 1, most of Study 2, and all of Studies 3 and 4 (Table 1).

The water quality at the six sites where zooplankton were collected differed substantially (Table 2). Conductivity was lowest at S1 and generally three times higher at SR and NR. Sites S3 and S4 were close to characteristics at S1, whereas S5 was similar to SR and NR. Chlorides and sulfates widely varied between sites, especially Study 3 where chlorides were 12- to 13-fold higher and sulfates 4- to 5-fold higher at SR and NR than at S1 (Table 2).

Mean water temperature was 17.3 °C in Study 1, 17.7 °C in Study 2, 18.2 °C in Study 3, and 18.9 °C in Study 4. Mean dissolved oxygen concentrations ranged from 7.6 to 7.8 mg/L in the four studies.

3.2. Elemental residues

Selenium concentrations in water at the six sites also varied greatly (Table 4). Reference water from S1 and at the S3 and S4 sites was consistently low in selenium concentration (<2.4 μg/L) during the four studies. Selenium concentrations in water from three other sites were higher than the reference site (S1). Measured selenium concentrations were slightly higher at S5 (0.6–3.1 μg/L), and substantially higher at SR (14–22 μg/L) and NR (57–107 μg/L).

Table 4
Concentrations of dissolved selenium (μg/L) in various waters at Ouray NWR from which zooplankton were collected to feed razorback sucker. Concentrations less than the LOD are reported as less than the LOD value for the analysis date

Date	Study no.	Site					
		S1	S3	S4	S5	SR	NR
05/22	1	0.7	0.4	0.8	1.9	21	78
05/23	1	<0.4	0.4	<0.4	3.1	21	107
05/28	1	<0.4	0.8	0.5	0.6	18	65
06/03	2	<0.6	<0.6	<0.6	0.6	22	75
06/11	3	<0.6	<0.6	<0.6	1.4	18	71
06/17	4	<1.1	<1.1	<2.4	<2.4	14	67
06/23	4	<1.1	<1.1	0.3	<2.4	14	57

Selenium concentrations in zooplankton from the S1 reference site ranged from 2.3 to 3.5 μg/g (Table 5). Concentrations were slightly higher in S3 and S4 (2.4–6.7 μg/g), substantially elevated at S5 (12–26 μg/g), and high at SR and NR (44–96 μg/g). Selenium concentrations decreased 32–53% in zooplankton over time at sites S1, S3, S4, and S5 probably due in large part to the flooding of these ponds with water from the Green River; whereas they decreased only 10% at SR and increased at NR where flooding was not a factor. Selenium concentrations in water at these sites did not change as much as selenium concentrations in zooplankton. Selenium concentrations in aquatic macroinvertebrates followed a pattern similar to those in zooplankton: lowest at sites S1, S3, and S4, intermediate at S5, and highest at SR and NR (Table 6).

Concentrations in water of Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Sb, Si, Sn, Ti, Tl, V, Zn, and Zr were close to or less than the LOD. Only four elements out of 31 measured by ICP showed a pattern of elevation across sites similar to that of selenium measured by AA-HG (Table 7). Concentrations of B, Li, Na, and Sr were lowest in S1, intermediate in S3, S4, and S5, and highest in SR and NR.

Concentrations in zooplankton of Ag, Bi, Co, Mo, Sb, Sn, and Tl were close to or less than the LOD. Only

Table 5
Mean, standard error (in parentheses), and number of samples (in brackets) of selenium (μg/g dry weight) in zooplankton collected from various sites at Ouray NWR and fed to razorback sucker

Study no.	Site					
	S1	S3	S4	S5	SR	NR
1	3.5 (0.4)[a] [7]	6.7 (0.5)[c] [8]	5.0 (1.0)[b] [8]	25.7 (1.5)[d] [6]	95.9 (5.5)[f] [11]	44.2 (2.3)[e] [10]
2	2.5 (0.3)[a] [14]	5.1 (0.4)[b] [9]	3.5 (0.7)[a] [14]	22.8 (1.4)[c] [5]	90.6 (5.9)[e] [13]	48.8 (3.4)[d] [13]
3	2.3 (0.2)[a] [10]	—	—	—	86.7 (7.3)[c] [10]	49.8 (4.3)[b] [10]
4	—	4.5 (0.3)[b] [14]	2.4 (0.4)[a] [14]	12.0 (1.0)[c] [14]	—	—
Geometric mean	2.7	5.4	3.5	19	91	47.5

Sites within a study with the same letter are not significantly different ($P \leqslant 0.05$; ANOVA and LSD).

Table 6
Mean, standard error (in parentheses), and number of samples (in brackets) of selenium (μg/g dry weight) in aquatic invertebrates (adult: Ad., larvae: Lar.) and tadpoles collected from various sites at Ouray NWR

Animal	Site					
	S1	S3	S4	S5	SR	NR
Dytiscidae Ad.	2.4 (-) [1]	—	3.9 (-) [1]	—	70.3 (2.4) [8]	132.4 (9.1) [2]
Lar.	2.1 (0) [2]	—	1.6 (-) [1]	8.5 (-) [1]	—	—
Haliphidae	—	—	—	—	55.0 (-) [1]	63.6 (28.2) [2]
Helodidae	—	—	—	—	—	114.1 (-) [1]
Hydrophilidae Ad.	1.9 (0.8) [2]	—	2.4 (-) [1]	5.3 (2.0) [2]	27.6 (7.8) [3]	15.5 (-) [1]
Lar.	2.5 (0.6) [2]	4.8 (0.3) [2]	1.3 (0.2) [2]	7.6 (-) [1]	2.5 (-) [1]	—
Snail	—	—	—	—	31.9 (1.4) [6]	27.3 (0.1) [2]
Boatman	—	—	1.9 (-) [1]	—	—	31.4 (11.2) [2]
Amphipod	—	—	—	—	—	26.1 (1.2) [2]
Backswimmer	—	—	—	—	81.6 (3.5) [7]	14.4 (-) [1]
Damselfly nymph	—	—	1.9 (-) [1]	—	109.7 (2.6) [3]	—
Tadpole	—	—	—	—	168.7 (10.2) [5]	—

These invertebrates were too large to feed to razorback sucker.

two elements, Sr and Zn, out of 30 measured by ICP showed a pattern of elevation across sites similar to that of selenium (Table 8). Several elements had higher concentrations in zooplankton from S1 than other sites: As was 2 times higher than S4, Cd 3–5 higher except at S4, Ni 2–7 higher, and Pb 2 higher. Chromium was

Table 7
Mean, standard error (in parentheses, $n = 5$) of elements (mg/L) in water collected from various sites at Ouray NWR

Element	Site					
	S1	S3	S4	S5	SR	NR
B	0.09 (0.02)	0.12 (0)	0.18 (0)	0.27 (0.03)	0.45 (0.01)	0.42 (0.01)
Li	0.02 (0)	0.02 (0)	0.04 (0)	0.05 (0.01)	0.09 (0)	0.08 (0)
Na	42 (5)	64 (6)	92 (2)	196 (29)	306 (6)	280 (6)
Sr	0.47 (0.04)	0.58 (0.02)	0.84 (0.04)	1.33 (0.15)	1.37 (0.05)	1.69 (0.05)

elevated at NR, V at S3 and NR, and Zn elevated at SR and NR.

3.3. Study 1

All fish died in Study 1 after 25 days of exposure (Fig. 3). No fish were available for measurement of growth or selenium residue analysis. The survival curves and median time to death were significantly different among sites (Table 9). Median time to death was shortest in fish fed zooplankton from S1 and longest for SR and NR.

3.4. Study 2

The majority of fish died in Study 2 after 12 days of exposure, including fish fed invertebrates from the S1 reference site (Fig. 4). The survival curve and median time to death for larvae fed zooplankton from S5 were significantly less than the other five sites, which were not different from each other (Table 9). Concentrations of selenium in zooplankton ranged from 2.5 μg/g at S1 to 91 μg/g at SR (Table 5). Whole-body residues of selenium in fish ranged from 8.7 to 94 μg/g (Table 10). Fish fed invertebrates from the S1 reference site contained 14.3 μg/g (Table 10). The whole-body concentrations of selenium in larvae from Study 2 were somewhat variable, as shown by the standard error, due in part to the very small-sized samples available (0.1–0.5 mg dry weight) and the imprecision of weighing submilligram samples.

3.5. Study 3

Survival was 100% in all treatments through 9 days of exposure, but almost 50% mortality or more occurred in the following 7 days (Fig. 5). The survival curves and median time to death were not different among (Table 9). Concentrations of selenium in zooplankton were 2.3 μg/g in S1, 50 μg/g in NR, and 87 μg/g in SR (Table 5). Whole-body residues of selenium in fish followed the same order of progression as in invertebrates and were 3.7 μg/g in the S1 treatment, 33 μg/g in the NR treatment, and 39 μg/g in the SR treatment (Table 10). Fish total length, weight, and condition factor were not different among sites (Table 11).

3.6. Study 4

Survival was 100% through 7 days in the S3 and S4 treatments, but only through 5 days in the S5 treatment (Fig. 6). By Day 20, survival in all treatments was decreased to 22–27%. The survival curves and median time to death were not different among sites (Table 9). Selenium concentrations in zooplankton ranged from 2.4 μg/g in S4 to 12 μg/g in S5 (Table 5). Similar to Study 3, whole-body selenium residues in fish followed the same order as in zooplankton and were lowest in treatment S4 (3.6 μg/g), intermediate in S3 (3.9 μg/g), and highest in S5 (5.9 μg/g) (Table 10). Fish weight, but not total length or condition factor, was significantly reduced in larvae fed zooplankton from S5 (Table 11).

3.7. Fish studies

The Pearson correlation (r) for the relation between selenium concentrations in zooplankton and those in larval razorback sucker for Studies 2, 3 and 4 combined was 0.71 ($P = 0.02$, $n = 10$). There was no consistent relation between concentrations of selenium or other elements in zooplankton and survival of larval razorback sucker in the four studies.

3.8. Supplemental feeding study

Survival at the end of 20 days of feeding brine shrimp was 95–97% for 5-day-old larvae, 90–95% for 10-day-old larvae, and 97–100% for 24-day-old larvae (Table 12). There were no differences in survival between feeding groups or between age groups; however, fish total length and weight were significantly different between feeding groups (Table 12). Larvae fed 80 organisms/fish were significantly larger than larvae fed either 20 or 40 organisms/fish, and larvae fed 40 organisms/fish were significantly larger larvae fed 20 organisms/fish.

Table 8
Mean, standard error (in parentheses), and number of samples (in brackets) of elements (μg/g dry weight) in zooplankton collected from various sites at Ouray NWR and fed to razorback sucker

Element	Site					
	S1	S3	S4	S5	SR	NR
Al	1810 (700) [5]	3920 (2300) [5]	1530 (580) [5]	1290 (410) [5]	3060 (2000) [5]	5260 (1220) [5]
As	12 (9) [3]	<4	5 (1) [5]	<4	<4	<4
B	12.4 (5.9) [4]	9.4 (4.0) [5]	8.5 (3.0) [5]	5.8 (1.5) [5]	13.2 (5.7) [4]	7.7 (2.0) [4]
Ba	54 (12) [5]	98 (46) [5]	260 (150) [5]	74 (25) [5]	149 (60) [5]	80 (16) [5]
Be	0.08 (0.06) [3]	0.13 (0.12) [5]	0.04 (0.02) [5]	0.03 (0.02) [5]	<0.03	0.10 (0.06) [4]
Ca	24520 (5820) [5]	35440 (4430) [5]	47490 (17900) [5]	31820 (2510) [5]	61120 (14580) [5]	67080 (9350) [5]
Cd	5.3 (2.8) [5]	1.7 (0.5) [5]	4.1 (1.7) [5]	1.3 (0.4) [5]	1.1 (0.4) [3]	1.7 (0.7) [5]
Cr	3.0 (0.9) [3]	4.1 (1.8) [5]	3.5 (1.2) [5]	2.4 (0.5) [5]	3.3 (1.2) [3]	9.1 (3.1) [5]
Cu	93 (14) [5]	60 (6) [5]	98 (16) [5]	62 (17) [5]	63 (9) [5]	48 (13) [5]
Fe	1620 (450) [5]	3480 (1870) [5]	1690 (540) [5]	2550 (670) [5]	2510 (1460) [5]	3580 (820) [5]
K	8580 (1310) [5]	5930 (1060) [5]	8130 (930) [5]	5750 (920) [5]	5660 (630) [5]	4680 (660) [5]
Li	2.2 (0.6) [5]	4.1 (2.2) [5]	2.5 (0.7) [5]	1.7 (0.5) [5]	3.6 (2.1) [5]	4.7 (1.1) [5]
Mg	2260 (270) [5]	3540 (1120) [5]	3020 (780) [5]	2160 (260) [5]	3170 (960) [5]	3460 (460) [5]
Mn	760 (290) [5]	356 (100) [5]	592 (115) [5]	1710 (1130) [5]	293 (82) [5]	290 (72) [5]
Na	4100 (730) [5]	3630 (710) [5]	4850 (690) [5]	3900 (190) [5]	3970 (860) [5]	3220 (160) [5]
Ni	16.4 (11.7) [4]	8.6 (3.1) [5]	5.5 (1.7) [5]	2.5 (0.9) [5]	2.5 (0.8) [3]	4.0 (1.4) [4]
P	11300 (1380) [5]	10640 (2120) [5]	9270 (1360) [5]	13200 (920) [5]	9760 (1830) [5]	10910 (480) [5]
Pb	14.8 (6.6) [5]	9.7 (2.0) [5]	15.0 (4.8) [5]	7.4 (1.0) [5]	10.0 (2.4) [4]	7.8 (1.3) [4]
Si	660 (220) [5]	1330 (490) [5]	660 (240) [5]	660 (170) [5]	850 (260) [5]	2240 (580) [5]
Sr	113 (27) [5]	193 (31) [5]	315 (138) [5]	243 (27) [5]	521 (115) [5]	443 (65) [5]
Ti	49 (19) [5]	116 (68) [5]	52 (20) [5]	41 (14) [5]	91 (57) [5]	158 (39) [5]
V	3.7 (1.2) [4]	8.4 (4.7) [5]	4.0 (1.2) [5]	2.8 (0.7) [5]	4.4 (2.0) [4]	9.5 (2.3) [5]
Zn	260 (60) [5]	153 (14) [5]	244 (61) [5]	351 (162) [5]	806 (619) [5]	1170 (830) [5]

4. Discussion

4.1. Survival and growth

Fish mortality in Study 1 did not begin until Day 5 of feeding selenium-laden zooplankton when fish age was 10 days posthatch. This mortality was similar to that in Study 2, in which mortality occurred at Day 2 of feeding when fish were 12 days posthatch. Mortality in Studies 3 and 4 occurred later at 5–10 days of feeding, suggesting more tolerance to selenium at an advanced life stage (24- and 28-day old). However, about 50% mortality occurred consistently after 10 to 15 days exposure in all treatments (Study 1, 10–15 days; Study 2, 6–12 days; Study 3, 13–15 days; Study 4, 14–16 days) (Figs. 3–6). These results were similar to those in two studies conducted with 5-day-old razorback sucker fed zooplankton with various selenium concentrations collected from three sites near Grand Junction, Colorado (Hamilton et al., 2001a, b). In those studies mortality started at about 5–8 days after feeding began and 50% mortality or higher occurred between 10 and 15 days of dietary exposure.

Starvation was probably not a cause of reduced survival in the present study because larvae were fed prior to and during the studies and mortality occurred in a shorter time period than observed by Papoulias and Minckley (1990) for starved fish. Papoulias and Minckley (1990) found that the median time to 50% mortality in starved larval razorback sucker was 24–25 days posthatch. They also reported that larval razorback sucker first fed beginning 7 days posthatch had 100% survival and those beginning feeding 11 or 15 days posthatch had 90% survival until larvae were 50 days posthatch. Larvae used in Studies 3 and 4 were held in the hatchery and feed Biokyowa B-250, which has been recommended for use in rearing larval razorback sucker because it results in high survival rates (78% reported by Tyus and Severson, 1990; 96% reported Severson et al., 1992).

Steve Severson (USFWS, Ouray NFF, Vernal, UT) observed fish on June 1 in Studies 1 (Day 11) and 2 (Day 6) when the feeding rate was 20 zooplankton/fish and noted their stomachs looked full. Likewise, prior to daily feedings of zooplankton, uneaten live zooplankton from the previous day were observed in the exposure beakers, thus suggesting larval razorback sucker were eating less than what was available. Nevertheless, the feeding rate was doubled to 40 zooplankton/fish after June 1.

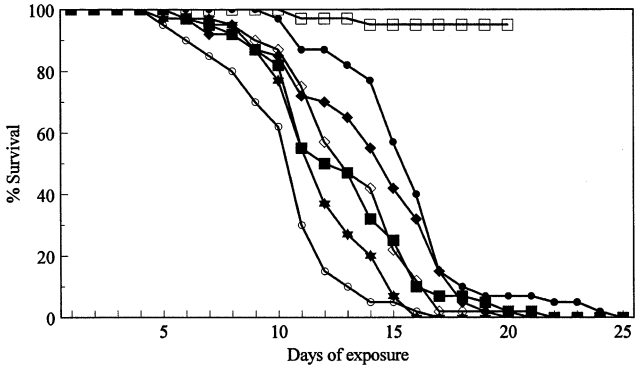

Fig. 3. Survival of 5-day-old razorback sucker fed zooplankton collected from various sites at Ouray NWR for 25 days in Study 1 (S1, ○; S3, ◇; S4, ■; S5, ★; SR, ●; NR, ◆), and 5-day-old larvae fed 40 brine shrimp in the supplemental feeding study (BS, □).

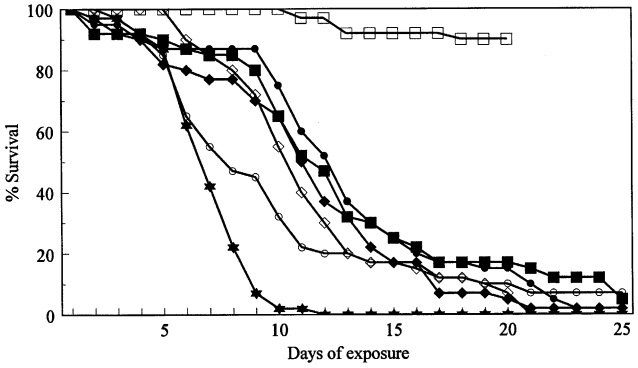

Fig. 4. Survival of 10-day-old razorback sucker fed zooplankton collected from various sites at Ouray NWR for 25 days in Study 2 (S1, ○; S3, ◇; S4, ■; S5, ★; SR, ●; NR, ◆), and 10-day-old larvae fed 40 brine shrimp in the supplemental feeding study (BS, □).

Table 9
Mean and standard error (in parentheses) of the estimated median time to death (days) of razorback sucker fed zooplankton from six sites at Ouray NWR

Study no.	Site					
	S1	S3	S4	S5	SR	NR
1	10.2 (0.4)^{a}	12.8 (0.4)^{bc}	13.0 (0.5)^{bc}	11.4 (0.4)^{ab}	15.3 (0.5)^{d}	13.9 (0.5)^{cd}
2	10.4 (0.8)^{b}	10.3 (0.7)^{b}	12.5 (0.9)^{b}	5.9 (0.4)^{a}	12.0 (0.9)^{b}	10.6 (0.8)^{b}
3	16.4 (0.7)^{a}	—	—	—	16.3 (0.8)^{a}	15.1 (0.7)^{a}
4	—	16.6 (1.0)^{a}	15.4 (0.9)^{a}	15.2 (0.9)^{a}	—	—

For each row, letters in common are not significantly different ($P \leqslant 0.05$).

Table 10
Mean, standard error (in parentheses), and number of samples (in brackets) of selenium (μg/g dry weight) in razorback sucker at the conclusion of Studies 2, 3, and 4 (no fish were available in Study 1)

Study no.	Site					
	S1	S3	S4	S5	SR	NR
2	14.3 (4.2) [3]	—	8.7 (2.5) [2]	—	52.8 (-) [1]	93.8 (-) [1]
3	3.7 (0.2)[a] [3]	—	—	—	39.1 (1.7)[b] [3]	32.6 (5.5)[b] [3]
4	—	3.9 (0.1)[a] [4]	3.6 (0.2)[a] [4]	5.9 (0.2)[b] [4]	—	—

For each study, letters in common are not significantly different ($P \leqslant 0.05$).

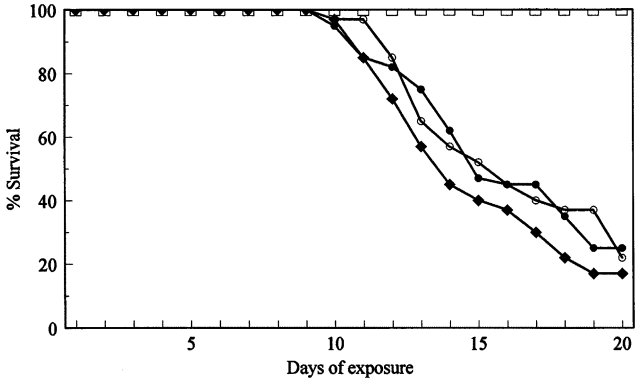

Fig. 5. Survival of 24-day-old razorback sucker fed zooplankton collected from various sites at Ouray NWR for 20 days in Study 3 (S1, ○; ●; NR, ◆), and 24-day-old larvae fed 40 brine shrimp in the supplemental feeding study (BS, □).

Feeding 20 zooplankton/fish (125 zooplankton/L) initially and 40 zooplankton/fish (250 zooplankton/L) through the majority of Studies 1 and 2 and all of Studies 3 and 4 should preclude starvation stress. Papoulias and Minckley (1990) reported in their laboratory study conducted at 18 °C that 30–60 nauplii of brine shrimp (*Artemia salina*) 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 high, medium, or low fertilized ponds with invertebrate densities of 43, 24, and 12 zooplankton/L, respectively. Our feeding rates of 125 or 250 zooplankton/L were 3 to 6 times higher than their pond with the highest number of zooplankton, which was highly fertilized and probably had a broad array of food items. Our supplemental feeding study confirmed that as little as 20 food organisms per fish per day would maintain a high survival rate. Even 24-day-old larvae fed 20 organisms per fish in the supplemental feeding study, half the rate in Study 3, had 97% survival.

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 larval razorback sucker in Lake Mohave, which had a low 1.55 zooplankton/L (reported as 1554 zooplankton/m^3), or in Arizona Bay backwater, which had 0.38 zooplankton/L (reported as 377 zooplankton/m^3). The zooplankton densities in the field studies by Papoulias and Minckley (1992) and Marsh and Langhorst (1988) encompassed all possible sizes, whereas the zooplankton used in our study were preselected to be <0.425 mm, which would have represented only a portion of the various sizes present in a field situation. Thus, their measured density would have overestimated the number of zooplankton because it would have included zooplankton too large for larval fish to consume.

The zooplankton density in the present study (125 or 250/L) was 8–17 times greater than the 15 zooplankton/L, which included all possible sizes, reported by Cooper and Severn (1994) for Sheppard Bottom pond 3. By increasing the density of zooplankton and preselecting small sizes in the present study, the likelihood that larval razorback sucker would successfully consume food was increased substantially. This approach also reduced the energy cost to larvae for searching and capturing prey. In natural wetlands, fish would have unlimited access 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.

A concentration response between survival and selenium or other elemental concentrations in zooplankton was not observed in this study. Apparently a threshold for toxic stress was reached and exceeded in the larval razorback sucker. Consequently, larvae in all treatments died at about the same time, i.e., 5–10 days after feeding exposure. However, in Study 1, the median time to death of larvae fed zooplankton from SR and NR was significantly longer than for larvae fed zooplankton from S1, which suggested an antagonistic

Table 11
Mean, standard error (in parentheses), and number of samples of total length (mm), weight (mg), and condition of razorback sucker in Studies 3 and 4 ($n = 4$ for each study and site)

Study and measure	Site					
	S1	S3	S4	S5	SR	NR
Study 3						
Length	13 (1)[a]	—	—	—	13 (0)[a]	13 (1)[a]
Weight	8 (0)[a]	—	—	—	8 (0)[a]	9 (0)[a]
Condition	0.45 (0.04)[a]	—	—	—	0.38 (0.01)[a]	0.41 (0.03)[a]
Study 4						
Length	—	14 (0)[a]	13 (0)[a]	13 (0)[a]	—	—
Weight	—	12 (0)[a]	9 (1)[ab]	9 (0)[b]	—	—
Condition	—	0.45 (0.01)[a]	0.40 (0.03)[a]	0.38 (0.03)[a]	—	—

For each study and measure, letters in common are not significantly different ($P \leqslant 0.10$).

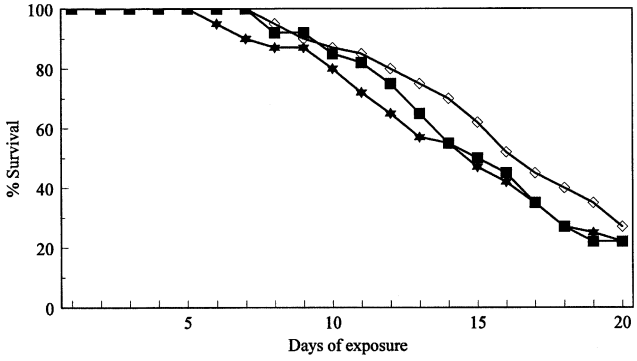

Fig. 6. Survival of 28-day-old razorback sucker fed zooplankton collected from various sites at Ouray NWR for 20 days in Study 4 (S3, ◇; S4, ■; S5, ★).

interaction between elements present in zooplankton. If a typical concentration response had been present, larvae fed zooplankton from SR and NR would have died first because concentrations of selenium and other elements were highest in SR and NR. However, because median time to death was significantly delayed in the SR and NR treatments, it seems that a stress threshold of some sort was exceeded in the S1, S3, S4, and S5 treatments before it was exceeded in SR and NR. This observation implies that a food-derived stress caused the differences in survival because all fish were held in the same water, which had a low selenium concentration ($<1.1\,\mu g/L$). Alternatively, the delayed mortality in the SR and NR treatments could have been a result of an interaction between S1 water and zooplankton from SR and NR.

The delayed mortality observed in Study 1 was similar to that in a study where 5-day-old razorback sucker were fed zooplankton collected from North Pond (Walter Walker State Wildlife Area near Grand Junction, CO), which had the highest concentrations of selenium (39 μg/g) and other elements compared to two other locations that were tested (Hamilton et al., 2001a).

Table 12
Mean and standard error (in parentheses) of survival, total length (mm), weight (mg), and condition of razorback sucker fed live brine shrimp in a supplemental feeding study ($n = 4$ for each fish age and feeding rate)

Fish age (day)	Measure	Feeding rate (organisms/fish)		
		20	40	80
5	% Survival	97 (2)[a]	95 (5)[a]	95 (3)[a]
	Length	10.8 (0)[a]	11.9 (0)[b]	12.9 (0.2)[c]
	Weight	4.6 (0.4)[a]	6.6 (0.3)[b]	9.6 (0.3)[c]
	Condition	0.38 (0.03)[a]	0.40 (0.01)[a]	0.46 (0.01)[a]
10	% Survival	92 (7)[a]	90 (7)[a]	95 (3)[a]
	Length	10.7 (0.1)[a]	12.0 (0.1)[b]	13.0 (0.1)[c]
	Weight	5.6 (0.4)[a]	8.5 (0.1)[b]	12.9 (0.4)[c]
	Condition	0.46 (0.01)[a]	0.52 (0.01)[b]	0.63 (0.02)[c]
24	% Survival	97 (2)[a]	100 (0)[a]	97 (2)[a]
	Length	13.6 (0.1)[a]	14.2 (0.1)[b]	15.4 (0.1)[c]
	Weight	9.8 (0.2)[a]	13.5 (0.2)[b]	20.2 (0.4)[c]
	Condition	0.39 (0.01)[a]	0.46 (0.01)[b]	0.54 (0.01)[c]

Measures within a fish age with the same letter are not significantly different ($P \leqslant 0.05$).

Delayed mortality was also observed in a second study conducted with razorback sucker larvae using zooplankton and water collected from three sites near Grand Junction, Colorado (Hamilton et al., 2001b). In that study larvae fed zooplankton from Horsethief east wetland (average 6.0 μg/g selenium) had mean predicted time to 90% mortality of 6 days in 24-Road water ($<1\,\mu g/L$ selenium), 8 days in Horsethief water (1.6 μg/L), 10 days in Abode Creek water (3.4 μg/L), and 14 days in North Pond water (13.3 μg/L), thus revealing a delayed mortality in treatments with increasing concentrations of waterborne selenium and other elements. In the same study, larvae held in North Pond water had mean predicted time to 90% mortality of 31 days when fed Horsethief zooplankton (6 μg/g selenium), 35 days

when fed Adobe Creek zooplankton (32 μg/g), and 43 days when fed North Pond zooplankton (52 μg/g), thus revealing delayed mortality in treatments with increasing concentrations of dietary selenium and other elements.

Larval razorback sucker in Studies 3 and 4 grew to 12–14 mm total length by 44–48 days posthatch. This growth is comparable to that reported by Papoulias and Minckley (1990) for laboratory-reared larvae, which had 14–16 mm total length at 50 days posthatch. Growth of larvae in Studies 3 and 4 was also similar to that in the supplement feeding study with 24-day-old larvae, which had 14–15 mm total length at 44 days posthatch. Even though the feeding rates in the supplement feeding study were fourfold different (20 versus 80 food organisms/fish in the low and high treatments), the difference between feeding treatments in total length was only 13%. In contrast to Studies 3 and 4, Papoulias and Minckley (1992) reported larvae reared in fertilized ponds for 42 days posthatch had total lengths of 16–19 mm, and Tyus and Severson (1990) reported total lengths of 15–18 mm in 45-day-old razorback sucker larvae fed dry diets with the best survival and 16–22 mm in larvae fed different dry diets giving the best growth, but poorer survival. Likewise, Marsh and Langhorst (1988) reported that larval razorback sucker grew up to 16 mm in a Lake Mohave backwater in about 6 weeks, but larvae in Lake Mohave reservoir showed no growth beyond their initial size of 10.6 mm.

Weight of larvae was not affected in Study 3, but was reduced in Study 4 in fish fed zooplankton from S5. Weights of larvae in the present study were close to those of larvae in the supplemental feeding study that were fed either 20 or 40 organisms/fish. However, weights of larvae in Studies 3 and 4, and even in larvae fed 80 organisms/fish in the supplement feeding study, were lower than those of Papoulias and Minckley (1992) who reported 25 mg for 6-week-old larvae in ponds with invertebrate densities of 12 organisms/L, 37 mg in ponds with 24 organisms/L, and 45 mg in ponds with 43 organisms/L. Their results suggest that larvae in the present study and in the supplemental feeding study did not grow as well as those in ponds where a greater variety of foods including algae, sessile organisms, benthic organisms, and detritus would be available. Other studies with larval razorback sucker did not report weights (Papoulias and Minckley, 1990; Marsh and Langhorst, 1988).

4.2. Selenium

Of the elements measured in zooplankton, only selenium seemed elevated sufficiently to have caused the reduced survival of larval razorback sucker. In the present study, S1 was considered the reference site because it was believed to be relatively uncontaminated as evidenced by low selenium concentrations in waterbird eggs (Stephens et al., 1992; Peltz and Waddell, 1991) and fish (Waddell and Wiens, 1994). However, selenium concentrations in larval razorback sucker fed zooplankton from S1 were elevated in Study 2 (14.3 μg/g) and close to the toxic threshold for adverse affects in larval fish (4 μg/g; Lemly, 1993, 1996; Hamilton, 2002, 2003) in Study 3 (3.7 μg/g). This lower value is two times higher than selenium concentrations typically reported in control fish from laboratory studies with either water or diet exposure or reference fish from field studies ($\leqslant$2.0 μg/g; Hamilton, 2002, 2003). It now seems clear that the S1 site was contaminated sufficiently with selenium in the food chain to result in elevated residues in larvae above those typically found in uncontaminated reference sites.

There was a substantial difference in selenium concentrations in older larvae in Studies 3 and 4 compared to concentrations in younger larvae in Study 2. Although selenium concentrations in zooplankton from various sites were slightly less in Studies 3 and 4 compared to Study 2, the difference was too small to account for the relatively large difference in fish residues. Bennett et al. (1986) reported a somewhat similar response in fathead minnow (*Pimephales promelas*) larvae fed selenium-laden rotifers. They found 9-day-old larvae accumulated selenium concentrations of 61 μg/g after 7 days exposure, whereas 17-day-old larvae accumulated only 52 μg/g after 9 days exposure. Even though the test with 17-day-old larvae was 2 days longer, they accumulated less selenium, and Bennett et al. (1986) suggested that the increase in larvae weight decreased the tissue selenium concentration. Part of the reason for the different whole-body residues may be due to body size, which affects kinetic rate constants for chemical uptake, and the concomitant dramatic differences in surface area/volume ratios between fish of different size (Rand et al., 1995).

Selenium concentrations of 4 μg/g or more in whole body of young fish exposed through dietary or waterborne exposures have been associated with adverse effects (Lemly, 1993, 1996; Hamilton, 2002, 2003). Waterborne exposure requires higher selenium exposure concentrations than dietary exposures to generate similar whole-body residues. However, once whole-body selenium reaches a threshold concentration (i.e., 4 μg/g), regardless of exposure route, adverse effects will occur. This threshold was exceeded in 7 out of the 10 residues in larval razorback suckers in the present study (Table 10), and the remaining three residues (3.6, 3.7, and 3.9 μg/g) also were close to the proposed threshold for toxic effects of selenium in fish. Thus, all larval razorback sucker were affected and, therefore, seem to be as sensitive as the three other species with the lowest whole-body residues of selenium associated with adverse effects, i.e., rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*Oncorhynchus tshawytscha*),

and bluegill (*Lepomis macrochirus*) (reviewed in Hamilton, 2002, 2003).

In the present study, larval razorback sucker accumulated selenium from zooplankton containing 2.3 μg/g or more (correlation $r = 0.70$), which is close to the toxic dietary threshold of 3 μg/g proposed for fish (Lemly, 1993, 1996; Hamilton, 2002, 2003). In the present study, all zooplankton exceeded this selenium threshold at S3, S5, SR, and NR, 2 out of 3 zooplankton samples exceeded the threshold at S4, and 1 out of 3 samples exceeded it at S1 (Table 5). The three zooplankton samples that did not exceed this threshold were, nevertheless, very near it, i.e., 2.3, 2.4, and 2.5 μg/g.

The whole-body selenium concentrations in larvae that were associated with mortality in the present study (⩾3.6 μg/g) were lower than those in two other studies with larval razorback sucker (Hamilton et al., 2001a, b). In those studies ⩾5.4 μg/g in larvae during the 1996 study and ⩾6.1 μg/g in larvae during the 1997 study were associated with decreased survival. Likewise, the dietary selenium concentration in zooplankton that was associated with mortality in the present study (⩾2.3 μg/g) was also lower than those in two other razorback sucker studies where zooplankton containing 4.6 μg/g selenium were associated with rapid mortality of larvae during the first 10–15 days of dietary exposure (Hamilton et al., 2001a, b).

4.3. Other elements

Other elements may have induced stress in razorback sucker larvae in the present study, especially in fish fed zooplankton from S1 in Study 4 where zooplankton contained elevated concentrations of As, Cd, Cu, Ni, and Pb. Furthermore, Sr concentrations in zooplankton from various sites followed a pattern similar to that of selenium; it was lowest in S1, intermediate in S3, S4, and S5, and highest in SR and NR. Vanadium was also elevated in zooplankton from the various sites, especially S3 and NR, and Zn was elevated in zooplankton from SR and NR. The greater survival in larvae fed zooplankton from SR and NR than from S1 in Study 1 may have been due to antagonistic interactions between selenium and other elements in zooplankton from SR and NR.

Strontium concentrations in water and zooplankton in the present study were similar to those reported by Stephens et al. (1988, 1992) and Peltz and Waddell (1991) in algae, aquatic invertebrates, and fish in S3, S5, SR, and NR. Strontium concentrations have been reported to be elevated in water and fish associated with impacts from irrigation activities (Nakamoto and Hassler, 1992), acidification (Moreau et al., 1983), and uranium mine drainage (Nichols and Scholz, 1989). Finger et al. (1994) conducted several on-site tests with irrigation-impacted waters, including three sites at Ouray NWR, and reported Sr was one of six elements (Se, B, Co, Cu, Li, Sr) that were highly correlated with mortality of test fish and invertebrates. Although Sr values were numerically large in these studies, there is little known about its toxic effects.

Vanadium concentrations in water were below the LOD, but elevated in zooplankton (2.8–9.5 μg/g) in the present study. The V concentrations in zooplankton in the present study are higher than those reported by Peltz and Waddell (1991) for mixed invertebrate samples in NR (0.4–8.4 μg/g), SR (0.5 μg/g), S5 (1–2 μg/g), or S3 (1.6–2.4 μg/g), but less than those in chironomids from the same sites (11–17 μg/g). Hilton and Bettger (1988) reported that V dietary toxicity to juvenile rainbow trout occurred at concentrations less than 10 μg/g, but above 1.2 μg/g in the control treatment. They concluded, based on their research with dietary selenium toxicity, that dietary V was at least as toxic, if not more toxic, than dietary selenium. Because V concentrations in zooplankton in the present study were close to toxic concentrations, V may have contributed to the reduced survival of razorback sucker.

4.4. Food chain

The low selenium concentrations in water at S1, S3, S4, and S5, but elevated concentrations in invertebrates in the present study, were similar to the observations of Schroeder et al. (1988), Skorupa and Ohlendorf (1991), Hallock and Hallock (1993), and Hamilton et al. (2001a, b). They reported that selenium at low water concentrations (0.5–3 μg/L) could be taken up by aquatic organisms and accumulated to concentrations toxic to fish and wildlife. For example, algae rapidly uptake selenium from water (Besser et al., 1993; Foe and Knight, 1985; Nassos et al., 1980; Riedel et al., 1991) as do other aquatic plants (Ornes et al., 1991). Algae typically took up maximal concentrations in 3–24 h, whereas the floating plants took about 1 week to accumulate maximal concentrations. Zooplankton also rapidly take up selenium from the water and accumulate it with no adverse effects on reproduction except at very high selenium concentrations (>400 μg/L) (Foe and Knight, 1985; Halter et al., 1980; Nassos et al., 1980; Reading and Buikema, 1983; Salki et al., 1985), and also show no effects on seasonal abundance of zooplankton in exposures up to 100 μg/L of selenium (Salki et al., 1985).

One unusual aspect of the study was that selenium concentrations in water were 3–5 times higher at NR than SR, but selenium concentrations in zooplankton were 2 times higher at SR than NR. The low selenium concentration in zooplankton at NR was probably because water was flowing substantially through the beaver pond site and zooplankton were not able to

accumulate selenium from water to the same concentrations as those in SR where there was no outflow.

The food chain in the six sites used to collect zooplankton to feed larval razorback sucker in the present study was contaminated with selenium as evidenced by the elevated selenium residues in zooplankton, other aquatic invertebrates, and larval razorback sucker. Although selenium concentrations in water were low at some sites, previous selenium loading in ecosystem components other than water (i.e., phytoplankton, zooplankton, aquatic plants, detritus, and sediments) or rapid uptake and bioaccumulation of low water concentrations was apparently sufficient to maintain selenium at elevated concentrations despite freshwater inputs from the Green River.

4.5. Ouray NWR

Part of the selenium loading in the food chain in S3 came from two seeps on the western edge of S3, which contained 25 and 73 μg/L selenium (Stephens et al., 1992; Peltz and Waddell, 1991). Groundwater moves from the draw above North Roadside Pond down gradient through Sheppard Bottom to the Green River. Selenium in seeps is from groundwater, which in a well at S3 contained 3200 μg/L selenium in November 1988 and 9300 μg/L in June 1989. Other selenium loading came from surface flow such as in S5 where flows in 1989 contained 48 μg/L in April and 17 μg/L in June. This selenium loading in Sheppard Bottom was diluted by flows from the Green River, which, for example, lowered selenium concentrations in 1987 in S3 to <1–3 μg/L and in S5 to 2–4 μg/L. These data were similar to those of the present study in that selenium concentrations were <2 μg/L in both S3 and S5, yet zooplankton from S3 contained selenium concentrations of 4.5–6.7 μg/g (geometric mean 5.4 μg/g) and from S5 contained 12–26 μg/g (geometric mean 19 μg/g). Stephens et al. (1992) and Peltz and Waddell (1991) reported that selenium concentrations in invertebrates were elevated, but varied widely in NR (12–49 μg/g) and SR (12–71 μg/g).

In March and April 1994, water flowed from S5 into S3 and a water sample taken on the east side of S3 (opposite side from the interconnecting water control structure with S5) contained 12 μg/L selenium (Carol Wiens, USFWS, written communication). After S3 was flushed with river water, water samples on the east side of S3 in May, June, and July contained <1 μg/L selenium and a sample in August contained 1 μg/L. These data reveal that water concentrations of selenium can change rapidly. Yet, at the time water contained <1 μg/L, aquatic invertebrates sampled in May contained 63.7 μg/g of selenium (70% Megaloptera, 20% Tricoptera, and 10% Odonata) from the northwest corner of S3 near the location of the interconnecting water control structure with S5; 9.0 μg/g in chironomids and 11.3 μg/g in zooplankton from the east side; and 13.5 μg/g in mixed insects and zooplankton and 15.2 μg/g in zooplankton from the west side (Carol Wiens, USFWS, written communication). These concentrations of selenium in zooplankton are about two to three times higher than those in the present study for S3 (Table 4) and exceed the proposed dietary toxic threshold of 3 μg/g. Similar to the present study, these data demonstrated that aquatic invertebrates in the food chain can have high selenium concentrations while water concentrations are low.

Cooper and Severn (1994) reviewed environmental conditions at Ouray NWR and concluded that the refuge wetlands would be a good site for rearing larval fish such as endangered razorback sucker because of the high densities of water-column Cladocera and Copepoda, especially in bullrush communities in Leota Bottom and Sheppard Bottom. Cooper and Severn (1994), however, had limited water chemistry data (only five samples were collected) that showed selenium was not detected in four samples and was 4 μg/L in a groundwater sample in a cottonwood riparian forest in Leota Bottom. No water chemistry samples were taken in Sheppard Bottom, nor were any of the extensive reports dealing with contaminant issues at Ouray NWR referenced such as Stephens et al. (1988, 1992) and Peltz and Waddell (1991). Consequently, the conclusion of Cooper and Severn (1994) that floodplain wetlands such as Sheppard Bottom would make ideal habitat for larval razorback sucker was inappropriate because it failed to account for potential adverse effects from contaminants through the abundant food chain they found in the refuge wetlands.

5. Conclusions

The collection of razorback sucker larvae from shorelines immediately downstream of suspected spawning areas in the Green River indicates spawning success (Tyus, 1987); however, recruitment has been very limited (Lanigan and Tyus, 1989; Gutermuth et al., 1994; Modde, 1996; Modde et al., 1996). Factors such as introduced predator species, altered habitat for adults and larvae, reduced water temperature, decreased turbidity, and altered seasonal and annual flow patterns have contributed to the decline of razorback sucker and inhibited recovery (USFWS, 1987).

This study demonstrated that relatively low selenium concentrations in the food chain, in combination with other elements in water and zooplankton resulted in low or no survival of larval razorback sucker. Waterborne concentrations of selenium in four sites at Ouray NWR (S1, S3, S4, and S5) were lower than the national water quality criterion for the protection of aquatic life, i.e.,

the 4-day average concentration not to exceed 5 μg/L (USEPA, 1987). However, exposure to dietary selenium concentrations of 2.3 μg/g or greater was sufficient to elevate whole-body residues to near the threshold where adverse effects have been reported in some other fish species. Larvae are typically much more susceptible to contaminant stresses than older fish because of their higher metabolic rate, higher surface to volume ratio, and incompletely developed metabolic pathways necessary for detoxification of xenobiotics (Rand et al., 1995). Delayed mortality in treatments with the highest concentrations of selenium in either food or water suggested an antagonist interaction between selenium and other elements. Mortality of larval razorback sucker was most rapid in the treatments where selenium concentrations were in the range of 3–5 μg/g and uninfluenced by interactions with other elements. Thus, lack of recruitment of razorback sucker in the Green River may result from or be linked with contaminant stresses from selenium and other elements in water and food organisms.

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