SELENIUM AND OTHER TRACE ELEMENTS IN WILD LARVAL RAZORBACK SUCKERS FROM THE GREEN RIVER, UTAH

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EXECUTIVE SUMMARY

Contaminant investigations of the middle Green River, Utah, have documented selenium contamination at sites receiving irrigation drainage. The middle Green River provides critical habitat for four endangered fishes including the largest extant riverine population of endangered razorback sucker. Although 2,175 larval razorback suckers were collected from the river between 1992 and 1996, very few juveniles have been captured within recent decades. Selenium concentrations were measured in larval razorback suckers collected from five sites in the middle Green River to assess the potential for adverse effects on recruitment of larvae to the juvenile stage and the adult population. Larvae from all sites contained selenium concentrations at or above the proposed toxic threshold of 4 μg/g for adverse biological effects in fish, derived from several laboratory and field studies with a wide range of fish species. At two sites, Cliff Creek and Stewart Lake Drain, selenium concentrations in larvae increased over time as fish grew, whereas selenium concentrations decreased as fish grew at Sportsmans Drain. Evaluation of a 279-larvae composite analyzed for 61 elements demonstrated that selenium, and to a lesser extent vanadium, were elevated to concentrations reported to be toxic to a wide range of fish species. Elevated selenium concentrations in larval razorback suckers from the five sites suggests that selenium contamination may be widespread in the middle Green River, and that survival and recruitment of larvae to the juvenile stage may be limited due to adverse biological effects. Selenium contamination may be adversely affecting the reproductive success of endangered razorback sucker.

Key words: Green River, razorback sucker, endangered fish, selenium, vanadium, inorganics, food chain, growth, residue

INTRODUCTION

Investigations conducted by the National Irrigation Water Quality Program (NIWQP) in the middle Green River basin, Utah, by the U.S. Fish and Wildlife Service (USFWS), U.S. Geological Survey, and Bureau of Reclamation from 1986 to 1989 detected elevated concentrations of selenium in various samples of water, sediment, and biota (Stephens et al. 1988, 1992, Peltz and Waddell 1991). Selenium concentrations were especially elevated in the areas of Ashley Creek, Stewart Lake Waterfowl Management Area, and Ouray National Wildlife Refuge (NWR). Water samples from various locations in Ashley Creek below the sewage lagoon near Vernal had selenium concentrations ranging from 25 to 150 μg/L, and at the mouth were 59 to 78 μg/L. At Stewart Lake outlet, selenium concentrations ranged from 1 to 12 μg/L. At Ouray NWR, high concentrations of selenium occurred at North Roadside Pond, South Roadside Pond, and in shallow groundwater in the Sheppard Bottoms area where samples from wells have contained up to 9,300 μg/L.

The middle Green River typically has relatively low selenium concentrations. However, because of concern about how selenium concentrations in the Green River are influenced by elevated selenium from Ashley Creek, Stewart Lake, and Sheppard Bottom at Ouray NWR as a result of surface and subsurface drainage, the U.S. Geological Survey collected water samples for selenium analysis along transects across the river at 13 sites from above Stewart Lake to Ouray NWR in 1991 and 1992. Concentrations of selenium ranged from <1 to 2 μg/L in 1991 and from <1 to 3 μg/L in 1992 (D. Stephens, personal communication, 1996). The highest concentrations during each year occurred below the confluence of Ashley Creek and Stewart Lake outlet with the Green River.

Elevated selenium concentrations have also been detected in fish from the Green River (Lowe et al. 1985, Stephens et al. 1988, 1992, Schmitt and Brumbaugh 1990, Peltz and Waddell 1991). From 1978 through 1987, 25 composite fish samples (four species) were collected near Browns Park, Echo Park, Stewart Lake Drain/Ashley Creek area, and Ouray NWR for trace element analysis (Waddell and Wiens 1994b). In 1991, 15 composite fish samples (four species) were collected from Jensen, Stewart Lake Drain/Ashley Creek area, Leota Bottom, Sheppard Bottom, and Ouray (Waddell and Wiens 1994b). In these 40 samples, concentrations of selenium ranged from 1.9 to 49 μg/g and variation was determined to be strongly correlated with fish location. Selenium concentrations were highest in fish from the Stewart Lake Drain/Ashley Creek area and Jensen areas where 82% of the samples exceeded the proposed toxicity threshold of 4 μg/g derived from several laboratory and field studies with a variety of fish species (Lemly 1993). These two river reaches receive surface water known to contain high selenium concentrations, and likely subsurface water sources. Waddell and Wiens (1994b) concluded that their data demonstrated some temporal variation. Selenium concentrations were higher in 1991 samples than in the five previous collection years (1978, 1980, 1984, 1986, 1987).

The middle Green River provides critical habitat for endangered Colorado squawfish (*Ptychocheilus lucius*), razorback sucker (*Xyrauchen texanus*), bonytail (*Gila elegans*), and

humpback chub (*Gila cypha*). The largest extant riverine population of razorback suckers in the upper Colorado River basin occurs in the middle Green River, but recent collections of razorback sucker larvae in the lower 210 km of the Green River strongly suggests localized reproduction (Muth et al. 1997). The Green River is currently the focus of a major habitat restoration program within the Recovery Implementation Program for the Endangered Fishes of the Upper Colorado River Basin.

Attempts to captive rear wild-caught razorback sucker larvae have produced mixed results. In 1994, approximately 50 larval razorback suckers were collected using light traps from Millard Canyon (river kilometer [rk] 53.9) or the Anderson Bottom-Bonita Bend area (rk 49.9-50.7) in the lower Green River and taken to the Larval Fish Laboratory for rearing. All but two of these larval fish died within 2 weeks (Muth and Wick 1997), even though proven culture techniques were used. Potential cause or contributing factor for this mortality include handling stress due to the long transportation period from the lower Green River to the Larval Fish Laboratory. Because of concerns raised by NIWQP studies about contaminant problems in the middle Green River (Stephens et al. 1988, 1992, Peltz and Waddell 1991), the observed mortality may have been due to contaminant accumulation from food organisms or waterborne exposure prior to larval fish being light-trapped or from deposition in the eggs from adult exposure prior to spawning, however, no contaminant residues were measured. In contrast, wild razorback sucker larvae collected in 1996 from the middle Green River and reared in a pond near Vernal, Utah, with few other fish species present had relatively good survival (29.3%, B. Haines, personal communication, 1996).

The purpose of this study was to determine the concentrations of selenium in larval razorback suckers previously collected from the middle Green River by light trapping by the Larval Fish Laboratory or the Colorado River Fishery Project (USFWS, Vernal, Utah). Most larval razorback suckers collected in light traps were probably ≤ 1 month posthatching (10-15 mm total length). Even though spawning habitats for razorback sucker have been speculated to be limited and the critical life stages are those from fertilized egg through the first year (Miller et al. 1982), 1,735 larvae were collected during 1992-1996 in the middle Green River and 440 larvae collected in the lower Green River (Muth et al. 1997). However, no juveniles over 1 year old have been found in the upper Colorado River basin, which includes the Green River. In contrast, Modde et al. (1996) suggested that some recruitment must be occurring because their population estimates for razorback sucker in the middle Green River could detect no significant decrease in population between 1982 and 1992. They characterized the population as precariously small but dynamic. The amount of selenium that larvae might be accumulating and the resulting effects are currently unknown.

METHODS AND MATERIALS

Study sites

Larval razorback sucker were collected between May 6 and June 13, 1994, by light traps

(Muth 1995) from five nursery habitat sites on the Green River: Cliff Creek, Stewart Lake Drain, Sportsmans Drain, Greasewood Corral at Ouray NWR, and inlet of Old Charlie Wash (Figure 1 and 2). Cliff Creek is a tributary on the east side of the river, Stewart Lake Drain and Sportsmans Drain are outlet canals on the west side of the river, and Greasewood Corral is a side channel (perhaps an old ox bow) and the inlet to Old Charlie Wash is a canal, both on the east side of the river. Collections in the middle Green River were made by the Larval Fish Laboratory or the Colorado River Fishery Project. Collections for razorback sucker larvae were part of a study to assess reproduction, distribution, and movements of mainstem razorback suckers in the middle Green River conducted under the Five-Year Flaming Gorge Research Program.

All nursery habitat sites where razorback sucker have been collected were low or zero-velocity habitats connected to the main channel during times of high flow in spring or early summer. As flows increase and habitats flood, eddies are formed near the mouths of these habitats, which serve to transport larvae from the main channel and into these habitats. After collection, larvae were preserved in ethanol and held in 20-ml glass vials at room temperature.

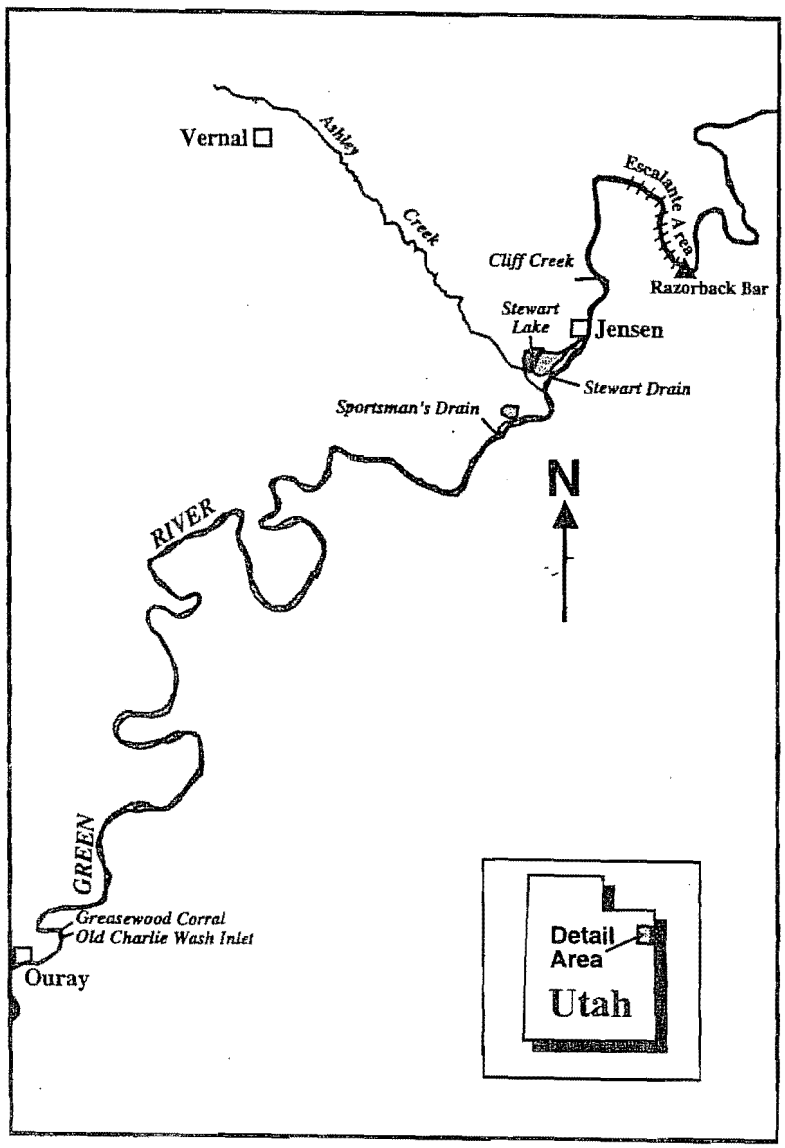
Determination of selenium in larvae

Originally, analysis of 10 individual razorback sucker larvae from each of the five sites on the Green River was requested. The 50 larvae were contained in 19 vials. Unfortunately, neutron activation analysis was performed on composites of the larvae present in the shipment vials. Consequently, 19 analyses were conducted instead of the 50 requested. Some analyses were accomplished on one larva and other analyses on composites of 2 to 10 larvae. Larvae and ethanol samples were analyzed at the Environmental and Contaminants Research Center (ECRC; formerly Midwest Science Center) and at the University of Missouri Research Reactor (MURR), both in Columbia, MO.

Neutron activation was used for the analysis of larvae because of the extremely small sample mass. All sample preparation prior to neutron activation analysis was conducted by the ECRC. Each vial of larvae was transferred from its original container into a small polyethylene vial (0.7 g capacity) provided by MURR. Vials were precleaned by stepwise washing with acetone, nitric acid, and deionized water. Each larva or composite of larvae was positioned and pressed flat against the vial bottom with a clean glass rod. All vials were left open and placed in the tray chamber of a Virus 20-SRC lyophilizer and frozen to -30°C. Samples were lyophilized to constant weight because lyophilization greatly reduces ^{19}O in the irradiated sample and significantly enhances measurement precision. Dried larva or composite larvae weights averaged 2.6 mg (range from 0.8 to 8.0 mg). After recording of final sample weight, an expandable, clean polyethylene plug was inserted into the vial, against which the vial lid was compressed shut. The polyethylene plug served to maintain constant sample geometry. Samples were transported to MURR for the determination of the radionuclide Se^{77m} (McKown and Morris 1978).

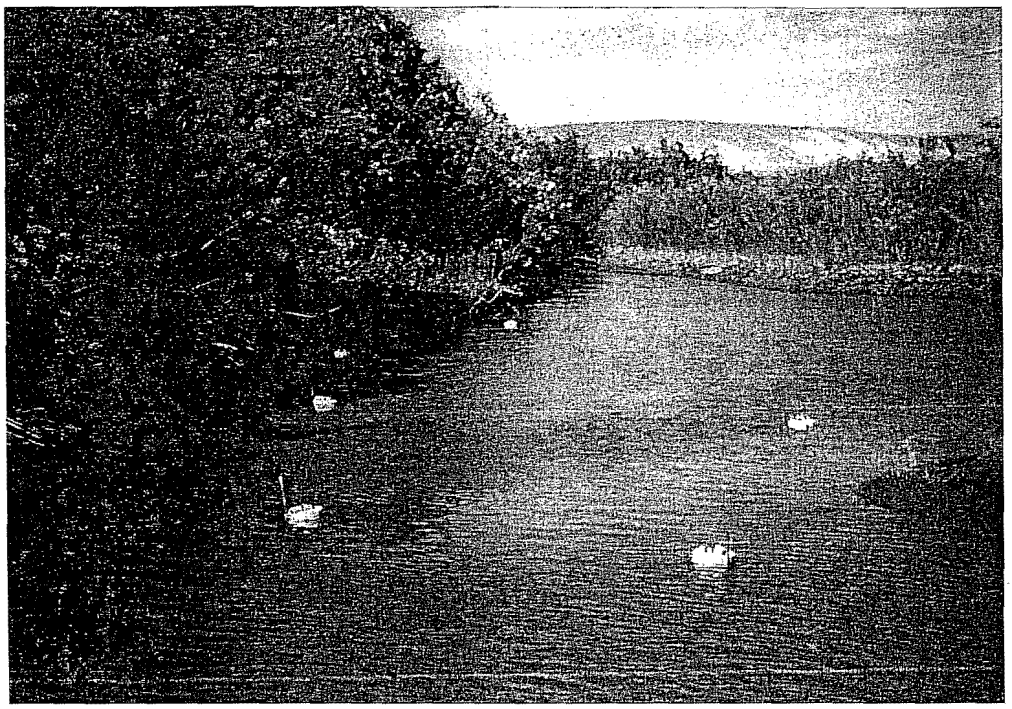
Because the major contributions of gross activity in irradiated tissue are produced from sodium and chlorine, standard solutions containing normal concentrations of selenium, sodium,

Figure 1. Map of middle Green River showing location of sampling sites for light trapping of larval razorback sucker.



0 4 8 MILES
0 4 8 KILOMETERS

Figure 2. Light trap deployment patterns at Cliff Creek and Stewart Lake Drain.



Cliff Creek

Stewart Lake Drain

and chlorine were prepared for the larval tissue. Irradiation standards were prepared by placing small aliquots of the standard solutions onto cellulose. Samples and standards were successively placed in a shuttle rabbit and irradiated for 5 seconds at a thermal neutron flux of about 8×10^{14}n $\times$ cm$^{-2} \times$ sec^{-1}. The pneumatic transfer facility had a delivery time to the counting station of about 7 seconds. The returned shuttle rabbit was opened quickly, and the sample vial was transferred to a 45-cm^3 germanium-lithium gamma ray spectrometer system. All samples were analyzed by a 5-second irradiation, 15-second decay, and 20-second count, with a sample to detector distance giving less than 10% deadtime at the analyzer (about 3 cm). Selenium standards and quality control samples were analyzed in the same manner. National Institute of Standards and Technology (NIST) 1577 (bovine liver) standard reference material was analyzed by MURR as quality control checks on accuracy and precision. 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.

Samples of the ethanol preservative and a composite sample of larval razorback suckers were analyzed for 61 elements by inductively-coupled plasma-mass spectrometry (ICP-MS). Seven vials containing ethanol only (no larvae) were combined, referred to as clean ethanol, and analyzed as one sample. Ethanol from in 26 vials that originally contained larvae was combined, referred to as dirty ethanol, and analyzed as one sample. One composite of 279 razorback suckers was formed from larvae collected between May 19 and June 6 at Stewart Lake Drain. Guts and otoliths of these larvae were removed and retained at the Larval Fish Laboratory for other determinations. The larval composite was placed in a 20-ml scintillation vial and lyophilized to a constant dry weight. The clean and dirty ethanol samples were each placed into a 250-ml Zymark TurboVap II tube and the samples evaporated with a TurboVap water bath at 50°C and nitrogen vortexing to approximately a 1-ml endpoint. Each ethanol liquid was transferred to a Questron bomb vessel (Teflon) by rinsing with 5 ml concentrated nitric acid. The lyophilized fish larvae composite sample (0.091 g) was also transferred to a Questron bomb to which 5 ml concentrated nitric acid was added. The bombs were sealed and placed in a CEM microwave oven which proceeded through a six-step program to acid digest the samples. After the bombs were cooled for 2 hours in a freezer, the resulting digestate liquid was transferred to a polyethylene bottle and diluted to 100 ml. The diluted digestate matrix was 5% nitric acid. Quality control samples carried through the procedure included ethanol blanks, spiked ethanol, four nitric acid digestion blanks, and spiked nitric acid blank.

All digestates were analyzed for elements using a PE/SCIEX Elan 6000 ICP-MS. The operation was conducted in Totalquant mode, which is an exhaustive semi-quantitative scan across the mass spectral range for 61 elements. Accuracy of the semi-quantitative scan is typically ±30% to ±50 % better, i.e., lower detection limits, than regular ICP analysis depending on analyte mass. Elemental response factors were adjusted prior to analysis with a certified reference solution (High-Purity Trace Metals in Drinking Water; High Purity Standards, Charleston, SC). This same solution was analyzed every 10 samples to provide an estimate of accuracy throughout the sample analysis. NIST 1643d (water) certified reference solution was analyzed as a laboratory control sample. Beryllium (80 μg/L), rhodium (10 μg/L), and bismuth

(10 μg/L) were used as internal standards to correct for instrumental drift. Elemental concentration values obtained for dirty ethanol were blank corrected by subtracting clean ethanol results. Similarly, the spiked ethanol was background corrected by subtracting results obtained from two ethanol blanks. Background correction for the larvae digestion came from the four nitric acid digestion blanks.

Statistics

The weighted mean was calculated for selenium concentrations in individual larvae and composites of larvae by weighting the concentration in the composite by the number of larvae in the composite. The Pearson correlation coefficient for the relation between fish total length and whole-body selenium concentrations was determined using Statistical Analysis System programs (SAS 1990).

RESULTS

Neutron activation and ICP-MS analyses

Results from analysis of four samples of NIST 1577 (bovine liver) standard reference material by neutron activation were all within the certified range, and method precision was 6.3% percent relative standard deviation. The limit of detection for the Se^{77m} method was 15 ng/g dry weight or 0.45 ng of selenium. The accuracy, precision, and limit of detection checks by MURR were all based on 48-mg samples. Consequently, the accuracy and precision of selenium measurements in the larvae samples may have been lower because their sample weights ranged from 0.8 to 8.0 mg.

For ICP-MS analysis of fish larvae, detection limits were categorized as follows: <1 μg/g: Li, Sc, Ga, Ge, As, Rb, Y, Nb, Ag, Sb, Cs, Th, U, and 27 rare-earth elements; <10 μg/g: V, Cr, Co, Ni, Se, Zr, Mo, Cd, Sn, and Pb; <50 μg/g: Ti, Mn, Cu, and Ba; >200 μg/g: Na, Mg, Al, K, Ca, Fe, and Zn. Recovery of 11 elements (Cr, Ni, Cu, Zn, As, Se, Cd, Sn, Sb, Hg, Pb) spiked into ethanol ranged from 70 to 135% for most elements. Severe losses were indicated for arsenic and selenium, whereas contamination was indicated for lead. Recoveries of these same 11 elements spiked into a blank solution prior to microwave digestion ranged from 94 to 108%, but contamination was apparent for tin, mercury, and lead. Analysis of High-Purity Trace Elements in Drinking Water analyzed after every 10 samples showed close agreement with certified values. Analysis of 24 elements in NIST 1643d (water) reference material resulted in recoveries of 80 to 111% for 20 elements, but were low for boron and iron and high for selenium and molybdenum.

There seemed to be little loss of elements from larvae into the ethanol storage medium. Only calcium, sodium, and zinc concentrations were elevated in dirty ethanol compared to clean ethanol. There was scant selenium in either the clean or dirty ethanol. Consequently, storage of larvae in ethanol did not seem to alter inorganic concentrations in larvae.

Selenium in larvae

Selenium concentrations in larvae from the five sites fell within a relatively narrow range of 2.24 μg/g at Cliff Creek to 7.42 μg/g at Stewart Lake Drain (Table 1). In general, fish collected at later dates were larger than those collected earlier (Table 1, Figure 3). For larvae collected at Cliff Creek and Stewart Lake Drain, selenium concentrations in larvae seemed to increase over time as fish grew, whereas they decreased at Sportsmans Drain and fluctuated at Greasewood Corral (Figure 4). Selenium concentrations in larvae were positively correlated to fish total length at Cliff Creek (R=0.65, P=0.16), Stewart Lake Drain (R=0.996, P=0.05), and Greasewood Corral (R=0.77, P=0.13), but not at Sportsmans Drain (R=-0.46, P=0.54). For the sites with positive correlations plus the one data point for Old Charlie Wash, the correlation coefficient for the combined data was R=0.69 (P=0.01).

The selenium concentration measured by ICP-MS in the 279-larvae composite sample from Stewart Lake Drain was 8.0 μg/g (Table 2), which was close to the weighted mean of the three samples from the same site measured by neutron activation, i.e., 5.8 μg/g. Several inorganics in the ICP-MS scan seemed elevated and included cadmium, copper, lead, selenium, vanadium, and zinc.

DISCUSSION

Larval abundance

During 1992-1996, 1,735 razorback sucker larvae were collected from main-channel or nursery habitats of the middle Green River, with considerable variation between years: 20 in 1992, 292 in 1993, 1,217 in 1994, 32 in 1995, and 174 in 1996 (Muth et al. 1997). Of these larvae, 40% were captured in the Escalante reach, 41% in the Jensen reach, and 20% in the Ouray reach. In the Escalante reach, over 90% of the larvae were collected at Cliff Creek. In the Jensen reach, 83% were collected at Stewart Lake Drain and 6% at Sportsmans Drain. In the Ouray reach, 85% were collected at Greasewood Corral and 14% at Old Charlie Wash inlet. The larvae evaluated in the present study were taken from the 1994 collection and were most likely representative of those in the middle Green River.

Natural recruitment of larvae to the juvenile life stage seems to be limited. Only 8 juveniles were collected in the Colorado River between Moab and Dead Horse Point, Utah, in 1962-1964 (Taba et al. 1965), 2 within Ouray NWR in 1993 (Utah Division of Wildlife Resources, unpublished data), 11 in Leota Bottom at Ouray NWR in 1994 (Modde and Wick 1997), 28 in Old Charlie Wash in 1995 (Modde 1996), 45 in Old Charlie Wash in 1996 (Modde 1997), and 2 in the lower Green River near Hell Roaring Canyon in 1991 (Gutermuth et al. 1994). Collection of juvenile razorback sucker at Old Charlie Wash was remarkable because it was dominated by nonnative predators and competitors (Modde 1997).

The suitability of wetlands in the Ouray area were evaluated in 1991 with 1-2 year old

Table 1. Location and dates of collection, individual and mean total length (mm; standard error in parentheses; n=10 for each location), and individual, composite, and weighted mean selenium concentration (μg/g dry weight and standard error in parentheses) analyzed by neutron activation of wild larval razorback sucker from five sites in the middle Green River.

Location	River kilometer (mile)	Collection date	Individual/ Total length (mm)	Composite number	Selenium (μg/g)
Cliff Creek	487.5	May 16	11.3	1	2.24
	(302.8)	May 19	11.5	2	3.82
		May 30	12.0	3	3.59
		June 2	11.0	4	3.90
		June 2	11.2	4	-
		June 2	11.2	4	-
		June 13	11.8	5	5.29
		June 13	11.0	6	5.42
		June 13	14.5	6	-
		June 13	15.8	6	-
	Mean		12.1		4.29
	(SE)		(0.5)		(0.34)
Stewart Lake Drain	481.7	May 23	11.0	1	4.47
	(299.2)	May 23	11.0	1	-
		May 23	11.6	1	-
		May 23	12.0	1	-
		May 23	12.5	1	-
		May 30	12.0	2	7.04
		May 30	12.2	2	-
		May 30	12.5	2	-
		May 30	14.0	2	-
		June 2	13.0	3	7.42
	Mean		12.2		5.79
	(SE)		(0.3)		(0.44)
Sportsmans Drain	477.4	May 23	11.4	1	6.11
	(296.5)	May 23	11.5	1	-
		May 23	11.5	2	5.46
		May 23	11.8	2	-
		May 23	12.4	3	4.88
		May 23	13.0	3	-

Table 1. Continued.

Location	River kilometer (mile)	Collection date	Individual/ Total length (mm)	Composite number	Selenium (μg/g)
		May 30	12.5	4	4.26
		May 30	10.6	4	-
		May 30	11.6	4	-
		May 30	12.4	4	-
	Mean		11.9		4.99
	(SE)		(0.2)		(0.24)
Greasewood Corral	405.4 (251.9)	May 23	11.3	1	2.80
		May 26	11.3	2	5.34
		May 26	11.6	2	-
		May 26	12.5	2	-
		May 26	12.7	2	-
		May 30	11.7	3	3.20
		June 2	12.2	4	4.20
		June 6	11.9	5	4.40
		June 6	12.1	5	-
		June 6	12.5	5	-
	Mean		12.0		4.48
	(SE)		(0.2)		(0.29)
Old Charlie Wash Inlet	405.4 (251.8)	May 26	11.5	1	5.84
		May 26	11.7	1	-
		May 26	11.8	1	-
		May 26	11.9	1	-
		May 26	11.9	1	-
		May 26	12.0	1	-
		May 26	12.0	1	-
		May 26	12.0	1	-
		May 26	12.0	1	-
		May 26	12.5	1	-
	Mean		11.9		5.84
	(SE)		(0.1)		(-)

Figure 3. Total length (mm) of larval razorback sucker collected at five sites in the middle Green River.

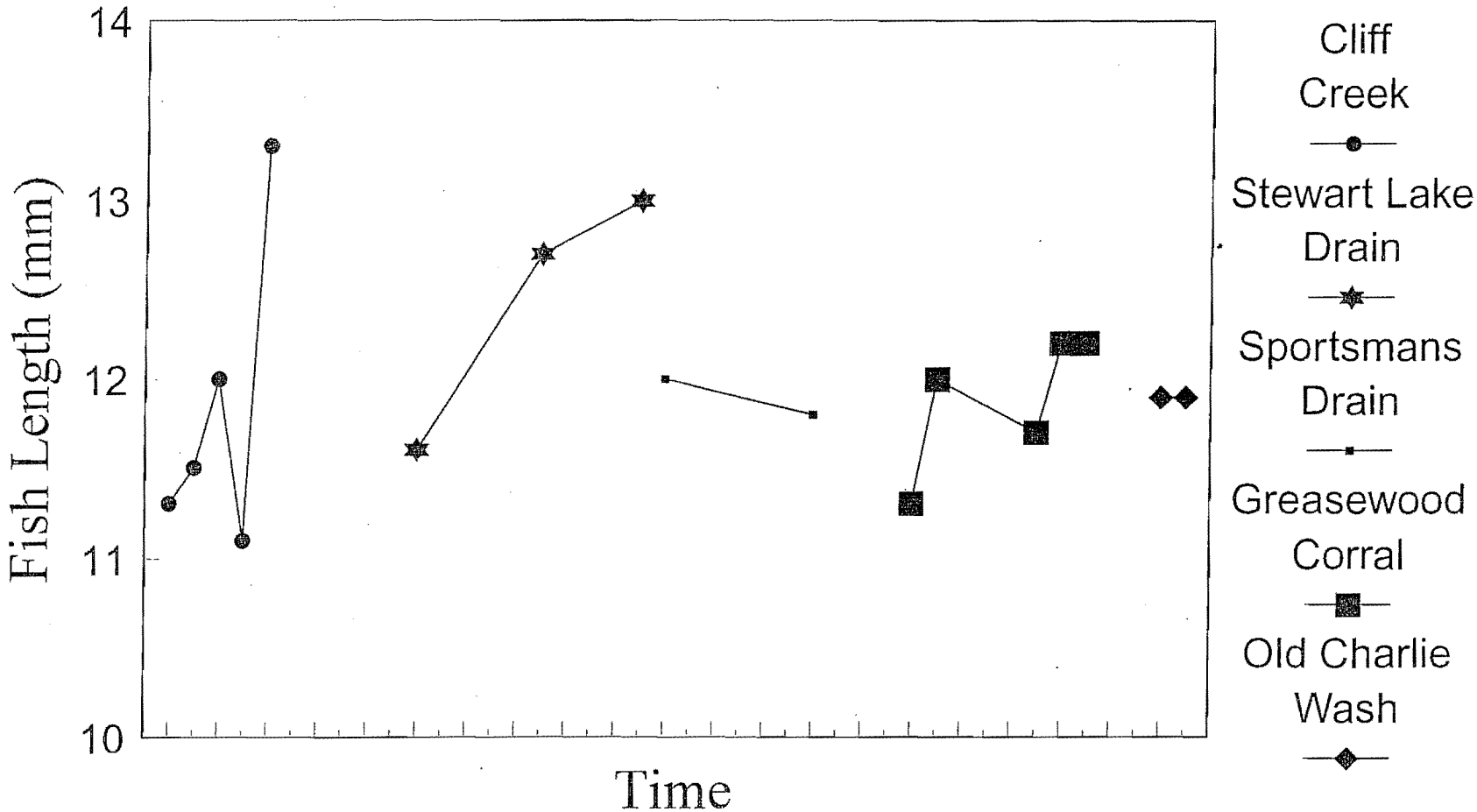

14
13
12
11
10
Fish Length (mm)
Time
Cliff Creek
Stewart Lake Drain
Sportsmans Drain
Greasewood Corral
Old Charlie Wash

Figure 4. Selenium concentrations (μg/g) in larval razorback sucker collected at five sites in the middle Green River.

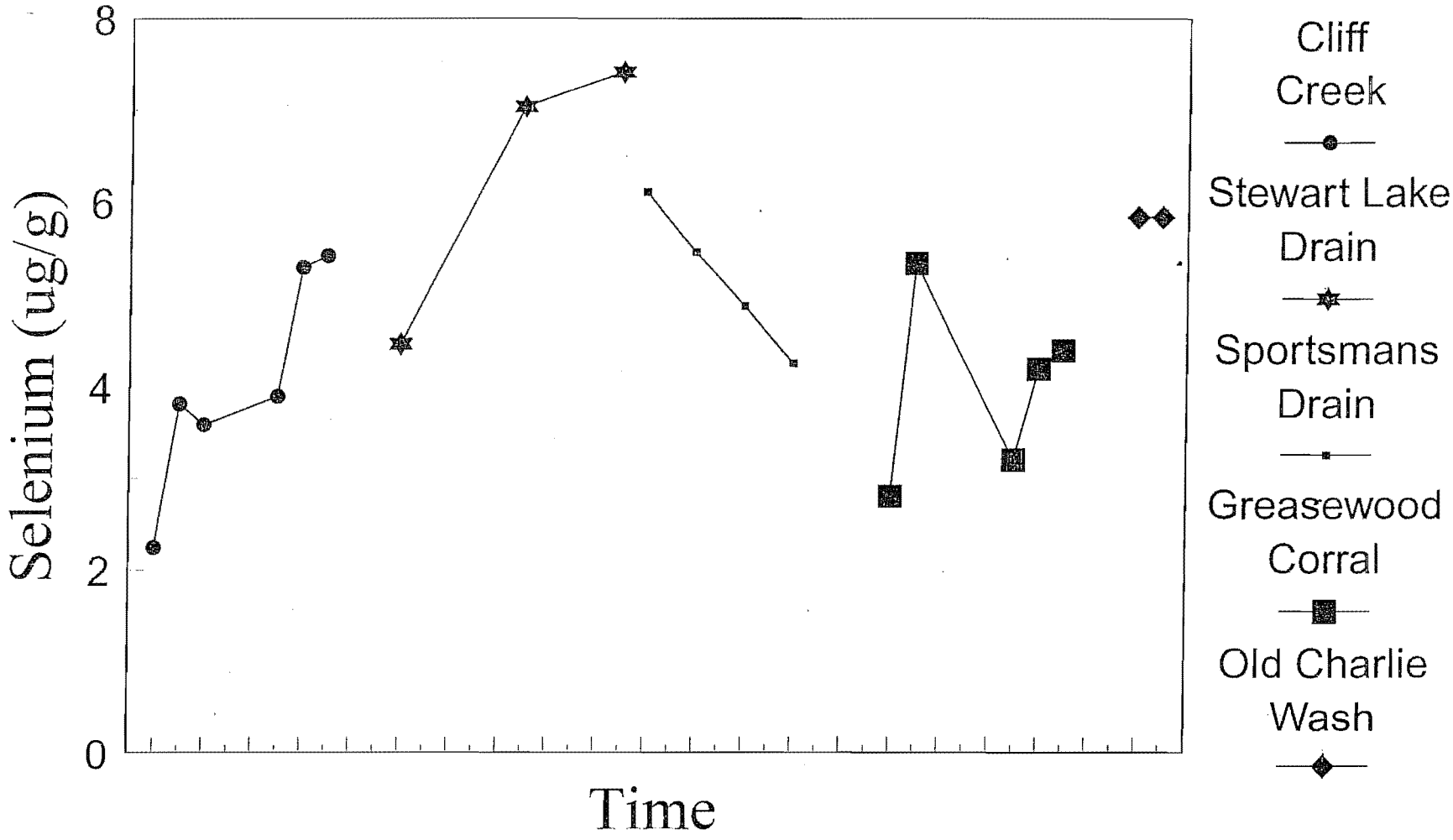

8
6
4
2
0
Selenium (ug/g)
Time
Cliff Creek
Stewart Lake Drain
Sportsmans Drain
Greasewood Corral
Old Charlie Wash

Table 2. Concentrations of 61 elements (μg/g dry weight; n=1) analyzed by inductively-coupled plasma-mass spectroscopy in wild larval razorback suckers (mean total length 11.5 mm, standard error 0.04, n=279) from Stewart Lake Drain between May 19 and June 6 in the middle Green River, and in clean and dirty ethanol (mg/L; n=1) used to store larval at the Larval Fish Laboratory.

Element	Fish (μg/g)	Ethanol (mg/L)	
		Clean	Dirty
Al	640	0.110	0.062
Ag	0.052	0.00002	0.00001
As	0.50	0.00001	0.00006
Au	0.003	0.00005	0.00002
Ba	17	0.001	0.001
Ca	10028	0.116	0.356
Cd	4.9	0.00002	0.00043
Ce	0.56	0.00003	0.00005
Co	2.5	0.0001	0.0002
Cr	4.6	0.004	0.012
Cs	0.055	ND[1]	ND
Cu	40	0.002	0.003
Er	0.011	ND	ND
Eu	0.011	ND	ND
Dy	0.033	ND	ND
Fe	246	0.027	0.097
Ga	0.25	0.00003	0.00002
Gd	0.044	ND	ND
Ge	0.011	ND	ND
Hf	0.033	0.00013	0.00002
Ho	0.011	ND	ND
In	ND	ND	ND
Ir	ND	ND	ND
K	260	0.018	0.140
La	0.30	0.00001	0.00002
Li	0.346	0.0002	0.0006
Lu	ND	ND	ND
Mg	4196	0.012	0.113
Mn	34	0.001	0.003
Mo	1.7	0.0002	0.0001
Na	662	0.897	3.610
Nb	0.044	ND	0.00001

Table 2. Continued.

Element	Fish (μg/g)	Ethanol (mg/L) Clean	Ethanol (mg/L) Dirty
Nd	0.25	ND	0.00002
Ni	5.0	0.003	0.006
Os	ND	ND	ND
Pb	3.8	0.001	0.001
Pd	ND	ND	ND
Pr	0.077	ND	0.00001
Pt	0.25	0.00003	0.00001
Rb	0.86	0.00001	0.00011
Re	ND	ND	ND
Ru	ND	ND	ND
Sc	0.011	ND	ND
Se	8.0	0.001	0.001
Sb	0.16	ND	0.00002
Sm	0.044	ND	ND
Sn	1.8	0.004	0.004
Ta	ND	ND	ND
Tb	ND	ND	ND
Te	ND	ND	ND
Th	0.088	0.00001	0.00001
Ti	33	0.001	0.002
Tl	0.022	ND	ND
Tm	ND	ND	ND
U	0.29	0.00001	0.00001
V	1.7	0.0005	0.00009
W	ND	0	0
Y	0.15	0.00001	0.00002
Yb	0.022	ND	ND
Zn	299	0.008	0.029
Zr	1.1	0.006	0.001

[1]ND=not detected; value of 0.00000 resulting from TotalQuant determination (semi-quantitative analysis).

razorback suckers held in cages at three wetland sites (Old Charlie Wash, Leota Bottom, Sheppard Bottom), three backwater sites (Haymaker, Woods, West Bend), and the Ouray Native Fish Facility as the reference site (Modde and Wick 1997). The best survival occurred at the hatchery and worst at the backwater site, where fish had negative growth during the 3-4 week study. This study showed that subadults could do well in wetlands with substantial food organisms, but not in backwater areas with low food organism densities.

Selenium at sites

Concentrations of selenium in fish were reported by Waddell and Wiens (1994b) for five reaches of the middle Green River. They found selenium concentrations in fish were low at Browns Park (1.9-3.2 μg/g) and Echo Park (2.7-4.2 μg/g), elevated at Jensen (4.6-21 μg/g, geometric mean 10.8, n=3) and Stewart Lake Drain/Ashley Creek area (3.1-49 μg/g, geometric mean 12.1, n=8), and slightly elevated at Leota Bottom (3.6-5.7 μg/g, geometric mean 4.6, n=3), Sheppard Bottom (3-5.7 μg/g, geometric mean 4.3, n=3), and Ouray (2.5-7.6 μg/g, geometric mean 3.5, n=18). Concentrations in larval fish in the present study were similar in that the lowest selenium concentration was in larvae collected upstream of Stewart Lake Drain at Cliff Creek (2.24 μg/g), the highest at Stewart Lake Drain (7.04 and 7.42 μg/g) with lower concentrations at the downstream sites.

There is no information on selenium concentrations in water, sediment, or biota for Cliff Creek in the NIWQP investigations or USFWS contaminant investigations. Waddell and Wiens (1994a) reported that the Brush Creek drainage, located adjacent and north of the Stewart Lake Drain/Ashley Creek area, had a substantial selenium contamination problem in biota, and was contributing to selenium loading in the Jensen reach of the river. Brush Creek is about 2.5 km upstream of Cliff Creek. Selenium concentrations in larvae collected at Cliff Creek increased over time and were positively correlated with increasing fish total length, thus indicating that larvae were accumulating selenium. However, Cliff Creek does not receive irrigation drainage, but at its confluence with the Green River, would be subject to selenium input from the river, especially selenium loading from Brush Creek.

Selenium contamination of Stewart Lake and its outflow have been well documented by Stephens et al. (1988, 1992) and Peltz and Waddell (1991), and was probably responsible for the elevated selenium concentrations in larvae collected at the Stewart Lake Drain site in the present study. Stephens et al. (1988) reported selenium concentrations in June and August, 1986, in the outflow water were 7 μg/L and in April and August, 1987, were 6 and 10 μg/L, respectively. Selenium in sediments of the outflow in 1986 were 5.1 μg/g. Selenium concentrations in fish collected in 1986 from the south side of Stewart Lake were 16 μg/g in black bullhead (*Ameiurus melas*), 23 μg/g in common carp (*Cyprinus carpio*), and 26 μg/g in green sunfish (*Lepomis cyanellus*). Stephens et al. (1992) and Peltz and Waddell (1991) also reported elevated selenium concentrations in 1988-1989 in outflow water (2-12 μg/L) and in fish (11-25 μg/g). They also reported selenium concentrations in aquatic invertebrates collected from the south side of Stewart Lake close to the outlet were 10-16 μg/g in three mixed invertebrate sample, 13.5 μg/g in a

corxid sample, and 27 μg/g in a predominantly chironomid sample. All of the selenium concentrations in aquatic invertebrates were substantially elevated, and above the toxic threshold of 3 μg/g proposed by Lemly (1993) for food organisms consumed by fish and wildlife. The Stewart Lake Drain/Ashley Creek area is the most selenium contaminated site in the Green River.

In 1992, an on-site toxicity test was conducted using water collected from Stewart Lake Drain with 3-day-old fathead minnow (*Pimephales promelas*), 40-60-day-old razorback sucker, and 24-hour-old *Ceriodaphnia* (Finger et al. 1994). No appreciable mortality occurred in the 10-day tests with fathead minnow or razorback sucker (10% mortality), but there was a 30% mortality and impaired reproduction in the *Ceriodaphnia* test. During these tests, selenium concentrations in outflow water ranged from 3 to 5 μg/L, which is far less than had been measured at other times. The lack of effects on razorback sucker is not surprising because the older life stage tested would have been more tolerant of contaminant stresses than if the test were conducted with an earlier, more sensitive life stage (Rand and Petrocelli 1985). Moreover, their study involved only waterborne exposure, which for selenium would have caused less stress than if a dietary selenium toxicity test had been conducted (Lemly and Smith 1987, Lemly 1993).

In contrast, acute toxicity tests with young razorback sucker (7-29 days old), Colorado squawfish (8-15 days old), and bonytail (4-19 days old) tested with a mixture of nine inorganics simulating the environmental ratios and concentrations in Stewart Lake Drain and tested a reconstituted simulating the middle Green River showed that the three species were very sensitive to the inorganic mixture (Buhl and Hamilton 1996). They compared the acute toxicity values with measured concentrations in Stewart Lake Drain and derived a high hazard, which suggested a high potential for adverse effects in long-term exposures. In two 90-day chronic toxicity tests, one with razorback sucker and the other with bonytail, fish were exposed to a similar nine-element mixture simulating Ashley Creek and tested in reconstituted middle Green River water (Hamilton et al. 1998). Adverse effects such as reduced growth, reduced swimming performance, and reduced survival were observed at concentrations within a factor of 4-8 times environmental concentrations, which confirmed the high hazard derived from the acute tests, and further demonstrated a high hazard existed.

The elevated selenium concentrations in larvae collected at Sportsmans Drain may be linked to the elevated selenium concentrations in the adjacent water impoundment reported Stephens et al. (1988, 1992) and Peltz and Waddell (1991). They referred to the impoundment as Marsh 4720 (4720 refers to the elevation of the marsh), but the site has also been called Little Stewart Lake or Sportsmans Lake by others, and is identified as the Unitah Sportsmans Club Lake in the Green River Wilderness Desolation River Guide (Evans and Belknap 1992) and by Muth et al. (1997). Sportsmans Lake receives irrigation tailwaters, but does not receive irrigation return flows by way of subsurface drains. The lake also receives surface inflow from the Lower Union Canal whose source of water is Ashley Creek near Highway 40 (below the Vernal sewage lagoons). This water contains 40-140 μg/L of selenium and is used to irrigate adjacent agricultural fields. Stephens et al. (1988) reported selenium concentrations in 1986 in

Sportsmans Lake were 31 μg/L in water, 4.2 μg/g in sediment, 7 μg/g in black bullhead, and 19 and 21 μg/g in common carp. Stephens et al. (1992) and Peltz and Waddell (1991) reported elevated selenium concentrations in 1988 were 26-130 μg/L in inflow water and 7 μg/L in the main marsh, 11-41 μg/g in aquatic invertebrates (Hemiptera), 41 μg/g in a mixed sample predominated by chironomids, and up to 37 μg/g in fish. All of these selenium concentrations were substantially elevated probably due to inflow of high selenium water from Ashley Creek via Lower Union Canal. The lower selenium concentration in the main marsh water compared to the inflow water was similar to reports by others who have found rapid uptake of selenium from water into aquatic plants (Allen 1991, Ornes et al. 1991), algae (Besser et al. 1993, Foe and Knight 1985, Nassos et al. 1980, Riedel et al. 1991), sediments and periphtyton (Graham et al. 1992), and bacteria and detritus (Bender et al. 1991, Presser et al. 1994). Outflow or seepage of marsh water, detrital matter, or food organisms with elevated selenium into Sportsmans Drain may have allowed some of the larval razorback sucker to accumulate selenium in the present study. Nevertheless, based on the negative correlation between fish total length and selenium concentrations in larvae, it seems the larvae at Sportsmans Drain were probably depurating selenium from their tissues. The higher selenium concentrations in small larvae may have come from deposition in female gonads at spawning.

There is no information on selenium concentrations in water, sediment, or biota for Greasewood Corral in the NIWQP investigations or USFWS contaminant investigations. Larvae collected at this site seemed to be slowly accumulating selenium, slower than larvae collected at Cliff Creek and Stewart Lake Drain, as evidenced by the positive correlation between fish total length and selenium concentrations in larvae. The only source of selenium loading at Greasewood Corral would be from the river, either upstream from the Stewart Lake Drain/Ashley Creek area or from Sheppard Bottom, which has been documented to have high selenium concentrations in surface and groundwater, aquatic and benthic invertebrates, sediments, and wildlife (Stephens et al. 1988, 1992, Peltz and Waddell 1991). Larvae in the present study were collected in low or zero velocity habitats, which are the most vulnerable to selenium uptake and cycling in biota (Lemly and Smith 1987).

The elevated selenium in larvae at Old Charlie Wash inlet may have resulted from exposure to elevated selenium concentrations from riverine sources similar to Greasewood Corral. In Old Charlie Wash, Wiens and Waddell (1996) reported aquatic invertebrates collected by light trap contained 1.7 to 4.1 μg/g in 1993 and a mean of 4.1 μg/g (n=4) in 1994. Four out of five samples in 1993 and all samples in 1994 were within the level of concern range [2-3 μg/g] proposed by the Interagency Technical Teams for Phase 4 Remediation Planning for the Kendrick and Middle Green River Projects, NIWQP (Table 4 in Stephens et al. 1997). These selenium concentrations in Old Charlie Wash were derived from riverine sources via the inlet canal, because Old Charlie Wash does not receive surface or subsurface irrigation drainage. Wiens and Waddell (1996) concluded that Old Charlie Wash may not be suitable as a juvenile razorback sucker rearing area due to the number of samples found at levels of concern for selenium. Nevertheless, 28 young of year razorback sucker were collected there in 1995 (Modde 1996), and 45 were collected in 1996 (Modde 1997); however, selenium residues were not

measured in the juveniles or other ecosystem components.

Selenium in larvae

The selenium concentrations in the larvae in the present study may have come in part from the spawning adults. Waddell and May (1995) reported that selenium concentrations in muscle plugs from all the adult razorback suckers caught at Stewart Lake outlet (1 fish) and Ashley Creek (9 fish; 11.9 - 54.1 μg/g), and 3 out of 12 caught at Razorback Bar (11.5 - 32.0 μg/g) had greater than 8 μg/g. Selenium concentrations in muscle tissue equal to or greater than 8 μg/g have been implicated in reproductive failure in fish (Lemly and Smith 1987). Stephens and Waddell (1998) reported selenium concentrations in muscle plugs from additional wild razorback suckers collected from the Green River. Selenium concentrations in 7 out of 12 additional fish caught in the Escalante Bar-Razorback Bar area were 8 μg/g or higher (8 - 46.2 μg/g), whereas an additional eight fish caught in the Old Charlie Wash area of the Green River all had concentrations of 5 μg/g or less (3.1 - 5.0 μg/g). Their combined data shows that 10 out of 26 adults from the Escalante Bar-Razorback Bar area, 10 out of 10 adults from the Stewart Lake Drain/Ashley Creek area, and none out of 9 from the Old Charlie Wash area had selenium concentrations above 8 μg/g, which is the proposed threshold for adverse reproductive effects in fish based on muscle concentrations. The elevated selenium concentrations in fish from the Stewart Lake Drain/Ashley Creek area may have been a result of adults temporarily using the Stewart Lake Drain/Ashley Creek area at some point prior to spawning. Consequently, some of the larvae evaluated in the present study may have come from adults with elevated amounts of selenium in their tissues.

When the Green River flooded in 1995, one adult razorback sucker was captured in Stewart Lake, and in 1997 during the flooding four adult razorback suckers and two Colorado squawfish were captured in the lake (E. Peterson, personal communication, 1997). Between 1979 and 1986, Tyus (1987) captured large numbers of adult razorback suckers just prior to spawning at the confluence of Stewart Lake Drain and Ashley Creek with the Green River. He also reported that five adults captured at Ashley Creek moved downstream to Old Charlie Wash to use that flooded bottomland. Tyus and Karp (1990) reported finding additional adults in the lower 0.8 km of Ashley Creek in 1987-1989, and that other researchers (P. Holden and L. Crist) had found 56 adults in the Ashley Creek- Jensen area between 1978 and 1980. Post-spawning use by adult razorback sucker of the Stewart Lake Drain/Ashley Creek mixing zone with the Green River has also been observed (Waddell and Wiens 1992). Modde (1993) reviewed adult razorback sucker captures between 1975 and 1991 and concluded that the Stewart Lake Drain/Ashley Creek area was regularly used by razorback suckers in both high and low flow water years. He also concluded that because of the documented high selenium concentrations at the Stewart Lake Drain/Ashley Creek area in water, sediments, and biota reported by Stephens et al. (1988, 1992), and Peltz and Waddell (1991), "it is likely that a significant portion of the remaining razorback sucker population in the middle Green River have been exposed to selenium contamination." Selenium concentrations in muscle plugs from adults confirm this conclusion (Waddell and May 1995, Stephens and Waddell 1998).

The selenium concentrations in some of the wild adults reported by Waddell and May (1995) and Stephens and Waddell (1998) were substantially higher than those in adult razorback suckers held for a year at three sites with varying amounts of selenium in water and food near Grand Junction, CO, as part of a reproduction study (unpublished data). In that study, adults at the reference site, Horsethief Canyon State Wildlife Area, had selenium concentrations in muscle plugs ranging from 4.4 to 5.2 μg/g (means of 2-9 fish at each sampling). At a second site, Adobe Creek, where selenium concentrations in water ranged from 2 to 10 μg/L and in zooplankton from 14 to 52 μg/g, selenium in muscle plugs increased from 3.9 μg/g at stocking to 12 μg/g a year later. At a third site, North Pond at Walter Walker State Wildlife Area, where selenium concentrations in water ranged from 4 to 14 μg/L (2 months prior to stocking the site had 115-133 μg/L) and in zooplankton from 21 to 40 μg/g (2 months prior to stocking, invertebrates at the site had up to 66 μg/g), selenium in muscle plugs increased from 4.1 μg/g at stocking to 17 μg/g a year later. Forty percent (18 out of 45) of the adults sampled by Waddell and May (1995) and Stephens and Waddell (1998) had selenium concentrations equal to or higher than the fish held at the Adobe Creek and North Pond sites, even though those fish were held at these two elevated selenium environments for a year and they had no opportunity to move to low selenium environments. The higher selenium in a substantial portion of the fish reported by Waddell and May (1995) and Stephens and Waddell (1998) suggests that some adults choose, or are forced due to lack of uncontaminated habitat, to use habitat with high selenium in water, food organisms, or both. It also suggests that wild razorback sucker can accumulate substantial amounts of selenium in their tissues even though they were free to move throughout the Green River in search of suitable habitat. Modde (1993) reviewed the capture records for razorback suckers and concluded that the Stewart Lake Drain/Ashley Creek area was regularly used by adults, especially in low flow years when contaminant effects from selenium would not be ameliorated by dilution with river water as in high flow years.

Hamilton and Waddell (1995) reported that selenium concentrations in eggs of wild adult razorback suckers collected from Razorback Bar in 1992 were 3.7 to 10.6 μg/g. These concentrations were within the range of those reported in eggs of razorback sucker near Razorback Bar in 1988 (4.9 μg/g, Peltz and Waddell 1991) and in 1992 (28 μg/g, Waddell and Wiens 1992). Hamilton and Waddell (1994) concluded that selenium concentrations in eggs were sufficiently elevated to suspect reproductive problems that may be contributing to the decline of razorback sucker in the upper Colorado River basin. In the reproduction study with adult razorback suckers held at three sites in Grand Junction, CO, the mean selenium concentration in eggs from fish at the reference site was 6.5 μg/g, whereas at the two sites with elevated selenium, eggs contained 46 μg/g at Adobe Creek and 38 μg/g at North Pond (unpublished data). Adverse effects in larvae hatched from those eggs were observed such as reduced growth and survival, and a variety of deformities. Because the muscle plugs in these captive held adults contained lower selenium concentrations than in 40% of the adults sampled by Waddell and others, it seems reasonable to assume that eggs and the resulting larvae from wild adults would probably contain elevated selenium concentrations, which would result in reduced survival of larvae in the environment.

Selenium concentrations in eggs could result in slightly higher concentrations in hatched larvae because the chorion membrane probably would not contribute much selenium to the egg, yet would contribute mass. Selenium in fish eggs is carried as part of the yolk precursor proteins, lipovitellin and phosvitin, and is incorporated into egg immunoglobulin and vitellogenin and transferred to yolk molecules (Kroll and Doroshov 1991).

The selenium in larvae in the present study may also have come from waterborne and dietary uptake of selenium. Although larvae were probably less than a month old at the time of capture by light trap, they would have been exposed to selenium and other inorganics in water since hatching, and in food organisms since initiation of feeding at about 4-5 days old. Several reports have documented elevated selenium concentrations in water and biota at Brush Creek, Stewart Lake Drain, Ashley Creek, Sportsmans Lake, and Sheppard Bottom, which have contributed selenium loading to the middle Green River (Stephens et al. 1988, 1992, Peltz and Waddell 1991, Waddell and Wiens 1994a). This selenium loading has resulted in elevated selenium in adults and contributed to the exposure of larvae and their concomitant accumulation of selenium.

Selenium concentrations in larvae from several of the collection sites increased over time. At Cliff Creek, larvae collected on May 16 had the lowest selenium, those collected between May 19 and June 2 had intermediate concentrations, and those collected on June 13 had the highest concentration (Table 1, Figure 4). The same pattern of increasing selenium concentration in larvae over time occurred in collections at Stewart Lake Drain. At Greasewood Corral, larvae in the earliest collection on May 23 had lower selenium concentrations than those collected later. At Sportsmans Drain, which had only two collections, selenium concentrations were lower in larger larvae than in smaller larvae and were lowest at the last collection date. No pattern was apparent at Old Charlie Wash, which had one collection. These results suggest that larvae at Cliff Creek, Stewart Lake Drain, and Greasewood Corral were accumulating selenium from the environment, but those at Sportsmans Drain were depurating selenium.

A similar pattern of increasing selenium in larvae over time seems to have occurred in larvae from Stewart Lake Drain analyzed by ICP-MS (Table 2). The selenium concentrations in larvae from Stewart Lake Drain that were analyzed by neutron activation contained 4.47 μg/g on May 23, 7.04 μg/g on May 30, and 7.42 μg/g on June 2 with a weighted mean of 5.79 μg/g. In the 279-larvae composite analyzed by ICP-MS, the sample was composed of 110 larvae collected on May 23 or earlier (39%) and 169 larvae were collected on May 26 or later (61%) and contained 8.0 μg/g of selenium. These later larvae were larger, and presumably older, than the larvae caught earlier (Table 3). Larvae captured on May 18 had total length 11.0 mm, whereas those on June 5 were 12.1 mm. Thus, the larvae captured in June were feeding and growing, and probably accumulated more selenium from food organisms and water over time than the apparently younger larvae captured in May. Residues are a result of exposure concentration and exposure time, and if time increases, but exposure concentrations stay constant, residues will increase (Rand and Petrocelli 1985). Applying this residue accumulation scenario to the current study, as razorback sucker larvae grew older while selenium concentrations in food organisms

Table 3. Mean total length (mm, standard error and number of samples for each collection period in parentheses) of wild larval razorback suckers collected from Stewart Lake Drain adjacent to the middle Green River and analyzed as a composite sample by ICP-MS.

Date	Total length (mm)
May 18	11.0 (0.1, 5)
May 19	11.1 (0.1, 9)
May 22	11.4 (0.1, 5)
May 23	11.3 (0, 90)
May 26	11.4 (0.3, 7)
May 30	11.6 (0.1, 113)
June 1	11.9 (0.2, 30)
June 2	11.6 (0.2, 5)
June 5	12.1 (0.2, 15)

stayed constant, residues increased as observed in the samples analyzed by neutron activation. Consequently, it seems logical that because the majority of the 279-larvae composite was composed of larger, and presumably older larvae with slightly greater selenium residues, the composite sample should have had more selenium than the weighted mean of the three composite samples analyzed by neutron activation (5.72 vs 8.0 μg/g).

Selenium concentrations in larvae were higher than concentrations in fish from control treatments in laboratory studies with either water, diet, or combined water and diet exposures, or reference sites in field studies (0.4-2.0 μg/g; Table 4). One unusual laboratory study reported that larval bluegill (*Lepomis macrochirus*) in the control treatment contained selenium concentrations of 3.3 μg/g, but were fed a commercial diet containing only 0.8 μg/g (Coyle et al. 1993). However, they also reported that the brine shrimp nauplii they fed to larvae from 5 days posthatch to 30 days posthatch contained selenium concentrations of 2.7 μg/g. In their study, selenium concentrations in adult reproductive tissue of control fish were 1.5-2 μg/g and in eggs was about 1.5 μg/g. Thus, larvae must have accumulated selenium from the brine shrimp to reach a residue of 3.3 μg/g because the commercial diet only contained 0.8 μg/g. Coyle et al. (1993) reported that up to 5 days posthatch, larval survival was >90%, but after 3 days of feeding brine shrimp, survival of control larvae decreased dramatically and was less than 25%. They concluded that the high mortality of larvae in their study was due to starvation resulting from unsuccessful transition between endogenous and exogenous feeding. Nevertheless, the 3.3 μg/g selenium residue in control fish was substantially higher than the typical range of selenium concentrations in control or reference fish.

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 (Table 5). Although some of the waterborne exposures may seem high in Table 5, the main point of the table is the values for whole-body residue and the resulting adverse effect. From Table 5, waterborne exposure requires higher selenium exposure concentrations than dietary exposures to generate similar whole-body residues. However, once whole-body selenium reaches a certain toxic threshold concentration (i.e., 4 μg/g), regardless of exposure route, adverse effects will occur. Based on the literature given in Table 5 and information from several other laboratory and field studies with a variety of fish species, Lemly (1993) recommended that whole-body residues of selenium in fish of 4 μg/g, regardless of exposure route, be taken as the toxic threshold for adverse effects. This toxic threshold was equaled or exceeded in the residues in larval razorback sucker collected from the five sites in the present study (Table 1).

One of the cited studies in Table 5 involved feeding larval razorback sucker selenium-laden zooplankton collected from sites in Sheppard Bottom at Ouray NWR (Hamilton et al. 1996). In that study, 5-, 10-, 24-, and 28-day-old larvae tested in four experiments experienced nearly complete mortality in 20-25 days after feeding on zooplankton from three to six sites with selenium concentrations ranging from 2.3 to 96 μg/g. Whole-body residues in these fish ranged from 3.6 to 94 μg/g. The range of residues in larvae show the variation that can occur in surviving fish, but from a toxicological standpoint, adverse effect concentrations are always

Table 4. Selenium concentrations in control fish from laboratory studies and reference fish from field studies.

Study type and selenium concentration	Species[1]	Whole-body selenium (μg/g)	Reference
Laboratory			
Water (μg/L)			
20	Bluegill	1.0	Cleveland et al. 1993
≈0	Bluegill	0.4-0.8[2]	Lemly 1982
≈0	Largemouth bass	0.8[2]	Lemly 1982
0.4	Rainbow trout	0.7[2]	Hodson et al. 1980
<0.4	Striped bass	1.1-1.4	Saiki et al. 1992
0.2	Chinook salmon	1.2-1.4	Saiki et al. 1992
0.3-1.4	Chinook salmon	1.1-2.0	Hamilton & Wiedmeyer 1990
Diet (μg/g)			
0.7	Bluegill	1.0	Cleveland et al. 1993
0.8+2.7[3]	Bluegill	3.3	Coyle et al. 1993
1.25	Rainbow trout	0.6	Hilton et al. 1980
1.0	Chinook salmon	0.8-1.0	Hamilton et al. 1990
0.4	Fathead minnow	1.8	Ogle & Knight 1989
Field			
Water (μg/L)			
<2	Fathead minnow	1.2[2]	Schultz & Hermanutz 1990
≈0.2	Striped bass	1.3-1.9	Saiki & Palawski 1990 Cutter 1989
<1	Bluegill	1.6[2]	Hermanutz et al. 1992
Diet (μg/g)			
1.3[4]	Striped bass	4.4[2]	Couglan and Velte 1989
-	Fish	0.4-2.4[2]	Pakkala et al. 1972 Pillay et al. 1974

[1]Bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*Oncorhynchus tshawytscha*), fathead minnow (*Pimephales promelas*), striped bass (*Morone saxatilis*).
[2]Reported as wet weight and converted to dry weight assuming 75% moisture.
[3]Dry diet contained 0.8 μg/g and brine shrimp nauplii contained 2.7 μg/g.
[4]Golden shiner (*Notemigonus crysoleucas*).

Table 5. Selenium concentrations in young fish exposed to selenium in the diet or water and adverse effects observed.

Exposure route, species (See Table 4, footnote 1), and weight (g)	Selenium exposure concentration (diet: μg/g; water: μg/L)	Selenium form	Exposure period (day)	Whole-body selenium (μg/g)	Effect	Reference
Diet						
Rainbow trout						
79	9	Selenite[2]	294	NG[10]	Mortality	Goettl & Davies 1978
1.3	13	Selenite[3]	80	5.2[11]	Mortality & reduced growth	Hilton et al. 1980
0.6	11-12	Selenite[3]	112	4.0-4.5[12]	Kidney damage	Hilton & Hodson 1983
Chinook salmon						
4.2	26[1]	SLD[4]	34	8.4[1]	Reduced migration	Hamilton et al. 1986
~1	9.6	SLD[4]	90	6.5	Mortality	Hamilton et al. 1990
	9.6	SEM[5]	90	5.4		
~1	5.3	SLD[4]	90	4.0	Reduced growth	Hamilton et al. 1990
	18.2	SEM[5]	90	10.8		
Fathead minnow						
0.12	20	Mix[6]	56	5.4	Reduced growth	Ogle & Knight 1989
0.0001	55-70	Rotifer[7]	7-9	43-61	Reduced growth	Bennett et al. 1986
Striped bass						
251	39	Fish[8]	80	15[1,13]	Mortality	Coughlan & Velte 1989

Table 5. Continued.

Exposure route, species (See Table 4, footnote 1), and weight (g)	Selenium exposure concentration (diet: μg/g; water: μg/L)	Selenium form	Exposure period (day)	Whole-body selenium (μg/g)	Effect	Reference
Bluegill						
2.8	54	Mayfly[9]	44	31[1,13]	Mortality	Finley 1985
0.3	6.5	SEM[5]	60	4.2[14]	Mortality	Cleveland et al. 1993
Razorback sucker						
≈0.005	2.3-4.5	Zooplankton[15]	30	3.6-14.3	Mortality	Hamilton et al. 1996
Water						
Rainbow trout	47	Selenite	60	5.2[1]	Mortality	Hunn et al. 1987
Chinook salmon	69	Mix[16]	60	3.8	Mortality	Hamilton et al. 1986; Hamilton & Wiedmeyer 1990
Chinook salmon	143	Mix[17]	60	4.9	Reduced growth	Hamilton et al. 1986; Hamilton & Wiedmeyer 1990
Chinook salmon	67	Mix[18]	60	4.5	Mortality & reduced growth	Hamilton et al. 1986; Hamilton & Wiedmeyer 1990

[1]Reported as wet weight and converted to dry weight assuming 75% moisture.
[2]Selenite: selenite incorporated in standard Colorado trout diet.
[3]Selenite: selenite incorporated in a casin-torula yeast trout diet.

Table 5. Continued.

[4]SLD: western mosquitofish (*Gambusia affinis*) collected from San Luis Drain, CA, used as fish meal portion in an Oregon moist pellet diet.
[5]SEM: selenomethionine incorporated into an Oregon moist pellet diet.
[6]Mix: 25% selenomethionine, 25% selenate, and 50% selenite incorporated in a fish food diet.
[7]Rotifer: rotifers fed selenium-laden algae.
[8]Fish: red shiners (*Notropis lutrensis*) sieved weekly from Belews Lake, NC, where they were chronically exposed to 10 μg/L selenium and food-chain selenium under natural conditions.
[9]Mayfly: burrowing mayfly nymphs (*Hexagenia limbata*) collected from Belews Lake, NC.
[10]NG: not given.
[11]Derived from figure 2 in Hilton et al. (1980).
[12]Carcass.
[13]Muscle tissue.
[14]Derived from figure 3 in Cleveland et al. (1993).
[15]Zooplankton: zooplankton collected from Sheppard Bottom ponds 1, 3, and 4 at Ouray NWR, UT.
[16]Mix: 3,023 μg/L boron, 96 μg/L molybdenum, 69 μg/L selenium, and water simulating the San Joaquin River, CA.
[17]Mix: 6,046 μg/L boron, 193 μg/L molybdenum, 143 μg/L selenium, and water simulating the San Joaquin River, CA.
[18]Mix: 2,692 μg/L boron, 92 μg/L molybdenum, 67 μg/L selenium, and well water at Yankton, SD.

linked with the lowest treatment or residue concentration associated with the observed adverse affect, i.e., LOAEL [lowest observed adverse effect level] (Rand and Petrocelli 1985). In the Ouray study, mortality occurred concurrently in larvae fed zooplankton from the six sites, which suggests that a toxic threshold was exceeded. The concentrations of selenium in zooplankton and larvae from the three least selenium contaminated sites (2.3 to 4.5 μg/g in zooplankton and 3.6 to 14.3 μg/g in larvae) were close to or higher than the toxic thresholds (3 μg/g in diet and 4 μg/g in whole-body) proposed by Lemly (1993) and supported by the results of studies summarized in Table 5.

As with any normal, bell-shaped distribution of responses in a group of individuals to a stimulus, some individuals will be adversely affected at low levels of stimulus, i.e., selenium residues less than the mean response, whereas some individuals will not be adversely affected until levels of stimulus are higher than the mean, i.e., selenium residues greater than the mean response. One misconception of threshold concentrations is that once a threshold is exceeded, all organisms are adversely affected on an equal basis. Toxic thresholds are usually based on the response of the most sensitive species tested, but there may be other untested species with greater sensitivity and others with less sensitivity to a stressor. Likewise, within a species, sensitivity is usually greatest in very young life stages, and within a life stage, sensitivity will vary among individuals. Consequently, threshold values should be used with caution.

Some fish in the Ouray study (Hamilton et al. 1996) died with lower whole-body concentrations of selenium (assumes that fish dying in a treatment have the same toxicant concentration as fish still a live in that treatment) than selenium concentrations in live, wild larvae collected in the present study. Adverse effects may have been occurring in the wild larvae, but no measurements were accomplished to determine if that was occurring. The wild larvae were collected from a demographically open population where loss of larvae due to predation and competition from non-native fish, contaminant effects, or other stresses associated with a wild, free-flowing river could be masked by movement of larvae from one backwater area to another. Similarly, spawning of adult razorback sucker over several days (Tyus and Karp [1990] reported ripe females over a 2 to 15 day period; Valdez et al. [1982] reported ripe adults over a 2-week period) could have added larvae to the population at a time when losses might be occurring due to predation, competition, contaminants, or other stresses. Figure 3 and Table 1 show that composite 4 from Cliff Creek, composite 2 from Sportsmans Drain, and composite 3 from Greasewood Corral were composed of smaller larvae than in earlier composite samples from the respective location. This occurrence of smaller larvae at later collection times suggests young larvae were being added to the cohort. Selenium concentrations in larvae followed this pattern of larval size (Figure 4).

The wild larvae collected in the present study were survivors from wild spawners in a free flowing river and as such had survived a variety of stresses before arriving at the locations where they were collected by light trapping. On the other hand, larvae used in the Ouray study were spawned from hatchery-held adults that were induced to spawn by injection of hormones. Larvae in the Ouray study came from two spawns, whereas the wild larvae probably came from several

adults that had spawned under natural conditions. Studies with Pacific salmon (*Oncorhynchus* sp.) showed that wild fish had higher survival rates than hatchery-reared fish (Felton et al. 1990). In a similar vein, the hatchery-spawned larval razorback sucker may have been less fit to deal with stresses than wild-spawned larvae, consequently, larvae used in the Ouray study might have been more sensitive to stressors than wild larvae. Nevertheless, based on the available literature summarized in Table 5, whole-body residues measured in wild razorback sucker larvae were comparable to residues in other species where adverse effects on survival and growth were observed.

Other inorganics in larvae

A few inorganics seemed elevated in the ICP-MS scan of the composite sample of larvae from Stewart Lake Drain. The concentration of cadmium in larvae was 4.9 μg/g, which was greater than the maximum concentration of 0.8 μg/g reported in the National Contaminant Biomonitoring Program (NBCP) for 315 composite samples (47 taxa) of whole-body fish collected from 109 stations nationwide in late 1984-early 1985 (Schmitt and Brumbaugh 1990). Values in the NCBP were given as wet weight tissue concentrations and were converted to dry weight concentrations by assuming 73.8% moisture in whole-body fish tissue, which was the average for the percent moisture in 315 fish samples collected as part of the NCBP (Schmitt and Brumbaugh 1990). The concentration of copper in larvae was 40 μg/g, which was greater than the 85th percentile value of 3.82 μg/g, but less than the maximum concentration of 88.2 μg/g in the NCBP. The 85th percentile is an arbitrary value used to identify values that are substantially above the nationwide median and possibly of concern, although the 85th percentile concentration has no toxicological significance. The concentration of lead in larvae was 3.8 μg/g, which was greater than the 85th percentile value of 0.84 μg/g, but less than the maximum value of 18.6 μg/g in the NCBP. The concentrations of selenium in larvae were 5.8 and 8.0 μg/g, which were greater than the 85th percentile value of 2.8 μg/g and close to the maximum value of 8.8 μg/g in the NCBP. The concentration of zinc in larvae was 299 μg/g, which was greater than the 85th percentile value of 131 μg/g, but less than the maximum value of 452 μg/g in the NCBP. The concentration of arsenic in larvae was not elevated compared to the NCBP data. Mercury and strontium were not measured in the larvae, but have been reported as inorganics of concern in water, sediment, or biota in the middle Green River (Stephens et al. 1988, 1992, Peltz and Waddell 1991, Finger et al. 1994, Hamilton et al. 1996).

Based on the literature, cadmium, copper, lead, and zinc residues in the larvae do not seem elevated to levels of concern. Mount et al. (1994) investigated each of these four inorganics individually in the diet with 33-day-old rainbow trout (*Oncorhynchus mykiss*) for 60 days. He reported no effects on survival or growth at residue concentrations in fish of 6.8 μg/g for cadmium, 36 μg/g for copper, 10 μg/g for lead, and 303 μg/g for zinc. Each of these concentrations were close to or greater than the concentrations in larvae for the present study.

Vanadium concentrations in larvae (1.7 μg/g) were close to those reported by Hilton and Bettger (1988) where adverse effects occurred in rainbow trout. They reported a vanadium

residue of 2.05 μg/g was present in rainbow trout that had reduced growth and reduced feeding response. Consequently, vanadium seems to be the only element, other than selenium, elevated sufficiently to be of concern.

SUMMARY

The range of selenium in larvae in the present study shows some had less than 3 μg/g as a whole-body residue, which suggests that if larvae from adults with low selenium residues drift to a relatively low selenium area, they probably would survive to the juvenile life stage. In fact, this scenario apparently happened at Old Charlie Wash as evidenced by the collection of 28 young of year razorback sucker in 1995 (Modde 1996) and 45 in 1996 (Modde 1997). However, if larvae start out with low selenium residues, such as occurred at Cliff Creek and Stewart Lake Drain, and then accumulate selenium through waterborne and dietary exposures to concentrations greater than the toxic threshold of 4 μg/g, there seems little likelihood of their survival to the juvenile stage. On the other hand, larvae at Sportsmans Drain started with elevated selenium concentrations, which were subsequently depurated as larvae grew, suggesting that if larvae can reach a relatively clean nursery area, they can reduce their selenium burden. Nevertheless, the widespread presence of elevated selenium residues, i.e., >4 μg/g, in larval razorback sucker from Cliff Creek to Old Charlie Wash inlet suggests that widespread selenium contamination of the middle Green River is occurring and may be adversely affecting the reproductive success of the endangered razorback sucker.

Other recent articles have also concluded that selenium concentrations in the Colorado River are elevated sufficiently in water, food organisms, and fish tissue to suggest selenium is causing adverse effects in razorback sucker and possibly other fish (Hamilton and Waddell 1994, Waddell and May 1995, Hamilton et al. 1996, Hamilton 1998, Stephens and Waddell 1998).

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