Rapid communication

Commentary: selenium study on endangered razorback sucker is flawed

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Abstract

The razorback sucker (*Xyrauchen texanus*) is listed as federally endangered throughout its range. A massive recovery effort by the Recovery Implementation Program for Endangered Fish Species in the Upper Colorado River Basin has focused its efforts in the upper Colorado River. The upper Colorado River basin also has two locations that have been identified by the National Irrigation Water Quality Program as having substantial selenium contamination. Selenium is toxic to fishes, affecting reproductive success. Thus, there is concern about potential effects of selenium on the endangered razorback sucker. Two sets of studies have investigated the effects of selenium on razorback suckers, but study results are conflicting. This commentary evaluates studies that claim selenium is not a problem for razorback sucker. We find that study bias was so pervasive that purported conclusions were unwarranted. Contaminated control water, older life stages of fish tested, lack of methodology for analysis of selenium in water, diet, or fish, use of rotifer food, low feeding rates, low growth rates of fish, and improper storage of site waters resulted in an apparent erroneous linkage of high selenium in whole-body residues with no adverse effects.
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1. Introduction

Many studies have been conducted on the endangered razorback sucker (*Xyrauchen texanus*) under the auspices of the Recovery Implementation Program (RIP) for Endangered Fish Species in the Upper Colorado River Basin. The RIP is a partnership composed of federal, state, and private organizations, and agencies of Colorado, Utah, and Wyoming (e.g., Tyus, 1998; Bestgen et al., 2002). There also have been a substantial number of studies on selenium related to irrigation activities conducted under the National Irrigation Water Quality Program (NIWQP), which is a Department of the Interior (DOI) intradepartmental program evaluating DOI irrigation projects (e.g., Engberg et al., 1998; Skorupa, 1998a; Seiler et al., 2003). The number of studies investigating the interaction of razorback suckers and selenium has been limited to two separate groups, Beyers and Sodergren (1999, 2001a, b, 2002) and Hamilton et al. (2001a, b, 2002a–c, 2004a–d). Somewhat conflicting conclusions were reached by these two groups: Beyers and Sodergren studies generally concluded selenium was not a problem for razorback sucker, and the Hamilton et al. studies concluded selenium was adversely impacting razorback sucker.

The RIP was formally established in 1987 and has five recovery elements: habitat management, habitat development and maintenance, stocking of native fish species, nonnative species and sportfishing management, and research, monitoring, and data management (USFWS, 1987). Concerns about water quality issues were not a recovery element, but discussed under habitat management. The NIWQP was established in 1985 and investigates trace constituents in drainwater from DOI irrigation projects in the western 17 states, including

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National Wildlife Refuges and other wetlands for which DOI has statutory authority (Engberg, 1998). Two major remediation efforts involving millions of dollars of effort by the NIWQP are located in the Gunnison, Uncompahgre, and Grand valleys, the main stem upper Colorado River in western Colorado, and the middle Green River in northeastern Utah. These latter two locations are the primary areas where the RIP has focused efforts at recovering razorback sucker and other endangered fish.

The US Fish and Wildlife Service (USFWS) has acknowledged that the impact of selenium on endangered fish is a concern in the Grand Valley area of Colorado, and supports the NIWQP's effort to move ahead with selenium remediation projects (USFWS, 1998, 2002b). The RIP recently acknowledged that selenium may be contributing to the decline of endangered fishes and could impact endangered razorback sucker (USFWS, 2002a), noting that NIWQP was implementing remediation projects to reduce selenium levels in areas of critical habitat. The RIP also states that, if necessary, it will identify actions to reduce deleterious concentrations of selenium contamination to minimize adverse effects on razorback sucker reproductive success and survival of young. However, the RIP has not identified decision criteria for determining the necessity of identifying actions to reduce selenium impacts and is not actively pursuing water quality issues, especially potential contaminant problems like selenium that might be influencing recovery efforts. In contrast, NIWQP believes selenium is currently impacting endangered fish and has sought an acknowledgment from the RIP that selenium was an important concern. Several meetings between the two programs in recent years have not completely resolved their differences of opinion.

This dichotomy of positions is reflected in the acceptance of the research investigations. Research summarized in the three Beyers and Sodergren reports was funded by the RIP and the NIWQP, and the reports were accepted by both. In contrast, research summarized in the two Hamilton et al. reports was funded by the RIP, but not fully accepted by the RIP because members of the Biology Committee expressed continued serious concern about the validity of the conclusions and recommendations, which they believed were not supported by the data in the reports. Thus, the RIP concluded that the two reports do not need to be considered in recovery efforts (UCREFRP, 2001).

The RIP is no stranger to controversy. The goal of the RIP is to reestablish self-sustaining populations of four endangered fish species while allowing continued development of water (USFWS, 1987). On first reading, this goal seems to have conflicting components—recover endangered fish, which requires instream flows and habitat types, yet allow continued water development,

i.e., removal. These seemingly conflicting goals have been noted by others (Hartman, 1997; Brower et al., 2001). Brower et al. (2001) reviewed the RIP and concluded that the consensus-based program was vulnerable to control by special interests and may be driven by bureaucratic procedural goals rather than species recovery. The review was met with criticism (Harmon, 2001; Daily Sentinel, 2001).

This commentary provides a technical critique of reports by Beyers and Sodergren (1999, 2001a, b, 2002) and raises important scientific concerns about the interpretation of study results and the possible misleading implications for future recovery efforts on behalf of the endangered Colorado River fishes.

2. Experimental design concerns

There were insufficient references and information given on measurement of water quality, source of brine shrimp cysts and culture methods, or use of dilution water with elevated selenium concentrations (Beyers and Sodergren, 1999, 2001a, b, 2002). The absence of this information precludes other researchers from repeating the experiments.

One of the fundamental components of the methods section of scientific reports and papers is describing the methods used and giving citations for published methods. Although Beyers and Sodergren (1999, 2001a, b, 2002) report several water quality characteristics, they do not give any citations for the methods employed.

The source and culture of brine shrimp is an important consideration in the culture of larval fish. ASTM (1992), the standard practice for using brine shrimp nauplii for food for test animals in aquatic toxicology, notes a wide variation in the quality of brine shrimp cysts due to commercial supplier and geographic source that affect their suitability for fish culture. Trace elements including selenium, fatty acids, nauplii size, and other factors influence the suitability of nauplii for use as fish food organisms (Olney et al., 1980; Petrucci et al., 1995; Leger et al., 1986; Cowgill et al., 1987; ASTM, 1992). For example, Hamilton et al. (2001b) reported that brine shrimp nauplii from Colombia, South America, contained elevated concentrations of arsenic, and the interaction of arsenic to reduce the toxic effects of selenium has been documented in birds and mammals (reviewed in Hamilton et al., 2001b). Elevated arsenic residues in brine shrimp nauplii fed to larval razorback sucker were a concern raised in explaining the incongruence between elevated selenium in larvae and the lack of adverse effects from selenium (Hamilton et al., 2001a, b).

Another concern was the concentration of selenium in control water from a well used in two 28-day chronic

toxicity studies. Beyers and Sodergren (1999) reported control water contained 5.35 μg/L selenium, and Beyers and Sodergren (2001b) reported control water contained 6.12 μg/L. In a third study Beyers and Sodergren (2001a) used control water containing <1 μg/L selenium. The two higher concentrations (5.35 and 6.12 μg/L) were above the US Environmental Protection Agency (USE-PA) criterion for protection of aquatic life of 5 μg/L (USEPA, 1987). Yet, there is no discussion of the importance of control water containing selenium concentrations greater than the federal criterion.

In the study where control water contained 6.12 μg/L, control fish contained 3.3 μg/g selenium, which probably came from waterborne exposure and not dietary exposure because selenium concentrations in algae were <0.2 μg/g and in rotifers were <0.7 μg/g (algae and rotifers were cultured in water containing <1 μg/L selenium) (Beyers and Sodergren, 2001b). In the study where control water contained 5.35 μg/L, control fish contained 1.2 μg/g selenium, which probably came from waterborne exposure and not dietary exposure because selenium concentrations in algae were 0.4 μg/g and in rotifers were 0.3 μg/g (Beyers and Sodergren, 1999). The whole-body selenium residue in one study (3.3 μg/g) but not the other (1.2 μg/g) was above typical background selenium concentrations in fish from laboratory and field studies, which are ≤2 μg/g (Maier and Knight, 1994; Hamilton et al., 2000b).

2.1. Fish age

It is important to use customary methods in fish feeding studies because fish are most sensitive at earlier life stages. Beyers and Sodergren (1999, 2001a, b) cite ASTM (1990) for the method for their early life stage studies, whereas Beyers and Sodergren (2002) cite ASTM (1995). ASTM (1990, 1995) describes water-only exposures and recommends starting early life stage fish studies with ≤48-h-old embryos. However, Beyers and Sodergren (1999, 2001a, b, 2002) used a water and dietary exposure instead of a water-only study, and started their studies with 12-, 27-, or 41-day-old razorback sucker larvae or 11-day-old flannelmouth sucker (*Catostomus latipinnis*) larvae.

Beginning feeding of larval razorback sucker at 10–12 days old may have caused a starvation stress. Beyers and Sodergren (2001a, b) reported that larvae were fed live brine shrimp nauplii or rotifers starting at about 10–12 days posthatch, or 2–31 days prior to using the fish in experiments. They reasoned that 10–12 days posthatch was the appropriate time to begin feeding by citing Muth et al. (1998) who examined the stomachs of 11–18 mm total length razorback sucker larvae collected from the middle and lower Green River between 1992 and 1996. Muth et al. (1998) did not age the larvae whose stomachs they exampled, but the smallest larvae

examined were 11 mm and could have been anywhere from a few to 10 days posthatch because newly hatched larvae have total lengths of 7–10.7 mm (Minckley and Gustafson, 1982; Snyder and Muth, 1990; Papoulias and Minckley, 1992).

In contrast, other researchers have reported that larval razorback sucker start feeding as early as 5 days posthatch. Tyus and Severson (1990) started feeding razorback sucker at 5 days posthatch, and Papoulias and Minckley (1992) started feeding razorback sucker at 7 days posthatch. Papoulias and Minckley (1992) reported that within 1 day of stocking (age 8 days posthatch) larvae had phytoplankton, diatoms, and detritus in their stomachs, and that the following day (age 9 days posthatch) these same food items plus rotifers, nauplii, cladocerans, invertebrate eggs, and chironomids were found in larvae stomachs. Minckley and Gustafson (1982) reported that 9-day-old razorback sucker larvae fed on ground aquarium fish food (Tetramin). Toney (1974) reported that razorback sucker at 6 days posthatch swam to the surface and fed on baby food, i.e., strained beef liver. In the studies by Papoulias and Minckley (1992) and Minckley and Gustafson (1982) it is unknown, but probable, that larval razorback sucker would have started feeding earlier if food had been presented to the larvae at an earlier age. Hamilton et al. (2001a, b) started dietary exposures of razorback sucker at 5 days posthatch at which time larvae were actively feeding.

Papoulias and Minckley reported that the point of irreversible starvation, and subsequent mortality, was between 19 and 23 days posthatch, whereas, the median time to 50% mortality for unfed larvae was between 24 and 25 days. Thus, not feeding larvae between 5 (time of first feeding reported by others) and 10–12 days posthatch probably resulted in a starvation stress, which probably was linked to the reduced growth exhibited in the three studies by Beyers and Sodergren (1999, 2001a, b), as discussed below.

Starting early life stage studies with 11-, 12-, 27-, or 41-day-old fish probably missed important life stages that were sensitive to contaminant stresses. Beyers and Sodergren (2001a, b) reasoned that magnitude of bias in comparing the sensitivities of 11-, 12-, 27-, and 41-day-old fish was small because Hamilton (1995) reported that razorback sucker ranging in age from 10 to 186 days had similar sensitivity to dissolved selenium. However, the Hamilton (1995) studies were 96-h acute toxicity tests using waterborne selenite or selenate, and not dietary exposures. Nevertheless, Beyers and Sodergren (2001a, b) assumed that there was no age-related sensitivity to selenium that was strongly dependent on route of exposure. In contrast, Mayer and Ellersieck (1986) reviewed a database of 410 chemicals tested with 66 species, and reported that 83% of the time, the sensitivity of fish decreased with development and

increased size. Dwyer et al. (1999) used this explanation, in part, to discuss the greater sensitivity of 3-day-old fathead minnow (*Pimephales promelas*) to a mixture of five chemicals (carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin in equitoxic proportions) in 7-day exposures compared to the other species tested at mostly 5–7 days old, which included Colorado pikeminnow (*Ptychocheilus lucius*), razorback sucker, bonytail (*Gila elegans*), and Gila topminnow (*Poeciliopsis occidentalis*). Consequently, by initiating their dietary selenium exposures with razorback sucker larvae 12, 27, or 41 days old or flannelmouth sucker 11 days old, Beyers and Sodergren (1999, 2001a, b) missed exposing larvae at a very important early life stage that would have been more sensitive than older life stages.

2.2. Residue analysis

The acceptability of residue analysis is contingent upon appropriate methods and procedures because accuracy and precision can vary widely and without this information the validity of measured concentrations cannot be ascertained. Beyers and Sodergren (1999, 2001a, b) give limited information on chemical analysis of selenium concentrations in water, algae, rotifers, and larval fish, and do not mention the volume or weight of the samples collected, how samples were collected, preserved, or stored, or how samples were prepared before analysis. They do not give methods used to measure selenium concentrations, nor quality assurance/ quality control measures used during the analyses. Beyers and Sodergren (2001a) report concentrations of 17 trace elements measured in test waters and Beyers and Sodergren (2001b) report concentrations of 16 trace elements measured in test waters, but neither report mentions how those elements were measured. In two reports, Beyers and Sodergren (2001a, b) note that fish samples were analyzed at North Carolina State University in Nuclear Services, Department of Nuclear Engineering, which implies that neutron activation was probably the method for measurement of selenium in larvae.

Knowing the analysis method for selenium and other elements is important because there are several methods that can be used such as atomic absorption-graphite furnace (AA-GF), atomic absorption-hydride generation (AA-HG), inductively coupled plasma (ICP) spectroscopy, ICP-mass spectroscopy (ICP-MS), fluorometric method, differential pulse cathodic stripping voltammetry, or neutron activation. Although all of these methods have been used to measure selenium in water or tissues (Lemly, 1982; Palmer, 1998; Tamari, 1998), some are excellent (AA-HG), whereas others are poor (AA-GF, ICP, ICP-MS) in large part due to high limit of detection values.

It is also important to know the precision and bias of the analytical method employed. Many ASTM guides include the statement that, "When appropriate, reagent blanks, recoveries, and standards should be included whenever samples are analyzed." Analysis of blanks is necessary to determine if contamination is present in reagents or from handling. Analysis of triplicate sample preparation and analysis is necessary to determine if consistent sample handling occurred during preparation, digestion, and analysis. Analysis of various recoveries is necessary because (1) recovery of the element of interest from standard reference material (National Institute of Standards and Technology, National Research Council of Canada, National Bureau of Standards, or some other national or international institution) is necessary to determine if the digestion and analysis procedure accurately measured elemental concentrations, (2) recovery from samples spiked prior to digestion is necessary to determine if the digestion procedure altered the amount of spiked element, and (3) recovery from digested samples spiked prior to analysis is necessary to determine if there were interferences from other components such as calcium, magnesium, chloride, or others.

The lack of analytical chemistry methodology casts doubt on the accuracy and precision of selenium residues reported in Beyers and Sodergren (1999, 2001a, b, 2002). For example, there was a 3-fold difference in selenium residues in control fish exposed to similar concentrations of selenium in water, algae, and rotifer: whole-body selenium residues were 3.33 μg/g in 69-day-old larvae exposed for 28 days to 6.12 μg/L in control water (<0.18 μg/g in algae and <0.7 in rotifers; Beyers and Sodergren, 2001b) compared to whole-body residues of 1.16 μg/g in 40-day-old larvae exposed for 28 days to 5.35 μg/L in control (0.41 μg/g in algae and 0.35 μg/g in rotifers; Beyers and Sodergren, 1999). As a second example, Beyers and Sodergren (2001b) reported that 100 μg/L waterborne selenate resulted in 3 μg/g in algae and 4.6 μg/g in rotifers in a static renewal system, which does not compare favorably with Dobbs et al. (1996) who reported 100 μg/L waterborne selenate resulted in about 50 μg/g in algae and 45 μg/g in rotifers using a flowthrough chemostat system. Likewise, Beyers and Sodergren (2001b) reported 200 μg/L waterborne selenate resulted in 6 μg/g in algae and 8 μg/g in rotifers in a static renewal system, which does not compare favorably with Dobbs et al. (1996) who reported 200 μg/ L waterborne selenate resulted in about 100 μg/g in algae and 70 μg/g in rotifer. Although there could be biological factors influencing these residue values, the lack of analytical methodology also must be considered as a possible source of data variability.

As another example, Beyers and Sodergren (1999) used waterborne selenium concentrations of 5.35–27.2 μg/L, but selenium residues in control and

treatment fish whole-body ranged from 0.69 to 1.40 µg/g, which reveals little uptake of selenium from water or dietary sources (algae contained selenium concentrations of 0.41–1.46 µg/g and rotifers contained 0.35–1.40 µg/g; both were cultured in water containing up to 20 µg/L selenium, thus suggesting little uptake of selenium by these organisms). In contrast, Beyers and Sodergren (2001b) had waterborne selenium concentrations of 6.12 µg/L in control water and selenium residues in whole-body were 3.33 µg/g in razorback sucker and 3.43 µg/g in flannelmouth sucker, which suggests uptake of selenium from water or diet sources growing in the exposure chambers (control algae contained selenium concentrations of <0.2 µg/g and control rotifers contained <0.7 µg/g; both were cultured in water with <1 µg/L selenium). The contrast in uptake of selenium in fish from water in these two studies may be related to analytical chemistry. There are several reports of low waterborne selenium (1–4 µg/L) resulting in elevated selenium in aquatic invertebrates (4–9 µg/g) (Holland, 1979; Schroeder et al., 1988; Peltz and Waddell, 1991; Stephens et al., 1992; Butler et al., 1994; Hamilton et al., 1996; Lemly, 1997).

Moreover, Beyers and Sodergren (2001a, 2002), which used site waters containing elevated waterborne selenium, reported there was substantial accumulation of selenium in razorback sucker in the site water/control diet exposures. In the Orchard Mesa water/control diet treatment (algae contained <0.2 µg/g and rotifers contained <0.7 µg/g, both were cultured in water with <1 µg/L selenium), selenium concentration in water was 5.4 µg/L and larvae contained 4.4 µg/g. In the North Pond 50% diluted water/control diet treatment, selenium concentrations in water were 10.6 µg/L and larvae contained 10.8 µg/g. In the North Pond water/control diet treatment, selenium concentrations in water were 20.3 µg/L and larvae contained 14.4 µg/g. The elevated residues in larvae must have come from either waterborne exposure or food chain accumulation through algae or detritus growing in the exposure chambers. It seems unusual that a similar uptake of selenium in larvae did not occur in the Beyers and Sodergren (1999) study, if analytical chemistry was appropriate.

2.3. Feeding rates and use of rotifers

The quantity of food in the Beyers and Sodergren (1999, 2001a, b) studies may have been a factor in the slow growth of razorback sucker larvae. In the first study, the number of rotifers fed per fish was 886 per day (Beyers and Sodergren, 1999). In that study slow growth of razorback sucker was noted and one explanation was that the ration was too small for optimal growth. In the follow-up studies, the number of rotifers fed per fish was either 759 (Beyers and Sodergren, 2001a, 2002) or 914 (Beyers and Sodergren,

2001b), thus indicating little difference in feeding rates. These numbers of rotifers per fish are relatively low compared to the findings of Kestemont and Awaiss (1989) who reported that the best growth of newly hatched gudgeon (*Gobio gobio*, 4.8 mm length, 0.5 mg weight) was achieved when they were fed 2500 rotifers per larva per day during the first week of rearing and up to 5500 rotifers per larva per day during the fourth week. Howell (1973) reported that newly hatched plaice (*Pleuronectes platessa*, 7 mm length) consumed 85 rotifers per day and larvae 56 days old consumed about 1400 rotifers per day. The feeding rates of Beyers and Sodergren (1999, 2001a, b) who started their 28-day studies with larvae aged 12, 27, and 41 days old (final ages 40, 55, and 69 days old) were about three times lower than those of Kestemont and Awaiss (1989).

The nutritional value of rotifers has been compared with other live foods used in larval fish culture. Hutchinson and Williams (1994) reported that rotifers were a good initial food for the first day or two in feeding tests with larval fathead minnows, but the best growth of larvae occurred with early feeding of brine shrimp nauplii. Banner and Van Arman (1973) reported that a mixture of rotifers, brine shrimp, and limnoplankton fed to sacfry bluegill (*Lepomis macrochirus*) resulted in 100% mortality in 2–3 days, whereas feeding only brine shrimp resulted in >90% survival after 8 days. Others have also stated that rotifers are a good first food for larval fish, but fish growth was reduced if larvae were reared too long on rotifers before switching to larger prey such as brine shrimp or dry diet (Kestemont and Awaiss, 1989). Howell (1973) reported that larvae of plaice and sole (*Solea solea*) grew more when fed brine shrimp nauplii than fed rotifers.

The caloric energy in rotifers is comparable to that in cladocerans and brine shrimp (Schindler et al., 1971; Watanabe et al., 1983). However, the substantial difference in size between rotifers and other live foods such as some cladocerans and brine shrimp nauplii means that larval fish would need to consume more rotifers at an earlier life stage (thus expending more energy) than the numbers of cladocerans and brine shrimp nauplii consumed at an older life stage. This supposition is supported by Bengtson et al. (1999) who reported that 13-day-old summer flounder (*Paralichthys dentatus*) consumed 301 rotifer per day compared to 23-day-old larvae, which consumed 59 brine shrimp per day.

Important details of rotifer culture in Beyers and Sodergren (1999, 2001a, b, 2002) are missing. The authors cite methods for rotifer culture in Hoff and Snell (1989), but that publication gives a variety of culture approaches for marine and freshwater rotifer culture and techniques for enrichment of rotifer cultures with essential highly unsaturated fatty acids important to rearing of larval fish. If the rotifer culture did not

include enrichment with fatty acids, the nutritional value of the rotifers to larval fish may have been lower than needed for adequate growth (Watanabe et al., 1983; Opstad et al., 1989). However, the lack of details for the rotifer culture in Beyers and Sodergren (1999, 2001a, b, 2002) preclude determining if rotifer culture techniques were adequate, especially concerning enrichment with fatty acids, and if the resulting rotifers were nutritionally sufficient for use in the toxicity studies with larval fish.

In fact, Beyers and Sodergren (1999, 2001a, b, 2002) do not describe how the algae culture was quantified prior to feeding the rotifer culture, nor how the rotifer culture was quantified prior to feeding larval fish in the test exposures.

2.4. Residue concentrations

Selenium concentrations in whole-body of larvae in the high selenium treatments in the three Beyers and Sodergren studies (1999, 2001a, b, 2002) are given in Table 2. Interestingly, no adverse effects were observed despite elevated selenium residues in several treatments. In contrast, positive effects of increased mass or total length occurred in four treatments with moderate residues, which was believed due to site water constituents. These results conflict with adverse effects reported in selenium exposure studies in the literature (reviewed by Hamilton, 2003). A substantial number of laboratory and field selenium studies generally revealed that whole-body residues in the 4–5 μg/g range were associated with adverse effects in young fish (Hamilton, 2003). Adverse effects such as reduced survival, growth, or some other measurement have also occurred in a variety of fish species with residues between 5 and 10 μg/g, including endangered fish in the Colorado River such as razorback sucker and bonytail (Hamilton, 2003). Specific to razorback sucker, whole-body selenium residues of 3.6–8.7 μg/g (Hamilton et al., 1996), 5.4 μg/g (Hamilton et al., 2001a), and 6.1 μg/g (Hamilton et al., 2001b) were linked with adverse effects of dietary selenium in separate studies. Thus, it is remarkable that elevated selenium residues in razorback suckers reported by Beyers and Sodergren (2001a, b, 2002) did not result in adverse effects.

2.5. Use of site waters

Selenium concentrations may have declined in stored waters. Beyers and Sodergren (2001a, 2002) used unfiltered site water collected from four sites in their algae-rotifer food chain exposure of larval razorback sucker. During their 28-day study, site water was collected on April 28, May 10, and May 17, and stored in open barrels at room temperature. The replacement rate of water in the algae exposures was once every 3.3–5 days and in the rotifer exposures it was every 2 days.

Storing the unfiltered site water in open barrels at room temperature over a 7- to 11-day period between collections would encourage growth of bacteria and plankton and loss of selenium from the water column. Two USEPA publications give specific guidance for collecting natural waters for use in toxicity tests (Weber, 1993; Lewis et al., 1994). Both publications state that, "Unless the samples are used in an on-site toxicity test the day of the collection, they should be chilled and maintained at 4 °C until used to inhibit microbial degradation, chemical transformation, and loss of highly volatile toxic substances." The USEPA guides state that water samples can be used for 24- and 48-h renewals if stored at 4 °C with minimum headspace, and that first use of the water samples must occur within 36 h of collection.

Selenium could be readily lost from solution while being held in open barrels at room temperature. Selenium in water is rapidly taken up by algae (Sandholm et al., 1973; Nassos et al., 1980; Foe and Knight, 1986; Riedel et al., 1991; Besser et al., 1993) and aquatic plants (Allen, 1991; Ornes et al., 1991). Typically, algae took up maximal concentrations in 3–24 h, whereas floating plants took about 1 week to accumulate maximal concentrations. Graham et al. (1992) reported that selenium rapidly disappeared from the water column in a pond study and correspondingly increased in sediments and biota, especially periphtyton. One component of the sediments is the detrital layer, which is partly composed of bacteria. Bender et al. (1991) reported that selenium was rapidly removed from the water column by bacteria and cyanobacteria and incorporated into a detrital-like mat composed of anaerobically processed grass clippings. In their experiment initial selenium concentrations of 40,000 μg/L were undetectable after 27 days of microbial activity.

The selenium concentrations measured in site waters are probably unreliable because they represent concentrations at the time of collection and not those in water after storage at room temperature in open barrels over 7- to 11-day periods. This potential for selenium loss from storage water seems to be supported by the low selenium concentrations in cultures of algae and rotifers reported by Beyers and Sodergren (2001a, 2002). For example, they reported that Orchard Mesa water contained 5.43 μg/L of selenium, and the corresponding selenium concentrations in algae were 1.55 μg/g and in rotifers were 4.83 μg/g. In the Grand Valley of Colorado close to where Beyers and Sodergren (2001a, 2002) collected their samples, Hamilton et al. (2001a) reported that at Adobe Creek waterborne selenium concentrations averaged 3.8 μg/L and zooplankton selenium concentrations averaged 28.5 μg/g, and at North Pond water selenium concentrations averaged 9.5 μg/L and zooplankton selenium concentrations averaged 27.1 μg/g. In Beyers and Sodergren (2001a), 20.3 μg/L

Table 1
Comparison of razorback sucker growth and selenium residues in control treatments among studies

Age at measurement (days posthatch)	Weight (mg)	Total length (mm)	Diet	Se concentration water (µg/L)/diet (µg/g)	Se residue (µg/g)	Reference[a]
25	6.6	12	Brine shrimp[b]	<1/2.7	NG[c]	A
35	8.5	12	Brine shimrp[b]	< 1/2.7	NG	A
35	8.6	12	Brine shrimp[b]	<1/2.7	3.6	B
35	11	14	Brine shrimp[b]	<1/3.2	5.2	C
36	34	17	Biokyowa + Bozeman[d]	<1/NG	NG	B
37	440	35	Biodiet[e] + brine shrimp[f]	<3/1.7–2.0 + 2.7	1.6	D
40	5.4	11.5	Algae-fed rotifers	5.35/0.3	1.2	E
42	NG	20	Tetramin	NG	NG	F
42	15–23	14–16	Zooplankton	NG	NG	G
44	14	14	Brine shrimp[b]	<1/2.7	NG	A
~50	NG	16–20	Zooplankton	NG	NG	H
55	10.5	13.5	Algae-fed rotifers	<1/<0.7	2.3	I
56	51–91	20–22	Zooplankton	NG	NG	G
57	NG	23	Zooplankton	NG	NG	J
59	NG	27	Tetramin	NG	NG	F
63	81–138	22–25	Zooplankton	NG	NG	G
67	1400	51	Biodiet only	<3/1.7–2.0	1.1	D
69	12.0	15.1	Algae-fed rotifers	6.12/<0.7	3.3	K
69	NG	35	Tetramin	NG	NG	F
78	51	35	Biokyowa + Bozeman[d]	<1/NG	NG	B
97	2850	63	Biodiet only	<3/1.7–2.0	1.0	D
108	2180	56	Biokyowa + Bozeman[d]	<1/NG	NG	B
134	3680	68	Biokyowa + Bozeman[d]	<1/NG	1.0	B

[a] A, Hamilton et al., 1996; B, Hamilton et al., 2001a; C, Hamilton et al., 2001b; D, Hamilton et al., 2000a; E, Beyers and Sodergren, 1999; F, Minckley and Gustafson, 1982; G, Papoulias and Minckley, 1992; H, Marsh and Langhorst, 1988; I, Beyers and Sodergren, 2001a; J, Papoulias and Minckley, 1990; K, Beyers and Sodergren, 2001b.

[b] 40 nauplii per larvae per day in 1600-mL of test water.

[c] Not given.

[d] Biokyowa 250 first week, mixture of Biokyowa 250 and Bozeman diet (Bozeman Fish Technology Center, Bozeman, MT) second week, Bozeman diet third week to Day 89, mixture of Bozeman diet and Tilapia-based diet Days 90–96; Tilapia-based diet Days 97–134; cultured at 24-Road Fish Hatchery.

[e] Biodiet: from BioProducts, Warrenton, OR.

[f] Ad libitum for larvae aged 5–37 days old.

of selenium in water from North Pond should correspondingly have produced approximately 58 µg/g selenium in rotifers if rotifer uptake was similar to other zooplankton. However, the investigators reported that rotifers contained a selenium concentration of 21.8 µg/g. Moreover, a 50% dilution of North Pond water containing 10.6 µg/L selenium resulted in more selenium in algae (3.74 µg/g) than in algae (2.30 µg/g) cultured in full-strength North Pond water containing 20.3 µg/L (Beyers and Sodergren, 2001a, 2002).

2.6. Growth of larval razorback sucker

Slow growth of fish in a study may indicate poor rearing conditions. The lack of growth of fish in Beyers and Sodergren (1999, 2001a, b, 2002) probably compromised growth as an endpoint for determining adverse effects from waterborne and dietary exposure to selenium. Larvae in studies conducted by Beyers and Sodergren (1999, 2001a, b, 2002) had slow growth compared to other studies with larval razorback sucker (Minckley and Gustafson, 1982; Marsh and Langhorst, 1988; Papoulias and Minckley, 1990, 1992; Hamilton et al., 2000a, b, 2001a) (Table 1). At hatch, razorback sucker are typically 7–9 mm (Snyder and Muth, 1990), although others have reported 7.3 mm (Minckley and Gustafson, 1982) and 9.4–10.7 mm (Papoulias and Minckley, 1992). Hamilton et al. (2001b) reported that 5-day-old razorback sucker had 11 mm total length and 4 mg weight. In comparison, 40-day-old larvae in Beyers and Sodergren (1999) had 11.5 mm total length and 5.4 mg weight at the end of the study (initial total length was 10.6 mm at start of study), which was substantially less than those reported by Papoulias and Minckley (1992) for 42-day-old larvae (15–23 mg weight and 14–16 mm total length). Other comparisons of larval weight and total length at various larvae ages reported in Beyers and Sodergren (1999, 2001a, b, 2002) show that growth was substantially less than would be expected compared to those reported in Papoulias and

Minckley (1990, 1992), Minckley and Gustafson (1982), Marsh and Langhorst (1988), and Hamilton et al. (2001a, b). In contrast, razorback sucker are apparently capable of rapid growth when fed Biodiet and brine shrimp nauplii ad libitum from 7 days posthatch to 37 days posthatch (Hamilton et al., 2000a) or when fed a sequence of Biokyowa, Bozeman diet, and Tilapia-based diet (Hamilton et al., 2001a). Beyers and Sodergren (1999) suggested that the slow growth of larvae was due to low quantity of rotifers fed. There are two other possible explanations for the slow growth of larvae.

First, the quality of the food offered might have been poor. Dabrowski (1984) reviewed the literature on feeding of fish larvae and stated that zooplankton were the natural food of fish larvae. He further stated that "zooplankton can be used as a model for the formulation of artificial feed," and went on to discuss problems in the culture of fish larvae associated with formulating nutritionally adequate, artificial dry diets, and rearing intensive monocultures of live invertebrates for feeding fish. Razorback sucker larvae seem to be opportunistic feeders and are capable of using zooplankton, benthic organisms, algae, and detritus as food, sometimes selectively and other times in proportion to the abundance of the item. Marsh and Langhorst (1988) found that stomachs of razorback sucker larvae (~16 mm total length) in Arizona Bay backwater in Lake Mohave, California and Arizona, contained mostly *Bosmina* (Cladoceran), rotifers, and copepoda. They also reported that larvae showed a positive selection for cladocerans, especially *Bosmina*, and strong negative selection for rotifers. In a more detailed report of their research with razorback sucker larvae, Langhorst and Marsh (1986) reported that larvae used all available habitats (limnetic, benthic, and macrophytic) as evidenced by the presence in larvae stomachs of *Macrothrix*, a cladoceran associated with vegetation. Gut analysis of larval razorback sucker stocked into, and collected 1 week later, from a backwater in the Salt River, indicated that chironomid larvae were the dominant food (J.E. Brooks, USFWS, personal communication, cited in Bestgen, 1990). Papoulias and Minckley (1992) reported that razorback sucker larvae (age 7 days posthatch) were eating sessile diatoms, phytoplankton, and detritus the first day after stocking in ponds, and by Day 2 larvae were also eating rotifers, nauplii, cladocerans, insect eggs, and chironomids. Muth (1995) examined gut contents of razorback sucker larvae collected in low velocity habitats of the middle and lower Green River, UT, and reported similar items were consumed including rotifers, chironomid larvae, filamentous and colonial algae, diatoms, and organic debris. In the Beyers and Sodergren studies, feeding one type of organism such as rotifers may not have been a balanced diet compared to feeding a variety of organisms.

Second, growth could have been reduced by selenium exposure. Some detrital material derived from fish excretions, uneaten rotifers, and airborne debris (including bacteria, fungi, and algae) was probably present in the exposure containers. In most laboratory chronic toxicity studies, algae and periphyton typically grow on the walls of the exposure containers. Consequently, walls of exposure containers must be routinely brushed to dislodge the build up of material, and the material removed by siphoning. The Beyers and Sodergren reports do not mention cleaning exposure containers. More importantly, the base water for one experiment contained 5.35 µg/L of selenium (Beyers and Sodergren, 1999) and base water in a second experiment contained 6.12 µg/L (Beyers and Sodergren, 2001b), which could have contributed to the food chain exposure. Selenium uptake in larvae in the studies may have come from dietary uptake of algae, periphyton, and detritus growing on the walls or bottom of the exposure containers. Selenium is readily accumulated from water into algae (Nassos et al., 1980; Foe and Knight, 1986; Riedel et al., 1991; Besser et al., 1993), periphyton (Graham et al., 1992), and detritus (Bender et al., 1991). Algae, periphyton, and detritus are consumed by razorback sucker larvae (Papoulias and Minckley, 1992). If not cleaned, this additional route of exposure and selenium uptake would have resulted in additional accumulation of selenium by larvae in some treatments.

Beyers and Sodergren (2001b) reported that the mass of larval fish was significantly increased by exposure to selenium in water and diet ($P = 0.0019$) using regression analysis, but they did not state which species, razorback sucker or flannelmouth sucker, or both, had significant differences. Inspection of Fig. 2 in Beyers and Sodergren (2001b) showed very little variation in mass values across treatments for either species, and Table 2 in Beyers and Sodergren (2001b) revealed an increase of 3.3% for razorback sucker (12.0 mg in control larvae versus 12.4 mg in the high treatment which had the largest larvae), and 10.8% for flannelmouth sucker (16.6 mg in control larvae versus 18.4 mg in the high treatment which had the largest larvae). Using the USEPA's Toxicity Data Analysis software (Weber et al., 1989; Lewis et al., 1994), which incorporates analysis of variance and either Dunnett's test (equal replicates) or Bonferroni's t test (unequal replicates), there was no significant difference in mass of larval razorback sucker, whereas for flannelmouth sucker, significant differences in mass occurred in the 51 µg/L water/2.0 µg/g diet treatment and the 190 µg/L water/8.2 µg/g diet treatment, but not in the intermediate 99 µg/L water/4.6 µg/g diet treatment. Considering that increased weight occurred inconsistently in two treatments and not in an intermediate treatment and the magnitude of difference in mass was 1.5–1.8 mg in flannelmouth

Table 2
Selenium residues in whole-body fish after 28 days of exposure and effects

Study	Species	Se concentration water (μg/L)/diet (μg/g)	Se residue (μg/g)	Effect[a]
Beyers and Sodergren (1999)	Razorback sucker	27/1.4	1.4	None
Beyers and Sodergren (2001b)	Razorback sucker	190/5.6	12.9	None
	Flannelmouth sucker	190/5.6	10.2	Increased mass
Beyers and Sodergren (2001a, 2002)	Razorback sucker	<1/<0.7 (DeBeque)	3.0	Increased mass
		<1/2.1 (DeBeque)	5.4	None
		5.4/<0.7 (Orchard Mesa)	4.4	Increased mass
		5.4/4.4 (Orchard Mesa)	11.0	None
		11/<0.7 (North Pond 50% dilution)	10.8	Increase mass and total length
		11/12 (North Pond 50% dilution)	41.1	None
		20/<0.7 (North Pond)	14.4	Increased mass and total length
		20/22 (North Pond)	42.0	None

[a]Based on results of USEPA Toxicity Data Analysis (Weber et al., 1989; Lewis et al., 1994).

sucker, the increase in mass seems to be biologically unimportant.

Beyers and Sodergren (2001a, 2002) reported that fish mass was significantly greater in all site water and control diet treatments compared to the control water and control diet treatment, which was confirmed using the USEPA Toxicity Data Analysis software. They also reported that fish mass and total length were significantly smaller in fish in the site water and site diet treatments compared to the site water and control diet treatment, and concluded that the effects were due to cocontaminants in the diet and not selenium exposure. However, analysis of data using the USEPA software did not detect any significant differences. The lack of significant differences seems justified by inspection of the data: control larvae had 13.5 mm total length and larvae in site water and site diet treatments had 13.7, 13.5, 13.5, and 13.7 mm (spread of 0.2 mm) total length, and control larvae had 10.5 mg mass and larvae in treatments had 10.7, 10.2, 10.1, and 11.2 mg (spread of 0.7 mg). Differences in statistical approaches between Beyers and Sodergren (2001a, 2002) and use of the USEPA Toxicity Data Analysis probably explain differences in the significance of statistical results. Overall, there seemed to be no adverse effects observed in any of the treatments.

The limited growth of larval razorback sucker and possibility of poor nutrition from the rotifer diets in Beyers and Sodergren (1999, 2001a, b, 2002) should have increased the susceptibility of the larvae to stress from selenium toxicity and resulted in effects on survival and growth. It is unclear how larvae could grow little in control and exposure treatments, compared to other studies with razorback sucker larvae, yet not show adverse effects even if unrelated to selenium toxicity.

On the other hand, Beyers and Sodergren (2001a, 2002) reported that growth of larval razorback sucker was enhanced in the site water treatments compared to the formulated control water treatment. They give three references to support the statement that formulated waters lack trace elements essential for survival and growth of biological organisms (Cowgill et al., 1986; Girling and Garforth, 1989; Keating et al., 1989); however, these three references are for daphnia and not fish. USEPA methods recommend formulated waters with various hardnesses for fish toxicity tests using survival and growth as measurement endpoints (Weber, 1993; Lewis et al., 1994). ASTM methods (ASTM, 1990, 1995) also allow the use of formulated waters of various hardnesses in fish studies, but ASTM (1996) expresses concerns about their use with daphnia.

3. Flawed conclusions

Beyers and Sodergren (2001a, 2002) conclude that selenium concentrations of <20.3 μg/L in water, <21.8 μg/g in diet, and <42 μg/g in whole-body did not negatively affect larval razorback sucker. The selenium literature has several major review papers that generally state that waterborne concentrations of 2–5 μg/L or greater, dietary concentrations of 3 μg/g or greater, or whole-body concentrations of 4 μg/g or greater are harmful to fish (Lemly, 1986, 1996, 1999, 2002; Maier et al., 1987, 1988; Maier and Knight, 1994; Skorupa et al., 1996; Skorupa, 1998b; Hamilton and Lemly, 1999; Hamilton, 2002, 2003). Although the results and conclusions of Beyers and Sodergren (2001a, 2002) were substantially different than the

selenium literature, there is little or no discussion of why their results were different than the results of the majority of selenium toxicity tests with fish.

Beyers and Sodergren (2001b) report that their results were similar to two other selenium food-chain studies using algae, rotifer, and fathead minnow (Bennett et al., 1986; Dobbs et al., 1996). The main similarity between the three selenium food chain studies was that they incorporated algae and rotifers, but adverse effects only occurred at very elevated whole-body residues in fathead minnow in Bennett et al. (1986) and Dobbs et al. (1996), not in razorback sucker in Beyers and Sodergren (1999, 2001a, b, 2002). Bennett et al. (1986) reported that fish fed rotifers containing 55 µg/g selenium had reduced growth and whole-body residues of 61 µg/g. Dobbs et al. (1996) reported that fish fed rotifers containing 40–60 µg/g selenium had reduced growth and whole-body residues of about 76 µg/g. All three studies had residues substantially higher than effect concentrations in other studies with fathead minnows (Schultz and Hermanutz, 1990; Hermanutz, 1992), but not in laboratory studies with fathead minnow (Brooks et al., 1984; Ogle and Knight, 1989) and in razorback sucker larvae (Hamilton et al., 1996, 2001a, b, 2002c, 2004a, d). Perhaps there is something unique about an algae–rotifer–fish food-chain selenium exposure that results in effects on growth or survival at relatively high selenium residues in fish.

One Beyers and Sodergren (2001a) report was published in the scientific literature (Beyers and Sodergren, 2002), but none of the concerns expressed here were addressed in that paper (e.g., analytical methodology, storage of site water, high selenium residues, but no adverse effects, nor was age of test fish given). In both of those publications there seems to be a conflict between the statements in the abstract or executive summary and the conclusions sections. The abstract states that, "Lack of detection of adverse effects from exposure (in the present study) does not imply that razorback sucker populations are not affected by increased environmental selenium concentrations. There are a variety of factors not included in this investigation that may influence sensitivity of razorback sucker to selenium." In contrast, the conclusion states that, "our data suggest that biologically significant effects on survival and growth of larval razorback sucker will not occur in nursery habitats with selenium at or below these levels" (20.3 µg/L in water and 21.8 µg/g in diet). The latter statement is in conflict with the majority of the selenium literature, and goes beyond the boundaries of their experiments, which presumably started with larvae from clean eggs, that were fed clean food until age 27 days posthatch, exposed for no more than 28 days, and assuming reliable analytical chemistry.

3.1. Selenium in the Colorado River basin

Prior to the completion of the Beyers and Sodergren studies and the Hamilton et al. studies, Hamilton (1998) reviewed numerous sources of information concerning endangered fish, irrigation activities, and the documentation of selenium contamination in the upper and lower Colorado River basin. He concluded that selenium concentrations were sufficiently elevated to be causing reproductive problems in endangered fish such as the razorback sucker. In a follow-up paper, he reviewed historical data on selenium concentrations in the upper and lower basins, along with historical records and reviews of the occurrence of native, later endangered fish, and presented a hypothesis that suggested selenium contamination from irrigated agriculture in the 1890–1910 period caused the decline of native fish in the upper basin in the 1910–1920 period and in the lower basin in the 1925–1935 period (Hamilton, 1999). Although many of the studies reviewed in Hamilton (1998, 1999) documented elevated selenium concentrations in various aquatic components, none of the studies assessed biological effects in endangered fish from exposure to selenium or other inorganic elements.

3.2. Selenium and endangered fish

The overall general conclusion of Beyers and Sodergren (1999, 2001a, b, 2002) was that selenium exposure in water and diet did not cause adverse effects in endangered razorback sucker or the closely related flannelmouth sucker, which is also native to the Colorado River. These reports have been accepted by the RIP, are listed at the RIP web site (www.r6.fws.gov/crrip), and thus, will be used in considerations of RIP directions. The results of these studies lent support to the RIP's position that contaminants are not affecting endangered fish such as razorback sucker, and should remain a lower priority for the recovery of endangered fish. In contrast, the Hamilton et al. (2001a, b, 2002c, 2004d) studies were conducted between 1995 and 1998 and concluded that selenium exposure primarily in diet (⩾ 4.6 µg/g) adversely affected larval razorback sucker survival at whole-body selenium concentrations of 5.4–6.1 µg/g. These results closely match those in selenium studies with other fish where selenium residues in the 4–6 µg/g range were linked with adverse effects (Hilton et al., 1980; Hilton and Hodson, 1983; Gatlin and Wilson, 1984; Hamilton et al., 1986, 1990, 1996, 2000a; Hunn et al., 1987; Ogle and Knight, 1989; Hamilton and Wiedmeyer, 1990; Cleveland et al., 1993). The Hamilton et al. (2001a, b) reports were not fully accepted by the RIP, and therefore, will not be considered in recovery efforts, and have not been listed at the RIP web site. Unacceptance of the two reports, however, does not mean the results and conclusions of

the research are invalid. Parts of the two reports have been published (Hamilton et al., 2002a–c), and three other papers are in press (Hamilton et al., 2004b–d).

The NIWQP has actively undertaken measures to remediate concerns about selenium in the upper Colorado River and Green River because the program managers believe that the elevated selenium concentrations in the aquatic ecosystem of the upper Colorado River are impacting the recovery of endangered fish. Selenium is listed (section 303(d) of the Clean Water Act) as an element of concern for the impairment of numerous segments of the Gunnison, Uncomphagre, and lower Colorado rivers and several of their tributaries in Colorado including critical habitat for endangered fish (State of Colorado, 2003a, b). These concerns about selenium in western Colorado have resulted in stakeholder groups forming the Gunnison Basin Selenium Task Force and the Grand Valley Selenium Task Force to address selenium concerns (Lolholm, 2000).

In light of the continuing concern for selenium issues in the upper Colorado, Gunnison, and Green rivers, the remediation efforts of the NIWQP, the agreement of the results of the Hamilton et al. studies with the selenium literature, and the numerous concerns in the Beyers and Sodergren studies, it seems that selenium's impact on endangered fish in the Colorado River should be an important issue to the RIP. The RIP is relying on the NIWQP to undertake remediation efforts of selenium's impact on endangered fish, but the NIWQP had a budget cut of 46% in 2003 and expects more budget cuts in 2004 (Borchardt, 2003). Although the USFWS has acknowledged concerns about selenium in the recovery of endangered fish (USFWS, 1998, 2002b), the RIP has taken the position of not actively supporting water quality remediation, and in general, participants do not believe that selenium is a threat to endangered fish (UCREFRP, 2003). It is unclear how the RIP intends to recover endangered fish and satisfy recovery goals for razorback sucker and Colorado pikeminnow in the upper Colorado River without considering effects from selenium contamination and reassessing the scientific merits of the Beyers and Sodergren reports.

Acknowledgments

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