

Utah State University

DigitalCommons@USU

---

All U.S. Government Documents (Utah Regional Depository)

U.S. Government Documents (Utah Regional Depository)


---

2-2000

## Selenium Poisoning of Wildlife and Western Agriculture: Cause and Effect

N. E. Korte

Follow this and additional works at: <https://digitalcommons.usu.edu/govdocs>

 Part of the [Environmental Sciences Commons](#), and the [Terrestrial and Aquatic Ecology Commons](#)

---

### Recommended Citation

Korte, N. E., "Selenium Poisoning of Wildlife and Western Agriculture: Cause and Effect" (2000). *All U.S. Government Documents (Utah Regional Depository)*. Paper 88.  
<https://digitalcommons.usu.edu/govdocs/88>

This Report is brought to you for free and open access by the U.S. Government Documents (Utah Regional Depository) at DigitalCommons@USU. It has been accepted for inclusion in All U.S. Government Documents (Utah Regional Depository) by an authorized administrator of DigitalCommons@USU. For more information, please contact [digitalcommons@usu.edu](mailto:digitalcommons@usu.edu).



ORNL/TM-2000/58

# **Selenium Poisoning of Wildlife and Western Agriculture: Cause and Effect**

N. E. Korte

Environmental Sciences Division  
Publication No. 4979



ENVIRONMENTAL SCIENCES DIVISION  
**SELENIUM POISONING OF WILDLIFE AND WESTERN AGRICULTURE:  
CAUSE AND EFFECT**

**N. E. Korte**  
Environmental Sciences Division  
Oak Ridge National Laboratory

Environmental Sciences Division  
Publication No. 4979

Date Published: February 2000

Prepared for the  
U.S. Department of Energy  
Office of Science  
Budget Activity Number 26 42 00 00 0

Prepared by  
OAK RIDGE NATIONAL LABORATORY  
Oak Ridge, Tennessee 37831  
managed by  
LOCKHEED MARTIN ENERGY RESEARCH CORP.  
for the  
U.S. DEPARTMENT OF ENERGY  
under contract DE-AC05-96OR22464

Available electronically from the following source.

**Web site:** [www.doe.gov/bridge](http://www.doe.gov/bridge)

Reports are available in paper to the public from the following source.

U.S. Department of Commerce  
National Technical Information Service  
5285 Port Royal Road  
Springfield, VA 22161

**Telephone:** 1-800-553-6847

**TDD:** 703-487-4639

**Fax:** 703-605-6900

**E-mail:** [orders@ntis.fedworld.gov](mailto:orders@ntis.fedworld.gov)

**Web site:** [www.ntis.gov/ordering.htm](http://www.ntis.gov/ordering.htm)

Reports are available in paper to U.S. Department of Energy (DOE) employees, DOE contractors, Energy Technology Data Exchange (ETDE) representatives, and International Nuclear Information System (INIS) representatives from the following source.

Office of Scientific and Technical Information  
P.O. Box 62

Oak Ridge, TN 37831

**Telephone:** 865-576-8401

**Fax:** 865-576-5728

**E-mail:** [reports@adonis.osti.gov](mailto:reports@adonis.osti.gov)

**Web site:** <http://www.osti.gov/products/sources.html>

This report was prepared as an account of work sponsored by an agency of the United States government. Neither the United States government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States government or any agency thereof.

# CONTENTS

	Page
LIST OF FIGURES.....	v
LIST OF TABLES .....	vii
EXECUTIVE SUMMARY .....	ix
1. INTRODUCTION.....	1
1.1 BACKGROUND.....	3
1.2 PROJECT SCOPE.....	3
1.3 AREA OF STUDY .....	4
1.4 THE ENDANGERED FISH .....	4
1.5 FRAMING THE CONTROVERSIES .....	5
1.5.1 Historical Accounts of Decline of the Endangered Species .....	5
1.6 DISCUSSION .....	10
2. SELENIUM CYCLING IN THE ENVIRONMENT.....	11
2.1 SOURCE .....	11
2.2 BIOGEOCHEMISTRY.....	12
2.2.1 Cycling in Wetlands .....	13
2.3 DISCUSSION .....	13
3. BIOTRANSFORMATIONS OF SELENIUM.....	15
3.1 TOXICITY TO FISH .....	16
3.1.1 Acute Toxicity.....	17
3.1.2 Chronic Toxicity .....	17
3.2 TOXICITY TO BIRDS.....	20
3.3 DISCUSSION .....	22
4. HABITAT, NONNATIVE FISH, AND LIFESTYLE.....	22
4.1 HABITAT .....	22
4.2 NONNATIVE FISH.....	25
4.3 FISH CHARACTERISTICS.....	26
4.3.1 Razorback Sucker.....	26
4.3.2 Colorado Pikeminnow (Squawfish) .....	27
4.3.3 Flannelmouth Sucker.....	27
4.4 DISCUSSION .....	28
5. STANDARDS.....	28
5.1 HABITAT CONSIDERATIONS.....	28
5.2 CHRONIC CRITERIA.....	29
5.3 ACUTE CRITERIA .....	30
5.4 DISCUSSION .....	30

	<b>Page</b>
6. RECOMMENDATIONS .....	30
6.1 SELENIUM UPTAKE AND ACCUMULATION .....	31
6.2 RELATIVE SENSITIVITY OF VARIOUS FISH SPECIES .....	32
6.3 BIOINDICATORS/BIOMARKERS.....	32
6.3.1 Deformities and Lesions .....	35
6.4 INHERITED RESISTANCE. ....	35
6.5 SOURCES.....	35
7. REFERENCES.....	36

## LIST OF FIGURES

Figure		Page
1	Upper Colorado Basin.....	2
2	Large squawfish (pikeminnow) caught in the Green River (Quartarone 1995).....	9
3	Predominance areas for dissolved selenium species in response to pH and redox potential (Masscheleyn and Patrick 1993) .....	14
4	Low flow in the Gunnison River (Burdick 1997) .....	24
5	Relationship of disability and impairment .....	33

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
1	List of interviewees.....	4
2	Selenium concentration guidelines .....	29
3	Selenium contents of water, sediment, zooplankton, and razorback sucker eggs at three sites in the Grand Valley (Holley 1999) .....	31
4	Bioindicators measured at various levels of biological organization.....	33
5	Bioindicators representative of the stress zones shown in Fig. 5.....	34



## EXECUTIVE SUMMARY

This project examined the hypothesis that selenium contamination is not the principal cause of the decline of endemic fish species in the Upper Colorado Basin. Activities employed to test this hypothesis included a reconnaissance of locations altered by recent road construction, a re-interpretation of available literature regarding selenium toxicity, and the interpretation of unpublished data obtained from the Upper Colorado Basin Fish Recovery Program. The project demonstrates that most of the evidence implicating selenium is circumstantial.

Specifically, this research demonstrates that neither the historical record nor the technical literature consistently supports the emphasis given selenium toxicity. For example, many locations in the intermountain region have elevated selenium in water and sediments without obvious consequences for wildlife. Consequently, biological and geochemical studies are required to understand the cycling, relative abundance, and bioavailability of selenium and other constituents so that causal agents in the Upper Colorado Basin can be identified with greater certainty.

The project also demonstrates the need for subcellular indicators of selenium poisoning. Unfortunately, most potential biomarkers are not specific for selenium. A potential candidate based on the mammalian literature is glutathione peroxidase (GPx) activity and/or the cellular levels of reduced glutathione and hydrogen selenide (Greeley, M.S., Oak Ridge National Laboratory, personal communication, Jan. 22, 1999). Evidence of increased lipid peroxidation and related glutathione peroxidase activity has been found in aquatic birds at sites such as Kesterson (Hoffman and Heinz 1988; Ohlendorf et al. 1988). Selenium has an antimutagenic effect so genomic research is not feasible. However, the activity of the GPx gene could be explored as a potential selenium biomarker. Little is known regarding the mechanistic relationships between GPx and selenium, which of itself is an area where additional research would provide important information.

Studies also are needed with the endangered fish and other species to develop predictive tools regarding the manner in which selenium cycles geochemically and biologically in riverine and backwater environments. Finally, hydrological investigations and modeling are needed to further examine the coordination of dam operation and water diversions to determine whether modifications can provide the habitat necessary to ensure survival of the endangered species.

The performance of this project has developed the relationships and knowledge required to advance proposals in the specific areas just described. Within the Environmental Sciences Division (ESD), a seminar and several group discussions were held to identify team members for proposals. Potential funding sources within and outside of the U.S. Department of Energy (DOE) have been identified, and ESD staff members are approaching these informally. The preparation of one or two proposals is anticipated during FY 2000. Finally, the journals *Bioscience* and *Reviews in Fisheries Science* were selected as appropriate venues for publishing this work, and a manuscript is in preparation.

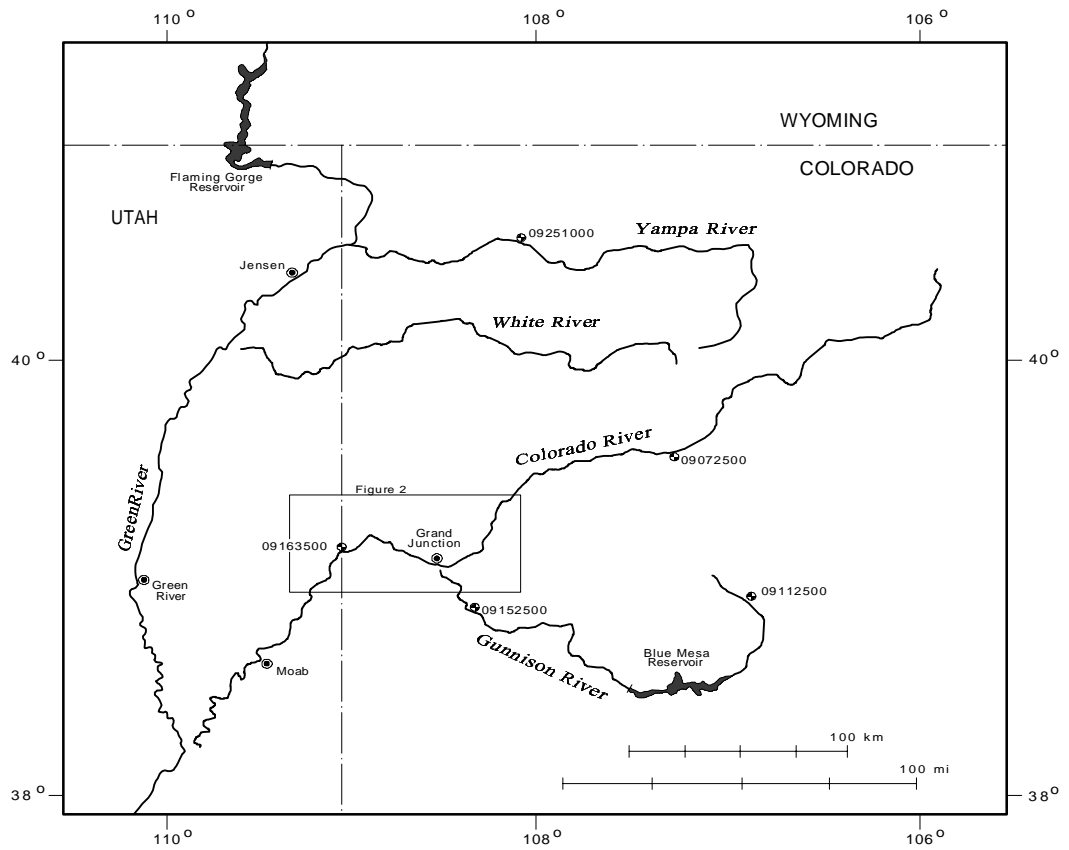
## 1. INTRODUCTION

Selenium toxicity is the presumed cause of population declines in many fish and avian species in the western United States. In particular, avian deformities at the Kesterson National Wildlife Refuge in California resulted in an emphasis on selenium toxicity in irrigated regions of the intermountain West (Presser 1994; Seiler 1998). Populations of many fish species also collapsed at Kesterson, and based on a well-documented case at Belew's Lake in North Carolina (Lemly 1985), it was assumed that selenium was responsible.

Recent data published in the veterinary literature, however, indicates that historical research with selenium and livestock cannot be duplicated, and that the reported effects, in some circumstances, can be attributed to other causes. The veterinary research questions the use of tissue plugs and total selenium analyses as a means of identifying selenium poisoning in any species. This research further suggests that some presumably affected species may be adapted to high levels of selenium. Accordingly, the purpose of this project was to reinterpret the available literature in the context of data available from local fish recovery programs in the Upper Colorado Basin (Fig. 1).

Assessing the decline and the attempted recovery of endangered fish in the Upper Colorado Basin is a complex undertaking because it is an interdisciplinary scientific problem in a multi-jurisdictional political environment. The latter refers not only to local and national politicians but also to the various government agencies involved, including the Fish and Wildlife Service (FWS), the Geological Survey (GS), the Bureau of Reclamation (BOR), and various state agencies.

There is little doubt that there were more and bigger fish with a wider distribution before human activities impacted the Upper Colorado Basin. There is also no disagreement regarding the fact that endangered fish in the Upper Colorado Basin have been harmed by both pollution and habitat modification. Within these broad categories, however, are several interrelated subcategories. Addressing these specific subcategories is where the problems arise. For example, no one disputes the fact that nonnative fish species prey on native fish (see Sect. 4.2). In addition, no one disputes that the populations of some of the nonnative fish are enhanced both by their ability to thrive in poor-quality water and their adaptation to the current river regime (see Sect. 4.2). Conflicts arise, however, on where to place emphasis on the endangered species recovery. Should the nonnative fish be poisoned? Should more funds go to removing selenium and other chemicals from agricultural drainage? Should emphasis be placed on returning the river to a flow regime that more closely mimics that under which the endangered fish evolved? These scientific questions are further complicated by political realities. Each of the three possibilities just mentioned has some political baggage. Poisoning nonnative fish is opposed by those who consider the native species to be "trash" fish or who utilize the nonnatives as sport fish. Cleaning up agricultural drainage is popular to those pursuing population increases and further land development because it creates jobs by bringing in federal dollars for lining ditches and canals. Blaming either selenium or nonnatives is favored by those pursuing further water development because it diverts emphasis from being placed on restoring the flow and physical characteristics of the rivers. The controversies extend to the natural resource agencies themselves. All agree that there is value in habitat reclamation, but there is considerable difference of opinion within and among the agencies regarding where and how recovery emphasis should be placed.



**Fig. 1. Upper Colorado Basin.**

## **1.1 Background**

This project was justified based on a belief that entrenched, existing programs are causing institutional resistance to consolidating and integrating historical and new research into a comprehensive description of the linkages among selenium, water development, and wildlife. Selenium toxicity is presumed to be the primary cause of avian teratogenesis at the Kesterson National Wildlife Refuge and some other locations in the western United States. Selenium toxicity also has been implicated in the collapse of several populations of endangered fish (Bureau of Reclamation 1998). The premise for ascribing the poisoning to selenium has its origin, in part, on the belief that a livestock disease called blind staggers was caused by selenium (Beath et al. 1953). High concentrations of selenium in animal tissue and the abundance of seleniferous plants were related to blind staggers. Consequently, selenium tissue concentrations have been used to guide recent investigations and programs related to the recovery of endangered fish such as the Colorado River squawfish (now pikeminnow) and the razorback sucker. The role of selenium, however, is disputed as shown by recent publications advocating a much higher standard for protection of aquatic life in western streams (Canton 1999).

Research published in the veterinary literature suggests that blind staggers in cattle is not caused by selenium but by sulfate and other factors associated with high salt/alkaline soils (O'Toole and Raisbeck 1995). This research also shows that selenium causes sterility but is not teratogenic to livestock. Other work shows that pronghorn, indigenous to high selenium locales, tolerate relatively high levels of selenium in their blood, and that mallards confined to a high selenium diet would starve rather than eat the food (Raisbeck et al. 1996). Taken together, these data suggest that the original premise implicating selenium could be overemphasized. Moreover, the data suggest that indigenous, endangered fish in the intermountain West also could be relatively tolerant of selenium. Indeed, the fish recovery programs are guided to some extent by selenium analyses of tissue plugs, a practice that the veterinary research refutes in mammals (O'Toole and Raisbeck 1995).

Researchers have also been monitoring and reporting changes within the Colorado River system (Van Steeter and Pitlick 1998). Recent published reports show that the Colorado River, in the Grand Valley, has lost approximately 30% of its average flow in the past 40 years. The average width of the river has decreased by approximately 60 ft in the same time period. Aerial photos show that the complexity of the habitat associated with the river also has declined at a significant rate. Furthermore, the preceding documented changes do not address the considerable alterations to the flow regime (suppressing spring runoff, prolonging summer/fall runoff, decreasing sediment load, decreasing summer temperature, increasing winter temperature) caused by water diversions and dams.

## **1.2 Project Scope**

This project was supported from the Seed Money Fund of the Laboratory Directed Research and Development Program at Oak Ridge National Laboratory. The project funding of approximately \$14,000 resulted in a limited scope that included a comprehensive literature search, in-depth interviews with researchers in the field (Table 1), and a field survey of habitat in the Grand Valley. Those contacted are listed in Table 1.

**Table 1. List of interviewees**

Name	Agency/affiliation	Date interviewed	Phone/ in person
Dan Beyers	Colorado State University	6/29/99	Phone
Donal O'Toole	University of Wyoming	6/4/99	Phone
Merl Raisbeck	University of Wyoming	7/2/99	Phone
Richard Krueger	U.S. Fish and Wildlife Service/ Grand Junction, Colorado	6/25/99, 6/29/99	In person
Barbara Osmundson	U.S. Fish and Wildlife Service/ Grand Junction, Colorado	6/25/99	In person
Pat Arbeiter	Bureau of Reclamation/ Grand Junction, Colorado	6/29/99	Phone
George Kidd	Northwest Fisheries/ Grand Junction, Colorado	5/25/99	In person
A. Dennis Lemly	U.S. Forest Service/ Blacksburg, Virginia	8/17/99	Phone
Ralph Seiler	U.S. Geological Survey/ California	6/26/99	Phone
Kathy Holley	U.S. Fish and Wildlife Service/ Grand Junction, Colorado	5/25/99	In person
Tim Modde	U.S. Fish and Wildlife Service/ Vernal, Utah	7/2/99	Phone
Frank Pfeiffer	U.S. Fish and Wildlife Service/ Grand Junction, Colorado	9/22/99	Phone
Steve Hamilton	U.S. Geological Service/ Yankton, South Dakota	6/18/99	Phone

### **1.3 Area of Study**

The Upper Colorado Basin is shown in Fig. 1. While a larger area is shown, this report emphasizes findings in the Grand Valley, that reach of the Colorado River that flows through Grand Junction, Colorado, and the adjacent communities of Palisade, Loma, and Mack.

### **1.4 The Endangered Fish**

The fish species of principal interest in the upper basin are the Colorado pikeminnow (formerly squawfish) and the razorback sucker. Most of the emphasis has been on the razorback sucker because the pikeminnow is still reproducing and recruiting to the adult population. Two other endangered species of concern in the Colorado River are the humpback chub and bonytail. All of the discussion in this report, however, relates to the razorback sucker (often called a humpback sucker by fishermen) and the Colorado pikeminnow or squawfish.

## **1.5 Framing the Controversies**

The controversies can be illustrated in a number of ways, but a specific example is the issue of whether Kesterson-like avian deformities have been found in the Grand Valley. Lemly (1999) states that such deformities have been found and quotes Seiler (1998). Seiler indicates that the information came from biologists working in the Grand Valley (Seiler 1999). The individuals named by Seiler, however, state that only one such nest has been found (Krueger 1999; Osmundson 1999). These researchers emphasize their belief that the reason there are not more incidents is the overall lack of reproduction in the area, not that selenium levels are insufficient to cause toxicity. Nevertheless, this finding that reported avian deformities represent a small number of incidents, is supported by another researcher who interviewed persons knowledgeable about other locations where selenium problems are suspected (Raisbeck, M.F., University of Wyoming, personal communication, July 2, 1999). As would be expected, the low number of incidents causes doubt regarding the severity of selenium toxicity. Similar circumstances exist with respect to the endangered fish. Deformed fish were found at Belew's Lake, but there is apparently no evidence of deformed fish in the Upper Colorado Basin.

Selenium is considered by some to be a major factor in the population declines of the Colorado pikeminnow and razorback sucker because of high concentrations found in adult fish and in water and sediments in rearing areas. Laboratory research has demonstrated that selenium affects these endangered species, but as will be discussed in subsequent sections, much of this research is controversial.

### **1.5.1 Historical Accounts of Decline of the Endangered Species**

An important aspect of determining the reasons for the decline of the endemic fish in the Upper Colorado Basin is an evaluation of their historical distribution. Because of the nature of the regional geologic environment (see Sect. 2) and the habits of the endangered species (see Sect. 4), it is likely that in presettlement times, native fish were occasionally exposed to concentrations of selenium that are considered elevated based on present-day standards for the protection of aquatic life. It is also apparent that fish populations were exposed to waters with higher than present-day concentrations of selenium for most of the twentieth century. For these reasons, relating the decline of the fish to historical exposure may provide important insight. Of particular interest is whether the decline occurred substantially before or after about 1950. Although water development began in the early 1900s, the most extensive alterations of the Colorado River through the Grand Valley occurred after 1950. In other words, the predominant effect on the endangered species prior to 1950 was human harvest and selenium exposure, whereas habitat loss and changes in the timing and magnitude of peak flows became most severe after 1950.

#### **1.5.1.1 Selenium Exposure**

It is known that water quality has always been a problem in the Upper Colorado Basin. Grand Junction newspaper reports from the late 1890s stated that there would never be settlement and growth unless better sources of water were located. Such statements suggest that the river water was often saline. Present-day research demonstrates a general correlation of dissolved solids with selenium. Nonetheless, without chemical analyses, these reports also may be referring to the high level of other dissolved salts and suspended sediment.

The endangered fish species have been exposed to selenium from irrigation runoff since 1915 with the completion of the first major irrigation project. The primary irrigation projects were completed prior to 1920 (Butler et al. 1996), and selenium concentrations initially were much higher than those observed now. For example, as reviewed by Hamilton (1998), “Historically, selenium concentrations measured in four irrigation drains in the Grand Valley in the 1930s were 320 to 1050  $\mu\text{g/L}$ , whereas two new drains near Mack, Colorado (about 32 km downstream from Grand Junction), contained 1980  $\mu\text{g/L}$  in 1934 and 2680  $\mu\text{g/L}$  in 1935 (Anderson et al. 1961). In 1936, selenium concentrations in the Colorado River at Cameo, Colorado, contained 0 to 1  $\mu\text{g/L}$ , whereas downstream of irrigation influences, but above the Gunnison River, the Colorado contained 0 to 10  $\mu\text{g/L}$  (mean 4  $\mu\text{g/L}$ ). At the same time, the Gunnison River at its mouth contained 5 to 55  $\mu\text{g/L}$  (mean 22.5  $\mu\text{g/L}$ ). Anderson et al. (1961) also reported that at one point in the 1930s the Gunnison River at Grand Junction contained 80  $\mu\text{g/L}$  and the Colorado River at Grand Junction contained 30  $\mu\text{g/L}$ .” Finally, it is worth noting that the present-day populations of the endangered fish are not significantly different above or below Cameo despite the fact that selenium exposure above Cameo, as described above, is much less.

Present-day selenium concentrations in the Grand Valley are much lower because most of the readily available selenium already has been leached from the soils. Nevertheless, about 64% of the water samples collected from the lower Gunnison and about 50% of samples from the Colorado River near the Colorado-Utah state line exceeded the 5  $\mu\text{g/L}$  Environmental Protection Agency (EPA) criterion for the protection of aquatic life. This investigation also noted that “almost all selenium concentrations in samples collected during the nonirrigation season from Mancos Shale areas exceeded the aquatic life criterion” (Butler et al. 1996).

Similar data are reported by Stephens and Waddell (1998) who examined the selenium content of streams in the Green River Basin. One generalization made by these investigators serves as a useful reference point when examining data from backwaters. Stephens and Waddell (1998) note that backwaters that received water primarily from the river were not high in selenium, while those backwaters receiving water from shallow groundwater or irrigation drains contained potentially toxic concentrations of Se. A few examples from their report are the following:

- Bitter Creek, near the town of Green River (Wyoming) has up to 10  $\mu\text{g/L}$  of selenium.
- In the Little Snake River drainage, tributaries had up to 3  $\mu\text{g/L}$ , and the river itself had typically less than 5  $\mu\text{g/L}$ .
- In the Yampa River, the headwaters are in gneiss and granite, but near Craig, Colorado, there are cretaceous outcrops. Several small streams near Craig have 24 to 300  $\mu\text{g/L}$  of selenium and “account for much of the 13 to 17  $\mu\text{g/L}$  measured in the Yampa downstream of Craig.”
- At Maybell, Colorado, the Yampa has 0 to 11  $\mu\text{g/L}$  with a mean of about 2  $\mu\text{g/L}$ .
- The White River at Meeker, Colorado, has selenium concentrations that occasionally exceed 5  $\mu\text{g/L}$ . The White, at its confluence with the Green, has been as high as 8  $\mu\text{g/L}$ . Other creeks draining cretaceous formations into the White River are mentioned.
- Tributaries to the Blacks Fork River have concentrations as high as 12  $\mu\text{g/L}$ .

Although enhanced by irrigation in some areas, these data indicate that selenium exposures have always occurred in the region.

The report by Hamilton (1998) also notes that the fish congregated in irrigation drainages that exposed them to high levels of selenium that he believes harmed their reproduction. However, reports that the fish used these areas go back to the 1920s (Quartarone 1995) indicating that such locations probably have been used since irrigation began in 1915.

Considering the selenium concentrations observed in the early and present-day historical accounts of irrigation water, it is worthwhile to consider data reported for an exposure in North Carolina. Concentrations of selenium of 150 to 200  $\mu\text{g/L}$  flowed into Belew's Lake raising the concentration in the lake to 8–22  $\mu\text{g/L}$  (Skorupa 1998). Immediate effects were observed, including drastic declines in fish populations and increased numbers of deformed fish. However, as described above, higher concentrations of selenium were observed in the Upper Colorado Basin, including within the rivers themselves, and neither deformed fish nor immediate population declines were observed. (More discussion of contaminant levels that affect the fish is provided in Sects. 3, 5, and 6.)

Hamilton (1998) suggests that the fish staged in the irrigation ditches before making their spawning run and probably absorbed significant selenium. An alternate consideration is that water levels and dilution would have been highest prior to the spawning run. The fish spawn in response to spring floods when the selenium should have been diluted such that exposure may not have been as great. According to Kidd (WestWater Engineering Inc. 1996), important rearing habitat for razorback suckers “had a source of water other than the Colorado River itself, and did not depend entirely upon the river flow to maintain their water level.” Based on the comments of Stephens and Waddell (1998) above, that could mean that selenium concentrations were typically highest in their preferred habitats where they were formerly abundant.

#### **1.5.1.2 Abundance and Decline of the Fish**

The timing of the decline of the fish populations is debatable and apparently varies from one river system to another and, perhaps, from one section of the river to another. A report reviewed by Hamilton (1998) indicates that the endangered fish were rare in the Upper Colorado Basin after 1930, but in previous decades had been numerous (Dill 1944). On the other hand, a fisheries biologist who commonly collected baitfish from backwaters in the Upper Colorado Basin reports that razorback suckers were numerous and had to be “kicked out of the way” when wading through some flooded bottomlands in the early 1970s (Kidd, G., Northwest Fisheries, personal communication, May 25, 1999). Two 24-hour net sets caught 16 razorbacks at this location in 1974. Single net sets, after road construction altered this location in 1975 and 1976, resulted in six and zero razorbacks, respectively (Kidd 1977). According to Mr. Kidd, razorbacks were spawning and various age classes were present between 1971 and 1980, observations were verified by another researcher (WestWater Engineering Inc. 1996). A member of the recovery program also stated that there were frequent reports that razorbacks and squawfish were common in the early 1970s (Pfeiffer, F., U.S. Fish and Wildlife Service, personal communication, Sept. 22, 1999). If the pikeminnow (squawfish) and razorback sucker were relatively common—if not abundant in the early 1970s in the Grand Valley—the question that arises is whether the populations at this time were only big enough to be noticed but not viable. In other words, was the apparent persistence of the fish long after high selenium exposures had begun a result of their longevity, or did the habitat changes in the 1970s deliver the critical blow to their populations?



Kidd (WestWater Engineering Inc. 1996) reports that construction of the interstate highway, which caused the loss of an area known as the Colorado River Overflow, upstream of Grand Junction, near Debeque, was a major blow to razorbacks because that was the best remaining spawning area. The report conveying this information also notes that “The decline of endangered fish in the Gunnison River had occurred prior to Mr. Kidd’s arrival in Western Colorado in 1969” but “Prior to 1975, the nonnative fish populations had not yet reached the critical levels which seem to have precluded successful regeneration of most endemic fish.” (WestWater Engineering Inc. 1996).

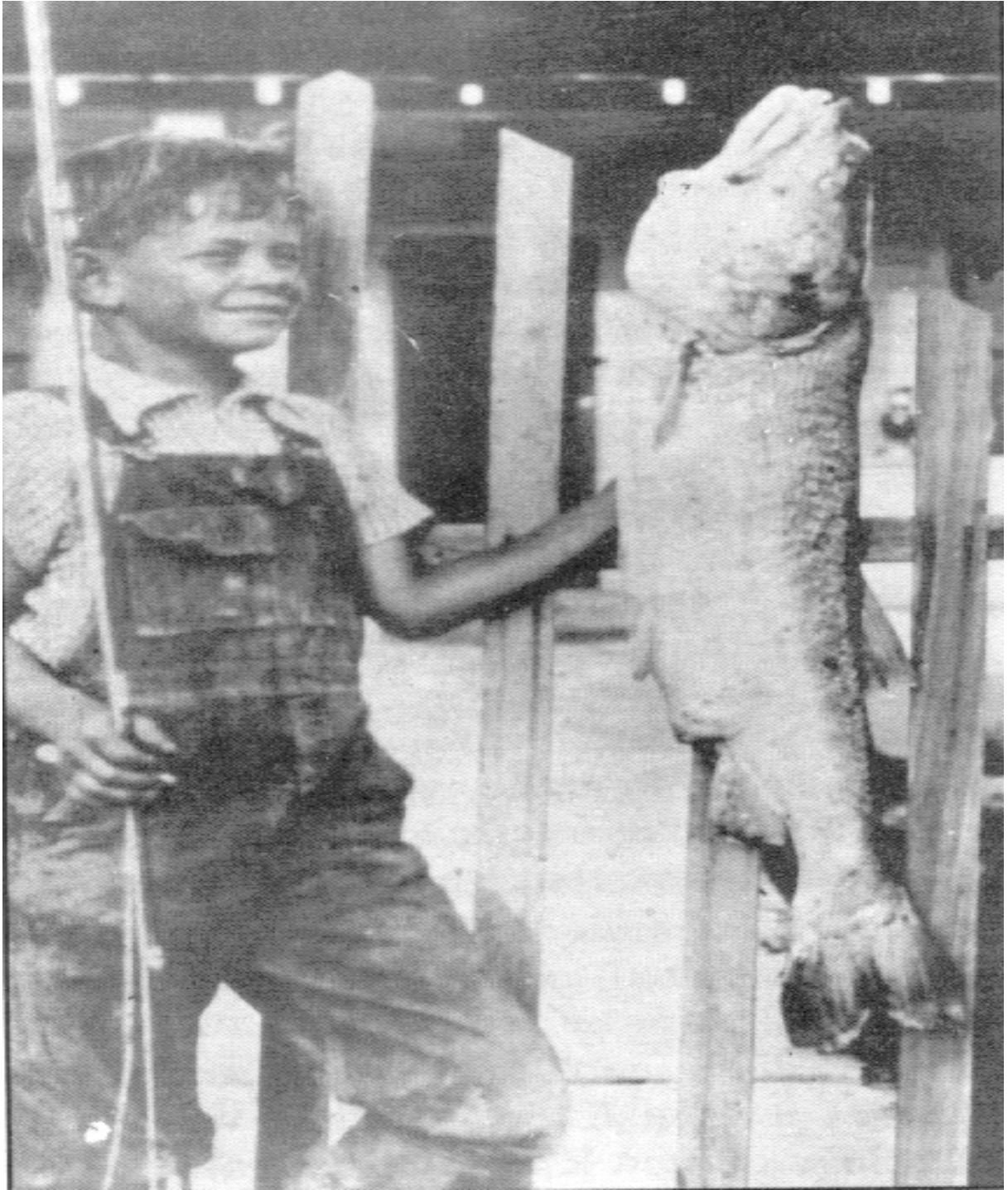
The flow regime of the Colorado River has not changed much since 1975, but prior to 1974, razorback suckers apparently were common between the Highline Canal and Debeque, Colorado (both just east of Grand Junction), and were wiped out by a gravel pit mine and the loss of the aforementioned Colorado River Overflow (WestWater Engineering Inc. 1996). Kidd also notes that razorbacks were relatively common through the early 1980s in the Grand Valley, but that the flood of 1983 and 1984 destroyed three additional important spawning areas (Kidd, G., Northwest Fisheries, personal communication, May 25, 1999).

Interviews with long-time residents (Quartarone 1995) also have been used to suggest that the fish declined prior to 1950. For instance, Hamilton (1998) quotes the latter source by saying: “In the late 1940s and early 1950s, many of those with fishing experience dating back to the 1920s stated that these fish were becoming rare in the Upper Colorado Basin.” But the specific examples supporting this statement in Quartarone’s report were all from the Dolores, a river severely affected by pollution from mining operations.

With respect to the Gunnison River, an interview with a man who netted fish for his mink farm noted that razorbacks were common until the mid-1950s, but were basically extirpated prior to the big water projects on the Gunnison (WestWater Engineering Inc. 1996). It was also concluded in the latter report that squawfish probably were never abundant in the Gunnison, a conclusion that contradicts at least one historical account that described experiences in the 1950s with a fish trap on the lower Gunnison: “...we’d go down there and there’s times you couldn’t even pick the damn thing up there’d be so many squawfish in it” (Quartarone 1995). It was also concluded that the decline of razorback suckers in the Gunnison was more directly related to the loss of important spawning and rearing areas because of bank armoring and channelizing rather than to the decrease in river flow or interruption of migration routes (WestWater Engineering Inc. 1996).

Squawfish reportedly declined in the Green River from the 1930s until Flaming Gorge Dam was constructed (Quartarone 1995). One man was quoted as saying that his father told him in the early 1950s that squawfish were rare and that he formerly saw more of them (Quartarone 1995). This comment, however, is contradicted by another interviewee who recalled catching squawfish on the Green “as big as a junior high kid” (Fig. 2) in the early 1950s (Quartarone 1995). The piscicide rotenone was used in the Green in the early 1960s, and although not abundant, squawfish and razorbacks were found. Older residents in the area said the endangered fish were mostly gone before the rotenone incident (Quartarone 1995).

There is also indication that other forms of pollution contributed to declines of the pikeminnow and razorback sucker in the Green River. For example, a retired game warden described a 1957 float trip on the Green River through the town of Green River, Wyoming: “...as soon as you get past Green River we were finding ducks and geese and everything else dead on the shores from



**Fig. 2. Large squawfish (pikeminnow) caught in the Green River (Quartarone 1995).**

the oil and pollution in the river.” Gravel operations, dumping of raw sewage, and dumping of waste from railroad yards were all cited by interviewees as affecting the endangered species (Quartarone 1995).

In addition to the personal statements of some interviewees regarding abundance and decline, there is also the fact that use of the endangered species in the 1930s and 1940s is frequently mentioned with little use or notice of them after that time. During the 1950s, however, there also was a significant decline in subsistence living. The entire country was in a post-war economic expansion, and western Colorado was undergoing a uranium boom that provided an enormous boost to the economy. Several quotes from Quartarone (1993) suggest that people stopped using the fish as times got better:

- “. . .in the Depression years you ate what you could get. . . .”
- “. . .we fished while the war was going on. . . .”
- One interviewee fished near Palisade, Colorado, “. . .between the years 1944 to 1949. . . .” and reported, “By god we’d catch a bushel basket full of them suckers. . .they were the humpback, some of them. . . .”
- Another interviewee reported catching humpbacks upstream from Moab, Utah, in the early 1960s. “Every time I went I probably caught three or four suckers and two or three squawfish. I’d say about half of them would be the humpback. . . .”
- Another anecdote records catching squawfish and razorback suckers in the 1950s on a farm along the Gunnison River. This speaker went on to say, “The humpbacks, there was lots of humpback suckers when we were kids. . .Back in the ’50s, late ’40s. . . .”
- Another quote indicating that fishing pressure was less in the 1950s is the following: “No, they didn’t disappear that fast you know. . .From the time we fished to say in the ’40s. . .when the war was going on, they was still around. . . .”
- There was even a published report in 1947 that indicated that squawfish were increasing: “Field reports indicate that this species of large minnow is on the increase. . . .”

## **1.6 Discussion**

There is much contradiction and many information gaps in the historical record regarding the loss of endangered fish in the Upper Colorado Basin. Moreover, several factors contributed to the decline. Documented factors include subsistence harvest and loss in irrigation ditches when fish were trapped after a flood (Quartarone 1995). Such losses, however, are probably not responsible for the catastrophic decline evident today. Other factors believed responsible include exposure to selenium, predation by nonnative species, and habitat alterations including loss of rearing areas and barriers to migration.

The endemic species were always exposed to selenium, but concentrations increased significantly beginning in 1915. Nevertheless, the fish remained relatively abundant for at least 30 more years and, at some locations, into the 1970s. Habitat alterations occurred throughout the 1900s

becoming more pronounced beginning in the 1950s and culminating with the major dam and road construction projects in the 1970s. These habitat alterations also were favorable to the proliferation of nonnative species that competed with the native fish.

Interviews with long-time residents provided some insight but no clear record regarding the decline of the native fish. The fish were used for subsistence in the 1930s and 1940s, a practice that ceased in the 1950s. By that time, the endemic species were considered “trash” fish as emphasis was placed on providing lakes and streams for trout fishing. This attitude was exemplified by the use of rotenone in the Green River during the 1960s.

In summary, the fish began a long-term decline sometime in the 1930–1950 period. With the advent of the 1970s, either that long-term decline became evident or the newly completed dam projects and road construction projects provided the mortal blow to the populations.

## **2. SELENIUM CYCLING IN THE ENVIRONMENT**

### **2.1 Source**

Because of the problems at Kesterson, the source of selenium in irrigation water in California’s San Joaquin Valley has been studied extensively (Martens and Suarez 1997). The explanation applicable to the San Joaquin is believed to explain selenium availability in other regions as well (Seiler 1998). In general, the primary sources of selenium in irrigation waters in the western United States are pyrite-rich cretaceous marine sediments. Selenium substitutes for sulfur in pyrite ( $\text{FeS}$ ) forming ferroselite ( $\text{FeSe}$ ). Selenium also substitutes for sulfur in chalcopyrite ( $\text{CuFeS}_2$ ). Supporting this explanation is the finding that selenium migration is inhibited in fractured rocks because of reactions with pyrite (Yllera et al. 1996).

The initial stages in mobilizing selenium are erosion of selenium-containing pyrites into alluvial fans and debris flows. As rainwater and snowmelt seep through these alluvial fans and debris flows, there is oxidation of the pyrites, a process manifested by acid sulfate seeps that contain high concentrations of selenium. The water from the acid seeps is neutralized by the soils lower in the valleys where the high pH and oxidizing conditions mobilize selenium. Because of the semi-arid climate, leaching and migration of the selenium is ephemeral and evaporite minerals are formed. In these evaporite deposits, selenium is associated with Na, Mg, and Ca. Two common selenium-containing evaporite minerals are mirabilite, a sodium sulfate with ten waters of hydration, and bloedite, a sodium/magnesium sulfate with four waters of hydration. In these and similar highly soluble minerals, selenate substitutes for sulfate. These minerals provide an instant source of mobile selenium when wetted. Meanwhile, the continued upslope erosion continues to add selenium to the system (Presser 1994).

Soils in the San Joaquin Valley were too saline for agriculture until a series of drains were installed. The irrigation water, therefore, leaches salts from the fields and into the drains that conduct them to collection areas, such as Kesterson (Presser 1994; Seiler 1998). The highest concentrations of salts usually occur where the groundwater flows upward, either in response to local topography or seasonal cessation of irrigation (Fio 1997).

## 2.2 Biogeochemistry

The particular form of selenium is important in predicting its toxicity and mobility. Selenite, because it is typically taken up more quickly than selenate, is more toxic. Apparently, passive adsorption to cell walls is an important aspect of the initial uptake (Bowie et al. 1996). For example, selenite uptake by phytoplankton was much faster than selenate uptake. Over the long term, however, selenite and selenate uptakes were similar and related to their concentration in the water. Apparently, bacteria and phytoplankton are primarily responsible for selenium's cycling in biota, however, toxicity to lower trophic levels requires selenium concentrations one to two orders of magnitude above what is found in the environment (Bowie et al. 1996). Indeed, algae and bacteria can incorporate selenium in their cells to levels more than 100 times greater than background (Masscheleyn and Patrick 1993). Higher trophic levels, such as fish, do not take up as much selenium from water and obtain most of their exposure from food (Bowie et al. 1996).

Several studies have examined selenium mobility in soils. For example, laboratory column studies have demonstrated that selenate moved as fast or faster than sulfate although some was retained in the soil (Alemi et al. 1988a). Indeed, when leaching alkaline soils, the addition of sulfate increases the leaching and availability of selenate (Brown and Carter 1969). Another study examined the application of selenium as a sodium salt to soil columns. Selenite was rapidly sorbed at all pH values. In contrast, selenate was mobile at all pH values and was completely leached with less than three pore volumes. Less than one pore volume was needed at pH 7–9 (Alrichs and Hossner 1987). In general, therefore, above pH 7.5, selenate sorption is virtually nonexistent (Masscheleyn and Patrick 1993).

Because of the differences in mobility and toxicity, the rate at which selenite or other reduced forms are converted to selenate is an important issue. Fortunately, much is known regarding selenium's biogeochemical cycling. For example, the Eh/pH diagram shown in Fig. 3 demonstrates that selenate is the dominant form in oxidizing waters. Indeed, most selenates are too soluble to persist in soils (Elrashidi et al. 1987). The Eh/pH diagram also shows that selenite and hydrogen selenite dominate in reducing systems. Selenium-VI is reduced to selenium-IV at +200 to +300mv and to elemental selenium or metal selenides below +50mv. Thus, although not as soluble as selenate, selenite and hydrogen selenite contribute to soluble selenium in soils at moderate redox potentials (Elrashidi et al. 1987). Finally, hydrogen selenide is the dominant form in highly reducing systems. In summary, the Eh/pH diagram suggests that under oxidized and moderately reduced conditions, selenium solubility is governed by adsorption-type mechanisms rather than by precipitation/dissolution reactions.

Selenium oxyanions can serve as electron acceptors, much as happens with sulfate reduction and Fe and Mn reduction. In other words, direct bacterial mineralization of organic matter occurs by selenate reducers that use selenium as an energy source (Presser 1994). Because of such biological conversions, the selenium species one would expect based on thermodynamics are not always present (Masscheleyn and Patrick 1993). For example, it has been demonstrated that adding nitrate inhibits selenate reduction because the organisms utilize nitrate as an electron acceptor in preference to selenium (Benson 1998).

During microbial assimilation, oxidized selenium species are reduced to various selenium-II compounds, such as seleno-amino acids. Organic forms of selenium are similar to those of sulfur and include seleno-amino acids (e.g., selenocysteine and selenomethionine), methyl selenides and methyl selenones (Dungan and Frankenberger 1999, also see Sect. 3). Organically complexed selenium is reported to be a significant fraction of total soil selenium in some systems. As shown

in Fig. 3, selenium reduction can occur under moderately reducing conditions (+100 to +400 mv). Under these same conditions, organic acid oxidation occurs (Masscheleyn and Patrick 1993). Thus, depolymerization or mineralization to inorganic selenium is a source of selenium for aquatic organisms and plant-root uptake (Masscheleyn and Patrick 1993).

Conversion in soils has been reported as relatively slow because of inhibition by stable ferric selenites (Geering et al. 1968). However, another study, which also referred to selenium oxidation as slow, reports that the conversion is biotic and yields both selenate and selenite (Losi and Frankenberger 1998).

In contrast to the slow oxidation, reduction of oxidized forms can occur in a matter of hours (Losi and Frankenberger 1998). Guo et al. (1999) reports that selenate reduction occurs within about 30 hours and that the reduced forms are stable even under extreme leaching conditions. Another study reports that selenate reduction is fast, within a day typically, and is microbially mediated. These investigators also report that the reduction potential is correlated to the amount of selenium present and not the amount of organic matter (Zhang and Moore 1997). Adding organic matter in column studies does cause selenate reduction on the column. The latter experiment also shows that more sorption seems to occur with intermittent leaching (Neal and Sposito 1991).

### **2.2.1 Cycling in Wetlands**

In wetlands where organic matter accumulates, some selenium can be expected to be present in an inorganic form, chiefly selenite, sorbed to inorganic minerals, primarily Fe-oxyhydroxides, or covalently bonded to organic matter. When wetlands are reduced further, the Fe may dissolve releasing the selenium. A removal mechanism in wetlands is the microbial production of dimethyl diselenide. Dimethyl diselenide is very insoluble in water and has a very high vapor pressure, so it is not persistent (Masscheleyn and Patrick 1993). Studies of pond sediments from Kesterson indicate that the selenium in the ponds and drains was mostly selenium-II, but that selenium would be available if the sediments dried out (Martens and Suarez 1997). An experiment was conducted in which it was shown that selenium was immobile in untreated sediments, but air drying and freeze drying followed by water leaching mobilized it. The data from this experiment indicate that the highest concentrations were found almost immediately (Alemi et al. 1988b).

Apparently, approximately 99% of the selenium in the ponds at Kesterson is the red, monoclinic elemental form. Rapid reoxidation of selenium was observed in some of the previously ponded sediments. Sixty percent was reoxidized to a mixture of selenium-IV and selenium-VI in unamended sediments within two days of sampling, but if organic matter was added, the reoxidation did not occur, at least over the 2-day period. Reoxidation occurred merely with exposure to air (Tokunaga et al. 1996). The latter results seem to contrast with those of Losi and Frankenberger (1998) who report that reoxidation is biotic and slow. Finally, another study reports that the reduction occurred only in the top 1 cm of sediment (Tokunaga et al. 1997).

### **2.3 Discussion**

Selenium cycling in soils and lower trophic levels has been studied extensively. The inorganic behavior of selenium appears to be well defined, but the combined geochemical/biochemical cycling is less well established. Uncertainties remain with respect to the structures of selenium metabolites in biological material and with respect to the kinetics regarding its remobilization from sediments. The latter issue is of great concern. An improved ability to predict selenium

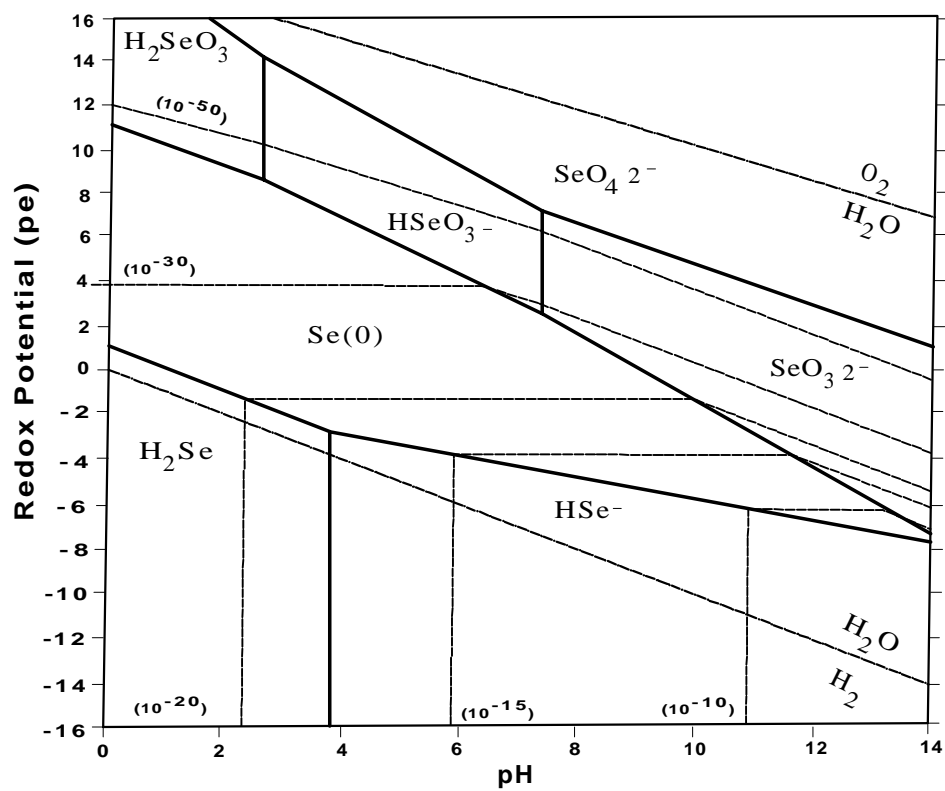


Fig. 3. Predominance areas for dissolved selenium species in response to pH and redox potential (Masscheleyn and Patrick 1993).

availability in sediments would assist in determining the long-term toxicity at selenium-impacted lakes and wetlands.

### 3. BIOTRANSFORMATIONS OF SELENIUM

Section 2 discussed some of the microbially mediated redox reactions of selenium. This section discusses specific transformations within organisms and the mechanisms of toxicity.

The complexity of selenium biochemistry is illustrated by recent research that shows how selenium deficiency affects reproduction in mammals. The investigators note that proteins often lead “multiple lives” depending upon their environment, but it is rare for a protein to act as a soluble enzyme under some circumstances and as an insoluble structural component in others. Apparently, a selenium-containing protein in sperm does behave in this fashion, which explains why selenium deficiency causes infertility. Apparently sperm with low selenium break in the middle (Ursini et al. 1999).

Selenium is similar to sulfur biochemically, and cells do not discriminate between the two when carrying out protein synthesis. Substitution of selenium for sulfur disrupts the normal chemical bonding, which results in improperly formed and dysfunctional proteins and enzymes. Selenium is also necessary for proper formation and functioning of glutathione peroxidase which is a major cellular antioxidant enzyme. The enzyme protects cell membranes from damage of lysis due to lipid peroxidation (Lemly 1998).

Primary producers transform selenium into selenoamino acids that are incorporated into selenoproteins (Brix et al. 1997). The latter investigators demonstrate that microbial communities accumulate selenium and that uptake of selenite is favored over selenate. Further, these investigators note that microbial uptake explains the high concentrations of selenium in midges collected from seleniferous locations.

It has also been demonstrated that aquatic microphytes volatilize selenium in the form of alkylselenides (Fan and Higashi 1997). For instance, a laboratory study shows that a filamentous cyanophyte mat cultured from waters of a large agricultural drainage basin in California volatilized selenium from selenite. Nearly 100% selenium removal occurs in the basin, and it was speculated that this is the reason. Selenite was incorporated into proteins in the form of selenomethionine at selenium concentrations of 5000  $\mu\text{g/L}$  or higher in the laboratory. This reaction, however, has not been verified in the field (Fan et al. 1998).

Although the structures of several selenium-containing metabolites have been identified (Fan et al. 1988), in general, little is known about specific selenium biotransformations. For this reason, research has often been conducted with selenomethionine. One study, however, found only ultra-trace levels of this compound in the primary producers surveyed, suggesting that it may not always be a good surrogate for biological exposure (Fan and Higashi 1997).

Another complexity with respect to field research is that other ions affect selenium uptake. For example, research shows that silicate and phosphate enhance the uptake of selenium by *Chlamydomonas reinhardtii* by a factor of three to five (Riedel et al. 1997). Other commonly occurring ions tested show little effect. Another study indicates that Mo, Hg, Cr, and As can inhibit microbial volatilization of selenium (Karlson and Frankenberger 1988). Data showing the



effect of other ions have not been obtained at many field locations suggesting that solution and sediment composition may be a factor in some of the seemingly contradictory evidence regarding the concentration of selenium that causes toxicity.

Indeed, the effect of other toxic ions on selenium toxicity is one of the more controversial issues. Unlike the Belew's Lake circumstances, elevated selenium in the Upper Colorado Basin and other western locations is accompanied by high concentrations of other ions. For example, Stephens et al. (1992), in a study of Stewart Lake and Ouray National Wildlife Refuge in Utah, found that boron and zinc concentrations, as well as selenium, were frequently greater than aquatic protection standards. Selenium concentrations ranged to 140 µg/L, with most samples having between 50 and 100 µg/L. Uranium was also elevated in some samples. Whether, and how much, the other potentially toxic ions affect selenium toxicity is unknown.

Stephens and Waddell (1998) report boron concentrations up to 700 µg/g in aquatic plants in a study on the Uintah and Ouray Indian Reservations. In addition, 54% of the water samples exceeded 750 µg/L, which is the Utah standard for protection of agricultural crops. Some locations exceeded 5000 µg/L—1000 times the current United States EPA criterion of 5 µg/L. Dissolved oxygen fluctuated widely, temperatures were high, and the total dissolved solids (TDS) exceeded 1200 mg/L in 40% of the samples. Vanadium was also elevated. Determining the role of selenium under such conditions is difficult, although elevated concentrations were reported for fish (8.3 µg/g) and invertebrates (~10 µg/g).

Definite conclusions regarding selenium accumulation are also difficult to draw from data from Western Colorado's Uncompahgre Project area. Soils on the cretaceous Mancos formation had high total- and water-extractable selenium, but only 5 of 128 samples of alfalfa grown on these soils had selenium concentrations that exceeded a recommended dietary limit for livestock. Of the constituents evaluated in the program, selenium caused the highest proportion of samples to exceed guidelines for fish, but Cu, Zn, B, and V concentrations also frequently exceeded aquatic protection limits (Butler et al. 1996). These investigators stated that irrigation may account for 75% of the selenium in the Colorado River near the Colorado/Utah state line. Nonetheless, toxicity tests on irrigation-drainage water from five streams indicated no significant toxicity differences between control and test samples.

### **3.1 Toxicity to Fish**

Selenium's toxicity to fish is well established from the episode at Belew's Lake and a similar unpublished occurrence at Roger's Quarry in Oak Ridge, Tennessee (Southworth, G.R., Oak Ridge National Laboratory, personal communication, September 1999). At both locations, selenium leaching from fly ash disposal resulted in the appearance of deformed fish. Adaptation of data from these incidents to other sites, particularly those in the western United States, remains controversial.

Symptoms of selenium deficiency in fish include reduced growth, anemia, exudative diathesis, muscular dystrophy, and increased mortality (Lemly 1998). With respect to specific uptake mechanisms, Lemly (1982) reports that the "major uptake pathway appears to be erythrocyte transport mediated by active oxo-groups on the selenium anion, with subsequent exchange at preferential sites. High apportionment into the spleen and heart may be influenced by differential formation of selenoproteins."

A study of fish from two Texas lakes evaluated hepatic concentrations of selenium. Selenium was nearly four times higher in sunfish from contaminated Martin Lake (7.6 ug/g) than in fish from a reference lake (2.1 ug/g). Sunfish with elevated levels of hepatic selenium had substantial alterations in the liver, including necrosis, cytoplasmic vacuolation, and Kupffer cell proliferation. The ovaries of mature fish collected from Martin Lake frequently had atretic follicles, abnormally shaped follicles, connective tissue hypertrophy, asynchronous oocyte development, and an overall reduction in the number of developing oocytes. These abnormalities were not detected in the population from the reference lake. This article does not give concentrations in water, sediment, or food, but notes that a reduced amount of selenium is still being discharged into the lake (Sorensen 1988).

Lemly (1983) showed that ventilation frequency and pectoral fin activity of bluegills and largemouth bass increased significantly when the selenium water concentration increased from 5 to 10  $\mu\text{g/L}$ . Such effects led to an increased need for oxygen, which demonstrates why selenium's toxicity is greater in colder water (Lemly 1993a, 1997a).

### **3.1.1 Acute Toxicity**

Although concentrations of selenium in the environment are seldom acutely toxic, a review of acute toxicity is appropriate because the data provide an estimate of the relative sensitivity of the various species. Acute toxicity data have been reviewed by Mauk and Brown (1999) who made the following comments: "Studies have determined that 96-h LC50 values range from 1 to 35 mg/L for fish species (e.g., Klaverkamp et al. 1983; Niimi and LaHam 1976; Sato et al. 1980). For coolwater fish species similar to walleye, Kalverkamp et al. (1983) report a 75.5-hour LC50 of 11.1 mg/L for northern pike and a 10-day LC50 of 4.8 mg/L for yellow perch." Those published values were similar to the 12 mg/L 96-h LC50 reported by Mauk and Brown (1999) for walleye.

Hamilton and Buhl (1996) reviewed their previous toxicity testing with the pikeminnow and razorback sucker and noted that both species from the Green River were "significantly less sensitive to selenate than to selenite." However, in San Juan River water, the pikeminnow had the same selenate to selenite relationship (the 96-h LC50 values were 88 mg/L for selenate and 20.7 for selenite), but the razorback sucker showed similar sensitivity to both forms (the 96-h LC50 was 15.9 mg/L for selenate versus 11.3 mg/L for selenite). These data suggest that there are unknown factors affecting the toxicity of the selenium species. The selenite 96-h LC50 was similar for the flannelmouth sucker which, like the pikeminnow, showed approximately half as much sensitivity to selenate.

### **3.1.2 Chronic Toxicity**

Chronic selenium toxicity to fish remains a controversial issue. One reason is that adult fish can tolerate relatively high levels of selenium in their tissues with no apparent ill effects. Dietary selenium sufficient to load eggs beyond teratogenic thresholds (diet of 5–20 ug/g) does not affect health or survival of parent fish. Apparently adults, because of their lipid reserves and larger livers, can withstand the selenium, but when reproducing, the female draws on these lipid reserves and passes the selenium to her progeny. Hatchability is not affected, but survival of the larvae is severely compromised (Lemly 1998). Tissue concentrations of 6–12 ug/g in fillets and 20–40 ug/g in visceral tissues are sufficient to be presumptive of teratogenesis. This report also

notes that the relationship between the whole-body concentration of selenium and the prevalence of teratogenic deformities in fish is exponential, such that relatively small changes in selenium can cause large changes in the incidence of teratogenesis.

As noted above, with respect to concentrations in water, one researcher reports toxic effects on bluegill at slightly more than 5 µg/L whereas other research shows no effect with razorback suckers at 15 µg/L. Similarly, Crane et al. (1992) report that approximately 50% of a yellow perch population disappeared during a 550-d pond experiment with a selenium concentration of 25 µg/L. Reproduction also was diminished at this concentration, but no obvious effects were observed at 10 µg/L.

It is generally believed that food chain concentrations, rather than water concentrations, are a better predictor of selenium toxicity. The data from the Uncompahgre Project area support this contention, because several locations where selenium was elevated in fish had no detectable selenium in the water (Butler et al. 1996).

A study by Hamilton et al. (1996) with razorback suckers demonstrates the difficulty in developing unequivocal selenium criteria for protection of the endangered species. The study was designed to obtain a dose-response curve, but instead there was 100% mortality, and the median time to death was actually longer from the high selenium locations. The investigators suggest that synergistic and antagonistic effects with other inorganics explain these observations, but no studies were done to identify these effects.

Hamilton and Buhl (1996) discuss the difficulty of interpreting a study such as theirs by citing another project with larval bluegill that had unexpected results (Coyle et al. 1993). The latter study concluded that the fish died of starvation. However, Hamilton et al. (1996) state, "Because high survival of larval fish [it was > 90%] fed brine shrimp nauplii during typical fish culture operations had been observed, and brine shrimp can contain as much as 2.7 µg/g Se, this concentration in aquatic invertebrates, by itself and in the absence of other biological, chemical, or physical stressors, is probably not sufficient to cause adverse effects in larval fish. This suggests that in the present investigation the adverse effects observed in studies 2, 3, and 4 in larvae-fed food organisms from S1 (selenium conc of 2.3 and 2.5 µg/g) or S4 (selenium conc of 2.4 µg/g) may not have been due to selenium alone but rather to the mixture of inorganics, including Se, in the food organisms or S1 water. Adverse effects observed in the other treatments and in fish fed food organisms from S1 and S4 that contained 3.5 µg/g or more of selenium probably due primarily to Se." These conclusions are based on research that indicates that 4 µg/g of selenium in food will have toxic effects on fish, regardless of the species (Lemly 1993b). The latter study noted that 3 µg/g was a toxic threshold for fish species. The implications of these interpretations, however, are significant because the final conclusion was that razorbacks are as susceptible as rainbow trout, Chinook salmon, and bluegill, which are the most sensitive fish species (Hamilton et al. 1996).

The only inorganic implicated, besides selenium in the latter study, was vanadium, which was also present in zooplankton at concentrations believed to be toxic. Hamilton et al. (1996) quote Hilton and Bettger (1988) who "concluded that dietary vanadium was at least as toxic, if not more toxic, than dietary selenium." Another possible factor is reported by Saiki et al. (1993) who attributes adverse effects at Kesterson to high concentrations of major ions in atypical ratios and high concentrations of sulfate.

Boron may also contribute to toxicity in many western locations because it is frequently elevated wherever selenium is high. The possibility of interactive effects between boron and selenium has been suggested (Hoffman et al. 1988).

Boron has a role in the calcium cycle and in respiratory processes and the utilization of carbohydrates. According to recent guidelines, fish reproductive tissues are affected at concentrations on the order of 1 mg/L (USDOI 1998). Boron in water becomes a problem at about 10 mg/L for crops and plants, at 13mg/L for aquatic invertebrates, at 25 mg/L for fish, at 20 mg/L for bird eggs, at >30 mg/L for waterfowl, and at >80 mg/L for mammals (USDOI 1998). Dietary boron in concentrations up to 1,000 ug/g was not teratogenic to mallards, and hatching success was unaffected by boron concentrations up to 300 ug/g.

Saiki et al. (1993) evaluated boron in the food chain in conjunction with selenium. The concentration of boron recommended for protection of sensitive aquatic life was exceeded in several locations, but not by as much as selenium. Fingerling fish were affected by some high-boron tile water, but effects of other ions could not be ruled out.

Boron was elevated in plants but did not seem to biomagnify. No effects were observed for molybdenum. This study also demonstrated that, although much variation was present, the highest concentrations of selenium occurred in detritus, mosquitofish, and chironomid larvae. Lower concentrations were measured in other invertebrates and fishes. Filamentous algae generally contained the lowest concentrations of selenium measured in biota. Compared with biota, water and sediment contained little selenium. This study also demonstrated that boron and selenium were often, but not always, correlated to conductance, turbidity, pH, total alkalinity, total dissolved solids (TDS), and total hardness.

Fish tissue concentrations have often been used as diagnostic for selenium poisoning, although researchers agree that eggs are a better indicator. Nevertheless, concentrations in tissue suggest differences in selenium uptake either among species or related to different environments. For example, at Belew's Lake, it was reported that 5–10 ug/g of selenium in muscle was an indicator that the bullheads, carp, and green sunfish were sterile (Lemly 1985). However, green sunfish and carp are reproducing at Sweitzer Lake in western Colorado, and tissue concentrations of 15–25 ug/g have been measured in the sunfish, with 30–40 ug/g having been measured in carp (Butler et al. 1991). It is also of interest to note that western Colorado's Crawford Reservoir, a lake known for its overabundance of yellow perch, yielded a perch fillet with 16 ug/g dry weight of selenium (Butler et al. 1994). In a later report in the Grand Valley, fish samples were frequently found exceeding the 5–10 ug/g cited above by Lemly (1985). Butler et al. (1994) also noted that the "selenium concentrations in whole-body fathead minnows in the middle Grand Valley ranged from 8 ug/g dry weight to 22 ug/g dry weight and exceeded known toxicity concentrations to fathead minnows of 6 ug/g dry weight." Butler et al. (1994) listed two citations (Ogle and Knight 1989; Schultz and Hermanutz 1990) for the 6 ug/g toxicity figure—a value that is low in comparison with Lemly's finding at Belew's Lake as discussed above (1985). Such data clearly indicate that there are unsettled issues regarding species sensitivity and the concentrations of selenium at which fish are affected.

Values of selenium in fish eggs also provide some confusing results. Lemly (1996) has stated that >20 ug/g represents a high hazard for reproductive impairment in fish. A study by Hamilton et al. (1997) showed that egg concentrations from fish in some Grand Valley backwaters were as high as 30–40 ug/g. Nevertheless, larval survival was 70% if the larvae were transferred to

reference water and fed brine shrimp. Survival was near zero when the fish were fed site food that ranged in concentration from 4.5–37 ug/g. Larvae from broodstock that were fed food with 4.6 ug/g of selenium also showed much lower survival (approximately 20–80% survival in three trials)—a survival rate lower than for the larvae hatched from the high-selenium eggs and fed brine shrimp. The data are interesting because they suggest that the concentration in food is the determining factor in survival, not the concentration in the eggs and larvae. Of course, larval fish high in selenium would typically be exposed to high-selenium food sources as well. Nevertheless, the data have implications regarding both the toxicological mechanism and the setting of standards for protection of aquatic species.

### **3.2 Toxicity to Birds**

Serious deformities in aquatic birds at Kesterson led to the recognition of elevated selenium in western irrigation projects. As with fish, however, much is unknown regarding uptake and specific transformations within the various species. For example, Hoffman and Heinz (1988) report that the mode of action of embryotoxicity and teratogenesis of selenium on mallards is uncertain. Evidence of increased lipid peroxidation and related glutathione peroxidase activity were found by Hoffman and Heinz (1988) and in coots from Kesterson by Ohlendorf et al. (1988). Selenium-caused oxidant-induced stress or damage and increased lipid peroxidation has been identified in mammals (Dougherty and Hoekstra 1982), and increases in organic solvent-soluble lipofuchsin pigments of the liver as well as glutathione peroxidase activity were identified in mice (Csallany et al. 1984).

Hoffman and Heinz (1988) also demonstrate that selenomethionine was much more toxic than selenite, probably because uptake is much greater. Harmful effects were observed when feed of 10 and 25 ug/g of selenium as selenite or selenomethionine was provided. Similarly, Ohlendorf (1997) states that dietary concentrations of 6 ug/g and higher will affect sensitive species.

Most studies do not report a water concentration that is considered harmful. Nolan and Clark (1997), however, state that irrigation drain water containing 3–20 µg/L selenium is hazardous to some aquatic birds under some conditions, but that greater than 20 µg/L is hazardous to most aquatic birds under most conditions. Concentrations greater than 20 µg/L are common in the Grand Valley, but as described in Sect. 1.5, obvious effects have not been identified.

There are considerable differences in species' sensitivity to selenium. For example, ducks are more sensitive than stilts, which are more sensitive than avocets—the latter being relatively insensitive to selenium (Ohlendorf 1997). Grebes also have less susceptibility than most other waterfowl (Hoffman et al. 1988). According to Ohlendorf (1997), ducks exhibit deformities at a 10% rate when egg concentrations are approximately 20 ug/g, stilts at 35 ug/g, and avocets at 75 ug/g. Concentrations in this range are found in the Grand Valley and in waters associated with the Uncompahgre Project. For example, mallard egg selenium concentrations were as high as 17 ug/g, but most were less than 10 ug/g. Five coot eggs, however, exceeded 20 ug/g of selenium (Butler et al. 1994). Another study in the Grand Valley also reports coot eggs with concentrations high enough to indicate risk from selenium (Butler et al. 1996). Only one incident of Kesterson-like deformities has been reported in the area. The low incidence of identified deformities may be the result of low overall reproduction (Osmundson, D.B., U.S. Fish and Wildlife Service, personal communication, June 25, 1999).

Other field data in the area show avian concentration of selenium. For example, coots at Stewart Lake and Ouray National Wildlife Refuge (NWR) had liver selenium concentrations of 32 ug/g which, based on results from Kesterson, was sufficient to cause deformities. Indeed, deformed coots led to closure of Ouray NWR for a while. This investigation shows that selenium, zinc, and boron all were elevated in waterfowl and fish compared with mean concentrations elsewhere. Both the zinc and selenium in coot livers was similar to Kesterson (Stephens et al. 1992). Selenium in the 30 ug/g range was also found in mallard livers at Sweitzer Lake (Butler et al. 1991), but no nests were found to evaluate reproductive impairment. Concentrations of selenium in livers of ducks, grebes, and coots in wetlands of the Uncompahgre Project also had elevated (17 to 49 ug/g) selenium (Butler et al. 1994), but once again, direct evidence of reproductive failure or deformities was absent.

An element of controversy with respect to avian toxicity is the natural rate of teratogenesis. Ohlendorf (1997) assumes low (~0.5%) rates compared with those of O'Toole and Raisbeck (1998), who quote several references that indicate that the rate of normal teratogenesis in avian species is higher (0.6 to 4.2% in mallards).

O'Toole and Raisbeck (1998) question much of the historical mammalian research on selenium. They note that there is little doubt that selenium was a problem at Kesterson and the Kendrick site in Wyoming, but they question the ubiquity of selenium poisoning. For example, O'Toole and Raisbeck state, "we do not consider that either numbers (concentrations of selenium in tissues) or lesions (morphological analysis) alone generate an assured diagnosis of selenosis." They also disagree that craniofacial abnormalities are a unique indicator of selenium poisoning, saying that a variety of chemicals, physical factors, and inherited defects cause the same problems in chickens and turkeys.

Selenium is apparently not teratogenic to mammalian species. When toxicosis was induced, heart lesions were observed in mammals but not in birds. O'Toole and Raisbeck also do not believe that selenium causes central nervous system problems in mammals and note that none have been reported in birds. O'Toole and Raisbeck also take note of the prevailing theory of "oxidative stress as the pivotal biochemical lesion in selenosis." Although they cite several references supporting this statement, they believe that "the link between oxidant stress and epithelial lesions remains to be demonstrated." These investigators, however, cite some of their own data that are at least suggestive of the same hypothesis.

O'Toole and Raisbeck (1998) believe that whole blood concentrations of selenium >10 ug/ml are suspicious for selenosis and note that "affected birds may have no lesions other than emaciation, a common nonspecific feature of many diseases of free-ranging waterfowl." O'Toole and Raisbeck also agree that egg concentrations of >3 ug/g are a probable indicator of selenosis but question what they call a "focused necropsy approach" or just looking for selenium. Other analyses and better microbiology are needed. "What is missing in many field studies is a correlation between tissue concentrations and disease syndromes and/or lesions that match credible descriptions of selenosis in waterfowl." For example, they believe that teratogenesis should have been observed at Stillwater, yet it was not. Moreover, O'Toole and Raisbeck believe that one ought to be able to produce in the lab what is observed in the field—a belief disputed by Skorupa (1998).

As with fish, effects of boron on avian species have been evaluated. Stephens and Waddell (1998) note that boron is not a problem for birds unless the diet contains 1000 ug/g. One study shows, however, that the combined toxicity of boron-selenium mixtures to ducklings was

approximately additive, particularly when the nutritional status of the birds was compromised (Hoffman et al. 1991). This finding suggests that the variable reports from the field may be the result of multiple stressors.

### **3.3 Discussion**

Much research is needed to explain the apparent contradictions in the technical literature. In particular, the mechanism by which selenium concentrates in adult fish tissues and then affects reproduction is not well established. Moreover, the effects of other ions on this mechanism and species-to-species sensitivity are poorly defined. Carefully controlled laboratory studies also are needed to determine the effects of other stressors (other ions, temperature, etc.) on selenium cycling and fish reproduction.

## **4. HABITAT, NONNATIVE FISH, AND LIFESTYLE**

### **4.1 Habitat**

Habitat change has contributed to the loss of the squawfish and razorback sucker. As listed by McAda and Kaeding (1991), changes to the rivers include:

- A significant decrease in the spring runoff has occurred. Peak discharges have decreased by 45% in the Colorado and Gunnison Rivers and by 20% in the Green River (Irving and Burdick 1995). The duration of flooding has also been reduced, all by transmountain diversions and reservoir storage. A related problem is that zooplankton, the primary food of razorback suckers, do not produce well in turbid rivers. The backwaters are more productive. Although the overall mass of sediment conducted by the rivers is now less, the rivers remain turbid at the same time the lower flow limits the number of backwaters that were formerly available. These facts suggest that starvation may sometimes be a problem for the pikeminnow and razorback suckers (Irving and Burdick 1995). Finally, spawning locations have become silted over and are rarely washed free because the peak flows are insufficient to move the sediment.
- Channels that formerly were braided have been lost. The decrease in habitat and habitat complexity has eliminated many locations favored by the endangered species.
- Because water is taken from the bottom of the reservoirs, lower temperatures at the spawning sites have been noted. The lower temperatures eliminate habitat because the fish need certain temperatures as spawning cues and for their most efficient metabolism. Moreover, there are indications that lower temperatures keep the young-of-the-year (YOY) too small. The fish are not able to grow adequately, rendering them more vulnerable to predation and other problems, such as disease and parasites (Kaeding and Osmundson 1988). The lower temperature has also eliminated habitat. For example, approximately 40 miles of additional habitat could be obtained if the Aspinall unit discharged slightly warmer water into the Gunnison. Similarly, 14 miles of additional habitat could be obtained in the Colorado River if a diversion structure, such as the one now in place at the Redlands Dam, were constructed. The habitat is suitable as far as DeBeque, Colorado, but this reach of river is inaccessible to

the fish (Osmundson, D.B., U.S. Fish and Wildlife Service, personal communication, June 25, 1999).

Burdick (1997) argues that habitat requirements on the Gunnison River have not been adequate for the survival of the endangered fish. Some of the problems cited in this study are:

- The depth below the Redlands Dam often dropped to less than 1 ft (Fig. 4). Indeed, flow ceased at times in this reach. Based on this finding, a minimum flow requirement of 300 cfs has been established to maintain 1 ft in depth at all times.
- Flows of 15,000 cfs were common before the Aspinall unit was completed but have only occurred four times since the project was completed in 1965.
- The Aspinall unit has also caused a temperature decrease in the summer and increase in the winter.

The most comprehensive view of habitat changes was conducted by Pitlick and co-workers (1999). Using data from unregulated rivers, the East River which flows into the Gunnison River and the Yampa River which flows into the Green River, they concluded that pre-1950 and post-1950 annual peak discharges and mean annual discharges are not statistically different. Their study showed that changes to the Gunnison and Colorado Rivers were frequent and widespread, but that the period of the 1950s to 1970s was especially significant. Peak discharges and mean annual sediment loads were reduced significantly during this period.

Because tributaries still contribute as much fine sediment as pre-1950, the lower flows now allow more sediment to deposit, causing the channel to become narrower and less complex. It is estimated that one-fourth of the sidechannel and backwater habitat has been lost in the Colorado since 1937 (Pitlick et al. 1999). Loss of habitat on the Gunnison River was not considered as severe by these authors. However, the Redlands Dam has prevented migration of the fish since 1918. Thus, pikeminnow might have spawned, drifted downstream, and then were unable to return. Indeed, the comment that pikeminnow were “never” abundant in the Gunnison (see Sect. 1) may be a result of the 1918 dam.

In order to recover the pikeminnow and razorback sucker on the Gunnison, one researcher made the following comment: “Key to this restoration effort is recommending and implementing streamflows that will mimic the historical hydrograph to increase the magnitude and lengthen the duration of spring flows to create and provide riverine habitat for native fishes and at the same time control the smaller nonnative fishes in warmwater reaches of the Gunnison River.” Razorbacks were then specifically mentioned as needing floodplain habitat—the need is for wetlands adjacent to the river corridor that were formerly inundated but no longer are (Burdick 1995). The appropriate floodplain habitat for razorbacks has been described as having a source of water other than the river itself with temperatures 5° to 10° degrees warmer. Such ponds would also be 5 acres or greater in surface area with a depth of 3 to 5 ft and a length of at least 1500 ft (WestWater Engineering Inc. 1996).

The overriding effect of habitat restoration may be evident based on observations related to re-operation of Flaming Gorge Dam on the Green River. Re-operation consisted of releasing flows that more closely mimicked historical circumstances. The number of pikeminnow encountered after re-operation was implemented is approximately three times what it was before. No effect





**Fig. 4. Low flow in the Gunnison River (Burdick 1997).**

has been observed for the razorback sucker, apparently because floods have been insufficient to provide sufficient bottomland habitat (Modde, T., U.S. Fish and Wildlife Service, personal communication, July 2, 1999).

Unfortunately, restoring the hydrograph will be almost impossible on the Colorado, because the river is influenced by too many dams and diversions. Coordinating the entire system of reservoirs and diversions would be very difficult technically and politically. In addition, flushing flows, such as those used in the Grand Canyon, would be more difficult to implement because the Gunnison and Colorado are gravel-bed rivers, whereas in the Grand Canyon, the river bottom is predominantly sand.

## **4.2 Nonnative Fish**

The role of nonnative fish is no less controversial than the other aspects involved with recovering the endemic endangered species. As noted in Sect. 1, blaming nonnatives is preferred by those favoring population and land development because poisoning nonnative fish has no effect on the water diversions needed to promote population growth.

Researchers G. Kidd (Northwest Fisheries, personal communication, May 25, 1999) and T. Modde (Fish and Wildlife Service, personal communication, July 2, 1999) both stated that while they believe nonnatives are a problem, nonnatives are not sufficient of themselves to account for the loss of the native species. Supporting this belief are data from electroshocking studies performed on the Gunnison River. In the 2.2 mile reach below the Redlands diversion dam, almost 7000 fish were identified (Burdick 1997). Flannelmouth suckers were the most common fish (33.9%) followed by bluehead suckers (31.9%), common carp (13.4%) and roundtail chub (12.6%). Only one northern pike was caught and only 85 (1.3% ) channel catfish. On the San Juan River, an examination of the digestive tracts of 529 likely, nonnative predators collected from backwaters where native larval fish were present concluded that, "predation by nonnative fishes does not appear to greatly reduce recruitment for these species" (Brandenburg and Gido 1999).

Another project characterized fish in the Gunnison River from the confluence of the north and south forks as far as the Redlands Dam. Twenty-four species were found: 7 natives, 14 nonnative species, and 3 hybrids. Endemics found included squawfish, humpback chub, and flannelmouth sucker. Native nonendemics included bluehead sucker, mottled sculpin, and speckled dace. Overall, 80% of the fish caught were natives. Trout constituted only 5% of the catch and no channel catfish were found. Twenty northern pike were caught, but these were thought to be from Paonia reservoir (Burdick 1995). The low percentage of nonnatives collected in the Gunnison River is, in part, a consequence of the electrofishing method which captures bigger fish and can significantly underrepresent small nonnatives that may prey heavily on larval, native fish. It is also possible that the Redlands Dam, which has been in place since 1918, may have inhibited the establishment of nonnatives to some degree. Nonnative fish species are more abundant than native fish in the Colorado River. Similar to the Gunnison River study, however, most fish electrofished were native species, primarily bluehead and flannelmouth suckers (D.B. Osmundson 1999).

The most serious predation problems, however, may occur in backwater rearing areas where nonnatives, such as largemouth bass, green sunfish, and red shiner, prey on the young of the endangered species. Nevertheless, it is not entirely clear why flannelmouth suckers and other native fish continue to thrive while the pikeminnow and, particularly, the razorback sucker do not. One explanation that has been advanced with respect to razorback suckers is related to their habit of spawning so early in the spring. Perhaps their larvae are the first to enter the backwaters in the spring as the water warms, and the nonnatives are especially ravenous. For that reason, the razorbacks could all be consumed, but when the other larval fish enter the nursery areas, there is significant survival because there is now enough prey to overwhelm the predators with their sheer numbers (Pfieffer, F., U.S. Fish and Wildlife Service, personal communication, Sept. 22, 1999).

Alternately, flannelmouths spawn in smaller streams (Hamilton and Buhl 1996) and may have more available habitat.

The proliferation of nonnative fish species has been “documented and shown to be related to habitat alteration and river flows” (Hawkins and Nesler 1991). This same study states that predation has mostly been documented with channel catfish and that “conclusive statements about competition could not be made.” The well-documented incidents occurred after stocking or in confined areas. Interviews with local fisheries professionals conclude that “habitat changes, primarily flow-related, were considered a primary problem of greater magnitude than introduced fishes,” and some form of habitat manipulation was considered the most likely way to improve the lot of native fishes (Hawkins and Nesler 1991). A literature review by these investigators indicates that the high spring floods that formerly occurred favored natives over nonnatives. Nonetheless, they also cite a study by Osmundson (1987) that shows in ponds along the Colorado River, largemouth bass prefer squawfish and have a big effect on survival of young fish. Similarly, young razorback suckers are vulnerable to predation and do little in terms of predator avoidance. Green sunfish are especially adept at devouring razorbacks (Tyus and Saunders 1996).

In summary, competition from and predation by nonnative fish species is a significant factor regarding the survival and recovery of populations of the pikeminnow and razorback sucker. This competition, however, is aided by changes in the habitat, changes that both favor the nonnative species and harm the endangered ones. There is also no question that the nonnative fish are now a permanent factor. However, as noted, many researchers believe that, despite increased predation from nonnatives, the endangered fish can thrive if there is sufficient habitat. Unfortunately, the habitat/nonnative fish problem is so intertwined that the relative importance of each factor probably cannot be determined.

### **4.3 Fish Characteristics**

There has been a great deal of study of the characteristics of the endemic fish species in the Upper Colorado Basin. These studies provide important insight regarding how habitat changes and exposure to selenium may affect the distribution and recovery of the pikeminnow and razorback sucker.

#### **4.3.1 Razorback Sucker**

The timing of spring flows seems to be an important cue for attracting and concentrating spawning adults, and the magnitude of flows may influence post-spawning movement (Modde and Wick 1997). Modde and Irving (1998) observed that razorback suckers move early in the

hydrograph. Apparently, razorbacks spawn over several weeks because larvae have been collected for an extended period of time for several years (1992–1996) in the Green River (Muth et al. 1998). Thus, razorbacks spawn in spring and drift into flooded bottomlands during their first summer while feeding primarily on zooplankton. Razorback suckers prefer backwaters and gravel pits during spawning, otherwise, they use pools and slow runs (Osmundson et al. 1995). The latter study demonstrates that razorbacks in the Grand Valley area preferred different habitats for rearing and spawning than for foraging.

#### **4.3.2 Colorado Pikeminnow (Squawfish)**

In contrast to razorback suckers, pikeminnows prefer eddys and deep pools (Osmundson et al. 1995). They apparently have more spawning site fidelity than razorbacks and spawn on the descending rather than the ascending limb of the hydrograph (Modde 1999).

Floods are important to the success of the pikeminnow. “Higher-than-average reproductive success during the mid-1980s may be related to the moderately high spring flows of this period following the exceptionally high flood years of 1983 and 1984.” These investigators cited several other studies that indicated that more YOY were found after high flow years (Osmundson and Burnham 1996).

Based on studies in the Colorado River, all native fish except the pikeminnow produce more larvae and YOY during years with high peak flows, while the pikeminnow has its highest reproduction the year after the high flows (McAda and Ryel 1999). The floods are believed to clear silt from spawning beds which prepares them for efficient use the following year. In the latter study, the most abundant fish caught were nonnatives: red shiner, sand shiner, and fathead minnow; but their relative abundance was less after a high spring runoff. This study also reported that reservoirs providing high summer runoff are harmful to the native fish because the larval fish are flushed too far downstream (McAda and Ryel 1999). This is important because studies have shown that the primary productivity of the Colorado River is much higher above Westwater Canyon (near the Colorado-Utah border) than below. Hence, fish transported too far downstream are in an environment with diminished food supplies or increased predation (Lake Powell). These fish migrate downstream as part of their natural life history, but releases from upstream reservoirs may prevent their return to the more productive environment they prefer as adults.

#### **4.3.3 Flannelmouth Sucker**

Some discussion of the flannelmouth sucker is warranted because it is a native endemic species that is doing reasonably well in comparison with the closely related razorback sucker. A significant difference is that flannelmouths can live in small drainages, whereas squawfish and razorback suckers are fish of large rivers (Hamilton and Buhl 1996). There is also research that indicates flannelmouths were found in all habitats and razorbacks only in slow water. In other words, flannelmouths are found in both high and low gradient reaches and smaller tributaries, whereas razorback suckers are found primarily in alluvial sections of large rivers.

McAda (1977) reported that flannelmouth and razorbacks have similar reproduction requirements in terms of temperature, flow, depth, and substrate, but that razorbacks historically made extensive spawning migrations which were later blocked by dams. That finding may not be consistent with more recent research regarding the loss of spawning grounds and the fact that razorbacks spawn in Lake Mohave.

Finally, sensitivity to pollutant stress may be different. Flannelmouths are found in the Dolores, but razorbacks are not, which suggests a greater tolerance for flannelmouths to pollution and altered river flows. Likewise, Hamilton and Buhl (1996) cite several studies in which flannelmouths contained less selenium than other fish in the same habitat.

#### **4.4 Discussion**

The endangered endemic species of the Upper Colorado Basin evolved under highly specific conditions of flows, temperature, turbidity, and competition. These conditions have been radically altered by water development, pollution, and the introduction and proliferation of nonnative fish. Indeed, the controversies involved with recovery of the native endemic fish are undoubtedly the result, in part, to the complexity of changes that have occurred. Nevertheless, anecdotal evidence and published reports suggest that habitat change is the most important factor in the decline of the fish. Changes in river operation have interfered with spawning cues, such as water temperature and the timing, magnitude, and duration of flow. Diversions have removed sediment, prevented flushing flows, and greatly reduced peak flows. These changes have created habitat that favors nonnative species while harming the endemic fish. Moreover, reports from field researchers indicate that conditions that mimic the historical hydrograph are directly beneficial to the fish. For example, reoperation of Flaming Gorge Dam resulted in an immediate, positive response from the pikeminnow in the Green River, and the floods of 1983 and 1984 resulted in a noticeable increase in recruitment of both pikeminnow and razorback suckers (Modde, T., U.S. Fish and Wildlife Service, personal communication, July 2, 1999). In addition, the critical blow for the razorback sucker in the Colorado River has been related to road construction in the 1970s and ironically to the floods of 1983 and 1984 which eliminated the remaining important spawning areas. These observations all occurred in the presence of nonnative species and suggest that restoring the natural hydrograph is more important than removing nonnatives.

### **5. STANDARDS**

This section provides a brief review of established criteria for selenium associated with aquatic habitats. Additional information on chronic and acute toxicity to fish was provided in Sect. 3.

#### **5.1 Habitat Considerations**

Because of the effect on habitat acquisition, determining selenium criteria for aquatic protection is a critical issue for the Upper Colorado Basin's fish recovery program. The recovery program includes habitat acquisition, but in addition to having the appropriate physical attributes, the selenium standard must also be satisfied. Finding habitat sufficiently low in selenium has been difficult and is a source of controversy within the recovery program as a whole. The face of the conflict is characterized by those who are fearful of "acquiring another Kesterson" versus those who may acknowledge harmful effects of selenium but believe that habitat should be acquired anyway because some reproduction will occur. In other words, the latter view is that "some reproduction is better than no reproduction."

Guidance considered for habitat acquisition is outlined in the National Irrigation Water Quality Program (NIWQP 1998). This document includes Table 2 that is used to consider whether mitigation of selenium may be necessary.

## 5.2 Chronic Criteria

Table 2 indicates use of a waterborne criterion of 2 µg/L which is more restrictive than the current United States EPA criterion of 5 µg/L. Others, however, believe that the standards do not have to be so rigorous for selenium in western waters. For example, Adams et al. (1997) state that toxic effects are clearly site-specific, saying the range of harmful concentrations was 1–37 µg/L for birds and 2–32 µg/L for fish. Similarly, Canton and Van Derveer (1997) report that “a number of streams in Colorado were found to contain waterborne selenium concentrations that consistently exceeded the current United States EPA chronic criterion of 5 µg/L and often exceeded the acute criterion of 20 µg/L,” and “no biological impact was observed.” These authors conclude that waterborne selenium concentrations are a poor predictor of biological effects and recommend establishment of criteria based on sediment concentrations.

Furthermore, these reports recommend that sediment-based criteria be related to selenium’s relationship with total organic carbon (TOC) in the sediment. TOC concentrates the selenium and enhances its bioavailability. Accordingly, Van Derveer and Canton (1997) and Van Derveer (1997) propose sediment selenium criteria of 2.5 ug/g as a threshold for sediment based on predicted effects and selenium concentrations of 4 ug/g as the observed threshold for fish and wildlife toxicity.

**Table 2. Selenium concentration guidelines**

Medium	No effect <sup>a</sup>	Level of concern <sup>b</sup>	Toxicity threshold <sup>c</sup>	Comments/ explanation
Water (µg/L)	<1	1–2	>2	Peterson and Nebeker 1992
Sediment (mg/kg, dw)	<2	2–4	>4	Lemly and Smith 1987
Diet (mg/kg, dw)	<2	2–3	>3	Lemly 1993b
Waterbird eggs (mg/kg, dw)	<3	3–8	>8	No-effect level from Lemly 1993a/Toxicity threshold from Skorupa and Ohlendorf 1991
Fish, whole-body (mg/kg, dw):				
Warm water species	<3	3–4	>4	Lemly 1993b
Cold water species	<2	2–4	>4	

<sup>a</sup>No effect = Concentrations lower than this value produce no discernible adverse effects on fish or wildlife and are typical of background concentrations in uncontaminated environments.

<sup>b</sup>Level of concern = Concentrations in this range rarely produce discernible adverse effects but are elevated above typical background concentrations.

<sup>c</sup>Toxicity threshold = Concentrations above this value appear to produce adverse effects on some fish and wildlife species.

Site-specific differences in the toxicity of selenium could be the result of several factors. The most significant of these may be that the conversion of selenate and selenite to their more toxic organic forms occurs much faster in lentic (standing-water) systems than in flowing-water systems. It is for this reason that Lemly (1999) proposed selenium criteria based on the concept of a hydrologic unit. The premise of this approach is that concentrations of selenium that are seemingly harmless in a river may be quite detrimental in an associated off-channel backwater area or reservoir. Based on this concept, the most sensitive portion of an interconnected

hydrologic system is used for setting the standard. Obviously, except in rare cases, this approach justifies the lowest toxic levels that have been reported. This is the approach used in Table 2.

### 5.3 Acute Criteria

Canton (1999) reviews acute criteria and notes that the LC50 tests for selenium toxicity are quite variable for species, ranging by a factor of 10 for *daphnia* and 20 for fathead minnows. Canton (1999) also notes that in virtually all cases in the literature where direct comparisons are made, selenate is less toxic than selenite. Data reviewed in this study suggest raising the acute criterion from the current standard of 20 µg/L to ~220 µg/L for selenite and ~410 µg/L for selenate.

### 5.4 Discussion

In summary, selenium criteria for protection of aquatic life are significant factors regarding whether or not to acquire habitat. Whether the criteria are appropriate, particularly for the endangered fish (see Sect. 3), is a matter of speculation. The problem of selecting appropriate criteria for selenium is exacerbated by differences in productivity between lentic and lotic habitats and the lifestyle of the endangered species. Finally, as mentioned in Sect. 3, other ions may have a significant effect on selenium toxicity. Unraveling these interconnected factors will be difficult, but the payoff in terms of applying a proper emphasis to fish recovery and river operation would be quite significant.

## 6. RECOMMENDATIONS

The justification for this project was the belief that there are opportunities to perform biological, geochemical, and hydrological research to resolve the issues surrounding selenium poisoning and the recovery of endangered fish in the Upper Colorado Basin. This report demonstrates the need for such investigations. For example, the approaches used for determining whether selenium poisoning has occurred are controversial, and many locations in the intermountain region have elevated selenium in water and sediments with no apparent consequences for wildlife. Similar concentrations at the Kesterson site in California, resulted in severe avian deformities. Consequently, biological and geochemical studies are required to understand the cycling, relative abundance, and bioavailability of selenium and other constituents so that causal agents can be identified with greater certainty. Similarly, hydrological investigations and modeling are needed to further examine the present coordination of dam operation and water diversions to determine whether modifications can provide the habitat necessary to ensure survival of the species now at risk.

The purpose of this section, therefore, is to provide specific recommendations for additional research. As described in the previous sections, interrelated factors have caused the decline of the Colorado pikeminnow and razorback sucker. Nevertheless, to ensure that the recovery programs have an appropriate emphasis will require resolution of the uncertainties regarding the effects of selenium. That selenium harms fish is certain, but the relative emphasis of the recovery program regarding the three primary factors of selenium, nonnative species, and habitat loss is in dispute.

The volume of publications on selenium suggests that most of the important research has been completed. This review, however, demonstrates that highly significant questions remain. Moreover, the social and economic significance of these questions will only increase as

population and recreation needs exert more pressure on western water supplies. Irrigation is such a major demographic and economic factor in the inter-mountain West, and water usage is so interrelated with politics and development, that the controversy is probably only in its infancy. Such conditions provide a significant opportunity to perform research that will more appropriately direct water development and species recovery for several decades. The resolution of these controversies has implications throughout the semi-arid regions of the United States. Any research, therefore, that succeeds in focusing funds such that endangered species recovery is enhanced can save millions of dollars.

### 6.1 Selenium Uptake and Accumulation

The following data illustrate the uncertainties regarding selenium exposure in backwaters in the Grand Valley (Holley, K., U.S. Fish and Wildlife Service, personal communication, Jan. 22, 1999). Water and sediment concentrations are not included in the table, because the detection limits were high enough that both water (<50 µg/L) and sediment (<10 ug/g) concentrations could have constituted a “high” hazard according to Lemly (1995). In fact, the sediment concentrations for the Walker Wildlife site were greater than 50 ug/g, exceeding by an order of magnitude the 4 ug/g which Lemly considers a “high” hazard.

**Table 3. Selenium contents of water, sediment, zooplankton, and razorback sucker eggs at three sites in the Grand Valley (Holley, K., U.S. Fish and Wildlife Service, personal communication, Jan. 22, 1999)**

Location	Zooplankton (ug/g, dry weight)	Razorback sucker eggs (ug/g, dry weight)
Horsethief Wildlife Area	15 (N=2)	7.7 (N=7)
Adobe Creek	28 (N=4)	44.5 (N=6)
Walker Wildlife Area	43 (N=4)	40 (N=5)

Any discussion of the data in Table 3 must be tempered by the small number of samples. Nonetheless, the data are problematic for the following reasons:

Sediment concentrations of 50 ug/g (Walker Wildlife) and less than 10 ug/g (Adobe Creek) produced similar concentrations in zooplankton and essentially the same selenium concentration in eggs of razorback suckers.

The zooplankton (Lemly’s criteria is greater than 5 ug/g for a high hazard) at all three sites constituted a high hazard, but the fish egg concentrations at Horsethief would only be rated “low.”

At Belew’s Lake, the site from which the selenium aquatic protection criteria were derived (see Sects. 3 and 5), the water-to-sediment concentration factors were nearly 1000 (calculated as ug/g sediment divided by µg/L water concentration). Thus, if the Walker Wildlife water concentration is 50 µg/L (it was reported as less than 50 µg/L), then this concentration factor would be in the same range. However, at Belew’s Lake, the sediment-to-plankton concentration factor was greater than two (Lemly 1985). The Walker Wildlife and Horsethief data show concentration factors of less than one—a potentially significant finding because larval razorback suckers feed primarily on zooplankton.



These data, therefore, argue against the generalizations that are typically made regarding the aquatic protection criteria used to guide the recovery program (see Sect. 5). Research is needed to determine why there are significant differences in bioconcentration from one site to the other. At present, the only factor that has been suggested is total organic carbon (Canton and Van Derveer 1997), but there is no quantitative information to support this idea. As discussed in previous sections, other inorganic ions may affect selenium uptake and accumulation, and species-to-species variability may be greater than has typically been supposed.

## **6.2 Relative Sensitivity of Various Fish Species**

The relative sensitivity of the fish species to selenium is not well established. As noted in Sect. 3, there are apparently significant differences in sensitivity when comparing one avian species with another, circumstances that are similar for mammalian species. Also needed are some definitive studies that show how and whence adult concentrations of selenium are transferred to the progeny. To fully address the question of sensitivity and mechanism, investigations are needed with a variety of species. It is recognized that work of this type is under way, but the scope must be expanded to address fully all of the uncertainties.

Another item that is lacking is a bioaccumulation model that can be used to simulate quantitatively how selenium is mobilized from sediment and through the aquatic system. Such research would enable water, sediment, and food concentrations to be used to predict a body burden in fish. This latter need is highlighted by the controversy in the literature as shown in papers by Canton (1999), Van Derveer and Canton (1997), and Lemly (1999) regarding standards for aquatic protection (see Sect. 5). The controversy has as its basis the fact that rivers have different characteristics than the ponds and lakes from which most selenium standards are derived.

In the Upper Colorado Basin, there has also been no examination of a “winter stress syndrome” as postulated by Lemly (1997a). Such a problem appears plausible in the Grand Valley because the onset of cold temperatures coincides with the end of the irrigation season. Once irrigation ceases, water tables rise and selenium concentrations in many backwater ponds are at their highest. At this time, the oxygen consumption and lipid content of the fish should be checked as a means of monitoring metabolic status. Whether or not winter stress explains why razorback suckers are not observed as adults has not been evaluated in the Grand Valley.

## **6.3 Bioindicators/Biomarkers**

Table 4 shows bioindicators for various levels of biological organization. For selenium and the endangered species, the indicators used are primarily from the population and community columns. Total selenium analyses are used for individual fish, but little has been done with the biochemical, physiological, or histopathological indicators. For example, specific biochemical and histopathological indicators can be measured in fish and birds from areas where selenium impacts are suspected to determine if, in fact, effects in these organisms are caused by selenium. In addition, these studies can be supported or validated with laboratory exposures to determine if similar responses occur under controlled conditions.

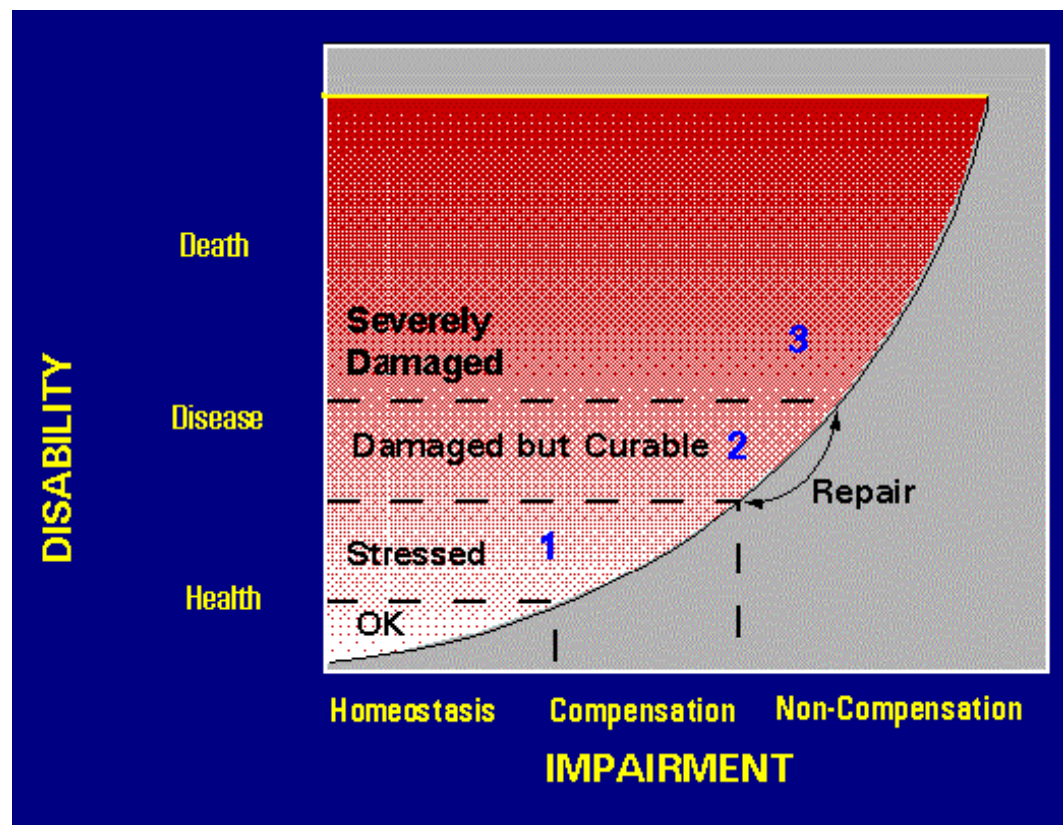
Figure 5 and Table 5 show that the bioindicators presently used are relatively insensitive. Figure 5 conceptually shows three stress zones for a population exposed to a stressor. Table 5 displays the sensitivity of the various bioindicators that might be used to evaluate or identify the stressor. Considering that reproduction and/or population-level parameters are used as indicators

**Table 4. Bioindicators measured at various levels of biological organization**

Biochemical	Physiological	Histopath	Individual	Population	Community
<sup>a</sup> MFO enzymes	Creatinine	Necrosis	Growth	Abundance	Richness
Bile metabolites	Transamin. Enzymes	Macrophage aggregate	Total body lipid	Size & age distribution	Index biotic integrity
DNA integrity	Cortisol	Parasitic lesions	Organo-Indices	Sex ratio	Intolerant species
Stress proteins	Triglycerides	Functional parenchyma	Condition factor	Bioenergetic parameters	Feeding types
Antioxidant enzymes	Steroid hormones	Carcinomas	Gross anomalies (lesions, etc.	Reproductive integrity	

<sup>a</sup>MFO, mixed function oxygenase

Source: [http://www.esd.ornl.gov/programs/bioindicators/con\\_fiel.htm](http://www.esd.ornl.gov/programs/bioindicators/con_fiel.htm)



**Fig. 5. Relationship of disability and impairment**

Source: [http://www.esd.ornl.gov/programs/bioindicators/con\\_org.htm](http://www.esd.ornl.gov/programs/bioindicators/con_org.htm)

**Table 5. Bioindicators representative of the stress zones shown in Fig. 5**

Zone 1 Most sensitive	Zone 2 Moderately sensitive	Zone 3 Least sensitive
Detoxification enzymes	Selected histopathologies	Population-level parameters
DNA damage	Immune system indicators	Community-level parameters
Bile metabolites	Bioenergetics (lipids)	Alterations in sex ratio
Antioxidant enzymes	Condition indices	Food-web alterations
Selected blood chemistries	Growth	Trophic-level relationships
Stress proteins	Reproductive parameters	

Source: [http://www.esd.ornl.gov/programs/bioindicators/con\\_org.htm](http://www.esd.ornl.gov/programs/bioindicators/con_org.htm)

of selenium poisoning, it is evident that the methods now used are no more than moderately sensitive. Specific biomarkers that would provide an indicator in the “most sensitive” column would greatly improve the ability to identify selenium exposure as a cause of impairment of sensitive fish populations.

A biomarker is a normal, subcellular process that acquires an abnormal value as a result of some outside stressor (McCarthy and Shugart 1990). Thus, a biomarker yields a quantitative distinction between individuals exposed to a specific stressor and those who are not exposed. What is provided by a biomarker, therefore, is an indicator of cause (i.e., selenium exposure) and effect (impaired reproduction, population declines, etc.). Biomarkers measurable at a molecular level respond rapidly to a stressor and are quickly evident although difficult to interpret at the population level. In contrast, biological indicators that have ecologically relevant endpoints, such as species diversity or population size, become evident too late to have preventative value (Braune et al. 1999). Establishing biomarkers for selenium effects on fish is needed in order to link the population declines to a causal agent and to assist in evaluating the effects of other potential stressors. Indeed, work evaluating multiple stressors may assist in determining relative effects of selenium and habitat deficiencies.

Selenium affects all components of the immune system, so there are a host of potential biomarkers. Unfortunately, most are nonspecific for selenium. Diminished plasma and erythrocyte levels have been used as selenium biomarkers, but as noted, they are relatively nonspecific. A potential candidate from the mammalian literature, however, is glutathione peroxidase (GPx) activity and/or the cellular levels of reduced glutathione and hydrogen selenide (Greeley, M.S., Oak Ridge National Laboratory, personal communication, Jan. 22, 1999). Evidence of increased lipid peroxidation and related glutathione peroxidase activity has been found in aquatic birds at sites such as Kesterson (Hoffman and Heinz 1988; Ohlendorf et al. 1988).

Selenium has an antimutagenic effect so genomic directions are not feasible. However, there is the possibility that the activity of the GPx gene could be explored as a potential selenium biomarker. Little is known regarding the mechanistic relationships between GPx and selenium, which of itself is an area where additional research could provide important insight (Greeley,

M.S., Oak Ridge National Laboratory, personal communication, Jan. 22, 1999). An important aspect of this research could be the development of an immunoassay technique to provide a rapid and inexpensive method for evaluating the amount of selenium stress.

### **6.3.1 Deformities and Lesions**

Another puzzling issue is the lack of teratogenic deformities observed in the Upper Colorado Basin. Lemly (1997b) has proposed that at tissue concentrations of approximately 40–50 ug/g, 25% of a population of centrarchids will show teratogenic deformities. Deformities were also noted at the Roger's Quarry site in Oak Ridge, Tennessee (Southworth et al. 2000). The reason that deformities have not been reported for fish in the Upper Colorado Basin requires elucidation.

Similarly, work should be performed with respect to evaluating the presence or absence of selenium-specific lesions. Such lesions were observed at levels of 8 ug/g in a Texas study (Sorenson 1988). As noted elsewhere in this report, tissue concentrations as high as 100 ug/g have been observed in apparently healthy fish from Sweitzer Lake. Indeed, tissue concentrations higher than 8 ug/g have been reported from many locations in the Upper Colorado Basin, but no lesions have been reported. Abnormalities in flannelmouth suckers in the San Juan River were suggested as being potentially related to chemical contamination (Hamilton and Buhl 1996), but no specific link has been determined.

### **6.4 Inherited Resistance**

In conjunction with work on multiple stressors, research regarding resistance to selenium is needed. Recent genetic studies have demonstrated that populations of fish exposed to mercury can develop tolerance within a few generations (Nadig et al. 1998). Knowledge of whether selenium tolerance can increase in successive generations has significant implications with respect to fish recovery and future irrigation diversions. A useful location to begin such research might be with an evaluation of fish from Sweitzer Lake where a robust population of Green Sunfish are present even though tissue samples have contained as much as 100 ug/g of selenium. It has been speculated, therefore, that this population has developed a tolerance to selenium. Perhaps this population of fish could be studied to determine how that tolerance is expressed biochemically.

### **6.5 Sources**

More work should be done with selenium sources in the Upper Colorado Basin. As reported by Oldfield (1998), feedlot runoff may contain 50 to 150 µg/L of selenium. There are several feedlots in the Grand Valley, and although these could not be a sufficient source to support the observed levels in the streams and ditches, they may be a locally common source that should be evaluated.

Section 2 indicated that selenium geochemistry is relatively well understood. However, more study is needed to determine whether selenium cycling in the Upper Colorado Basin has the same characteristics of the circumstances found at Kesterson. The data in Table 3 appears to show unexpected relationships with respect to bioaccumulation. This anomaly may be the result of different geochemical conditions. Thus, data from the various Grand Valley sites should be reviewed to determine whether there are significant geochemical differences when compared with Kesterson and other more extensively studied sites. Such a review may result in needs for additional laboratory studies of selenium's biogeochemical cycling.

## 7. REFERENCES

- Adams, W. J., K. V. Brix, A. Fairbrother, and R. Cardwell. 1997. Understanding selenium in the aquatic environment. In *Understanding Selenium in the Aquatic Environment*, sponsored by Kennecott Utah Copper, organized by W.J. Adams, Salt Lake City, March 6-7, 1997.
- Ahlich, J. S., and L. R. Hossner. 1987. Selenate and selenite mobility in overburden by saturated flow. *J. Environ. Qual.* 16:95-98.
- Alemi, M. H., D. A. Goldhamer, M. E. Grismer, and D. R. Nielson. 1988a. Selenate transport in steady-state, water-saturated soil columns. *J. Environ. Qual.* 17:608-613.
- Alemi, M. H., D. A. Goldhamer, M. E. Grismer, and D.R.Nielson. 1988b. Elution of selenium from contaminated evaporation pond sediments. *J. Environ. Qual.* 17:613-618.
- Anderson, M. S., H. W. Lakin, K. C. Beeson, F. F. Smith, and E. Thacker. 1961. Selenium in Agriculture. *Agriculture Handbook No. 200*, U.S. Department of Agriculture, Washington, D.C.
- Beath, O. A., C. S. Gilbert, H. F. Eppson, and I. Rosenfeld. 1953. Poisonous plants and livestock poisoning. *Univ. Wyo., Agric. Exp. Stn. Bull.* 324:1-94.
- Benson, S. M. 1998. Influence of nitrate on the mobility and reduction kinetics of selenium in groundwater systems. In W.T. Frankenberger and R.A. Engberg (eds.), *Environmental Chemistry of Selenium*. Marcel-Dekker Inc., New York.
- Bowie, G. L., J. G. Sanders, G. F. Riedel, C. G. Gilmour, D. L. Breitburg, G. A. Cutter, and D. B. Porcella. 1996. Assessing selenium cycling and accumulation in aquatic ecosystems. *Water Air Soil Pollut.* 90:93-104.
- Brandenburg, W. H., and K. B. Gido. 1999. Predation by nonnative fish on native fishes in the San Juan River, New Mexico and Utah. *Southwest. Nat.* 44:392-394.
- Braune, B., D. Muir, B. DeMarch, M. Gamberg, K. Poole, R. Currie, M. Dodd, W. Duschenko, J. Eamer, B. Elkin, M. Evans, S. Grundy, C. Hebert, R. Johnstone, K. Kidd, B. Koenig, L. Lockhart, H. Marshall, K. Reimer, J. Sanderson, and L. Shutt. 1999. Spatial and temporal trends of contaminants in Canadian Arctic freshwater and terrestrial ecosystems. *Sci. Total Environ.* 230:145-207.
- Brix, K., R. Cardwell, D. Lester, B. Adams, and B. Coldrick. 1997. Selenium bioaccumulation in macro-invertebrates in Kennecott wetlands. In *Understanding Selenium in the Aquatic Environment*, sponsored by Kennecott Utah Copper, organized by W.J. Adams, Salt Lake City, March 6-7, 1997.
- Brown, M. J., and D. L. Carter. 1969. Leaching of added Se from alkaline soils as influenced by sulfate. *Proc. Soil Sci. Soc. Amer.* 33:563-565.
- Burdick, B. D. 1995. Ichthyofaunal studies of the Gunnison River, Colorado, 1992-1994. *Aspinall Unit Umbrella Studies, Recovery Program Project No. 42*. U.S. Fish and Wildlife Service. Grand Junction, Colo.

Burdick, B. D. 1997. Minimum flow recommendation for passage of Colorado squawfish and razorback sucker in the 2.3 mile reach of the lower Gunnison River: Redlands diversion dam to the Colorado River confluence. Recovery Program Project No. 57. U.S. Fish and Wildlife Service. Grand Junction, Colo.

Bureau of Reclamation. 1998. Selenium Update. Newsletter No. 2. National Irrigation Water Quality Program, Grand Junction, Colo.

Butler, D., R. Krueger, B. Osmundson, A. Thompson, and S. K. McCall. 1991. Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Gunnison and Uncompahgre River Basins and at Sweitzer Lake, West-Central Colorado, 1988-1989. Water-Resources Investigation Report 91-4103. U.S. Geological Survey, Denver.

Butler, D. L., W. G. Wright, D. A. Hahn, R. P. Krueger, and B. C. Osmundson. 1994. Physical, chemical, and biological data for detailed study of irrigation drainage in the Uncompahgre Project Area and in the Grand Valley, West-Central Colorado, 1991-1992. Open-file report 94-110. U.S. Geological Survey, Denver.

Butler, D. L., W. G. Wright, K. C. Stewart, B. C. Osmundson, R. P. Krueger, and D. W. Crabtree. 1996. Detailed study of selenium and other constituents in water, bottom sediment, soil, alfalfa, and biota associated with irrigation drainage in the Uncompahgre Project Area and in the Grand Valley, West-Central Colorado, 1991-1993. Water-Resources Investigation Report 96-4138. U.S. Geological Survey, Denver.

Canton, S. P., and W. Van Derveer. 1997. Selenium toxicity to aquatic life: An argument for sediment-based water quality criteria. *Environ. Toxicol. Chem.* 16:1255-1259.

Canton, S. P. 1999. Acute aquatic life criteria for selenium. *Environ. Toxicol. Chem.* 18:1425-1432.

Coyle, J. J., D. R. Buckler, C. G. Ingersoll, J. F. Fairchild, and T. W. May. 1993. Effect of dietary selenium on the reproductive success of bluegills (*Lepomis macrochirus*). *Environ. Toxicol. Chem.* 12:551-565.

Crane, M., T. Flower, D. Holmes, and S. Watson. 1992. The toxicity of selenium in experimental freshwater ponds. *Arch. Environ. Contam. Toxicol.* 23:440-452.

Csallany, A. S., L. C. Lu, and B. Z. Menken. 1984. Effect of selenite, vitamin E, and N-N-diphenyl-p-phenylenediamine on liver organic solvent-soluble lipofuchsin pigments in mice. *J. Nutr.* 114:1582-1587.

Dill, W. A. 1944. The fishery of the lower Colorado River. *Calif. Fish Game* 30:109-211.

Dougherty, J. L., and W. G. Hoekstra. 1982. Stimulation of lipid peroxidation in vivo by injected selenite and lack of stimulation of selenate. *Proc. Soc. Exp. Biol. Med.* 169:209-215.

Dungan, R. S., and W. T. Frankenberger, Jr. 1999. Microbial transformations of selenium and the bioremediation of seleniferous environments. *Biorem. J.* 3:171-188.

- Elrashidi, M. A., D. C. Adriano, S. M. Workman, and W. L. Lindsay. 1987. Chemical equilibria of selenium in soils: A theoretical framework. *Soil Sci.* 144:141-152.
- Fan, T., and R. Higashi. 1997. Why is Se toxicology complex? In *Understanding Selenium in the Aquatic Environment*, sponsored by Kennecott Utah Copper, organized by W.J. Adams, Salt Lake City, March 6-7, 1997.
- Fan, T. W., R. M. Higashi, and A. N. Lane. 1998. Biotransformations of selenium oxyanion by filamentous cyanophyte-dominated mat cultured from agricultural drainage waters. *Environ. Sci. Technol.* 32:3185-3193.
- Fio, J. L. 1997. Geohydrologic effects on drainwater quality. *J. Irrig. Drain. Eng.* May/June, pp. 159-164.
- Geering, H. R., E. E. Cary, L. H. P. Jones, and W. H. Allaway. 1968. Solubility and redox criteria for the possible forms of selenium in soils. *Proc. Soil Sci. Soc. Amer.* 32:35-40.
- Guo, L., W. T. Frankenberger, and W. A. Jury. 1999. Evaluation of simultaneous reduction and transport of Se in saturated soil columns. *Water Resour. Res.* 35:663-669.
- Hamilton, S. J., and K. J. Buhl. 1996. Hazard assessment of inorganics, singly and in mixtures, to flannelmouth sucker in the San Juan River, New Mexico. Final report to U.S. Bureau of Reclamation for the San Juan Recovery Implementation Program. Salt Lake City.
- Hamilton, S. J., K. J. Buhl, F. A. Bullard, and S. F. McDonald. 1996. Evaluation of toxicity to larval razorback sucker of selenium-laden food organisms from Ouray NWR on the Green River, Utah. Final Report. U.S. Fish and Wildlife Service, Colorado River Recovery Implementation Program, Denver.
- Hamilton, S. J., K. J. Buhl, F. A. Bullard, K. M. Holley, and F. K. Pfeiffer. 1997. Evaluation of contaminant impacts on razorback sucker in restored floodplain habitats and remediation techniques. In *Understanding Selenium in the Aquatic Environment*, sponsored by Kennecott Utah Copper, organized by W.J. Adams, Salt Lake City, March 6-7, 1997.
- Hamilton, S. J. 1998. Selenium effects on endangered fish in the Colorado River Basin. In W.T. Frankenberger and R.A. Engberg (eds.), *Environmental Chemistry of Selenium*. Marcel-Dekker Inc., New York.
- Hawkins, J. A., and T. P. Nesler. 1991. Nonnative fishes of the Upper Colorado River Basin: An issue paper. Larval Fish Laboratory, Colorado State University, Fort Collins, Colo.
- Hilton, J. W., and W. J. Bettger. 1988. Dietary vanadium toxicity in juvenile rainbow trout: A preliminary study. *Aquat. Toxicol.* 12:63-71.
- Hoffman, D. J., and G. H. Heinz. 1988. Embryotoxic and teratogenic effects of selenium in the diet of mallards. *J. Toxicol. Environ. Health* 24:477-490.
- Hoffman, D. J., H. M. Ohlendorf, and T. W. Aldrich. 1988. Selenium teratogenesis in natural populations of aquatic birds in central California. *Arch. Environ. Contam. Toxicol.* 17:519-525.

- Hoffman, D. J., C. J. Sanderson, L. J. Lecaptain, E. Cromartie, and G. W. Pendleton. 1991. Interactive effects of boron, selenium, and dietary protein on survival, growth, and physiology in mallard ducklings. *Arch. Environ. Contam. Toxicol.* 20:288-294.
- Irving, D. B., and B. D. Burdick. 1995. Reconnaissance inventory and prioritization of existing and potential bottomlands in the Upper Colorado River Basin, 1993-1994. Final Report. U.S. Fish and Wildlife Service, Grand Junction, Colo.
- Kaeding, L. R., and D. B. Osmundson. 1988. Interaction of slow growth and increased early-life mortality: A hypothesis on the decline of Colorado squawfish in the upstream regions of its historic range. *Environ. Biol. Fishes* 22:287-298.
- Karlson, U., and W. T. Frankenberger. 1988. Effects of carbon and trace element addition on alkylselenide production by soil. *Soil Sci. Soc. Am. J.* 52:1640-1644.
- Kidd, G. 1977. An investigation of endangered and threatened fish species in the Upper Colorado River as related to Bureau of Reclamation Projects. Final Report. 1977. Northwest Fisheries Research, Clifton, Colo.
- Klaverkamp, J. F., D. A. Hodgins, and A. Lutz. 1983. Selenite toxicity and mercury-selenium interactions in juvenile fish. *Arch. Environ. Contam. Toxicol.* 12:405-413.
- Lemly, A. D., and G. J. Smith. 1987. Aquatic cycling of selenium: Implications for fish and wildlife. Fish and Wildlife Leaflet 12. U.S. Fish and Wildlife Service, Washington, D.C.
- Lemly, A. D. 1982. Response of juvenile centrarchids to sublethal concentrations of waterborne selenium. I. uptake, tissue distribution, and retention. *Aquat. Toxicol.* 2:235-252.
- Lemly, A. D. 1983. A simple activity quotient for detecting pollution-induced stress in fishes. *Environ. Technol. Lett.* 4:173-178.
- Lemly, A. D. 1985. Toxicology of selenium in a freshwater reservoir: Implications for environmental hazard evaluation and safety. *Ecotoxicol. Environ. Saf.* 10:314-338.
- Lemly, A. D. 1993a. Metabolic stress during winter increases the toxicity of selenium to fish. *Aquat. Toxicol.* 27:133-158.
- Lemly, A. D. 1993b. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. *Environ. Monit. Assess.* 28:83-100.
- Lemly, A. D. 1995. A protocol for aquatic hazard assessment of selenium. *Ecotoxicol. Environ. Saf.* 32:280-288.
- Lemly, A. D. 1996. Assessing the toxic threat of selenium to fish and aquatic birds. *Environ. Monit. Assess.* 43:19-35.
- Lemly, A. D. 1997a. Role of season in aquatic hazard assessment. *Environ. Monit. Assess.* 45:89-98.



- Lemly, A. D. 1997b. A teratogenic deformity index for evaluating impacts of selenium on fish populations. *Ecotoxicol. Environ. Saf.* 37:259-266.
- Lemly, A. D. 1998. Pathology of selenium poisoning in fish. In W.T. Frankenberger and R.A. Engberg (eds.), *Environmental Chemistry of Selenium*. Marcel-Dekker Inc., New York.
- Lemly, A. D. 1999. Selenium transport and bioaccumulation in aquatic ecosystems: A proposal for water quality criteria based on hydrological units. *Ecotoxicol. Environ. Saf.* 42:150-156.
- Losi, M. E., and W. T. Frankenberger, Jr. 1998. Microbial oxidation and solubilization of precipitated elemental Se in soil. *J. Environ. Qual.* 27:836-843.
- McAda, C. W., and L. R. Kaeding. 1991. Physical changes in the Gunnison and Colorado Rivers resulting from construction of the Aspinall Unit and related projects, with hypotheses to assess the effects of the endangered fishes. Final Report. 1991. U.S. Fish and Wildlife Service, Grand Junction, Colo.
- McAda, C. W., and R. J. Ryel. 1999. Distribution, relative abundance, and environmental correlates for age-0 Colorado pikeminnow and sympatric fishes in the Colorado River. Recovery Program Project No. 45. U.S. Fish and Wildlife Service, Grand Junction, Colo.
- McAda, C. W. 1977. Aspects of the life history of three catostomids native to the Upper Colorado River Basin. M.S. thesis. Utah State University, Logan, Utah.
- McCarthy, J. F., and L. R. Shugart (eds.). 1990. *Biomarkers of Environmental Contamination* pp. 3-14. Lewis Publishers, Chelsea, Minn.
- Martens, D. A., and D. L. Suarez. 1997. Selenium speciation of marine shales, alluvial soils, and evaporation basin soils of California. *J. Environ. Qual.* 26:424-432.
- Masscheleyn, P. H., and W. H. Patrick, Jr. 1993. Biogeochemical processes affecting selenium cycling in wetlands. *Environ. Toxicol. Chem.* 12:2235-2243.
- Mauk, R. J., and M. L. Brown. 1999. Acute toxicity of sodium selenite to juvenile walleye. *Bull. Environ. Contam. Toxicol.* 63:188-194.
- Modde, T., and E. J. Wick. 1997. Investigations of razorback sucker distribution movements and habitats used during spring in the Green River, Utah. Recovery Program Project Number 49. Final Report. U.S. Fish and Wildlife Service, Vernal, Utah.
- Modde, T., and D. B. Irving. 1998. Use of multiple spawning sites and seasonal movement of razorback sucker in the Green River, Utah. *North Am. J. Fish. Manage.* 18:318-326.
- Muth, R. T., G. B. Haines, S. M. Meismer, E. J. Wick, T. E. Chart, D. E. Snyder, and J. M. Bundy. 1998. Reproduction and early life history of razorback sucker in the Green River, Utah, and Colorado, 1992-1996. Final Report No. 34, submitted to the Recovery Implementation Program for the Endangered Fish Species in the Upper Colorado River Basin. U.S. Fish and Wildlife Service, Denver.
- Nadig, S. G., K. L. Lee, and S. M. Adams. 1998. Evaluating alterations of genetic diversity in sunfish populations exposed to contaminants using RAPD assay. *Aquat. Toxicol.* 43:163-178.

- National Irrigation Water Quality Program (NIWQP). 1998. Phase IV remediation planning for the lower Gunnison River Basin and Grand Valley. Grand Valley Backwater/Bottomland Component, July 28, 1998. U.S. Bureau of Reclamation, Grand Junction, Colo.
- Neal, R. H., and G. Sposito. 1991. Selenium mobility in irrigated soil columns as affected by organic carbon amendment. *J. Environ. Qual.* 20:808-814.
- Niimi, A. J., and Q. N. LaHam. 1976. Relative toxicity of organic and inorganic compounds of selenium to newly hatched zebrafish (*Brachydanio rerio*). *Can. J. Zool.* 54:501-509.
- Nolan, B. T., and M. L. Clark. 1997. Selenium in irrigated agricultural areas of the western United States. *J. Environ. Qual.* 26:849-857.
- Ogle, R. S., and A. W. Knight. 1989. Effects of elevated foodborne selenium on growth and reproduction of the fathead minnow (*Pimephales promelas*). *Arch. Environ. Contam. Toxicol.* 18:795-803.
- Ohlendorf, H., A. Kilness, J. Simmons, R. Stroud, D. Hoffman, and J. Moore. 1988. Selenium toxicosis in wild aquatic birds. *J. Toxicol. Environ. Health* 24:67-92.
- Ohlendorf, H. 1997. Toxicological effects of selenium on birds. In *Understanding Selenium in the Aquatic Environment*, sponsored by Kennecott Utah Copper, organized by W.J. Adams, Salt Lake City, March 6-7, 1997.
- Oldfield, J. E. 1998. Environmental implications of uses of selenium with animals. In W.T. Frankenberger and R.A. Engberg (eds.), *Environmental Chemistry of Selenium*. Marcel-Dekker Inc., New York.
- Osmundson, D. B., P. Nelson, K. Fenton, and D. W. Ryden. 1995. Relationships between flow and rare fish habitat in the '15 mile reach' of the Upper Colorado River. U.S. Fish and Wildlife Service, Grand Junction, Colo.
- Osmundson, D. B., and K. P. Burnham. 1996. Status and trends of the Colorado squawfish in the Upper Colorado River. Recovery Implementation Program. U.S. Fish and Wildlife Service, Grand Junction, Colo.
- Osmundson, D. B. 1987. Growth and survival of Colorado squawfish (*Ptychocheilus lucius*) stocked in riverside ponds, with reference to largemouth bass (*Micropterus salmoides*) predation. M.S. thesis. Utah State University, Logan, Utah.
- Osmundson, D. B. 1999. Longitudinal variation in fish community structure and water temperature in the Upper Colorado River: Implications for Colorado pikeminnow habitat suitability. Report A. Recovery Implementation Program Project No. 48. U.S. Fish and Wildlife Service, Grand Junction, Colo.
- O'Toole, D., and M. F. Raisbeck. 1995. Pathology of experimentally induced chronic selenosis (alkali disease) in yearling cattle. *J. Vet. Diagn. Invest.* 7:364-373.

O'Toole, D., and M. F. Raisbeck. Magic numbers, elusive lesions: Comparative pathology and toxicology of selenosis in waterfowl and mammalian species. pp. 355-395. In W.T. Frankenberger and R.A. Engberg (eds.), Environmental Chemistry of Selenium. Marcel-Dekker Inc., New York.

Peterson, J. A., and A. V. Nebeker. 1992. Estimation of waterborne selenium concentrations that are toxicity thresholds for wildlife. Arch. Environ. Contam. Toxicol. 23:154-162.

Pitlick, J., M. Van Steeter, B. Barkett, R. Cress, and M. Franseen. 1999. Geomorphology and hydrology of the Colorado and Gunnison Rivers and implications for habitats used by endangered fishes. Report for Recovery Implementation Program Project No. 44-B. Department of Geography, University of Colorado, Boulder, Colo.

Presser, T. 1994. The Kesterson effect. Environ. Manage. 18:437-454.

Quartarone, F. 1995. Historical accounts of Upper Colorado River Basin endangered fish. Edited by Connie Young. Final Report. September 1995. U.S. Fish and Wildlife Service, Denver.

Raisbeck, M. F., D. O'Toole, R. A. Schamber, E. L. Belden, and L. J. Robinson. 1996. Toxicologic evaluation of a high-selenium hay diet in captive pronghorn antelope. J. Wildl. Dis. 32:9-16.

Riedel, G., J. Sanders, C. Gilmour, C. Goulden, R. Sanders, D. Breitburg, and R. Osman. 1997. Selenium interactions in aquatic ecosystems and effects on fish populations. In Understanding Selenium in the Aquatic Environment, sponsored by Kennecott Utah Copper, organized by W.J. Adams, Salt Lake City, March 6-7, 1997.

Saiki, M. K., M. R. Jennings, and R. H. Wiedmeyer. 1993. Toxicity of agricultural subsurface drainwater from the San Joaquin Valley, California, to juvenile Chinook salmon and striped bass. Trans. Am. Fish. Soc. 121:78-93.

Sato, T., Y. Ose, and T. Sakai. 1980. Toxicological effect of selenium on fish. Environ. Pollut. 21A:217-224.

Schultz, R., and R. Hermanutz. 1990. Transfer of toxic concentrations of selenium from parent to progeny in the fathead minnow (*Pimephales promelas*). Bull. Environ. Contam. Toxicol. 45:568-573.

Seiler, R. L. 1998. Prediction of lands susceptible to irrigation-induced selenium contamination of water. In W.T. Frankenberger and R.A. Engberg (eds.), Environmental Chemistry of Selenium. Marcel-Dekker Inc., New York.

Skorupa, J. P., and H. M. Ohlendorf. 1991. Contaminants in drainage water and avian risk thresholds. pp. 345-368. In A. Dinar and D. Zilberman (eds.), The Economics and Management of Water and Drainage in Agriculture. Kluwer Academic Publishers, Boston.

Skorupa, J. P. 1998. Selenium poisoning of fish and wildlife in nature: Lessons from twelve real-world experiences. In W.T. Frankenberger and R.A. Engberg (eds.), Environmental Chemistry of Selenium. Marcel-Dekker Inc., New York.

- Sorensen, E. 1988. Selenium accumulation, reproductive status, and histopathological changes in environmentally exposed redear sunfish. *Arch. Toxicol.* 61:324-329.
- Southworth, G. R., M. J. Peterson, and M. G. Ryon. 2000. Long-term increased bioaccumulation of mercury in largemouth bass follows reduction of waterborne selenium. *Chemosphere* (in press).
- Stephens, D. W., B. Waddell, and J. B. Miller. 1988. Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Middle Green River Basin, Utah, 1986-1987. U.S. Geological Survey, Water Resources Investigations Report 88-4011. Salt Lake City.
- Stephens, D. W., and B. Waddell. 1998. Field screening of water quality, bottom sediment, and biota associated with irrigation on the Uintah and Ouray Indian reservation, eastern Utah, 1995. U.S. Geological Survey, Water Resources Investigations Report 98-4161. Salt Lake City.
- Stephens, D. W., and B. Waddell. 1998. Selenium sources and effects on biota in the Green River Basin of Wyoming, Colorado, and Utah. In W.T. Frankenberger and R.A. Engberg (eds.), *Environmental Chemistry of Selenium*. Marcel-Dekker Inc., New York.
- Tokunaga, T. K., I. J. Pickering, and G. E. Brown, Jr. 1996. Selenium transformations in ponded sediments. *Soil Sci. Soc. Am. J.* 60:781-790.
- Tokunaga, T. K., G. E. Brown, I. J. Pickering, S. R. Sutton, and S. Bajt. 1997. Selenium redox reactions and transport between ponded waters and sediments. *Environ. Sci. Technol.* 31:1419-1425.
- Tyus, H. M., and J. F. Saunders III. 1996. Nonnative fishes in the Upper Colorado River Basin and a strategic plan for their control. Final Report, Cooperative Agreement, No. 14-48-0006-95-923. U.S. Fish and Wildlife Service. Denver.
- United States Department of the Interior (USDOI). 1998. Guidelines for interpretation of the biological effects of selected constituents in biota, water, and sediment. National Irrigation Water Quality Program Information Report #3. November 1998. Bureau of Reclamation, Denver.
- Ursini, F., S. Heim, M. Kiess, M. Maiorino, A. Roveri, J. Wissing, and L. Flohe. 1999. Dual function of the selenoprotein PHGPx during sperm maturation. *Science* 285:1393-1396.
- Van Derveer, W., and S. P. Canton. 1997. Selenium sediment toxicity thresholds and derivation of water quality criteria for freshwater biota of western streams. *Environ. Toxicol. Chem.* 16:1260-1268.
- Van Derveer, W. 1997. The role of total organic carbon in controlling selenium bioavailability and deriving water quality criteria. In *Understanding Selenium in the Aquatic Environment*, sponsored by Kennecott Utah Copper, organized by W.J. Adams, Salt Lake City, March 6-7, 1997.
- Van Steeter, M., and J. Pitlick. 1998. Geomorphology and endangered fish habitats of the Upper Colorado River 1. Historic changes in stream flow, sediment load, and channel morphology.

Water Resour. Res. 34:287-302.

WestWater Engineering Inc. 1996. Endangered fish in the Upper Colorado River, observations from 1970 through 1995-George T. Kidd, 2516 Foresight Circle, Grand Junction, Colo.

Yllera, de Llano, A., G. Bidoglio, A. Avogadro, P. N. Gibson, and P. Rivas Romero. 1996. Redox reactions and transport of Se through fractured granite. J. Contam. Hydrol. 21:129-139.

Zhang, Y., and J. N. Moore. 1997. Reduction potential of selenate in wetland sediment. J. Environ. Qual. 26:910-916.

**INTERNAL DISTRIBUTION**

- |                                    |  |
|------------------------------------|--|
| 1. M. Adams, 1505, MS-6036         | 11. D. E. Reichle, 4500N, MS-6253                  |
| 2. J. H. Cushman, 1059, MS-6422    | 12. T. P. Sjoreen, 4500N, MS-6251                  |
| 3. D. E. Fowler, 1505, MS-6035     | 13. G. R. Southworth, 1505, MS-6036                |
| 4. M. S. Greeley, 1505, 6038       | 14. A. J. Stewart, 1505, MS-6036                   |
| 5. S. G. Hildebrand, 1505, MS-6037 | 15-17 Central Research Library, 4500N,<br>MS-6191  |
| 6. G. K. Jacobs, 1505, MS-6035     | 18-19. Laboratory Records-OSTI, 4500-N,<br>MS-6285 |
| 7. Y. I. Jager, 1505, MS-6036      | 20. Laboratory Records-RC                          |
| 8. P. Kanciruk, 1507, MS-6407      |  |
| 9. J. M. Loar, 1505, MS-6036       |  |
| 10. T. E. Myrick, 1000, MS-6296    |  |

**EXTERNAL DISTRIBUTION**

21. E. G. Cumesty, Assistant Manager for Laboratories and Site Manager, Department of Energy, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831-6269
22. Jerry Elwood, Acting Director, Environmental Sciences Division, ER-74, Department of Energy, 19901 Germantown Road, Germantown, MD 20874
23. J. P. Giesy, College of Natural Science, Department of Zoology, Michigan State University, 203 Natural Science Building, East Lansing, MI 48824-1115
24. Kathy Holley, U.S. Fish and Wildlife Service, Colorado River Fish Recovery Program, 764 Horizon Drive, Grand Junction, CO 81506
25. George Kidd, Northwest Fisheries Research, 3361 G. Road, Route 1, Clifton, CO 81520
26. Rick Krueger, U.S. Fish and Wildlife Service, Ecological Services, 764 Horizon Drive, Grand Junction, CO 81506
27. A. A. Lucier, National Council of the Paper Industry for Air and Stream Improvement, Inc., P.O. Box 13318, Research Triangle Park, NC 27709-3318
28. Michael C. MacCracken, Director, Office of the U.S. Global Change Research Program, Code YS-1, 300 E Street, SW, Washington, DC 20546
29. Tim Modde, U.S. Fish and Wildlife Service, 266 West 100 North , Suite 2, Vernal, UT 84078-2042

**EXTERNAL DISTRIBUTION continued**

30. Pat Nelson, U.S. Fish and Wildlife Service, 134 Union Boulevard, No. 670, Lakewood, CO 80228
31. Barbara Osmundson, U.S. Fish and Wildlife Service, Ecological Services, 764 Horizon Drive, Grand Junction, CO 81506
32. Donal O'Toole, Department of Veterinary Sciences, University of Wyoming, Laramie, WY 82072-3404
33. Frank Pfeiffer, U.S. Fish and Wildlife Service, Colorado River Fish Recovery Program, 764 Horizon Drive, Grand Junction, CO 81506
34. Merl Raisbeck, Department of Veterinary Sciences, University of Wyoming, Laramie, WY 82072-3404
35. L. Robinson, Director, Environmental Sciences Institute, Florida A&M University, Science Research Facility, 1520 S. Bronough Street, Tallahassee, FL 32307
36. J. M. Tiedje, University Distinguished Professor and Director, Center for Microbial Ecology, Michigan State University, 540 Plant and Soil Sciences Building, East Lansing, MI 48824
37. Bruce Waddell, U.S. Fish and Wildlife Service, Ecological Services, 145 East 1300 South, Lincoln Plaza, Suite 404, Salt Lake City, UT 84115