SELENIUM AND WATER QUALITY IN THREE WETLAND TYPES ALONG THE LOWER COLORADO RIVER - IMPERIAL NATIONAL WILDLIFE REFUGE, ARIZONA

by

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I dedicate this work and significant step forward in my life to the memory of my father, Juan R. Prieto Jr. My father a policeman, was killed on the job on February 2, 1993. His sudden loss was such a tragedy to my family, that the effects are still being felt today. On the other hand, the tremendous outpouring of support from the community underscored the existence of an honorable and successful life and career. It was then that I began to realize what success meant.

My father didn’t just ponder leading, he set an example. He made God a part of his everyday life. He contributed himself to something much larger than himself. He gave back when possible. He remembered his family and friends and spent time with them. He had fun and didn’t take himself too seriously. He was my beacon in life and I didn’t realize it until he was gone.

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ABSTRACT

Wetlands along the Lower Colorado River are divided into three types based on degree of connectivity to the river mainstem: connected lakes, pseudo seeps, and true seeps. In general, water quality and selenium levels in biota decrease with decreased connectivity to the river, i.e., true seeps have the poorest water quality and lowest selenium burdens, connected lakes have water quality equal to the river and the highest selenium levels, and pseudo seeps have water quality slightly improved over that in connected lakes and lower selenium levels. Based on selenium standards developed by Lemly (1995), selenium concentrations in fish but not crayfish were sufficiently elevated to pose bioaccumulation problems, though none were apparent in our study. Of the three wetland types, pseudo seeps have the most desirable combination of high water quality and modest selenium levels. Additionally, pseudo seeps may harbor the highest biodiversity of the three wetland types present in the LCR. The lack of apparent problems from elevated selenium levels raises many questions concerning selenium dynamics and cycling in the LCR. Management requires knowledge of selenium levels in individual wetland types and an understanding of selenium cycling.
INTRODUCTION

Prior to dam construction, the Lower Colorado River flooded seasonally and replenished floodplain wetlands. Today however, dams and diversions have eliminated seasonal floods changing the way the Colorado River and its floodplain function hydrologically and ecologically. The Lower Colorado River (LCR) and its floodplain are highly modified systems. During the period of 1936-1938, Hoover, Parker, and Imperial Dams were completed on the LCR. Under Public Law 469, June 28, 1946, the U. S. Bureau of Reclamation (USBR) was authorized to carry out the policy of, “controlling the Colorado River and modifying, straightening and rectifying the channel thereof” (Beland 1952).

Currently channels and wetlands along the LCR are being filled in by the deposition of both organic matter (from extensive marsh vegetation) and sediment due probably to the absence of floods and continued upstream scouring (USBR 1984). If these trends of wetland shrinkage continue unabated, many existing wetlands will likely disappear. Maintenance of wetlands is important to the Fish & Wildlife Service (FWS). The progressive conversion of some wetlands to terrestrial communities has led the LCR National Wildlife Refuge Complex to plan to dredge channels from the river to these wetlands. Although dredging may improve access for fish and sportsmen, it may also change the dynamics of selenium in the systems.

Selenium levels are elevated in water, sediment, and biota from various aquatic environments along the LCR (Radtke et al. 1988, Rusk 1991, King et al. 1993, Lusk
1993, Bell-McCaulou 1993, Martinez 1994, Ruiz 1994, Welsh and Maughan 1994, Villegas 1997). In addition selenium levels are higher in aquatic animals and fish-eating birds from backwater lakes (those connected to riverine circulation) in LCR than from those in hydrologically isolated seep lakes (Lusk 1993, Martinez 1994). Selenium bioaccumulation takes place to a greater extent in wetlands receiving direct riverine flow than those that receive only groundwater input from the river aquifer system (seep lakes) or rainfall. Accumulation rates are important because selenium can cause malformations in birds and fish, affect reproductive success, and is directly toxic at high levels to a variety of aquatic vertebrates (Ohlendorf et al. 1986, 1988; Presser and Ohlendorf 1987, Cleveland et al. 1993, Coyle et al. 1993, Lemly 1993a). Thus wetland construction (channel dredging) might mobilize selenium that is currently trapped in the sediment, as well as increase Se in biota through increased hydrological connection with the Colorado River.

Lusk (1993), Martinez (1994), and others suggest there are two primary types of wetlands (backwaters) associated with the LCR Valley: 1) seep lakes that receive only groundwater from the river aquifer system and limited rainwater inputs; and 2) backwater lakes that are directly connected to riverine flow. Water quality varies widely between these two types of wetlands. Additionally, water quality often varies widely among seep lakes. Some seep lakes support few if any fish.

Research Objectives

This study inventoried and classified backwater wetlands and defined how each
type functioned at normal riverine flows (high summer and low winter flows). The goal was to develop a model of wetland function along the LCR as defined by: 1) the degree of connectivity to the river; 2) selenium levels in fish as a function of riverine connection; and 3) the level of selenium uptake in crayfish. Specifically, the intent of my study was to determine if “clean biota” placed in various wetland types uptake selenium to different levels as a function of connectivity to the river. I tested whether the process of selenium bioaccumulation was fundamentally different in the three types of wetlands. My null hypothesis was that selenium bioaccumulation levels in biota would not differ significantly among wetland types. If this null hypothesis is correct, then opening up the wetlands along the LCR would have no effect on selenium uptake or mobility. Ultimately, I hope to further the general knowledge regarding LCR wetland ecology and assist in the future management and restoration of these wetlands.
SELENIUM BACKGROUND

Selenium (Se) is a widely distributed metalloid that exists in the same valence states as sulfur (-2, 0, +4, and +6) (Cutter 1985, Coyle et al. 1993). The four oxidation states of Se include: selenide ($\text{Se}^2$), elemental selenium ($\text{Se}^0$), selenite ($\text{SeO}_3^{2-}$), and selenate ($\text{SeO}_4^{2-}$) (Balistrieri and Chao 1987). Natural weathering processes, biomethylation and anthropogenic activities are the principle mechanisms and sources for selenium entering aquatic systems (Maier et al. 1988). Selenium averages 0.05 $\mu$g/g (ppm) in the earth's crust, ranges from <0.01 to 30 $\mu$g/l (ppb) in major U.S. rivers, and averages 0.09 $\mu$g/l in seawater (Eisler 1985). Selenium is a trace element and normally present in water at 0.1-0.3 $\mu$g/l (Lemly 1985b) concentrations.

Much higher Se concentrations have been found in areas receiving anthropogenically mobilized Se. Irrigation drain water from seleniferous soils, runoff from deposits of coal fly ash, oil refinery wastewater, sulfide ore mining, and various manufacturing process are known sources of Se (Lemly 1985b, Ohlendorf et al. 1986, Saiki 1986, Maier et al. 1988, Skorupa et al. 1996). Waterborne Se in the western United States ranges from 1 to 400 $\mu$g/l in areas in Colorado and Utah, to 1,400 $\mu$g/l in some impoundments in California (Presser 1994, Presser et al. 1994) and up to 6,000 $\mu$g/l in evaporation ponds from the Tulare Lake Drainage District, California (Maier and Knight 1994). Water used to transport or settle Se-rich fly ash particles, scrubber sludge, or other waste products from coal-fired power plants frequently contains elevated (3-22 $\mu$g/l) Se concentrations (Lemly 1985a, Maier et al. 1988). Oil refinery waste water effluents
contain about 10-30 μg/l Se concentrations (Skorupa et al. 1996). Public health may be threatened if humans consume Se-contaminated water, fish, and game (Fan et al. 1988). In the United States, about 4.6 million kg of Se are released annually into the environment: 33% from combustion of fossil fuels, 59% from industrial sources, and 8% from municipal wastes (Eisler 1985).

*Selenium Nutrition & Toxicity* - Selenium is an essential micronutrient in animal nutrition (Schwartz and Foltz 1957, Stadtman 1980). Dietary inorganic Se requirements in fish range from 0.07 μg/g dry feed in rainbow trout (*Salmo gairdneri*) (Hilton et al. 1980) to 0.28 mg/kg of diet, in channel catfish (*Ictalurus punctatus*) (Gatlin and Wilson 1984, Wang and Lovell 1997). Organic Se requirements range from 0.09 to 0.11 mg/kg in the diet of channel catfish (Wang and Lovell 1997). As a nutrient in the diet of fish, concentrations of about 0.1-0.5 μg/g dry weight are generally required (Hodson and Hilton 1983, Sorenson 1991, Eisenberg 1993). One of the most important features of Se ecotoxicology is the very narrow margin between nutritionally optimal and potentially toxic dietary exposures for vertebrate animals (Wilber 1980, 1983).

Selenium is necessary for proper formation and functioning of glutathione peroxidase, a major cellular antioxidant enzyme (Bell et al. 1986). Symptoms of Se deficiency in fish include reduced growth, muscular dystrophy, anemia, exudative diathesis, anemia, and mortality (Bell et al. 1985, Gatlin et al. 1986). At concentrations above normal physiological requirements, Se can be toxic. Although the exact mechanism is not fully understood, substitution of Se for sulfur in amino acids results in
the production of unstable enzymes and proteins that interfere with normal metabolic pathways (Stadtman 1980). Sulfur to sulfur linkages are necessary for the proper function of protein molecules. Se substitution disrupts the normal chemical bonding thereby resulting in improperly formed and dysfunctional proteins and enzymes (Reddy and Massaro 1983, Sunde 1984).

Excessive Se in fish can cause a wide variety of detrimental effects at the biochemical, cellular, and organ levels (Sorensen 1986). At 1-5 μg/l Se can bioaccumulate in aquatic food chains and become highly toxic to fish and wildlife (Skorupa et al. 1996, Lemly 1997c). Outwardly the effects of excessive Se in fish include: 1) edema-swollen and distended abdomen due to the accumulation of fluid, 2) exophthalmus-bulging eyes due to accumulation of fluid in the eye sockets, and 3) cataracts. These anomalies are nonteratogenic in origin but selenium-induced. They appear to be reversible and can occur in both juvenile and adult fish (Lemly 1993a, 1997a).

Teratogenesis (congenital malformations) can occur because of elevated Se levels in the diet. Dietary Se can be passed from parents to offspring in the eggs, where it can be teratogenic and cause complete reproductive failure. Teratogenic malformations include: 1) lordosis-concave curvature of the lumbar part of the spine, 2) kyphosis-convex curvature of the thoracic part of the spine, 3) scoliosis-lateral curvature of the spine, 4) missing or deformed fins, gills, opercle, and eyes; 5) mis-shaped head and mouth defects. These are true teratogenic effects that include all the morphologic,
biochemical, and functional abnormalities due to Se that occur during embryonic
development. They are considered irreversible, and manifest themselves before or at

Selenium in the Aquatic Environment - Selenium from both natural and
anthropogenic sources enters surface waters as a mixture of several chemical species.
The predominant forms however, are the highly soluble Se(+4/IV) and Se(+6/VI) which
form selenite, SeO_3^{2-} and selenate, SeO_4^{2-} salts respectively (Besser et al. 1993). Selenite
and selenate are the two principle inorganic Se species (Maier et al. 1988, Ogle et al.
1988, Masscheleyn and Patrick 1993). These forms are biotransformed into organic
species by primary producers. Selenite is more readily reduced and further metabolized
than selenate (Ogle et al. 1988, Hamilton and Buhl 1990, Brasher and Ogle 1993). Thus
the source of Se is potentially important. Coal fly ash effluent and oil refinery
wastewater contain primarily selenite and irrigation drain water selenate (Besser et al.
1993).

Elemental Se is stable, insoluble in water, and poorly assimilated by aquatic
organisms. It is not considered a hazard due to its low bioavailability (Maier et al. 1988).
Selenium as selenide occurs in aquatic systems in both organic and inorganic forms.
Inorganic complexes form naturally in anaerobic environments and generally form
insoluble metal selenides (Maier et al 1988, Maier and Knight 1994). Organic selenides,
Se(-2/-II), including Se-amino acids (selenocysteine and selenomethionine), Se-proteins,
methylated selenides (dimethyl selenide and dimethyl diselenide), and other Se-
substituted analogs of organosulfur compounds are produced by biological reduction of selenite (Chau et al. 1976, Maier et al. 1988, Besser et al. 1993) and usually occur at lower concentrations in water than inorganic Se species (Besser et al. 1993).

Toxicity of Se to aquatic biota is affected by aqueous speciation and by Se dynamics in aquatic food chains. Dissolved selenite and selenate (waterborne Se) are not directly toxic to aquatic organisms when water is the only exposure route (Lemly 1993b, Skorupa et al. 1996) whereas, organoselenium compounds may contribute significantly to Se toxicity despite their lower concentrations in water (Ogle et al 1988, Besser et al 1993). Once Se becomes incorporated into aquatic food chains, apparently the issue of chemical speciation is not an important interpretive factor (Skorupa et al. 1996). Se in natural aquatic systems seems to have a toxicity profile very similar to selenomethionine, an organic form (Hamilton et al. 1990, Heinz et al. 1996).

Four primary pathways for Se exist in aquatic systems: 1) it can be absorbed or ingested by organisms, 2) it can bind or complex with particulate matter, 3) it can remain free in solution, or 4) it can be released to the atmosphere through volatilization (Lemly and Smith 1987, Lemly 1997c). Selenium can be removed from solution and sequestered in sediments through the natural process of chemical and microbial reduction of selenate to selenite. Such immobilization is thought to occur more effectively in slow moving or still-water habitats. Estimates suggest that up to 90% of the total Se in an aquatic system may be in the upper few centimeters of sediment and detritus (Lemly and Smith 1987).

Se in sediments, is very important to the long-term habitat quality. There are
many chemical and biological processes in aquatic habitats that mobilize this Se into food chains. Some of these include: 1) oxidation and methylation of inorganic and organic Se by plants and microorganisms, 2) bioturbation of sediments by invertebrates and vertebrates, 3) physical perturbation and chemical oxidation associated with water circulation and mixing, and 4) uptake of Se by plants, invertebrates, fish, and wildlife (Lemly and Smith 1987, Lemly 1997c).

There are two processes of bioaccumulation: bioconcentration and biomagnification (Ogle et al. 1988). Bioconcentration is the direct uptake of selenium from water or sediment across respiratory or epidermal surfaces. Bioconcentration primarily involves selenite and selenate, the dominant waterborne species in most aquatic systems. Organisms can also accumulate selenium via biomagnification, which is uptake and accumulation through the food chain. Biomagnification involves both organic Se compounds such as selenomethionine as well as inorganic species. Increasingly however, evidence suggest that Se in food chains does not conform to the strictest definition of biomagnification. Selenium concentrations do not necessarily increase through the trophic hierarchy of aquatic food chains. This lack of true biomagnification of Se is thought to occur because of biotransformation and other metabolic process during ecological cycling (Maier and Knight 1994). Bioaccumulation is the combined result of both processes - bioconcentration and biomagnification (Hansen et al. 1993).

Uptake mechanisms indicate that selenate is taken up via the sulfate membrane carrier and organic Se is taken up via the methionine membrane carrier. Both processes
are active and can be modified by environmental conditions (light, pH, and temperature). Selenite apparently has an independent uptake mechanism that is specific for this ion (Ogle et al. 1988). Selenite is accumulated to a greater extent than selenate and is readily reduced and metabolized to form organic selenium compounds (selenium amino-acids) (Ogle et al. 1988).

Metabolically two pools for Se exist, an inorganic pool with a short half-life and an organically-bound pool with a much longer half-life (Ogle et al. 1988). This difference in Se exposure and toxicity via water and/or food chain components can be viewed as two functional groups (Bertram and Brooks 1983). Fish exposed to waterborne Se reached tissue equilibrium levels within 30 days but fish exposed to food-chain Se took more than 11 weeks to reach equilibrium (Lemly 1982, Ogle et al. 1988).

In lake mesocosm studies biomagnification appeared to be greater than bioconcentration (Rudd et al. 1980, Klaverkamp et al. 1983). At low waterborne Se levels, biomagnification is more important than bioconcentration, but this trend diminishes as waterborne Se levels are increased (Turner and Swick 1983).

_Selenium in Fish & Wildlife_ - Selenium was recognized as toxic to fish as early as the 1930's (Ellis et al. 1937). Early estimates of Se toxicity to aquatic life were based on the effects of waterborne exposure. Acute or chronic toxicity of inorganic Se species to freshwater fish and invertebrates has been reported at concentrations of 55 to 75 μg/l. Adams and Johnson (1981) reviewed the toxicity of inorganic Se and recommended 52 μg/l as a water-quality criterion for the protection of aquatic life. In contrast, Se amino-
acid (selenomethionine) is acutely toxic to fish and invertebrates at concentrations as low as 10μg/l (EPA 1987, Besser et al. 1993).

Differences in toxicity of aqueous Se species are reflected in differences in bioconcentration by aquatic organisms. Most published accounts for selenite and selenate toxicity in fish range from 2 to 16 μg/l (Lemly 1985a, Baumann and Gillespie 1986). In agricultural irrigation drain water in the western U.S., Se may accumulate to toxic levels when waterborne concentrations are as low as 0.5 to 3 μg/l (Stephens et al. 1988, Saiki 1990, Skorupa and Ohlendorf 1991). Organoselenium compounds can be bioconcentrated when water concentrations are as low as 0.5 to 0.8 μg/l (Lemly 1993b).

Preferential uptake of selenomethionine relative to inorganic Se species has been reported in algae (Sandholm et al. 1973, Kiffney and Knight 1990), daphnids (Besser et al. 1989, 1993) and fish (Sharma and Davis 1980, Besser et al. 1993). Metabolic stress caused by winter weather can increase the susceptibility to Se poisoning of birds (Heinz and Fitzgerald 1993) and fish (Sorenson 1991, Lemly 1993c). Subchronic levels of Se exposure may increase the susceptibility to otherwise benign pathogens due to Se-induced immune dysfunction (Fairbrother and Fowles 1990). Up to thirty percent teratogenesis has been found in fish populations whose tissue concentrations contained 40-50 μg/g Se (Lemly 1993a).

*Waterborne & Dietary Selenium* - Increasingly, evidence has emerged that toxicity of Se correlates more with dietary than waterborne exposure (Sandholm et al 1973, Rudd et al. 1980, Bertram and Brooks 1983, Lemly 1985b, Woock et al. 1987,
Coughlan and Velte 1989, Hamilton et al. 1990 and Harrison et al. 1990). The decline of a Centrarchid fishery in Belews Lake, North Carolina, receiving Se-laden process water from a coal fired power plant, occurred at water borne Se concentrations averaging 10 \( \mu g/l \) (Lemly 1985b) over a two year period. Similarly, a fisheries decline due to Se contamination occurred in Martin Lake, Texas (Sorensen and Bauer 1984). Lemly and Smith (1987) reviewed studies on the toxicity to bluegills (\textit{Lepomis macrochirus}) of combined dietary (15-73 \( \mu g/g \) dry weight) and waterborne Se (5-22 \( \mu g/l \)) concentrations from contaminated sources. They found effects ranging from deformity to reproductive failure and mortality, with up to 100% mortality within 7 to 61 days for some purely dietary studies. Dietary Se-methionine is more toxic to fish than dietary inorganic Se (Woock et al. 1987, Coughlan and Velte 1989).

More recently toxic effects have been documented in fish consuming diets containing 6.5 to 33 \( \mu g/g \) Se. These concentrations are similar to those in prey organisms from Se-impacted habitats (Woock et al. 1987, Coughlan and Velte 1989, Coyle et al. 1993). Levels as low as 3-4 \( \mu g/g \) in diets are thought to be toxic to rainbow trout (Hilton et al. 1980) and impair waterfowl reproduction (Heinz et al. 1989). In the San Joaquin Valley of California, elevated Se concentrations (14-17 \( \mu g/g \)) of food-chain organisms have been associated with fish population declines (Hamilton et al. 1986, Saiki et al. 1992) and reproductive failure in nesting birds (Ohlendorf et al. 1986). Gillespie and Baumann (1986) found that aqueous Se concentrations of 8 to 12 \( \mu g/l \) produced reproductive failure of bluegill through the dietary transfers of Se.
Currently, waterborne Se concentrations in the range of 1.0-5.0 μg/l are known to bioaccumulate to toxic levels in food chains (Skorupa and Ohlendorf 1991, Besser et al. 1993). Teratogenic deformities increase rapidly at about 10 μg/g Se in fish eggs (Coyle et al. 1993, Lemly 1997a). The most important aspect of Se residues in aquatic food chains is the dietary source of Se (Lemly 1993b) and the consensus of research studies is that most of the Se in fish tissues is from dietary sources (Lemly 1985a, Woock et al. 1987, Hamilton et al. 1990, Besser et al. 1993, Coyle et al. 1993, Saiki et al. 1993, Lemly 1997a).

*Selenium Regulation* - In 1987 the U. S Environmental Protection Agency (US EPA) established an ambient-water-quality criterion (4 day average, not to exceed) of 5 μg/L (US EPA 1987) in an attempt to limit the accumulation of Se in the aquatic food chain. However the EPA criteria does not address the specific forms of selenium in water, i.e., “..... the variety of forms of selenium in ambient water and the lack of definitive information about their relative toxicity to aquatic species” (US EPA 1987). Yet, it is increasingly apparent that there are important differences in toxicity and bioaccumulation between the Se forms (Ogle et al. 1988, Ingersoll et al. 1990, Brasher and Ogle 1993). The US EPA is currently considering lowering the freshwater criterion for Se to 2.0 μg Se/l (Maier and Knight 1994).

*Selenium in the Lower Colorado River* - In the LCR conditions that influence concentrations of Se are: 1) a nearby geologic source; 2) low rainfall and high evaporation (Engberg 1995); and 3) for all practical purposes a closed drainage route; the
LCR only flows to the Gulf of California in years of abundant rainfall or snowfall, i.e., 1997 (per. observation). With the exception of floods, virtually all Colorado River water is allocated. No planned flows have reached the Colorado River Delta since 1935, only the floods of 1983, 1984, and 1986 (Ohmart et al. 1988). The consensus is that much of the Se in the Lower Colorado River comes from weathering of natural geologic sources (Cretaceous shale formations) and hydrologic transport to the river from the upper reaches of the Colorado River watershed (Presser 1994, Presser et al. 1994) (Figure 1).

However, other potential sources contributing to the Se burden in the Lower Colorado River may include irrigation drain water, mine tail water, and coal-fired electric generating stations in the upper Colorado River basin (Radtke et al. 1988, Welsh and Maughan 1994). In the LCR Radtke et al. (1988) found that waterborne Se concentrations were lower at stations under the direct influence of agricultural discharges. Bottom sediments at all stations exceeded geometric means of soils in the western United States from 2-30 times. Biological sampling indicated that Se is the inorganic constituent of greatest concern for the protection of fish and wildlife.

Fish collected at all stations in the LCR exceeded the waterfowl diet goal of 3.0 ppm Se set by the U.S. Bureau of Reclamation and U.S. Fish and Wildlife Service for Kesterson NWR (Radtke et al. 1988, Deason 1989). King et al. in (1993) found Se concentrations in all fish were greater than the National Contaminant Biomonitoring Program's 85th percentile and 53% of the fish samples exceeded the minimum threshold
Figure 1. Colorado River Watershed and Source of Selenium from natural geologic sources (Cretaceous Shale Formations).
concentration where Se effects fish reproduction. Additionally, as cited by Presser et al. (1994), Kepner and others (1993) found elevated levels of Se in the livers (25.3 μg/g DW) and eggs (12.5 μg/g DW) of the endangered Yuma clapper rail and mean Se levels of 4.2 μg/g in crayfish, a main component of the rails diet.

Selenium in the LCR is not accumulated in all biotic pathways. Selenium bioaccumulates in benthic insects, fish, and fish-eating birds (Welsh and Maughan 1994). However, Se has not been found to concentrate in plants or bioaccumulate in plant-eating insects and birds (Lusk 1993, Martinez 1994). Additionally, there is variation in levels to which Se accumulates in the different environments along the LCR (Lusk 1993, Martinez 1994). In general, biota from connected backwaters contained Se levels >5 ppm DW and biota from seep lakes contained Se levels <2 ppm DW (Lusk 1993, Martinez 1994, Welsh and Maughan 1994). Selenium concentrations in the LCR are at toxicity thresholds for sensitive species and Se inputs from any source should be reduced to minimize the potential for Se-induced problems (Welsh and Maughan 1994).
STUDY SITE

Background - Imperial NWR (INWR) is located in the southwestern corner of Arizona and eastern California (Figure 2). The LCR in the U.S. stretches for 276 river miles from Davis Dam near Laughlin, Nevada, south to the International boundary between the U.S. and Mexico. The LCR was once known as "La Palma de la Mano de Dios" (the palm of God's hand) to the Native/Mexican people (Jaeger 1957), suggesting that the region where the Gila River and the LCR intersect was highly productive. The river forms the boundary between California and Arizona. INWR straddles the LCR from a point 7 river miles upstream of Imperial Dam to roughly 28 river miles upstream from this point. The refuge covers parts of Imperial County, California and Yuma and LaPaz Counties, Arizona. INWR is the south-most of four national wildlife refuges along the LCR. INWR encompasses over 45 km of the LCR and contains 4,324 ha of wetlands. Additionally it contains 5,630 ha of desert, mountains and washes, and roughly 96 ha of cropland (Baca et al., 1994). INWR was created in 1941 as a refuge and breeding ground for migratory birds and other wildlife.

The creation of Imperial Dam in 1938, 7 km downstream of INWR, flooded lowland riparian areas. Over time the river has formed natural levees (by erosion and deposition) along its banks. This process has been augmented by channelization, river straightening, and filling of backwaters in some areas by the USBR, and by the artificial rise and fall of this highly regulated river. Backwaters formed by the levees may be connected to the mainstream by a natural break in the levee: such wetlands are herein
Figure 2. Study Site—Imperial National Wildlife Refuge on Lower Colorado River near Yuma, AZ.
termed connected lakes. The amount of water passing through these breaks may be influenced by specific flow conditions, or more often, by human activity. Some backwaters do not have obvious connections to the river. Others appear to be isolated from the river by natural levees. Isolated backwaters are thought to be recharged by groundwater seepage from the river aquifer system or storm runoff: such wetlands are herein referred to as true seeps. Wetlands filled by runoff and seepage from the river have what is considered degraded water quality. They have very warm water, low dissolved oxygen, and a high salt content due to evapoconcentration (USBR 1984, Ponder 1975, Ohmart et al. 1988, Lusk 1993, Prieto per. obser.). The degree of connection between the river and the various backwaters is an important factor affecting water quality, fisheries, and ecological productivity (Saiki 1976, Kennedy 1979, Holden et al. 1986, Lusk 1993, Prieto per. obser.). In general, local waters (Imperial reservoir and larger backwaters) are considered eutrophic and holomictic (Hallock 1973).

The flow of the LCR is highly regulated by dams, reservoirs and diversions. These man-made structures cause fluctuations in the level of the river which in turn affects aquatic communities (Ponder 1975, Saiki 1976, Kennedy 1979, Ohmart et al. 1988). Generally water is stored in upstream reservoirs during the fall and winter, as a result river flows decrease. Upstream water storage creates the lowest annual water levels in the backwaters. In the spring and summer water levels are augmented by the release of water for agricultural use from upstream reservoirs. Water release for agriculture creates the highest annual water levels in the backwaters. In unusual years, (e.g., 1997 the
second year of this study) accumulation of snow followed by spring thaw in the upper reaches of the Upper Colorado River watershed in Nevada, Utah, Wyoming, and Colorado requires the release of water during the winter in anticipation of the storage required to contain the snowmelt. In years such as 1997 the river maintains a somewhat constant level year-round and may flow to the Gulf of California.

Weather - The general study area is arid and hot (Metzger et al. 1973). High temperatures produce average pan evaporation rates of 355.6 cm/yr (Kennedy 1979), average lake evaporation rates of 218 cm/yr (Lusk 1993) and average annual evaporation rates of 225 cm/year (Radtke et al. 1988). Approximately 68% of this annual evaporation occurs from May to October (Kennedy 1979). Precipitation averages 6.6 cm/yr (Lusk 1993) and is extremely variable (>40%), the highest variation in North America (Van Royan 1954). Water temperatures in backwaters along the LCR can exceed 30°C in the summer (Ponder 1975, Saiki 1976, Kennedy 1979, Prieto per. observation). Air temperatures range from 4 to 21 °C in the winter and 23 to 41°C during the summer (Ohmart et al. 1988). Ambient air temperatures exceed 32°C over 160 days of the year (Ohmart et al. 1988, Villegas 1997). The average daily illuminance is approximately 500g cal/cm² (Hallock 1973). These conditions combine to create a precipitation/potential evapotranspiration ratio less than 0.03% and make this area south from Cibola NWR to the international boundary one of two hyperarid deserts in North America (Cole 1990). The other hyperarid region is the Mohave Desert’s Death Valley.
**Geology** - The area surrounding the riparian corridor is characterized geomorphically by mountains, hills, piedmont slopes, dissected uplands, river terraces and mesas, sand dunes and flood plains (Metzger et al. 1973, McDonald and Loeltz 1976, Barmore et al. 1980). The mountains are low (to 2000 ft/600 m), northwest-trending and separated by extensive desert plains. The mountains and hills are composed of dense crystalline rock and hard volcanic rock of pre-Tertiary age. Piedmont slopes are characterized by desert pavement, and the remaining uplands, terraces, dunes and flood plains by fluvial gravel, sand, silt, alluvium and eolian deposits from the extensive former delta plain of both the Colorado River and the Gila River (Barmore et al. 1980). Today much of this former delta supports agricultural production.

**Soils** - General soil units occurring on the refuge and local area include: 1) Ligurta-Cirstobal-Carrizo; and 2) Laposa-Rock outcrop. The Ligurta-Cirstobal-Carrizo group in generally characterized as deep, nearly level, well to excessively drained, gravelly to very gravelly soils on alluvial fans, low terraces, and flood plains. The Laposa-Rock outcrop are moderately deep, steep, well drained, extremely gravelly soils, and rock outcrops on hills and mountains (Barmore et al. 1980). Soil complexes from lowest to highest elevation include: Lagunita loamy sand at elevations from 75 to 600 ft (22.5-180 m), Carrizo very gravelly sand at elevations from 100 to 1200 ft (30-360 m), Ligurta-Critobal complex with 2 to 6% slopes and elevations from 250 to 1300 ft (75-390 m), Torriorthents-Torrifluvents complex with 1 to 50% slopes and elevations from 400 to 1200 ft (120-360 m), and Laposa-Rock outcrop complex with 15 to 75% slopes ranging
in elevation from 400 to 2000 ft (120-600 m) (Barmore et al. 1980).

The parent material of these soils includes various kinds of bedrock and alluvium including granite, schist, gneiss, andesite, rhyolite, basalt, and sandstone. In general the soils can be characterized as deep, alkaline, fine and moderately fine textured except on flood plains which, have medium to coarse textured soils. Locally on some flood plains, high water tables exist (Barmore et al. 1980).

Vegetation - The river, backwaters and flood plains (riparian corridor) are part of what is considered the southern reaches of the Sonoran riparian deciduous forest community, and the uplands on both sides of the LCR are Sonoran desert scrub (Ohmart et al. 1988, Brown 1994). Today this riparian area can be characterized as a disclimax dominated by salt cedar (Tamarix spp.) (per. observation). The river channel is comprised of medium to course shifting sands and the banks are lined by the dominants saltcedar (Tamarix chinensis) and common reed (Phragmites australis). Other riparian species include Gooding willow (Salix goodingii), velvet mesquite (Prosopis velutina), screwbean mesquite (Prosopis pubescens), Fremont cottonwood (Populus fremontii), arrowweed (Tessarea servicea), bulrush (Scirupus californicus), and cattails (Typha latifolia). Submergents include sago pondweed (Potamogeton pectinatus), coontail (Ceratophyllum demersum), water milfoil (Myriophyllum exalbescens), and spiny naiad (Najas marina).

Backwaters have a detrital-silt substrate underlain by sand and gravel and are surrounded by dense, monotypic stands of cattails, bulrush, or giant reed depending on
water depth and slope. The predominant submergent is spiny naiad with lesser amounts of sago pondweed, water milfoil, macroalgae (*Chara* spp.) and coontail along the edges. True seeps support no submerged aquatic macrophytes and are lined almost exclusively by cattails.

Almost every underwater surface is covered with a layer of yellowish-brown, slimy aufwuchs, i.e., the combined assemblage of epipellic, epiphytic, and epizoic periphyton and suctorian ciliates (Cole 1994). During the summer, floating mats of filamentous algae (mostly Cyanophyta) (Kennedy 1979) are present in many of the backwaters. Seeps lakes contained benthic algae and fresh water sponges that may be unique to these wetlands (pers. observation).
MATERIALS AND METHODS

Initial Wetland Survey

Site Selection - In the summer and fall of 1996 (July 31 through November 3), I collected water quality data from 22 backwaters occurring within the boundaries of INWR on the LCR (Figure 3). The purpose was to characterize the wetlands according to water quality parameters, degree of connectivity to the river, fish species, and to allow us to better design the experimental selenium aspect of this study.

These preliminary data suggested that there were three rather than two wetland types in the LCR (see results). Therefore, in the spring of 1997 I chose a subsample of nine backwater lakes based on water quality measures obtained in 1996. I selected three each of the apparent three types of wetlands for further detailed study. I continued to sample water quality and fish from these wetlands. I also conducted a selenium bioaccumulation experiment in these wetlands.

Species Selection - I sampled wild fish of the genus Lepomis and more specifically the bluegill (Lepomis macrochirus) when possible. The family Centrarchidae is sensitive to Se contamination (Lemly 1982, 1985a, b, 1993a, c) and Se data on sunfish are available from other studies (Baumann and Gillespie 1986, Gillespie and Baumann 1986, Saiki and May 1988, Welsh 1992, Coyle et al. 1993, Lusk 1993). Bluegill were common in most of the wetlands along the LCR. Mosquitofish (Gambusia affinis) were collected in some of the seeps, often being the only fish species available. Fortunately, there is Se data for mosquitofish also (Saiki 1987, Saiki and Lowe 1987, Lusk 1993).
Figure 3. Lower Colorado River backwaters from river mile (RM) 72.8 to RM 43.2 including Imperial NWR. The symbol designates the 9 wetlands in the experimental study. \( \star \) or \( \ast \) designates the 22 wetlands in the water quality survey.
Both fish species are eaten by predatory fish and birds (Hallock 1973, Saiki 1976, Minckley 1982, and Martinez 1994).

Crayfish for the experimental study of selenium uptake were purchased from a commercial vendor in Sanger, California, J & J Aquafarms. I used red swamp crayfish (Procambarus clarkii) due to its commercial availability, naturalized presence in the LCR, and importance as a food source for predatory fish and birds (Minckley 1982). Additionally, there are some contaminants data for the red swamp crayfish (Lusk 1993, Saiki et al. 1993). Collectively, these species are not highly mobile and thus likely have remained at their collection sites long enough for body burdens of Se to equilibrate with prevailing environmental concentrations (Saiki et al. 1992).

Sample Collection and Preparation

*Water Quality Sampling* - Water quality was measured initially upon entering a new wetland in 1996 and generally once a week at each designated wetland in 1997. In 1997 water quality was measured weekly for the duration of the bioaccumulation experiment. These measurements were taken randomly adjacent to one of the cages containing crayfish. Water quality parameters were measured near the surface. Only dissolved oxygen was measured near the sediment-water interface. Meters were calibrated each week before entering the field.

*Water Quality Equipment* - A Hach model 2100P portable turbidimeter was used to measure turbidity in nephelometer units (NTU). A Hach model EC20 portable pH/ISE meter with a model 50205 combination pH electrode (gel-filled) and thermometer was
used to measure hydrogen-ion concentration (pH) and temperature (°C). A YSI model 30 hand-held salinity, conductivity, and temperature meter was used to measure salinity (ppt), temperature (°C), conductivity in microseimens (μS/cm) and milliseimens (mS/cm) per centimeter at ambient temperature or standardized to 25°C. Conductivity data were collected in microseimens standardized to 25°C to permit direct comparisons. A YSI model 58 dissolved oxygen meter was used to measure dissolved oxygen in mg/l.

Fish Sampling - Fish were collected with cast nets and fyke nets (trap net). Dip nets and minnow traps were used to catch mosquitofish. Cast nets and dip nets were actively used during the day to catch fish. Fyke nets and minnow traps were set near dusk and raised as soon as possible the following morning. Minnow traps were baited with canned dog food. Fish samples were collected from the wetlands in 1996 and 1997. One composite of whole-body samples (including gut contents) of each fish species (bluegill and mosquitofish) was the goal per wetland. The use of whole fishes can yield variable contaminants data (Braumbaugh and Kane 1985, Saiki et al. 1992). However, our rational to use whole fish is that food items are consumed whole by piscivorous birds and fish.

Fish Sampling Gear - Cast nets were multi-filament bait cast nets with a radius of 4ft (1.2 m) and 1/4 inch (0.83 cm) square mesh (Memphis Net and Twine Co., Inc.). Fyke nets (H. Christiansen Co.) were of 3/4 inch (1.86 cm) mesh netcoat-treated net, with two 3 x 4 ft (0.9 x 1.2 m) rectangular conduit metal frames. 5 steel hoops, 2 throats one each on the first and third hoop, and a 50 ft (15 m) lead of 3/4 inch (1.86 cm) mesh. The
lead was of 3 ft (0.9 m) depth, tied on 1/4 inch (0.83 cm) polypropylene rope with SB-2 floats at 48 inch (120 cm) intervals and 50# lead line. Dip nets (Memphis Net and Twine Co., Inc.) were standard duty square 14 by 15 inch (35 x 37.5 cm) nets, with a plastic guard on front, 1/8 inch (0.31 cm) square nylon knotless mesh, 24 inch (60 cm) deep with long handles. Minnow traps (Memphis Net and Twine Co., Inc.) were of galvanized steel with 1/4 inch (0.63 cm) wire mesh, 17 1/2 long by 9 inch diameter (43.75 x 22.5 cm). Small fish could enter from either end.

Crayfish Experimental Materials and Sampling - Cages to house crayfish during the Se bioaccumulation experiment were one meter square and approximately 40 cm high. The cage frames were made using 1 1/4 inch (3.13 cm) PVC pipe. These frames were then covered with 1/4 inch (0.63 cm) grid plastic netting commonly called vexar mesh (Memphis Net and Twine Co., Inc.) made of high density polyethylene. Netting was cut and secured to the PVC pipe using nylon safety ties. There was a door on the top side of the cages to allow weekly collection of crayfish. Three cages containing approximately 2.5 gallons (9.5 l) of organic material (plant material collected in situ), 7-8 pieces of PVC pipe approximately 6-9 inches (15-22.5 cm) long for shelter, and 7-8 crayfish per cage were placed into each of the designated wetlands. A total of 27 cages were used in the experiment. Crayfish in each cage were followed with the intent of collecting 7 weeks of data. However, low water quality in some of the seeps precluded me from reaching this goal.

Sample Handling & Processing - Whole body composites of fish (minimum 3
sunfish and/or 30-40 mosquitofish) were collected from each of the nine wetlands in 1996 and 1997. Whole body individual crayfish were collected from each wetland on a weekly basis during the bioaccumulation experiment in 1997. Whole-body samples included the contents in the gut. Specimens were kept on wet ice (max. 6 hr) in standard 1 gallon size zip-lock bags and then frozen upon reaching refuge headquarters. Samples remained frozen until processing. In total, 151 samples (131 crayfish and 19 fish) were collected. Five of the crayfish samples were composites of 3 crayfish. These composite crayfish samples were used to establish the pre-experiment Se content (baseline Se levels), prior to stocking crayfish in the wetlands.

Prior to shipment to a contract laboratory for Se analysis, samples were weighed, measured, identified, labeled and packaged on clean aluminum foil. Style 42 white latex surgical gloves, gamma sterilized, were used when handling all samples. The aluminum foil and latex gloves were replaced for each sample, i.e., used only once. Samples were placed in labeled, six ounce puncture-proof Whirl-Pak plastic bags sterilized by the ethylene-oxide process. These bags were placed inside an identical but larger 18 oz. bag, also labeled, to comply with lab requirements and identify discrete samples. To prevent cross-sample contamination, all tools and instruments were distilled water rinsed between use on each specimen. Chemical rinse was in distilled-deionized water followed by acetone and hexane. Tools and instruments were allowed to air dry. This procedure was repeated between preparation of each sample. Samples were then stored in a commercial freezer until analysis.
Samples were shipped in a heavy Styrofoam cooler (approx. 5 cm thick walls) with a 1:1 sample to dry ice ratio. The dry ice was wrapped in newspaper to avoid direct contact with samples. The excess space was filled with crumpled newspaper to avoid shifting and damage of contents. This cooler was placed into a heavy cardboard box, properly labeled and shipped overnight Federal Express to the designated lab for analysis. A copy of the original catalog submitted to the Patuxent Analytical Control Facility and a copy of the authorization to ship were included.

**Laboratory Sample Analysis**

All biological samples were prepared and analyzed for moisture and selenium content by Environmental Trace Substance Laboratory, University of Missouri, 101 USBM Bldg., 1300 North Bishop Ave., Rolla, MO 65409-0530. However, due to the duration of the study and the timing of collection of biological samples, not all samples were analyzed at the same time, nor were all samples stored in the freezer for the same length of time. Analytical procedures are impossible to duplicate for samples submitted on different dates. This variance is termed the “batch effect.” Batch 1 consists of the first year’s fish samples and the crayfish used in the bioaccumulation experiment. Batch 2 consists of the second year’s fish samples.

*Homogenization & Moisture Content* - Large tissue samples, such as whole fish or crayfish, were first run through a meat grinder one or more times depending on the size of the sample. An aliquot of the ground sample was weighed and frozen. For samples of sufficient size, moisture was determined by placing a weighed aliquot in a Fisher Isotemp
oven and drying at 103-105 °C. The dried sample was then reweighed and the data entered into a computer program to generate the % moisture. Samples too small for oven-dried moisture determination had the % moisture calculated from the moisture lost during freeze-drying. For these smaller samples the entire sample was weighed, frozen, then placed in a Labconco Freeze Dryer 8 until the moisture had been removed. These data were entered into a computer program to generate an estimate of the % moisture. All dried samples were then weighed and further homogenized using a blender, or Spex Industries, Inc. Model 8000 mixer/mill with tungsten-carbide vial and balls in preparation for selenium analysis. The dry-blending facilitates acid digestion, a prerequisite to hydride-generation atomic absorption spectrophotometry for hydrogen selenide.

Preparatory Acid Digestion - Approximately 0.5 g of homogenized sample described above was weighed and placed into a freshly cleaned 100 ml quartz Kjeldahl flask. Samples containing a high percent of silica were digested in 100 ml Teflon beakers. Slowly 15 ml of concentrated sub-boiled HNO₃ and 2.5 ml of concentrated sub-boiled HClO₄ were added. If the foaming became excessive, the container was cooled in a beaker of cold water. After the initial reaction had subsided, the sample was placed on low heat until the evolution of dark red fumes had ceased. Gradually, the heat was increased until the HNO₃ began refluxing; samples were allowed to reflux overnight. Refluxing overnight decreased the chance for charring during the reaction with HClO₄. After refluxing, the heat was gradually increased until the HNO₃ had been driven off, and the reaction with HClO₄ had occurred. When dense white fumes from the HClO₄ were
evident, the samples were removed from the heat and allowed to cool. Two ml of
conzentrated sub-boiled HCl were then added. The flasks were placed on heat and
warmed until the contents started to boil. Containers were removed from the heat, and 5-
10 ml of deionized water were added. Samples were allowed to cool. They were then
diluted using deionized water in a 50 ml volumetric flask and transferred to a clean,
labeled, 2 oz. polyethylene bottle.

_HGAAS Selenium Analysis_ - Selenium content was determined using hydride-
generation atomic absorption spectrophotometry (HGAAS). A Varian VGA-76 hydride
generation accessory was mounted on either a Perkin-Elmer Model 603 AA or Model
3030 (B) AA. Electrodeless discharge lamps (EDL) were used. Appropriate instrument
and EDL settings were taken from the instrument manuals. The burner mount for a
Perkin-Elmer Model 10 Hydride generator was modified slightly to hold the Varian
quartz cell. The cell was aligned in the light path of the burner chamber and a very lean
flame was used for heating the cell. The stock solution was 50% v/v sub-boiled HCl and
0.6% NaBH₄ in 0.5% NaOH for selenium analysis by hydride formation. First however,
the samples, as prepared above by acid digestion, were diluted in 10% v/v sub-boiled
HCl.

In HGAAS, the stock solution of hydrochloric acid, sodium tetraborohydride, and
sodium hydroxide is then added to the diluted acidified sample. It is here where selenium
has presumably been entirely converted to its selenite form prior to forming gaseous
hydrogen selenide. Samples were then analyzed by taking an integrated reading for 3
seconds after the plateau was reached for the sample. This plateau occurred approximately 45 seconds after the sampling tube was placed in the sample. Gaseous hydrogen selenide is stripped from the solution and atomized in the quartz cell. The spectra absorbed are compared to known absorption spectra and then related back to concentration in solution and in the sample.

Standards were prepared by dilution of Fisher 1000 ppm stock in 10% v/v sub-boiled HCl in the range of 0 to 20 ppb. The instrument was standardized to read directly in ppb using S1=5.00 and S2=20.00. Standardization was checked by reading other standards such as 2.00, 10.00, and 15.00 ppb and an instrumental quality control sample of known value. If the standards and quality control were acceptable, the detection limit was determined by reading the zero standard 10 times. Twice the standard deviation of the mean was used as the detection limit. Standardization was checked every 8-15 samples and approximately 10% of the samples were checked by additions to monitor batch effects. Batch effects were usually not significant with the VGA-76. The data were corrected for drift of the standard curve and entered into the AA calculation program. This program corrected for blank, dilution, sample weight, sample volume and recorded the data in the LIMS database for report generation. Duplicates, blanks, reference materials and spiked samples were similarly analyzed. Concentrations of inorganic chemicals (selenium) in biotic samples were reported on a dry-weight (dry-wt) basis and a wet-weight (wet-wt) basis in part-per-million (ppm).
**Analytical Quality Control and Assurance**

Quality control procedures were supplied as a written report by the U.S. Fish and Wildlife Service Patuxent Analytical Control Facility (PACF). Quality assurance includes the evaluation of analytical precision, as measured by duplicate sample analysis, and laboratory accuracy, as measured by spike recovery. Blanks and analysis of standard reference materials (dogfish liver) are also used to assure quality control and assessment.

Laboratory analytical precision was deemed acceptable by PACF. The evaluation of precision was applied to all samples analyzed by the laboratory and not just specific batches. The accuracy for the recovery of samples spiked with known amounts of selenium and the certified values of standard reference materials were also considered acceptable by PACF. Acceptable recovery ranges for all samples submitted to a laboratory are from 85-115% for Se-spiked materials (Moore 1990).

**Experimental Design, Statistical Methods and Data Treatment**

*Water Quality Data* - Summer 1996 water quality data consisted of a one time visit to 22 wetlands on INWR. Hence, data were visually inspected and explored simply by sorting data in various ways to verify basic data trends. In the spring of 1997 a subset of nine of the same wetlands were repeatedly sampled over time. These data were treated as a repeated measures design with any given water quality parameter as the dependant variable and wetland type (three classes) and time as the independent variables. Hence, the data collected from the nine wetlands visited repeatedly in the spring of 1997 were used exclusively for statistical analysis.
Water quality parameters were rigorously tested for two basic reasons: 1) to serve as a monitor of water quality conditions that might potentially affect the Se bioaccumulation experiment; and 2) as a means to test differences in water quality in the 3 types of wetlands found in the exploratory wetland inventory of 1996. Hence, the hypothesis regarding water quality parameters is two-fold: 1) that there would be significant changes in water quality thus potentially confounding our experiment; and 2) that measured water quality parameters may not be consistent for the purpose of differentiating the proposed wetland types.

Conversely if we found little difference in parameters over time, this result would provide additional credence for the Se bioaccumulation experiment by suggesting consistent environmental conditions during the experiment. Additionally, little difference in a given water quality parameter over time would support the fact that certain designated water quality parameters are sufficiently consistent within wetland types to serve as tools in identifying wetland types.

Water quality data were tested for normality using the Shapiro-Wilk W Test. Most water quality data (μS/cm@25°C, salinity, pH, and turbidity) were transformed using a natural log (base e) transformation, $X' = \log_e (X)$ to normalize the variance. Only temperature data was not transformed due to its acceptable distribution. Dissolved oxygen (DO) data were converted to percent saturation. Approximately 26% of the DO data were in excess of 100%. Thus, a square root arcsine transformation which is commonly used for percent data (Zar 1996), was not used due to a significant loss in
sample size (those > 100%). This loss of data and lack of enhancement of data distribution were considered unacceptable. Thus, DO data were simply used as percent saturation. Transformed and other data had skewness and kurtosis coefficients close to zero, thus approaching normality and allowing the use of parametric testing.

A Repeated Measures ANOVA was used to determine if water quality parameters varied through time within a given wetland type, and if wetland types differed significantly from each other in a given parameter. Water quality parameters were measured once weekly in each wetland over 13 weeks during the spring of 1997. However, due to the lack of sufficient error degrees of freedom in the repeated measures ANOVA model for water quality (13 weeks by 3 wetland types replicated 3 times), I reduced the 13 weeks into four groups of water quality data across time. Hence, I used an average of weeks 0 through 3 for group one, an average of weeks 4-6 for group two, weeks 7-9 comprised group three, and weeks 10-12 comprised group four. This arrangement used the entire data set and satisfied the minimal required degrees of freedom.

Where a water quality parameter remained relatively constant over time (i.e., there was no significant “time effect” in the repeated design), I collapsed the values over time and used single-factor ANOVA to examine individual water quality parameters between wetland types, including the river. When significant differences were found between wetland types, the Tukey-Kramer HSD Test was used to make multiple comparisons of means. Differences between means were considered significant at $P \leq 0.05$. Arithmetic
means of summarized water quality data are presented in tabular form to permit comparisons between wetlands.

Principal component analysis (PCA) was used to transform (7) intercorrelated water quality parameters into a new combined set of uncorrelated factors to help visualize and explain variation between the wetland types. These factors, in combination with univariate graphs and multivariate biplots, were used to aid in determining those water quality parameters most useful in distinguishing between wetland types in the LCR.

**Fish Data** - Data on Se concentration in fish were tested for normality using the Shapiro-Wilk W Test. Data were transformed using a natural log (base e) transformation plus one, \( X' = \log_e (X+1) \), to normalize the variance and to avoid zero values following transformation. The transformed data had skewness and kurtosis coefficients close to zero, thus approaching normality and allowing the use of parametric testing.

Selenium concentrations for each fish species (bluegill and mosquitofish) were first evaluated between years using a Student t-test. Upon finding no significant difference in Se concentrations by years, annual data were then combined for further analysis. However, prior to analyzing differences between wetland types, I first had to develop a statistical relationship between selenium levels in the two species from wetlands where they co-occur.

To obtain a measure of equivalency of selenium levels in mosquitofish and bluegill, I used selenium data from Lusk (1993) for three wetlands where the two species are sympatric (Butler, Island, and Devils Lakes), as well as from Clear Lake (this study).
I then used a Pearson product-moment pairwise correlation to determine the strength of linear relationships of Se concentrations between fish species. Use of the Pearson product-moment pairwise correlation aided in the interpretation of results of subsequent ANOVA for the differences in selenium levels in fish from different wetland types.

After accounting for different species using the Pearson product-moment correlation and assuming that associated differences between Se levels in the various wetlands was due primarily to wetland type and not species or trophic levels, annual data were pooled for further analysis. These data were then examined using single-factor ANOVA to evaluate Se levels in fish between the three wetland types. When significant differences were found in fish Se levels, the Tukey-Kramer HSD Test was used to make multiple comparisons of means. Differences between means were considered significant at P < 0.05.

*Crayfish Bioaccumulation Experiment* - The crayfish bioaccumulation experiment was a longitudinal repeated measures design with Se concentration as the dependant variable measured over time. Independent variables were time and wetland type (three designations). Individual wetlands were nested within wetland type, i.e., wetland[wetland type]. This is a repeated measures design because the same wetlands were repeatedly sampled over time. Nine wetlands were randomly selected from the larger group of 22 wetlands sampled in year one (1996). Included were three wetlands of each of three types: connected lakes and two types of seep lakes. This design permitted testing the hypothesis that there is a fundamental difference in the levels of Se uptake in crayfish.
held experimentally in the three types of wetlands.

To test this hypothesis three cages were placed in each of nine wetlands along the shoreline in about 3-4 ft (0.9-1.2 m) of water. Each cage contained 7-8 crayfish. One crayfish was collected from each cage every week for a total of seven weeks. This design gave a total of 27 cages, nine representing each wetland type and seven weeks of data from each of the cages. Selenium in test crayfish was measured prior to the beginning of the study, and weekly thereafter for seven weeks during the course of the experiment. This design permitted testing for differences due to the type of wetland. Further, the use of replicate cages per wetland per week permitted a measure of the variation within each wetland type. The design allowed determination of whether treatment (Se uptake by wetland type) differences were large enough to be judged biologically important. This selenium bioaccumulation experiment was conducted during the spring of the year, March 17 through June 14, 1997.

In cases where individual crayfish were lost due to vandalism or escapes, additional crayfish were marked and replaced according to the experimental design. Replacement of lost or dead crayfish meant running the bioaccumulation experiment five weeks longer than the seven weeks initially intended in some cases. Hence there was a time lag of up to five weeks for the completion of data collection from the first cage to the last cage.

*Crayfish Data* - Data were tested for normality using the Shapiro-Wilk W Test. Data were transformed using a natural log (base e) transformation plus one, \( X' = \log_e \).
(X+1), to normalize the variance and to avoid zero values following transformation. The transformed data had skewness and kurtosis coefficients close to zero, thus approaching normality and allowing the use of parametric testing.

A Repeated Measures ANOVA was used to determine if Se levels increased in crayfish overtime, and if there were significant differences in Se concentrations in crayfish between the wetland types. When significant differences were found, orthogonal contrasts was used to make multiple comparisons of means. Differences between means were considered significant at \( P \leq 0.05 \). Starting Se concentrations at week 0 were not used in the repeated measures ANOVA because they constituted a pooled estimate of starting Se levels and not Se levels from specific wetlands. No elemental concentrations were below detection limits, thus I made no additional manipulation of sample data to facilitate the comparisons of means. All statistical calculations of data were made using JMP statistical software (SAS Institute Inc., Cary, NC, USA) (Sall and Lehman 1996).

Individual crayfish within a cage were uniquely marked to permit tracking individuals for analysis of growth to help assess if experimental conditions were meeting the basic needs of crayfish. However, multiple molts within a cage in a given week often made it impossible to track every individual. Thirty-four percent of crayfish were identifiable at both beginning and end of the experiment. Paired crayfish weight data of these individuals were tested using a Paired t-test.

**General Data Considerations** - In order to compare our data to relevant literature, Se concentration results are reported as part-per-million dry-wt (ppm DW) which is
directly comparable to the concentration microgram per gram (µg/g) dry wt. Dry weight values allow for the standardization of sample moisture content (Saiki et al. 1992, Ruiz 1994). Statistical comparisons were all made using ppm DW concentrations. Wet-weight (WW) concentrations can be calculated by multiplying the DW by 1 minus the percent sample moisture expressed as a decimal. If sample moisture content is not available, 70-75% moisture is usually assumed (Lemly and Smith 1987, Ruiz 1994, Villegas 1997). To facilitate the comparison of these data to others the following formulae are provided:

\[
\text{WW concentration} = \text{DW concentration} \times (1 - \% \text{ moisture of sample})
\]

\[
\text{DW concentration} = \frac{\text{WW concentration}}{(1 - \% \text{ moisture of sample})}.
\]

Selenium levels are analyzed and reported using arithmetic means following the recommendations of Parkhurst (1998). Where appropriate geometric means in parenthesis follow reported arithmetic means. Selenium data tables present the arithmetic mean plus or minus 1 standard error, and the geometric mean and range of the logarithm-transformed data for comparison with previously reported data. Geometric means were calculated as the antilogarithm of the arithmetic mean of the logarithms of the data (Zar 1996). The confidence interval for the geometric mean can be calculated by taking the natural logarithm of the geometric mean then adding and subtracting the error factor (Lusk 1993).

Geometric means are reported in contaminants data rather than arithmetic means because they are less influenced by extreme outliers and hence provide a more conservative estimate of central tendency (Saiki and Palawski 1990). However,
geometric means may not allow for the most accurate determination of potential effects due to the very steep toxicity threshold for Se in the aquatic environment (Lemly 1996a).

Additionally, geometric means are always smaller than the arithmetic mean unless all numbers in the data set are identical. This bias is a result of the curvature of the logarithmic function, which downplays larger values relative to smaller ones. As a result, geometric means are of little use for mass balance analyses and should not be used for regulatory purposes (Parkhurst 1998).
RESULTS

Water Quality and Classification of Wetlands

Water Quality 1996 - Water quality data and qualitative observations made in 1996 from the 22 backwaters along the LCR strongly suggested the presence of three wetland types (Table 1). The initial separation of connected lakes (formerly backwaters) and true seeps (formerly seeps) was readily apparent by looking at conductivity and salinity. Those wetlands with conductivities and salinity similar to the river (≤ 1275.0 μS/cm@25°C) and (≤ 0.6 ppt) were undeniably connected lakes and those with very high conductivities and salinities (≥ 3000.0 μS/cm@25°C) and (≥ 2.0 ppt) were true seeps. Physically these two wetland types are easy to differentiate. Connected lakes are obviously and directly connected to the river mainstem. There is a direct flow of water from the river to the connected lakes via a channel often wide enough for entry with a small boat. True seeps are not directly connected to the river and can occur at distances varying from a few meters (adjacent) up to 100 m from the river. Their conductivities generally exceed 2000 μS/cm@25°C. Connected lakes and true seeps have generally been accepted as the types of wetlands along the LCR.

The third type of wetland became apparent initially through aesthetic qualities and later through subtle differences in measured water quality parameters. For example, in 1996 though I was not measuring turbidity, it was very apparent that certain wetlands had very clear water, unlike the other two wetland types. In 1997 it became apparent that conductivities and salinities of these “new wetlands” were intermediate between those of
Table 1. Water quality data from initial survey of 22 wetlands on Imperial NWR, summer 1996 presented in order of increasing specific conductivity and salinity. All parameters were measured within the top 5 cm of water.

<table>
<thead>
<tr>
<th>Wetland Name</th>
<th>Numerical*</th>
<th>New**</th>
<th>Temperature °C</th>
<th>S. Conductivity μS/cm@25°C</th>
<th>Salinity ppt</th>
<th>D. Oxygen mg/l</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZ Backwater</td>
<td>A64.5</td>
<td>CL</td>
<td>27.9</td>
<td>1123.0</td>
<td>0.6</td>
<td>11.6</td>
<td>9.29</td>
</tr>
<tr>
<td>AZ Seep</td>
<td>A63.4</td>
<td>PS</td>
<td>24.7</td>
<td>1218.0</td>
<td>0.6</td>
<td>7.9</td>
<td>7.87</td>
</tr>
<tr>
<td>Cabin Lake</td>
<td>A65.4</td>
<td>CL</td>
<td>26.4</td>
<td>1210.0</td>
<td>0.6</td>
<td>12.1</td>
<td>8.46</td>
</tr>
<tr>
<td>Hidden Lake</td>
<td>A62.3</td>
<td>CL</td>
<td>28.1</td>
<td>1221.0</td>
<td>0.6</td>
<td>8.5</td>
<td>8.46</td>
</tr>
<tr>
<td>AZ Seep</td>
<td>A62.1</td>
<td>PS</td>
<td>30.6</td>
<td>1229.0</td>
<td>0.6</td>
<td>7.4</td>
<td>8.03</td>
</tr>
<tr>
<td>AZ Backwater</td>
<td>A63.2</td>
<td>CL</td>
<td>24.6</td>
<td>1231.0</td>
<td>0.6</td>
<td>8.3</td>
<td>7.74</td>
</tr>
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<td>Calif. Seep</td>
<td>C64.01</td>
<td>PS</td>
<td>27.4</td>
<td>1250.0</td>
<td>0.6</td>
<td>8.0</td>
<td>7.74</td>
</tr>
<tr>
<td>Calif. BW</td>
<td>C66.6</td>
<td>CL</td>
<td>27.5</td>
<td>1250.0</td>
<td>0.6</td>
<td>9.5</td>
<td>8.05</td>
</tr>
<tr>
<td>Long Lake</td>
<td>A62.53</td>
<td>CL</td>
<td>26.8</td>
<td>1251.0</td>
<td>0.6</td>
<td>7.4</td>
<td>7.95</td>
</tr>
<tr>
<td>Island Lake River</td>
<td>A65.9</td>
<td>CL</td>
<td>26.8</td>
<td>1256.0</td>
<td>0.6</td>
<td>8.2</td>
<td>8.24</td>
</tr>
<tr>
<td>Calif. BW</td>
<td>C67.1</td>
<td>CL</td>
<td>26.8</td>
<td>1267.0</td>
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<td>6.3</td>
<td>7.50</td>
</tr>
<tr>
<td>Cable Lake</td>
<td>A63.7</td>
<td>CL</td>
<td>26.8</td>
<td>1288.0</td>
<td>0.6</td>
<td>7.8</td>
<td>8.24</td>
</tr>
<tr>
<td>Calif. Seep</td>
<td>C66.3</td>
<td>PS</td>
<td>26.2</td>
<td>1264.0</td>
<td>0.6</td>
<td>6.6</td>
<td>7.52</td>
</tr>
<tr>
<td>Calif. Seep</td>
<td>C64.4</td>
<td>PS</td>
<td>27.0</td>
<td>1318.0</td>
<td>0.7</td>
<td>10.2</td>
<td>8.71</td>
</tr>
<tr>
<td>Clear Lake</td>
<td>A62.5</td>
<td>PS</td>
<td>30.2</td>
<td>1386.0</td>
<td>0.7</td>
<td>8.8</td>
<td>8.89</td>
</tr>
<tr>
<td>Growout Lake</td>
<td>A56.43</td>
<td>PS</td>
<td>27.3</td>
<td>1524.0</td>
<td>0.8</td>
<td>6.7</td>
<td>8.26</td>
</tr>
<tr>
<td>House Pond</td>
<td>A59.85</td>
<td>PS</td>
<td>29.5</td>
<td>1786.0</td>
<td>0.9</td>
<td>7.1</td>
<td>8.18</td>
</tr>
<tr>
<td>Calif. Seep</td>
<td>C64.1</td>
<td>TS</td>
<td>25.6</td>
<td>3918.0</td>
<td>2.1</td>
<td>6.5</td>
<td>8.17</td>
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</table>
Table 1. Continued.

<table>
<thead>
<tr>
<th>Wetland Name</th>
<th>Numerical*</th>
<th>New**</th>
<th>Temperature °C</th>
<th>S. Conductivity μS/cm@25°C</th>
<th>Salinity ppt</th>
<th>D. Oxygen mg/l</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butler Lake</td>
<td>A61.55</td>
<td>TS</td>
<td>30.0</td>
<td>5330.0</td>
<td>2.8</td>
<td>7.4</td>
<td>8.55</td>
</tr>
<tr>
<td>Calif. Seep</td>
<td>C63.8</td>
<td>TS</td>
<td>26.7</td>
<td>7950.0</td>
<td>4.4</td>
<td>11.9</td>
<td>8.39</td>
</tr>
<tr>
<td>Ag. Lake #2</td>
<td>A58.9</td>
<td>TS</td>
<td>25.2</td>
<td>13270.0</td>
<td>7.6</td>
<td>3.8</td>
<td>8.31</td>
</tr>
<tr>
<td>McAllister L.</td>
<td>A61.0</td>
<td>TS</td>
<td>33.7</td>
<td>19820.0</td>
<td>11.7</td>
<td>13.1</td>
<td>8.72</td>
</tr>
</tbody>
</table>

** Legend (wetland type): CL=connected lake, PS=pseudo seep, and TS=true seep.
1-Not shown in Figure 3, Lower Colorado River Backwaters. River mileage designation given in this study.
2-Shown in Figure 3 as part of Clear Lake, presently separate.
3-This PS, now a separate wetland, was formerly a cove of Martinez Lake (A56.4).
connected lakes and true seeps, i.e., new wetlands had conductivities \( \geq 1276.0 \) and \( \leq 1790.0 \, \mu S/cm@25^\circ C \) and salinities \( \geq 0.7 \) and \( \leq 1.0 \) ppt. This "new type of wetland" was not connected to the river mainstem as were connected lakes and did not have the same water quality as true seeps.

This "new type of wetland" was adjacent to the river, but had no apparent connection to the river mainstem. However, water quality measurements strongly suggested that there was exchange with the river despite having no obvious surface connection. Thus, it became very apparent that wetland water quality was directly influenced by the degree of connectivity to the river mainstem, whether above or below ground. The term "pseudo seep" was coined to designate these "new" wetlands. Hereafter, I will categorize the wetlands based on decreasing connectivity to the river - connected lakes (CL), pseudo seeps (PS), and true seeps (TS).

*Water Quality 1997* - Water quality data collected in 1997 during repeated visits to nine wetlands along the LCR are summarized in Table 2. A repeated measures ANOVA table summarizing statistical data analysis of water quality parameters through time and between wetland types comprises Table 3. In Table 3 significant p-values are highlighted in bold.

Temperature within the three wetland classes did not vary significantly over time. However, there was a gradual warming trend observed in all of the wetlands. There was a trend for temperatures to increase from the river mainstem (RM), to CL, PS and TS \((R^2=0.63)\). Mean temperatures ranged from 21.3 to 24.6\(^\circ C\) respectively. There was no
Table 2. Arithmetic mean ± 1 SE (below) of water quality parameters from selected experimental wetlands on LCR, Imperial NWR, Spring 1997.

<table>
<thead>
<tr>
<th>Wetland Name</th>
<th>Numerical Designation</th>
<th>Wetland Classification</th>
<th>Temp °C</th>
<th>S Conductivity μS/cm@25C</th>
<th>Salinity ppt</th>
<th>T-DO % saturation</th>
<th>B-DO % sat</th>
<th>pH</th>
<th>Turbidity NTU</th>
</tr>
</thead>
<tbody>
<tr>
<td>McAllister L.</td>
<td>A61.0</td>
<td>TS</td>
<td>23.4</td>
<td>4940.4</td>
<td>2.68</td>
<td>82</td>
<td>17</td>
<td>8.00</td>
<td>45.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.9</td>
<td>77.4</td>
<td>0.05</td>
<td>14</td>
<td>5</td>
<td>0.07</td>
<td>7.39</td>
</tr>
<tr>
<td>Butler L.</td>
<td>A61.55</td>
<td>TS</td>
<td>24.5</td>
<td>13944.2</td>
<td>8.07</td>
<td>84</td>
<td>11</td>
<td>8.40</td>
<td>53.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>149.0</td>
<td>0.09</td>
<td>10</td>
<td>3</td>
<td>0.03</td>
<td>4.52</td>
</tr>
<tr>
<td>Ag. L. #2</td>
<td>A58.9</td>
<td>TS</td>
<td>25.9</td>
<td>11140.4</td>
<td>6.23</td>
<td>72</td>
<td>10</td>
<td>7.96</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.2</td>
<td>191.7</td>
<td>0.12</td>
<td>15</td>
<td>5</td>
<td>0.04</td>
<td>2.18</td>
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<td>Growout L.</td>
<td>A56.4x</td>
<td>PS</td>
<td>25.2</td>
<td>1923.1</td>
<td>0.96</td>
<td>72</td>
<td>58</td>
<td>7.39</td>
<td>2.28</td>
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<td></td>
<td></td>
<td></td>
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<td>48.0</td>
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<td>4</td>
<td>7</td>
<td>0.04</td>
<td>0.20</td>
</tr>
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<td>Az Seep</td>
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<td>PS</td>
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<td>1175.2</td>
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<td>87</td>
<td>72</td>
<td>7.47</td>
<td>3.37</td>
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<tr>
<td></td>
<td></td>
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<td>6.1</td>
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<td>5</td>
<td>5</td>
<td>0.09</td>
<td>0.38</td>
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<tr>
<td>Clear L.</td>
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<td>PS</td>
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<td>94</td>
<td>68</td>
<td>7.88</td>
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<td></td>
<td></td>
<td></td>
<td>0.8</td>
<td>26.0</td>
<td>0.01</td>
<td>6</td>
<td>6</td>
<td>0.34</td>
<td>0.66</td>
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<td>Long L.</td>
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<td>CL</td>
<td>22.3</td>
<td>1162.6</td>
<td>0.60</td>
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<td>60</td>
<td>7.32</td>
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<td>5</td>
<td>0.07</td>
<td>2.43</td>
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<td>Cabin L.</td>
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<td>CL</td>
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<td>0.59</td>
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<td>4.3</td>
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<td>2</td>
<td>0.06</td>
<td>0.44</td>
</tr>
<tr>
<td>Cable L.</td>
<td>A63.7</td>
<td>CL</td>
<td>24.1</td>
<td>1203.1</td>
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<td>7.8</td>
<td>0.00</td>
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<tr>
<td>River</td>
<td>-----</td>
<td>----</td>
<td>21.3</td>
<td>1124.5</td>
<td>0.58</td>
<td>99</td>
<td>----</td>
<td>7.89</td>
<td>8.83</td>
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<td>1.1</td>
<td>6.1</td>
<td>0.01</td>
<td>3</td>
<td>----</td>
<td>0.06</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Table 3. Results of repeated measures ANOVA for water quality parameters in three wetland types on LNWR. $\lambda = \text{Wilks Lambda}$; df = degrees of freedom; $F = F$ statistic; $P = \text{probability associated with } F$. 

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Table 3. Results of repeated measures ANOVA for water quality parameters in three wetland types on INWR. \( \lambda \) = Wilks Lambda; \( df \) = degrees of freedom; \( F \) = F statistic; \( P \) = probability associated with \( F \).

<table>
<thead>
<tr>
<th>Source</th>
<th>( \lambda )</th>
<th>( df )</th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dissolved Oxygen - Top</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects Effects:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (4 time periods)</td>
<td>0.252</td>
<td>3.4</td>
<td>3.95</td>
<td>0.109</td>
</tr>
<tr>
<td>Time x Wetland Type</td>
<td>0.420</td>
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<td>0.72</td>
<td>0.643</td>
</tr>
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<td>Between Subjects Effects:</td>
<td></td>
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<td>Wetland Type</td>
<td>0.919</td>
<td>2.6</td>
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</tr>
<tr>
<td><strong>Dissolved Oxygen - Bottom</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects Effects:</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (4 time periods)</td>
<td>0.294</td>
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<td>3.21</td>
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</tr>
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<td>Between Subjects Effects &amp; Contrasts:</td>
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<td>Wetland Type</td>
<td>0.064</td>
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<td>43.58</td>
<td>0.0003</td>
</tr>
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<td>0.991</td>
<td>1.6</td>
<td>0.06</td>
<td>0.822</td>
</tr>
<tr>
<td>(CL&amp;PS) vs TS</td>
<td>0.064</td>
<td>1.6</td>
<td>87.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>pH</strong></td>
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<tr>
<td>Within Subjects Effects:</td>
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<td></td>
</tr>
<tr>
<td>Time (4 time periods)</td>
<td>0.059</td>
<td>3.4</td>
<td>21.44</td>
<td>0.006</td>
</tr>
<tr>
<td>Time x Wetland Type</td>
<td>0.191</td>
<td>6.8</td>
<td>1.72</td>
<td>0.234</td>
</tr>
<tr>
<td>Between Subjects Effects &amp; Contrasts:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wetland Type</td>
<td>0.293</td>
<td>2.6</td>
<td>7.23</td>
<td>0.025</td>
</tr>
<tr>
<td>CL vs PS</td>
<td>0.971</td>
<td>1.6</td>
<td>0.18</td>
<td>0.688</td>
</tr>
<tr>
<td>(CL&amp;PS) vs TS</td>
<td>0.296</td>
<td>1.6</td>
<td>14.27</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Standardized S. Conductivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects Effects:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (4 time periods)</td>
<td>0.105</td>
<td>3.4</td>
<td>11.33</td>
<td>0.020</td>
</tr>
<tr>
<td>Time x Wetland Type</td>
<td>0.026</td>
<td>6.8</td>
<td>6.96</td>
<td>0.008</td>
</tr>
<tr>
<td>Between Subjects Effects &amp; Contrasts:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wetland Type</td>
<td>0.869</td>
<td>2.6</td>
<td>31.49</td>
<td>0.0007</td>
</tr>
<tr>
<td>CL vs PS</td>
<td>0.860</td>
<td>1.6</td>
<td>0.974</td>
<td>0.362</td>
</tr>
<tr>
<td>(CL&amp;PS) vs TS</td>
<td>0.088</td>
<td>1.6</td>
<td>62.00</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
### Table 3. Continued - Repeated Measures ANOVA of water quality parameters.

<table>
<thead>
<tr>
<th>Source</th>
<th>λ</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salinity</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Within Subjects Effects:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (4 time periods)</td>
<td>0.262</td>
<td>3.4</td>
<td>3.75</td>
<td>0.117</td>
</tr>
<tr>
<td>Time x Wetland Type</td>
<td>0.080</td>
<td>6.8</td>
<td>3.38</td>
<td>0.057</td>
</tr>
<tr>
<td>Between Subjects Effects &amp; Contrasts:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wetland Type</td>
<td>0.085</td>
<td>2.6</td>
<td>32.11</td>
<td><strong>0.0006</strong></td>
</tr>
<tr>
<td>CL vs PS</td>
<td>0.879</td>
<td>1.6</td>
<td>0.82</td>
<td>0.400</td>
</tr>
<tr>
<td>(CL &amp; PS) vs TS</td>
<td>0.086</td>
<td>1.6</td>
<td>63.39</td>
<td><strong>0.0002</strong></td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects Effects:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (4 time periods)</td>
<td>0.011</td>
<td>3.4</td>
<td>120.80</td>
<td><strong>0.0002</strong></td>
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<tr>
<td>Time x Wetland Type</td>
<td>0.335</td>
<td>6.8</td>
<td>0.97</td>
<td>0.499</td>
</tr>
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<td>Between Subjects Effects:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wetland Type</td>
<td>0.584</td>
<td>2.6</td>
<td>2.14</td>
<td>0.199</td>
</tr>
<tr>
<td><strong>Turbidity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects Effects:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (4 time periods)</td>
<td>0.146</td>
<td>3.4</td>
<td>7.79</td>
<td><strong>0.038</strong></td>
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<tr>
<td>Time x Wetland Type</td>
<td>0.344</td>
<td>6.8</td>
<td>0.94</td>
<td>0.516</td>
</tr>
<tr>
<td>Between Subjects Effects &amp; Contrasts:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wetland Type</td>
<td>0.168</td>
<td>2.6</td>
<td>14.85</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>CL vs PS</td>
<td>0.572</td>
<td>1.6</td>
<td>4.48</td>
<td>0.078</td>
</tr>
<tr>
<td>(CL &amp; PS) vs TS</td>
<td>0.192</td>
<td>1.6</td>
<td>25.21</td>
<td><strong>0.002</strong></td>
</tr>
</tbody>
</table>

1 - 4 time periods equals a total of 12 weeks.
significant difference in mean temperatures between the three wetland types and the river.

Standardized specific conductivity in microseimens per centimeter at 25°C ($\mu$S/cm@25°C) within the three wetland classes did vary significantly over time (Table 3). This significant “time effect” was due to a slight increase in conductivity in TS over the twelve weeks (Figure 4). There were highly significant differences in mean standardized specific conductivity between TS and the other two types of wetlands (Table 3).

Salinity roughly paralleled the results of specific conductivity but with somewhat less significance perhaps due to the less accurate measurements (Table 3). Additionally, there was a strong trend for both conductivity and salinity to increase from the RM, to CL, and PS with a major rise in TS ($R^2 = 0.92$). Mean conductivities ($\mu$S/cm@25°C) ranged from 1124 in the river mainstem and 1165 in connected lakes to 1547 in pseudo seeps and 9144 in true seeps. Mean salinities ranged from 0.58/0.59 ppt for the river and connected lakes to 0.78 ppt for pseudo seeps. Average salinity in true seeps was far higher at 5.12 ppt. Conductivity and salinity increased with decreasing connectivity to the river (Figure 5a & 5b).

Surface and bottom dissolved oxygen (DO % saturation) did not vary significantly over time within the wetland types (Table 3). There were significant differences in mean bottom DO between the wetlands, but not in top DO. Bottom DO differed significantly between TS and the other two wetland types (Table 3). Bottom DO means were 65 and 66 % saturation for CL and PS respectively but were 13 % saturated for TS. Riverine
Figure 4. Weekly mean standardized specific conductivity (μS/cm@25°C) and 1 SE in wetland classes. CL = connected lakes, PS = pseudo seeps, and TS = true seeps.
Figure 5a & 5b. Mean standardized specific conductivity (μS/cm@25°C) (5a), and salinity (5b) of wetland types along the LCR, INWR, 1997. Error bars are 1 SE. RM= river mainstem, CL= connected lake, PS= pseudo seep, and TS= true seep.
bottom DO readings were not taken.

Hydrogen-ion concentrations (pH) did vary significantly over time within wetland types (Table 3). There was a gradual trend for declining pH values over the course of the experiment (Figure 6a). Significant differences in mean pH existed between TS and the other two wetland types (Table 3). Considering only the wetlands, there was a trend of increasing pH with decreasing riverine connectivity (Figure 6b). Mean pH values were 7.89 for the river mainstem, 7.48 for connected lakes, 7.6 for pseudo seeps, and 8.12 for true seeps.

Turbidity (NTU) decreased between time periods 1 and 3, then increased from time period 3 and 4 in all wetlands types over the course of the experiment (Figure 7a) (Table 3). Significant differences in mean turbidity existed between TS and the other two wetland types (Table 3). Mean turbidity values were 3.48 NTU for pseudo seeps, 8.03 NTU for connected lakes, 8.52 NTU for river mainstem, and 30.25 NTU for true seeps (Figure 7b).

A PCA of the entire water quality data set of three wetland types revealed two major patterns of variation in the water quality parameters (Tables 4 & 5). The first principal component (PC1) separated wetlands with low conductivities, higher than average bottom DO, and low turbidities (CL and PS) from wetlands with high conductivities, low bottom DO, and high turbidities (TS). Along the PC1 axis, true seeps ordinated positively and were tightly and uniquely grouped. In contrast CL and PS ordinated negatively, were tightly grouped, and could not be separated from each other
Figure 6a. pH values over time as analyzed in repeated measures ANOVA. TP 1 = time period one etc., CL = connected lake, PS = pseudo seep, and TS = true seep.

Figure 6b. Mean pH and 1 SE of wetland types along the LCR, INWR, 1997. RM = river mainstem, CL = connected lake, PS = pseudo seep, and TS = true seep.
Figure 7a. Turbidity values over time as analyzed in repeated measures ANOVA. TP 1 = time period one, etc., CL = connected lake, PS = pseudo seep, and TS = true seep.

Figure 7b. Mean turbidity and 1 SE of wetland types along the LCR, INWR, 1997. RM = river mainstem, CL = connected lake, PS = pseudo seep, and TS = true seep.
### Table 4. Correlation matrix of original water quality variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>μS/cm@25°C</th>
<th>T-DO %sat</th>
<th>B-DO %sat</th>
<th>pH</th>
<th>Turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>μS/cm@25°C</td>
<td>1.0000</td>
<td>-0.0740</td>
<td>-0.7652</td>
<td>0.5494</td>
<td>0.6800</td>
</tr>
<tr>
<td>T-DO %sat</td>
<td>-0.0740</td>
<td>1.0000</td>
<td>0.3505</td>
<td>0.3307</td>
<td>-0.0222</td>
</tr>
<tr>
<td>B-DO %sat</td>
<td>-0.7652</td>
<td>0.3505</td>
<td>1.0000</td>
<td>-0.2566</td>
<td>-0.5837</td>
</tr>
<tr>
<td>pH</td>
<td>0.5494</td>
<td>0.3307</td>
<td>-0.2566</td>
<td>1.0000</td>
<td>0.5320</td>
</tr>
<tr>
<td>Turbidity</td>
<td>0.6800</td>
<td>-0.0222</td>
<td>-0.5837</td>
<td>0.5320</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

### Table 5. Principal components factor loading table of water quality variables.

<table>
<thead>
<tr>
<th>Principal Comp</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigen Value</td>
<td>2.7230</td>
<td>1.3703</td>
<td>0.3880</td>
<td>0.3647</td>
<td>0.1539</td>
</tr>
<tr>
<td>Percent</td>
<td>54.4607</td>
<td>27.4064</td>
<td>7.7597</td>
<td>7.2948</td>
<td>3.0783</td>
</tr>
<tr>
<td>Cum %</td>
<td>54.4607</td>
<td>81.8672</td>
<td>89.6269</td>
<td>96.9217</td>
<td>100.000</td>
</tr>
<tr>
<td>Eigen Vectors:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uS/cm@25°C</td>
<td><strong>0.56206</strong></td>
<td>-0.0249</td>
<td>-0.26526</td>
<td>-0.28594</td>
<td>0.72893</td>
</tr>
<tr>
<td>T-DO %sat</td>
<td>-0.05330</td>
<td><strong>0.78646</strong></td>
<td>-0.58819</td>
<td>0.16056</td>
<td>-0.08305</td>
</tr>
<tr>
<td>B-DO %sat</td>
<td><strong>-0.49654</strong></td>
<td>0.35877</td>
<td>0.47330</td>
<td>0.13192</td>
<td>0.61913</td>
</tr>
<tr>
<td>pH</td>
<td>0.40911</td>
<td><strong>0.49877</strong></td>
<td>0.54446</td>
<td>-0.45739</td>
<td>-0.27968</td>
</tr>
<tr>
<td>Turbidity</td>
<td><strong>0.51704</strong></td>
<td>0.05808</td>
<td>0.25145</td>
<td>0.81599</td>
<td>0.01490</td>
</tr>
</tbody>
</table>
(Figure 8). PC1 accounted for 54.5% of the variability in water quality data.

On the second principal component (PC2), surface DO and pH loaded strongly. PC2 separated wetlands with relatively higher surface DO (% saturation) and average to lower pH (CL and PS) from wetlands with relatively lower surface DO and higher than average pH (TS). Along the PC2 axis, CL, PS, and TS were relatively poorly separated. CL and PS were more tightly grouped than TS and clustered around the mid-point (0) of the PC2 axis. TS were less tightly grouped but also centered on the axis mid-point (Figure 8). PC2 accounted for approximately 27.4% of the variability within the water quality data. PC1 and PC2 combined accounted for 82% of the variance in the original water quality data.

*Wetland Classification* - Variation in water quality coupled with the degree of connection to the river mainstem strongly suggest that there are three rather than two wetland types along the LCR. Backwaters with obvious connections to the river are basically, *connected lakes (wetlands)*. The term connected lakes is more accurate than backwater lakes because all wetlands are collectively referred to as backwaters in the LCR Valley. Connected lakes have water quality measurements virtually identical to the river. Seep wetlands have no obvious connections to the river and can be divided into two types - true seeps and pseudo seeps. *True seep wetlands* are isolated, have high evaporation rates, relatively higher water temperatures, low bottom dissolved oxygen, relatively high turbidities, and considerably elevated conductivities suggesting that evaporation greatly exceeds subterranean recharge through the river-aquifer system.
Figure 8. Principal Component 1 and 2 for water quality parameters in three wetland types INWR, LCR 1997.
**Pseudo seep wetlands** have water quality measurements resembling river water with minor differences in conductivity, salinity, pH, temperature, and turbidity. Generally, water quality was slightly improved from river water in pseudo seeps. The conductivity in pseudo seeps coupled with no visible surface connection suggest a direct but subsurface connection to the river.

**Selenium in Fish as a Function of Wetland Type**

Nineteen composite fish samples were collected, nine in 1996 and ten in 1997. The samples for 1996 included 6 composites of bluegill, three each from CL and PS, and 3 composites of mosquitofish from TS, one from each of the wetlands. In 1997 the same number and types of samples were collected with the addition of one composite of mosquitofish from Clear Lake, a PS. Bluegills were not found in TS, and mosquitofish were limited and difficult to catch in PS and very scarce in CL. Thus, the comparisons use bluegill for CL and PS, and mosquitofish for TS. I used a Pearson product moment pairwise correlation to aid in the interpretation of whether observed differences in selenium levels are due to wetland type or fish species.

Selenium concentrations between years for individual species were not significantly different for mosquitofish in TS (df=5, t=-1.942, p=0.11) or bluegill in PS (df=4, t=0.81, p=0.46), or for bluegill in CL (df=4, t=-0.45, p=0.68). Arithmetic means (geometric means in parenthesis) (μg/g Se DW) were 1.40 (1.36) ppm DW for mosquitofish and 7.09 (6.98) ppm DW for bluegill combining across years.

However, there were significant differences in Se concentrations between
mosquitofish and bluegill (df = 7, t=-10.33, p<0.0001). A Pearson product-moment pairwise correlation comparing Se concentrations in mosquitofish and bluegill in four wetlands where the species coexist (Lusk 1993) found selenium levels in the two species were positively and significantly correlated in log transformed data (n=8, coef. =0.90, p=0.0026) and in raw data (n=8, coef.=0.82, p=0.012) (Table 6).

There were statistically significant differences in Se concentrations between mosquitofish in TS and bluegills in the other two wetland types (ANOVA, df=8, F=49.21, p=0.0002). Mean Se concentrations in fish by wetland type were: 7.45 (7.43) ppm DW for bluegills in CL, 6.72 (6.56) ppm DW for bluegill in PS, and 1.40 (1.36) ppm DW for mosquitofish in TS (Table 7). Because the Se concentrations in bluegills and mosquitofish are equivalent at a probability of 0.90 in wetlands where they co-occur (Table 6). I interpret the results of the ANOVA test to mean that fish in CL and PS have significantly higher selenium than those in true seeps and that this significant difference is due to wetland type rather than fish species. Basic statistical parameters relative to Se concentrations in bluegill are given in Table 8. The trend was for a decrease in Se concentrations in fish with decreased connectivity to the river mainstem.

Selenium in Crayfish Over Time and as a Function of Wetland Type

Crayfish Samples - There were three practical problems with the crayfish experiment: 1) True Seep water quality, 2) vandals, and 3) general loses. Crayfish did not survive in the TS beyond the first week. Consequently, TS are not represented in our crayfish Se bioaccumulation experiment (Table 9).
Table 6. Combined raw data of fish selenium concentrations (Lusk (1993) and this study) for Pearson product-moment pairwise correlation analysis.

<table>
<thead>
<tr>
<th>Wetland Name</th>
<th>Numerical Designation</th>
<th>Wetland Type</th>
<th>Mosquitofish Se μg/g DW</th>
<th>Bluegill Se μg/g DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butler Lake</td>
<td>A61.55</td>
<td>TS</td>
<td>3.88</td>
<td>2.56</td>
</tr>
<tr>
<td>Island Lake</td>
<td>A65.9</td>
<td>CL</td>
<td>10.11</td>
<td>8.25</td>
</tr>
<tr>
<td>Island Lake</td>
<td></td>
<td></td>
<td>10.95</td>
<td>8.60</td>
</tr>
<tr>
<td>Island Lake</td>
<td></td>
<td></td>
<td>10.13</td>
<td>11.21</td>
</tr>
<tr>
<td>Devils Lake</td>
<td>C62.9</td>
<td>PS*</td>
<td>10.90</td>
<td>8.75</td>
</tr>
<tr>
<td>Devils Lake</td>
<td></td>
<td></td>
<td>7.46</td>
<td>9.53</td>
</tr>
<tr>
<td>Devils Lake</td>
<td></td>
<td></td>
<td>12.49</td>
<td>9.59</td>
</tr>
<tr>
<td>Clear Lake</td>
<td>A62.5</td>
<td>PS</td>
<td>4.90</td>
<td>4.80</td>
</tr>
</tbody>
</table>

*I have no conductivity data from this wetland however, the inlet was silted in on August 1996. Therefore, I assume this is a PS.

Table 7. Arithmetic mean ± SE (geometric mean) and range of Se concentrations (ppm DW) in fish by year and wetland type on INWR, Lower Colorado River.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluegill</td>
<td>7.10</td>
<td>7.83</td>
<td>7.30</td>
<td>6.13</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>±0.64</td>
<td>±1.21</td>
<td>±0.95</td>
<td>±1.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(7.04)</td>
<td>(7.64)</td>
<td>(7.17)</td>
<td>(5.92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*N</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Range</td>
<td>6.0-8.2</td>
<td>5.8-10.0</td>
<td>5.6-8.9</td>
<td>4.0-7.0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Mosquitofish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.09</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.21</td>
<td>±0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.06)</td>
<td>(2.18)</td>
</tr>
<tr>
<td>*N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8-1.5</td>
<td>1.4-2.2</td>
</tr>
</tbody>
</table>

*Combined Yrs. | 7.45 | 6.72 | 1.40 |
|               | ±0.51 | ±0.97 | ±0.23 |
|               | (7.43) | (6.56) | (1.36) |

*N=composite samples of 3 fish, one from each of the three lakes in each wetland class.
Table 8. Arithmetic Se concentrations in bluegill from the LCR, INWR, 1996 and 1997.

<table>
<thead>
<tr>
<th>Wetland Type</th>
<th>Mean (µg/g)</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>Upper 95% C.I</th>
<th>Lower 95% C.I</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>6.72</td>
<td>1.70</td>
<td>0.69</td>
<td>8.50</td>
<td>4.94</td>
<td>2.88</td>
</tr>
<tr>
<td>CL</td>
<td>7.45</td>
<td>1.55</td>
<td>0.63</td>
<td>9.10</td>
<td>5.84</td>
<td>2.41</td>
</tr>
</tbody>
</table>

Table 9. Crayfish used in the Se bioaccumulation experiment. LCR, INWR, 1997.

<table>
<thead>
<tr>
<th></th>
<th>CL</th>
<th>PS</th>
<th>TS</th>
<th>*Baseline</th>
<th>Total Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Individuals</td>
<td>63</td>
<td>63</td>
<td>63</td>
<td>5</td>
<td>194</td>
</tr>
<tr>
<td>Losses</td>
<td>3</td>
<td>0</td>
<td>63</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>Samples Analyzed</td>
<td>60</td>
<td>63</td>
<td>0</td>
<td>5</td>
<td>128</td>
</tr>
</tbody>
</table>

*Baseline samples were composites of 3 crayfish each, analyzed to establish the Se content (baseline Se levels) of crayfish prior to stocking in the wetlands.

by confinement and competition for available resources, we compared crayfish weights
Crayfish persisted in CL and PS. However, it took up to 13 weeks to collect 7 weeks of data from all of the cages. Three cages, 1 in Cable Lake and 2 in Long Lake, were repeatedly vandalized, and other cages experienced infrequent unexplained losses. These losses required that I replace the lost crayfish and “start the experiment over” for replacement animals in specific cages. Cages were moved to deeper locations in an effort to minimize vandalism.

**Crayfish Weights** - To assess whether crayfish might have been adversely affected by confinement and competition for available resources, I compared crayfish weights before and after the experiment. Crayfish grew significantly larger from their mean starting weights in both CL (df=24, t=-3.06, p=0.005) and PS (df=16, t=-3.93, p=0.0006). However, crayfish in PS gained an average of 4g, those in CL only gained 1g (Table 10). This difference in weight gain in the two types of wetlands was marginally statistically significant (df=39, t=-1.81, p=0.08).

**Bioaccumulation Experiment** - Crayfish began the selenium bioaccumulation experiment with an average (arithmetic mean) selenium concentration of $0.54 \pm 0.06 \mu g/g$ DW. During weeks 1 and 2 the crayfish rapidly took up selenium (Figure 9a). The peak in selenium levels occurred at week 3 for pseudo seeps ($2.18 \pm 0.16 \mu g/g$ DW), and week 6 ($3.21 \mu g/g$ DW) for connected lakes. At week 3, Se levels in PS exceeded those of CL for the only time during the experiment. Following week 3, Se levels continued to increase in CL, whereas Se levels declined in pseudo seeps (Figure 9a). Figure 9b illustrates weekly geometric means in comparison to arithmetic mean Se concentrations.
Figure 9a. Weekly arithmetic mean and 1 SE of Se concentrations (ppm DW) in experimental crayfish as analyzed in repeated measures ANOVA. CL = connected lakes and PS = pseudo seeps.
Figure 9b. Weekly arithmetic mean and geometric mean of Se concentrations (ppm DW) in experimental crayfish as analyzed in repeated measures ANOVA. CL(GM) = connected lakes (geometric mean), PS (GM) = pseudo seep (geometric mean), CL (AM) = connected lakes (arithmetic mean), and PS (AM) = pseudo seeps (arithmetic mean).
Table 10. Arithmetic mean crayfish weights (g) ± SE before and after the Se bioaccumulation experiment by wetland type LCR, INWR, 1997.

<table>
<thead>
<tr>
<th>W. Type</th>
<th>Before Wt</th>
<th>n</th>
<th>After Wt</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>13.09±0.43</td>
<td>63</td>
<td>14.12±0.54</td>
<td>60</td>
</tr>
<tr>
<td>PS</td>
<td>12.42±0.44</td>
<td>63</td>
<td>16.54±0.67</td>
<td>63</td>
</tr>
</tbody>
</table>

Table 11. Results of repeated-measures ANOVA of Selenium levels in crayfish held experimentally in replicate cages in three wetlands each of two wetland types, connected lakes (CL) and pseudo seeps (PS), on INWR.

<table>
<thead>
<tr>
<th>Source</th>
<th>λ</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within - Subjects Effects:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (7 weeks)</td>
<td>0.073</td>
<td>6.6</td>
<td>12.74</td>
<td>0.003</td>
</tr>
<tr>
<td>Time x Wetland[wetland type]</td>
<td>0.154</td>
<td>24.22</td>
<td>0.65</td>
<td>0.845</td>
</tr>
<tr>
<td>Time x Wetland Type</td>
<td>0.432</td>
<td>6.6</td>
<td>1.32</td>
<td>0.374</td>
</tr>
<tr>
<td><strong>Between - Subjects Effects:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wetland Type</td>
<td>0.694</td>
<td>1.11</td>
<td>4.84</td>
<td>0.050</td>
</tr>
<tr>
<td>Wetland[wetland type]</td>
<td>0.424</td>
<td>4.11</td>
<td>3.73</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Between - Subjects Contrasts:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connected Lakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL vs (CaL &amp; CbL)¹</td>
<td>0.632</td>
<td>1.11</td>
<td>6.38</td>
<td>0.028</td>
</tr>
<tr>
<td>Pseudo Seep Wetlands</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS - CL vs (GP &amp; A62.1)²</td>
<td>0.589</td>
<td>1.11</td>
<td>7.67</td>
<td>0.018</td>
</tr>
</tbody>
</table>

1 - LL = Long Lake, CaL = Cabin Lake, and CbL = Cable Lake.
2 - CL = Clear Lake, and GP = Growout Pond
in crayfish. The bioaccumulation of selenium over time in experimental crayfish is reflected in the statistically significant “time effect” (p = 0.003) in the repeated-measures ANOVA (Table 11).

The between-subjects test for the nesting effect in the experimental design (specific wetlands being nested within a wetland type) was statistically significant at p = 0.037 (Table 11). Between-subjects contrasts revealed that within both wetland types, one of the wetlands differed from the other two replicates in the level of selenium bioaccumulation in crayfish. Crayfish in Long Lake (a connected lake) and those in Clear Lake (a pseudo seep) had lower selenium levels than other wetlands in their respective wetland types (p = 0.028 and p = 0.018 respectively).

Most importantly in terms of the objectives of this study, selenium levels in crayfish were found to be significantly higher on average in connected lakes than in pseudo seeps (p = 0.05, Table 11, wetland type effect). These differences in the levels of selenium bioaccumulation as a function of wetland type (and hydrologic connection with the river) are particularly apparent in weeks 4-7 (Figure 9). Thus, despite variation between replicates within a wetland type, crayfish in connected lakes still accumulated more selenium on average than did those in pseudo seeps (significant “wetland type effect”, Table 11). Arithmetic means, SE, geometric means in parenthesis, and the range of Se concentrations in experimental crayfish by week are given in (Table 12). Table 13 summarizes basic statistical parameters for the same data averaged over time.
Table 12. Weekly arithmetic mean ± SE (geometric mean) and range of Se concentrations (ppm DW) in experimental crayfish by week and wetland type LCR, INWR, 1997.

<table>
<thead>
<tr>
<th>Type</th>
<th>Wk 0</th>
<th>Wk 1</th>
<th>Wk 2</th>
<th>Wk 3</th>
<th>Wk 4</th>
<th>Wk 5</th>
<th>Wk 6</th>
<th>Wk 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>1.46</td>
<td>1.70</td>
<td>1.82</td>
<td>2.73</td>
<td>2.59</td>
<td>3.21</td>
<td>2.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.18</td>
<td>±0.26</td>
<td>±0.29</td>
<td>±0.41</td>
<td>±0.35</td>
<td>±0.69</td>
<td>±0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.42)</td>
<td>(1.66)</td>
<td>(1.66)</td>
<td>(2.65)</td>
<td>(2.40)</td>
<td>(2.79)</td>
<td>(1.94)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>*5</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Range</td>
<td>0.3-0.7</td>
<td>0.9-2.6</td>
<td>1.0-3.3</td>
<td>0.7-3.1</td>
<td>1.4-4.7</td>
<td>1.1-3.8</td>
<td>1.5-6.8</td>
<td>0.8-6.0</td>
</tr>
<tr>
<td>PS</td>
<td>1.25</td>
<td>1.72</td>
<td>2.18</td>
<td>2.06</td>
<td>2.09</td>
<td>1.85</td>
<td>1.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.13</td>
<td>±0.24</td>
<td>±0.16</td>
<td>±0.37</td>
<td>±0.20</td>
<td>±0.37</td>
<td>±0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.19)</td>
<td>(1.59)</td>
<td>(2.13)</td>
<td>(1.73)</td>
<td>(2.00)</td>
<td>(1.59)</td>
<td>(1.47)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>*5</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Range</td>
<td>0.3-0.7</td>
<td>0.6-1.6</td>
<td>0.9-3.0</td>
<td>1.6-3.1</td>
<td>0.4-3.7</td>
<td>1.1-3.1</td>
<td>0.8-4.3</td>
<td>0.5-2.5</td>
</tr>
</tbody>
</table>
*Week 0=baseline composite samples.


<table>
<thead>
<tr>
<th>Wetland Type</th>
<th>Mean μg/g</th>
<th>N</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% C I Upper</th>
<th>95% C I Lower</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>1.83 (1.65)</td>
<td>63</td>
<td>0.81</td>
<td>0.10</td>
<td>2.04</td>
<td>1.63</td>
<td>0.66</td>
</tr>
<tr>
<td>CL</td>
<td>2.25 (1.95)</td>
<td>60</td>
<td>1.31</td>
<td>0.17</td>
<td>2.60</td>
<td>1.92</td>
<td>1.73</td>
</tr>
</tbody>
</table>
DISCUSSION

LCR Wetland Classification

*Connected Lakes* - The river is the main source of water for all backwaters along the LCR (Metzger et al. 1973; Wilson and Owen-Joyce 1994). In the LCR from the east end of Lake Mead to Laguna Dam, the river and the underlying and adjacent river aquifer form a complex, hydraulically connected ground-water and surface-water flow system. In this system the Colorado River is the source for virtually all recharge to the river aquifer in the LCR (Wilson and Owen-Joyce 1994).

There are three wetland types, (connected lakes, pseudo seeps, and true seeps) rather than the two (seep lakes and backwater lakes) previously described (Lusk 1993, Martinez 1994). Selenium burdens and water quality in connected lakes are virtually identical to those in the river but there is considerable variation among connected lakes. Characteristics of CL are influenced first by river flow but also by the number, location, and size of connection(s) to the river. Thus, CL are more dynamic than other wetland types primarily due to increased water flow.

Previous authors have recognized considerable variation in the characteristics of backwaters but failed to categorize this variation. Saiki (1976) and Kennedy (1979) found that diurnal water levels in backwaters fluctuated according to the amount of contact with the river. Kennedy (1979) described No Name Lake as a backwater lake. However, water levels only fluctuated 5 cm when diurnal fluctuations in the river were 120 cm. Under my classification, No Name Lake would be a PS. Saiki (1976) reported
similar (≤15 cm) diurnal water level fluctuations for Deer Island Lake. Deer Island Lake is apparently also a PS where a culvert has been installed (Saiki 1976).

Connected lakes, true seeps and pseudo seeps differ in nutrient loads. Kennedy (1979) found that high flushing rates prevented nutrient loading of sediments in connected lakes. He determined that in backwaters with high flushing rates, nutrients were resuspended in the water column and lost to the river by bioturbation or agitation of sediments by wind and water, and by wind and water movement of floating mats of plants and epibenthic algae out of the backwater and into the river. Additionally, the feeding activities by coots and moorhens breaks apart mats of vegetation and makes plants available for export to the river (per. observation).

Kennedy (1979) reported that the smaller the entrance to the backwaters, the lower the fish diversity. He attributed low fish diversity to low flushing rates, warmer summer temperatures and cooler winter temperatures. Apparently, shad are absent or only present at low densities in backwater that are closed to the river (Ponder 1975, Kennedy 1979). In general, CL’s that are the most open to the river are more reminiscent of riverine (lotic) systems and those least open to the river exhibit more lentic or lake-like conditions.

Water retention times in the wetlands in my study, though not directly measured, can be inferred from a consideration of standardized conductivity in combination with information from previous research. Water retention times varied from rapid turnover and short retention times (days) in CL, to primarily ground water inputs from the river...
aquifer system with low turnover and long retention times (months to a year or more) for true seeps. Pseudo seeps are intermediate in retention times and turnover and receive river water via seepage through the levee, through beaver openings running from the river to the backwater through the levee, and perhaps via ground water inputs through the soil matrix. Hence, turnover and retention time for PS appears to be in the order of weeks to several months. Kennedy (1979) estimated that CL, A-7, A-7B, and A-10 had flushing rates, of 5.7, 3.7, and 18.5 days respectively, and Saiki (1976) estimated Deer Island Lake flushed every 244.4 days.

The size and location of connections to the river affect public use of CL but do not seem to affect Se burdens in fish. Previous studies (Lusk 1993, Martinez 1994) and my data suggest that CL are virtually identical to the river mainstem in Se burdens in the biota. Long Lake was the only observed exception to this trend in this study. Yuma Wash drains surface water into Long Lake (Figure 3). During my study there was a major spring thunderstorm (first week in June 1997) which provided much runoff to Long Lake. I attribute the relatively lower Se concentrations in crayfish from Long Lake (Table 10) to dilution of river water by surface runoff from Yuma wash.

There are several reasons why biota from connected backwaters contain the highest levels of Se. Sediments ≤ 63 micrometers in diameter and with high organic content may act as a sink for Se (Radtke et al. 1988). In general CL are lacustrine as compared to the river and have fine-grained bottom sediments with a larger percentage of organic material than the sandy riverine sediments (Radtke et al. 1988). Under conditions
of periodic inflow, fine-grained sediments can reduce Se concentrations in overlying waters (Besser et al. 1989) and make the system sensitive to Se accumulation (Lemly and Smith 1987). Accumulation of selenium in sediments could result in a reservoir of Se that may be remobilized by biological activity or changes in physical or chemical conditions. However, because these connected backwaters receive constant fresh river water inflows, waterborne Se is never really lowered as it is in systems with periodic inflow. In addition, constant perturbation of sediments by wind and animals may keep Se levels relatively high in these CL systems.

**True Seeps** - True seep (TS) wetlands are the most hydrologically isolated from the river of any floodplain wetlands in the LCR Valley. True seeps have high evaporation rates, high water temperatures, low dissolved oxygen, high turbidities, and considerably elevated conductivities suggesting limited exchange with the river. Kennedy (1979) found a few areas where DO was depressed near the bottom sediments and attributed these low oxygen levels to accumulations of organic detritus and/or poor water exchange with the river. Hallock (1973) found that conductivity increased in the more isolated backwaters and that conductivities in lakes not connected to the river reached 40,000 micromhos/cm. In smaller TS, evaporation often exceeds subterranean recharge and these wetlands dry up during the summer. e.g., A61.5 located between Butler and McAllister Lakes (Figure 3). In general. TS are farther from the river than the other wetland types, although at least two (C63.8 and C64.1) were adjacent to the river mainstem.
True seeps were thought to be recharged by ground water or storm runoff (Lusk 1993). Incidental observations of water levels in TS confirm an indirect connection to the river via ground water contained within the river-aquifer system; levels rise and fall as a function of river water level with a time lag of approximately 1-2 days (pers. observation). The location of a wetland in the floodplain may explain the apparent relationship between water levels and water quality and those of the river. The distance of a TS from the river mainstem combined with the permeability of the intervening alluvium to groundwater flow are likely the most important factors affecting the conductivity of water in TS wetlands.

Ground water levels as measured in monitoring wells in Parker Valley (Parker-Blythe-Cibola Area) beginning approximately 30 km upriver from the northern border of INWR, have lower fluctuations the greater the distance from the river. Water levels in observation wells 30 to 60 m from the river fluctuated about half as much as the river and lagged 3-5 hrs behind river stages. Ground water 300 m from the river fluctuated about one-twentieth the daily amplitude of the river and lagged 6-8 hours behind river changes (Metzger et al. 1973). Therefore, water levels in TS that are a considerable distances from the river, still fluctuate in relation to water levels in the river.

Selenium concentrations in biota from TS are consistently lower than levels in other wetland types (Lusk 1993, Martinez 1994, and this study). However, few organisms seem to survive in these seeps, although Kennedy (1979) found that reduced DO did not necessarily mean reduced numbers of invertebrates. In TS larger fish species
were not found and crayfish did not survive the bioaccumulation experiment. However, an important benefit of TS in addition to food items having lower Se concentrations, is the site fidelity of certain aquatic birds and their broods (Martinez 1993).

*Pseudo Seeps* - Water quality in PS wetlands closely resembled that in the river and in connected lakes. There were, however, minor increases in conductivity, temperature, and salinity and decreases in turbidity, pH, and Se burdens. Although there is no obvious surface connection between PS and the river, there is exchange with the river. Water appears to pass directly from the river mainstem through the levees separating PS from the river, but this source is perhaps amplified by the river-aquifer recharge. Kennedy (1979) in his assessment of No Name Lake (a PS in my classification) concluded that water must seep through the levee between No Name Lake and the river. In addition, there are beaver tunnels directly linking some PS with the river (per. observation). Passage of water through beaver and perhaps muskrat burrows may significantly augment the recharge of certain pseudo seeps, e.g., wetland A62.1 and Clear Lake (Figure 3).

In general, backwater wetlands provide more stable bottom substrates and are therefore more productive than the river (Kennedy 1979). Bell-McCaulou (1993) found Asiatic clams (*Corbicula fluminea*) were significantly larger in backwater sites than in river sites and concluded that warmer water, lower flow rates, and higher phytoplankton production were possible explanations. In the present study, pseudo seeps had the highest biological diversity and water quality of any of the backwater wetlands. Kennedy (1979)
determined that Deer Island Lake (a PS) had the most diverse assemblage of benthic invertebrates, and invertebrates associated with plants of the lakes he and Saiki (1976) studied. He attributed this increased diversity to low flushing rates. This hypothesis of low flushing rates positively affecting productivity and diversity is supported in the work by Brook and Woodard (1956) and Dickman (1969).

Selenium concentrations in crayfish and fish from PS were consistently below those in connected lakes. Previously, researchers have made no distinction between pseudo seeps and connected lakes. Therefore the dynamics of Se in PS is not well understood and is in need of additional research. Perhaps the crayfish Se bioaccumulation experiment needs to be extended over a longer period or compared between seasons. However, one can formulate hypotheses and suggest how selenium functions in PS by comparing conditions and Se levels in biota from the better known wetland types (CL and TS).

Tools Required for Wetland Differentiation - Conductivity, dissolved oxygen, pH, and turbidity are the most useful parameters for distinguishing between wetland types. Conductivity, the single most definitive variable for practical field use, readily separates the two wetland types (PS and TS) with no visible riverine connection. True seeps had conductivities in excess of 3000 $\mu$S/cm whereas conductivity in PS closely resembles that of river water and connected lakes. There is generally no difficulty in classifying CL’s since they are directly accessible via boat from the river.

Use of my classifications (CL, PS, and TS) in addition to the system proposed by
Holden et al. (1986), which uses a letter code to denote the State and a number to denote river mile, will facilitate work along the LCR. These two classification systems in combination incorporate many of the observations by Saiki (1976), Kennedy (1979), and Ohmart et al. (1988). They recognize that wildlife values increase with decreased public use, that vegetation within these backwaters is influenced by water quality parameters which in turn are influenced by wetland characteristics, and lastly, that seepage across levees or river-aquifer sources maintains water levels in some wetlands.

My data do not suggest that flushing backwaters or connecting backwaters to the river maximizes aquatic productivity or reduces selenium in biota from backwaters. In fact, Villegas (1997) found that flow-through of waters at Cibola Lake on Cibola NWR did not lower Se levels nor did it improve water quality as measured by conductivity, pH, temperature, and alkalinity. Importantly, the present study supports the hypothesis that backwaters with intermediate water turnover (pseudo seeps) are the most productive, have the highest water quality, and lower Se levels in crayfish as compared to connected lakes.

**Selenium in Biota**

*Background* - Maier and Knight (1994) report a range of 0.5-2.0 ppm Se in invertebrates for national background concentrations. In a Se-normal aquatic environments (i.e., one with naturally occurring Se) in Colorado, Birkner (1978) reported Se concentration in invertebrates averaged 2.3-4.2 ppm Se. In the San Joaquin River system crayfish averaged 0.5-0.9 ppm Se at sites with ≤1.0 ppb waterborne Se (Saiki et
al. 1993). At Se-normal sites of the Lower Colorado River, crayfish contained 0.6-2.5 ppm Se and averaged 1.5 ppm (Welsh and Maughan 1994). National monitoring programs have revealed that most species of fish average less than 4 ppm Se in whole body samples (Schmitt and Braumbaugh 1990). At several Se-normal sites (≤1 ppb Se) in the San Joaquin River of California, Saiki et al. (1993) found mosquitofish, bluegill, and bass usually averaged ≤2 ppm whole body Se. Sunfish sampled at confirmed Se-normal sites in the Lower Colorado River averaged 1.6-2.4 ppm whole body Se (Welsh and Maughan 1994). Reproductive failure of sensitive fish occurs at Se levels as low as 7-10 ppm in gonads/eggs (Lemly 1993a). Dietary exposures as low as 3-8 ppm Se (organic) have been demonstrated to impair normal juvenile survival and/or development in salmonids (Hamilton et al. 1990) and centrarchids (Cleveland et al. 1993, Lemly 1993a).

Se concentrations of concern for protection of fish and wildlife have been suggested by Maier and Knight (1994) (Table 14). Safe concentrations have not been associated with adverse effects in fish and wildlife whereas toxic exposures have been associated with adverse effects (Maier and Knight 1994).

<table>
<thead>
<tr>
<th></th>
<th>Safe</th>
<th>Toxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>&lt;2.0 µg/l</td>
<td>&gt;2.7 µg/l</td>
</tr>
<tr>
<td>Dietary</td>
<td>&lt;3.0 µg/g</td>
<td>&gt;4.0 µg/g dry wt.</td>
</tr>
<tr>
<td>Tissue</td>
<td>&lt;3.0 µg/g</td>
<td>&gt;4.5 µg/g dry wt.</td>
</tr>
</tbody>
</table>

Lemly (1993b) recommends the following tissue levels of concern for the overall
health and reproductive vigor of freshwater fish: whole-body 4 μg/g and ovary and eggs 10 μg/g, 3 μg/g as the toxic threshold for dietary Se. and 2 μg/l for waterborne Se concentrations to be considered highly hazardous to the health and long-term survival of fish and wildlife. However, it should be noted that for these levels of concern (Lemly 1993b; Maier and Knight 1994), it was not specifically indicated whether the values reflected raw data (arithmetic values) or logarithm transformation (geometric means (values)).

LCR Fish & Crayfish - Selenium concentrations arithmetic means (geometric means) for individual species were higher for bluegill (7.09 (6.98) ppm DW Se) than for mosquito fish (1.40 (1.36) ppm DW Se). Selenium levels in bluegills exceed levels at which adverse effects are known to occur (≥4.5 μg/g).

Se concentrations generally decrease in fish living in backwaters with decreased connectivity to the river mainstem. Se concentrations were significantly lower in biota from TS than those in other wetland types. Arithmetic means (geometric means) for biota in each type of wetland were: CL 7.45 (7.43) ppm DW Se (bluegill), PS 6.72 (6.56) ppm DW Se (bluegill), and TS 1.40 (1.36) ppm DW (mosquitofish). These data meet or exceed the adverse effect criteria for fish (bluegill and mosquitofish) as combined food items, and for whole-body bluegill, but not for mosquitofish from true seeps.

Baseline Se concentrations (0.54 μg/g) in crayfish used in the bioaccumulation experiment were well within tolerance levels listed by Maier and Knight (1994) for national background concentrations. Crayfish in CL and PS essentially paralleled each
other in the levels of Se uptake per week. Arithmetic means (geometric means) peaked in week 3 at 2.18 (2.13) ppm DW Se for PS and in week 6 at 3.21 (2.79) ppm DW Se for CL over the seven week period. These high values (3.21 (2.79) \mu g/g and 2.17 (2.13) \mu g/g Se DW) however, fall within the safe level of (<3.0 \mu g/g DW) for dietary items.

Selenium decreased with decreased connectivity to the river mainstem. The difference in Se concentrations between crayfish in CL versus PS was statistically significant (Table 11), but requires further study to determine if such differences are biologically significant.

However, there are reasons to suggest that these differences may, in fact, be biologically significant. First, my estimates of selenium levels in crayfish in both wetland types may be conservative for two reasons: 1) higher than usual water levels likely lessened the distinctions between wetland types by forcing more water into seep wetlands through the river aquifer system; and 2) some research suggests that selenium levels in biota in summer may exceed those is spring (Saiki and Lowe 1987). Secondly, based on the very small threshold between selenium levels considered safe in biota versus those considered potentially detrimental (Maier and Knight 1994: Table 14, this document) one can argue that pseudo seep wetlands are a relatively safer haven for resident biota in terms of river-borne selenium than are connected lakes, even if only by a small amount. Finally, the fact that caged crayfish gained 4 times as much weight in pseudo seeps than in connected lakes (4 vs 1 gram: individual on average) further suggests that there are real ecological differences in the two wetland types.
Other studies on INWR have reported Se concentrations in free-ranging crayfish from 1.5 to 35.8 µg/g DW, with a geometric mean of 7.7 µg/g (Lusk 1993). It is possible that the crayfish in this study were limited to feeding primarily on detritus and did not have access to the same full range of available biota that they would have had they been free-ranging. It is also possible that past studies conducted during the summer and this study completed during the spring reflect seasonal differences in Se levels.

The connectivity of wetlands to the river mainstem strongly affects water quality, and increased water quality appears to positively affect subjectively-assessed biological diversity. Selenium concentrations in fish and crayfish are also affected by connectivity to the river. In the LCR, water quality and Se levels in biota vary with the degree of connectivity to the river, e.g., TS have the lowest Se levels and lowest water quality, PS have intermediate Se levels and the highest water quality, and CL the highest Se concentrations and intermediate water quality.

**Potential Factors Affecting Data Variation** - Weather during the course of the selenium bioaccumulation experiment was consistently moderate and Spring-like. Additionally, the repeated measures analysis of water quality parameters strongly suggested that changes in water quality over the course of the experiment were modest. Therefore, I assumed that the 5 week difference in actual start and finish for some crayfish in this experiment was not likely a factor confounding the Se bioaccumulation experiment. Lemly (1985b) reports significant within-year fluctuation in waterborne Se concentrations and (Saiki and Lowe 1987) report of seasonal fluctuation in Se
concentrations in biota. Lemly observed highest concentrations during mid to late summer and lowest concentrations during mid-winter to early-spring, and Saiki and Lowe observed highest Se concentrations in biota during the summer and lowest concentrations during the spring. This bioaccumulation experiment in the LCR was confined entirely to spring so that seasonal differences would not confound the data.

**General Management Strategies**

*Past Management* - Beland (1952) concluded that river channel dredging significantly decreased the value of sport fisheries in the LCR. He concluded that draining adjoining backwaters, eliminating riparian vegetation, eliminating eddies and holes in the river mainstem, increasing turbidity, increasing bank erosion and reducing spawning areas caused the decline in fisheries.

He concluded, “Since little can be undertaken in this area to rectify the losses to the fishery, it is recommended that the Bureau of Reclamation compensate for damages by improving fishing conditions in other portions of the river when the need arises, wherever feasible, and that dredging operations be modified so that maximum area of lake and slough habitat remains open to the river.”

*Current Management Considerations* - The long-term maintenance of wetlands along the LCR is a high priority with the Fish and Wildlife Service. However, there are several types of wetlands along the LCR and each wetland type contributes to the larger ecosystem. These wetlands differ in both water quality and Se levels in the biota. It is also likely that these wetlands occur on a continuum bounded by CL on one end and TS
on the other. Further, individual wetlands may not be permanent members of a given wetland type. Rather, wetlands may change categories, as for example a connected lake may become a pseudo seep when emergent vegetation grows across an opening formerly connecting the wetland directly to the river. Maintaining the current diversity of wetlands provides a variety of habitats and water quality options.

Management proposals, however, are to open selected PS and TS to the river to improve sport fisheries and water quality. Opening seep wetlands might improve sport fisheries and standard water quality parameters in specific cases, but would also mobilize Se that is currently trapped in the sediment, and increase exposure of wetland biota to selenium. Selenium exposure can result from two factors associated with opening up wetlands; disturbance of sediments and increased hydrological connection with the Colorado River.

Currently most public use takes place in connected lakes. These wetlands also make up the single largest category, approximately (41%), of wetlands along the LCR on Imperial National Wildlife Refuge. Under current conditions, natural physical barriers "manage public access" without written regulations and enforcement. Public access would be very difficult to manage if all wetlands were opened to the river. Natural barriers also protect some areas for wildlife.
CONCLUSIONS

My results indicate that there are three wetland types in the LCR: connected lakes, pseudo seeps, and true seeps. Each of the wetlands have unique water quality features and ecological functions based on the degree of connectivity to the river and Se levels in biota. Selenium levels in bluegill in CL and PS exceed those that are known to cause adverse effects. Specifically, whole-body Se concentrations in bluegill exceed levels known to impair reproduction and cause population declines. Selenium levels in mosquitofish in TS are below levels known to cause adverse effects. Similarly, mean Se concentrations for crayfish held experimentally in cages in CL and PS fall below threshold levels for dietary food items. The model of wetland function in the LCR derived from the present study indicates that lowered water quality (increased conductivity) and relatively low Se concentrations in biota are tied to decreased connectivity to the river mainstem. Thus my results support the hypothesis that the process of Se bioaccumulation is fundamentally different in the three types of wetlands.

There were no obvious impacts of elevated Se levels on aquatic biota in wetlands along the LCR. However, none of the selenium studies to date has been specifically designed to quantify population-level effects of selenium. Welsh (1992) found little evidence that elevated selenium levels had any population level effects in the fish on Cibola National Wildlife Refuge. However, he did find that selenium may be one of a suite of factors affecting the overall abundance of fish. Additionally, waterborne Se levels and/or Se levels in biota may vary seasonally or over time (Lemly 1985b, Saiki and
Lowe 1987, Allen 1991, Velinsky and Cutter 1991, and Villegas 1997). Metabolic stress is known to increase the susceptibility to Se poisoning in birds (Heinz and Fitzgerald 1993) and fish (Sorsenson 1991, Lemly 1993c). Subchronic levels of Se exposure is known to increase the susceptibility to otherwise benign pathogens due to Se-induced immune dysfunction (Fairbrother and Fowles 1990). In the LCR selenium may be part of a suite of factors that explain observed summer mortality of juvenile fish (per. observation).

Additionally, Se in certain situations and/or chemical species of Se can be greatly accumulated by aquatic invertebrates and some fish with no apparent ill effects. Examples of this phenomenon include algae (Kiffney and Knight 1990), daphids (Besser et al. 1993), and fish (Ogle and Knight 1989). However, toxic effects may not be related to absolute body concentrations, but may be apparent only when the rate of uptake of contaminants (Se) exceeds the rates of physiological/biochemical detoxification and/or excretion (Rainbow 1996). All studies of selenium to date in the LCR have measured absolute body concentration.

The dynamics of total dissolved Se (Skorupa et al. 1996) in the three wetland types requires additional study in the LCR. Sampling of gravid ovarian tissue, larval stages or ichthyoplankton of bluegill and other species would help to determine whether there are actual impacts of elevated Se levels on the reproduction of biota from backwater wetlands (Lemly 1993b, 1997b, Skorupa et al. 1996). Rates of wetland loss/persistence need to be more thoroughly addressed. There is a need for quantification of wetland
losses in the LCR and not merely a perception of such losses.

Wetlands can act as natural sinks for the removal of water column contaminants (Allen 1991, Wrigley and Toerien 1988). Selenium is thought to be removed from solution and sequestered in the sediments. Estimates suggest that up to 90% of the total Se in an aquatic system may be in the upper centimeters of sediment and detritus (Lemly and Smith 1987). However, there is a need to distinguish between competing hypothesis for lower Se levels in biota in seep wetlands. Is less Se reaching these seep wetlands? Is selenium naturally present in Colorado River water being removed from the water in the aquifer by soil sorption or microbiological processes? Additionally, do the roots of riparian vegetation, i.e., tamarisk, remove selenium from the soil solution thereby preventing less selenium from reaching seep wetlands?

Biomethylation of Se is considered an important process by which some wetlands rid themselves of selenium (Cooke 1985, Cooke and Bruland 1987, Calderone et al. 1990, Thompson-Eagle and Frankenberger 1989, 1990, and 1991, Masscheleyn and Patrick 1993, Fatoki 1997). Is biomethylation causing Se to be lost to the atmosphere rather than entering wetland biota particularly in seep wetlands? Additionally, it is thought that increased biodiversity and enhanced wetland productivity may also play a role in reducing the effects of selenium (Rudd and Turner 1983a, 1983b, Lemly and Smith 1987, Skorupa et al. 1996). Is enhanced productivity resulting in lower Se per individual, particularly in pseudo seep wetlands?

Decreases in water quality and Se concentrations are clearly tied to decreased
connectivity to the river mainstem. Connecting true seeps to the river mainstem will increase water quality and improve sport fisheries, but will also increase Se levels in the biota in these wetlands. Connecting pseudo seeps to the main river will probably increase Se levels in biota only marginally. However, even a marginal increase may be biologically significant because the difference between safe and potentially toxic levels is so small (Table 14). Additionally, connecting pseudo seeps to the main river may reduce wetland productivity, wetland biodiversity and perhaps ecological processes that dissipate selenium.

Selenium concentrations in several wetlands in the LCR are at toxicity thresholds for sensitive species and Se inputs from any source should be reduced to minimize the potential for Se-induced impacts. Historic water flows, specifically scouring floods, which presumably periodically reduced Se levels, are not likely to be restored in the LCR. Almost all the water in the river has been appropriated and use is tightly regulated. More water for wetlands means less for agriculture and municipal use. Human water demands are pushing nature’s hydrological system to the limit in the LCR and change will not occur easily, if at all. In light of the present situation, serious thought should be given to maintaining the current diversity of wetlands until we better understand their respective functions.
LITERATURE CITED


Lemly, A.D. 1993c. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology 27:133-158.


