

THE CHRONIC TOXICITY OF DIETARY AND WATERBORNE SELENIUM
TO ADULT COLORADO PIKEMINNOW (*PTYCHOCHEILUS LUCIUS*) IN A
WATER QUALITY SIMULATING THAT IN THE SAN JUAN RIVER.

Final Report

November 24, 2000

Prepared for:
San Juan River Basin
Recovery Implementation Program
Biology Committee and
National Irrigation Water Quality Program

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LIST OF KEY WORDS

Colorado pikeminnow
 Survival
 Growth
 Reproduction
 Selenium
 Dietary exposure
 Waterborne exposure
 Muscle plug
 Accumulation
 Depuration

ACKNOWLEDGMENTS

We thank Buddy Jensen, Dexter National Fish Hatchery, Dexter, NM, for providing the fish and Roger Hamman for his valuable assistance in spawning the fish. We also thank F. Art Bullard for the in-house selenium analyses; Marvin Ehlers, Susan Muehlbeier, Heather Hamilton, and Susan McDonald for their technical assistance in conducting the test; and Dr. Mark Ellersieck, University of Missouri, Columbia, MO, for his valuable assistance and guidance with the statistical analyses. The authors also thank Dr. Michael Petersen, Keller-Bliesner Engineering, Logan, UT, for his comments on the reproduction data analyses. This research was funded by the San Juan River Recovery Implementation Program and the National Irrigation Water Quality Program.

EXECUTIVE SUMMARY

Investigations conducted by U.S. Department of the Interior agencies have documented elevated concentrations of selenium in water, sediment, and biota at various locations in the San Juan River basin. The San Juan River provides critical habitat for two endangered fishes, Colorado pikeminnow (*Ptychocheilus lucius*, formally named Colorado squawfish) and razorback sucker (*Xyrauchen texanus*) and concern has been raised about the effects of selenium on these native fishes. At present, little is known about the sensitivity of Colorado pikeminnow to long-term selenium exposures. The objective of this study was to determine the effects of dietary and waterborne selenium on survival, growth, and reproduction of adult Colorado pikeminnow under controlled laboratory conditions.

Adult Colorado pikeminnow (16 years old) were obtained from Dexter National Fish Hatchery, Dexter, NM, and exposed to one of six combined dietary and waterborne selenium treatments for 155 days and then held under control conditions for 90 days to allow for selenium depuration. The treatments consisted of three dietary concentrations (2.2 [control], 7.3, 11.8 µg/g dry weight, as seleno-DL-methionine) and two waterborne concentrations (control and 5µg/L, as a 6:1 ratio of selenate:selenite). Growth was measured on all fish and muscle plugs taken from females at test initiation and every 30 days thereafter. The fish were tested in reconstituted water designed to simulate the water quality of the San Juan River, near Shiprock, NM. The test water was routinely monitored for selenium concentrations and general water quality characteristics.

The fish were artificially spawned after 154 days of exposure and the number of eggs expressed, egg diameter and weight, and selenium residues in eggs were determined. The eggs produced were monitored for survival, hatchability, hatch time, and deformities in a separate test

system. The resulting larvae were held for 30 days and monitored for survival, growth, and selenium residues. The egg and larval studies were conducted in clean reconstituted test water.

Survival and growth of adults were not affected by the combined dietary and waterborne selenium exposures for 155 days. Adults of both sexes lost weight during the first 30 days of the study and during the depuration period. The rate of weight loss was higher in females than in males. Regardless of sex, none of the fish exhibited a significant weight gain during the study compared to their weights at day 0. Spawning success of females was low and highly variable among selenium treatments. Only 10 of 35 females expressed eggs and spawning success was limited to one female in three treatments, two females in two treatments and three females in one treatment. All males expressed milt during the spawning trials, but 6 of 19 males expressed less than 20 ml. None of the reproductive metrics (eggs released, hatchability, egg size, incidence of deformities, and survival and growth of larvae) were correlated with dietary or waterborne selenium exposure concentrations of the female parent. However, due to the lack of replicate spawns for half of the treatments, we were not able to draw unequivocal conclusions concerning the effects of these dietary and waterborne selenium exposures on reproduction of Colorado pikeminnow.

Selenium concentrations in muscle plugs of adults were strongly correlated with dietary selenium concentrations, but not with waterborne selenium, and concentrations seemed to reach an equilibrium after 120 days of exposure. The reduction in muscle plug selenium residues after 90 days of depuration was minimal, which indicated that selenium accumulated in muscle was slowly eliminated from the fish. Bioaccumulation factors for selenium in muscle tissue (compared to the diet) were less than one (range, 0.17 to 0.60) and were inversely related to dietary selenium concentrations.

Eggs and 1-day posthatch larvae contained significantly greater selenium concentrations than the female parent (about 2- to 5-fold) and these concentrations were strongly correlated with dietary exposure of the parent. These results indicated that selenium assimilated from the diet of the female parent was transferred to the eggs in a concentration-dependent manner. Selenium concentrations in larvae exposed to clean water and diet for 30 days were 20 to 76% lower than in newly hatched larvae, and the magnitude of reduction was related to initial whole-body selenium concentrations.

The biological significance of selenium residues observed in eggs and larvae are difficult to interpret due to the lack of replicate spawns for three treatments and the small sample size. Hatchability of eggs containing 1.8-11.6 $\mu\text{g/g}$ selenium averaged 84% and the incidence of deformities in the resulting larvae averaged 13%. Survival of larvae containing 2.8-13.4 $\mu\text{g/g}$ selenium at hatch averaged 87% after 30 days. Overall survival of progeny from the 10 spawns average 72%. Selenium concentrations in eggs (9.8 to 11.6 $\mu\text{g/g}$) produced by females with muscle tissue selenium residues of 2.5-3.0 $\mu\text{g/g}$ fall in the low to moderate hazard category for potential selenium-induced reproductive impairment in fish. However, the female with the highest muscle selenium concentration of 5.2 $\mu\text{g/g}$ did not spawn, so the effect (if any) of this accumulated selenium residue from the high selenium diet (11.8 $\mu\text{g/g}$) on reproduction could not be determined. Consequently, the hazard posed by parental exposures to dietary selenium concentrations ≤ 12 $\mu\text{g/g}$ and waterborne selenium concentrations ≤ 8 $\mu\text{g/L}$ to wild Colorado pikeminnow cannot be adequately assessed from the reproduction data obtained in this study and additional research is needed to determine the dietary selenium threshold concentration for reproductive impairment in these fish.

INTRODUCTION

After the discovery of contaminated irrigation return waters in the San Joaquin Valley of central California in the early 1980s (Ohlendorf et al. 1986, Saiki 1986), the Department of the Interior (DOI) initiated the National Irrigation Water Quality Program (NIWQP) in 1985 to identify other irrigation drainages in the western United States with similar water quality problems (Feltz and Engberg 1994, Engberg 1998). These investigations focused on DOI irrigation projects where the receiving water was a national wildlife refuge or had the potential to impact migratory birds or endangered species. The San Juan River, located in northwestern New Mexico and southeastern Utah, was identified as one area needing further study.

Five DOI irrigation projects on the San Juan River return water by overland flow, seepage, or subsurface tile drains to the main stem or to the ground-water system in the San Juan River basin. Extensive irrigation outside DOI projects influences water quality in the tributaries and main stem of the San Juan River (Blanchard et al. 1993). It is estimated that irrigation return flows, after completion of the Navajo Indian Irrigation Project, may comprise about 15% of the annual flow of the San Juan River (USGS et al. 1989).

The San Juan River provides critical habitats for two federally-listed endangered fish species, Colorado pikeminnow (*Ptychocheilus lucius*, formally named Colorado squawfish) and razorback sucker (*Xyrauchen texanus*; USFWS 1994), and two species of concern, flannelmouth sucker (*Catostomus latipinnis*) and roundtail chub (*Gila robusta*; USFWS 1996). A small reproducing population of Colorado pikeminnow exists in the San Juan River in the area from its confluence with the Animas River near Farmington, NM, downstream to Lake Powell (USFWS 1990).

Radiotelemetry studies of Colorado pikeminnow in the San Juan River suggest that

spawning is limited to an area known as the “Mixer” and that prior to spawning, adults stage at the confluence of the Mancos River and San Juan River located about 11.6 km downstream of the Mixer (Ryden and Pfeifer 1995, Miller 1994). Adult Colorado pikeminnow use a variety of habitats, including runs, riffles, eddies, shorelines, and backwaters and their habitat use is dependent on season, hydrology, temperature, and availability (USFWS 1990, 1994). Adults seem to prefer backwaters and flooded bottomlands during the runoff period (Tyus 1990) and are commonly found in shoreline habitats during mid-to-late summer and winter (Tyus and Karp 1989). These habitats occur along the San Juan River at or below areas where tributaries or irrigation flows enter the main stem river.

A DOI irrigation drainwater investigation of the San Juan River from the Hammond Project Diversion to the mouth of the Mancos River found that concentrations of selenium and other inorganic elements were elevated in water, sediment, and biota at various locations, usually associated with irrigation drainage (Blanchard et al. 1993). Whole-body selenium concentrations in fish collected from the San Juan River, which are potential food items for adult Colorado pikeminnow, ranged from 1.3 to 8.3 µg/g dry weight (Blanchard et al. 1993). Waterborne selenium concentrations in the same region of the San Juan River ranged from <1 to 4 µg/L (Blanchard et al. 1993). A separate DOI investigation of the San Juan River from the Four Corners to Mexican Hat, UT, and tributaries in Utah and Colorado also reported elevated selenium concentrations in some samples (Butler et al. 1995). As a result of these investigations, concern has been raised about the potential impact of elevated selenium concentrations in water and food-chain organisms on native Colorado pikeminnow.

Very little is known about the sensitivity of Colorado pikeminnow, and other listed fish species, to long-term waterborne and dietary selenium exposures. Previous toxicological studies

with endangered fishes of the Colorado River basin determined their sensitivity to lethal waterborne concentrations of selenium and other inorganic elements found in irrigation drainwater (Hamilton 1995, Buhl and Hamilton 1996, Hamilton and Buhl 1997). However, the bioaccumulation and toxic effects of selenium in fish result primarily from the consumption of selenium-laden food (Lemly and Smith 1987, Woock et al. 1987, Hamilton et al. 1990, Coyle et al. 1993, Maier and Knight 1994). Consequently, the hazard potential of selenium to these fish should be based on selenium concentrations in their tissues and food, rather than on waterborne concentrations (Lemly 1993).

Objective

The objective of this study was to determine the effects of combined dietary and waterborne selenium exposures on adult Colorado pikeminnow in a water quality simulating that in the San Juan River. We exposed the adults to selenium via diet and water in the laboratory for 155 days followed by a 90-day depuration period and monitored survival, growth, reproduction, and selenium residues in muscle tissue. Hatching success of eggs and survival and growth of larvae produced by these adults were evaluated. In addition, selenium residues in the eggs and larvae were measured.

METHODS AND MATERIALS

Test Fish

Fifty-eight adult Colorado pikeminnow (16 years old) were transported from Dexter National Fish Hatchery (DNFH), Dexter, NM, to our laboratory in a distribution truck equipped with two hauling tanks (1,230 and 1,419 L) supplied with pure oxygen. The fish were hatched in 1981 (DNFH Lot no. 81DXGRCR) and each fish was tagged with an internally implanted passive integrated transponder (PIT) by personnel at DNFH before transport to our laboratory.

Upon arrival, each fish was identified by reading its PIT tag and impartially stocked into 757-L fiberglass circular tanks. Two females and one male were stocked into each of 18 exposure tanks, with one exception, and four extra fish (two of each sex) were placed in a separate culture tank. Two males were accidentally stocked in one replicate tank of treatment 2 (defined below). This error occurred because one of these males was misidentified as a female by personnel at DNFH and was not discovered until the spawning trials were initiated. The tanks were calibrated to hold 662 L of water using a center-mounted standpipe and sleeve. Prior to stocking, about two-thirds of the well water in each exposure tank was drained and replaced with water from tanks on the distribution truck. Water temperature in exposure tanks at the time of stocking (13-14°C) was within 1-2°C of the shipping water (12-13°C).

On the day after arrival, the fish were treated with 0.5% salt solution to help them recover from the handling stress (B. Jensen, U.S. Fish and Wildlife Service, personal communication). Food-grade NaCl was added directly to each exposure tank and the fish were maintained under static conditions for about 7 hours. Water flow was then restored to the tanks and the salt concentration was gradually diluted with well water. None of the fish exhibited any overt signs of stress (i.e., surfacing or lethargy) during or after the salt treatment.

The fish were held in well water maintained at ambient temperature for 3 weeks before starting the study. Water flow to the tanks was provided by an intermittent flow-through diluter system calibrated to deliver 2 L of well water to each tank every 15 minutes. All tanks were aerated continuously using large air stones supplied with compressed air from an oil-less air compressor. Mean (SD) characteristics of the holding water were as follows: hardness, 1,065 (6) mg/L as CaCO₃; alkalinity, 299 (2) mg/L as CaCO₃; pH, 7.9 (0.1); conductivity, 2,093 (257) µmhos/cm @25°C; un-ionized ammonia, 0.041 (0.022) mg/L as N; and dissolved oxygen (DO),

7.2 (1.1) mg/L. Mean daily water temperatures (averaged across all tanks) during the 3-week acclimation period ranged from 13.1 to 15.9°C.

Prior to starting the test, fish were fed Silver Cup Brood 6.35-mm (¼ inch) dry pellets (Nelson and Sons Inc., Murray, UT) 3-4 times daily at a rate of about 1.0% body weight per day. This diet contained 1.37 µg/g selenium dry weight (average of duplicate analysis) and 7.9% moisture. Each tank was siphoned daily after the last feeding to remove uneaten food, fecal material, and detritus.

Test Water

All fish were tested in a nonstandardized reconstituted water designed to simulate the major water quality characteristics (without the inorganic contaminants) of the San Juan River at Shiprock, NM, Station ID 09368000 on November 5, 1985 (Table 1; Beal and Gold 1987). This date was selected because it was a year with average river flows. The test water was prepared by adding appropriate amounts of calcium chloride, calcium sulfate dihydrate, magnesium carbonate, sodium bicarbonate, and potassium bicarbonate to deionized water in large (5,678-L or 11,355-L) polyethylene blending tanks. Each blending tank was fitted with a recirculating pump to mix and aerate the water. To facilitate the dissolution of magnesium carbonate, carbon dioxide (CO₂) gas was bubbled in the tank for about 5 minutes after the salt was added. The water was then vigorously aerated with compressed air for least 2 hours to drive off the excess CO₂. Each tank of test water prepared was analyzed for general water quality characteristics prior to use in the study to insure that the water quality parameters were within 10% (11% for magnesium) of their desired concentrations, except for chloride. Chloride concentrations in the blending tanks were about 2.2 times higher than that measured in the San Juan River because of the type of mineral salts used to prepare the water. The flow of reconstituted test water to the

Table 1. Water quality characteristics of reconstituted San Juan River water used in chronic toxicity test with Colorado pikeminnow.

Parameter (unit)	San Juan River ^a	Measured values (mean and SD in parentheses)		
		Blending tanks	Adult exposure tanks	Embryo-larval exposure tanks
pH (standard units)	7.5	7.4 (0.4)	7.5 (0.2)	7.8 (0.1)
Hardness (mg/L as CaCO ₃)	150	145 (2)	145 (2)	151 (4)
Calcium (mg/L)	43	44 (1)	45 (1)	45 (1)
Magnesium (mg/L)	9.2	8 (1)	8 (1)	10 (1)
Sodium (mg/L)	22	22 ^b	--	--
Potassium (mg/L)	1.9	2 ^b	--	--
Alkalinity (mg/L as CaCO ₃)	89	85 (2)	74 (8)	88 (2)
Chloride (mg/L)	5.9	13 (0.5)	24 (5)	36 (19)
Sulfate (mg/L)	89	88 (3)	90 (4)	89 (6)
Conductivity (µmhos/cm @25°C)	360	395 (6)	439 (15)	474 (62)
Un-ionized ammonia (µg/L as N)	0.26 ^c	--	5.6 (7.7)	3.5 (6.0)
Dissolved oxygen (mg/L)	9.5	--	6.8 (0.7)	7.4 (0.5)
n	--	171	192 ^d	56

^aWater quality in San Juan River at Shiprock, NM, on November 5, 1985; Station ID 09368000 (Beal and Gold 1987).

Table 1. Continued.

^bNominal concentration based on the amount of bicarbonate salt added to the tank.

^cCalculated from measured values of total ammonia (0.040 mg/L as N), pH (7.5), and temperature (11.5°C) using the equations of Emerson et al. (1975).

^d_n = 4,122 for dissolved oxygen concentrations in adult exposure tanks.

exposure tanks was started 2 days before introducing the selenium-spiked water.

Adult Exposure

Adults were exposed to one of six combinations of dietary and waterborne selenium for 155 days (last day of spawning trials) and then held for 90 days under control conditions to allow for selenium depuration. To minimized handling, the adults were tested in the same diluter system in which they were initially stocked (described above). The experimental treatments were assigned using a balanced 3 x 2 factorial design with three dietary concentrations and two waterborne concentrations. Treatment designation for the combined dietary and waterborne selenium concentrations is summarized in the text-table below:

Waterborne selenium ($\mu\text{g/L}$)	Dietary selenium ($\mu\text{g/g}$ dry weight)		
	Control	5	10
Control	Treatment 1	Treatment 3	Treatment 5
5	Treatment 2	Treatment 4	Treatment 6

Each treatment had three replicate exposure tanks, which were the experimental units, and two females and one male were stocked in each tank (except for one replicate of treatment 2 as discussed above). Thus, six females (five in treatment 2) and three males (four in treatment 2) were exposed to each treatment. The exposure tanks (total of 18) were arranged in three rows of six, with all treatments present in each row. The assignment of treatments to the tanks followed a randomized block design, with rows serving as the blocks (ASTM 1992).

For the dietary exposure component, fish were fed a commercial semi-moist salmon diet fortified with seleno-DL-methionine to achieve nominal selenium concentrations of 0, 5, or 10 $\mu\text{g/g}$ (dry weight). The dietary selenium concentrations were based on proposed dietary threshold concentrations of 3, 5, and 10 $\mu\text{g/g}$ (Lemly 1993, 1996a, Lemly and Smith 1987,

USDOI 1998) for reproductive impairment in sensitive fish. Also, the dietary concentrations tested here were representative of those in potential food items of adult Colorado pikeminnow in the San Juan River (whole-body fish tissue, 1.3-8.3 $\mu\text{g/g}$; Blanchard et al. 1993).

Seleno-DL-methionine was used because it was found to be a good model for natural forms of selenium in food-chain organisms, based on bioaccumulation and toxicity studies with fish (Bryson et al. 1985, Hamilton et al. 1990). The test diets were formulated by BioProducts Inc. (Warrington, OR) using standard production techniques. The two selenium-fortified diets were prepared by adding the desired amount of seleno-DL-methionine ($\text{C}_5\text{H}_{11}\text{NO}_2\text{Se}$), obtained from Sigma Chemical (St. Louis, MO), to the dextran binder and then thoroughly mixing the binder into the normal BioDiet "Brood" dough prior to pelletization. The control diet was prepared as the normal BioDiet Brood dough, but without seleno-DL-methionine added to the dextran binder. The dough was extruded into 6-mm pellets and vacuumed sealed with nitrogen in plastic bags. The diets were shipped by ground transport to our laboratory. Upon arrival, duplicate 10-g samples were taken from each bag of diet for total selenium analysis and the diets were stored in a freezer at or below -18°C . All diets were analyzed for total selenium residues prior to use in the study.

For the waterborne exposure component, fish were exposed to nominal selenium concentrations of 0 or 5 $\mu\text{g/L}$. The waterborne concentration of total selenium (5 $\mu\text{g/L}$) was based on the current water quality Criterion Continuous Concentration of 5 $\mu\text{g/L}$ (USEPA 1987). Because selenate and selenite usually occur simultaneously in surface waters (USEPA 1987), a 6:1 ratio of selenate:selenite in the water was used to better approximate environmental conditions. This ratio is representative of that found in selenium contaminated irrigation drainwater entering the Kesterson National Wildlife Refuge in California (Presser and Barnes

1984). Stock solutions of selenium were prepared in deionized water using sodium selenate (Na_2SeO_4) obtained from Johnson Matthey (Ward Hill, MA) and sodium selenite (Na_2SeO_3) obtained from Aldrich Chemical (Milwaukee, WI) at a 6:1 ratio of selenate:selenite. The selenium stock solution was delivered to the diluter system by automated pipettes (Micromedic Systems, Horsham, PA).

To document exposure concentrations during the adult study, 100 ml of water were collected from all exposure tanks on days 0, 15, 75, 120, 149, 166, and 180 of the study and from one replicate of each treatment on days 215 and 245 of the study for analysis of total selenium. Water samples were collected in 125-ml acid-cleaned plastic bottles, preserved in 1% instra-analyzed HCl (J.T. Baker, Phillipsburg, NJ), and frozen until analysis.

The selenium-fortified diets were introduced on the day after the selenium-spiked water was introduced and this dietary exposure was considered as day 1 of the study. Daily rations of test diets were adjusted periodically during the early part of the study to minimize the amount of uneaten food in the tanks. Fish were fed at a rate (based on total weight of fish in each tank) of 1.1% for days 1-23, 1.7-2.0% for days 24-40, 1.5% for days 41-60, 1.75% for days 61-90, and 2.0% for days 91-245 of the study. Fish were fed three times a day during the first 2 weeks and five times a day thereafter. Food was withheld on days when growth was measured.

On the day the waterborne exposure began and every 30 days thereafter, all fish were identified from the PIT tag, weighed (nearest g, A&D model HW-10KA2 scale, A&D Engineering, Milpitas, CA), measured for total length (nearest mm), and examined for general health. Also, a muscle plug was taken from each female for selenium residue analysis at these sampling intervals. The plugs, which include the skin and scales, were taken from the mid-dorsal area about 1-2 cm below the dorsal fin, using a sterile 5-mm biopsy punch (Miltex

Instrument Co., Lake Success, NY). Each muscle plug was transferred to a plastic vial (Nunc CryoTube, Kamstrup, Denmark) using a stainless steel forceps and then placed in a freezer.

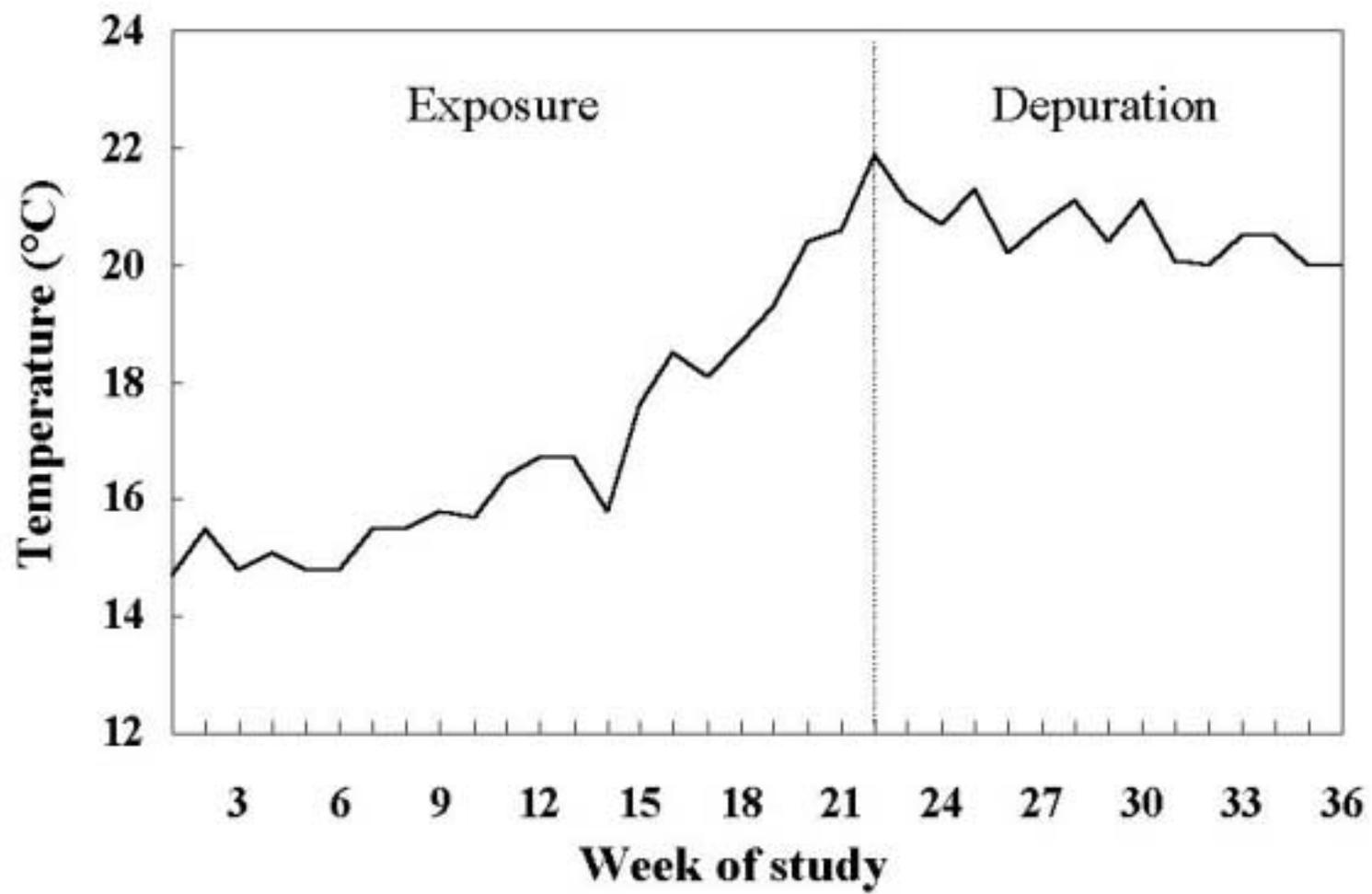
Water temperature regimes were designed to simulate those at DNFH in an attempt to maintain the reproductive cycle of the test fish in synchrony with adults of the same year class that have been spawned successfully at DNFH. Water temperature in adult exposure tanks was maintained at ambient laboratory temperature, which varied from 13°C in January at the start of the study to 23°C in June when the spawning trials were initiated. Weekly water temperatures, averaged across all tanks, are shown in Figure 1. This temperature regime was similar to that in holding ponds and raceways used for culturing Colorado pikeminnow broodstock at DNFH (R. Hamman, U.S. Fish and Wildlife Service, written communication).

All fish were exposed under a photoperiod that simulated conditions at Shiprock, NM, from January to September (Appendix A). The light was provided by a combination of wide-spectrum and cool-white fluorescent bulbs controlled by a timer. The photoperiod was adjusted on the first day of each month.

All adult exposure tanks were siphoned each day to remove uneaten food, fecal material, and detritus. After siphoning, the water in each tank was filtered continuously for at least 2 hours to remove suspended material (primarily disintegrated food pellets) and increase the dissolved oxygen concentration. Filtration was accomplished by using a submersible pump (1.7 amp, Little Giant Pump, Oklahoma City, OK) fitted with a nylon hose that conveyed the water to a 25- μ m polypropylene bag filter (Filter Specialists, Michigan City, IN) suspended over the tank. The tanks were covered with nylon netting (48-mm mesh), except during filtration, to prevent fish from jumping out of the tanks.

Figure 1. Average weekly water temperatures (n=18) in adult Colorado pikeminnow exposure tanks during the 155-day selenium exposure period and 90-day depuration period.

Dashed vertical line indicates week of spawning trials.



Spawning Trials

After water temperatures reached 20°C (day 135 of study), five males were examined for spawning characteristics. Four of them had developed tubercles on the head, operculars, and pelvic fins. The tuberculated males were checked for the presence of milt, but none of them expressed a milky fluid. By day 140 of study, all of the males had tubercles and over 25% of them expressed a milky fluid when handled. On day 144 of study, 16 females were examined and all of them had acquired tubercles on the head and their abdominal region appeared to be distended. On day 153, all adults were examined by the Assistant Manager, DNFH, for spawning characteristics and over one-half of the females appeared to be in condition for ovulation if given a hormone injection (R. Hamman, U.S. Fish and Wildlife Service, written communication). Consequently, the decision was made to induce spawning by hormone injection (described below).

Spawning trials occurred on days 154 and 155 of study. Females that did not express eggs on day 154 were checked for ripeness on day 155 and females that did not release eggs after the second spawning trial were considered to be nonspawners. No further attempts were made to induce ovulation in the nonspawning females because they were judged to be incapable of spawning this year (R. Hamman, U.S. Fish and Wildlife Service, written communication). After the spawning trials were completed, the selenium treatments were discontinued and the depuration period began.

The procedures used to spawn the fish followed those of Hamman (1981). Briefly, on day 153 of the study, all males were injected intraperitoneal (IP) with human chorionic gonadotropin hormone (Chorex, Hyrex Pharmaceuticals, Memphis, TN) at 660 IU/kg body weight and all females were injected IP with carp pituitary (Stoller Fisheries, Spirit Lake, IA) at

4.4 mg/kg body weight after growth measurements and muscle plugs were taken. The sexes were then separated by placing all males of a given treatment in one tank and all females of the same treatment in the two remaining tanks for that treatment.

Starting with the control fish, individual males were anesthetized in a bath containing tricaine methanesulfonate (MS-222, Argent Chemical, Redmond, WA) at 100 mg/L and then manually stripped. The milt was collected in 50-ml plastic centrifuge tubes and placed in an ice bath. The fish was placed in a tank of clean test water (i.e., without selenium) until it regained equilibrium and then it was returned to the exposure tank. After all males of a given treatment were stripped, the females were checked. Each female was anesthetized as above and an attempt was made to manually strip the eggs. If the female expressed eggs, they were collected in a 2-L stainless steel bowl containing about 100 ml of sperm extender solution prepared by adding 12 g of Dilueur 523 (Sanofi Sante Nutrition Animale, Libourne, France) to 1 L of clean test water at 22-23°C.

We intended to cross the two females in a given tank with the male from the same tank. If the male did not express a sufficient amount of milt, the eggs were fertilized with sperm from a male of the same treatment. Milt from one male was added to the eggs and the gametes were gently stirred with a large feather for about 1 minute, during which about 250 ml of clean test water was added to activate the sperm. After fertilization, about 250-500 ml of a bentonite clay slurry was added to the bowl and stirred for about 1 minute to counteract adhesiveness and prevent clumping of the eggs. The egg mass was divided between two medium size fine-mesh dip nets and thoroughly rinsed in clean test water. The eggs were then submerged in a container of clean test water to water-harden for at least 1 hour.

After water-hardening, total number of eggs expressed by each female was estimated

using the gravimetric method for enumeration (Piper et al. 1982). Briefly, the entire lot of eggs was blotted with cloth towels to remove most of the excess water and then weighed (nearest g). Three subsamples of eggs (about 300-700 eggs) were counted and weighed (nearest 0.001 g) to determine average egg weight. Total number of eggs expressed was calculated by dividing the total weight of the spawn by the average egg weight. The eggs used to determine average egg weight were placed in Whirl-Pak bags and frozen until analysis for whole-egg selenium residues. Egg diameters (long axis, nearest 0.01 mm) were measured on groups of 25 eggs collected from each spawn using an ocular micrometer (Reichert model 426C filar micrometer eyepiece, Reichert Scientific Instruments, Buffalo, NY) and stereoscope.

The remaining eggs from each spawn were transferred to three acrylic standard hatching jars placed in a glass aquarium (73-cm long, 30-cm wide, 30-cm deep) with a water depth of about 21 cm (about 46 L of water). The aquaria were placed in a water bath maintained at $21 \pm 1^\circ\text{C}$ and received test water from an intermittent flow-through diluter at a rate of 1 L every 15 minutes. A small submersible pump (1.1 amp, Little Giant) fitted with latex tubing, glass tees, and glass pipets was used to deliver water from the aquaria to the three hatching jars. Water flow to the jars was regulated by clamps on the latex tubing and was adjusted to gently roll the eggs. Water in each aquaria was aerated using air stones supplied with compressed air.

Progeny Tests

Eggs

For the hatchability study, a sample of 100 fertilized eggs was collected from each spawn at 1 day postspawn and stocked into egg incubation cups. To facilitate daily observations on the condition of the embryos, only 25 eggs were placed in an incubation cup and four incubation cups were used. Fertilized eggs were considered as those undergoing active embryological

development and devoid of any visible abnormalities. All eggs were examined under a stereoscope at 30X magnification prior to stocking. The egg incubation cups were 220-ml glass jars with the bottoms cut off and replaced with 0.285-mm mesh polypropylene screen. The cups were suspended in glass aquaria (described above) from a rocker arm apparatus connected to a 0.3 amp motor, which continuously oscillated the cups in the water column and was positioned so that the eggs were completely submerged at all times. The aquaria were divided into three chambers, one large chamber (37-cm long, 30-cm wide, 30-cm deep) where the four egg cups were suspended and two smaller growth chambers (36-cm long, 15-cm wide, and 30-cm deep) where the resulting larvae were tested. Air stones supplied with compressed air were placed in each chamber. The aquaria were placed in a water bath maintained at $21 \pm 1^\circ\text{C}$ and received test water from an intermittent flow-through diluter at a rate of 1 L every 15 minutes. The fish were tested under a photoperiod of 14 hours light:10 hours dark.

The number of live and dead eggs were recorded daily and all dead eggs were removed with a glass pipet. When hatching commenced, the number of eggs hatched and the number of live, deformed, and dead larvae were recorded daily and all dead larvae removed. The criteria for death were whitening of the embryo or yolk in the eggs and absence of a heart beat (under 30X magnification) in the larvae. The types of deformities recorded followed the descriptions given in Scudder et al. (1988) and Bantle et al. (1990). For each spawn, the time to onset and completion of hatch was recorded to the nearest day and the average day to hatch was calculated using the formula of Scudder et al. (1988) as follows:

$$\text{Average day to hatch} = \frac{\sum_{i=1}^n E_i X_i}{M}$$

where E_i = number of eggs hatched on day X_i , n = number of days eggs incubated, and M = total number of eggs hatched.

Larval survival and growth

After all viable eggs hatched, 15 larvae from two egg cups were stocked into each growth chamber (30 larvae per growth chamber and 60 larvae per spawn). Two growth chambers were used to facilitate daily observations on the condition of the larvae. The larvae were held for 30 days in clean test water at $21 \pm 1^\circ\text{C}$ and fed live nauplii of brine shrimp (*Artemia* sp.) three times a day. Mortality and overt abnormal behavior were monitored daily and total length (nearest mm) and weight (nearest 0.001 g) were measured at the end of the 30-day test period. Overall survival of the progeny from a given spawn was calculated as the product of percent survival of embryos and larvae in the egg cups at the time of thinning (when larvae were stocked into growth chambers) times larval survival from thinning to the end of the test (ASTM 1992).

Progeny residues

Eggs not used in the hatchability and larval survival and growth studies were used in the progeny residue study. After the eggs hatched, the larvae were transferred from the hatching jars to the aquaria (described above). The larvae were held for 30 days in clean test water at $21 \pm 1^\circ\text{C}$ and fed live nauplii of brine shrimp and BioDiet Starter (BioProducts Inc., Warrenton, OR) *ad libitum*. Mortality was recorded daily, and at 1-, 16-, and 30-days posthatch, composite samples of fish were collected from each aquarium for whole-body selenium residues. The fish were euthanized with MS-222, weighed, packed in Whirl-Pak bags, and frozen until analysis.

Radio-tagging

On days 229 and 330 of study, radio transmitters (Advanced Telemetry Systems, Isanti, MN) were surgically implanted in the body cavity of 13 fish by personnel of the Colorado River

Fishery Project (U.S. Fish and Wildlife Service, Grand Junction, CO). These fish were later used in radiotelemetry studies in the Colorado River System. Three additional fish served as sham controls and received the same surgical procedure as the implanted fish, but without a transmitter.

Water Quality

During the adult exposure and depuration periods, general water quality characteristics were monitored in one set of replicate exposure tanks per week (Table 1). Calcium, total alkalinity, and total hardness were measured using titration methods of APHA (1995). Magnesium was determined indirectly by the difference between total hardness and calcium. Total ammonia was measured using an Orion model 95-12 ammonia electrode with an Orion model 901 ionalyzer (Orion Research 1990). Un-ionized ammonia was calculated from measured values of total ammonia, pH, and temperature using the equations of Emerson et al. (1975). Chloride was measured using the mercuric nitrate titration method of Hach (1992). Conductivity (corrected to 25°C) was measured with a YSI (Yellow Springs Instruments, Yellow Springs, OH) model 31 conductivity bridge with a cell constant of $K=1.0 \pm 1\%$. Sulfate was determined by the turbidimetric method described in APHA (1995) using Hach SulfaVer 4 reagent (Hach 1992) and a Turner Designs (Mountain View, CA) model 40 nephelometer. Turbidity was measured using the same nephelometer following standard methods (APHA 1995). Total suspended solids, fixed suspended solids, and volatile suspended solids were determined according to standard methods (APHA 1995). Dissolved oxygen (DO) was measured in all exposure tanks at least once a day with a YSI model 58 dissolved oxygen meter. Water temperature was measured daily in each tank using stainless steel thermometers.

In the hatchability and larval survival and growth study, general water quality

characteristics including DO were measured weekly in all exposure aquaria as described above. During the progeny residue study, the same water quality parameters were measured twice in three exposure aquaria. Water temperature in the progeny studies was monitored daily in one aquarium using a mercury thermometer.

Selenium Analyses

Total selenium concentrations in water, diet, eggs, and larvae were determined by hydride generation atomic absorption spectroscopy (HGAAS) using a Perkin-Elmer Corporation (Norwalk, CT) model 3300 atomic absorption spectrophotometer equipped with a model MHS-10 mercury hydride generator. Operational procedures for HGAAS closely followed those of Perkin-Elmer (1978, 1982).

Water samples were thawed under refrigeration and then subjected to a two-step digestion procedure modified from Presser and Barnes (1984). Briefly, the samples were prepared for analysis by adding 1 ml of 5% potassium persulfate and 0.4 ml of in-situ-analyzed HCl (J.T. Baker) to 20 ml of sample in a 100-ml beaker. Duplicate digestions were performed on each sample. The samples were digested on a hotplate and the digestate was diluted to 100 ml with 8% HCl prior to analysis. Each digestate was analyzed in duplicate and the mean concentration recorded.

Duplicate samples of the diet, eggs, and larvae were freeze dried to a constant weight for moisture determination using a Virtis (Gardiner, NY) 3-L vacu-freeze condenser sentry system. The dried samples were placed in 100-ml beakers and subjected to a combined nitric acid wet digestion and magnesium nitrate dry ash procedure (Siu and Berman 1984). The digestates were diluted to 100 ml with 10% HCl prior to analysis. Each digestate was analyzed in duplicate and the mean concentration recorded.

Muscle plugs were analyzed for selenium by neutron activation analysis at the University of Missouri Research Reactor (MURR), Columbia, MO, using a gamma-ray spectrophotometer with a Tennelec 244 amplifier coupled to a Nuclear Data 599 loss-free counting module and a Nuclear Data 581 ADC. Samples were prepared for analysis at the Columbia Environmental Research Center (CERC), Columbia, MO, according to the methods described in Waddell and May (1995). Determination of radionuclide $\text{Se}^{77\text{m}}$ followed the methods of McKown and Morris (1978).

Quality Control

Quality control measures for HGAAS analysis of selenium included the preparation and analysis of triplicate samples (to measure precision), digested sample spikes (to measure loss of selenium), analysis spikes (to measure matrix effects), procedural blanks (to measure contamination and determine detection limits), and standard reference material (to measure accuracy). The standard reference materials (SRM) used were water from the National Institute of Standards and Technology (SRM 1643d, Gaithersburg, MD) and dogfish tissue from the National Research Council of Canada (DORM-2, Ottawa, Canada).

Consistent sample handling during preparation, digestion, and analysis was indicated by the low mean percent relative standard deviations, which ranged from 3.7 to 6.1% (Table 2). Recoveries of selenium in digested spiked samples and analysis spikes were excellent (97-103%), which indicated no significant loss of selenium from the original sample during the digestion procedure and no significant interference from the other matrix components. Selenium concentrations in all procedural blanks (n=16) were lower than the calculated limit of detection. The analysis of reference materials were within acceptable ranges established by the CERC (B. Brumbaugh, CERC, personal communication) and were within 10% of certified values, which

Table 2. Quality control results for selenium analysis of water, diet, and tissue samples by hydride generation atomic absorption spectroscopy.

Measure	Matrix ^a		
	Water	Diet	Tissue
Limit of detection ($\mu\text{g/L}$ or $\mu\text{g/g}$) ^b	0.63 (0.35) [8]	0.100 (0.0390) [5]	0.064 (0.004) [3]
Method precision (%RSD) ^c	6.0 (2.4) [8]	6.1 (2.3) [5]	3.7 (1.9) [3]
Digested spikes (% recovery) ^d	97 (8) [16]	102 (9) [10]	97 (5) [6]
Analysis spikes (% recovery) ^e	101 (6) [8]	98 (9) [5]	103 (12) [3]
Reference water ($\mu\text{g/L}$, NIST SRM 1643d) ^f	11.9 (0.5) [8]	--	--
Reference material ($\mu\text{g/g}$, NRCC DORM-2) ^g	--	1.36 (0.11) [5]	1.19 (0.02) [3]

^aData are means with SD in parentheses and N in brackets.

^bLimit of detection = $3(\text{SD}_b^2 + \text{SD}_s^2)^{0.5}$, where SD_b is standard deviation of blank and SD_s is standard deviation of low-level sample.

^c%RSD = percent relative standard deviation calculated as $\text{SD}/\text{Mean} \times 100$ for a triplicate determination of a representative sample for each sample run.

^dSamples spiked with seleno-DL-methionine at the beginning of chemical preparation.

^eDigested samples spiked with sodium selenite at instrument to check for matrix interference.

^fNIST SRM 1643d = National Institute of Standards and Technology Standard Reference Material Trace Elements in Water 1643d; certified concentration = 11.26-11.60 $\mu\text{g/L}$, recommended concentration = 10.13-12.76 $\mu\text{g/L}$ (based on in-house quality control/quality assurance program at the Columbia Environmental Research Center, B. Brumbaugh, Columbia, MO, personal communication).

^gNRCC DORM-2 = National Research Council of Canada dogfish muscle tissue DORM-2; certified concentration = 1.31-1.49 $\mu\text{g/g}$, recommended concentration = 1.18-1.64 $\mu\text{g/g}$ (based on in-house quality control/quality assurance program at the Columbia Environmental Research Center, B. Brumbaugh, Columbia, MO, personal communication).

indicated that the procedures accurately measured the selenium concentrations in these samples.

For neutron activation analysis of selenium in muscle plugs, the accuracy and precision of the irradiation method was checked by MURR by irradiating 11 samples of National Institute of Standards and Technology Bovine Liver SRM 1577a. All 11 samples (mean = 1.14 µg/g Se) were within the certified range (1.0-1.2 µg/g Se) and method precision for these 11 replicate tissue analyses was 4.2% relative standard deviation. The limit of detection was 0.015 µg/g selenium.

Statistical Analyses

All statistical analyses were performed using procedures in the Statistical Analysis System (SAS Institute 1990) software package. Growth data for adult fish were analyzed using a split plot in space and time analysis of variance (ANOVA), with treatment as the main plot, sex as the subplot within treatment, and day of study as the time variable, along with all possible interactions (Steel and Torrie 1980). Condition factor (K) was calculated by the formula of Anderson and Gutreuter (1983) as follows:

$$K = \frac{\text{weight (g)} \times 100,000}{\text{total length (mm)}^3}$$

Reproduction data for the females and subsequent biological data for the resulting progeny were analyzed as a 3 x 2 factorial ANOVA with 3 levels of diet treatment and 2 levels of water treatment. Selenium residue data for adult muscle plugs were analyzed using a split plot in time ANOVA, with treatment and sample day as the main effects. When significant F values were obtained, differences between pairs of means were determined with Fisher's least significant difference (LSD) test.

Pearson's correlation coefficients were calculated to explore for possible relations among

size (total length, weight, and condition factor) of the female, number of eggs expressed, egg diameter, and egg weight; and among hatchability, average day to hatch, incidence of larval abnormalities, survival and growth of larvae, overall progeny survival, parental dietary and waterborne selenium concentrations, and selenium tissue residues. Data expressed as percentages were arcsine-square root transformed prior to analysis. Stepwise regression analyses were used to determine the relations of selenium concentrations in adult muscle plugs, eggs, and larvae to dietary and waterborne selenium exposure concentrations of the adults. For correlation and regression analyses, individual spawns were the experimental unit. Statistical results were accepted as significant when $P \leq 0.05$.

RESULTS

Exposure Conditions

Mean selenium concentrations in test diets used during the exposure period were 2.18, 7.28, and 11.83 $\mu\text{g/g}$ dry weight (Table 3). Control diets used during the depuration period contained 2.20 $\mu\text{g/g}$ selenium dry weight. Mean (SD) percent moisture in the diets were 22.3 (0.3)% for the control diets (exposure and depuration periods, $n=14$) and 20.5 (0.3)% for the selenium-fortified diets (low and high selenium diets, $n=10$). When the mean selenium concentration in the control diet was subtracted from the measured values in the two selenium-fortified diets, selenium concentrations in these diets were within 3.5% of nominal values.

During the exposure period, mean waterborne selenium concentrations in treatments with control water and selenium-fortified diets (treatments 3 and 5) were about 9-12 times higher than that in the control treatment (treatment 1). In treatments with selenium-spiked water (treatments 2, 4, and 6), mean measured selenium concentrations were 1.1-1.6 times higher than the nominal

Table 3. Measured selenium (Se) concentrations (mean and SD in parentheses) in test diets and water used to expose adult Colorado pikeminnow.

Treatment ID	Nominal Se concentration		Measured Se during exposure period		Measured Se during depuration period	
	Diet ($\mu\text{g/g}$) ^a	Water ($\mu\text{g/L}$) ^b	Diet ($\mu\text{g/g}$)	Water ($\mu\text{g/L}$)	Diet ($\mu\text{g/g}$)	Water ($\mu\text{g/L}$)
1	0	0	2.18 (0.07)	0.15 (0.09)	2.20 (0.06)	0.21 (0.17)
2	0	5.0	2.18 (0.07)	5.44 (0.56)	2.20 (0.06)	1.37 (1.80)
3	5.0	0	7.28 (0.05)	1.28 (0.72)	2.20 (0.06)	0.26 (0.11)
4	5.0	5.0	7.28 (0.05)	5.72 (1.40)	2.20 (0.06)	0.27 (0.16)
5	10.0	0	11.83 (0.34)	1.73 (1.00)	2.20 (0.06)	0.32 (0.14)
6	10.0	5.0	11.83 (0.34)	7.94 (1.51)	2.20 (0.06)	0.32 (0.27)
N			5	15	9	8

^aDry weight.

^bTotal selenium as a 6:1 ratio of selenate:selenite.

value (5 µg/L). These results indicated that the diets and possibly the feces and urine were indirectly adding selenium to the water.

During the depuration period, waterborne selenium concentrations in all tanks were at or below 0.90 µg/L within 11 days, except for two replicates of treatment 2. Measured selenium concentrations in these replicate tanks were 1.55 and 5.65 µg/L at day 11 of depuration and 0.95 and 1.25 µg/L at day 25 of depuration. At 60 and 90 days of depuration, selenium concentrations in the replicate with 5.65 µg/L at day 11 of depuration were at or below 0.75 µg/L. Selenium concentrations in the other replicate tank of treatment 2 for the same periods were 0.20-0.30 µg/L. The reason for these high selenium values in two replicates of treatment 2 is not known, but it seems likely that there was reduced water flow from the diluter to these tanks during the first month of the depuration period.

Water Quality

Mean water quality characteristics in exposure tanks during the adult exposure were within 10% of measured values in the blending tanks, except for alkalinity, chloride, and conductivity (Table 1). The high chloride and conductivity values in the adult exposure water, compared to those in blending tanks, may be partly due to the dissolution of uneaten food as the diets were formulated to contain 2.57% NaCl by weight (T. Brown, BioProducts, personal communication). The reason for the reduced alkalinity in the adult tanks compared to the blending tanks is not known. Typically, DO concentrations decreased after feeding began and remained below the prefeeding concentrations until the tanks were siphoned and filtered.

Mean (SD) concentrations of measured solids in adult exposure water were 19.8 (10.3) mg/L for total suspended solids, 2.8 (6.6) mg/L for fixed suspended solids, and 17.2 (10.6) for volatile suspended solids. Mean (SD) turbidity of the same water was 6.9 (3.7) NTU. These

parameters were highly variable, as evidenced by the large coefficients of variation (mean/SD x 100) that ranged from 52 to 236%. The variation in these parameters was partly due to differences in the amount of food added to the tanks and in daily cleaning efficiencies (siphoning and filtering). Any direct adverse effects of suspended solids on the fish were believed to be minimal because measured concentrations in the tanks (range 4-77 mg/L) were below concentrations considered to be a protective of salmonids against gill damage (80-100 mg/L, Wedemeyer 1996).

For the larval exposures, all measured parameters, except for chloride, magnesium, and conductivity, were within 10% of measured values in the blending tanks (Table 1). The high chloride, magnesium, and conductivity values, relative to those in blending tanks, were probably due to the high salt concentration of the brine shrimp solution fed to the larvae. Brine shrimp were hatched and cultured in reconstituted sea water at about 35 g/L salinity, prepared by dissolving Instant Ocean (Aquarium Systems, Mentor, OH) dehydrated sea salts in deionized water.

Adult Exposure

Mortality

One male and eight females died while they were at our laboratory from December 16, 1996 to September 21, 1997 (Appendix B). However, there were no mortalities during the exposure phase of the study. The one male mortality occurred during the acclimation period, 16 days before the study began. All female mortalities occurred during or after the depuration phase of the study. Among the female mortalities, three were spawners and five were nonspawners. The mortality of the spawners was probably due to handling stress associated with artificial fertilization, because all three females died within 6 days after being spawned. One of the

nonspawners died because it had jumped out of the tank during the night. The mortality of three nonspawners was probably due to complications from surgery, two of the fish received radio transmitters and the other fish was a sham control for the surgical implantation procedure. The last mortality occurred 8 days after the study ended and may have been partly due to an injury it received during the last growth sampling period. These results indicated that the dietary and waterborne selenium concentrations tested did not adversely affect the survival of adult Colorado pikeminnow.

Initial size

Analysis of growth data at day 0 showed that there were no significant differences in initial weight, total length, or condition factors of adults among treatments (Table 4). Size differences between the sexes were obvious and significant; females were about 1.7 times heavier ($F=71.95$, $P<0.01$) and 1.2 times longer ($F=141.13$, $P<0.01$) than the males. Average condition factors at day 0 were also significantly higher in females ($F=8.14$, $P=0.04$) compared to the males.

Growth

Analysis of growth data revealed that there were no differences in weight, total length, or condition factors of adults among treatments and no significant interaction of treatment with sex, day, or sex and day for these growth parameters during the 245 day study (Table 5). However, there was a significant effect of sex and day on all three growth parameters and there was a significant interaction between sex and day for weight. This interaction indicated that changes in weight over time were different for females and males. Because there were no treatment effects or any significant interactions with treatment, the data were pooled across treatments to examine differences in growth over time. Mean growth metrics for fish in each treatment are given in Appendices C-E.

Table 4. Initial size and condition (mean and SD in parentheses)^a of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

ID	Selenium treatment ^b		Total length (mm)		Weight (g)		K ^c	
	Diet (µg/g)	Water (µg/L)	Female	Male	Female	Male	Female	Male
1	2.18	0.15	658 (39)	567 (25)	2,147 (527)	1,338 (248)	0.74 (0.06)	0.73 (0.04)
2	2.18	5.44	648 (24)	586 (34)	2,172 (342)	1,516 (297)	0.79 (0.06)	0.75 (0.03)
3	7.28	1.28	704 (58)	589 (38)	2,878 (1,069)	1,440 (294)	0.80 (0.07)	0.70 (0.06)
4	7.28	5.72	676 (45)	568(25)	2,437 (499)	1,307 (141)	0.78 (0.06)	0.71 (0.02)
5	11.83	1.73	669 (55)	571 (17)	2,494 (770)	1,443 (137)	0.81 (0.06)	0.78 (0.08)
6	11.83	7.94	688 (33)	602 (6)	2,440 (491)	1,624 (104)	0.74 (0.07)	0.74 (0.05)

^an=6 for females and n=3 for males, except in treatment 2 where n=5 for females and n=4 for males.

^bMean measured concentrations with diet concentrations based on dry weight.

^cCondition factor, calculated according to Anderson and Gutreuter (1983)

Table 5. Results of split plot in space and time analysis of variance (ANOVA) comparing growth of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Source of variation ^a	Growth parameter					
	Total length		Weight		Condition factor	
	F ratio	P value	F ratio	P value	F ratio	P value
Treatment (5) ^b [10] ^c	1.58	0.25	0.86	0.54	1.34	0.32
Sex (1) [12]	133.06	<0.01	112.57	<0.01	5.92	0.03
Day (8) [16]	30.56	<0.01	13.22	<0.01	13.18	<0.01
Treatment x Sex (5) [12]	0.93	0.50	1.85	0.18	1.69	0.21
Treatment x Day (40) [80]	0.67	0.92	0.54	0.98	0.74	0.86
Sex x Day (8) [96]	1.88	0.07	4.56	<0.01	1.92	0.07
Treatment x Sex x Day (40) [96]	0.56	0.98	0.94	0.58	1.35	0.12

^aTreatment = dietary and waterborne selenium concentrations, see Table 3; Day = day of study when growth was measured, see text; Sex = male or female.

^bDegrees of freedom.

^cDegrees of freedom for Mean Square error term.

Weights of females at days 30, 58, 184, 215, and 245 (but not at days 90, 120, and 153) were significantly lower than those at day 0, and their weight at day 245 was lower than those at all other days (Table 6). These results showed that females lost weight during the first 58 days of exposure and during the depuration period following the spawning trials. For males, only mean weight at day 245 was lower than that at test initiation. Regardless of sex, none of the fish exhibited a significant weight gain during the study relative to day 0. At day 153, only 16 of 35 (46%) females and 10 of 19 (53%) males had weights numerically equal to or higher than those at test initiation. Differences in body weight at day 153 compared to day 0 ranged from -7.5 to 17.1% in females and -13.8 to 12.2% in males.

Differences in total length of males and females over time were small, but were statistically significant (Table 6). In both sexes, total length generally increased from day 58 to day 120 of exposure. However, the largest average increase in length from day 0 was only 10 mm for females and 8 mm for males. Moreover, some of the variation in total length may be partly due to errors in measuring the fish. The fish were not anesthetized during growth sampling, and when handled, some fish would struggle continuously and were not in a relaxed condition on the measuring board. This factor was probably not a source of error in measuring body weights, because only stable readings (usually obtained within 10 seconds) were recorded.

Condition factors for both sexes at all sample days were significantly lower than those at day 0, except for males at days 30 and 153 (Table 6). As was observed for weight, the magnitude of reduction in condition factors during the depuration period compared to day 0 was greater in females than in males. Although some of the variation in condition factors may be partly attributable to errors in measuring total length (discussed above), the effect of these errors on the trends observed was believed to be minimal.

Table 6. Size and condition (least square means pooled across treatment)^a of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Day of study	Total length (mm)		Weight (g)		K ^b	
	Female	Male	Female	Male	Female	Male
0	674 ^C	581 ^D	2,427 ^A	1,448 ^A	0.776 ^A	0.734 ^A
30	675 ^C	581 ^D	2,328 ^B	1,414 ^{AB}	0.744 ^C	0.717 ^{AB}
58	674 ^C	581 ^D	2,328 ^B	1,392 ^{AB}	0.745 ^C	0.705 ^{BC}
90	679 ^B	584 ^{BCD}	2,414 ^A	1,430 ^{AB}	0.755 ^{BC}	0.715 ^B
120	684 ^A	589 ^A	2,454 ^A	1,455 ^A	0.751 ^{BC}	0.711 ^{BC}
153	679 ^B	584 ^{CD}	2,429 ^A	1,437 ^{AB}	0.763 ^B	0.721 ^{AB}
184 ^c	680 ^B	587 ^{ABC}	2,322 ^B	1,407 ^{AB}	0.726 ^D	0.695 ^{CD}
215 ^c	684 ^A	589 ^A	2,295 ^B	1,397 ^{AB}	0.705 ^E	0.682 ^{DE}
245 ^d	674 ^C	588 ^{AB}	2,116 ^C	1,366 ^B	0.682 ^F	0.672 ^E
SE ^e	1.1	1.6	21	28	0.005	0.006
n	35	19	35	19	35	19

^aWithin a column, means with the same upper case letter are not significantly different ($P \leq 0.05$).

^bCondition factor, calculated according to Anderson and Gutreuter (1983).

^cFor females at days 184 and 215; SE = 1.2 mm for total length, 22 g for weight, and 0.005 for K, n=32.

^dFor females at day 245; SE = 1.3 mm for total length, 24 g for weight, and 0.005 for K, n=29.

^ePooled standard error of least square mean.

Due to the significant effect of day on growth, absolute growth rates (AGR) were calculated for each exposure period and analyzed using the same ANOVA procedures described above. Absolute growth rates based on body weight (AGR-W, g/d) and total length (AGR-L, mm/d) were calculated for each period according to Ricker (1979) as follows:

$$\text{AGR-W (g/d)} = \frac{W_j - W_i}{D_j - D_i} \qquad \text{AGR-L} = \frac{L_j - L_i}{D_j - D_i}$$

where W_j = body weight at end of period, W_i = body weight at beginning of period, L_j = total length at end of period, L_i = total length at beginning of period, D_j = day of study at end of period, and D_i = day of study at beginning of period.

Absolute growth rates based on body weight varied significantly among exposure periods ($F=5.16$, $P<0.01$), but not among treatments ($F=0.16$, $P=0.97$). As was observed for weight, AGR-W differed between sexes ($F=6.59$, $P=0.02$) and there was a significant interaction between sex and day ($F=2.70$, $P=0.01$). Because there was no effect of treatment, AGR-W were pooled across treatments (Table 7). Mean AGR-W for fish in each treatment are given in Appendix F. Adults of both sexes exhibited negative growth during the first 30 days of the study (mean AGR-W, -3.31 and -1.12 g/d) and during the depuration period (mean AGR-W, -2.57 to -0.30 g/d; Table 7). The largest mean AGR-W for both sexes occurred from days 58 to 90 (1.18 and 2.67 g/d) and following this peak, the AGR-W in females decreased significantly to day 184. For three of eight periods, average daily weight loss in females was at least 1.5 g/d more than in males and these differences were significant or close to being significant. The ability to detect statistical differences in AGR-W at the 5% level of probability was probably hampered by the large variability in the data; differences between minimum and maximum AGR-W ranged from 10.82 to 43.79 g/d for females and 3.40 to 5.77 g/d for males.

Table 7. Absolute growth rate (least square means pooled across treatment)^a based on total length (AGR-L) and body weight (AGR-W) of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Exposure period (d)	AGR-L ^b (mm/d)		AGR-W ^c (g/d)		P value ^d
	Female	Male	Female	Male	
0-30	0.02	<0.01	-3.31 ^D	-1.12 ^C	0.01
30-58	-0.01	0.01	0.01 ^C	-0.79 ^{BC}	0.31
58-90	0.14	0.09	2.67 ^A	1.18 ^A	0.06
90-120	0.18	0.15	1.32 ^B	0.83 ^{AB}	0.53
120-153	-0.16	-0.15	-0.74 ^C	-0.54 ^{ABC}	0.80
153-184 ^e	0.06	0.10	-2.54 ^D	-0.99 ^C	0.06
184-215 ^e	0.14	0.09	-0.87 ^C	-0.30 ^{ABC}	0.48
215-245 ^f	-0.08	-0.04	-2.57 ^D	-1.05 ^C	0.07
SE ^g	0.016	0.022	0.462	0.627	
n	35	19	35	19	

^aAbsolute growth rates for each period calculated according to Ricker (1979).

^bDifferences in AGR-L between sexes were not significant; see text and Appendix 7 for mean comparisons.

^cFor each sex, AGR-W values with the same upper case letter are not significantly different ($P \leq 0.05$).

^dProbability values for least square mean comparisons between sexes.

^eFor females at period from day 153-184 and day 184-215; pooled SE = 0.017 mm/d for AGR-L and 0.497 g/d for AGR-W, n=32.

^fFor females at period from day 215-245; pooled SE = 0.018 mm/d for AGR-L and 0.530 g/d for AGR-W, n=29.

^gPooled standard error of least square means.

There were no differences in AGR-L among treatments ($F=1.08$, $P=0.43$) or between sexes ($F=0.35$, $P=0.56$), but AGR-L differed among exposure periods ($F=36.45$, $P<0.01$) and there was a significant interaction between treatment and period ($F=2.04$, $P=0.01$), which indicated that changes in AGR-L over time were not the same among treatments. Consequently, LSD comparisons of AGR-L were made using treatment means pooled across sex, rather than mean AGR-L for each sex pooled across treatments as is given in Table 7. Results of these comparisons showed that although the rank order of AGR-L varied among treatments (Appendix G), the largest AGR-L for fish in each treatment occurred between days 90 and 120 (range 0.10 to 0.22 mm/d) and the smallest or second smallest AGR-L occurred during the following period from days 120 to 153 (range -0.20 to -0.10 mm/d; Appendix G).

Reproductive response

Only 10 of 35 females (29%) spawned and spawning success was highly variable among treatments. The control and treatments 2 and 6 had only one female spawn, treatments 4 and 5 had two females spawn, and the treatment 3 had three females spawn. It was highly unlikely that any of the females spawned in the exposure tanks because no eggs were ever observed during daily siphoning and filtering procedures. All males expressed milt during the spawning trials, but 6 of 19 males (32%) expressed less than 20 ml, compared to at least 50 ml for the other 13 males. Results of the factorial ANOVA tests did not detect any significant treatment effects (dietary selenium, waterborne selenium, and diet and water interaction) on number of eggs expressed, egg size (diameter and weight), hatchability, time to hatch, or on survival, growth, and deformities of resulting larvae (Appendix H). However, the reproductive data are problematic due to the lack of replicate spawns for half of the treatments. The Mean Square error term in the ANOVA tables had only two degrees of freedom and the power of these tests

(which is the probability of detecting a difference when one really exists) was close to zero (M. Ellersieck, University of Missouri-Columbia, written communication). Inferences drawn from tests with such low power are highly questionable and extreme caution is advised when making interpretations of these data. (Zar 1996, M. Ellersieck, University of Missouri-Columbia, written communication). Consequently, we do not believe that the reproductive data are complete enough to draw unequivocal conclusions concerning the effects of the dietary and waterborne selenium exposures tested in this study on reproduction of Colorado pikeminnow.

For the 10 female spawners, number of eggs expressed per female averaged 95,171 and ranged from 57,874 to 121,414 (Table 8). The average (and range) number eggs per gram body weight was 37 (22-46) and the average (and range) number of eggs per millimeter total length was 140 (84-165). Egg diameter and egg weight averaged 2.43 mm and 5.9 mg and ranged from 2.36 to 2.53 mm and 5.3 to 6.6 mg, respectively. There were no significant correlations between the size of female (total length or weight) and number of eggs per female or egg diameter. Egg weight was correlated with total length of female ($r=0.622$, $P=0.05$) and was nearly correlated with weight of the female ($r=0.620$, $P=0.06$). Neither egg diameter nor egg weight was correlated with number of eggs per female. As expected, egg diameter was correlated with egg weight ($r=0.799$, $P=0.01$). None of these measures of reproduction were correlated with selenium exposure concentrations of the female parent.

Due to problems with the egg incubation system, most of the eggs not used in the hatchability study were lost before viability measurements were made. Consequently, fertilization rates and total egg viability of the spawns could not be estimated.

Table 8. Reproduction data for Colorado pikeminnow females spawned at day 154 of exposure to selenium in the diet and water.

Fish ID ^c	Selenium treatment ^a		Size at day 153			Reproductive output			Egg ^b	
	Diet (µg/g)	Water (µg/L)	Weight (g)	Total length (mm)	K ^d	Eggs per female	Eggs per g body weight	Eggs per mm total length	Diameter (mm)	Weight (mg)
1A-2	2.18	0.15	1,974	632	0.78	85,733	43	136	2.37 (0.12)	5.3 (0.4)
2A-10	2.18	5.74	2,686	668	0.90	72,227	27	108	2.42 (0.08)	5.6 (0.1)
3A-20	7.28	1.21	2,717	702	0.79	98,141	36	140	2.36 (0.16)	5.8 (0.2)
3B-22	7.28	1.02	2,367	662	0.82	103,392	44	156	2.37 (0.12)	5.6 (0.1)
3C-26	7.28	1.62	2,463	688	0.76	109,850	45	160	2.49 (0.12)	5.7 (0.2)
4A-29	7.28	5.95	2,123	631	0.85	97,578	46	155	2.41 (0.11)	5.7 (0.2)
4B-31	7.28	5.82	2,628	692	0.79	57,874	22	84	2.51 (0.16)	6.6 (0.4)
5A-38	11.83	2.38	2,372	666	0.80	107,342	45	161	2.46 (0.13)	6.0 (0.1)
5B-41	11.83	1.63	3,605	734	0.91	121,414	34	165	2.53 (0.12)	6.5 (0.4)
6B-50	11.83	8.12	3,120	723	0.83	98,155	31	136	2.42 (0.14)	5.8 (0.2)

^aMean measured concentrations in exposure tank with diet concentrations based on dry weight.

^bMean and SD in parentheses, n=25 for egg diameter and n=3 for pooled egg weight.

^cPrefix denotes treatment ID (1 to 6, see Table 3) and replicate tank (A to C).

^dCondition factor, calculated according to Anderson and Gutreuter (1983).

Progeny

Hatchability and abnormalities

Hatchability for eight of the 10 spawns was 87% or higher and averaged about 84% across all spawns (Table 9). The low hatchability of 43% for eggs produced by female 4B-31 was due primarily to a fungal infection that occurred in all four egg cups at 4 days postspawn. Average time to hatch ranged from 4.2 to 4.7 days and over 99% of the eggs hatched at 4 or 5 days postspawn. Survival of the resulting larvae during the hatching period was greater than 95%. There were no significant correlations among hatchability, time to hatch, larval survival, and adult selenium exposure concentrations. Moreover, hatchability was not correlated with number of eggs per female or size of the eggs.

The incidence of larvae with abnormalities ranged from 7.0 to 19.3% and was not correlated with adult selenium exposure, hatchability, or larval survival (Table 9). The lowest incidence of abnormalities occurred in larvae hatched from the group of eggs infected with fungus. This observation indicated that embryos with deformities may have been more sensitive than normal embryos to the fungus infection. The most commonly observed abnormalities were scoliosis (lateral curvature of spine) and cardiac edema, which occurred in 9.4 and 6.2% of the larvae, respectively. Other abnormalities observed (% occurrence in larvae) included abdominal edema (1.5%), microcephaly (abnormally small head, 1.5%), microphthalmia (abnormally small eyes, 1.1%), and kyphosis (dorsal curvature of spine, 0.7%).

Larval survival and growth

Survival of larvae after 30 days ranged from about 73 to 98% and averaged about 87% across all spawns (Table 10). Most of the mortalities (62% of all mortalities) occurred within 5

Table 9. Hatchability, survival, and abnormalities of Colorado pikeminnow eggs and larvae produced by adults exposed to selenium in the diet and water for 154 days prior to spawning.

Female ID ^b	Adult selenium exposure ^a		Progeny			
	Diet (µg/g)	Water (µg/L)	Hatch of fertile eggs (%)	Average day to hatch	Larval survival (%) during hatch	Larvae with abnormalities (%)
1A-2	2.18	0.15	87.0	4.7	100	12.6
2A-10	2.18	5.74	94.0	4.3	98.9	12.8
3A-20	7.28	1.21	97.0	4.7	100	12.4
3B-22	7.28	1.02	87.9	4.2	97.7	13.8
3C-26	7.28	1.62	69.3	4.7	95.7	12.9
4A-29	7.28	5.95	93.0	4.7	97.8	12.9
4B-31	7.28	5.82	43.0 ^c	4.3	100	7.0
5A-38	11.83	2.38	88.0	4.3	100	19.3
5B-41	11.83	1.63	91.0	4.5	100	12.1
6B-50	11.83	8.12	91.0	4.2	100	12.1

^aMean measured concentrations in exposure tank with diet concentrations based on dry weight.

^bPrefix denotes treatment ID (1 to 6, see Table 3) and replicate tank (A to C).

^cEgg mortality due to fungus infection.

Table 10. Survival and growth of Colorado pikeminnow larvae produced by adults exposed to selenium in the diet and water for 154 days prior to spawning.

Female ID ^c	Adult selenium exposure ^a		Larvae							Overall progeny survival ^e (%)
	Diet (µg/g)	Water (µg/L)	Survival (%) at			Growth ^b				
			Swimup (Day 5)	Day 10	Day 30	Total length (mm)	Weight (mg)	K ^d	n	
1A-2	2.18	0.15	100	83.3	80.0	20.1 (1.1)	55.3 (7.1)	0.68 (0.06)	44	69.6
2A-10	2.18	5.74	98.3	98.3	95.0	19.6 (1.1)	53.3 (6.9)	0.71 (0.08)	51	88.4
3A-20	7.28	1.21	100	96.7	93.3	19.7 (1.4)	50.6 (9.1)	0.65 (0.06)	53	90.5
3B-22	7.28	1.02	95.0	93.3	91.7	19.6 (1.3)	53.2 (7.6)	0.70 (0.07)	52	78.8
3C-26	7.28	1.62	88.3	85.0	85.0	19.4 (1.0)	51.1 (7.8)	0.69 (0.06)	47	56.4
4A-29	7.28	5.95	98.3	93.3	90.0	20.0 (0.8)	54.3 (7.2)	0.68 (0.05)	52	81.9
4B-31	7.28	5.82	97.5	97.5	97.5	20.2 (0.9)	56.1 (7.0)	0.68 (0.05)	37	41.9
5A-38	11.83	2.38	100	78.3	78.3	19.9 (0.9)	52.1 (6.7)	0.66 (0.05)	45	68.9
5B-41	11.83	1.63	98.3	78.3	73.3	20.3 (1.1)	58.8 (9.3)	0.70 (0.06)	39	66.7
6B-50	11.83	8.12	95.0	86.7	85.0	19.8 (1.1)	52.6 (8.0)	0.68 (0.06)	42	77.4

^aMean measured concentrations in exposure tank with diet concentrations based on dry weight.

^bMean and SD in parentheses.

^cPrefix denotes treatment ID (1 to 6, see Table 3) and replicate tank (A to C).

^dCondition factor, calculated according to Anderson and Gutreuter (1983).

^eProduct of percent survival of embryos at the time when larvae were stocked into growth chambers times percent survival of larvae at 30 days.

days after the larvae had reached the swim-up stage (days 6-10 of larval study). In comparison, about 22% of all mortalities occurred during the first 5 days after hatch and about 17% of all mortalities occurred during the remainder of the test. Growth of larvae after 30 days was similar for all groups; differences in mean total length, weight, and condition factor of larvae across all spawns were ≤ 1.2 fold. The range of mean condition factors for larvae (0.65-0.71) overlapped that of the adult males at test initiation (0.70-0.78, Table 4). Larval survival at 30 days and growth were not correlated with each other or with parental selenium exposure.

Overall survival of the progeny from the nine spawns not infected with fungus ranged from about 56 to 90% (Table 10). For these spawns, differences in survival between embryos (eggs and newly hatched larvae in egg cups) and larvae (in growth chambers) were ≤ 1.3 -fold and these differences were not statistically significant (paired T-test; $T=0.746$, $P=0.477$). The low overall survival of progeny from the spawn infected with fungus (42%, from female 4B-31) resulted from the high fungus-induced mortality of the eggs (57%).

Selenium residues

Muscle plugs

Although muscle plugs were taken from all females at each sample period, only muscle plugs from one female in each tank (which included the 10 spawners) were analyzed, except for one replicate of treatment 2. For treatment 2, muscle plugs from the misidentified male were analyzed. For one replicate of treatments 3 and 5, muscle plugs from the other female in these tanks were analyzed for selenium at days 153, 184, 215, and 245 due to mortalities of the spawners during the depuration period.

Analysis of variance testing of selenium residues in muscle plugs (Table 11) revealed a significant treatment effect ($F=5.31$, $P=0.01$), significant time effect ($F=33.53$, $P<0.01$), and

Table 11. Selenium concentrations (least square means, $\mu\text{g/g}$ dry weight^a) in muscle plugs of adult female (and one male in treatment 2) Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Selenium treatment ^b			Day of study ^c								
Treatment ID	Diet ($\mu\text{g/g}$)	Water ($\mu\text{g/L}$)	0	30	58	90	120	153	184	215	245
1	2.18	0.15	1.2 ^A _w	1.2 ^B _w	1.2 ^C _w	1.3 ^C _w	1.2 ^C _w	1.2 ^D _w	1.2 ^E _w	1.3 ^D _w	1.2 ^C _w
2	2.18	5.44	1.1 ^A _w	1.2 ^B _w	1.2 ^C _w	1.2 ^C _w	1.2 ^C _w	1.2 ^D _w	1.4 ^{DE} _w	1.4 ^D _w	1.2 ^C _w
3	7.28	1.28	1.1 ^A _y	1.1 ^B _y	1.4 ^{BC} _{xy}	1.8 ^B _{wx}	1.9 ^B _w	1.9 ^C _w	1.7 ^{CD} _{wx}	1.9 ^C _{wx}	1.7 ^{BC} _{wx}
4	7.28	5.72	1.3 ^A _z	1.3 ^{AB} _z	1.5 ^{ABC} _{yz}	1.9 ^B _{xy}	2.2 ^B _{wx}	2.1 ^{BC} _{wx}	2.2 ^{BC} _{wx}	2.4 ^{BC} _w	2.1 ^B _{wx}
5	11.83	1.73	1.1 ^A _z	1.7 ^A _y	1.9 ^A _y	2.8 ^A _x	3.5 ^A _w	3.5 ^A _w	3.5 ^A _w	3.8 ^A _w	3.5 ^A _w
6	11.83	7.94	1.0 ^A _z	1.1 ^B _z	1.7 ^{AB} _y	2.0 ^B _{xy}	2.3 ^B _{wx}	2.5 ^B _w	2.3 ^B _{wx}	2.4 ^B _{wx}	2.1 ^B _{xy}

^aPooled SE = 0.16 $\mu\text{g/g}$ and n=3 for all means, except for treatment 2 at all days (pooled SE = 0.19 $\mu\text{g/g}$, n=2), treatments 3 and 5 at day 153 (pooled SE = 0.14 $\mu\text{g/g}$, n=4) and treatment 3 at days 184, 215, and 245 (pooled SE = 0.20 $\mu\text{g/g}$, n=2).

^bMean measured concentrations for exposure period with diet concentrations based on dry weight.

^cMeans within a column sharing the same upper case letter are not significantly different ($P \leq 0.05$) and means within a row sharing the same lower case letter are not significantly different ($P \leq 0.05$).

significant interaction between treatment and day ($F=5.12$, $P<0.01$). The interaction of treatment and day indicated that selenium accumulation in muscle tissue of females over time was not the same across treatments. Multiple mean comparisons showed that muscle plug selenium concentrations in fish exposed to treatment 2 were similar to those of control fish (treatment 1) at all sample periods. At day 58 of exposure, selenium concentrations in muscle plugs of females exposed to the high selenium-fortified diet with or without selenium-spiked water were significantly greater than those of the controls. From day 90 to the end of the study, muscle plug selenium concentrations in females exposed to either selenium-fortified diet with or without selenium-spiked water were higher than those in control fish, except for fish in treatment 3 at day 245. Ranges in female muscle plug selenium concentrations after 153 days of exposure were 1.1 to 1.3 $\mu\text{g/g}$ for treatment 1, 1.2 to 1.3 $\mu\text{g/g}$ for treatment 2, 1.5 to 2.1 $\mu\text{g/g}$ for treatment 3, 2.0 to 2.3 $\mu\text{g/g}$ for treatment 4, 2.5 to 5.2 $\mu\text{g/g}$ for treatment 5, and 2.0 to 3.0 $\mu\text{g/g}$ for treatment 6. Selenium concentrations in muscle plugs of the one male fish from treatment 2 ranged from only 1.2 to 1.4 $\mu\text{g/g}$ across all sample days.

For fish fed the low selenium-fortified diets, there were no differences in muscle plug selenium concentrations between the control water and selenium-spiked water treatments at any of the sample periods (Table 11). For the high selenium dietary treatments, fish exposed to control water had higher muscle plug selenium concentrations than those exposed to 7.94 $\mu\text{g/L}$ selenium in the water from day 90 to the end of the study. These results indicated that waterborne selenium exposures of about 5-8 $\mu\text{g/L}$ for 153 days did not significantly influence selenium accumulation in epaxial muscle at dietary selenium concentrations of 2.18-11.83 $\mu\text{g/g}$.

Additional analysis using time-weighted average selenium exposure concentrations for

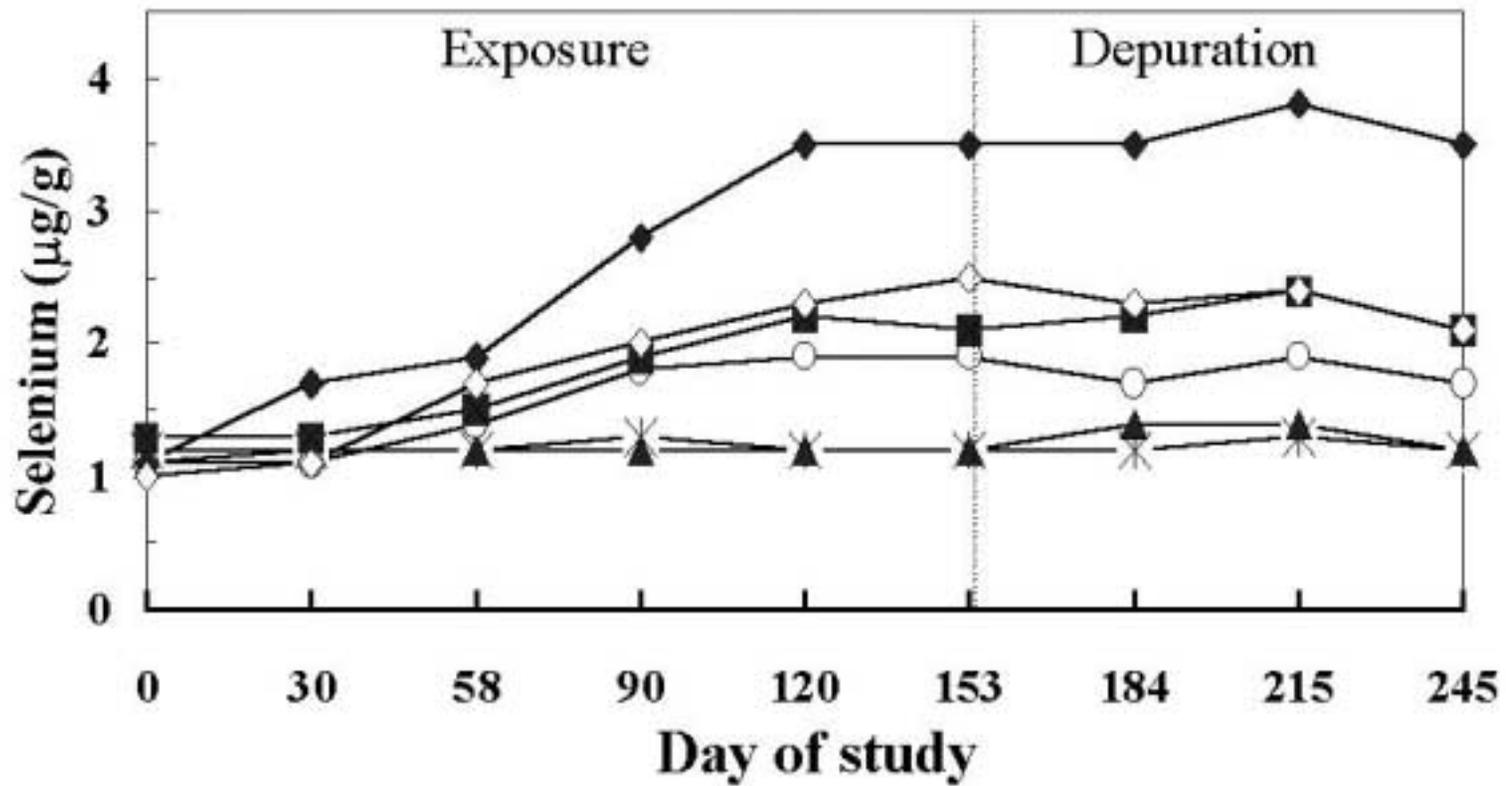
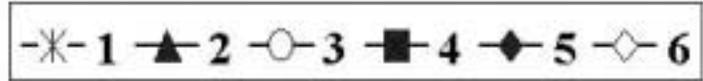
each exposure period and tank revealed that selenium concentrations in muscle plugs at days 58, 90, 120, and 153 were correlated with selenium in the diet ($r= 0.657-0.759$, $P<0.01$, $n=17-19$), but not with selenium in the water. The relation between selenium residues (based on dry weights) in muscle tissue of adult females at day 153 and mean dietary concentrations was described by the following equation ($r^2=0.573$, $P<0.01$, $n=19$): Muscle plug selenium ($\mu\text{g/g}$) = $0.755+ 0.184 \times$ dietary selenium ($\mu\text{g/g}$).

To better examine selenium residue dynamics in these fish, the time course of selenium accumulation was plotted (Figure 2). In contrast to females exposed to the control diets (treatments 1 and 2), selenium accumulation in muscle tissue appeared to reach a plateau at 90 days in females exposed to treatment 3 and at 120 days in females exposed to the treatments 4, 5, and 6. With the exception of fish from treatment 6, muscle plug selenium concentrations at day 245 (90 days of depuration) were similar to those at day 153 (Table 11). These results indicated that the loss of selenium from muscle tissue was minimal after 90 days of depuration.

Bioaccumulation factors (BAF, Rand 1995) for selenium in muscle tissue of individual fish at day 153 (compared to the diet) were considerably lower than unity, ranging from 0.17 to 0.60 and were negatively correlated with dietary selenium concentrations ($r=-0.821$, $P<0.01$, $n=19$). Mean selenium BAF for females exposed to the control diets (0.55-0.57) were about twice as high as those for females exposed to the two selenium-fortified diets (0.21-0.29). Bioconcentration factors (i.e., ratio of tissue to water selenium concentrations) were not calculated because the accumulated selenium residues in muscle were due primarily to selenium in the diet.

Figure 2. Mean selenium concentrations ($\mu\text{g/g}$ dry weight, $n=3$) in muscle plugs from adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period. Selenium treatment designations (diet:water) based on measured concentrations are: 1 = 2.18 $\mu\text{g/g}$:0.15 $\mu\text{g/L}$; 2 = 2.18 $\mu\text{g/g}$:5.44 $\mu\text{g/L}$; 3 = 7.28 $\mu\text{g/g}$:1.28 $\mu\text{g/L}$; 4 = 7.28 $\mu\text{g/g}$:5.72 $\mu\text{g/L}$; 5 = 11.83 $\mu\text{g/g}$:1.73 $\mu\text{g/L}$; 6 = 11.83 $\mu\text{g/g}$:7.94 $\mu\text{g/L}$.

Treatments



Eggs and larvae

Selenium concentrations in eggs and newly hatched larvae produced by females exposed to the two selenium-fortified diets were 2.9-5.4 times higher than those in muscle plugs of the female parent at day 153 of exposure (Table 12). In contrast, selenium concentrations in eggs and larvae from females exposed to the control diets were only 1.5-2.3 times higher than those in muscle plugs at day 153. Selenium concentrations in 1-day posthatch (dph) larvae were significantly higher than those in eggs (paired T-test, $T=4.08$, $P<0.01$, $n=8$), but the magnitude of difference was small (1.1-1.4 fold).

Whole-body selenium concentrations in larvae decreased progressively with age and the magnitude of reduction in selenium concentrations in 16- and 30-dph larvae was correlated with selenium concentrations in newly hatched larvae ($r=0.892$ and 0.850 , $P=0.02$, $n=6$ and 7 , respectively). Whole-body selenium concentrations in larvae with the highest residues at hatch (13.35 - 13.40 $\mu\text{g/g}$) were reduced by 75-76% after 30 days (3.20 - 3.30 $\mu\text{g/g}$); whereas larvae with the lowest residues at hatch (2.75 $\mu\text{g/g}$) were only reduced by 20% after 30 days (2.20 $\mu\text{g/g}$). Whole-body selenium concentrations in 30-dph larvae were within 22% of that in brine shrimp nauplii (2.7 $\mu\text{g/g}$ selenium).

There were no significant correlations between selenium residues in eggs and hatchability and deformed larvae or between selenium residues in 1-dph larvae and survival to swim-up and growth after 30 days. Overall survival was not correlated with selenium residues in eggs or 1-dph larvae. However, survival of larvae at 10 and 30 days was inversely correlated with their whole-body selenium residues at 1 dph ($r=-0.784$ and -0.726 , $P=0.02$ and 0.04 , $n=8$). The biological significance of these correlations is doubtful because of the small sample size ($n=8$) and the fact that one group of larvae with the second highest selenium residues at hatch (13.35

Table 12. Selenium concentrations in Colorado pikeminnow eggs and larvae produced by adults exposed to selenium in the diet and water for 154 days prior to spawning.

Female ID ^c	Adult selenium exposure ^a		Muscle plug at Day 153	Selenium concentration $\mu\text{g/g}^b$			
	Diet ($\mu\text{g/g}$)	Water ($\mu\text{g/L}$)		Eggs	1-dph ^d larvae	16-dph larvae	30-dph larvae
1A-2	2.18	0.15	1.2	1.80	-- ^e	--	--
2A-10	2.18	5.74	1.2	2.05	2.75	2.45	2.20
3A-20	7.28	1.21	2.1	6.55	7.65	5.25	2.55
3B-22	7.28	1.02	1.5	6.25	7.45	--	--
3C-26	7.28	1.62	1.9	6.45	7.40	5.15	2.80
4A-29	7.28	5.95	2.0	7.15	7.95	6.55	2.60
4B-31	7.28	5.82	2.3	6.70	7.55	--	2.60
5A-38	11.83	2.38	2.5	9.85	13.40	8.25	3.30
5B-41	11.83	1.63	2.9	11.45	--	--	--
6B-50	11.83	8.12	3.0	11.65	13.35	7.20	3.20

^aMean measured concentrations in exposure tank with diet concentrations based on dry weight.

^bDry weight.

^cPrefix denotes treatment ID (1 to 6, see Table 3) and replicate tank (A to C).

^ddph = days posthatch.

^eInsufficient number of fish available for analysis.

$\mu\text{g/g}$) had greater survival (85%) than the controls (80%) after 30 days.

Selenium concentrations in eggs and newly hatched larvae were strongly correlated with selenium in the diet ($r=0.988$ and 0.993 , $P<0.01$, $n=10$ and 8 , respectively) and muscle plugs ($r=0.956$ and 0.889 , $P<0.01$, $n=10$ and 8 , respectively) of the female parent, but not with selenium in water. To determine the best predictor of selenium residues in eggs and 1-dph larvae, the data were subjected to stepwise regression analysis using dietary selenium, waterborne selenium, and muscle plug selenium at day 153 as the independent variables. These analyses revealed that selenium in the diet was the best predictor of selenium residues in eggs ($r^2=0.976$, $P<0.01$, $n=10$) and larvae ($r^2=0.985$, $P<0.01$, $n=8$). Selenium residues in muscle plugs of the female parent were the second best predictor of those in eggs ($r^2=0.913$, $P<0.01$, $n=10$) and larvae ($r^2=0.791$, $P<0.01$, $n=8$). For the eggs, the addition of selenium in muscle plugs to the model only increased the r^2 (amount of variance explained) by 1.1% ($r^2=0.987$) and the addition of selenium in water to the model was not significant at $P=0.15$. For the larvae, the addition of selenium in muscle plugs and selenium in water to the model were not significant at $P=0.15$. Also, the addition of the variable selenium in eggs to the larval model was not significant.

Selenium residues (based on dry weights) in eggs were related to those in the diet and muscle tissue of the female parent by the following equations: Whole-egg selenium ($\mu\text{g/g}$) = $-0.178 + 0.940 \times \text{dietary selenium } (\mu\text{g/g})$ and whole-egg selenium ($\mu\text{g/g}$) = $-3.412 + 5.049 \times \text{muscle plug selenium } (\mu\text{g/g})$. The relation between selenium residues (based on dry weights) in 1-dph larvae and those in the parental diet and muscle tissue were described by the following equations: Whole-body selenium ($\mu\text{g/g}$) = $-0.356 + 1.130 \times \text{dietary selenium } (\mu\text{g/g})$ and whole-body selenium ($\mu\text{g/g}$) = $-2.900 + 5.497 \times \text{muscle plug selenium } (\mu\text{g/g})$.

These results demonstrated that selenium residues in progeny of Colorado pikeminnow

were attributable to dietary selenium exposures of the female parent and that waterborne selenium concentrations of 5-8 µg/L had little effect on selenium accumulation in the progeny. Selenium BAF (based on parental dietary selenium concentrations) averaged 0.91 (range, 0.83 to 0.98) for the eggs and 1.09 (range, 1.02 to 1.26) for 1-dph larvae.

DISCUSSION

To allow comparisons with published data, all selenium concentrations in diets and fish tissue are reported on a dry weight basis. Unless noted differently, selenium tissue concentrations reported on a wet weight basis with no moisture values were converted to approximate dry weight concentrations assuming 75% moisture for all tissues (wet weight concentration \div 0.25; Lemly 1993), except for ovaries, which were assumed to have 85% moisture (Gillespie and Baumann 1986).

Survival and Growth of Adults

Survival and growth of adult Colorado pikeminnow were not affected by the combined dietary and waterborne selenium exposures tested in our study. The waterborne selenium concentrations were not expected to be toxic because the nominal concentration selected was the current U.S. Environmental Protection Agency water quality criterion chronic concentration of 5 µg/L (USEPA 1987) and highest mean concentration of total selenium present in the water (8.12 µg/L, Table 8) was about one-tenth the chronic value of 88 µg/L (geometric mean of no observed and lowest observed effect concentrations of 60 and 130 µg/L based on survival) for selenite and rainbow trout (*Oncorhynchus mykiss*; USEPA 1987).

Reduced growth is usually one of the first metabolic indications of chronic stress in fish (Rand and Petrocelli 1985). However the lack of growth effects in adult fish during the study was not unexpected because of the relatively short exposure period (ca. 5 months) coupled with

slow growth rates of 16-year-old fish (R. Hamman, U.S. Fish and Wildlife Service, personal communication). Growth rates of adults in the present study were highly variable, ranging from -0.16 to 0.18 mm/d and -3.31 to 2.67 g/d (Table 7) and the negative and slow growth rates were probably related to the effects of transport and handling (discussed below in the section on spawning). Growth increments (based on total length) after 245 days (0.67 year) averaged 8.7 (range, -10 to 22) mm for females and 7.0 (range, -13 to 14) mm for males and are similar to reported annual growth increments of 11.2 mm for adults in the Green River (Tyus 1988, as cited in Tyus 1991) and 9.5 mm for fish longer than 549 mm in the Colorado River (Osmundson et al. 1997).

The lack of adverse effects on growth and survival at the dietary selenium concentrations tested in this study has also been observed in studies with reproductively mature bluegill (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*). Woock et al. (1987) reported reduced survival of adult bluegill exposed to 30 µg/g selenium in the diet (as selenite or seleno-DL-methionine) for 260 days, but did not observe these adverse effects in fish exposed to dietary selenium concentrations of 13 µg/g alone or combined with 10 µg/L selenite in the water. They also observed that fish fed the diet containing 30 µg/g selenium as selenite exhibited reduced weights and 37% of those fed the selenomethionine diet at 30 µg/g developed cataracts, which were not observed in the other test groups. Coyle et al. (1993) exposed adult bluegill to combinations of waterborne and dietary selenium for 140 days and observed no effects on survival and growth at waterborne concentrations up to 10 µg/L (as 6:1 selenate:selenite) and dietary concentrations up to 32 µg/g (as seleno-L-methionine). Ogle and Knight (1989) observed reduced weights in fathead minnow fed diets containing 20 and 30 µg/g selenium (as 25% seleno-L-methionine, 25% selenate, and 50% selenite), but not in those fed 5, 10, and 15

µg/g selenium, during the pre-spawning period.

In contrast to the adults, dietary selenium concentrations close to those tested in our study have been found to be toxic to juvenile salmonids and centrachids. Juvenile rainbow trout (0.6-1.3 g) fed diets containing 11 to 13 µg/g (as selenite) for 16-20 weeks exhibited reduced weight and survival (Hilton et al. 1980, Hicks et al. 1984). Hamilton et al. (1990) exposed swim-up fry of chinook salmon (*O. tshawytscha*) to two selenium diets (one containing naturally incorporated selenium and the other seleno-DL-methionine) and observed reduced survival at dietary selenium concentrations of ≥ 9.6 µg/g for both diets and reduced growth at ≥ 5.3 µg/g in the natural selenium diet and ≥ 18.2 µg/g in the selenomethionine diet after 90 days of exposure. Cleveland et al. (1993) reported that the dietary threshold concentration of selenium (as seleno-L-methionine) for juvenile bluegill based on reduced condition factors fell between 9 and 19 µg/g (reported as 6.5 and 13.0 µg/g wet weight, converted to dry weight assuming 31% moisture in Oregon moist diet, see Hamilton et al. 1990).

Spawning Trials

The reasons for the low number of females that ovulated in this study (10 of 35) are not known. Reproduction in fish is a complex process that is controlled by the coordinated actions of various hormones associated with the hypothalamus-pituitary-gonal axis, which in turn is regulated by sensory inputs, feedback actions of pituitary-gonadal hormones, and other hormones (Van Der Kraak et al. 1997). Each stage of the reproductive cycle, from gametogenesis to embryonic development, is stimulated and inhibited by a number of environmental variables including temperature, photoperiod, water flow, spawning substrate, and food availability (Billard et al. 1981, Donaldson 1990). Moreover, each stage is subject to the effects of stress (Donaldson 1990) and stress induced effects at one point in the reproductive

cycle may have a profound effect on reproduction success.

The low number of females that spawned in our study was probably related to the reproductive condition of the fish rather than to the procedures used to induce ovulation. In order for the injections of carp pituitary to successfully induce ovulation, other physiological conditions in the fish must be optimum for this activity (Schreck and Scanlon 1977). The artificial spawning techniques used in this study (Hamman 1981) have been used successfully in hatcheries to control reproduction of endemic cyprinids of the Colorado River basin. Hamman (1982, 1985, 1986) reported that he was able to induce ovulation in 8 of 9 (89%) humpback chub (*Gila cypha*), all 35 bonytail (*G. elegans*), and all 33 Colorado pikeminnow injected with carp pituitary and the number of eggs expressed per female averaged about 2,500 for humpback chub, 5,000 for 2-year-old bonytail, 16,500 for 3-year-old bonytail, and 72,000 for 9- and 10-year-old Colorado pikeminnow. Hamman (1982) also reported that all 7 male humpback chub used in the spawning trials produced milt with active sperm, but no data were given for males of the other two species. Due to the high value of these fish, none of the females in our study were biopsied nor necropsied to assess egg development and condition of the ovaries. However, Hamman (U.S. Fish and Wildlife Service, written communication) noted that some of the females in our study did not appear to be gravid at time of the spawning trials and would not ovulate.

The annual reproductive cycle of Colorado pikeminnow is not fully understood. Tyus (1990) suggested that ovarian development in feral females may commence 8-9 months before spawning migrations occur, with a long period of dormancy during the winter followed by maturation in the spring coinciding with warmer water, increasing flows, and altered water quality. When conditions are suitable, final maturation and spawning may occur within a short

period. Tyus (1990) also reported a personal communication with R. Hamman (U.S. Fish and Wildlife Service), in which egg development was observed in hatchery broodstock soon after spawning, which occurred in June. Based on these observations, the females in our study were probably undergoing ovarian development and the eggs may have been in the vitellogenic phase of growth at the time they were transported to our laboratory.

Based on radiotelemetry studies and recaptures of tagged fish in the Green and Yampa rivers, the reproductive cycle of Colorado pikeminnow seems to be synchronized with water flow and temperature, with spawning occurring as water flow decreased (3-8 weeks after peak discharge) and temperature increased to 19°C or higher (Tyus 1990). In addition, Tyus (1990) suggested that photoperiod may also be an important environmental cue influencing sexual maturation in these fish. In our study, the temperature and photoperiod regimes during the selenium exposure-prespawning phase were similar to those at DNFH (R. Hamman, U.S. Fish and Wildlife Service, written communication), where Colorado pikeminnow have been routinely spawned for at least 17 years.

The relative importance of fluctuating water flows on the reproductive cycle of hatchery-reared Colorado pikeminnow has not been determined independently of temperature and photoperiod. Adult Colorado pikeminnow reared in concrete raceways with recirculated water at a constant flow rate and in ponds have been spawned successfully at DNFH (R. Hamman, U.S. Fish and Wildlife Service, personal communication). The adults in our study were reared for 16 years in 0.32- and 0.41-hectare (0.8 and 1.0 acre) ponds, and fish of the same year class have been spawned successfully in 1996 and 1997 at DNFH (R. Hamman, U.S. Fish and Wildlife Service, personal communication). This information indicates that the absence of fluctuating water flows does not disrupt the reproductive cycle of hatchery-reared Colorado

pikeminnow, when the appropriate temperature and photoperiod regimes are present.

Consequently, the low spawning success of the females in our study was probably not related to these factors.

It has been shown that stresses associated with fish culture procedures such as handling, transport, and confinement can adversely affect the reproductive capacity of fish. The stresses associated with these activities may adversely affect growth and reproduction as a result of metabolic consequences or by acting directly or indirectly on the appropriate hormonal pathways (Pankhurst and Van Der Kraak 1997). Stress (usually manifested as an increase in plasma cortisol) generally results in a reduction in plasma levels of androgens and estrogens, which seems to be associated with ovarian atresia (Pankhurst and Van Der Kraak 1997). The effect of stress on metabolism is believed to be the result of energy allocation in fish; presumably a portion of the fish's energy budget is used to cope with the stress, which results in less energy available for growth and reproduction (Pankhurst and Van Der Kraak 1997). Stress related to aquaculture activity can also affect feeding behavior and result in a loss of appetite, which affects the nutrition state of the fish and may ultimately have adverse consequences for growth and reproduction (Schreck et al. 1997).

Based on the above discussion, it seems plausible that the stress associated with handling and transport of these fish from Dexter, NM, to Yankton, SD, in late November and their confinement in 1.22-m (4 ft) diameter circular tanks may have been a contributing factor to the low reproductive success in this study. Stresses induced by capture and confinement have been shown to inhibit ovulation in snapper (*Pagrus auratus*, Carragher and Pankhurst 1991) and increase the frequency of atresia in vitellogenic oocytes of red gurnard (*Chelidonichthys kumu*, Clearwater and Pankhurst 1997). The stresses associated with the transport and placement in

circular tanks of Colorado pikeminnow during a critical period of ovarian development may have disrupted or arrested egg growth and development in some of the females.

The production of eggs is an energetically expensive process, so factors that affect the nutrition state of females during vitellogenesis and maturation may have a marked effect on reproduction. During the first 58 days of the study, body weights of all but two females were lower than those at test initiation and AGR-W for females (pooled across treatments) averaged -3.31 g/d from day 0 to 30 and 0.01 g/d from day 30 to 58 (Table 7). To further investigate this effect on reproduction, we compared AGR-W between spawning and nonspawning females for each exposure period (Wilcoxon test, SAS 1990; Table 13). These comparisons revealed that during each of the five pre-spawning periods, the rate of weight loss was higher or rate of weight gain was lower in females that did not spawn compared to those that spawned. Although these differences were not statistically significant for four of five periods, the trend present in the data suggest that spawning females may have been in a better nutritional state during gametogenesis compared to nonspawning females. This supposition is in agreement with other studies on the effects of food deprivation on reproduction. Wallace and Selman (1980) reported that vitellogenic eggs in starved mummichog (*Fundulus heteroclitus*) failed to mature and Kjesbu et al. (1991) found that atresia of vitellogenic eggs in Atlantic cod (*Gadus morhua*) was inversely related to the nutritional status of the female.

The same pattern in weight loss was also observed in the males, based on the amount of milt expressed during the spawning trials. For this comparison, fish that expressed at least 20 ml of semen were judged to be a spawner and those that expressed less than 20 ml were judged to be a nonspawner. A comparison of AGR-W of males between spawners and nonspawners indicated that nonspawners lost more weight or gained less weight than spawners during each sample

Table 13. Comparison of absolute growth rates based on weight (g/d, mean and range across treatments)^a between Colorado pikeminnow spawners and nonspawners exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Exposure period (d)	Female		Male	
	Spawner	Nonspawner ^b	Spawner	Nonspawner ^b
0-30	-2.43 (-4.30 to -1.57)	-3.73 (-30.17 to 0.67)	-1.19 (-2.73 to 0.67)	-0.98 (-1.60 to -0.33)
30-58	0.09 (-3.71 to 1.93)	0.05 (-6.00 to 37.79)	-0.63 (-1.96 to 1.57)	-1.19 (-2.21 to 0.00)
58-90	3.50 (1.75 to 8.06)	2.51* (-2.00 to 31.22)	1.50 (-0.97 to 3.00)	0.21* (-1.31 to 1.94)
90-120	2.16 (-0.43 to 6.00)	0.94 (-4.83 to 7.33)	1.25 (-0.30 to 2.93)	-0.09* (-1.63 to 1.23)
120-153	-0.40 (-3.48 to 0.94)	-0.89 (-7.24 to 3.58)	-0.30 (-2.12 to 1.39)	-0.90 (-1.61 to 0.55)
153-184 ^c	-7.27 (-12.39 to -0.35)	-1.39* (-5.19 to 4.35)	-1.44 (-4.23 to 1.19)	-0.32* (-1.00 to 1.52)
184-215	0.90 (-1.45 to 5.45)	-1.21* (-8.13 to 5.71)	-0.46 (-2.71 to 1.06)	-0.17 (-1.42 to 0.84)
215-245	-3.70 (-8.33 to 1.23)	-2.28 (-7.90 to 5.57)	-0.80 (-3.13 to 2.27)	-1.53 (-3.50 to -0.20)

^aAbsolute growth rates for each period were calculated according to Ricker (1979).

^bValues with an asterisks are significantly different ($P \leq 0.05$) from the corresponding value for the spawners.

^cSpawning trials occurred on day 154 of study and depuration phase began on day 155.

period prior to the spawning trials (Table 13). These results suggest that spermatogenesis and spermiation may also have been affected by the nutrition state of the fish.

During the first 32 days after the spawning trials, average AGR-W for the spawners were markedly lower than those for the nonspawners (Table 13). The large negative growth rates exhibited by the spawners, compared to nonspawners, was partly due to weight loss associated with the release of their sex products at spawning. The weight of eggs shed by the females accounted for 14-27% of their body weight at day 153. However, the effects of handling stress on postspawn growth cannot be discounted because 76% of nonspawning females and 50% of nonspawning males exhibited negative growth (-5.19 to -0.13 g/d for females and -1.00 to -0.16 g/d for males) during the same period.

The loss of weight of adults in our study may have resulted from changes in feeding behavior, low water clarity, or a combination of both factors. Schreck et al. (1997), in their review of behavioral responses to stress, noted that handling stress in Pacific salmon (*Oncorhynchus* spp.) induced by frequently transferring fish between tanks resulted in reduced feeding and the duration of this effect was dependent on the severity of the stress. The negative growth rates of both sexes observed during the first 30 days of our study are consistent with altered feeding behavior induced by the stresses associated with their transport to Yankton. The low water clarity in the exposure tanks precluded any observations of feeding behavior during the study. Although the effect of low water clarity on feeding behavior cannot be discounted, the fish did consume the test diets as evidenced by the positive AGR-W for females during 3 of the 5 periods prior to the spawning trials (Table 7). Moreover, we believe that the test diets were acceptable to the fish because they were reared on pelleted feed (primarily Silver Cup 4.76-mm [3/16-inch] pellets, as adults) at DNFH (R. Hamman, U.S. Fish and Wildlife Service, personal

communication).

Stresses associated with handling have been implicated as a contributing factor to poor spawning success of Colorado pikeminnow at Willow Beach National Fish Hatchery (WBNFH), Boulder City, NV, and DNFH (R. Hamman, U.S. Fish and Wildlife Service, personal communication). Based on the results of this study and anecdotal evidence from over 20 years of culturing and spawning Colorado pikeminnow, R. Hamman (U.S. Fish and Wildlife Service, personal communication) recommended that broodstock (wild or hatchery-reared) should be transported to the intended spawning facility about one year prior to the spawning trials.

No adverse effects on spawning behavior in fish have been reported at the selenium concentrations tested in this study. Coyle et al. (1993) reported that selenium exposures up to 33.3 $\mu\text{g/g}$ in the diet and up to 10.1 $\mu\text{g/L}$ in water had no effect on spawning frequency or number of eggs per spawn of bluegill. Similarly, number of spawns and number of eggs released by fathead minnow were not affected following 98 days of exposure to comparable dietary selenium concentrations (Ogle and Knight 1989). However, Ogle and Knight (1989) reported that fathead minnow exposed to dietary selenium concentrations of 40 $\mu\text{g/g}$ and higher did not spawn. These dietary concentrations are at least 3 times higher than those tested in our study with Colorado pikeminnow.

Selenium in Adult Tissues

In the present study, the low waterborne concentrations of inorganic selenium (5-8 $\mu\text{g/L}$), at dietary exposures of 2.18-11.83 $\mu\text{g/g}$, did not contribute significantly to the acquired selenium residues in muscle tissue of adult females or to selenium deposition in the eggs (and larvae). A similar finding was observed for the data of Coyle et al. (1993). Based on visual inspection of their data, whole-body, carcass, and gonad selenium concentrations appear to be very similar

between adult bluegill exposed to the same selenium diet (0.8 µg/g), but to different waterborne selenium concentrations (0.6 or 8.4 µg/L). These results indicate that bioconcentration factors (ratio of tissue to water selenium concentration) for muscle tissue or eggs may be inappropriate at low waterborne concentrations of inorganic selenium, if the fish were exposed simultaneously to selenium in the diet. In contrast, selenium accumulation in whole fathead minnow and bluegill exposed to waterborne selenite and selenium-laden *Daphnia* sp. (fed selenium-enriched algae) was found to be additive (Bertram and Brooks 1986, Besser et al. 1993). However, most of the accumulated selenium in bluegill was from the food (Besser et al. 1993). Our findings for Colorado pikeminnow are in agreement with the consensus that dietary selenium is the primary source for selenium accumulation in fish (Sandholm et al. 1973, Cumbie and Van Horn 1978, Hilton et al. 1980, Lemly 1993, 1996a).

In the present study, selenium concentrations in muscle plugs increased in a concentration dependant manner after 58 days of exposure and seemed to stabilize within 120 days. Coyle et al. (1993) reported that selenium concentrations in carcass tissue (body tissue with gonads removed) of adult bluegill remained constant between 60 and 140 days of exposure to dietary selenium concentrations up to 33.3 µg/g selenium (seleno-L-methionine) and waterborne concentrations up to 10 µg/L selenium (1:6 selenite:selenate). Similarly, Gillespie et al. (1988) fed adult bluegill mealworms injected with Se⁷⁵ labelled seleno-L-methionine and reported that selenium concentrations in muscle tissue remained constant from 6 to 12 weeks of exposure. Ogle and Knight (1989) reported that whole-body selenium residues in fathead minnow fed up to 30 µg/g selenium (25% seleno-L-methionine, 25% selenate, and 50% selenite) were dose dependent, but equilibrium was attained within just 14 days. Concentration-dependent selenium accumulation has also been reported for whole-body tissues of chinook salmon fed

diets spiked with either seleno-DL-methionine or selenium-laden fish meal (Hamilton et al. 1990) and various tissues in rainbow trout fed diets spiked with selenite (Hilton et al. 1982). However, the supposition that equilibrium selenium concentrations in muscle tissue of adult Colorado pikeminnow were attained during the exposure phase of our study needs to be verified by longer exposures.

In contrast to laboratory studies using synthetic diets, Hamilton et al. (1999) reported that selenium concentrations in muscle plugs of adult razorback sucker had not reached equilibrium after 305 days of exposure to natural forms of waterborne and dietary selenium in two backwater sites on the Colorado River contaminated with irrigation drainwater. The apparent lack of equilibrium in muscle tissue selenium concentrations in razorback sucker (in their study) may have been partly due to fluctuating selenium concentrations in the water and likely food items during the exposure period. For example, waterborne concentrations differed by about 5- to 8-fold (1.5-11.6 $\mu\text{g/L}$ at the Adobe Creek site and 3.8-19.6 $\mu\text{g/L}$ at the Walter Walker site) and concentrations in zooplankton differed by about 2- to 4-fold (13.7-55.6 $\mu\text{g/g}$ at the Adobe Creek site and 20.3-36.6 $\mu\text{g/g}$ at the Walter Walker site) during the period when adult fish were present (Hamilton et al. 1999).

In our study, most of the accumulated selenium in muscle tissue of adult females fed the two selenium-fortified diets was retained after 90 days of depuration (Table 11). After 90 days of depuration, seven of eleven females showed a net reduction in muscle selenium concentrations (from those at day 153) that ranged from -33 to -4%. Hamilton et al. (1999) reported similar reductions in muscle plug selenium concentrations (-29 to -1%) after 66 days of depuration in four adult female razorback sucker previously held for 305 days in one of two selenium-contaminated backwater sites on the Colorado River. Slow depuration of selenium

from muscle tissue has also been observed for adult fathead minnow exposed to waterborne selenium (Adams 1976). Adams (1976) reported that the rate of selenium elimination from entire viscera (intestine, kidney, liver) in adult fathead minnow following a 96-day exposure to selenite was about 12 times faster than from muscle tissue based on average half-life values of 5.1 and 63 days, respectively.

Selenium BAF in muscle tissue were inversely related to selenium concentrations in the diet and mean BAF for females exposed to the control diets (0.55-0.57) were about twice as high as those for females exposed to the two selenium-fortified diets (0.21-0.29). The lower BAF in muscle tissue of females fed the two selenium-fortified diets compared to those fed control diet, may be partly due to less efficient assimilation or more efficient elimination of selenium at the higher dietary concentrations (Hilton et al. 1982, Hodson and Hilton 1983). Decreased BAF with increasing dietary concentrations of selenium has been reported for whole-body tissues of fathead minnow (Bertram and Brooks 1986, Ogle and Knight 1989) and carcass tissue of rainbow trout (Hilton et al. 1980, 1982). Calculated BAF for muscle tissue of striped bass (*Morone saxatilis*) were about an order of magnitude lower in fish fed selenium-contaminated prey for 80 days (0.36-0.42) compared to those fed uncontaminated prey (3.67; Coughlan and Velte 1989).

The BAF we obtained for muscle tissue of Colorado pikeminnow were similar to those reported for rainbow trout and fathead minnow exposed to comparable dietary selenium concentrations. Hilton et al. (1982) obtained selenium BAF of 0.54, 0.37, and 0.35 in carcass tissue of rainbow trout at dietary concentrations of 1.3, 3.7, and 13.1 $\mu\text{g/g}$ selenite, respectively. Calculated BAF for whole-body tissue of fathead minnow after 98 days of exposure to dietary concentrations of 5.2, 10.2, and 15.2 $\mu\text{g/g}$ selenium (as 25% seleno-L-methionine, 25% selenate,

and 50% selenite) were 0.53, 0.35, and 0.36, respectively (Ogle and Knight 1989). The low BAF of selenium in muscle tissue we observed for adult Colorado pikeminnow is consistent with the findings of field studies where selenium accumulation in whole-body and muscle tissue of fish was less than that in their potential prey items (Saiki et al. 1993, Hamilton et al. 1999).

In the present study, the selenium concentrations we observed in muscle tissue of female adults fed the two selenium-fortified diets (range, 1.5 to 5.2 $\mu\text{g/g}$) were lower than those linked with reduced survival, growth, and reproduction of adult bluegill (7.2-11.2 $\mu\text{g/g}$, reported as 1.8-2.8 $\mu\text{g/g}$ wet weight) exposed in artificial streams dosed with selenite (Hermanutz et al. 1992) and with reduced growth in adult fathead minnow (6.5-8.8 $\mu\text{g/g}$) exposed in the laboratory to dietary selenium (Ogle and Knight 1989). Our muscle plug selenium concentrations were also lower than muscle tissue residues linked with reduced survival of juvenile bluegill (20-32 $\mu\text{g/g}$, reported as 5.10-7.94 $\mu\text{g/g}$ wet weight) and striped bass (15 $\mu\text{g/g}$ reported as 3.8 $\mu\text{g/g}$ wet weight) fed selenium-laden prey items (Finley 1985, Coughlan and Velte 1989).

Reproductive Performance

The absence of replicate spawns in three of the six selenium treatments tested in our study compromised the validity of any statistical comparisons and conclusions drawn regarding the effects of these exposures and accumulated selenium residues on reproduction of Colorado pikeminnow. Although there were no measures of variability in the responses of fish to three of the parental exposure treatments, the reproductive performance of the 10 females that spawned was comparable to that of hatchery brood stock. The number of eggs manually spawned per gram body weight in our study ranged from 22 to 46 and overlaps that reported by Hamman (1986) for 9- and 10-year-old females (38 to 66 and 8 to 61, respectively) at DNFH. Egg diameters (2.4-2.5 mm) fell in the range of those obtained for wild females (1.5 to 2.5 mm), but

exceeded those for 6-year-old fish (1.5 to 2.0 mm) reared at WBNFH (Hamman 1981).

Hatchability data in our study cannot be compared directly with hatchery data because each egg was visually examined for viability and condition prior to use in our study, whereas those used in hatchery studies were not preselected for quality and were hatched in large batches. Hatchability estimates of eggs produced from 9- and 10-year-old females at DNFH were low, ranging from 15 to 20% and viability estimates for the same group of eggs ranged from 49 to 66% (Hamman 1986). The average hatch times in our study (4.2-4.7 days at $21 \pm 1^\circ\text{C}$) falls in the hatching period of 4 to 6 days for hatchery produced eggs incubated at $21\text{-}22^\circ\text{C}$ (Hamman 1986).

The incidence of deformities we observed in newly hatched larvae from eight of the ten spawns incubated at 21°C (12-14%) are close to that reported by Marsh (1985) for Colorado pikeminnow ova incubated at 20°C (11%). In the same study, the incidence of abnormal larvae hatched from eggs reared at 25°C was 26%. Marsh (1985) also observed low hatchability of eggs incubated at six temperatures; 2-27% at 20°C , 0-9% at 25°C , and 0% at 5, 10, 15, and 30°C .

Survival of Colorado pikeminnow larvae from eight of the ten spawns was at or exceeded the survival criterion for control fish ($\geq 80\%$) in the standard 7-day fathead minnow survival and growth test (Weber et al. 1989). Survival of larvae from the other two spawns were within 9% of this criterion. Moreover, overall survival of the progeny from six spawns met the control fish survival criterion of 70% for early life-stage toxicity tests with fathead minnow (ASTM 1992). Hamman (1981) observed survival rates of 76 to 85% for posthatch Colorado pikeminnow cultured for 98 days at DNFH. Growth of larvae after 30 days in our study was judged to be good in comparison to that of hatchery-reared fish. The range of average total length reported for 28-day-old Colorado pikeminnow (19-20 mm) reared in raceways at $23\text{-}24^\circ\text{C}$ and fed

zooplankton (Hamman 1981) was identical to that for larvae in our study.

Results of field and laboratory investigations have strongly indicated that selenium induced reproductive failure in fish was due to the transfer of foodborne selenium from the female parent to the eggs, which then causes abnormal development and mortality in the resulting embryos and larvae during yolk absorption (Gillespie and Baumann, 1986, Schultz and Hermanutz 1990, Coyle et al. 1993, Lemly 1993, 1996a). Although hatchability is a commonly used end point in reproduction studies (McKim 1985), it is not among the most sensitive responses for determining selenium toxicity in fish. Hatchability of bluegill and fathead minnow eggs were not affected by parental exposures to dietary selenium concentrations up to 33.3 µg/g (organic and inorganic selenium) with or without 8-11 µg/L inorganic selenium in the water (Woock et al. 1987, Ogle and Knight 1989, Coyle et al. 1993). Moreover, the hatchability of eggs produced by bluegill collected from a selenium-contaminated reservoir, where reproductive impairment in centrachids had been documented (Hyco Reservoir, NC), was similar to that of eggs produced by females collected from a reference site (Gillespie and Baumann 1986). In contrast, Hermanutz et al. (1992) reported reduced hatchability of bluegill eggs produced by adults exposed in outdoor experimental streams dosed at 10 and 30 µg/L selenium (as selenite). In a concurrent study in the same artificial streams, no adverse effects on the hatchability of fathead minnow eggs were observed (Schultz and Hermanutz 1990).

The study of Woock et al. (1987) was one of the first laboratory investigations to demonstrate that selenium-induced reproductive failure in bluegill resulted from reduced survival and increased incidence of abnormalities in larvae, which was related to parental exposures to dietary selenium. In their study, larvae produced from parents exposed to dietary selenium concentrations of 30 µg/g (seleno-DL-methionine or selenite) or 13 µg/g selenium

(seleno-DL-methionine) plus 10 µg/L selenite in the water exhibited reduced survival (0-75%) and a higher incidence of deformities (1-97%). Coyle et al. (1993) tested the same species with a comparable range of dietary and waterborne selenium concentrations and reported reduced survival in larvae (mean, 7%) from parental dietary exposures of 33.3 µg/g selenium (seleno-L-methionine), but not at 16.8 µg/g selenium or lower. Conversely, Ogle and Knight (1989) did not observe a reduction in survival of larval produced by fathead minnow exposed to dietary selenium concentrations of 5.2 to 29.5 µg/g selenium (25% seleno-L-methionine, 25% selenate, and 50% selenite).

Selenium in Eggs

Our finding that selenium residues deposited in the eggs were significantly greater than those in muscle tissue of the female parent is consistent with the generalized accumulation pattern of selenium in fish tissues. Although total accumulation of selenium in fish and its relative distribution in different tissues varies considerably among species, selenium is preferentially accumulated in the liver and kidney of most fish (Sato et al. 1980, Hilton et al. 1982, Lemly 1982, Kleinow and Brooks 1986, Hermanutz et al. 1992) and also in the ovary of sexually mature females (Cumbie and Van Horn 1978, Baumann and Gillespie 1986); whereas lower accumulation generally occurs in muscle tissue (Adams 1976, Sager and Cofield 1984, Gillespie et al. 1988).

The ratios of selenium concentration in eggs to muscle plugs of female parents we observed for Colorado pikeminnow (range, 1.5 to 4.2) fall in the range of those reported for razorback sucker (1.3 to 4.7) exposed to environmental concentrations of selenium for about 44 weeks in backwater habitats of the upper Colorado River (Hamilton et al. 1999). Moreover, Hamilton et al. (1999) also observed that selenium deposition in eggs was strongly correlated

with residues in muscle tissue ($r=0.85-0.96$, $P\leq 0.01$, $n=10-15$). In contrast, Hamilton and Waddell (1994) reported that selenium concentrations in wild razorback sucker eggs collected in the Green River, UT, were about 15-67% lower than those in muscle tissue of the female parent (egg to muscle plug ratios, 0.33-0.85). The reason for this disparity is not known; but one possible factor might be the sampling procedures used. The eggs collected in our study and by Hamilton et al. (1999) were manually stripped, fertilized, and mixed with milt prior to collection; whereas in the study of Hamilton and Waddell (1994) unfertilized eggs were collected directly from the female. It is possible that selenium deposition in eggs is not uniform and the first group of eggs expressed may have lower residues than the remaining eggs. The possibility that the sperm or milt contributed to the higher egg to muscle plug ratio cannot be discounted because selenium residues were not measured in unfertilized eggs or milt in our study or in that of Hamilton et al. (1999). However, Gillespie and Baumann (1986) reported that in 18 artificial crosses of bluegill with high or low selenium concentrations in gonads, edema and mortality occurred in larvae from high-selenium females (even when crossed with reference males), but not in high-selenium males (when crossed with reference females). Likewise, Bryson et al. (1984) reported similar results in artificial crosses of adult bluegill from selenium-impacted Hyco Reservoir, NC, and adults from a reference lake. Thus, selenium in fish embryos seems to be due primarily to selenium transferred to the ova and not to selenium in the milt. The conflicting results of Hamilton et al. (1999) and Hamilton and Waddell (1994) limits the utility of extrapolating selenium residues in eggs of razorback sucker from those in muscle plugs.

The highest selenium concentrations we observed in Colorado pikeminnow eggs (9.8-11.6 $\mu\text{g/g}$) following parental exposures to dietary and waterborne selenium were lower than those in eggs or ovaries linked with reproductive impairment in fish. In a laboratory

reproduction study with bluegill, Coyle et al. (1993) showed that larvae hatched from eggs containing about 42 $\mu\text{g/g}$ selenium failed to survive past the swim-up stage; whereas survival of larvae from eggs with about 7-23 $\mu\text{g/g}$ selenium was comparable to that of the controls. Ogle and Knight (1989) reported no adverse reproductive effects in fathead minnow with ovarian selenium residues between 7 and 11 $\mu\text{g/g}$ selenium. In one of the first studies that linked reproductive impairment in fish with high selenium residues, Gillespie and Baumann (1986) reported that larvae produced from bluegill with ovarian selenium concentrations of about 39-53 $\mu\text{g/g}$ (reported as 5.79-8.00 $\mu\text{g/g}$ wet weight) had a high incidence of edema and none of them survived to the swim-up stage. Schultz and Hermanutz (1990) reported increased edema and lordosis in larval fathead minnow developed from embryos containing about 16 $\mu\text{g/g}$ selenium (reported as 3.91 $\mu\text{g/g}$ wet weight). The embryos in their study were produced by adults exposed to naturally incorporated forms of selenium in artificial streams dosed with 10 $\mu\text{g/L}$ selenite for a year. Hermanutz et al. (1992) exposed bluegill for about 37 weeks in the same streams and reported reduced hatch and increased terata and mortality in larvae produced from ovaries with about 29 $\mu\text{g/g}$ selenium (reported as 4.4 $\mu\text{g/g}$ wet weight).

Confounding Factors

The low spawning success of the females limited the interpretation of the tissue residue data in that the female with the highest muscle plug selenium concentration at day 120 (5.0 $\mu\text{g/g}$) and day 153 (5.2 $\mu\text{g/g}$) did not spawn. This female was exposed to the high selenium diet (11.8 $\mu\text{g/g}$) in control water (treatment 5). Based on regression models relating selenium residues in eggs and larvae with those in muscle plugs of female parent at day 153, this female may have produced eggs and larvae containing 22.8 and 25.7 $\mu\text{g/g}$ selenium, respectively. These concentrations fall in the range of those in fathead minnow embryos (16 $\mu\text{g/g}$ reported as 3.91

µg/g wet weight) and bluegill larvae (28.2 µg/g) linked with increased deformities and reduced larval survival (Gillespie and Baumann 1986, Schultz and Hermanutz 1990). Moreover, this extrapolated selenium concentration in eggs (23 µg/g) exceeds proposed toxic threshold concentrations of 10-17 µg/g for fish eggs (Lemly 1993, 1996a, USDOJ 1998, DeForest et al. 1999). Based on Lemly's (1996b) selenium hazard assessment procedure, selenium concentrations in eggs at or above 20 µg/g are likely to cause complete reproductive failure in most fish species.

Selenium in Larvae

The reduction in whole-body selenium concentrations in larvae during our 30-day growth study was probably a function of both tissue dilution and elimination mechanisms in the fish. Although initial size measurements were not taken, daily observations revealed that the size of the fish increased progressively during the study. Consequently, tissue dilution was a contributing factor to the observed reduction in larval whole-body selenium residues. The supposition that larvae were regulating their selenium body burdens by elimination mechanisms was supported by the observation that the percent reduction in whole-body selenium concentrations after 30 days was inversely related to whole-body selenium concentrations at 1 dph, which suggests that elimination rates were faster at higher tissue burdens. The difference in selenium depuration between larvae and adults can also be partly explained by tissue dilution in the larvae along with differences in physiology and metabolism between the life stages (Bennett et al. 1986). Bennett et al. (1986) reported that depuration rates of selenium residues acquired from laboratory food-chain exposures were faster in larval fathead minnow than in adults.

Hazard Assessment

Both selenium-fortified diets fed to adult Colorado pikeminnow contained selenium

concentrations that exceeded the proposed dietary toxic threshold concentrations of 3 and 5 $\mu\text{g/g}$ (Lemly 1993, 1996a, Lemly and Smith 1987). However, exposures to these diets resulted in selenium concentrations in muscle tissue of adult females (1.5 to 5.2 $\mu\text{g/g}$) that were below Lemly's (1993, 1996a) proposed muscle tissue threshold concentration of 8 $\mu\text{g/g}$, based on the potential for reproductive failure in fish. We believe that comparisons of our selenium residues in muscle plugs to Lemly's muscle tissue threshold concentration are valid because Waddell and May (1995) demonstrated that muscle plugs taken from the mid-dorsal region of the fish (as was done here) provide an accurate assessment of selenium concentrations in adjacent musculature. Colorado pikeminnow females with selenium concentrations in muscle tissue up to 5.2 $\mu\text{g/g}$ did not exhibit reduced survival or growth (compared to the controls) and females that spawned (muscle plug concentrations, 1.2-3.0 $\mu\text{g/g}$) were able to produce viable progeny. These results demonstrate the importance of tissue residues in interpreting the effects of toxicant exposures.

Considering that reproductive success is the most sensitive biological response to selenium toxicity in fish, selenium residues in eggs are a key component in the hazard assessment of selenium (Lemly 1993, 1996a). Based on a review of selenium toxicity and bioaccumulation studies, Lemly (1993) proposed a toxic threshold concentration of 10 $\mu\text{g/g}$ in eggs or ovary of fish for reproductive failure. In a later report, Lemly (1995) developed a quantitative environmental hazard assessment protocol for selenium based on selenium residues in five ecosystem components, one of which was eggs or ovaries of fish. Lemly (1995) assigned a hazard ranking to egg residues as follows: no hazard for selenium concentrations at <3 $\mu\text{g/g}$, minimal hazard at 3-5 $\mu\text{g/g}$, low hazard at 5-10 $\mu\text{g/g}$, moderate hazard at 10-20 $\mu\text{g/g}$, and high hazard at >20 $\mu\text{g/g}$. In our study, the highest selenium concentrations in eggs (9.8 to 11.6 $\mu\text{g/g}$) straddle the low and moderate hazard rating.

According to Lemly (1996b), a moderate selenium hazard may result in substantial reproductive impairment in some species and little effect on others and a low selenium hazard would have a marginal effect on reproductive success in some sensitive species, but most species will not be affected. Colorado pikeminnow eggs that contained selenium residues up to 11.6 $\mu\text{g/g}$ hatched successfully and developed into larvae that exhibited good survival and growth. However, due to the low number of females that spawned, the reproduction data are insufficient ($n=1$ for three of six treatment groups, including the control) to draw firm conclusions regarding the effects of these selenium residues on reproduction of Colorado pikeminnow. Moreover, these interpretations are limited to reproductive effects in females with muscle tissue selenium residues at or below 3.0 $\mu\text{g/g}$.

Environmental Considerations

Because of their endangered status, few data are available on selenium accumulation in wild Colorado pikeminnow, especially for tissues other than muscle. The only reported selenium concentration in gonads found is 6.5 $\mu\text{g/g}$ for one fish collected from the upper Colorado River basin in 1982 (Peltz and Waddell 1991). This concentration falls in the range of those for eggs produced by females exposed to 7.28 $\mu\text{g/g}$ selenium in the diet (6.2-7.2 $\mu\text{g/g}$).

There are insufficient data to test our regression model that predicts selenium concentrations in eggs of Colorado pikeminnow from those measured in muscle plugs of the female parent. We only found data for one fish in which selenium concentrations were measured in muscle tissue and eggs. The reported selenium concentrations were 2.79 $\mu\text{g/g}$ in the muscle plug and 3.43 $\mu\text{g/g}$ in eggs of one adult collected from the San Juan River in 1991 (F. Pfeifer, U.S. Fish and Wildlife Service, written communication). In our study, adults with similar selenium residues in muscle plugs (2.5-2.9 $\mu\text{g/g}$) produced eggs that contained about 3 times

more selenium (9.85-11.45 $\mu\text{g/g}$) than in eggs from this one wild adult. The difference in the egg-to-muscle ratios for selenium between the wild fish (1.2) and our fish (3.9) may be partly due to the developmental stage of the eggs and the hypothesized mechanism by which selenium is deposited in eggs. The eggs analyzed for selenium in our study were spawned, whereas those in the wild fish were expected to be spawned in about month (F. Pfeifer, U.S. Fish and Wildlife Service, personal communication) and probably were not mature when collected. Selenium from the diet is believed to be incorporated into precursors of yolk proteins in the liver during vitellogenesis and then transported to the ovary where it is taken up by the developing eggs (Ogle and Knight 1989, Kroll and Doroshov 1991). Thus, it is reasonable to assume that eggs undergoing vitellogenesis would have lower selenium residues than those at maturation or spawning.

Simpson and Lusk (1999) reported selenium concentrations of 2.9-3.9 $\mu\text{g/g}$ in muscle plugs of four Colorado pikeminnow collected from the San Juan River in 1994. These concentrations fall in range of those for adult females (2.0-5.2 $\mu\text{g/g}$) fed the high selenium-fortified diet in our study. Moreover, the length (TL, 617-823 mm) and weight (2,000-5,510 g) of their fish bracket those of the females tested in our study. Simpson and Lusk (1999) also determined whole-body selenium residues in several other fishes and found that selenium concentrations varied considerably within (up to 72-fold) and among (up to 151-fold) species. However, the largest whole-body selenium concentrations they reported for rainbow trout (15.1 $\mu\text{g/g}$), small-body fish (14.3 $\mu\text{g/g}$), speckled dace (*Rhinichthys osculus*, 11.0 $\mu\text{g/g}$), and channel catfish (*Ictalurus punctatus*, 10.3 $\mu\text{g/g}$) closely bracket the highest dietary selenium concentration (11.8 $\mu\text{g/g}$) tested in our study. Although little is known about the food habitats of adult Colorado pikeminnow in the San Juan River, Osmundson et al. (1998) reported that the

diets of adult Colorado pikeminnow (TL, 400-550 mm) in the Upper Colorado River consisted primarily of small cyprinids (average standard length [SL], ≤ 85 mm) and white sucker (*Catostomus commersoni*; average SL, 70 mm). Adult Colorado pikeminnow have also been reported to prey on channel catfish (McAda 1983). These findings indicate that adult Colorado pikeminnow in the San Juan River are probably exposed to dietary selenium concentrations comparable to those tested in this study.

Osmundson et al. (2000) determined selenium concentrations in muscle plugs of 39 wild Colorado pikeminnow collected in 1994 from three stretches of the Colorado River within the Grand Valley, CO, and reported selenium concentrations ranging from 3.2 to 30.7 $\mu\text{g/g}$. In 38% of their fish, muscle tissue selenium concentrations exceed Lemly's toxic threshold value of 8 $\mu\text{g/g}$, and fish with the highest mean selenium residues were collected at Walter Walker State Wildlife Area (WWSWA), which is a high selenium site (Hamilton et al. 1999). Based on our results, it seems that wild Colorado pikeminnow collected at WWSWA were feeding on prey items that contained selenium concentrations markedly greater than that in our highest dietary treatment (11.8 $\mu\text{g/g}$). Over a 3-year period, mean selenium concentrations in muscle plugs of Colorado pikeminnow collected at WWSWA ranged from 9.4 to 16.6 $\mu\text{g/g}$ (Osmundson et al. 2000). Based on our diet to muscle plug regression model (muscle plug selenium = $0.755 + 0.184 \times \text{dietary selenium}$), extrapolated selenium concentrations in the diet of these wild fish range from about 47 to 86 $\mu\text{g/g}$. However, caution is advised for this extrapolation because muscle plug selenium concentrations for the wild fish are higher than those used to develop the model. Also, waterborne selenium cannot be discounted if the environmental concentrations are markedly higher than those tested in our study. These extrapolated selenium concentrations for prey items of wild Colorado pikeminnow at WWSWA fall in the range of those reported for live

minnows (22 to 87 $\mu\text{g/g}$) collected from a backwater site (North Pond, which is isolated from the river) located at WWSWA (Hamilton et al. 1999).

One implication of slow selenium depuration in adult Colorado pikeminnow is that residues accumulated by fish when they occupy selenium contaminated habitats may be retained during periods when they move into less contaminated habitats. This selenium retention may result in a progressive increase in tissue selenium concentrations over the life of the fish, that may eventually accumulate to toxic concentrations (not yet determined in adult Colorado pikeminnow). Osmundson et al. (2000) reported that some Colorado pikeminnow collected at WWSWA had maintained elevated selenium concentrations over a 3-year period. They also reported elevated muscle plug selenium concentrations in fish collected at other locations in the Colorado River, which may be partly due to the slow depuration of selenium accumulated from high-selenium habitats, such as WWSWA. Additional studies (i.e., longer exposure and depuration periods than those in this study) are needed to determine the utility of muscle plug selenium residues for assessing seasonal versus longer-term selenium exposures in the environment.

CONCLUSIONS

1. Exposures to mean dietary selenium concentrations of 2.18 (control), 7.30, and 11.83 $\mu\text{g/g}$ dry weight and mean waterborne selenium concentrations of 0.15 (control) to 7.94 $\mu\text{g/L}$ for 155 days did not adversely affect survival or growth of adult Colorado pikeminnow.
2. Selenium accumulation in muscle tissue of adults seemed to reach a plateau after 120 days of exposure and did not decrease significantly after 90 days of depuration. Selenium residues in adult muscle tissue after 90 days of exposure were strongly

correlated with dietary selenium concentrations, but not with waterborne selenium concentrations. Bioaccumulation factors for selenium in muscle plugs (1.1-5.2 $\mu\text{g/g}$ dry weight) based on selenium in the diet (2.2-11.8 $\mu\text{g/g}$ dry weight) ranged from 0.17 to 0.60 and were inversely related to dietary concentration.

3. The reproductive data are problematic due to the lack of replicate spawns for half of the treatments. Only 10 of 35 females (29%) expressed eggs and only one female in the control, lowest, and highest treatments spawned. Results of ANOVA testing indicated that there were no differences among treatments for any of the reproductive metrics analyzed, which included number of eggs expressed, egg weight and diameter, hatchability, time to hatch, and survival, growth, and deformities of larvae. However, the power of these tests (which is the probability of detecting a difference when one really exists) was close to zero and inferences drawn from tests with such low power are highly questionable. Moreover, the female with the highest muscle plug selenium concentration at day 153 (5.2 $\mu\text{g/g}$) did not spawn and the significance of this higher tissue residue on reproduction could not be evaluated.
4. Hatchability of eggs containing 1.8-11.6 $\mu\text{g/g}$ selenium (dry weight) averaged 84% and the incidence of deformities in the resulting larvae averaged 13%. Survival of larvae to 30-days posthatch containing 2.8-13.4 $\mu\text{g/g}$ selenium (dry weight) at hatch averaged 87%. Mean overall survival of progeny from the 10 spawns was 72%. Hatchability, incidence of deformities, larval growth, and overall progeny survival were not correlated with selenium residues in the eggs or muscle plugs of the female parent.
5. Selenium residues in eggs and newly hatched larvae were about 2-5 times higher than those in muscle tissue of the female parent and were strongly correlated with selenium in

the diet and muscle plugs of the adults. These results showed that selenium, taken up primarily from the diet, was transferred from the adult female to their progeny in a concentration-dependent manner. However, the hazard posed by these selenium residues to wild Colorado pikeminnow cannot be adequately assessed from the reproduction data obtained in this study and awaits further investigation.

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Appendix A. Photoperiods used in chronic toxicity study with adult Colorado pikeminnow.

	Daylight length (standard time) at Shiprock, NM ^a			Photoperiod in study	
	Sunrise a.m.	Sunset p.m.	Total daylight time (h:min)	Light (h:min)	Dark (h:min)
January 1	7:15	5:06	9:51	9:45	14:55
February 1	7:06	5:35	10:29	10:30	13:30
March 1	6:36	6:02	11:26	11:30	12:30
April 1	5:54	6:28	12:34	12:30	11:30
May 1	5:16	6:52	13:36	13:30	10:30
June 1	4:54	7:15	14:21	14:15	9:45
July 1	4:56	7:24	14:28	14:30	9:30
August 1	5:15	7:10	13:55	14:00	10:00
September 1	5:39	6:34	12:55	13:00	11:00
October 1	6:01	5:51	11:50	11:45	12:15
November 1	6:27	5:13	10:46	10:45	13:15
December 1	6:56	4:55	9:59	10:00	14:00

^aU.S. National Weather Service, Meteorologist-in-charge, Albuquerque, NM.

Appendix B. Individual mortalities of adult Colorado pikeminnow that occurred at the Yankton Ecotoxicology Research Station, Yankton, SD, from December 16, 1996, to September 21, 1997.

Date	Day of Study ^a	PIT tag number	Sex	Treatment ^b	Remarks
12-23-96	-16	7F7F1F047C	M	None	
06-15-97	158 (3)	7F7F1F132B (3A-20) ^c	F	3	spawned 06-11-97
06-15-97	158 (3)	7F7F1F201A (5B-41)	F	5	spawned 06-11-97
06-17-97	160 (5)	7F7F1F0F28 (3B-22)	F	3	spawned 06-11-97
08-11-97	215 (60)	7F7F1F1A29 (4A-28)	F	4	jumped out of tank
09-09-97	244 (89)	7F7F1F1906 (1B-4)	F	1	radio transmitter implanted 08-26-97
09-09-97	244 (89)	7F7F1E7F15 (3C-25)	F	3	radio transmitter implanted 08-26-97
09-11-97	245+1 (91)	7F7F1F0A5E (3B-23)	F	3	received sham surgery 08-27-97
09-18-97	245+08 (98)	7F7F1F1315 (2B-13)	F	2	injured during sampling 08-10-97

^aThe study was conducted for 245 days; fish were exposed to selenium for 155 days followed by a 90-day depuration period. The fish were held for 11 days after the study awaiting transport to Grand Junction, CO. Mortalities occurring after test completion are indicated by + sign. Values in parentheses give days in depuration period.

^bSee Table 3 for selenium treatments.

^cFish identification number in study.

Appendix C. Total length (mm, mean and SD in parentheses)^a of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Day of study	Selenium treatment ^b											
	1		2		3		4		5		6	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
30	658 (36)	565 (24)	651 (22)	584 (32)	703 (56)	591 (35)	678 (46)	569 (26)	669 (54)	572 (16)	687 (34)	602 (1)
58	660 (38)	569 (23)	651 (21)	585 (32)	701 (56)	590 (35)	676 (43)	567 (28)	668 (53)	570 (15)	688 (34)	604 (4)
90	662 (38)	570 (25)	652 (20)	588 (33)	710 (55)	593 (34)	682 (45)	571 (25)	673 (53)	574 (17)	692 (35)	605 (4)
120	668 (39)	575 (25)	657 (17)	591 (33)	715 (56)	596 (31)	689 (46)	577 (22)	676 (53)	577 (16)	698 (35)	611 (3)
153	666 (39)	571 (22)	652 (16)	590 (36)	710 (56)	589 (30)	682 (46)	571 (25)	670 (52)	572 (16)	691 (36)	606 (3)
184	665 (40)	576 (23)	654 (16)	591 (34)	720 ^{**} (61)	591 (31)	686 (48)	573 (25)	661 [*] (47)	577 (15)	695 (36)	609 (1)
215	668 (42)	577 (23)	657 (18)	595 (35)	727 ^{**} (64)	595 (24)	690 (47)	578 (24)	665 [*] (45)	578 (13)	699 (35)	611 (8)
245	660 [*] (45)	574 (26)	656 (20)	594 (37)	691 ^{***} (34)	592 (24)	680 [*] (44)	576 (24)	664 [*] (45)	577 (17)	697 (36)	612 (4)

^an=6 for females and n=3 for males; except for treatment 2 where n=5 for females and n=4 for males and for means footnoted as follows: * n=5, ** n=4, *** n=3.

^bSee Table 3 for selenium treatments.

Appendix D. Body weight (g, mean and SD in parentheses)^a of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Day of study	Selenium treatment ^b											
	1		2		3		4		5		6	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
30	2,097 (511)	1,328 (219)	2,118 (333)	1,471 (285)	2,807 (1,054)	1,406 (281)	2,232 (322)	1,285 (142)	2,453 (748)	1,405 (126)	2,265 (598)	1,574 (86)
58	2,040 (491)	1,299 (204)	2,081 (367)	1,454 (287)	2,798 (1,049)	1,382 (231)	2,191 (299)	1,262 (138)	2,462 (777)	1,384 (110)	2,404 (429)	1,551 (48)
90	2,111 (516)	1,373 (204)	2,067 (399)	1,479 (288)	2,906 (1,061)	1,419 (178)	2,403 (470)	1,279 (135)	2,570 (835)	1,405 (122)	2,438 (459)	1,591 (72)
120	2,125 (506)	1,406 (204)	2,111 (370)	1,516 (311)	2,927 (982)	1,403 (149)	2,453 (441)	1,296 (126)	2,663 (871)	1,428 (147)	2,450 (461)	1,641 (96)
153	2,107 (469)	1,405 (191)	2,128 (372)	1,513 (316)	2,886 (920)	1,369 (148)	2,468 (436)	1,294 (131)	2,599 (820)	1,389 (164)	2,400 (486)	1,619 (75)
184	2,008 (509)	1,354 (160)	2,113 (271)	1,521 (288)	2,928** (1,103)	1,334 (194)	2,336 (464)	1,300 (131)	2,324* (781)	1,389 (153)	2,348 (505)	1,515 (53)
215	2,022 (529)	1,356 (169)	2,106 (228)	1,468 (260)	2,911** (1,097)	1,335 (217)	2,271 (405)	1,307 (131)	2,270* (729)	1,391 (172)	2,339 (573)	1,501 (82)
245	1,895* (538)	1,315 (178)	2,050 (208)	1,448 (260)	2,299*** (454)	1,312 (134)	2,066* (315)	1,275 (113)	2,182* (734)	1,348 (131)	2,265 (534)	1,471 (73)

^an=6 for females and n=3 for males; except for treatment 2 where n=5 for females and n=4 for males and for means footnoted as follows: *n=5, **n=4, ***n=3.

^bSee Table 3 for selenium treatments.

Appendix E. Condition factor^a (mean and SD in parentheses)^b of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Day of study	Selenium treatment ^c											
	1		2		3		4		5		6	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
30	0.72 (0.06)	0.73 (0.03)	0.76 (0.06)	0.73 (0.04)	0.78 (0.09)	0.68 (0.05)	0.72 (0.08)	0.69 (0.02)	0.79 (0.06)	0.75 (0.08)	0.69 (0.12)	0.72 (0.04)
58	0.70 (0.06)	0.70 (0.04)	0.75 (0.07)	0.72 (0.04)	0.78 (0.08)	0.67 (0.01)	0.71 (0.08)	0.69 (0.03)	0.80 (0.07)	0.75 (0.07)	0.73 (0.05)	0.70 (0.02)
90	0.72 (0.06)	0.74 (0.03)	0.74 (0.09)	0.72 (0.04)	0.79 (0.08)	0.68 (0.05)	0.75 (0.05)	0.69 (0.03)	0.82 (0.08)	0.74 (0.09)	0.73 (0.06)	0.72 (0.02)
120	0.70 (0.06)	0.74 (0.02)	0.74 (0.08)	0.72 (0.04)	0.78 (0.06)	0.66 (0.04)	0.75 (0.06)	0.67 (0.03)	0.83 (0.09)	0.75 (0.10)	0.71 (0.06)	0.72 (0.03)
153	0.71 (0.06)	0.75 (0.02)	0.76 (0.09)	0.73 (0.05)	0.79 (0.06)	0.67 (0.04)	0.77 (0.05)	0.70 (0.06)	0.84 (0.08)	0.74 (0.10)	0.72 (0.07)	0.73 (0.03)
184	0.67 (0.04)	0.71 (<0.01)	0.75 (0.05)	0.73 (0.06)	0.76 ^{**} (0.09)	0.64 (0.02)	0.72 (0.04)	0.69 (0.06)	0.78 [*] (0.11)	0.72 (0.10)	0.69 (0.07)	0.67 (0.02)
215	0.66 (0.05)	0.70 (0.02)	0.74 (0.05)	0.69 (0.06)	0.73 ^{**} (0.07)	0.63 (0.03)	0.69 (0.04)	0.68 (0.04)	0.75 [*] (0.09)	0.72 (0.11)	0.67 (0.10)	0.66 (0.02)
245	0.65 [*] (0.04)	0.69 (0.04)	0.72 (0.06)	0.68 (0.05)	0.69 ^{***} (0.04)	0.63 (0.01)	0.66 [*] (0.05)	0.67 (0.06)	0.73 [*] (0.10)	0.71 (0.11)	0.66 (0.09)	0.64 (0.03)

^aCondition factor calculated according to Anderson and Gutreuter (1983).

Appendix E. Continued.

^bn=6 for females and n=3 for males; except for treatment 2 where n=5 for females and n=4 for males and for means footnoted as follows: * n=5, ** n=4, *** n=3.

^cSee Table 3 for selenium treatments.

Appendix F. Absolute growth rate based on body weight^a (g/d, mean and SD in parentheses)^b of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period.

Exposure period (d)	Selenium treatment ^c											
	1		2		3		4		5		6	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
0-30	-1.64 (0.83)	-0.32 (0.98)	-1.81 (0.67)	-1.47 (0.47)	-2.37 (0.85)	-1.11 (0.45)	-6.83 (11.43)	-0.74 (0.05)	-1.39 (1.86)	-1.29 (0.40)	-5.84 (8.67)	-1.67 (0.93)
30-58	-2.07 (0.93)	-1.05 (0.90)	-1.32 (1.74)	-0.62 (0.59)	-0.33 (1.59)	-0.87 (2.12)	-1.46 (3.15)	-0.81 (0.16)	0.35 (1.41)	-0.74 (0.64)	4.96 (16.12)	-0.83 (1.45)
58-90	2.22 (1.38)	2.33 (0.34)	-0.43 (1.52)	0.78 (1.48)	3.39 (2.68)	1.15 (2.22)	6.62 (12.09)	0.51 (0.50)	3.35 (2.50)	0.65 (0.63)	1.07 (1.66)	1.27 (1.39)
90-120	0.47 (1.00)	1.09 (0.13)	1.45 (1.31)	1.23 (0.90)	0.70 (3.54)	-0.53 (1.00)	1.67 (2.32)	0.57 (1.09)	3.11 (2.35)	0.79 (0.93)	0.38 (1.43)	1.67 (1.12)
120-153	-0.54 (1.56)	-0.02 (0.63)	0.53 (1.66)	-0.09 (1.60)	-1.25 (1.98)	-1.03 (0.27)	0.44 (1.74)	-0.06 (0.99)	-1.95 (3.01)	-1.19 (0.50)	-1.51 (1.33)	-0.67 (0.64)
153-184	-3.18 (5.52)	-1.67 (1.15)	-0.48 (4.04)	0.26 (1.67)	-4.19 ^{**} (2.64)	-1.13 (2.22)	-4.23 (2.91)	0.22 (0.39)	-2.37 [*] (5.42)	0.01 (0.53)	-1.68 (2.68)	-3.37 (0.76)
184-215	0.43 (2.81)	0.08 (1.32)	-0.23 (2.38)	-1.69 (1.10)	-0.56 ^{**} (1.43)	0.04 (0.76)	-2.12 (3.38)	0.23 (0.61)	-1.75 [*] (2.51)	0.05 (1.07)	-0.28 (3.58)	-0.46 (0.95)
215-245	-1.96 [*] (1.57)	-1.37 (1.15)	-1.87 (4.01)	-0.68 (0.61)	-2.42 ^{***} (6.95)	-0.78 (2.90)	-4.05 [*] (3.52)	-1.08 (0.76)	-2.93 [*] (1.97)	-1.42 (1.53)	-2.47 (1.41)	-0.98 (0.30)

^aAbsolute growth rate for each exposure period were calculated according to Ricker (1979).

Appendix F. Continued.

^bn=6 for females and n=3 for males except for treatment 2 where n=5 for females and n=4 for males and for means footnoted as follows: * n=5, ** n=4, *** n=3.

^cSee Table 3 for selenium treatments.

Appendix G. Absolute growth rate based on total length (least square means pooled across sex)^a of adult Colorado pikeminnow exposed to selenium in the diet and water for 155 days followed by a 90-day depuration period. Means sharing the same upper case letter within a treatment (column) are not significantly different ($P \leq 0.05$).

Exposure period (d)	Treatment ^b					
	1	2	3	4	5	6
0-30	-0.01 ^{CD}	0.01 ^{BC}	0.02 ^B	0.06 ^{BC}	0.02 ^{BCD}	-0.02 ^C
30-58	0.10 ^{AB}	0.03 ^{BC}	-0.05 ^{BC}	-0.08 ^D	-0.05 ^D	0.04 ^{BC}
58-90	0.04 ^{BC}	0.05 ^{BC}	0.18 ^A	0.15 ^{AB}	0.14 ^A	0.09 ^B
90-120	0.18 ^A	0.17 ^A	0.14 ^A	0.22 ^A	0.10 ^{AB}	0.20 ^A
120-153	-0.10 ^{DE}	-0.11 ^D	-0.19 ^D	-0.20 ^E	-0.16 ^E	-0.19 ^D
153-184	0.06 ^{BC}	0.05 ^{BC}	-0.01 ^{B**}	0.09 ^B	0.15 ^{A*}	0.11 ^{AB}
184-215	0.08 ^{BC}	0.11 ^{AB}	0.17 ^{A**}	0.15 ^{AB}	0.06 ^{ABC*}	0.10 ^{AB}
215-245	-0.14 ^{E*}	0.00 ^C	-0.16 ^{CD***}	-0.03 ^{CD*}	-0.04 ^{CD*}	-0.02 ^C

^aPooled standard error = 0.037 mm/d and n=9 for all means, except for those footnoted as follows: *Pooled SE = 0.039 mm/d and n=8; **Pooled SE = 0.041 mm/d and n=7;

***Pooled SE = 0.043 mm/d and n=6.

^bSee Table 3 for selenium treatments.

Appendix H. Results of 3 x 2 factorial analysis of variance (ANOVA) comparing reproductive parameters of adult female Colorado pikeminnow and their progeny. The adults were exposed to six selenium treatments, three levels in diet and two levels in water, for 154 days prior to spawning.

Parameter	Error mean square (2) ^b	Source of variation ^a					
		Diet (2) ^b		Water (1)		Diet x Water (2)	
		F ratio	P value	F ratio	P value	F ratio	P value
Female parent							
Eggs/adult	4.16 x 10 ⁸	1.09	0.48	1.26	0.38	0.06	0.94
Eggs/mm	1.12 x 10 ³	0.59	0.63	1.09	0.41	0.02	0.98
Eggs/g	125.94	0.21	0.83	1.13	0.40	0.21	0.82
Egg quality							
Diameter (mm)	0.0009	0.97	0.51	0.46	0.57	9.20	0.10
Weight (mg)	0.1550	0.28	0.78	0.01	0.92	1.55	0.39
Hatch (%)	0.0520	0.38	0.72	<0.01	0.96	0.94	0.52
Hatch day	0.0596	0.14	0.88	0.38	0.60	0.44	0.69
Larvae with deformities (%)	0.0024	0.92	0.52	0.44	0.58	0.22	0.82
Larval survival (%) at							
1-2 dph ^c	0.0038	0.65	0.61	0.17	0.72	0.12	0.90
5 dph	0.0023	0.01	0.99	2.09	0.28	0.68	0.59
10 dph	0.0042	8.28	0.11	8.61	0.10	3.09	0.24
30 dph	0.0073	4.31	0.19	4.23	0.18	0.93	0.52
Larval growth at 30 dph							
Total length (mm)	0.0317	0.07	0.93	1.27	0.38	6.56	0.13
Weight (mg)	3.4550	1.26	0.44	0.72	0.49	3.76	0.21
K ^d	0.0003	2.25	0.31	0.13	0.76	1.24	0.45

Appendix H. Continued.

^aSee Table 3 for dietary and waterborne selenium concentrations.

^bDegrees of freedom.

^cdph = days posthatch.

^dCondition factor, calculated according to Anderson and Gutreuter (1983).