Project Report:

Development of Methods for Laboratory Culture and Toxicity Testing of the Endangered Desert Pupfish, *Cyprinodon macularius,* and Evaluation of the Acute Toxicity of Selenium

by

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Abstract

We conducted a series of studies to evaluate methods for laboratory culture and toxicity testing with the endangered desert pupfish, *Cyprinodon macularius*. This species may be exposed to hazardous levels of selenium in agricultural drains and shoreline pools in the Imperial Valley of California and the Sonny Bono Salton Sea National Wildlife Refuge. Thirty-eight adult pupfish collected by the U.S. Fish and Wildlife Service and shipped to the Columbia Environmental Research Center adapted readily to laboratory culture in well water adjusted to 10 parts per thousand (ppt) salinity and produced numerous offspring. Offspring were successfully reared to sexual maturity to provide additional brood stock, and both second and third generation offspring were used for acute toxicity tests and for development of methods for chronic toxicity tests.

Acute (96-hr) toxicity tests were conducted in fresh water (conductivity 311 µS/cm) with newly-hatched larvae (<24 hr old) and 3- to 6-month old juveniles (average length 29 mm) desert pupfish, and with comparable age groups of fathead minnows (*Pimephales promelas*), a species that has been widely used as a laboratory test organism. Acute toxicity differed widely among three chemical forms of selenium (selenate, selenite, and selenomethionine) and between fish species and age groups. For both species, toxicity generally increased in the order: selenate < selenite < selenomethionine. In tests with all three selenium forms, desert pupfish were generally less sensitive than fathead minnows, which are among the most sensitive aquatic taxa to acute toxicity of waterborne selenium. Lowest median lethal concentrations (LC50) for pupfish were 33 mg/L for selenate, 22 mg/L for selenite, and 0.43 mg/L for selenomethionine. The ranking of sensitivity among selenium species differed somewhat among age groups of desert pupfish, as larvae were more sensitive than juveniles to selenite and juveniles were more sensitive to selenate and selenomethionine than larvae.

Newly-hatched pupfish performed well under typical test conditions for chronic toxicity

tests, with virtually no mortality during a 90-d study conducted at a salinity of 5 ppt. Pupfish larvae thrived on a diet of live brine shrimp, and were able to switch to a diet of laboratory-cultured aquatic oligochaetes (*Lumbriculus variegatus*) after 48 days. Pupfish fed a diet that included oligochaetes grew more rapidly than those fed only brine shrimp and reached reproductive maturity by the end of the test. Pupfish from the oligochaete diet treatment that were transferred to a breeding tank began spawning immediately and produced large numbers of eggs during a three-week observation period. Hatching success (90%) and larval survival (96%) were excellent. Results of these studies indicate that desert pupfish are highly suitable for laboratory culture and toxicity testing, and that the rapid sexual maturation of this species should facilitate completion of life cycle testing (egg-larvae-juvenile-adult-F1) in a period of six months or less. For chronic laboratory toxicity tests to be realistic simulations of selenium exposure in contaminated habitats, these tests should include exposure to selenium via live diets. However, additional methods development will be necessary to characterize the uptake and bioavailability of selenium into potential live diets, such as brine shrimp and/or oligochaetes.

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Introduction

This report summarizes the first phase of a series of studies intended to determine the effects of selenium on desert pupfish (*Cyprinodon macularius*) that inhabit Imperial Valley agricultural drains and shoreline pools at and in the vicinity of the Sonny Bono Salton Sea National Wildlife Refuge. A project proposal, submitted to the Carlsbad Office of the U.S. Fish and Wildlife Service (USFWS) by the Columbia Environmental Research Center of the U.S. Geological Survey (CERC; memo from Chris Ingersoll, November 8, 2002), listed a series of six tasks to accomplish the overall project goal:

(1) Development of methods for culturing pupfish;

(2) Development of methods for conducting chronic toxicity tests with pupfish;

(3) Development of methods for dosing selenium in the diet of pupfish;

(4) Conducting acute water-borne exposures with pupfish;

(5) Conducting early life-stage dietary exposures with pupfish; and

(6) Conducting reproductive dietary exposures with pupfish.

Results of this research will be used to determine if proposed management actions, specifically actions taken as part of the Imperial Irrigation District's Habitat Conservation Plan, are sufficient to conserve the desert pupfish in the Imperial Valley. The ultimate goal of this research will be to directly assess the potential effects of selenium on reproduction of desert pupfish in the Imperial Valley. The approach chosen for this research involves both acute and chronic toxicity testing. Acute toxicity tests will evaluate the sensitivity of desert pupfish to several chemical forms of selenium in water, and compare their sensitivity to that of a commonly-tested species, fathead minnow. Chronic toxicity tests will evaluate the chronic effects of dietary selenium exposure on survival, growth, and reproduction of desert pupfish and determine toxicity thresholds for selenium effects based on selenium concentrations in both diet

and tissues of desert pupfish.

Funds received during FY2001 through FY2003 allowed CERC to work on Tasks 1, 2, and 4 of the original proposal. This report addresses the three objectives of these tasks:

(1) Develop methods for laboratory culture of desert pupfish, including methods for spawning adult fish received from USFWS, hatching and rearing young, and raising these offspring to provide brood stock for future toxicity studies.

(2) Develop methods for conducting chronic toxicity tests with desert pupfish, including evaluation of test chambers and diets suitable for different life stages (newly-hatched, juvenile, and spawning adults).

(3) Conduct acute toxicity tests with newly-hatched and juvenile desert pupfish and fathead minnows with three chemical form of selenium: selenate, selenite, and selenomethionine.

Methods

Laboratory Culture of Desert Pupfish

Desert pupfish were held, spawned, and tested at CERC under provisions of an endangered species permit issued by USFWS (Permit TE021847-0; expires 7/18/2005). A total of 38 live desert pupfish (10 males and 28 females), were collected by USFWS from the wild population at the Dos Palmas Preserve (Rancho Dos Palmas, CA), and received at CERC on June 6, 2002. Nineteen fish perished during shipping due to a rupture in one of the shipping bags. At CERC, pupfish were held in well water (280 mg/L hardness) with salinity adjusted to 10 ppt with Instant Ocean® salt mix (Aquarium Systems, Mentor, Ohio). Temperature was maintained at 25° C. Three 40-liter aquaria were initially set up with sex ratios of 4 males to 10 females in one aquarium and 3 males to 9 females in two aquaria. Fish were fed brine shrimp (*Artemia* sp.) and

a flaked diet (Ziegler Bros., Gardener, Pennsylvania) *ad libitum*. Water flow was regulated by an automatic timer to provide one complete exchange in each aquarium daily. Foam biofilters were used in each aquarium to control ammonia. Artificial lights regulated day length in a cycle of 16 hours light:8 hours dark. Spawning 'huts', constructed of green nylon scouring pads (3M, St. Paul, Minnesota) provided a spawning substrate for the pupfish. Egg production was checked daily.

Acute Toxicity Tests

Acute (96-hr) water-only toxicity tests were conducted using methods adapted from standard methods (USEPA 1993, ASTM 2003a). Test conditions are summarized in Table 1. Tests were conducted with two life stages (newly-hatched larvae and juveniles) of desert pupfish and fathead minnows (from Aquatic Bio Systems, Fort Collins, Colorado). Pupfish larvae were tested less than 24 hours after hatching and minnow larvae were tested less than 48 hours post-hatch, due to the delay caused by shipping from the culture facility. Juvenile desert pupfish were tested at 3 to 6 months of age, with a mean total length of 29 mm and mean wet weight of 0.45 g. Juvenile fathead minnows of the same age range had mean total length of 33 mm and mean wet weight of 0.31 g.

Tests were conducted with three chemical forms of selenium: selenate (as sodium selenate); selenite (as sodium selenite), and seleno[DL]methionine (Sigma-Aldrich, St. Louis, Missouri). Exposure solutions were prepared in a reconstituted test water, designed to represent water quality conditions in the San Joaquin River in central California, which had been used for previous investigations of selenium toxicity in irrigation drainwater (Ingersoll et al. 1990). This test water was prepared by diluting CERC well water with deionized water to a strength of 25%, followed by addition of calcium sulfate ($CaSO_4$+$2H_2O$, 54 mg/L) and magnesium sulfate

($MgSO_4$+7H_2O, 78 mg/L). Final characteristics of this water were: conductivity, 311 µS/cm; hardness, 140 mg/L as $CaCO_3$; alkalinity, 80 mg/L as $CaCO_3$; sulfate, 70 mg/L; chloride, 6 mg/L, and pH, 8.0. Samples of test water were collected from selected test chambers and analyzed for selenium. Analyses were performed by flow-injection hydride-generation atomic absorption spectroscopy (May et al. 2001). A final report on the selenium analyses and associated quality assurance measures (May et al. 2003) has been completed and will be submitted to USFWS with this report.

Median lethal concentrations (LC50s) were used to compare acute toxicity among selenium form and between fish species and life stages. Whenever possible, LC50s were estimated using the trimmed Spearman-Karber method, with the 10% trimming level (Hamilton et al. 1977). When more than six selenium levels (including controls) were tested, LC50s were calculated using data from six selenium levels that best bracket on the 50% mortality level. Because selenium concentrations in test water were not analyzed for all exposure concentrations from all tests, LC50s for some tests with selenite and selenomethionine included selenium concentrations that were extrapolated from nominal concentrations using the average ratios of measured/nominal selenium concentrations. Average measured/nominal ratios (with standard deviation and number of analyses) were: selenite, 109% (SD=4%, N=10); and selenomethionine, 101 (SD=2%, N=8).

Chronic Toxicity Test Methods Development

We evaluated the suitability of standard methods (ASTM 2003b) for conducting chronic toxicity tests with desert pupfish. The evaluation consisted of a simulated (without toxicant) early life-stage toxicity test, followed by a simulated reproduction test. The early life-stage test (Table 2) was conducted in a proportional diluter, starting with eight groups of fifteen newly-hatched (<24-

h old) desert pupfish, with each group held in a 6.5-L chamber that received three volume-additions of test water daily. Test water was well water adjusted to a salinity of 5 ppt. For the first 48 days of the test, all groups of pupfish were fed live brine shrimp *ad libitum* (three times daily during the week, twice daily on weekends). After day 48, four of the eight replicate groups (brine shrimp treatment) continued to be fed brine shrimp and four groups (oligochaete treatment) were switched to a diet of live aquatic oligochaetes (*Lumbriculus vareigatus*), fed *ad libitum* twice daily. Survival and growth (length and weight) of pupfish were determined after 90 days, except growth measurements were only made on two of the four groups from the oligochaete treatment, with the other two groups reserved for an evaluation of their reproductive status. The reproduction test was conducted with 30 surviving pupfish (18 females, 12 males) from the oligochaete feeding treatment, which were transferred to larger (40-L) aquaria equipped with spawning substrates. Water quality and diets were the same as in the early-life stage test. Egg production was monitored daily, and eggs were removed daily during the first two weeks to document hatching success. Subsets of hatched larvae were held for up to 18 d to estimate larval survival rates.

Results and Discussion

Laboratory Culture of Desert Pupfish

Adult pupfish received at CERC adapted well to laboratory conditions. During a 160-day period, the initial cohort of 38 adults produced 8,419 eggs. For a subset of 60 spawns, average egg hatchability was 80%. A subset of 1040 of the resultant juveniles, with an average weight 0.45 grams, were used as test organisms for acute toxicity tests. Another group of offspring from the original brood stock were reared to sexual maturity to provide additional brood stock. These second-generation fish were held in 10 aquaria, with fish paired at a ratio of 6 females to 3 males.

Four spawning huts were placed into each aquarium. A total of 940 larvae from these spawns were used to provide newly-hatched (<24 hours old) larvae for use in the acute toxicity tests. Excess embryos and larvae were humanely euthanized. One first-generation female died of a parasitic intestinal nematode infection. Judging from the size of the nematode, the fish was likely infected by the parasite in the wild. No live pupfish were removed from the laboratory or released into the wild.

Acute Toxicity Tests

Acute toxicity of selenium form differed between fish species and life stages. Selenate had relatively low toxicity to both desert pupfish and fathead minnows (Table 3). Minnow larvae were most sensitive to selenate (LC50=24 mg/L) and pupfish larvae were least sensitive to selenate, with little mortality observed except at the highest test concentration (50% mortality at 97 mg/L). Selenate was equally toxic to juvenile pupfish and juvenile minnows. Selenite was less acutely toxic to desert pupfish than to fathead minnows (Table 4). LC50s for selenite toxicity to pupfish (22-40 mg/L) were about ten-fold greater than those for minnows (1.4-3.5 mg/L). Selenite was more toxic to pupfish larvae than to juveniles, but the opposite trend was observed in tests with minnow larvae and juveniles. The acute toxicity of selenomethionine varied widely among different species and life stages (Table 5). Pupfish larvae were extremely tolerant of selenomethionine, with less than 50% mortality at the highest concentration tested, 102 mg/L, whereas selenomethionine LC50s for the other three groups were all less than 1 mg/L. Juvenile pupfish (LC50=0.43 mg/L) were less sensitive to selenomethionine than either larval or juvenile minnows (LC50s=0.11 and 0.031 mg/L, respectively).

Desert pupfish were consistently less sensitive than fathead minnows to acute selenium

toxicity. Except for the similar sensitivity of juvenile pupfish and juvenile minnows to selenate, all three selenium forms were more toxic to larval and juvenile minnows than to corresponding pupfish age groups (Tables 3-5). For fathead minnows, differences in sensitivity of larvae and juveniles were small, relative to differences in toxicity among the selenium form. Pooled results of tests with both age groups of minnows indicated a consistent trend in the toxicity of the selenium form, with selenate least toxic and selenomethionine most toxic (Figure 1). In contrast, the overall ranges of pupfish LC50s for selenate, selenite, and selenomethionine overlapped broadly, and differences in sensitivity between pupfish age groups were as important as differences in toxicity among selenium form (Figure 1). For pupfish (as for minnows), there was no consistent trend for differences in toxicity of selenium form between larvae and juveniles. Comparison of the lowest pupfish LC50s for each selenium form, regardless of age group, suggests that the toxicity of selenate (LC50 = 33 mg/L) and selenite (LC50= 22 mg/L) differed relatively little, but that both of these inorganic form were much less toxic than the organic form, selenomethionine (LC50=0.43 mg/L).

Our results suggest that desert pupfish are not among the aquatic species that are most sensitive to acute toxicity of selenate and selenite. Canton (1999) reviewed the available literature on acute aquatic toxicity of selenite and selenate and generated rankings of genus mean acute values (GMAVs) for 26 genera exposed to selenite and for 18 genera exposed to selenate. These rankings can be expressed as a percentiles, which estimate the percent of genera with lower GMAVs and therefore greater sensitivity to acute toxicity. Average LC50s from our tests with two life stages of desert pupfish corresponded to the 81st percentile of GMAVs for aquatic taxa tested with selenate and the 87th percentile for selenite. In contrast, Canton (1999) listed fathead minnows as the most sensitive fish species tested for toxicity of both selenate and selenite, and average LC50s for our tests with fathead minnows corresponded to the 25th

percentile of aquatic taxa for selenate and the 58th percentile for selenite.

Selenomethionine was the most toxic form of selenium to both desert pupfish and fathead minnows. One or more LC50s from tests with both fish species were less than 1 mg/L (Table 5), much lower than any LC50s we determined for selenate or selenite (Tables 3 and 4). As was observed for selenate and selenite, toxicity of selenomethionine was greater for fathead minnows than for desert pupfish. The lowest selenomethionine LC50 for pupfish, 0.43 mg/L for juveniles, was an order of magnitude greater than the LC50 for selenomethionine toxicity to juvenile fathead minnows. In a remarkable contrast, selenomethionine had very low toxicity to newly-hatched pupfish (LC50 >102 mg/L). This large difference in toxicity between life stages suggest that physiological changes during development greatly affect selenomethionine uptake, metabolism, and/or toxicity to target organs. Although the larger cohort of pupfish tested were considered juveniles at the time of testing, results of subsequent studies (discussed below) suggest that the 'juvenile' pupfish tested (average length 29 mm) approached the size at which this species reaches sexual maturity under laboratory conditions. Relatively little acute toxicity data is available for selenomethionine, as this compound is not commonly analyzed and is unlikely to persist at high concentrations in surface waters (Besser et al. 1989, 1993). Other studies have reported wide differences in LC50s for toxicity of selenomethionine to other fish species and life stages. Juvenile striped bass, *Morone saxatilis,* were more sensitive than newly-hatched larvae, with acute LC50s as low as 0.011 mg/L (Duane Chapman, CERC; unpublished data). Other species were quite insensitive, with LC50s as high as >21.6 mg/L for juvenile chinook salmon, *Onchorhynchus tshawytscha* (Hamilton and Buhl 1990) and > 40 mg/L for zebrafish, *Brachydanio rerio* (Niimi and LaHam 1976). Selenomethionine has also been reported to be more toxic than selenate or selenite to aquatic invertebrates (Ingersoll et al. 1990).

The sensitivity of desert pupfish to acute toxicity of waterborne selenium may not

provide a reliable prediction of the potential for chronic selenium toxicity. Fathead minnows, which are consistently among the most sensitive fish species to acute selenium toxicity, are not highly sensitive to chronic selenium toxicity, whereas sunfishes (*Lepomis*) and Pacific salmon (*Onchorhynchus*), which are consistently among the most sensitive genera in chronic toxicity studies, are not highly sensitive to acute toxicity (Canton 1999; DeForest et al. 1999, Hamilton 2003). Chronic selenium toxicity in fish is typically caused by different exposure routes (selenium-contaminated diets) and chronic effects are expressed on different target organs (reproductive tract) than acute toxicity.

Evaluation of Methods for Early Life-stage and Reproduction Tests

Cohorts of newly-hatched desert pupfish performed very well under conditions typical of early life-stage toxicity tests. The newly-hatched fish fed readily on brine shrimp and only one mortality was observed out of the 120 test fish during the course of the 90-d study (Table 6). Results also suggest that a diet of oligochaetes was suitable for larger juvenile pupfish. Fish in the oligochaete feeding treatment had greater average total length of (31.9 mm) and greater average dry weight (0.179 g dry wt.) than the group that was fed only brine shrimp (30.6 mm and 0.143 g dry wt.). The use of the oligochaete diet would be advantageous for the design of chronic dietary toxicity tests, as the greater biomass and longer life cycle of oligochaetes, relative to brine shrimp, would facilitate production of the large quantity of selenium-dosed food required for tests with adult pupfish. Supplemental studies conducted at CERC suggest that desert pupfish can be switched to a diet of live oligochaetes at an earlier age (approx. 5 weeks) than was attempted in this study. Researchers at USEPA have developed methods for using metal-dosed oligochaetes in dietary toxicity studies with freshwater fish (Mount et al. 2000) and these methods could be adapted to dose *Lumbriculu*s with selenium for future dietary toxicity studies

with desert pupfish.

The suitability of conditions in the diluter was further indicated by the rapid onset of reproduction. Pupfish began to exhibit secondary sexual characteristics by the end of the 90-d early life-stage study, and dissections of females at the end of the test demonstrated maturing ovaries. In the reproduction study, the first eggs were observed on day 96 – days counted from the start of the early life-stage study -- and the number of eggs produced increased steadily until the test was terminated on day 117, with a peak of 103 eggs on day 113 (Figure 2). Over a three-week period, 18 females laid a total of 787 eggs, an average of 44 eggs per female. Eggs collected during the first two weeks of the study had consistently high hatching success (Figure 2) and a high larval survival. A survival rate of 96% was determined for a subset of 193 larvae that were held for at least 7 days post-hatch. Although it is not clear from the study how long egg production would continue, or how the fecundity of these young adult fish compares to that of older adult fish, the early onset of reproduction observed in this study should prove advantageous for reproductive toxicity studies.

Conclusions

1. Desert pupfish were easily propagated in the laboratory. The fish readily accepted typical laboratory diets, and thrived in our culture systems in well water fortified to a salinity of 5 ppt. About 200 adult pupfish are currently being held in well water without added salinity and appear to thrive under these conditions.

2. Desert pupfish were less sensitive to acute toxicity of three chemical forms of selenium in fresh water than were fathead minnows. For selenite and selenate, the selenium form that predominate in surface waters, average LC50s for pupfish were greater than those reported in the

literature for most aquatic taxa. Selenite was more toxic than selenate to both pupfish and minnows. Tests with selenomethionine produced the lowest LC50s (<0.5 mg/L) for both species.

3. Selenium toxicity differed between newly-hatched larvae and juveniles for both species, but neither life stage was consistently more sensitive. For pupfish, differences in sensitivity between life stages were as important as differences in toxicity between selenium form. The greatest difference between life stages was observed for pupfish exposed to selenomethionine, with juvenile pupfish more than 200-times more sensitive than larvae.

4. Exposure to acutely lethal concentrations of waterborne selenium is not likely to be a significant factor limiting recovery of desert pupfish in the Imperial Valley. Evaluation of toxic effects of chronic exposure to selenium in diet and water, including effects on reproduction and early life stages, is necessary for a realistic assessment of overall risks of selenium to desert pupfish populations.

5. Pupfish performed well under typical conditions used in for chronic toxicity tests. Newly-hatched larvae thrived on a diet of brine shrimp, and growth of juvenile pupfish was increased by providing a diet of oligochaetes halfway through the 90-d test period. After 90 d, some pupfish that were fed the oligochaete diet had reached sexual maturity. Egg production increased over time for fish between 90 and 120 days old, with consistently high rates of egg hatching and larvae survival. These results indicate that toxicity tests to determine the effects of selenium-contaminated diets over the full life cycle of desert pupfish could be conducted in a relatively short time period (< 6 months) without highly specialized test methods.

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Table 1. Summary of test conditions for conducting acute toxicity tests with desert pupfish (*Cyprinodon macularius*) and fathead minnows (*Pimephales promelas*). Methods adapted from ASTM (2003a) and USEPA (1993).

Parameter	Conditions
1. Exposure system	Static
2. Temperature	22° C
3. Toxicant(s)	Sodium selenate, sodium selenite, and selenomethionine
4. Dilution	60%; 5 or 6 concentrations and a control
5. Lighting	16 hr light:8 hr dark; light intensity of approx. 10-20 μE/m^2/s
6. Test/chamber volume	newly-hatched larvae: 250 mL in 1-L jars juveniles: 15 L in 19-L jars
7. Test water	Reconstituted 'San Joaquin River' fresh water (conductivity, 311 μS/cm; see Methods)
8. Water renewal	None
9. Size/age of organisms	newly hatched: <24 hr old juveniles: 3-6 months old
10. Organisms/chamber	10
11. Replication	2 per toxicant level
12. Feeding	None (juveniles held 48 h without food before test)
13. Aeration	None
14. Chamber cleaning	None
15. Water quality	Day 0, waterborne selenium; days 0 and 4, pH, alkalinity, hardness, dissolved oxygen; day 4, ammonia
16. Duration	96 h (4 d)
17. Endpoints	Survival (at 6, 12, 24, 48, 72, and 96 h)
18. Test acceptability	90% survival in controls

Table 2. Summary of test conditions for mock early life-stage toxicity test with desert pupfish (*Cyprinodon macularius*). Methods adapted from ASTM (2003b).

Parameter	Conditions
1. Exposure system	Intermittent-flow proportional diluter
2. Temperature	25±1° C
3. Toxicant(s)	None
4. Dilution	Not applicable
5. Lighting	16 hr light:8 hr dark; light intensity of approx. 10-20 μE/m^2/s
6. Test/chamber volume	6 L/6.5 L
7. Test water	CERC well water, adjusted to salinity of 5 parts per thousand
8. Water renewal	0.75 liter/chamber/h
9. Size/age of organisms	newly hatched larvae (<24 hr)
10. Organisms/chamber	15
11. Replication	4 replicates per feeding treatment
12. Feeding	0-48 d: live brine shrimp to all fish, fed 2-3 times/d 49-90 d: live oligochaetes (group O) or brine shrimp (group B)
13. Aeration	None
14. Chamber cleaning	Daily
15. Water quality	Daily: temperature Weekly: conductivity, salinity, pH, dissolved oxygen
16. Duration	90 d
17. Endpoints	Survival, growth (weight, length)
18. Test acceptability	Not applicable (methods development)

Table 3. Results of acute (96-hr) toxicity tests with desert pupfish and fathead minnows exposed to selenate. Median lethal concentrations (LC50) and 95% confidence intervals calculated using Trimmed Spearman-Karber method (Hamilton et al. 1977).

Measured Se conc. (mg/L)	Survival (%)			
	Pupfish (larvae)	Pupfish (juvenile)	Minnow (larvae)	Minnow (juvenile)
Control	100	100	100	100
7.3	97	100	100	97
13	93	100	67	83
21	100	97	73	83
35	100	37	30	53
55	83.3	17	3.3	0
97	50	3.3	0	0
LC50, mg/L (95% C.I.)	**≥97**	**33 (29-38)**	**24 (20-28)**	**33 (28-40)**

Table 4. Results of acute (96-hr) toxicity tests with desert pupfish exposed to selenite. Median lethal concentrations (LC50) and 95% confidence intervals calculated using Trimmed Spearman-Karber method (Hamilton et al. 1977).

Measured Se conc. (mg/L)	Survival (%)		Measured Se conc. (mg/L)*	Survival (%)	
	Pupfish (larvae)	Pupfish (juvenile)		Minnow (larvae)	Minnow (juvenile)
Control	100	100	Control	93	100
8.2	--	100	0.9	97	80
14	80	100	1.4*	97	67
23	47	97	2.4*	83	10
40	17	43	3.9*	33	0
67	0	13	6.5*	10	0
104	0	0	11	0	0
183	0				
LC50, mg/L	**22**	**40**		**3.5**	**1.4**
(95% C.I.)	**(17-29)**	**(35-45)**		**(3.1-4.1)**	**(1.2-1.8)**

* Some 'measured' values were estimated based on average [measured/nominal] ratio.

Table 5. Results of acute (96-hr) toxicity tests with desert pupfish and fathead minnows exposed to selenomethionine. Median lethal concentrations (LC50) and 95% confidence intervals calculated using Trimmed Spearman-Karber method (Hamilton et al. 1977).

Measured Se conc. (mg/L)*	Survival (%)	Measured Se conc. (mg/L)*	Survival (%)		
	Pupfish (larvae)		Pupfish (juvenile)	Minnow (larvae)	Minnow (juvenile)
Control	100	Control	100	97	100
8.3	100	0.056	100	100	23
13*	97	0.086	97	70	0
23*	87	0.13	100	20	0
37*	93	0.23*	100	17	0
62*	73	0.37	77	10	0
102	57	0.63	0	7	0
LC50, mg/L	**>102**		**0.43**	**0.11**	**0.031**
(95% C.I.)	**--**		**(0.40-0.47)**	**(0.09-0.13)**	**(0.03-0.04)**

* Some 'measured' values were estimated based on average [measured/nominal] ratio.

Table 6. Survival and growth of desert pupfish during mock 90-d early life-stage test. Total lengths were determined for each surviving fish; dry weights were determined in aggregate.

Measurement	Brine shrimp diet				Oligochaete diet	
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2
Number stocked	15	15	15	15	15	15
Number surviving	14	15	15	15	15	15
Mean total length,	30.4	30.9	30.6	30.4	31.8	32.0
mm (std. dev.)	(3.8)	(2.5)	(3.2)	(2.6)	(4.2)	(2.3)
Mean dry wt. , g	0.150	0.151	0.140	0.131	0.180	0.178

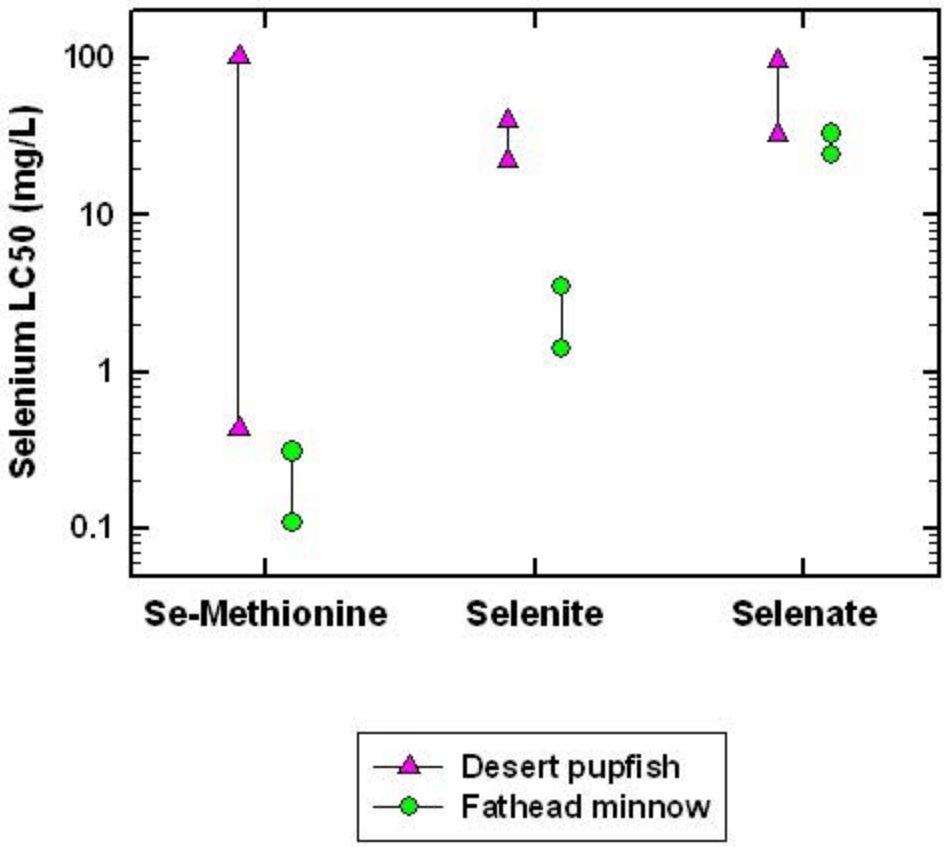

Figure 1. Acute toxicity of three forms of selenium to desert pupfish and fathead minnows. Median lethal concentrations (LC50s) from tests with larvae and juveniles of each fish species.

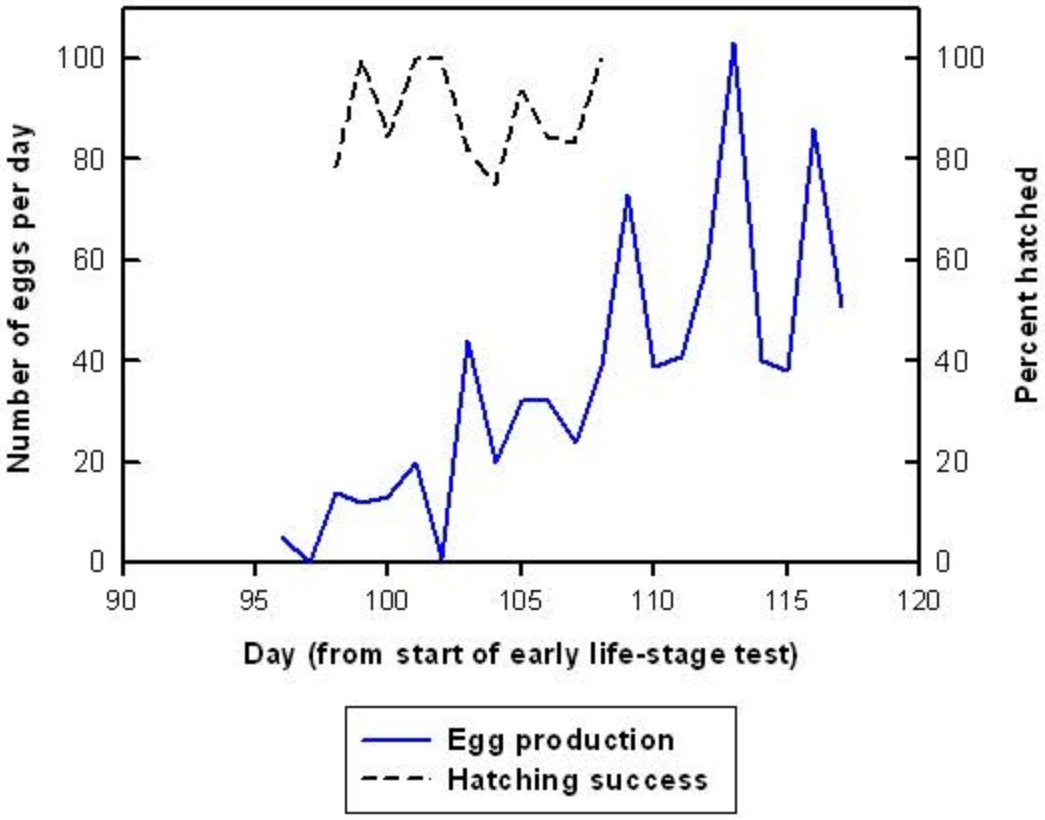

Figure 2. Egg production and egg hatching success during mock reproduction test with desert pupfish.