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Effects of Elevated Foodborne Selenium on Growth and Reproduction of the Fathead Minnow (*Pimephales promelas*)

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Abstract. Several field studies of selenium-contaminated lakes and reservoirs have indicated the possibility of selenium-induced reproductive failure in important populations of fish. These investigators have hypothesized that bioaccumulation of selenium through the food chain led to fish selenium levels high enough to elicit toxic responses. The present investigation was designed to determine the effects of elevated foodborne selenium on the fathead minnow (*Pimephales promelas*). Fish were fed a diet spiked with a mixture of inorganic (selenite and selenate) and organic (seleno-L-methionine) selenium and effects on growth and reproduction were determined. Growth was significantly inhibited at the highest selenium treatment levels evaluated (20 and 30 ppm Se). There were no significant treatment effects on any of the reproductive parameters measured. Reasons for the disparity between selenium-induced reproductive impairment observed in other species and apparent lack of impairment in fathead minnows may involve reduced bioaccumulation of selenium by minnows due to differences in gut morphology and physiology.

As with most animals, selenium (a natural trace element) is a required micronutrient for fishes (Poston *et al.* 1976; Gatlin and Wilson 1984; Gatlin *et al.* 1986; Bell *et al.* 1986). Additional beneficial effects in fish include amelioration of the accumulation and/or toxicity of mercury (Kim *et al.* 1977); Heisinger *et al.* 1979; Klaverkamp *et al.* 1983; Heisinger and Scott 1985) as well as reduction of the effectiveness of infectious bacteria (MacFarlane *et al.* 1986).

However, as early as 1957, elevated levels of selenium were being linked to drastic declines in fish populations (Barnhart 1957). This relationship was observed more recently in waters contaminated with selenium from coal ash pond effluent from power plant operations. Field monitoring of one such system at Belews Lake (North Carolina) revealed an absence of young-of-the-year for many fish species, despite the fact that adults had been observed spawning in the lake. A survey of trace elements in the water and biota indicated elevated levels of selenium and the investigators hypothesized that selenium bioaccumulation through the food chain (biomagnification) led to levels high enough in fish to impair reproduction (Cumbie and VanHorn 1978).

In Martin Creek Reservoir (Texas), selenium contamination from ash pond effluent was linked to a dramatic drop in fish biomass (excepting *Cyprinus carpio*) and corresponding alterations of fish community structure (Garrett and Inman 1984). In a subsequent study of Belews Lake, it was reported that of 20 species of fish originally present in the reservoir, 16 had been entirely eliminated (Lemly 1985). Again, these investigators hypothesized that biomagnification of selenium had led to impaired reproduction and fish population declines.

The objective of the present study was to investigate the effects of elevated foodborne selenium on the fathead minnow (*Pimephales promelas*). Fish were fed a diet spiked with a mixture of organic and inorganic selenium and effects on growth and reproduction were determined. While growth was significantly inhibited at the highest treatment levels evaluated (20 and 30 ppm Se), reproduction appeared to be unaffected. Potential causes of the disparity between effects on reproduction of different fish groups are discussed.

Table 1. Purified diet ingredients

Ingredients	*g/kg*
Vitamin-free casein	310
Wheat gluten	150
Dried egg white	40
Dextrin	272
Lecithin	80
Celufil	8
Bernhart-Tomarelli Salt Mix	30
Oil mix[a]	60
ADEK oil mix	10
Vitamin mix	40
Vitamin mix	*g/kg*
Thiamine HCl	5
Riboflavin	8
Nicotinic acid	26
Ca-d-Pantothenate	15
Pyridoxine HCl	3
Cobalamine	1
Folic acid	5
Biotin	1
Inositol-meso	180
L-ascorbic acid	125
PABA	30
BHA	1
Celufil	600
ADEK oil mix	*g/100 gm*
D-L Tocopherol	0.75
Menadione (K) oil	0.50
Vitamin A acetate	0.18
Cholecalciferol (D3)	0.025
Oil mix	98.5

[a] Oil mix consists of 1:1:1 mixture of cod liver oil, corn oil, and animal lard. All ingredients from U.S. Biochemical

Materials and Methods

Preparation of Diet

The diets were prepared by spiking a purified diet mix (Table 1) with a mixture of inorganic and organic selenium. Our intent was to use the predominant inorganic ions (selenate and selenite) and seleno-amino acids (selenocysteine and selenomethionine) equally as sources for the selenium in the spiked diets; however, a commercial source of uncontaminated selenocysteine (Se-cys) could not be found. As the possibility of hydrolysis of the selenohydryl moiety of Se-cys exists (Huber and Criddle 1967), it was decided to shift the Se-cys proportion to selenite (of the available selenium compounds, a likely product of such a hydrolysis reaction). Thus, the selected proportions of selenium from each source were as follows: 25% of selenium from seleno-L-methionine (Se-L-met), 25% from Na_2SeO_4, and 50% from Na_2SeO_3 (all obtained from Sigma Chemical). The selenium mixture was homogenized in dextrin which was added to each diet as appropriate to achieve the desired selenium levels. For both range-finding and final experiments, a control (unspiked) and five concentrations of selenium-spiked diets were prepared.

The prepared diet mix was extruded into 1.5 mm pellets which were blown-air (room temperature) dried to 5% moisture content. These pellets were crushed and particles passing through a 7.1 mesh/cm sieve and retained by an 11.8 mesh/cm sieve were used in this study. Diets were stored frozen until just prior to use. Samples of each diet were collected every 14 days for selenium content analysis.

Physical/Chemical System

Six 60-L glass aquaria were placed in each of two temperature-controlled (25° ± 2° C) water baths. Each aquarium was divided into four equal cells using perforated polyethylene tank dividers. A completely randomized block experimental design was used with the water baths serving as the blocks providing a total of eight replicates at each of six treatment levels. Lighting was provided by Vita-Lite (DuroTest) fluorescent lighting using the photoperiod schedule established by Benoit (1981) to induce spawning in fathead minnows.

Aerated campus (University of California, Davis) well water was used in all experiments. Temperature, pH, and dissolved oxygen were determined on one randomly-selected replicate from each treatment in each water bath every 48 hr. Alkalinity, water hardness, and NH_3-N were determined (APHA 1980) for samples from similarly selected replicates every seven days. In addition, 10 ml water samples were collected every seven days from one replicate per treatment in each bath, acidified with five ml of a 5:2 mixture of ACS reagent nitric and perchloric acids, and stored under refrigeration for subsequent selenium analysis. Water quality results are reported in Table 2.

Range-Finding Experiment

Range-finding foodborne selenium levels were established at 0.5 (control), 10, 20, 40, 80, and 160 ppm, dry weight. The f_1 generation of fish obtained from the Columbia National Fishery Research Laboratory (U.S. Fish & Wildlife Service) were used in this experiment. Using protocol similar to that described for the final experiment, effects of these diet levels on growth and spawning activity were determined.

At 80 and 160 ppm Se, fish fed the first day, developed severe edema within 24 hr, and were not observed feeding during the remainder of the experiment. Both treatments experienced substantial mortality, apparently due to starvation. While feeding and growth were reduced at 40 ppm Se, no mortalities attributed to starvation were observed; however, at this and the higher treatments, no spawning occurred. Feeding, growth, and spawning activity appeared normal at the lower selenium treatments.

At this time, information became available indicating that selenium-induced reproductive impairment in bluegill involved larval stages of the fish (R. B. Gillespie, pers. comm.). As it was deemed desirable to ensure that fish did spawn, nominal treatment selenium levels of 0 (control), 5, 10, 15, 20, and 30 ppm were selected for the final experiment. Selenium analysis of these diets revealed actual mean selenium levels of 0.4, 5.2, 10.2, 15.2, 20.3, and 29.5 ppm, respectively.

Final Experiment Protocol

F_2 generation fish were used in the first block of the final experiment. Fish obtained from a commercial supplier (Aquatic Research Organisms, Hampton, NH) were used in the second

Table 2. Wate quality characteristics. Mean ± SD

Temperature	25.7° ± 0.1°C
Dissolved Oxygen	8.48 ± 0.07 mg/L
pH	8.19 ± 0.06
Alkalinity (to pH 4.6)	247.4 ± 23.2 mg/L $CaCO_3$
Hardness	139.4 ± 17.4 mg/L $CaCO_3$
NH_3-N	27.5 ± 5.3 µg/L
Total Selenium	0.8 ± 0.3 µg/L

block, which was initiated three weeks later than block one. Ten randomly-selected fish (59–61 days old) were weighed to the nearest 0.01 g and placed in each cell. Fish were fed twice daily at 6% body weight per day, with wastes and uneaten food being removed 30 min after each feeding. Tanks were then flushed with two tank volumes of fresh water.

Every two weeks, fish were collected by net from each replicate and weighed for determination of treatment effects on growth. At this time, one fish from two of the four replicates in each block was removed and frozen for later selenium analysis to determine the bioaccumulation trends during this exposure period. On day 98, spawning substrates (10 cm half-sections of 10.1 cm PVC pipe) were placed in each cell. On day 105, fish were removed to individual 250 ml beakers and examined for sexual maturity with the best male and female (as evidenced by development of secondary sexual characteristics) being retained as the spawning pair for that replicate. The subsequent spawning period for each replicate extended to 30 days after the first spawning event for that replicate. Pairs which did not spawn by 14 days after the last spawning event in that block were declared non-spawners. At the end of each spawning period for each replicate, the fish were weighed and frozen for later dissection and collection of gonad and muscle tissues for selenium analysis.

Spawning substrates were inspected daily. When present, eggs were inspected for fertility and viable eggs counted. Samples of not more than 50 eggs from each spawn were incubated in flowing aerated water, inspected daily with dead eggs being removed, and percent hatch determined.

Ten larvae from each incubated brood were then transfered to glass test chambers containing 350 ml water. These jars were placed in water baths with temperature and lighting regulated as in the spawning tanks. Larval fish were fed newly-hatched brine shrimp *ad libitum* twice daily. Uneaten brine shrimp were removed at the end of each day. Approximately 80% of the water in each chamber was replaced every 48 hr. Larval fish were maintained for 14 days and percent survival determined.

Selenium Determinations

Fish whole body and tissue samples were rinsed with de-ionized water, blot-dried on a paper towel, and weighed to the nearest 0.01 g. These samples were then dried at 70°C in a drying oven for 60 hr and then weighed to the nearest 1.00 mg. Six ml of the 5:2 mixture of nitric and perchloric acids were added to each sample which, along with the water samples, were digested using gradually increasing temperatures which eventually boiled off the nitric acid. The digested samples (all at the Se^{6+} state) were reduced to the Se^{4+} state by adding three ml of 6N HCl and heating at 150°C for ten min. Selenium levels of each sample were determined by hydride generation with a Varian VGA-76 hydride generator coupled to a Perkin-Elmer Model 403 atomic absorption spectrophotometer. Accuracy of these determinations was validated by similarly analyzing National Bureau of Standards albacore tuna samples with recovery of 100 ± 9.6%. All selenium levels for diet and fish whole body and tissue samples are reported for dry weights.

Data Analysis

The general linear model (SAS Institute 1985) was used to determine the significance of block and treatment effect on measured parameters. The arcsin angular transformation was applied to the percent hatch and percent survival data in order to establish data normality; statistics on these data were back-transformed to report the mean and S.D. as percent. Multiple comparisons of means were made using Tukey's (HSD) test with alpha = 0.05.

Results

Significant growth occurred throughout the pre-spawning exposure period (Table 3). Fish in the second block grew faster than fish in the first block and the block effect was significant at all treatment levels. Foodborne selenium treatments up to 15 ppm did not significantly inhibit growth (relative to controls) at any time during this period. At 20 ppm, fish mean weights became signficantly less than controls from day 56 on. The decline in growth was most obvious at 30 ppm. By day 56, fish mean weights at 30 ppm were significantly less than all treatments except 20 ppm, a trend which continued throughout this pre-spawning period.

Selenium bioaccumulation (measured as whole body selenium level) during this period was rapid, with most treatments essentially reaching equilibrium within 14 days (Table 4). Block effect was not significant for any of the whole-body selenium comparisons. Whole body selenium levels were dose-dependent throughout this period, although there was considerable variability in the selenium levels between replicates (Table 4).

Comparative tissue selenium levels from the spawning pairs of fish were also dose-dependent (Table 5). Female gonads contained significantly higher levels of selenium than other tissues evaluated. Variability was most pronounced for male and female gonad tissues.

Considering that ovaries contained greater levels of selenium than other tissues, effects on reproduction might have been expected. However, selenium treatment had no significant effect on any of the reproductive parameters measured (Table 6). Number of spawns per pair were highly variable, and the least spawning seemed to occur at the control treatment (this apparent difference was not statistically significant). The numbers of eggs per spawn were

Table 3. Mean fathead minnow weights (g) over course of pre-spawning exposure period. Mean ± S.D. Sample size = 8 for all means

Selenium treatment	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98	Statistical significance
Control	0.12 ± 0.02 A;a	0.21 ± 0.02 B	0.30 ± 0.02 C;a	0.45 ± 0.04 D;a	0.63 ± 0.12 E;a	0.82 ± 0.15 F;a	1.04 ± 0.21 G;a	1.30 ± 0.22 H;a	$p < 0.0001$
5 ppm	0.12 ± 0.02 A;a,b	0.20 ± 0.02 B	0.28 ± 0.02 C;a,b	0.43 ± 0.03 D;a	0.59 ± 0.08 E;a,b	0.77 ± 0.10 F;a,b	0.96 ± 0.18 G;a,b	1.24 ± 0.20 H;a,b	$p < 0.0001$
10 ppm	0.12 ± 0.02 A;a	0.20 ± 0.03 B	0.29 ± 0.04 C;a	0.43 ± 0.04 D;a	0.58 ± 0.10 E;a,b	0.74 ± 0.10 F;a,b	0.95 ± 0.12 G;a,b	1.20 ± 0.11 H;a,b	$p < 0.0001$
15 ppm	0.11 ± 0.02 A;a,b	0.19 ± 0.03 B	0.28 ± 0.02 C;a,b	0.42 ± 0.04 D;a,b	0.58 ± 0.08 E;a,b	0.76 ± 0.11 F;a,b	0.94 ± 0.13 G;a,b	1.21 ± 0.14 H;a,b	$p < 0.0001$
20 ppm	0.12 ± 0.03 A;a	0.20 ± 0.03 B	0.28 ± 0.03 C;a,b	0.41 ± 0.04 D;a,b	0.53 ± 0.07 E;b,c	0.68 ± 0.08 F;b,c	0.85 ± 0.12 G;b,c	1.09 ± 0.16 H;b,c	$p < 0.0001$
30 ppm	0.11 ± 0.02 A;b	0.19 ± 0.03 B	0.25 ± 0.02 C;b	0.37 ± 0.03 D;b	0.47 ± 0.05 E;c	0.60 ± 0.06 F;c	0.73 ± 0.07 G;c	0.94 ± 0.07 H;c	$p < 0.0001$
Statistical significance	$p < 0.004$	$p < 0.05$	$p < 0.03$	$p < 0.002$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	

Row means with the same upper case letter are not significantly different
Column means with the same lower case letter are not significantly different

Table 4. Fathead minnow whole body selenium levels (μg/g) over course of pre-spawning exposure period. Mean ± S.D. (Sample size = 20 for means at Day 0, 4 for remaining means)

Selenium treatment	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84	Day 98	Statistical significance
Control	1.35 ± 0.51 A	1.40 ± 0.41 A,B;a	1.85 ± 0.17 A,B,C;a	1.78 ± 0.90 A,B,C;a	2.67 ± 1.13 C;a	2.52 ± 0.81 B,C;a	2.43 ± 0.95 B,C;a	1.76 ± 0.26 A,B,C;a	$p < 0.0001$
5 ppm	1.35 ± 0.51 A	2.35 ± 0.53 A,B;a,b	2.72 ± 0.59 B;a,b	2.37 ± 0.45 A,B;a	2.74 ± 0.84 B;a	3.79 ± 0.83 B;a,b	3.37 ± 1.33 B;a,b	2.78 ± 0.33 B;a	$p < 0.0001$
10 ppm	1.35 ± 0.51 A	3.40 ± 0.30 B;b,c	3.76 ± 1.29 B;b,c	3.54 ± 0.59 B;a,b	3.94 ± 0.68 B;a,b	4.26 ± 0.75 B;a,b	3.98 ± 0.83 B;a,b,c	3.42 ± 0.37 B;a	$p < 0.0001$
15 ppm	1.35 ± 0.51 A	3.86 ± 0.96 B;c	5.36 ± 0.97 B;c,d	5.25 ± 1.30 B;b,c	4.98 ± 1.33 B;a,b	4.69 ± 0.56 B;b	5.70 ± 1.07 B;b,c,d	5.40 ± 1.16 B;b	$p < 0.0001$
20 ppm	1.35 ± 0.51 A	5.30 ± 0.40 B;d	5.66 ± 1.33 B;d	6.70 ± 1.27 B;c,d	5.43 ± 0.90 B;b	7.02 ± 1.20 B;c	6.04 ± 1.54 B;c,d	6.58 ± 0.94 B;b,c	$p < 0.0001$
30 ppm	1.35 ± 0.51 A	6.79 ± 0.85 B;e	6.46 ± 0.97 B;d	8.03 ± 0.61 B;d	7.96 ± 1.35 B;c	8.06 ± 0.94 B;c	7.57 ± 0.66 B;d	7.46 ± 1.16 B;c	$p < 0.0001$
Statistical significance		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	

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also highly variable. Again, no treatment effect was observed. Percent hatch and percent larval survival were both very high and essentially equal for all of the treatments.

Discussion

Growth inhibition in fish is a commonly reported response to elevated selenium in the diet. In a laboratory food chain consisting of an alga, rotifers, and larval fathead minnows, Bennett *et al.* (1986) reported that 46-91 ppm Se (dry weight concentration in the rotifers) resulted in decreased larval growth after less than ten days. However, a multi-generational study with fathead minnows reported that growth was unaffected by foodborne selenium levels up to 8.8 ppm (Brooks *et al.* 1984). These results are consistent with our data (Table 3) in which growth was inhibited at selenium levels of 20 ppm or greater, but remained unaffected at selenium levels of 15 ppm.

Feeding experiments with bluegill indicated that at five ppm Se, diets spiked with Se-D,L-met, seleno-D,L-cystine (Se-D,L-cys), selenite, or naturally-contaminated plankton did not affect fish growth (Bryson *et al.* 1984). A subsequent study indicated that at 30 ppm Se (selenite), bluegill growth was significantly decreased when compared to lesser treatment levels or with similar selenium levels of Se-D,L-met (Woock *et al.* 1987). These results suggest that selenite is the most important selenium species involved in fish growth impairment.

Studies with salmonids report similar information. Growth of rainbow trout was inhibited by diets containing 10, 11.4, and 13 ppm Se (selenite) (Hilton *et al.* 1980; Hilton and Hodson 1983; Hicks *et al.* 1984). However, using a diet which incorporated fish from a selenium-contaminated site, Hamilton *et al.* (1986) reported that selenium levels up to 26 ppm did not result in significant decreases in growth. Assuming that the latter diet contained large amounts of organic selenium compounds relative to selenite (evidence presented later), then these studies provide additional information suggesting that growth impairment results from selenite.

It is not clear whether inhibition of fish growth is a direct or secondary effect of selenium. Significant reductions in feed:weight gain ratio have been reported (Hilton *et al.* 1980; Hilton and Hodson 1983; Hicks *et al.* 1984), however these studies did not measure the amount of food ingested, but only the amount of food provided the fish. Many studies have reported reduced feeding activity and/or refusal of food as a response to elevated dietary selenium (Hilton *et al.* 1980; Hilton and Hodson 1983; Hicks *et al.* 1984; Finley 1985; Hamilton *et al.* 1986; Woock *et al.* 1987). In the present study, fish provided food containing 80 ppm and 160 ppm Se in range-finding tests ceased feeding after the first day. In our final experiment, reduced feeding was obvious (as evidenced by the amount of uneaten food) at 30 ppm, and in general, fish at the higher treatment levels could be observed racing from food particle to food particle, 'mouthing' and rejecting many. We believe that reduced palatability of seleniferous food items plays a key role in decreased growth.

Whole-body selenium levels were dose-dependent and essentially reached equilibrium within the first 14 days (Table 4). Bertram and Brooks (1986) reported that whole-body selenium burdens of similarly aged fathead minnows had not reached equilibrium after 11 weeks. However, they offered selenium-contaminated food to the fish in the morning and a non-contaminated commercial food in the afternoon. It seems likely that 'dilution' of selenium treatment through the use of non-seleniferous food would significantly reduce selenium uptake, particularly if the fish were avoiding the seleniferous diet. An additional potential cause for this disparity may result from the fact that the laboratory food chain employed by Bertram and Brooks was based on selenate in the water. Incorporation of selenate into organic pathways is limited (Ogle *et al.* 1988). It is possible that rate-limited steps in this process might have resulted in reduced availability of organic selenium relative to that employed in our diet. If so, a more gradual accumulation, such as observed by Bertram and Brooks, might be expected.

In a recent review of U.S. monitoring data for selenium levels in fish, Baumann and May (1984) reported a geometric mean and 85th percentile of about 0.50 ppm and 0.85 ppm Se, wet weight, or about 2.0 and 3.4 ppm Se dry weight, respectively. Selenium levels of our control fish varied around the two ppm level, and fish at the five ppm Se treatment were still within the 85th percentile; however, fish at higher treatments had selenium equilibrium levels greater than the 85th percentile value. While our selenium treatments did result in unusually high selenium levels, the levels reported in our study remain low relative to those of fish from selenium-contaminated sites and/or other species in similar experiments.

For instance, Hamilton *et al.* (1986) reported that chinook salmon fed a diet at 26 ppm Se reached whole-body levels of 22 ppm, a level considerably

Table 5. Tissue selenium levels (μg/g) from spawning pairs of fathead minnows. Mean ± SD (n)

Selenium treatment	Female gonad	Female muscle	Male gonad	Male muscle	Statistical significance
Control	6.08 ± 1.67 (8) A;a	2.71 ± 1.38 (7) B;a	3.85 ± 2.10 (8) B;a	1.93 ± 0.51 (8) B;a	$p < 0.0001$
5 ppm	6.86 ± 1.76 (7) A;a,b	4.90 ± 1.76 (7) A,B;a,b	5.56 ± 2.16 (7) A,B;a,b	3.31 ± 0.77 (7) B;a,b	$p < 0.007$
10 ppm	7.59 ± 1.82 (7) A;a,b	5.30 ± 1.98 (6) A,B;a,b,c	5.66 ± 2.39 (8) A,B;a,b	4.31 ± 0.86 (8) B;b,c	$p < 0.02$
15 ppm	8.47 ± 1.72 (8) A;a,b,c	5.86 ± 1.77 (8) B;b,c	6.25 ± 1.01 (6) A,B;a,b	5.48 ± 1.50 (8) B;c,d	$p < 0.004$
20 ppm	9.11 ± 2.18 (7) A;b,c	6.47 ± 0.99 (7) B;b,c	5.95 ± 1.60 (8) B;a,b	6.66 ± 1.65 (8) B;d	$p < 0.002$
30 ppm	10.92 ± 1.62 (8) A;c	7.84 ± 1.41 (8) B;c	7.82 ± 1.10 (8) B;b	8.77 ± 1.22 (8) B;e	$p < 0.0002$
Statistical significance	$p < 0.0001$	$p < 0.0001$	$p < 0.003$	$p < 0.0001$	

Row means with same upper case letter are not significantly different
Column means with same lower case letter are not significantly different

Table 6. Effects of selenium treatment on reproduction. Mean + SD (n)

Selenium treatment	No. of spawns per pair	No. of eggs per spawn	Percent hatch	Percent survival (14 days)
Control	2.62 ± 1.77 (8)	32.00 ± 24.24 (6)	95.2 ± 18.1 (6)	92.3 ± 0.9 (6)
5 ppm	4.50 ± 2.00 (8)	35.51 ± 31.51 (8)	99.2 ± 3.1 (7)	93.0 ± 4.7 (7)
10 ppm	4.86 ± 1.95 (7)	63.65 ± 23.30 (7)	99.6 ± 0.3 (7)	90.2 ± 8.1 (7)
15 ppm	5.43 ± 1.62 (7)	38.16 ± 20.70 (7)	99.0 ± 1.6 (7)	87.4 ± 1.2 (7)
20 ppm	3.50 ± 2.78 (8)	43.99 ± 25.08 (8)	95.0 ± 14.7 (8)	91.0 ± 2.3 (8)
30 ppm	4.62 ± 1.68 (8)	48.86 ± 28.14 (8)	93.5 ± 15.6 (8)	93.4 ± 1.6 (8)
Statistical significance	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

higher than the 8 ppm observed in our experiment for fish feeding at 30 ppm. At Belews Lake, fathead minnow muscle levels (typically the tissue with the lowest selenium levels) were almost three-fold higher than the maximum whole-body levels we observed (Lemly 1985). In both examples, fish had eaten diets containing naturally biotransformed selenium species. Acknowledging that the proportions of selenium species used in our diet were arbitrary due to compromises resulting from what was commercially available, other problems also exist with this treatment approach. For instance, assimilation of methionine is greater when the amino acid is incorporated in peptides (Murai *et al.* 1981). If assimilation trends are similar for Se-met, then uptake from a diet supplemented with free Se-met would be expected to be less than uptake from a diet in which naturally biotransformed Se-met had been incorporated into peptides.

Additional problems arise concerning the levels of organic selenium in our diet. Bryson *et al.* (1984) reported that diets spiked with seleno-amino acids more closely resembled the bioaccumulation pattern resulting from diets supplemented with naturally-contaminated plankton. Other workers have observed that toxic effects from diets spiked with Se-met more closely resembled toxic effects associated with uptake from naturally biotransformed selenium (Woock *et al.* 1987; S. Hamilton, pers. comm.). It seems likely that seleno-amino acids

comprise a greater proportion of natural food items than was used in our diet. Given that these seleno-amino acids accumulate in fish to a greater degree than inorganic selenium compounds (Bryson *et al.* 1984; Kleinow and Brooks 1986), this may help explain why selenium bioaccumulation was relatively low in this experiment.

The tissue selenium levels that we observed (Table 5) follow the generally reported trend in which ovarian selenium levels are greater than testicular and/or muscle selenium levels (Cumbie and VanHorn 1978; Sager and Cofield 1984; Brooks *et al.* 1984; Lemly 1985; Baumann and Gillespie 1986; Kai *et al.* 1986). We hypothesize that this results from the nature of the vertebrate circulatory system interacting with gametogenesis in oviparous fish. In vertebrates, substances absorbed from the G.I. tract are delivered to the liver via the hepatic portal system prior to being distributed to the rest of the body. The liver is the site of vitellogenesis, which is the synthesis of egg yolk precursors (Ng and Idler 1983). Thus, when selenium is absorbed from food items, liver metabolic processes (including vitellogenesis) will have maximal exposure to the absorbed selenium compounds. The hepatocytes then incorporate these selenium compounds into the egg yolk precursors which are then incorporated into the yolk platelets of developing eggs. This hypothesis is supported by the finding that significant ovarian selenium accumulation coincides with the pre-spawning vitellogenic period (Bryson *et al.* 1984) and would explain why female gonads consistently accumulate selenium to such relatively high levels. However, this hypothesis needs further verification (*e.g.*, direct measurement of the incorporation of selenium into vitellogenin and egg yolk).

Despite the elevated ovarian selenium levels observed in this study, we found no detectable effects on the reproductive parameters evaluated (Table 6). Only two pairs of fish failed to spawn, and these were both control replicates. Bryson *et al.* (1984) also reported a lack of spawning in their control bluegill, although they do not suggest possible causes.

No significant treatment effects were observed in the number of eggs per spawn. The range of treatment means for this parameter was 32–64 eggs per spawn. These numbers are much lower than the 203–256 eggs per spawn reported in a similar study (Brooks *et al.* 1984). The previous study used spawning substrate similar to ours, but coated theirs with fine quartz sand. It may be that the smoothness of our uncoated substrates inhibited oviposition and/or adhesion of eggs to the substrate, either of which could explain our lower counts.

Both percent hatch and percent larval survival through 14 days were high in this study and selenium treatment had no apparent effect on either parameter. Brooks *et al.* (1984) also reported high percent hatch and larval survival with no selenium treatment effect.

The evidence obtained in this study indicates that fathead minnow reproduction was unaffected by the levels of foodborne selenium used in our study. However, recently published studies of bluegill verify the impaired reproduction hypothesized by previous field investigations. Gillespie and Baumann (1986) crossed male and female bluegill from selenium-contaminated Hyco Reservoir (North Carolina) with bluegill from an uncontaminated site in all possible combinations and reported that all crosses involving Hyco Reservoir females produced larvae that developed edema and did not survive to the swim-up stage. A subsequent laboratory study in which bluegill were fed diets spiked with different selenium compounds reported that treatment levels of 13 and 30 ppm Se (Se-D,L-met) and 30 ppm Se (selenite) resulted in significantly decreased larval survival, and larval edema was again reported (Woock *et al.* 1987). In both of these studies, reproductive failure resulted from abnormal larval development.

Woock *et al.* (1987) found that concurrent exposure to both foodborne levels of 13 ppm Se (Se-D,L-met) and 10 ppb Se (selenite) in the water resulted in a threefold increase in the percentage of larval deaths and a five-fold increase in the percentage of abnormal larvae relative to similarly fed fish in control water. Moreover, an analysis of the data from Brooks *et al.* (1984) indicates that selenium concentrations in fathead minnow eggs more than doubled when foodborne selenium was coupled with waterborne selenium. These studies indicate that waterborne selenium (coupled with foodborne selenium) may play a more important role in reproductive impairment than previously thought.

Although exposure to waterborne selenium was not included in this study, that does not explain the lack of reproductive impairment, because dietary treatment alone of 30 ppm Se (both as Se-D,L-met and selenite) resulted in reproductive impairment in the study by Woock *et al.* (1987). Thus, it appears that there may be some significant differences between fathead minnows and other taxonomic groups regarding selenium-induced reproductive impairment. Lemly (1985) reported that fathead minnows were able to establish a reproducing pop-

ulation in Belews Lake and that they (and cyprinids in general) contained much lower visceral selenium levels than other fish species. This suggests that the absence of selenium effect on minnow reproduction may involve decreased bioaccumulation relative to other species.

There are several aspects of cyprinid digestive biology that may explain the decreased selenium bioaccumulation. Cyprinids possess an undifferentiated G.I. tract that lacks a true stomach and/or pyloric caeca. Relative to other fish, particularly those possessing numerous pyloric caeca, minnows probably contain fewer and a less diverse assemblage of gut microflora. Metabolism of inorganic compounds into amino acids by gut bacteria increases the bioavailability of amino acids to host organisms (Whanger *et al.* 1978; Fong and Mann 1980; Hudman and Glenn 1984, 1985). Thus, fish with fewer gut microflora probably have less organic selenium available to them. Catfish, which have a true stomach but lack pyloric caeca, exhibit selenium toxicity and bioaccumulation to a lesser degree than centrarchids (Garrett and Inman 1984; Lemly 1985).

A related aspect involves differences in gut pH between these fish groups. Lacking a true stomach, cyprinids have a digestive system that is neutral-to-alkaline throughout, while fish with stomachs (such as catfish and sunfish) typically have very acidic conditions in the stomach (Norris *et al.* 1973; Page *et al.* 1976; Maier and Tullis 1984). Stability of the various selenium species is pH-dependent with acidic conditions favoring the stability of reduced selenium species whereas alkaline conditions favor the oxidized forms (Callahan *et al.* 1979). Thus, the alkaline gut conditions of fathead minnows would favor the occurrence of selenate while the acidic stomach conditions of other fish would favor selenides and selenite. As previously stated, selenate is less likely to enter into organic biochemical pathways relative to the reduced selenium species (Ogle *et al.* 1988). Therefore, other fish groups will have greater levels of organic selenium compounds available to them due to increased biotransformation potential in their G.I. tracts.

An additional aspect of gut pH differences involves the lability of the selenohydryl moiety of selenocysteine. Under acidic conditions (pH <5.24), this moiety is highly susceptible to hydrolysis reactions (Huber and Criddle 1967). In the neutral-to-alkaline minnow gut, hydrolysis of selenocysteine becomes possible. Because of diverse potential hydrolysis reactions, the results of these reactions are unclear. In any case, this is a difference between minnows and other fish (*e.g.*, catfish and sunfish) that may be important in selenium uptake and toxicity.

The results of our study demonstrate that while growth of fathead minnows is inhibited by elevated foodborne selenium, reproduction appears to be unaffected. Data from this and other studies suggest that the lack of reproductive impairment in fathead minnows may involve reduced selenium bioaccumulation relative to other fish groups. Possible reasons for this involve differences in gut morphology and physiology between the fish groups. Previous assertions (Ogle *et al.* 1988) that biotransformation and subsequent bioaccumulation are significantly different between selenate and selenite also help to explain these differences in selenium uptake.

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References

APHA (1980) Standard Methods for the Examination of Water and Wastewater, 15th ed. American Public Health Association, Washington DC

Barnhart RA (1957) Chemical factors affecting survival of game fish in a western Colorado reservoir. MS thesis, Colorado State University, Ft. Collins, CO

Baumann PC, May TW (1984) Selenium residues in fish from inland waters of the United States. In: The effects of trace elements on aquatic ecosystems. EPRI-EA-3322, Electric Power Research Institute, Palo Alto, CA, pp 7-1 to 7-38

Baumann PC, Gillespie RB (1986) Selenium bioaccumulation in gonads of largemouth bass and bluegill from three power plant cooling reservoirs. Environ Toxicol Chem 5:695–670

Bell JG, Pirie BJS, Adron JW, Cowey CB (1986) Some effects of selenium deficiency on glutathione peroxidase (EC 1.11.1.9) activity and tissue pathology in rainbow trout (*Salmo gairdneri*). Br J Nutr 55:305–311

Bennett WN, Brooks AS, Boraas ME (1986) Selenium uptake and transfer in an aquatic food chain and its effects on fathead minnow larvae. Arch Environ Contam Toxicol 15:513–517

Benoit, DA (1981) User's guide for conducting life-cycle chronic toxicity tests with fathead minnows (*Pimephales promelas*). US EPA Ecological Research Series EPA-600/8-81-011. US EPA, Washington, DC

Bertram PE, Brooks AS (1986) Kinetics of accumulation of selenium from food and water by fathead minnows. Water Res 20:877–884

Brooks AS, Bertram PE, Szmania DC, Seale DB, Boraas ME (1984) The effect of selenium on the reproductive potential of the fathead minnow. EPRI Research Project 1631-1, Final Report, Electric Power Research Institute, Palo Alto, CA

Bryson WT, MacPherson KA, Mallin MA, Partin WE, Woock SE (1984) Roxboro Steam Electric Plant Hyco Reservoir 1984 Bioassay Report. Carolina Power and Light Company, NC

Callahan MA, Slimak MW, Gabel NW, May IP, Fowler CF, Freed JR, Jennings P, Durfee RL, Whitmore FC, Maestri B, Mabey WR, Holt BR, Gould R. (1979) Water-related environmental fate of 129 priority pollutants. Vol. 1. EPA-440/4-79-029a. US EPA, Washington, DC

Cumbie PM, VanHorn SL (1978) Selenium accumulation associated with fish mortality and reproductive failure. Proc Ann Conf SE Assoc Fish Wildl Agencies 32:612–624

Finley KA (1985) Observations of bluegills fed selenium-contaminated *Hexagenia* nymphs collected from Belews Lake, North Carolina. Bull Environ Contam Toxicol 35:816–825

Fong W, Mann KH (1980) Role of gut flora in the transfer of amino acids through a marine food chain. Can J Fish Aquat Sci 37:88–96

Garrett GP, Inman CR (1984) Selenium-induced changes in fish populations of a heated reservoir. Proc Ann Conf SE Assoc Fish Wildl Agencies 38:291–302

Gatlin DM, Wilson RP (1984) Dietary selenium requirement of fingerling channel catfish. J Nutr 114:627–633

Gatlin DM, Poe WE, Wilson RP (1986) Effects of singular and combined dietary deficiencies of selenium and Vitamin E on fingerling channel catfish (*Ictalurus punctatus*). J Nutr 116:1061–1067

Gillespie RB, Baumann PC (1986) Effects of high tissue concentrations of selenium on reproduction by bluegills. Trans Am Fish Soc 115:208–213

Hamilton SJ, Palmisano AN, Wedemeyer GA, Yasutake WT (1986) Impacts of selenium on early life stages and smoltification of fall chinook salmon. Trans North Am Wildl Nat Res Conf 51:343–356

Heisinger JF, Hansen CD, Kim JW (1979) Effect of selenium dioxide on the accumulation and acute toxicity of mercuric chloride in goldfish. Arch Environ Contam Toxicol 8:279–283

Heisinger JF, Scott L (1985) Selenium prevents mercuric chloride ionduced osmoregulatory failure without glutathione peroxidase involvement in the black bullhead (*Ictalurus melas*). Comp Biochem Physiol 80C:295–297

Hicks BD, Hilton JW, Ferguson HW (1984) Influence of dietary selenium on the occurrence of nephrocalcinosis in the rainbow trout. *Salmo gairdneri* Richardson. J Fish Dis 7:379–389

Hilton JS, Hodson PV. Slinger SJ (1980) The requirement toxicity of selenium in rainbow trout (*Salmo gairdneri*). J Nutr 110:2527–2535

Hilton JW, Hodson PV (1983) Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). J Nutr 113:1241–1248

Huber RE, Criddle RS (1967) Comparison of the chemical properties of selenocysteine and selenocystine with their sulfur analogs. Arch Biochem Biophys 122:164–173

Hudman JF, Glenn AR (1984) Selenite uptake and incorporation by *Selenomonas ruminantium*. Arch Microbiol 140:252–256

——— ——— (1985) Selenium uptake by *Butyrivibrio fibrisolvens* and *Bacteroides ruminicola*. FEMS Microbiol Lett 27:215–220

Kai N, Ueda T, Takeda M, Kataoka A (1986) The levels of mercury and selenium in gonad of marlins from the Pacific Ocean. Bull Jap Soc Sci Fish 52:553–556

Kim JW, Birks E, Heisinger JF (1977) Protective action of selenium against mercury in Northern Creek chubs. Bull Environ Contam Toxicol 17:132–136

Klaverkamp JF, Hodgins DA, Lutz A (1983) Selenite toxicity and mercury-selenium interactions in juvenile fish. Arch Environ Contam Toxicol 12:405–413

Kleinow KM, Brooks AS (1986) Selenium compounds in the fathead minnow (*Pimephales promelas*) - I. Uptake, distribution, and elimination of orally administered selenate, selenite, and l-selenomethionine. Comp Biochem Phys 83C:61–69

Lemly D (1985) Toxicology of selenium in a freshwater reservoir: Implications for environmental hazard evaluation and safety. Ecotoxicol Environ Safety 10:314–338

MacFarlane RD, Bullock GL, McLaughlin JJA (1986) Effects of five metals on susceptibility of striped bass to *Flexibacter columnaris*. Trans Am Fish Soc 115:227–231

Maier KJ, Tullis RE (1984) The effects of diet and digestive cycle on the gastrointestinal tract pH values in the goldfish, *Carrasius auratus* L., Mozambique tilapia, *Oreochromis mossambicus* (Peters), and channel catfish, *Ictalurus punctatus* (Rafinesque). J Fish Biol 25:151–165

Murai T, Akiyama T, Nose T (1981) Use of crystalline amino acids coated with casein in diets for carp. Bull Jap Soc Sci Fish 47:523–527

Ng TB, Idler DR (1983) Yolk formation and differentiation in teleost fishes. In: Hoar WS, Randall DJ, Donaldson E (eds), Fish Physiology, Volume IXA. Academic Press, New York, pp 373–404

Norris JS, Norris DO, Windell JT (1973) Effect of simulated meal size on gastric acid and pepsin secretory rates in bluegill (*Lepomis macrochirus*). J Fish Res Board Can 30:201–204

Ogle RS, Maier KJ, Kiffney P, Williams MJ, Brasher A, Melton L, Knight AW (1988) Bioaccumulation of selenium in aquatic ecosystems. Lake Reservoir Management (in press)

Page JW, Andrews JW, Murai T, Murray MW (1976) Hydrogen ion concentration in the gastrointestinal tract of channel catfish. J Fish Biol 8:225–228

Poston HA, Combs GF, Leibovitz L (1976) Vitamin E and selenium inter-relations in the diet of Atlantic salmon (*Salmo salar*): gross, histological, and biochemical deficiency signs. J Nutr 106:892–904

Sager DR, Cofield CR (1984) Differential accumulation of selenium among axial muscle, reproductive and liver tissues of four warmwater fish species. Water Res Bull 20:359–363

SAS Institute (1985) SAS User's Guide: Statistics. Version 5. SAS Institute Inc, Cary, NC

Whanger PD, Weswig PH, Oldfield JE (1978) Selenium, sulfur, and nitrogen levels in ovine rumen microorganisms. J Animal Sci 46:515–519

Woock SE, Garrett WR, Partin WE, Bryson WT (1987) Decreased survival and teratogenesis during laboratory selenium exposures to bluegill *Lepomis macrochirus*. Bull Environ Contam Toxicol 39:998–1005

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