Fathead minnow responses to cadmium in effluent-dominated streams

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ABSTRACT

Stream flows in the southwestern U.S. are often influenced by effluent discharges. Cadmium, a non-point source contaminant in several north Texas streams, reportedly binds to and inactivates fish estrogen receptors. Our previous research identified that: 1.metal bioavailability is reduced in effluent dominated streams; and 2. City of Denton, TX effluent is seasonally estrogenic, corresponding to population demographic changes between fall and spring university semesters. To evaluate fish responses to cadmium and estrogenic municipal effluent mixtures, we exposed juvenile fathead minnows to 0, 5, 20, and 80 µg/L nominal Cd during fall 2001. The University of North Texas Stream Research Facility, outdoor lotic mesocosms with municipal effluent as source water, served as experimental units for a 90-day period. Effluent metal concentrations and Cd treatment levels were verified by AAS. Fish reared in the laboratory served as untreated organisms. Vitellogenesis, steroidogenesis, hepatic estrogen receptor, vertebral bone strength and calcium content, and fish swimming performance were evaluated on day 90. Although mortality increased with treatment level, survival was not significantly affected. Compared to laboratory fish, gonadosomatic index was reduced and hematocrit was increased in both males and females. Stream fish were larger than laboratory fish; however, male and female condition was reduced in all streams. Male vitellogenesis was observed in 0, 5 and 20 µg/L treatments, but not in 80 µg/L streams. Female vitellogenesis was affected in all streams with the greatest impairment in 80µg/L treatments. In addition, male critical swimming speed was reduced in 20 and 80 µg/L treatments. Such reduced swimming performance may result from alteration of calcium metabolism and compromised strength of caudal vertebrae.

RESEARCH RATIONALE

- Rivers in the southwestern and south-central U.S. are often greater than 90% return flow from water reclamation plants.
- We recently constructed the University of North Texas Stream Research Facility (UNTSRF) at the City of Denton, TX Pecan Creek Water Reclamation facility. City of Denton, TX final treated effluent serves as source water for outdoor experimental streams that consist of riffle and pool sections.
- Previous research identified Denton, TX municipal effluent as seasonally estrogenic (Hemming et al. 2001, Allen et al. 2001) due to steroid pharmaceuticals (Huggett et al. in review). Consequences of prolonged vitellogenesis in fish exposed to estrogenic effluents include reproductive impairment (Nichols et al. 1999) and skeletal calcium losses (Sumpter & Jobling 1995).
- Cadmium, a non-point source contaminant in north Texas stream, is an endocrine modulating compound (Foran et al. in press) that may inactivate fish estrogen receptors (Guevel et al. 2000).
- Prolonged sublethal Cd exposure results in hypocalcemia (Larsson 1975, Haux et al. 1988), bone calcium resorbtion and skeletal deformities (Bengtsson et al. 1975, Muramoto 1981). Alteration of vertebral integrity may affect fish swimming performance (Mayer et al. 1988).
- Effects of cadmium and estrogenic effluents on the endocrine function and calcium metabolism of aquatic biota have not been assessed under field conditions. This is of particular interest in systems affected by cadmium, and dominated by municipal effluent discharge.

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EXPERIMENTAL OBJECTIVES

- Evaluate effects of Cd and estrogenic municipal effluents on *Pimephales promelas* endpoints.

EXPERIMENTAL APPROACH

- 100 juvenile (14 day old) fathead minnows were placed in UNTSRF pool sections on 19 September 2001 (day –2) to acclimate. Concurrently, fish of same age were reared in the laboratory.
- Replicate streams were nominally dosed with 0, 5, 20, or 80 µg/L Cd using high precision peristaltic pumps from 21 September 2001 (Day 0) to 19 December 2001 (Day 90).
- Model stream metal concentrations (Cd, Cu, Zn, Ni) were sampled weekly and quantified by GFAAS (Table 1).
- City of Denton effluent water quality (source water for UNTSRF) was monitored hourly during the study period with a Hydrolab datasonde and alkalinity and hardness measures taken with metal grab samples (Table 2).
- Fish populations were removed from pools on day 90 and transported to the laboratory. Swimming performance was measured as critical swimming speed (U_{crit}) using a Brett (1964)-type swim tunnel; speed was quantified with a Marsh-McBurney flowmeter. $U_{crit} = u_1 + (t_1/t_2 \times u_2)$ where u_1 is the highest speed maintained for 1 minute, u_2 is the speed increment (10 cm sec^{-1}), t_1 is time swam at fatigue speed, and t_2 is the swimming period (1 min).
- Following swimming trials, 8 fish (4 males, 4 females) were sacrificed from each stream, tissues dissected and stored at –80ºC.
- Analysis for VTG and ER was performed on whole liver homogenates by SDS-PAGE and Western blotting. A monoclonal anti-VTG antibody was purchased from Cayman Chemical Co. that crossreacts with fathead minnows (Hemming et al. 2001). A primary antibody for estrogen receptor was developed in mouse by Affinity Bioreagents, Inc. (Stillwater, MN) against the DNA-binding domain of human ER (Foran et al. 2002). Hepatic protein content was determined using a bovine serum albumin standard and a Bio-Rad Protein Assay protein dye.
- Estradiol (E2) and testosterone (T) concentrations were determined from plasma by an enzyme immunosorbant assay (Munro & Lasley 1988).
- Analysis of treatment effects by ANOVA using SAS 8.2. Data are presented as untransformed means (±SE).

Table 1. Mean metal concentrations (±SD) in UNTSRF streams during 90 d study period.

Stream	Nominal Cd Level	Cd	Cu	Zn	Ni
3	0	**2.25 (±1.48)**	14 (±8.3)	39.1 (±9.04)	13.88 (±13.73)
11	0	**3.75 (±1.58)**	18.1 (±10.2)	49.1 (±27.7)	9.9 (±12.2)
4	5	**6.9 (±4.1)**	13.5 (±8.5)	81.1 (±15)	12.7 (±12.1)
12	5	**7.5 (±3.2)**	18.2 (±8.1)	40.3 (±6.76)	18.5 (±12.4)
2	20	**18.9 (±7.5)**	14.1 (±7.1)	152.4 (±45.6)	15.6 (±16.5)
7	20	**27 (±11.7)**	12.9 (±7.3)	418 (±151)	13.4 (±14.1)
5	80	**74.8 (±30.8)**	12.6 (±6.7)	46.8 (±31.3)	14.5 (±15.5)
9	80	**79.75 (±31.6)**	14.7 (±8.3)	324.8 (±1134)	13.9 (±14.2)

Figure 1. Effects 0, 5, 20 and 80 µg/L Cd on condition of male (p=0.15) and female (p=0.14) fathead minnows.

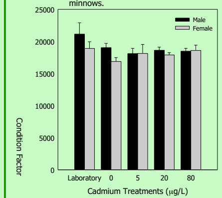

Figure 2. Effects 0, 5, 20 and 80 µg/L Cd on gonadosomatic index of male (p=0.004) and female (p=0.014) fathead minnows.

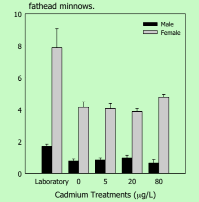

Figure 3. Effects 0, 5, 20 and 80 µg/L Cd on male (T, p=0.009; E2, p=0.008) and female (T, p=0.07; E2, p=0.03) fathead minnow plasma steroids.

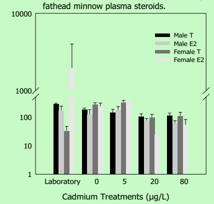

Figure 4. Effects 0, 5, 20 and 80 µg/L Cd on male (p= 0.11) and female (p=0.14) fathead minnow hepatic vitellogenin.

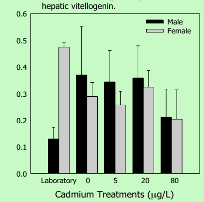

Figure 5. Effects 0, 5, 20 and 80 µg/L Cd on male (p = 0.49) and female (p = 0.19) fathead minnow hepatic estrogen receptor levels.

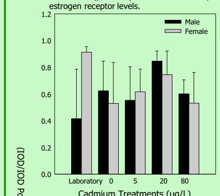

Figure 6. Effects 0, 5, 20 and 80 µg/L Cd on male (p= 0.13) and female (p=0.66) fathead minnow critical swimming performance.

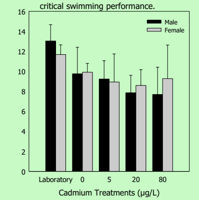

Table 2. Mean water quality parameters (±SD) of Denton, TX effluent during 90 d study.

pH	6.96 (±0.15)
Temperature (ºC)	24.5 (±1.64)
Dissolved Oxygen (mg/L)	6.65 (±1.07)
Specific Conductance (µS/cm)	715.72 (±28.9)
Alkalinity (mg/L, $CaCO_3$)	87.7 (±13.3)
Hardness (mg/L, $CaCO_3$)	139.6 (±9.03)

RESULTS AND CONCLUSIONS

- Condition reduced, though not significantly, in males and females (Figure 1).
- GSI significantly reduced in males and females (Figure 2), similar to previous studies (Nichols et al. 1999, Hemming et al. 2001).
- Male E2 and T significantly reduced observed at 20 and 80 µg/L levels (Figure 3).
- Female E2 and T significantly reduced by 20 and 80 µg/L Cd treatment levels (Figure 3).
- Vitellogenin (VTG) induced in male and reduced in female *P. promelas* (Figure 4).
- Hepatic estrogen receptor content higher in males and lower in females (Figure 5).
- Male, but not female, critical swimming performance affected by 20 and 80 µg/L Cd treatment levels (p=0.1307, Figure 5).
- Are cadmium effects on swimming performance related to steroid influences on calcium metabolism and vertebral bone strength?

CURRENT RESEARCH

- Evaluating *P. promelas* caudal vertebrae for effects of treatment structure on bone strength and calcium content.

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