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Effect of Selenium on Reproductive Behavior and Fry of Fathead Minnows

Mark Pyron and Thomas L. Beitinger

Division of Environmental Sciences, Department of Biological Sciences, University of North Texas, Denton, Texas 76203, USA

Although selenium is a necessary trace element (Stadtman 1979), it has the potential to cause sublethal and lethal effects in organisms (Adams and Johnson 1981). Anthropogenic selenium enters aquatic ecosystems from the disposal of solid wastes and from burning of fossil fuels, particularly coal (Gutenmann et al. 1976). Both field and laboratory studies have documented that fish bioaccumulate selenium (Adams and Johnson 1981; Sandholm et al. 1973). Declines in population densities have been observed along with high selenium body burdens in fishes from some North Carolina cooling reserviors (Cumbie and VanHorn 1978; Lemly 1985).

Watenpaugh and Beitinger (1985a) reported a 24-h median lethal concentration for fathead minnows (*Pimephales promelas*) exposed to water-borne selenate as 82-mg selenium/L in hardwater (USEPA 1975). These investigators found a highly significant inverse relationship between selenium concentration and upper temperature tolerance in this species. The highest selenium exposure concentration (ca. 75 % of 24-h LC50) reduced the Critical Thermal Maximum by nearly 6° C. However, similar exposures did not affect metabolic rate (Watenpaugh and Beitinger 1985b). Also, fathead minnows did not behaviorally avoid concentrations of selenate which would cause death within 24 h (Watenpaugh and Beitinger 1985c).

Since selenium has been shown to accumulate in gonads of fish with subsequent reproductive effects (Baumann and Gillespie 1986), we attempted to determine if exposure to water-borne selenium would adversely impact reproductive behavior in fathead minnows. Behavior, including courtship, is an important aspect of reproduction. Courtship includes behaviors which bring both genders to the spawning site and provides cues for species recognition, assessment of reproductive ability (Kreb and Davies 1981), and gender identification. Reproductive behavior of fathead minnows has been described in the field and the laboratory (McMillan and Smith 1974; Cole and Smith 1987); however, little is known of its stability or the effects of exposure to sublethal concentrations of selenium on reproductive behavior, the objective of this study.

Send reprint requests to T.L. Beitinger at the above address

MATERIALS AND METHODS

Fathead minnows, 6-mo post hatching from a culture maintained at North Texas State University were placed in 38-L aquaria containing aged tap water at 21°C. Water was filtered through activated charcoal and filter floss which were changed weekly. Fish were fed twice daily with newly-hatched, frozen brine shrimp (Artemia sp.) and supplemented with commercial flake food. Flourescent lighting was regulated to provide a LD 18:6 photoperiod. Ten fish per treatment group were exposed to selenium as sodium selenate for 24 h in aquaria containing 25 L of aerated, reconstituted hard water (USEPA 1975). Nominal concentrations were 0, 20, 30, and 60 mg Se/L. Two water samples were taken at the beginning and end of each exposure. Selenium was measured to an accuracy of 1 mg/L with a flame atomic absorption spectrophotometer (Perkin-Elmer 1982) which had been calibrated with known controls.

Behaviors were observed in 38-L aquaria containing split-PVC tubing in lengths of 8 cm for nest sites. Selenium-exposed test fish which appeared to be in reproductive condition (e.g., banding and tubercles in males, enlarged urogenital papillae in females) were placed in observation aquaria in pairs of one male and one female. Five white cloud minnows, Tanichtys albonubes, were added to each aquarium to provide a dither effect (Barlow 1977). Observations (1000-sec) were made between 0600 and 1000 h as fish appeared to be most active at this time. A total of 30 to 34 observation periods were made at each selenium concentration. Several behaviors originally described by McMillan and Smith (1974) and/or Cole and Smith (1987) were recorded:

1. Occurrences of the males and females in the territory;
2. Approach behavior - male approaches female;
3. Leading behavior - male swims from near the female directly to his territory and the female follows;
4. Lateral display - male moves in front of, or at right angles to the female and hovers, extending dorsal, caudal, anal, and pectoral fins;
5. Tailbeating - the lateral quiver of Cole and Smith (1987) and tailbeating of McMillan and Smith (1974);
6. Vibrating - the male and female come in close side-by-side contact and vibrate against each other (McMillian and Smith 1974);
7. Butting - the male moves toward the female and pushes at her with the snout.

RESULTS AND DISCUSSION

Measured concentrations of selenium in water samples were 66 mg/L selenium (nominal 60 mg/L), 36 mg/L selenium (nominal 30 mg/L), and 20 mg/L (nominal 20 mg/L). No variation greater than 1 mg/L was found in any of the water samples. Five fish exposed to 60 mg/L selenium died during the 24-h exposure, and the remaining fish in this group died within 24 h of exposure. In contrast, only a single mortality occurred in fish exposed to 30 mg/L selenium, and no

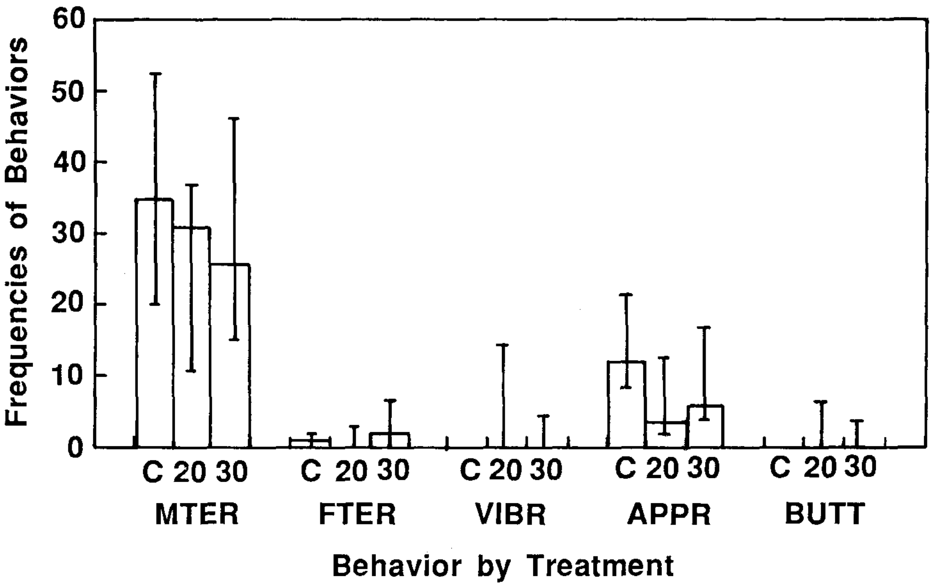

Figure 1. Frequencies of behaviors by territorial fathead minnows. Medians and interquartile ranges (vertical lines) are given. Groups are designated as C (control), 20 (20 mg/L selenium) and 30 (30 mg/L selenium). Behaviors observed were MTER (male in the territory), FTER (female in the territory), VIBR (vibrating behavior by the male and female), APPR (male approaches the female) and BUTT (male butts the female).

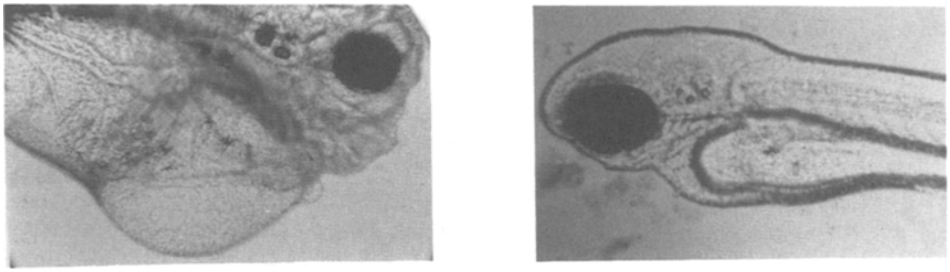

Figure 2. Typical edema seen in a larval fathead minnow whose parents were exposed to 20 mg/L selenium for 24 h (left panel) relative to a control larvae (right panel). 100 X magnification.

mortalities were observed in fish exposed to 20 mg/L selenium. Exposures to selenium at concentrations of 20 and 30 mg/L for 24 h in this study did not have a noticeable affect on the reproductive behaviors monitored. A total of 2013, 1227 and 1372 individual behaviors were observed in control, 20 mg Se /L and 30 mg Se / L, respectively. In general, all behaviors were present in both control and treated fish. Frequencies among the individual behaviors were highly different (Fig. 1); however, there was little variation within a behavior for fish in the three groups. The most commonly observed behavior was male-within-territory where median frequencies for the three groups ranged from 27 to 35. In contrast, median frequencies of female-within-territories were less than 2 in all three groups. The second most common behavior was approach (i.e., male approaches female). Median frequencies for this behavior ranged from 3 to 12. The other four behaviors had median frequencies of zero. Three of the behaviors, tailbeating, lateral display and leading, were not observed during trials.

Females in all groups released eggs which were fertilized by the territorial males, i.e., slight differences in the fequencies of the observed behaviors did not prevent reproduction. Hatching appeared to proceed normally in all three groups. Similar results were obtained by Gillespie and Baumann (1986) who reported that neither percentage fertilization nor hatch were different among 18 artificial crosses of bluegills with high and low Se body burdens. In addition none of the control larvae showed anatomical abnormalities. Nevertheless, nearly all larvae produced by fish exposed to 20 and 30 mg/L selenium had gross morphological abnormalities, in particular general edema (Fig. 2), similar to that of bluegill larvae of adults exposed to 9 to 12 ug/L selenium within a cooling-lake population as reported by Gillespie and Baumann 1986). Consistent with the findings of Baumann and Gillespie (1986) who reported that Se-exposed, edamatous bluegill larvae died before attaining the swim-up stage, none of edematous fathead minnow larvae survived longer than 7 days post-hatching. Since eggs and sperm were formed before fish were exposed to selenium, our exposures would have not affected gamete formation. Larvae must have been affected by eggs which had accumulated selenium. Gillespie and Baumann (1986) reported that ovarian tissues have a greater capacity to accumulate selenium than testicular tissues.

In summary, we found that fathead minnows exposed to acute, high concentrations of selenium demonstrate typical reproductive behaviors which resulted in gamate release and fertilization; however, the offspring tended to have edema and low survivability. These results suggest that the reproductive behavior of fathead minnow does not make a good bioassay model at least for selenium but survival and condition of the fry are more sensitive. Although the reproductive behavior of individual fathead minnow is quite variable, the overall behavior appears to be quite insensitive to water-borne selenium. Perhaps this stability is due to the importance of reproductive behavior in the survival of a species.

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