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Transfer of Toxic Concentrations of Selenium from Parent to Progeny in the Fathead Minnow (*Pimephales promelas*)

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Selenium, an essential trace element, may become concentrated in aquatic ecosystems to levels that are toxic to fish. Finley (1985) and Gillespie and Baumann (1986) have shown that selenium in overflow water from coal burning power plant settling basins contributed to a decline in fish populations. The leaching of selenium from the soil into water systems used for irrigation in highly seleniferous areas of the country poses another serious problem (Eisler 1985 and Saiki and Lowe 1987).

Gillespie and Baumann (1986) and Woock et al. (1987) demonstrated that female bluegill sunfish transfer selenium to their progeny. Research undertaken at the Monticello Ecological Research Station (MERS) has shown occurrences of edema and lordosis in the larvae of fathead minnows (Pimephales promelas) exposed to 10 ug/l selenium in experimental streams. The objective of this study was to determine whether the selenium levels within fathead minnow embryos in a semi-natural ecosystem resulted from direct uptake by the embryos following spawning, from female-to-progeny transferral, or from some combination of these two occurrences. This study was carried out by 1) collecting embryos and ovaries from fatheads raised in control and 10 ug/l selenium streams, 2) analyzing these fathead embryos and ovaries for selenium content, 3) analyzing the uptake of selenium over a 24 hour period by embryos from control streams when placed in 10 ug/l selenium water, and 4) comparing occurrences of edema and lordosis in the larvae.

MATERIALS AND METHODS

The Monticello Ecological Research Station (MERS), an EPA facility at Monticello, Minnesota was the location of this study. The site has eight experimental streams, each 520 meters long, with alternating pools and riffles every 30.5 meters. Water is pumped into the streams from the Mississippi River (Zischke et al. 1983). This study lasted from 9/1/87 until 9/1/88 and utilized four of the eight streams.

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Two streams were dosed continuously with sodium selenite (Na_2SeO_3) to achieve a concentration of 10 ug/l selenium and two streams served as controls. Selenium water concentrations were measured weekly in each stream by graphite furnace atomic absorption spectrometry (U.S. EPA method 270.2, 1983). The spectrometer was calibrated to solutions made up from Fisher Scientific standard selenium reference solution. Duplicate injections were made for all samples and replicate samples were taken at one location in each stream. The limits of detection were approximately 2 ug/l. Quality assurance sample analyses were conducted using U.S. EPA and NBS reference materials. Over 50 such analyses were made with results averaging within 10% of nominal values. Spike recoveries in river water averaged 90 ± 13%. Selenium water concentrations (mean ± standard deviation) at the head of the two 10 ug/l streams were 9.8 ± 1.2 ug/l (n=87) and 10.3 ± 1.7 ug/l (n=87). Selenium water concentrations in the two control streams were below detection levels.

Submerged spawning platforms were suspended from overhead wooden structures to allow the fatheads a surface on which to spawn (Zischke et al. 1983). The platforms were inspected daily for embryos, which were removed from the platforms with a metal spatula. Six embryo samples were collected from the dosed streams, and six samples were collected from control streams for selenium analysis. Each sample contained approximately 2000 embryos. Ten embryo samples each from the dosed and control streams were reared in mesh-bottom incubation cups in a proportional diluter (Mount and Brungs 1967). The incubation cups were oscillated in test chambers that contained river water and selenium concentrations similar to that in the respective test streams. Selenium concentration in the 10 ug/l chambers was 9.7 ± 2.6 ug/l, and selenium concentration in control chambers was below detection levels. The selenium concentration was analyzed three times during the summer months that larvae were being reared. Each sample, initially containing approximately 50 embryos, was observed every post-hatch day for edema and lordosis. The larvae were not fed, and observations continued till all larvae died. Maximum sample length was seven days.

Approximately 1050 embryos from fish reared in control streams were also collected to determine the amount of selenium embryos may accumulate during this stage of development. The results were used to help determine if the amount of selenium within embryos from the dosed streams was a result of uptake by the embryos themselves, or transferred to them through the female parent. The embryos were placed in a shallow pan of 10 ug/l selenium water for 24 hours. Since the platforms in the streams were inspected for embryos every 24 hours, the embryos in the pan were also left to accumulate selenium for 24 hours, and then removed for analysis. This simulated the maximum time that embryos from the dosed streams were exposed to selenium after spawning. An airstone was placed in the water to supply oxygen

for the embryos.

Just prior to termination of this study, the 10 ug/l selenium streams and control streams were seined to obtain adult females. Seven females from the dosed streams and eight from the control streams were selected for selenium analysis of the ovaries. Because a minimal weight of approximately 0.50 g was needed to accurately perform a selenium analysis on the embryos and ovaries, some ovaries from the dosed streams had to be combined. A total of four ovary samples from the dosed streams and eight ovary samples from the control streams were analyzed in this study.

Embryo and ovary samples were rinsed with de-ionized water, blotted dry with a paper towel, and placed in polystyrene petri dishes to be frozen for later analysis. At the time of analyses samples were thawed, ground-up, and digested using a nitric acid-hydrogen peroxide procedure (Martin et al. 1985). Analyses were conducted by graphite furnace atomic absorption spectrometery and reported as ug Se/g wet weight. Percent recovery of known selenium concentrations in the gonads was 76.6 ± 9.0%.

A t-test analysis was used to determine significant differences in selenium content between control and 10 ug/l embryos and ovaries. A t-test analysis was also used to determine significant differences in the occurrence of edema and lordosis in embryos reared in the proportional diluter. For statistical treatment, percentages were converted to (arcsine)(square root)(percentage/100). All statistical tests were conducted using Minitab, Inc. (Ryan et al. 1987).

RESULTS AND DISCUSSION

A higher incidence of edema and lordosis was found to occur in fathead minnow larvae from 10 ug/l streams versus larvae from control streams. The highest incidence was usually noted on day four. Two-sample t-tests revealed a significant difference existed between the incidence of edema and lordosis in larvae from fish in control and 10 ug/l streams (Table 1).

A significant difference also existed between the amount of selenium accumulated by embryos spawned in streams containing 10 ug/l selenium and embryos spawned in control streams (Table 2). Embryos from fish reared in the dosed streams contained selenium levels thirteen times higher than embryos from fish reared in the control streams (i.e., 3.91 ug/g vs. 0.31 ug/g). In addition, the selenium concentrations in embryos from fish reared in the dosed streams was considerably higher than the concentrations accumulated by embryos spawned in control streams but exposed to 10 ug/l selenium for 24 hours. Before exposure, the selenium concentration of the sample of control embryos used for this part of the study was 0.35 ug/g. After 24 hours of exposure the embryos had accumulated an additional

0.14 ug/g. Only one sample of control embryos was subjected to this procedure. Embryos from fish reared in the dosed streams had selenium concentrations twenty-eight times greater than that accumulated by control embryos over 24 hours (i.e., 3.91 ug/g vs. 0.14 ug/g).

Table 1. Incidence of edema and lordosis among fathead minnow (Pimephales promelas) larvae reared in incubation cups.

Selenium Concentration[a]	% Occurrence[b] Edema	Lordosis
Control	0.9±2.2	5.6±8.8
10 ug/l	24.6±36.1[c]	23.4±20.8[c]

[a]Concentration in water of source stream and incubation chambers
[b]Mean ± standard deviation of maximum incidence in each of 10 tests
[c]Incidence signficantly greater than control larvae, $p<0.05$

The mean selenium concentrations in ovaries from control stream fish was significantly lower than that in ovaries from fish subjected to 10 ug/l selenium (Table 2). Ovaries from the fish in the dosed streams contained more than seven times the selenium found in ovaries of fish from control streams (i.e., 5.89 ug/g vs. 0.77 ug/g).

Table 2. Selenium residues in fathead minnow (Pimephales promelas) embryos and ovaries.

Selenium Concentration in Source Streams	Residues (ug/g wet weight)[a] Embryos	Ovaries
Control	0.31±0.01	0.77±0.14
10 ug/l	3.91±1.87[b]	5.89±2.21[b]

[a]Mean ± standard deviation; number in parentheses represent number of samples analyzed
[b]Residues significantly greater than controls, $p<0.01$

The average selenium concentrations in embryos and ovaries were comparable. The mean selenium concentrations were similar for embryos and ovaries from control streams, and the selenium concentration in the embryos from fish exposed to the 10 ug/l selenium dosed streams closely matched the selenium concentration in those ovaries. Since the ovary normally does not contain significant amounts of selenium, detrimental effects on egg fertilization, embryonic development, or survival of

larvae may result from the accumulation of high concentrations of selenium (Cumbie and VanHorn 1978).

The hypothesis that selenium is passed from female to progeny in fathead minnows is supported by 1) the similarity in residues of selenium between embryos and ovaries within the same treatment, and 2) the lack of significant uptake of selenium by control embryos exposed to water containing 10 ug/l selenium. In addition, this passage of selenium has been found to result in toxic effects demonstrated by the increased incidence of edema and lordosis. Larvae exhibiting these characteristics are not expected to survive in natural systems.

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