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Effects of Methylmercury on Sperm and Egg Viability of Two Populations of Killifish (*Fundulus heteroclitus*)

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Abstract. Exposure of sperm of killifish (*Fundulus heteroclitus*) from a relatively clean area in Long Island (LI) to 0.01 mg/L methylmercury (meHg) in 15‰ sea water caused significant reduction of fertilization success. However, exposure of killifish sperm from polluted Piles Creek (PC) to either 0.01 or 0.05 mg/L meHg in 15‰ sea water prior to insemination had no effect on fertilization success. Exposure of LI killifish sperm to 0.05 mg/L meHg caused significant reduction in motility. However, PC killifish sperm showed no significant difference in motility between 0 and 0.05 mg/L meHg exposure. Exposure for 5 min to 0.05 mg/L meHg caused significant reduction in motility. These data indicate that meHg is less toxic to PC killifish sperm than LI killifish sperm. Exposure of PC and LI killifish sperm to 0.05 mg/L meHg for 15 min had no effect on sperm morphology. PC killifish sperm also showed higher (20 min) motility in 15‰ sea water than LI killifish sperm (10 min). Exposure of PC and LI killifish eggs up to 25 min to 0.05 mg/L meHg prior to fertilization had no effect on fertilization success.

Heavy metal pollution in the marine environment is a major problem because of the toxicity of these metals and their persistence and tendency to accumulate in organisms. Of all the heavy metals, mercury is one of the most toxic. In aquatic environments inorganic mercury (Hg) is often converted to methylmercury (meHg) by bacterial action (Weis and Weis 1982).

Deleterious effects of metals on sea urchin and fish fertilization have been previously addressed. Kobayashi (1971) found that fertilization success of sea urchin (*Hemicentrotus pulcherrimus*) was dose-dependent. The higher the concentrations of Hg, Cd, Zn, Ni, Pb, Cr, Mn, and Co, the lower the fertilization success. Pagano *et al.* (1982) reported that exposure (10 min) of sea urchin (*Paracentrotus lividus*) sperm to Cd (11.20 mg/L) suppressed fertilization capacity.

Preliminary US EPA (1972) data showed that sexual development of fathead minnows (*Pimephales promelas*) was arrested at 0.25 mg/L meHg. McIntyre (1973) reported that concentrations above 1.0 mg/L meHg markedly reduce viability of *Salmo gairdneri* sperm exposed for 30 min. Billard and Roubaud (1985) indicated that 1.0 mg/L Hg significantly decreased the fertilizing ability of rainbow trout (*S. gairdneri*) sperm.

Other studies have focused on pollutant effects on eggs prior to fertilization. Shaw and Brown (1971) found that Cu (1.0 mg/L) and Ni (1.0 mg/L) had no effect on fertilization success of *S. gairdneri*. Rosenthal and Alderdice (1976) found no effects on fertilization of Pacific herring (*Clupea harengus harengus*) eggs after exposures up to 10 mg/L Cd. However, delayed effects of exposure became noticeable when full-term grown eggs began to die prior to hatching. Similarly, Hall *et al.* (1984) reported that a standard organic-inorganic contaminant mixture did not prevent initial fertilization of striped bass (*Morone saxatilis*) eggs. However, a masked effect was observed later during the postlarval stages.

There is no information available on the effect of mercury on fish sperm motility; many authors have reported that pH and salinity can influence the sperm motility (Duplinsky 1982; Mohr and Chalanchuk 1985; Billard 1978).

The killifish, *F. heteroclitus*, is found in estuaries

along the Atlantic coast from Nova Scotia to Florida and is one resident species that has continued to survive in some of the highly polluted estuaries of the Hudson Raritan system.

Weis and Weis (1977a, 1977b) studied the effects of meHg and Hg on the embryonic development of killifish and found teratogenic effects after exposure to low concentrations of meHg or Hg. Weis *et al.* (1982) reported considerable variation in meHg susceptibility of killifish eggs from different females from a relatively unpolluted area in Montauk, Long Island, New York. However, in the population of Piles Creek (PC), a tributary of the Arthur Kill in Linden, NJ, an area heavily impacted by metal and oil pollution, very few females produced susceptible eggs, and most clutches were tolerant with respect to embryonic malformations (Weis *et al.* 1981). However, larvae and adults of PC were not similarly resistant to meHg. The embryonic meHg resistance which was shown in PC was no longer present in larvae, which were comparable to LI larvae (Toppin 1985; Weis *et al.* 1987). Adults from PC, rather than being tolerant, were stressed, weakened and more susceptible to pollutant stress (Renna 1982; Weis *et al.* 1987).

The effect of meHg on fertilization success and sperm motility of killifish has not been reported. This paper reports on the effects of meHg on eggs, sperm, sperm motility, and sperm morphology of two populations of killifish. The following locations were evaluated: (1) Piles Creek (PC), a polluted tidal creek near a heavily industrialized area in Linden, NJ; and (2) a Southampton Creek, adjacent to Bull Head Bay, Long Island (LI), NY. PC sediment mercury concentrations have been reported to be as high as 10.3 μg/g (Koepp *et al.* 1980); LI sediment mercury concentration was almost undetectable (Weis and Weis 1984).

Materials and Methods

Adult killifish were caught by minnow traps during the summers of 1984, 1985, and 1986 from Piles Creek (PC), Linden, NJ and Long Island (LI), NY. Salinities in these areas ranges from 20–25‰. Fish were acclimated 3–5 days at room temperature in 20‰ sea water (Instant Ocean) and a light:dark cycle of 14:10 hr.

The following experiments were made to evaluate the effect of methylmercury on sperm and eggs: 1. For the first series of experiments, eggs and sperm were obtained by stripping the fish. PC killifish sperm were placed into 15‰ artificial sea water with 0 (control), 0.01, and 0.05 mg/L meHg (I.C.N. Pharmaceuticals, Plainview, N.Y., dissolved in 0.2% $NaHCO_3$) for 2, 5, 10, 15 or 20 min prior to combination with freshly stripped eggs. Similarly, LI fish sperm were treated for 2, 3, 4, and 5 min (pilot studies had shown that the sperm of LI fish survive only 5–10 min in artificial sea water, so they were treated for 5 min prior to combination with newly stripped untreated eggs). 2. For a second series of experiments, PC and LI killifish eggs were placed in 0 (control), 0.01 or 0.05 mg/L meHg for 2, 5, 10, 15, 20 or 25 min prior to combination with freshly stripped untreated sperm. After 2 min, eggs were washed with 15‰ sea water 3–4 times in order to remove extra sperm and contaminants. Each exposure time and meHg concentration were considered as a separate experiment. Each utilized the sperm and eggs of a single male and female. Each experiment was replicated 5–10 times using males and females that were not used previously. Nominal concentrations were used for all of these experiments, since mercury concentrations were not measured in the test solutions. After 120–150 min, eggs were checked under a dissecting microscope for cleavage, which was used as an indicator of fertilization success. The effect of meHg either on sperm or eggs was measured by counting the percent of the total number of eggs that had been fertilized. After a week of incubation at 24°C and 15‰ salinity, the embryos reached stage 35–36 (Armstrong and Child 1965) and were examined under a dissecting microscope for malformations of the head, cardiovascular system, and skeletal system. The data were analyzed by using the Chi-square (Zar 1984). Results were significant at alpha = 0.05.

Sperm were stripped into 15‰ sea water with 0 (control) and 0.05 mg/L meHg, to determine the sperm motility of PC and LI killifish. For each concentration, approximately 0.2–0.3 ml of sperm from a single male were added to 2 ml test solution. Sperm were observed under a compound microscope (400×) 2, 5, 10, 15, and 20 min after hydration. The sperm motility in each treatment was graded with an arbitrary scale of 0–4: 4—all motile (rapid random movement), 3—most of them motile, few in circular movement, 2—less than 50% motile (circular or random), 1—less than 10% motile (circular or random), and 0—no motility. Evaluation of sperm motility was made in a "double blind" fashion. Each exposure time and mercury concentration were considered as a separate experiment. Each utilized the sperm of a single male. Each experiment was replicated 5–10 times, using male fish that were not used previously. The sperm motility data were analyzed with the non-parametric Mann-Whitney U test (Zar 1984). Results were significant at alpha = 0.05.

For morphological analysis, sperm were stripped from PC and LI males into 0 (control), 0.01 or 0.05 mg/L meHg for 2, 5, 10 and 15 min, and fixed in 2% glutaraldehyde (in 20‰ Instant Ocean solution). Sperm were processed on cover slips (15 mm diameter). Cover slips were soaked in 0.2% poly-l-lysin solution for a few minutes and air dried for 24 hours. One drop of glutaraldehyde fixed sperm was added. After 40 min, the cover slip was rinsed with 20‰ sea water; then a graded ethanol dehydration (30–100%) sequence was performed prior to critical point drying (Denton Dep 1) in CO_2. This procedure provided the best preservation of sperm morphology. After critical point drying, the cover slips were attached on the specimen holders by Duco cement and the edge of the cover slips were covered with silver nitrate to prevent spreading of the gold palladium during palladium coating. Specimens were coated with gold palladium and examined in SEM (AMRAY 12B) operated at 15 KV. Photographs were taken on polaroid Type 55 land film.

Results

Exposure of PC killifish sperm to 0.01 or 0.05 mg/L meHg for 5 min showed significant reduction of mean % fertilization success (Figure 1). Prolonged

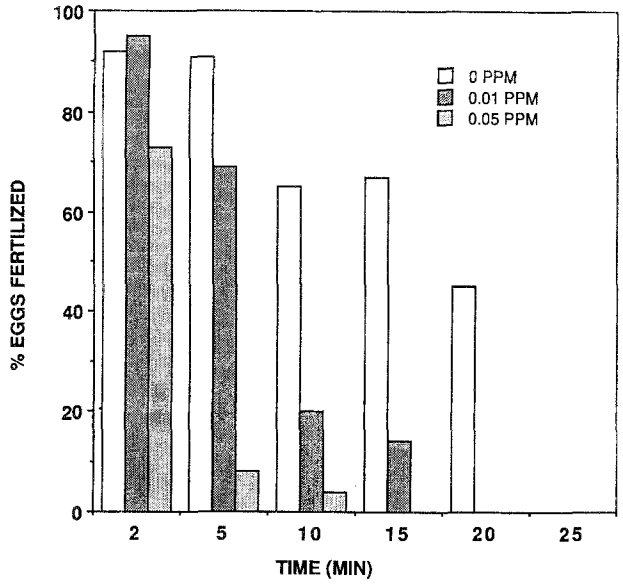

Fig. 1. Effect of methylmercury on mean % fertilization success of Piles Creek killifish eggs (Exposure of sperm to meHg prior to fertilization).

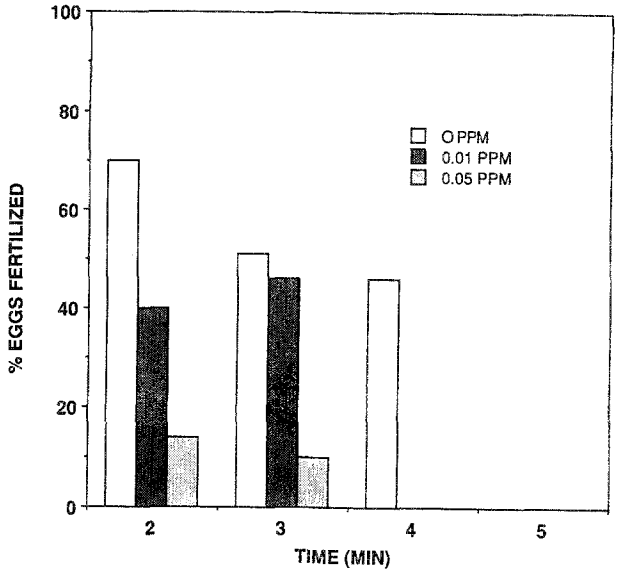

Fig. 2. Effect of methylmercury on mean % fertilization success of Long Island killifish eggs (Exposure of sperm to meHg prior to fertilization).

exposure and higher concentrations of meHg greatly reduced mean % fertilization success. Exposure of sperm to 0.05 mg/L meHg for 10 min showed 95% reduction of mean fertilization success. However, control sperm showed only 33% reduction of mean fertilization after 15 min of exposure (Figure 1).

Exposure of LI killifish sperm to 0.01 mg/L meHg for 2 min showed a significant reduction in mean % fertilization success. Since there was no significant difference between controls of PC and LI, these data indicate that meHg is more toxic to LI fish sperm than PC fish sperm (Figure 2).

Exposure of PC and LI sperm to 0 mg/L for 2 min did not cause any significant difference between PC and LI mean sperm motility. LI killifish sperm exposure (2 min) to 0.05 mg/L meHg caused significant reduction in mean sperm motility (Figure 3). PC sperm showed more tolerance to meHg in that there was no difference between 0 and 0.05 mg/L exposure for 2 min (Figure 4). However, exposure of PC killifish sperm for 5 min to 0.05 mg/L meHg caused a significant reduction in mean sperm motility. These data also indicate that meHg is less toxic to PC killifish sperm in terms of inhibiting motility. PC killifish sperm showed longer (20 min) mean motility in 15‰ sea water than LI killifish sperm (10 min) (Figures 3 and 4.).

The mature normal sperm consisted of ovoid head, a short mid-piece, and a long flagellum. In some views, the anterior end of the head appears slightly broader than the posterior end. There was no morphological difference between the PC control and LI control killifish sperm (Figures 5A and 6A). Exposure of PC and LI killifish sperm either in 15‰ sea water or 0.05 mg/L meHg up to 15 min did not have any effect on the morphology of the sperm (Figures 5B and 6B). Therefore, the reduction in fertilization success due to time and meHg exposure was probably due to the reduced motility.

Exposure of PC and LI eggs to 0.05 mg/L meHg up to 25 min prior to fertilization had no significant effect on fertilization success compared with control. However, after 15 min, control and treated eggs of both populations showed an equal decline in mean % fertilization success (Figure 7). Fertilized eggs developed normally.

Discussion

Although heavy metal concentrations in the creek water were not measured, we found high concentrations of heavy metal (11.23 mg/L, 5.78 mg/L, 623.47 mg/L, 627.78 mg/L of Hg, Cd, Cu, and Zn, respectively) in PC sediment; the concentrations in LI sediment were within normal ranges (0 mg/L, 0.46 mg/L, 41.00 mg/L, and 49.40 mg/L of Hg, Cd, Cu, and Zn, respectively). These fish release their gametes in the intertidal zone where the sediment pollutant concentrations are very high. Thus their gametes are at high risk of exposure to these pollutants.

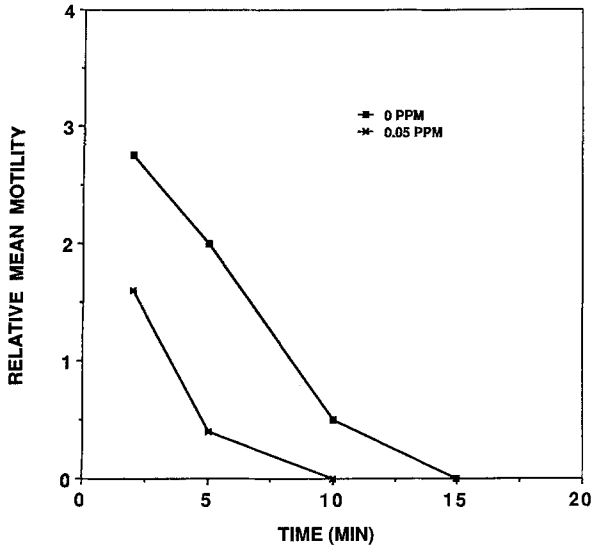

Fig. 3. Effect of methylmercury on relative mean sperm motility of Long Island killifish.

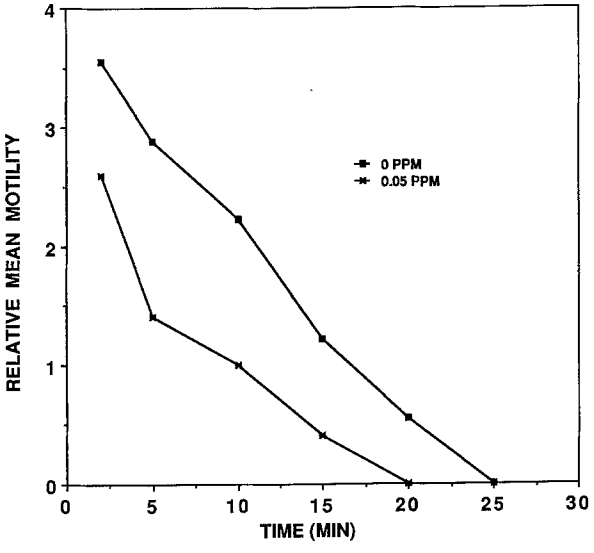

Fig. 4. Effect of methylmercury on relative mean sperm motility of Piles Creek killifish.

Several authors have indicated that populations living in metal-polluted environments become tolerant to metals. Brown (1976) reported that isopod (*Asellus meridianus*) tolerant to Pb and Cu could survive and accumulate concentrations that proved to be lethal to non-tolerant animals. This tolerance persisted into subsequent generations bred in the laboratory, indicating a genetic basis for the tolerance. Fraser *et al.* (1978) demonstrated resistance

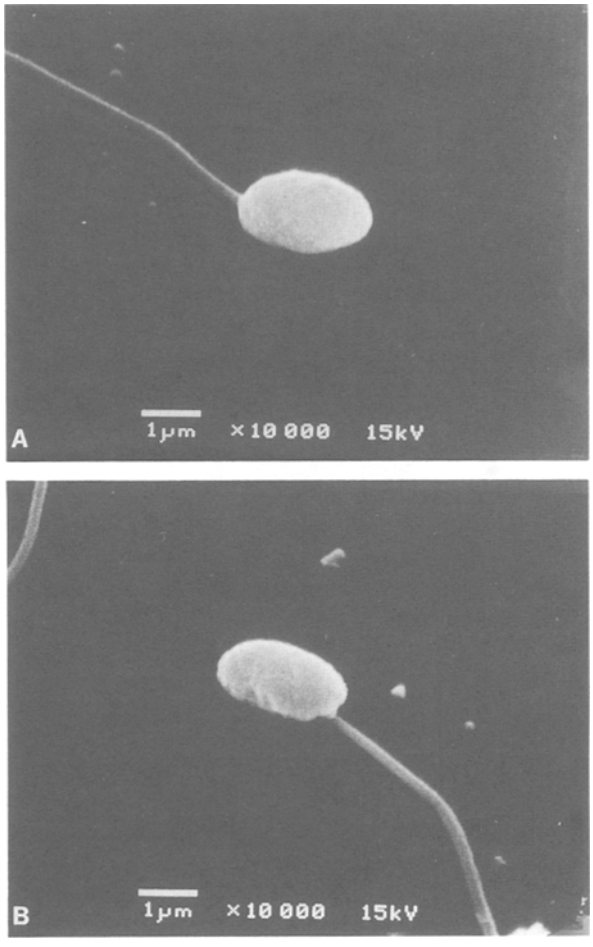

Fig. 5. (A) Control sperm of PC killifish. (B) 0.05 mg/L meHg treated sperm of PC killifish.

to Pb in the isopod (*A. aquaticus*) from sites with higher Pb concentrations. Levington (1980) suggested that pollution can act as a selective agent that causes the more tolerant genotypes to persist in polluted areas.

Killifish sperm sensitivity to meHg tested in this experiment appeared to be very different from those of other fish. McIntyre (1973) reported that concentrations greater than 1.0 mg/L meHg exposure (30 min) markedly reduced the sperm viability of steelhead trout. Billard and Roubaud (1985) also indicated that 1.0 mg/L Hg significantly decreased the fertilizing ability of rainbow trout sperm. Apparently, killifish sperm are more susceptible to meHg than those of steelhead trout and rainbow trout. The killifish is considered to be a very resilient species, but apparently this is not always the case. Organic Hg compounds are highly specific reagents for sulfhydryl groups and these groups are

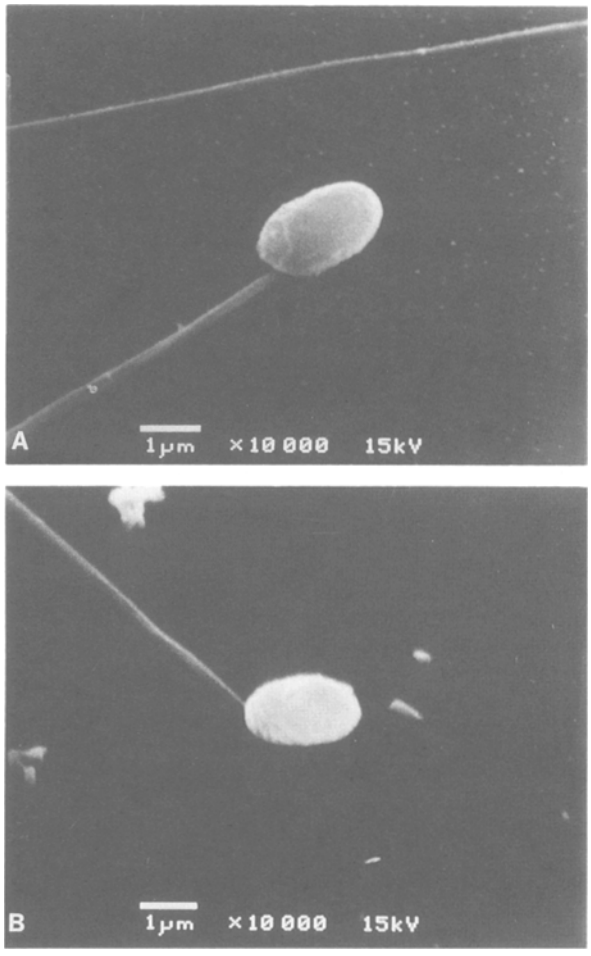

Fig. 6. (A) Control sperm of LI Killifish. (B) 0.05 mg/L meHg sperm of LI killifish.

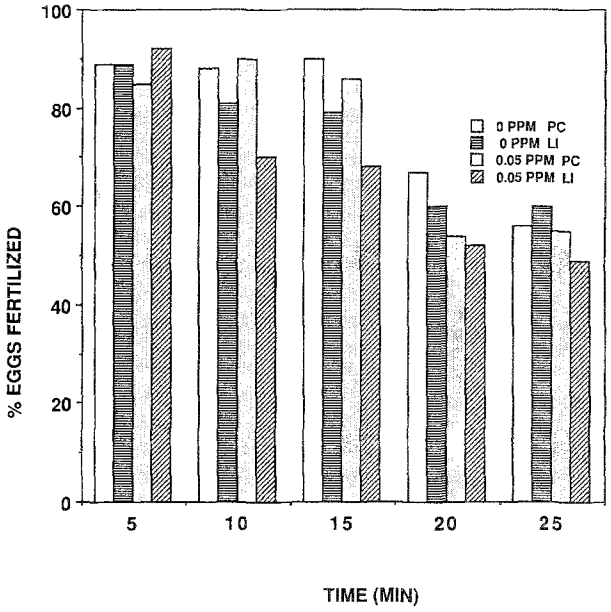

Fig. 7. Effect of methylmercury on mean % fertilization success of Piles Creek and Long Island killifish eggs (exposure of eggs to meHg prior to fertilization).

found in the membranes that encapsulate the nucleus, mid-piece, and tail of sperm (Nelson 1960). Mohamed *et al.* (1986) reported that meHg inhibits monkey's (*Macaca fascicularis*) sperm motility by inhibiting the microtubules, the motor system of the sperm. Abe *et al.* (1975) mentioned that meHg interfered with the microtuble dependent cellular functions such as neuroplasmic transport. Miura *et al.* (1978) and Imura *et al.* (1980) found that cytoplasmic microtubules in cultured glioma cells and spindles are disturbed by meHg exposure.

LI killifish sperm were more susceptible to meHg than those of PC killifish. This is in keeping with earlier data on this population. Weis *et al.* (1982) have shown that meHg was less toxic for PC embryos than that of LI embryos. The evidence indicates that the population has developed a tolerance to meHg. The tolerance of PC killifish sperm to meHg is somewhat surprising, since although the embryos are also tolerant to meHg, the male does not contribute to the tolerance of the embryos (Weis *et al.* 1982; Toppin 1985).

The amount of time that fish sperm remain motile is very crucial to the successful reproduction of that species. Since the micropyle of most fish ova remains open for a few minutes, the faster the sperm can reach an egg and fertilize it, the greater the chances are of reproduction. The results showed that PC and LI killifish sperm were motile up to 20 and 10 min, respectively. Several authors have recorded the sperm motility in white sucker (*Catostomus commersoni*), rainbow trout (*S. gairdneri*), northern pike (*Esox lucius*), and chain pickerel (*E. niger*) (Stewart 1926; Billard 1978; Duplinsky 1982) ranging from 20–137 seconds. Billard (1978) also noticed that guppy's (*Poecilla reticulata*) sperm was motile up to 40 min in physiological solution, however, in freshwater or sea water, the sperm was motile for only one min. Thus, although the sperm of killifish is more sensitive to meHg than other fish species, killifish sperm remain viable for a much longer time in the natural environment. The reason why they remain viable longer is not known. However, several authors have mentioned that higher concentration of phospholipid in the mid-piece of sea urchin sperm (Rothschild and Cleland 1952; Mohri 1963) and higher concentration of glycogen in the mid-piece of guppy's sperm sup-

plies energy for movement (Billard 1978). We have not measured the concentration of phospholipid or glycogen in killifish sperm.

Eggs fertilized by treated sperm or treated eggs fertilized by untreated sperm showed normal embryonic development when examined after one week of incubation. Hatching success or larval survival was not monitored, so some long term deleterious effects may have occurred. Pagano *et al.* (1982) reported that a short treatment of sperm (2 min) or of eggs (5 min) with up to 1,120 mg/L Cd failed to result in any detectable effect on cleavage or on differentiation of sea urchin eggs. Although Rosenthal and Alderdice (1976) found no effect of Cd (10 mg/L) treatment on fertilization success of *M. saxatilis;* delayed effects became noticeable prior to hatching.

There was no structural alteration observed by SEM in PC and LI killifish sperm after up to 15 min exposure either in sea water (15‰) or in 0.05 mg/L meHg. However, Billard (1978) found structural change (rupture of plasma membrane and mitochondria swelling) in sperm of rainbow trout after exposure in freshwater and 30‰ sea water.

The meHg treatment of PC and LI killifish eggs prior to fertilization did not prevent fertilization or cause any drastic effects on development. Sperm was far more sensitive to prolonged exposure than eggs. It is possible that some metals have a tanning effect on the egg's shell, limiting metal entry into the eggs (Billard and Roubaud 1985). Billard and Roubaud (1985) also reported that exposure (40 min) of *S. gairdneri* eggs to 1.0 mg/L Hg did not prevent fertilization. However, it caused greater damage to spermatozoa. Hall *et al.* (1984) found similar results in *M. saxatilis* after exposure of eggs to organic and inorganic contaminants prior to fertilization.

Generally, PC killifish sperm showed greater tolerance to meHg than LI killifish sperm. This is in agreement with the finding of Weis *et al.* (1982) that PC embryos were more resistant to meHg. Callahan and Weis (1983) found that fiddler crabs (*Uca pugnax*) from PC were less affected by meHg than crabs collected from Big Sheepshead Creek, a clean area. Moraitou-Apostolopouou *et al.* (1982) reported that small shrimp (*Palaemon elegans*) from a polluted estuary were more tolerant to Cd and Cr than those from a less polluted area. Bryan and Hummerstone (1971) stated that polychaetes (*Neries diversicolor*) from metal-contaminated sediments were more resistant to Cu and Zn than those from non-polluted sediments. Luoma (1977) stated that greater resistance to a toxicant in a population from one area as compared to another is direct evidence that the toxicant is exerting selective pressure at the first site.

Our data appear to indicate selective pressure by meHg on killifish at Piles Creek.

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