Marine Biology 65, 283–287 (1981)

MARINE BIOLOGY


# Methylmercury Tolerance of Killifish (*Fundulus heteroclitus*) Embryos from a Polluted *vs* Non-Polluted Environment

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## Abstract

We have previously found great differences in susceptibility to methylmercury (meHg) among batches of eggs produced by different females. This study compares the range and distribution of susceptibility in two populations of killifish, one from a creek in Montauk, New York, USA a rather pristine area, the other from Pile's Creek in Linden, New Jersey, an area heavily impacted by metal and oil pollution. After treatment with 0.05 ppm meHg, the distribution of craniofacial defects in embryos of the Montauk population ranged from very tolerant to very susceptible. The distribution of cardiovascular defects also ranged from very tolerant to very susceptible. Skeletal defects were prevalent in most batches of eggs. However, in the Pile's Creek population, very few females produced susceptible eggs, and most batches were tolerant with respect to the three types of malformations, especially the craniofacial defects. The ability to adapt to pollution is one reason that this species has remained abundant in such a highly polluted area. The ability of some species to adapt to chronic pollution by developing tolerance is a phenomenon that should be considered in choosing organisms for routine bioassay procedures, since the results will depend on the degree to which the population has become tolerant to the toxicant.

## Introduction

Mercury pollution of the marine environment is a serious problem because of its toxicity, persistence, and tendency to undergo food chain amplification. One major aspect of mercury pollution in aquatic ecosystems is the conversion of inorganic Hg to the more toxic methylmercury (meHg) by bacterial action.

Deleterious effects of Hg on fish reproduction (Kihlstrom, *et al.*, 1971; McIntyre, 1973) and development (Dial, 1978) have been noted. We have found Hg and meHg to be teratogenic to embryos of the killifish *Fundulus heteroclitus* (Weis and Weis, 1977 a, b). This species is readily obtainable, its embryos are easily maintained and have been described and staged (Armstrong and Child, 1965). It is one resident fish which has continued to survive in significant numbers in Newark Bay, the Arthur Kill, and portions of the Hackensack Meadowlands which are highly mercury-contaminated areas.

In our previous work (Weis and Weis, 1977 b) we found that exposure of developing eggs to 0.02–0.05 ppm meHg produced a variety of abnormalities, including skeletal defects, cardiovascular and craniofacial anomalies. The craniofacial defects reflect progressive convergence of the optic cups at the anterior end of the head, resulting in cyclopia or intermediate conditions of synophthalmia, reflecting progressive interference with induction of the forebrain. We have developed a craniofacial index (CFI) to quantify the degree of abnormality. The second day of development (gastrulation) was found to be the most sensitive period for production of these defects. This is the time in which forebrain induction is taking place. With incomplete forebrain induction, the lateral optic vesicles approach each other anteriorly and fuse.

The cardiovascular abnormalities involve failure of the heart chambers to differentiate properly. In severe cases, the heart remains a thin feebly beating tube, incapable of circulating blood. In the most severe cases, there is no discernable cardiac tissue at all. Accompanying the heart abnormality, areas of hemostasis and hemorrhage are often seen. Our cardiovascular index (CVI) allows quantification of this effect.

Skeletal defects include vertebral bends and stunting.

Our original methods involved pooling eggs from different females, then dividing them into experimental groups. When experiments were repeated, percentages of normal embryos and of mean CFI and CVI were not consistent. We suspected that this reflected variation in susceptibility within the population. Therefore, in subsequent experiments (Weis *et al.*, 1979) batches of eggs from dif-

0025-3162/81/0065/0283/$ 01.00

ferent females were kept separate, and great differences between batches were observed. At a given level of meHg the eggs from one female could be severely affected, while those from another female, collected at the same time from the same place and fertilized by the same male, could be mostly unaffected. These fish were all from Montauk, NY, a relatively pristine area. In view of studies revealing adaptation to metal pollution in populations that are chronically exposed (Lloyd, 1960; Bryan, 1976; Fraser, 1978; Saliba and Krzyz, 1976) the studies reported here were undertaken to compare the distribution of susceptibility in the Montauk population with that of a population from a highly polluted area, Pile's Creek, a tributary of the Arthur Kill, in heavily industrialized Linden, NJ. Mercury levels of 10.3 ppm have been measured in the sediments, as well as 2 400 ppm of Zn and 3 000 ppm of Pb (Koepp, *et al.*, 1980).

## Materials and Methods

*Fundulus heteroclitus* adults were collected by the use of traps in a tidal creek adjacent to Lake Montauk, NY or in Pile's Creek, NJ in June and July. Gravid females were stripped according to Costello *et al.* (1957) into fingerbowls of seawater and fertilized with milt stripped from males. Preliminary experiments with the Pile's Creek fish showed that they could not be successfully fertilized in water of 30‰ salinity, but could be fertilized well in water of 15‰ S. Montauk eggs were equally successful in 30‰ or 15‰ salinity, and 30‰ S was generally used. In setting up the fertilization procedure, large batches of eggs, when available, were divided into separate fingerbowls and fertilized by different males. Each group included controls and experimental dishes dosed with meHg (ICN Pharmaceuticals, Plainview, NY). If there were only enough eggs to study one concentration, 0.05 ppm was used. When additional eggs were available 0.03 ppm was used as well. Females were maintained in the laboratory so that in many cases a second stripping was possible at a later date with a different male. A total of 85 different Montauk females and 35 different males were used, to produce a total of 128 batches of eggs. A total of 63 Pile's Creek females and 26 males were used, to produce a total of 106 batches of eggs. The total number of eggs examined was about 10 000 from Montauk and 8 000 from Pile's Creek.

After stripping, eggs which initiated cleavage were placed in fingerbowls of dosed seawater (30‰ S for both populations). Daily changes for the first 4 d provided constancy in dosing until past the critical stages of development. A maximum of 50 eggs per 50 ml dish assured a better than 1 000:1 ratio of medium to eggs.

After a week of incubation at 24 °C the embryos reach stage 34–36 (Armstrong and Child, 1965) and were examined with stereomicroscopes for malformations of the head, cardiovascular system, and skeletal system. The results were quantified by use of the craniofacial index (CFI), in which each embryo's convergence of the eyes was graded on a scale of 0–6 (0 = normal, 1 = slight convergence, 2 = greater convergence to touching, 3 = synophthalmia, 4 = fused into one median eye with two lenses, 5 = cyclopia, 6 = anencephaly). In the cardiovascular index (CVI) 0 = normal heart, 1 = slight deficiency in structure or function, 2 = tube heart with contractions, 3 = tube heart without contractions, 4 = a poorly differentiated mass of cells with regular contractions, 5 = no discernable cardiac tissue (modified from Weis *et al.*, 1979). The mean CFI and CVI, and percent skeletal anomalies of different batches could then be calculated and compared.

Embryos representative of tolerance (CFI and CVI < 1) and susceptibility (CFI or CVI > 3) were preserved in glutaraldehyde and were prepared for atomic absorption spectroscopy by the cold vapor technique. Some batches of eggs were maintained longer to observe hatching, which was stimulated by changing the water.

## Results

In all groups, control eggs were either 100 percent or close to 100 percent normal. The average CFI of treated (0.05 ppm) batches from Montauk varied widely from close to 0 (resistant) to 6 (very susceptible). Fig. 1a shows the distribution of CFI in the Montauk population. The fish from Pile's Creek, on the other hand, were very resistant to

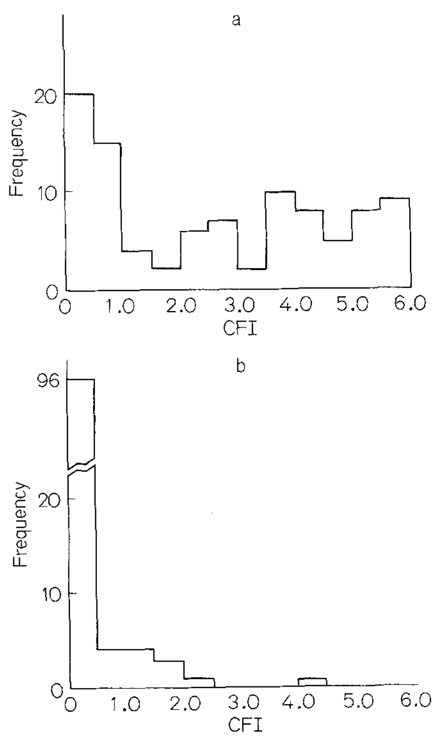


**Fig. 1.** *Fundulus heteroclitus.* (a) Distribution of CFI among treated (0.05 ppm meHg) batches of eggs from Montauk (Median value = 2.3). (b) Distribution of CFI among treated batches of eggs from Pile's Creek (Median value = 0.0). Chi-square for these distributions = 78.1, $P < 0.001$. The Y-axis, "Frequency," represents the number of batches having that particular response

the craniofacial defects produced by methylmercury, as seen in Fig. 1 b. The average CVI of Montauk eggs again varied from very tolerant to very susceptible (Fig. 2 a), whereas Pile's Creek embryos again tended to be resistant to the cardiovascular effects of the meHg (Fig. 2 b).

The percent skeletal anomalies in embryos from the Montauk population varied, but was generally high (Fig. 3 a), whereas the Pile's Creek embryos tended to be resistant (Fig. 3 b). There were, however, significant numbers of batches from Pile's Creek with high percentages of skeletal defects. These embryos, however, generally exhibited mild vertebral bends rather than the severe stunting seen in many of the Montauk embryos. To better quantify skeletal effects, in future work we will use a skeletal index to rate defects on a scale of severity comparable to CFI and CVI.

Among eggs treated with 0.03 ppm, the Pile's Creek eggs had virtually no anomalies, while the Montauk eggs varied from unaffected to rather severely affected.

Control eggs from Montauk hatched readily (80–100% success) by 12–15 d when the water was changed. Eggs from Pile's Creek, however, had a very low hatching rate (< 10%) in clean sea water. On the other hand, Pile's Creek eggs which had been incubated in 0.03 ppm meHg showed a much better (> 50%) hatching rate.

*Uptake:* Pooled tolerant eggs from Montauk took up $6.45 \pm 0.60$ ppm Hg, whereas susceptible eggs took up $7.17 \pm 0.67$ ppm. This difference is not significant. Pooled

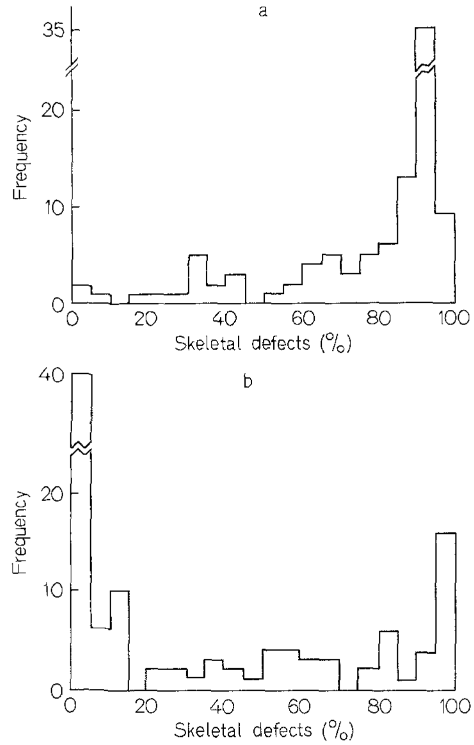


**Fig. 3.** *Fundulus heteroclitus.* (a) Distribution of percent skeletal anomalies among treated batches of eggs from Montauk (Median value = 83%). (b) Distribution of percent skeletal anomalies among treated batches of eggs from Pile's Creek (Median value = 24%). Chi-square for these distributions = 66.7, $P < 0.001$. The Y-axis, "Frequency," in all figures, represents the number of batches having that particular response

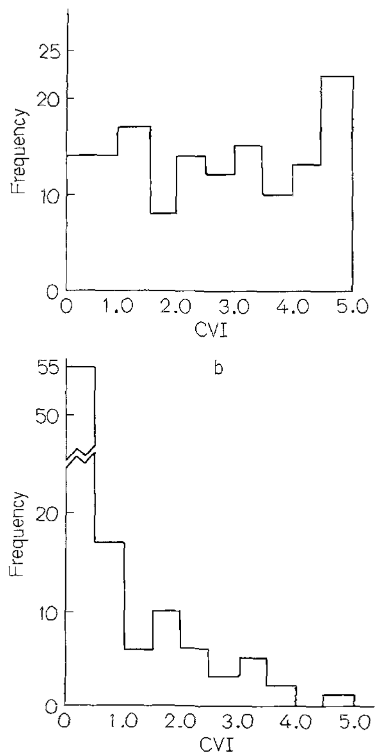


**Fig. 2.** *Fundulus heteroclitus.* (a). Distribution of CVI among treated batches of eggs from Montauk (Median value = 2.5). (b) Distribution of CVI among treated batches of eggs from Pile's Creek (Median value = 0.4). Chi-square for these distributions = 68.6, $P < 0.001$. The Y-axis, "Frequency," represents the number of batches having that particular response

tolerant eggs from Pile's Creek took up $5.7 \pm 0.5$ ppm, and susceptible eggs took up $5.4 \pm 0.0$ ppm. Therefore, Pile's Creek eggs (both resistant and susceptible) took up significantly less Hg than the susceptible Montauk eggs ($P = 0.05$), but not significantly less than the resistant ones.

## Discussion

Eggs from Pile's Creek fish were resistant to the teratological effects of 0.05 ppm methylmercury, and even had their hatching enhanced by 0.03 ppm. Similar enhanced hatching in response to Cd was observed by von Westernhagen *et al.* (1974) in herring (*Clupea harengus*). Montauk embryos ranged from resistant to very susceptible. The susceptible Montauk eggs incorporated higher levels of mercury than did the Pile's Creek eggs.

The only difference in the treatment of eggs in the two populations was that the Montauk eggs were fertilized in 30‰ S sea water and Pile's Creek eggs at 15‰ S. It might be possible that this different procedure could contribute to the different responses since Kinne (1962) has shown that the salinity in which fertilization and early development of *Cyprinodon macularius* occurs may influence growth of subsequent stages. However, the eggs in his experiments developed for 3 to 6 h in the spawning salinity and at a higher temperature (27 °C) and therefore had developed further than our *Fundulus heteroclitus* embryos which were at the 2-cell stage when transferred. Furthermore, when some Montauk eggs were fertilized in 15‰ S and transferred to 30‰ S for dosing (same procedure as for Pile's Creek fish), their response was the same as eggs from the same batch which had been at 30‰ salinity throughout. (We have previously demonstrated that incubation of Montauk killifish eggs with meHg at lowered salinity intensifies the teratological effects – Weis *et al.*, in press.) Therefore, the difference of salinity at fertilization is not responsible for the differential responses of the two populations to meHg.

The Montauk population is a much more variable one with regard to meHg tolerance. The presence of tolerant individuals allows this population to be in some sense "pre-adapted" to a potential influx of mercury pollution. The Pile's Creek population is much more homogeneous with respect to mercury tolerance.

Adaptation to metal pollutants in chronically exposed populations has been demonstrated for a variety of organisms (reviewed in Bryan, 1976). Moraitou-Apostolopoulou *et al.* (1979) showed differences in cadmium effects in clean *vs* polluted populations of the copepod, *Acartia clausi.* Also, Orciari (1979) demonstrated resistance to rotenone in golden shiners (*Notemigonus crysoleucas*) in a pond which had been repeatedly treated with rotenone.

Levinton (1980) discusses pollution as a selective agent in estuaries, and points out that adaptation of organisms to toxicants can cast doubt on the accuracy of routine bioassay procedures, since the outcome of the bioassay depends on the degree to which the population has become resistant to the toxicant.

Luoma (1977) discusses the potential use of toxicant resistance as a tool in assessing contaminant effects in natural ecosystems. He states that greater resistance to a toxicant in a population from one area, as compared to another area, is direct evidence that the toxicant is exerting selective pressure at the first site. He states that the level of resistance of the organisms reflects the degree of contamination of the environment. He further suggests that the presence of a toxicant-resistant population of one species in an ecosystem suggests that other species may also have been affected.

Rahel (1981) observed that common shiners (*Notropis cornutus*) from a zinc-polluted stream were not more zinc-resistant than populations of the same species from unpolluted streams. These data, plus a literature review, suggested to him that although fish have the genetic potential to evolve metal tolerance, they are unable to do so rapidly enough to survive in contaminated environments. Our results with killifish indicate otherwise. *Fundulus heteroclitus,* with its broad plasticity, is an "opportunistic" species with a high probability of adapting. Mitton and Koehn (1975) felt that the polygynous mating system of this species allows for rapid gene frequency changes, and therefore rapid evolutionary response to a variable environment. The frequent spawning of this species would have the same results. Whether a population will adapt or become eliminated in response to stress depends on the rapidity and severity of the stress in relation to the capacity of the population to adapt. It is probably the above-mentioned diversity and mating system which have allowed *F. heteroclitus* to survive in polluted environments like Pile's Creek, from which many other species have been eliminated.

In addition to the genetic adaptation discussed above, there is also the possibility of physiological acclimation. Individuals pre-exposed to low levels of a toxicant show greater tolerance to high levels than those individuals not pre-exposed. One mechanism by which individuals can become more metal-tolerant is by the synthesis of metal-binding proteins, or metallothioneins, which sequester the metal and reduce its toxicity to them (see review by Cherian and Goyer, 1978). It is unlikely that the embryos could manufacture their own protective proteins at an early enough stage to prevent the development of the craniofacial anomalies. Bowers *et al.* (1980) have shown that liver tissue is necessary for the protective effect, being the apparent source of the metal-binding proteins. The critical stages of craniofacial development occur before this time. However, it is possible that a metal-exposed female could incorporate metallothioneins or other metal binding compounds into her eggs, thus making them more resistant to metal toxicity throughout their development. The decreased uptake of mercury by the eggs from Pile's Creek fish can also contribute to their increased resistance.

Our present study does not address the question of whether mercury tolerance of killifish embryos is genetic or physiologically determined, but only shows increased tolerance in the population from the polluted area. It is likely that both genetic and physiological mechanisms are involved.

The ability to adapt to pollution is certainly advantageous to the killifish. If, however, high concentrations of contaminants are stored, these may be transmitted along the food chain to predatory species which may not be so adapted.

*Acknowledgments.* We would like to express our appreciation to Dr. J. C. Baiardi, Director of the New York Ocean Science Laboratory, Montauk, NY for his hospitality, to D. Kowalski, S. Tardif, and M. Renna for technical assistance. This research was supported by the US Dept. of Commerce NOAA Office of Sea Grant No. 04-7-158-44042, and by a grant from the Busch Memorial Fund of Rutgers University. Publication N. J. S. G.-82-71.

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Date of final manuscript acceptance: September 14, 1981.
Communicated by S. K. Pierce, College Park