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# Behavior and Brain Chemistry Correlates in Mummichogs (*Fundulus heteroclitus*) from Polluted and Unpolluted Environments

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## *ABSTRACT*

*The ability of* Fundulus *from a polluted environment to capture a prey organism, the grass shrimp (*Palaemonetes pugio*), was compared to that of* Fundulus *from an unpolluted reference site. Fish from the unpolluted site took significantly more prey ($p < 0.1$) during the first 5–10 min of each trial than fish from the polluted site.*

*Analysis of the neurotransmitters dopamine (DA) and serotonin (5-HT) and their metabolites, DOPAC, homovanillic acid (HVA), and 5-hydroxyindolacetic acid (5-HIAA) in cerebellums and medullas was performed by HPLC. Mummichogs from the polluted site had significantly lower concentrations of 5-HT and its metabolite 5-HIAA in their medullas, but not cerebellums, than fish from the reference site ($p < 0.05$). Concentrations of DA, DOPAC and HVA were not different between populations in either part of the brain. Neurotransmitter concentrations were shown to be independent of size and therefore, presumably, age.*

*Analysis of Hg in livers and brains of these mummichogs by atomic absorption spectrophotometry showed significant differences between the two populations for both organs. Earlier work showed that the behavioral deficit could be elicited by Hg; thus, this was the putative neurotoxic agent. However, there are other candidates which may contribute to neurotoxicity in the study site.*

## INTRODUCTION

Previous research has demonstrated deficits in growth and lifespan in mummichogs (*Fundulus heteroclitus*) from mercury-polluted Piles Creek in Linden, New Jersey, relative to conspecifics from an unpolluted reference site (Weis & Weis, 1989). This population also exhibited less ability to capture prey. This, in turn,

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might be connected to a reduction in aggressive behavior, induced by environmental contaminants such as methylmercury. Exposure of mummichogs to mercury at concentrations of 0.01 mg/l for two weeks produced a significant reduction in feeding rates (Weis & Khan, 1990). Mercury was found to induce hypoactivity in walleyes (*Stizostedion vitreum*) (Scherer *et al.*, 1975), but doses of mercury as low as 10 μg/l caused significant increases in activity in largemouth bass (*Micropterus salmoides*) (Morgan, 1979).

Sublethal doses of mercury have been shown to decrease levels of serotonin (5-HT) in rat pups (Taylor & Distefano, 1976), and reduced levels of 5-HT were associated with progressive loss of motor control in striped mullet (*Mugil cephalus*) (Thomas *et al.*, 1981). Exposure to lead caused an increase in feeding time and miscues and increased levels of 5-HT in fathead minnows (*Pimephales promelas*) (Weber *et al.*, 1991). But injecting the killifish *Fundulus grandis* with 5-HT induced lower levels of activity than saline-injected controls (Fingerman, 1976), and levels of 5-hydroxy-indolacetic acid (5-HIAA), a metabolite of 5-HT, were higher in arctic charr (*Salvelinus alpinus*) of low social rank, which corresponds to increased stress and lower levels of aggression (Winberg *et al.*, 1991).

This study was intended to show if differences in prey capture ability between mummichogs from polluted versus unpolluted environments were correlated with levels of brain neurotransmitters and/or mercury contamination.

## METHODS

Mummichogs were collected in minnow traps from Piles Creek (PC) and from a reference site near Tuckerton (TK) on the Great Bay estuary north of Atlantic City, New Jersey. After several days to permit the fish to acclimate to the laboratory, three fish per trial were placed in an experimental aquarium. These aquaria each contained three large rocks to provide shelter for the prey organisms. Twenty minutes later, ten prey organisms (the grass shrimp *Palaemonetes pugio*) were added to the aquarium. The fish were observed constantly for 30 min and the times of prey capture recorded. After one hour, the fish were removed from the aquarium. Ten trials were run for the PC and the TK groups.

To determine neurotransmitter levels, the hindbrains of 20 fish from each site were dissected out and the cerebellums and medullas homogenized with 0.1M perchloric acid and centrifuged. The supernatant was then analyzed by HPLC ($C_{18}$-reverse phase column, electrochemical detection) to determine levels of dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), 5-HT, and 5-HIAA. Analysis data, expressed as ng/g brain wet weight, were analyzed by *t*-test.

To determine mercury levels, livers and brains of fish from the same populations were analyzed for total Hg by cold vapor atomic absorption spectrophotometry. Data are given as μg/g and were analyzed by *t*-test.

## RESULTS

As shown in Fig. 1, the TK fish caught their prey faster than the PC fish, averaging more than two captures per aquarium during the first ten minutes. The

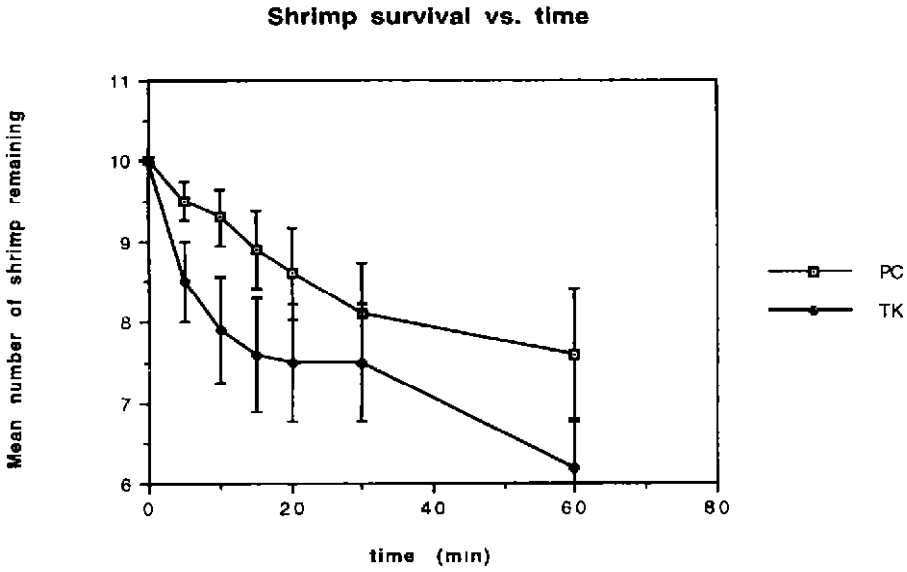


**Fig. 1.** Prey capture ability (mean ± SEM) of two populations of mummichogs over time, as indicated by number of prey shrimp remaining. PC = the Piles Creek population, TK = Tuckerton, the reference population.

PC fish, on the other hand, averaged less than one capture per aquarium over the same time period. Times to first capture varied widely. In four of the PC trials, the fish took no shrimp during the entire hour. In four others, they made their first captures in 3–4 minutes. In only one of the TK trials did the fish take no shrimp during the test period. In another, they made two captures within the first minute. The difference between the mean number of shrimp taken at any given time was analyzed by *t*-test. The differences at 5 and 10 min were found to be significant at the 90% level $t = 1.897$ and 1.980, respectively.

The results of the neurotransmitter study showed a significantly lower mean level of 5-HT ($423.6 \pm 99.53$ ng/g versus $544.2 \pm 176.1$ ng/g, $t = 2.667$, $p < 0.02$) and 5-HIAA ($66.15 \pm 15.66$ ng/g versus $87.25 \pm 37.34$ ng/g, $t = 2.330$, $p < 0.05$) in the medullas of the PC fish relative to the TK fish (Fig. 2). For medullary DA and DOPAC, there were no significant differences between populations, and none of the neurotransmitters were significantly different in the cerebellums of the two populations. HVA levels proved difficult to record reliably, and were not analyzed. There was no relationship between brain neurotransmitter levels and fish body weight in either population, although DOPAC and 5-HT levels varied much more than PA and 5-HIAA levels.

The mean liver Hg levels of the two populations were $0.076 \pm 0.027$ μg/g (TK) and $0.131 \pm 0.033$ μg/g (PC), for which $t = 4.950$ and $p < 0.001$. The mean brain Hg levels were $0.029 \pm 0.011$ (TK) and $0.066 \pm 0.043$ (PC), for which $t = 3.295$ and $p < 0.005$.

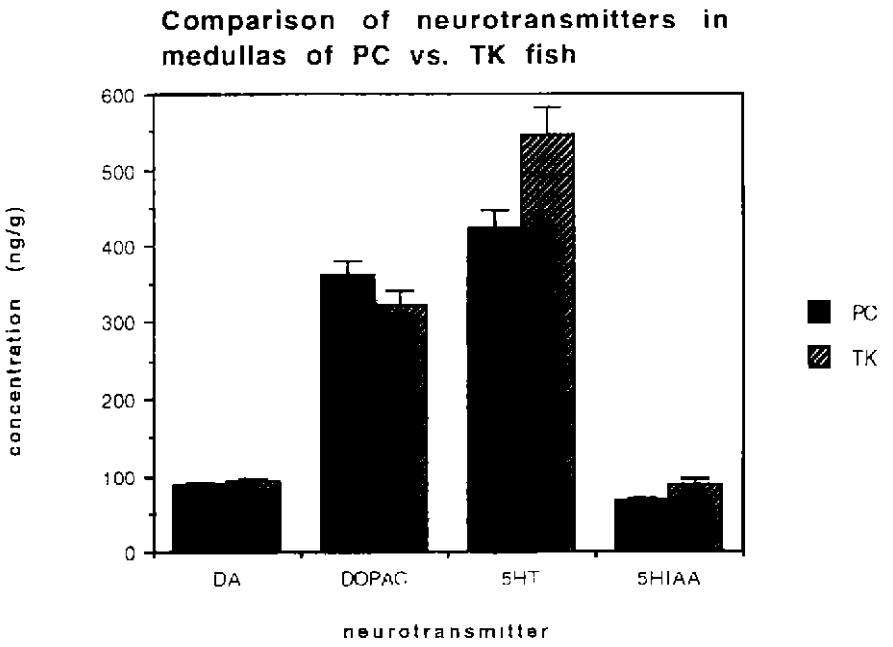


**Fig. 2.** Neurotransmitter levels in medullas of two populations of mummichogs (means ± S.D.). Significant differences were found between populations for serotonin (5-HT) and its metabolite, 5-hydroxyindolacetic acid (5-HIAA), but not for dopamine (DA) or its metabolite, dihydroxyphenylacetic acid (DOPAC). PC = the Piles Creek population, TK = Tuckerton, the reference population.

## DISCUSSION

The Piles Creek fish are poorer predators than the Tuckerton fish. They are less likely to attack prey and less likely to be successful when they do attack. They may suffer from a perceptual handicap which makes it harder for them to target prey, from difficulty in coordinating their movements, from a behavioral problem which reduces their aggressiveness, or from any combination of these factors. Further testing would be required to determine which, if any, of these is the reason for their weak performance. Also, not all PC fish were equally ineffective — as many performed well as performed poorly.

The reduced levels of 5-HT and 5-HIAA in the medullas of PC fish relative to TK fish suggests that 5-HT may be involved in some way in the poorer performance of the PC fish. Thomas *et al.* (1981) observed that reduced levels of 5-HT in mullet exposed to fluorene analogs were associated with a loss of motor control, but whether this is a cause or an effect is uncertain. Fingerman (1976), working with a much more closely related species, *Fundulus grandis*, found that high levels of 5-HT were associated with reduced activity.

Weis & Khan (1990) demonstrated similar behavioral changes in mummichogs which had been experimentally exposed to mercury. The finding in the present study of significant differences between the populations in brain and liver Hg suggests a causal relationship. However, there are other neurotoxic contaminants at PC, e.g. lead. Elevated Hg at Piles Creek may not be the only cause of the observed behavioral differences.

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