PSYCHOLOGY & NEUROSCIENCE

# Effects of methylmercury on electric organ discharges in the weak electric fish *Gymnotus sylvius*

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## Abstract

Methylmercury (MeHg) is present in the environment because of natural and anthropogenic causes. MeHg can reach the central nervous system (CNS) and cause neurological damage in humans and animals. Electric organ discharges (EODs) in the weak electric fish *Gymnotus sylvius* are produced by the electric organ and modulated by the CNS. These discharges are used for electrolocation and communication. The purpose of the present study was to investigate the effects of dietary MeHg exposure on EOD rate in *G. sylvius*. An oscilloscope was used to record the EOD rate. Two treatments were investigated: chronic MeHg administration (4 µg/kg MeHg every 2 days, with a total of nine dietary exposures to MeHg) and acute MeHg administration (a single dose of 20 µg/kg MeHg). The control data for both treatments were collected every 2 days for 18 days, with a total of nine sessions (day 1 until day 18). Data of fish exposed to MeHg were collected every 2 days, totaling nine sessions (day 19 until day 36). Chronic treatment significantly increased the EOD rate in *G. sylvius* ($p < .05$), especially with the final treatment (day 32 until day 36). Acute treatment resulted in an initial increase in the EOD rate, which was maintained midway through the experiment (day 26 until day 30; $p < .05$). The present study provides the first insights into the effects of MeHg on EODs in weak electric fish. The EOD rate is a novel response of the fish to MeHg administration.
**Keywords:** electric organ discharge (EOD), methylmercury, electroreception, novel response.

## Introduction

Mercury is present in the environment because of natural and anthropogenic events. Methylmercury (MeHg) is an organic form of mercury and the most toxic mercurial compound. The extensive use of MeHg

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in gold mining leads to aquatic pollution, which has been documented in Amazonian rivers (Akagi et al., 1995; Rabitto et al., 2011). MeHg is hazardous to wildlife and humans who consume contaminated fish. When MeHg reaches the aquatic environment, it can be incorporated into the food chain when absorbed by the gills or digestive tract of aquatic organisms (Pinheiro et al., 2000; Wang, Wong, & Wang, 2010; Dutton, & Fischer, 2011).

MeHg especially affects the nervous system, leading to recognized neurological diseases in humans and animals (Vilagi, Doczi, & Banczerowski-Pelyhe, 2000; Mieiro, Pereira, Duarte, & Pacheco, 2011). The mechanisms of MeHg neurotoxicity are still under investigation, but MeHg is known to disrupt protein synthesis by reacting with sulfhydryl (-SH) groups in enzymes and other small molecules such as glutathione (GSH; Bondy & Agrawal, 1980; Castoldi, Coccini, Ceccatelli, & Manzo, 2001; Farina, Rocha, Aschner, 2011). Alterations in GSH homeostasis can induce oxidative stress (Farina et al., 2011), leading to the oxidation of lipids, proteins, and DNA in mammals and

fish (Grotto et al., 2011; Vicari, Ferraro, Ramsdorf, Mela, Ribeiro, & Cestari, 2012). Glutamate dyshomeostasis in the central nervous system (CNS) is another neurotoxic effect of MeHg (Aschner, Yao, Allen, & Tan, 2000; Farina et al., 2011). MeHg inhibits astrocytic glutamate uptake and increases glutamate release, leading to elevated extracellular glutamate levels and MeHg-induced excitotoxicity (Allen, Shanker, Tan, & Aschner, 2002; Aschner et al., 2000; Farina et al., 2011). Glutamate is a major excitatory neurotransmitter in the CNS, and extracellular glutamate accumulation can provoke overactivation of $N$-methyl-D-aspartate (NMDA)-type glutamate receptors, leading to an increase in $Na^{2+}$ and $Ca^{2+}$ influx into neurons and consequently the induction of cell death pathways (Farina et al., 2011).

Neurological effects associated with sensorial and behavioral disturbances in fish have been observed with MeHg accumulation in the nervous system (Baatrup, 1991; Hawryshyn & Mackay, 1979). The species *Fundulus heteroclitus* was exposed to MeHg and presented impairments in prey-capture behavior (Smith & Weis, 1997). Such an effect was attributed to reduced serotonin levels, which was also seen in *Oreochromis niloticus* when exposed to the mercurial compound (Tsai, Jang, & Wang, 1995). Schooling behavior and delayed spawning were observed in *Notemigonus crysoteucas* (Webber & Haines, 2003) and *Pimephales promelas* (Hammerschmidt, Sandheinrich, Wiener, & Rada, 2002), respectively, when they were contaminated with MeHg. MeHg also induces oxidative stress, alterations in serotonin levels, and anxiogenic-like behavior in *Danio rerio* (Maximino et al., 2011). The olfactory and visual systems in fish are also vulnerable to mercurial compounds (Baatrup, 1991; Tanan et al., 2006). Reports also indicate that MeHg intoxication affects neurophysiology and sensory-motor coordination in fish.

*Gymnotus sylvius* is a weak electric fish that emits low-voltage electric pulses at a discharge rate of 20–70 Hz. The external morphology of this species is very similar to *G. carapo*, but it is biogeographically more restricted to central rivers in Brazil. In gymnotids, electric organ discharges (EODs) are used for electrolocation and communication (Hopkins, 1988; Stopa & Hoshino, 1999), which determines reproductive and non-reproductive interactions. The EOD is produced by electric organs situated along the lateral line, and the CNS is highly involved in the control of electric activity.

Despite the increased studies on the effects of MeHg in aquatic animals, no data are available about the effects of MeHg on electric activity in fish. The aim of the present study was to investigate the effects of dietary exposure to MeHg on EOD rate in *G. sylvius*, providing the first insights into the effects of MeHg on EODs in a weak electric fish.

## Materials and Methods

### Subjects

The experimental conditions and procedures were approved by the Research Ethics Committee of Universidade Estadual Paulista (UNESP) at the 12th Ordinary Meeting. *Gymnotus* fish, with an average weight of 29.7 ± 11.6 g and length of 15 ± 7 cm, were collected from the Tietê River, São Paulo state, Brazil. The fish were maintained under controlled laboratory conditions and kept individually in glass aquaria (.6 × .25 × .4 m) with hiding locations made of polyvinyl chloride tubes. The photoperiod (14 h light, 10 h dark), water temperature (23 ± 3°C), pH (7.3 ± .5), and aeration (5 ± .5 mg dissolved $O_2$) were controlled. Animals were fed daily with earthworms (*Lumbricus* sp.).

Cytogenetic analysis was conducted for species confirmation. The mitosis stimulation protocol was adapted to fish (Cole & Leavens, 1971; Oliveira, Almeida-Toledo, Foresti, & Toledo-Filho, 1988), and the mitotic chromosome preparation was described previously (Foresti, Oliveira, & Almeida-Toledo, 1993). The fish were anesthetized in an ice bath and sacrificed by transecting the spinal cord. All animals presented the 2n = 40 karyotype belonging to the species *G. sylvius*.

### Apparatus

The EOD recordings were conducted in the Electric Engineering Laboratory (UNESP, Bauru). The animals were transferred to the laboratory on the days of data collection and individually kept in an aquarium (.3 × .14 × .2 m) with a polyvinyl chloride tube and placed inside a wire-mesh Faraday cage. The EODs were detected by two electrodes made of 50-Ω impedance coaxial cables attached to the opposite ends of the hiding tube. A Tektronix digital oscilloscope (TDS2014) collected the signals from the electrodes and produced discharge rate curves that were stored on a computer. The EOD rates were obtained for each animal with minute-by-minute sampling for 30 continuous minutes per day. Therefore, 30 recordings were collected per fish per day.

### Drug

MeHg chloride ($CH_3HgCl_2$) was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). After the experiments, the contaminated material was acidified (pH < 2), neutralized in amide, and discarded for incineration.

### Procedure

The chronic and acute effects of ingested MeHg chloride on EOD rate were investigated. The fish were exposed to MeHg through the ingestion of contaminated earthworms. The earthworms were injected with MeHg using insulin syringes at the time the fish were fed. Oral exposure to MeHg has important environmental significance because it is similar to the most common human exposure and avoids the discomfort of injections (Farina et al., 2011) and handling stress.

To evaluate the cumulative effects of MeHg administration over 18 days (day 19 until day 36), five fish (29.3 ± 9.0 g, 15.3 ± 2 cm) were subjected to multiple doses of MeHg (chronic treatment). They were

exposed to MeHg every 2 days for 18 days, with a total of nine dietary exposures to MeHg. With each exposure, the fish were exposed to 4 µg/kg MeHg.

To evaluate the effect of methylmercury depuration over 18 days (day 19 until day 36), five fish (24.6 ± 13 g, 14.8 ± 2.5 cm) were subjected to a single dose of MeHg (acute treatment). They were exposed to 20 µg/kg MeHg.

Because all of the fish served as their own controls, the control data for both treatments were collected before dietary MeHg exposure. The control data for both treatments were collected every 2 days for 18 days (day 1 until day 18), with a total of nine sessions. In each session, the EOD recordings began immediately after a 5-min habituation period.

After the control EOD recordings, the fish were subjected to the acute and chronic experiments. The acutely treated animals were subjected to nine sessions of EOD recordings after MeHg administration, repeating the baseline protocol of the control sessions. The same occurred with the chronically treated animals, but the recordings were made on the interim days between dietary MeHg administration.

### Statistical analysis

The 30 EOD rate measurements collected for each fish per day were averaged into one measure. The individual mean EOD rate was analyzed by comparing the data before and after MeHg administration for each treatment regimen. Each animal had its EOD rate recorded repeatedly after or during the treatments. Data were analyzed by one-way repeated-measures analysis of variance (ANOVA). The significance level was 95% ($p < .05$).

## Results

### Chronic MeHg treatment

The one-way repeated-measures ANOVA indicated that chronic MeHg treatment altered the EOD rate in *G. sylvius* ($F = 5.01$, $df = 17$, $p < .001$). The EOD rate exhibited oscillations during treatment (Figure 1). According to the Tukey *post hoc* test, the EOD rate significantly increased during the last three recordings (day 32 until day 36) when the estimated cumulative dose reached 36 µg/kg.

### Acute MeHg treatment

One-way repeated-measures ANOVA indicated that acute MeHg treatment increased the EOD rate in *G. sylvius* ($F = 3.63$, $df = 17$, $p < .001$). From day 26 to day 30, the EOD rate significantly increased compared with the control recordings (Tukey test; $p < .05$; Figure 2). The prominent increase in EOD occurred on day 28 (Tukey test, $p < .05$).

## Discussion

The effects of dietary MeHg administration were investigated on EOD rate in the weak electric fish *G. sylvius* using two treatment regimens. The chronic treatment was conducted to evaluate the consequences of small but constant exposure to MeHg, simulating trophic accumulation. The acute treatment was
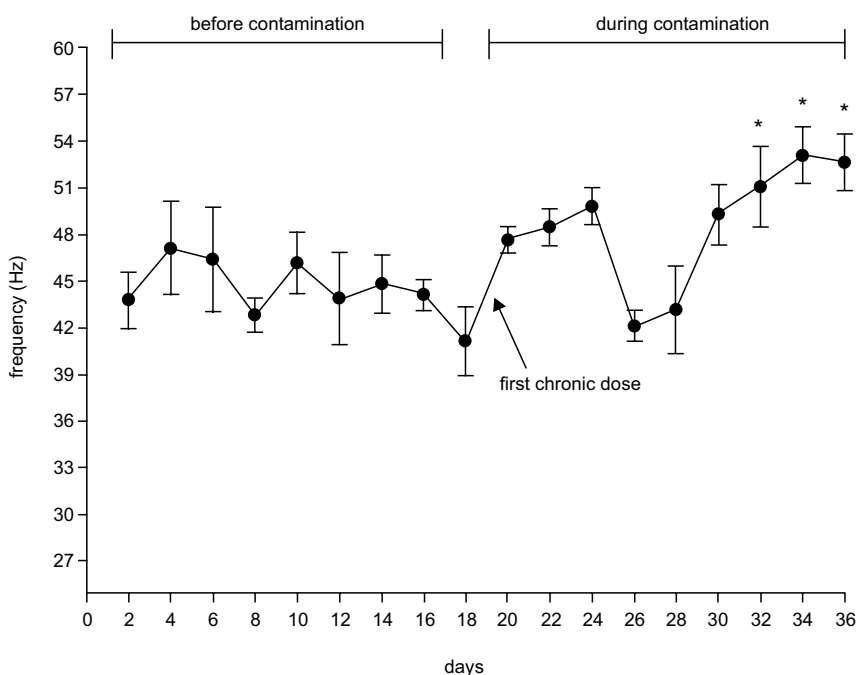


**Figure 1.** Mean electric organ discharge (EOD) rates (±SEM) in *Gymnotus sylvius* with chronic MeHg treatment. The control data were collected on the interim days for 18 days before MeHg administration (day 1 until day 18). The fish were then subjected to multiple doses of MeHg. They were exposed to MeHg every 2 days for 18 days, with a total of nine dietary exposures to MeHg (day 19 until day 36). In each exposure the fish received 4 µg/kg MeHg. The recordings were made on the interim days between dietary MeHg exposures. *$p < .05$, different from control recordings.
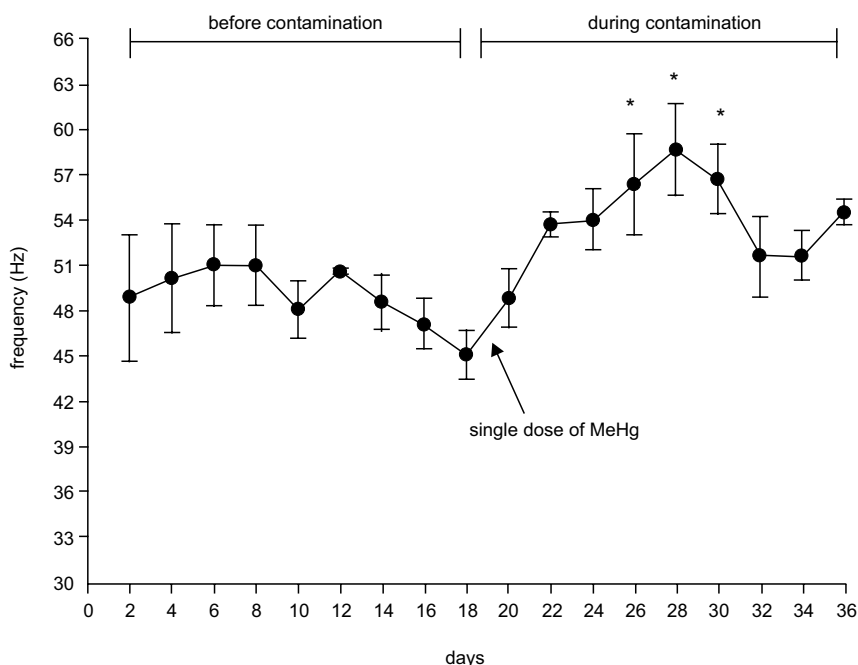
**Figure 2.** Mean EOD rates (±SEM) in *G. sylvius* with acute MeHg treatment. The control data were collected on the interim days for 18 days before MeHg administration (day 1 until day 18). The fish were then subjected to a single dose of 20 µg/kg MeHg. The EOD recordings were collected for nine sessions (day 19 until day 36) by repeating the baseline protocol for controls. *$p < .05$, different from control recordings.

conducted to evaluate the effects of an elevated dose of MeHg and post-depuration events. In both treatments, MeHg altered the modulation of EOD, leading to an evident increase in the discharge rate of electric pulses.

Chronic MeHg treatment increased the EOD rate in *G. sylvius*, especially during the final stages of treatment. The progressively increasing pattern may be attributable to dose-dependent MeHg accumulation. The sequential doses of MeHg at short time intervals can accumulate in the organism (Oliveira-Ribeiro, Belger, Pelletier, & Rouleau, 2002) because of biomagnification caused by the high assimilation and low elimination rate of MeHg in fish (Wang et al., 2010; Dutton & Fischer, 2011). Horizontal cells in the retina in *Hoplias malabaricus* also presented increased responsiveness as a consequence of low-dose MeHg exposure (Tanan et al., 2006).

The acute treatment indicated that the MeHg depuration period affected the modulation of electric pulses in *G. sylvius*. Although the MeHg concentration was not quantified in *G. sylvius* tissues, it likely reached the brain of the fish, leading to alterations in the neural control of the EOD rate. MeHg is totally retained in several fish species, including 7 days after depuration in *Oreochromis niloticus* (Wang et al., 2010), 9 days after feeding on worms contaminated with MeHg in *Fundulus heteroclitus* (Dutton & Fischer, 2011), and 15 days after depuration in *Salmo gairdneri* (Hawryshyn & Mackay, 1979). MeHg can accumulate in the brain in *S. gairdneri* (Hawryshyn & Mackay, 1979) and gills, viscera, skin, liver, brain, and muscle in *Fundulus heteroclitus*, which presents the highest concentration of MeHg after 9 days of dietary exposure (Dutton & Fischer, 2011).

The increase in the EOD rate observed with both treatments in the present study can be related to MeHg neurotoxicity that occurs through an excitotoxic mechanism. Electric organ discharges are modulated by a series of neural nuclei including the electrosensorius nucleus, diencephalic pre-pacemaker nucleus, and bulbar pacemaker nucleus in the brain as described in *G. carapo* (Correa & Hoffman, 1999). This circuit possesses γ-aminobutyric acid (GABAergic) and glutamatergic projections in which the respective neurotransmitters interact with $GABA_A$ and NMDA receptors. Glutamate increases the EOD rate, and GABA reduces the EOD rate in the gymnotiform electric fish *Hypopomus brevirostris* (Kawasaki & Heiligenberg, 1990). MeHg neurotoxicity is related to glutamate dyshomeostasis in the CNS, which provokes extracellular glutamate accumulation in astrocytes. This phenomenon occurs by inhibiting astrocytic glutamate uptake and increasing glutamate release (Allen et al., 2002; Aschner et al., 2000; Farina et al., 2011). If MeHg mediates excitotoxicity via accumulation of extracellular glutamate levels, then the increase in EOD rate in *G. sylvius* may be a consequence of MeHg-induced excitotoxicity. The fine-tuning of the EOD rate is crucial for electrical communication among *Gymnotus* species, which determines reproductive and non-reproductive interactions and affects social and ecological aspects (Gouvêa Junior, Stopa, Paula, & Hoshino, 2002).

In summary, dietary MeHg administration affected the EOD in the weak electric fish *G. sylvius*, inducing a significant dose- and depuration-dependent increase in the EOD rate. Future investigations are needed to elucidate the effects of MeHg on glutamate homeostasis in the brain in *G. sylvius* and the direct effects of MeHg on the electric organ in the fish. The present study points to the EOD rate as novel response of fish to MeHg

administration. Measuring EODs is inexpensive, useful, and non-invasive and may be used to monitor mercurial contamination in water and animals, especially in Amazonian rivers.

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