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Effects of Trophic Poisoning with Methylmercury on the Appetitive Elements of the Agonistic Sequence in Fighting-Fish (*Betta Splendens)*

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The aggressive display in *Betta splendens* is particularly prominent, and vital to its adaptation to the environment. Methylmercury is an organic variation of Hg that presents particularly pronounced neuro-behavioral effects. The present experiments aim to test the effect of acute and chronic poisoning with methylmercury on the display in *Bettas*. The animals were poisoned by trophic means in both experiments (16 ug/kg in acute poisoning; 16 ug/kg/day for chronic poisoning), and tested in agonistic pairs. The total frequency of the display was recorded, analyzing the topography of the agonistic response. The methylmercury seems to present a dose- and detoxification-dependent effect on these responses, with a more pronounced effect on motivity in acute poisoning and on emotionality in the chronic poisoning. It is possible that this effect could be mediated by alteration in the mono-amino-oxidase systems.
Keywords: methylmercury, aggression, emotional behavior, Betta splendens

El despliegue agresivo en la *Betta splendens* es especialmente prominente y es vital para su adaptación al medio ambiente. Metil-mercurio es una variación orgánica de Hg que presenta efectos neuro-conductuales especialmente pronunciados. Los experimentos actuales intentan poner aprueba el efecto de envenenamiento agudo y crónico con metil-mercurio sobre el despliegue en *Bettas*. Los animales fueron envenenados tróficamente en ambos experimentos (16 ug/kg e el envenenamiento agudo) y probados en parejas agonistas. Se registró la frecuencia total del despliegue, analizando la topografía de la respuesta agonista. El metil-mercurio parece presentar un efecto dependiente de la dosis y de la detoxificación sobre estas respuestas, con un efecto más pronunciado sobre la motilidad en el envenenamiento agudo y sobre la emotividad en el envenenamiento crónico. Posiblemente, este efecto podría mediarse por la alteración en los sistemas de mono-amino-oxidasa.
Palabras clave: metil-mercurio, agresión, conducta emocional, Betta splendens

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The aggressive display of the *Betta splendens* (Teleostei, Belontiidae) is very prominent, and Bronstein (1980, 1981a, 1982) suggested that it's reproductive and agonistic strategies are typical of many teleosts that utilize external fertilization in relation to the body of the female of the species. A species-specific agonistic sequence may be separated into appetitive, mating, and post-mating components (Klein, Figler, & Peeke, 1976). In the case of *Betta splendens*, the appetitive component that corresponds to the display has been the most studied. These appetitive components include saturation of body coloration, erection of the opercles, or gill cover, orientation and movement characteristics (Simpson, 1968). The mating-related components include biting, jaw blocking between opponents and striking with the tail (Simpson, 1968). An alteration in one of the appetitive components predictably alters the mating components (Klein, Figler, & Peeke, 1976; Bronstein, 1985).

The response to the display may be elicited by (a) the presentation of a subject of the same species in the same or another aquarium, (b) the presentation of a model, or (c) the presentation of a mirror (Meliska, Meliska, & Peeke, 1980). The vigor with which animals present their display, defined by the duration and frequency of the demonstration, is a reliable predictor of the animal's performance in a real combat situation (Evans, 1985) and in situations in which dominance is established (Baenninger, 1968; Meliska & Meliska, 1976; Meliska et al., 1975, 1980; Simpson, 1968). Rhoad, Kalat, and Lopfer (1975) showed that the agonistic display in *Bettas* is more vigorous when they are presented with a male of the same species, followed by a mirror image, a moving model, and finally, a stationary model.

In neural terms, the aggressive display of the *Betta splendens* was studied with ablation techniques and discrete lesions of the telencephalon (Marino-Neto & Sabbatini, 1983a, 1983b) and through the waterborne presentation of neurotransmitters (Baenninger, 1968). Baenninger exposed various fighting-fish to epinephrine and norepinephrine hydrically and observed their agonistic display, revealing suppression of the appetitive elements of the agonistic sequence of the *Betta*. Electrolytic lesions in the dorsomedial telencenphalon (Marino-Neto & Sabbatini, 1988; this region is probably homologous to the tetrapod amygdala: Braford Jr., 1995; Butler, 2000) appear to cause a decrease in the frequency and duration of various behaviors of the display and a greater stereotypy in the patterns of the display. Beyond this, the agonistic sequences are more dispersed among other behaviors, such as exploration and locomotion, with sudden returns to aggressive activity (Marino-Neto & Sabbatini, 1983a). The subjects' responses to mechanical stimuli applied to the wall of the aquarium were also altered, with an increase in jumps or "fast starts" and with a more rapid frequency in respect to these stimuli (Marino-Neto & Sabbatini, 1983b). Taken together, these results suggest that the dorsomedial telencephalon of the *Betta splendens* plays an important role in inhibitory control of the animal's attention and excitation system (cf. Flood et al., 1976, for a discussion of the function of the telencephalon of teleosts in attention and excitation). The other element altered by lesions of the dorsomedial telencephalon is the gradual inhibition of behavior after accustomization. Portavella et al.'s work (Portavella, Vargas, Torres, & Salas, 2002), using injuries to the dorsomedial telencephalon of the *Carassius auratus*, showed that emotional learning (inhibitory avoidance) is controlled by this structure. Thus, it is possible that the dorsomedial telencephalon is a necessary structure for the control of aggression and learning of avoidance in teleosts, functionally analogous to the amygdala in tetrapods (cf. Panksepp, 1998). This functional analog could, perhaps, be supported by a *de facto* homology of the structures (Bradford, 1995; Butler, 2000; Northcutt, 2006). In fact, the connection between aggression and anxiety has been made in various models (i.e., Miczek, Weerts, Vivian, & Barros, 1995) and, apparently, both are controlled by the serotoninergic system (Graeff, 2002). The serotoninergic neural systems exhibit consistency in the evolutionary chain of medial structures (Ma, 1997). The function of the serotoninergic systems was observed in the mediation of the aggressive response in species that included lobsters, ants, fish, and rodents (Berman, Tracy, & Coccaro, 1997; Blanchard, Griebel, Rodgers, & Blanchard, 1998; Lesch & Merschdorf, 2000).

Methylmercury (abbreviated as MeHg or CH_3Hg) is a highly toxic and bioaccumulative metal, with a profound effect on the nervous system, as much in humans as in other animals (Hartman, 1995). Mercury has been released in nature in an uncontrolled manner, principally through gold panning, and chlorine, caustic soda, and paper industries, among others. Fish, at the top of the food chain, appear to be the most affected by aquatic contamination (Azevedo, 2003). It has been demonstrated that organic and inorganic mercury deposits cause extensive effects on the central nervous system in fish of the teleost order (Baatrup, 1991). Methylmercury is present in great bioaccumulation in the aquatic environment (Azevedo, 2003), and trophic and hydric exposure are likely the greatest route of accumulation of mercury in fish (Harris & Bodaly, 1998; Regine, Gilles, Yannick, Alain, 2006). Although various studies have been conducted on the lethality of mercury, the neurotoxicology of methylmercury in sublethal doses is of interest from an ecological point of view, as the substance presents alterations that have a great effect on the reproductive capacity and survival of animals in the natural environment.

Few behavioral studies have been conducted using fish as models. In general, Methylmercury appears to affect swimming performance, learning, patterns of activity, and the abilities of foraging, reproduction, competition, and avoidance of predators in fish (e.g., Atchison, Henry, & Sandheinrich, 1987; Baatrup, 1991; Bernstssen, Aatland, & Handyc, 2003; Fjeld, Haugoen & Vlestad, 1998; Webber & Haines, 2003; Woebser, 1975). These consequences, in general, have effects on the likelihood of survival and reproductive ability of fish populations in the natural environment.

Concern over the neurobehavioral effects of methylmercury arose from reports of contamination in humans. In general, various symptoms of the effects on the central nervous system were reported. According to Azevedo (2003), ataxia (loss of coordination) is the first clinical indication of methylmercury contamination, but paresthesia, dysarthria, deafness, appearance of scotomas (blind spots), reduction of the visual field, blindness, alterations in olfactory and palate function, emotional alterations, muscular tremors, and perturbations of movement are also included. In addition to these effects, apraxia, astereognosia, and disturbance of active sensation were detected. Ninomiya et al. (2005) considered that the persistent somatosensory effects immediately after exposure to methylmercury are induced by injuries spread throughout the somatosensory cortex. The effects on the emotional balance in contaminated patients is correlated in studies that indicate alterations in the response to predators in fish (Smith & Weis, 1997), as well as in feeding behavior (Fjeld, Haugoen, & Vlestad, 1998), as much in exposure in the embryonic stage as in adults. Studies in our laboratory indicated that mercury alters the response to anxiety as much in mice as in fish of the *Danio rerio* (zebra fish) species (unpublished data). Besides the data on *Danio rerio*, facilitating certain generalizations, these are not complete for other species of fish, given the diversity of metabolism encountered among this class of vertebrates, especially the variation encountered among tropical and non-tropical fish, as tropical fish can exhibit physiological parameters very different from non-tropical fish.

In neurotoxicological terms, the effect of methylmercury has been described basically in mammals. Although it has been believed for a long time that absorption of methylmercury in the intracellular environment occurs through "molecular mimesis" (a process in which a given molecule is "confused" with another due to structural similarity), it has been shown (Hoffmeyer et al., 2006) that it is more likely that this process occurs through "mimesis" of the methylmercury molecule with the l-alpha portion of an amino acid. Mercury causes depletion of the principal cellular antioxidants, with high affinity for the thionic group (Azevedo, 2003), the functional group of the amino acid, cysteine. Beyond effects on the retinal cell (eg, Bonci et al., 2006), various other cells of the nervous system appear to be affected by exposure to the metal (Nascimento & Chasin, 2001). The observation that methylmercury induces oxidative stress in the brain of house mice (Yee & Choi, 1994) led Aschner et al. (1995, 1998, 2000) to consider that there are two critical components in the neurotoxicology of methylmercury: the increase in extracellular concentration of glutamate as a result of alterations in the astrocyte function (Aschner, Vitarella, Allen, Conklin, & Cowan, 1998), as well as direct interaction with the cysteine transporter, causing a decrease in the concentration of cysteine for synthesis of glutathione; and interference with the function of the astrocyte transporter of glutamate, causing an increase in the extracellular concentration of this neurotransmitter (Morken, Sonnewald, Aschner, & Syversen, 2005); this activity is modulated by adenosine (Aschner, Mullaney, Wagoner, Jr., Lash, & Kimelberg, 1995) and by glutathione (Kaur, Aschner, & Syversen, 2006). Moreover, the generation of species reactive to oxygen, a consequence of the altered production of arachidonic acid, must interfere with the removal of extracellular glutamate, inhibiting the activity of glutamate transporters. According to Juárez et al. (2005), altered activity of NMDA receptors may contribute to a sudden increase in the apoptosis of various cortical and subcortical cells. Allen et al. (Allen, El-Oqayli, Aschner, Syversen, & Sonnewald, 2001) claimed that the effect of methylmercury on the activity of astrocytes is mediated by the effect of this toxin on the mitochondria of the astroglia. In addition to glutamate, various other neurotransmitter systems were implicated in the neurotoxicology of mercury: muscarine and nicotine receptors (Bondy & Agrawal, 1980; Castoldi, Candura, Costa, Manzo, & Costa, 1996), nitric oxide (Kuo, Huang, & Lin-Shiau, 2002) serotoninergic (Hrdina, Peters, & Singhal, 1976), noradrenergic (Komulainen & Tuomisto, 1981; Gassó, Suñol, Sanfeliu, Rodríguez-Farré, & Cristòfol, 2000) and dopaminergic (Bondy & Agrawal; Komulainen & Tuomisto; Scheuhammer & Cherian, 1985; Faro et al., 1997, 2002; Daré et al., 2003) systems. The activity of monoamino-oxidase (MAO) also exhibits extensive alterations due to methylmercury toxicology (Chakrabarti et al., 1998). MAO inhibition by methylmercury is involved in the accumulation of serotonin in the nervous system and a decrease in the level of 5-hydroxi-indolacetic acid (Azevedo, 2003). According to Fonfría, Rodríguez-Farré, and Suñol (2001), a good part of the effects of methylmercury on neurotransmission can be explained in terms of the affinity between this toxin and the thiol group of proteins, peptides, and amino acids. Methylmercury also alters intracellular concentrations of Ca^{2+} in neuroblastomas that express the 5-HT_3 receptor (Hare & Atchison, 1995a), which led Hare and Atchison (1995b) to claim that methylmercury depolarizes the plasmatic membrane of these cells, causing an increase in the activation of the Na^+ and Ca^{2+} channels; Tarabova et al. (2006) observed that methylmercury inhibits the expression of the Cav3.1 of neuronal cells in the HEK 293 family. The sensitivity of the potassium channels to methylmercury is low (Yuan et al., 2005), in contrast to the extensive alteration of the calcium dynamics. Furthermore, Girault, Boudou, and Dufourc (1997) reported that methylmercury is connected to lipids in the cellular membrane, inducing a great number of disturbances in its structure. The temporal course of these neurotoxicological alterations by methylmercury is of some importance: the first effect to appear is the formation of an oxygen reactive species, followed by oxidative damage to the DNA, parallel to partial depolarization of the mitochondria (Belletti et. al., 2002). Given an alteration so pervasive on molecular systems, it is difficult to determine a general effect of mercury on any species, or, specifically, species of various phyla.

The present study aims at verifying the effect of trophic methylmercury poisoning on the agonistic display of the *Betta splendens*. Given that the aggressive elements are probably mediated by the same structures that control emotion, the results will be discussed in the light of the literature on the emotional effects of mercury and other studies conducted in our laboratory.

Experiment 1: Effects of purification of acute poisoning on the display in *Betta splendens*

Method

Subjects

Twelve adult *Bettas* (*Aqua Mundi*, Bauru/SP) were used for this experiment, experimentally ingenuous, kept isolated in individual tanks (14 × 6 × 11 cm), with controlled lighting (12/12 hrs, cycle beginning at 7:00 a.m.), average Ph level at 8.0 and average temperature of 22°C. The subjects weighed 0.6±0.05 mg.

The animals were kept previously in the laboratory for 15 to 30 days, until they were acclimated and adapted to the dietary regime, which during this time was composed of flocculated rations (*Mini Betta, Brazil*) and salt-water artemias.

The baseline of the subjects' display was obtained through observation of agonistic interactions, in separate aquariums, in daily sessions lasting 5 minutes. For this, two tanks were placed side by side, each of them containing a fish. The behaviors described in Table 1 were observed.

All behaviors were described in terms of frequency. The interactions were recorded, and the behaviors were transcribed using EthoLog software (Ottoni, 2000). Furthermore, an analysis of the duration of the entirety of the display was also made.

Table 1
Distinct Display Behaviors

Floating, without opening the fins
Resting at tank bottom
Emerging and swallowing air
Swimming slowly using pectoral fins
Undulating swimming pattern
"Shaking" the body
Opening the gill cover, without either horizontal or vertical display
Horizontal display
Vertical display
Arching the body
Charging the opponent
Retreating from opponent when becoming the target of attack/advance

The fish in the "contaminated" group were trophically contaminated, by ingestion of saltwater artemias, hydrically contaminated for 24 hours, at the ratio of 0.2 g of artemia/gram of fish, resulting in an initial contamination of 0.4 μg methylmercury/g /*Betta*. After a purification period (24, 48, and 96 hours), the fish were tested in agonistic pairs, in individual tanks adjacent to each other, the measures of the rate of occurrence of the behaviors were recorded in an ethogram of the complete display, determined above, of the molar components of the display (see results below) and the total activity (determined as the sum of the frequency of all of the behaviors).

Results

The results of the ethogram were analyzed at two levels: in terms of the frequency of the behaviors in Table 1, and total activity; and in a larger group, determined by factor analysis (principal components and varimax rotation with Kaiser normalization). The extraction of principal components reached rotation convergence at 6 iterations, generating three main extracted components. Table 2 and Figure 1 show the results of the analysis.

The first extracted components contains the behaviors of floating, resting at the bottom, breathing, shaking, arching, charging, and retreating, being, therefore, a component of the accessory elements of the display; the second components contains the behaviors of swimming with fins, undulating, and opening the gill covers, thus configuring a component of motility; the third components contains the behaviors of horizontal and vertical display, configuring the component of the display itself. These results corroborate the molar analysis of the literature (e.g., Bronstein, 1984). Figures 2 and 3 present the means and standard-deviations for the two levels of the analysis.

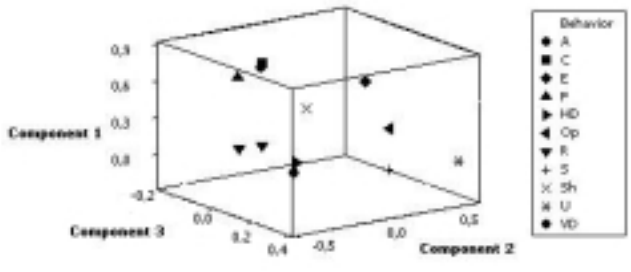

Figure 1. Rotated vectorial space of the components extracted with principal components.
Key: F: floating; R: resting at tank bottom; E: emerging and swallowing air; S: swimming slowly with pectoral fins; U: undulating swimming pattern; Sh: "shaking" the body; Op: opening gill cover; HD: horizontal display; VD: vertical display; A: arching body; C: charging; R: retreating.

Table 2
Rotated Component Matrix (Elements in Boldface are the Behaviors Belonging to that Component)

	Components		
Behaviors	1	2	3
Floating	**0.759**	–0.365	-3.515^{-3}
Resting at tank bottom	**0.168**	–0.353	5.768^{-2}
Emerging and swallowing air	**0.533**	0.524	-5.579^{-2}
Swimming slowly with pectoral fins	–0.224	**0.678**	0.201
Undulating swimming pattern	3.059^{-2}	**0.702**	0.348
Shaking the body	**0.395**	0.111	–0.188
Opening gill cover	0.113	**0.684**	–0.303
Horizontal display	-3.469^{-2}	4.809^{-2}	**0.817**
Vertical display	–0.106	2.225^{-2}	**0.760**
Arching body	**0.809**	–0.201	7.677^{-2}
Charging	**0.619**	0.146	–0.260
Retreating	0.16	–0.191	3.093^{-2}

Note. Component 1 = accessory elements of display; Component 2 = motility component; Component 3 = display proper.

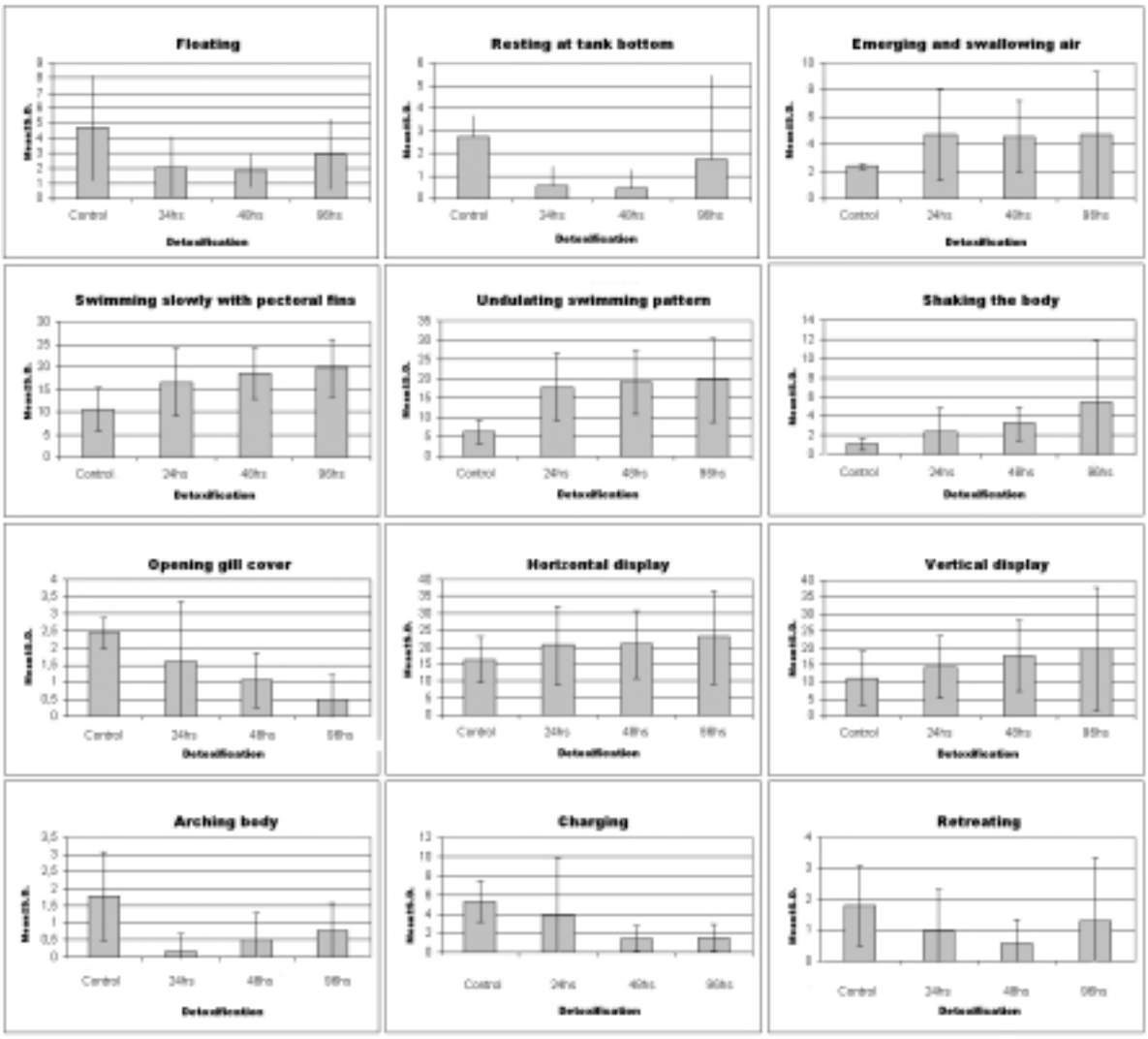

Figure 2. Frequencies ($M \pm SD$) of the behaviors observed as a function of the detoxification time in acute poisoning.

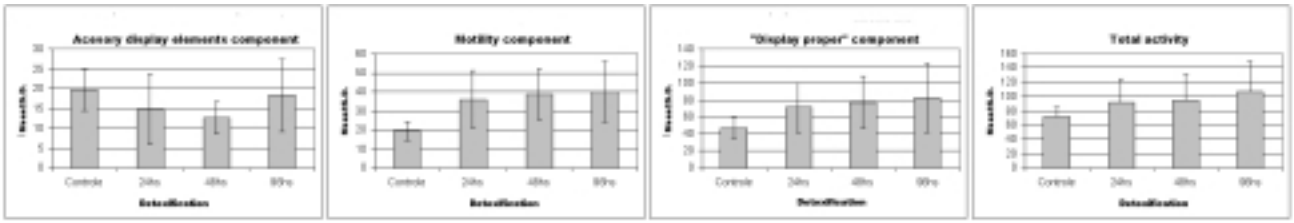

Figure 3. Frequencies (*M* ± *SD*) of the behaviors grouped by components of the factor analysis and of total activity in acute poisoning, as a function of detoxification time. The results of the factor analysis are found in Table 2 and Figure 1.

Table 3
Matrix of the Difference for the Independent Variable "Purification"

Dependent variable	Treatment	Treatment	Mean difference	Standard error	Significance
Floating	Control	24 Hours	2.585	0.808	.011
		48 Hours	2.863	0.808	.004
Resting at tank bottom	Control	24 Hours	2.184	0.676	.010
		48 Hours	2.295	0.676	.006
Swimming slowly with pectoral fins	Control	24 Hours	–6.085	2.036	.020
		48 Hours	–7.973	2.036	.001
		96 Hours	–9.196	2.036	< .001
Undulating swimming pattern	Control	24 Hours	–11.405	2.750	< .001
		48 Hours	–12.682	2.750	< .001
		96 Hours	–13.238	2.750	< .001
Shaking the body	Control	96 Hours	–4.310	1,.83	.003
Opening gill cover	Control	48 Hours	1.379	0.350	.001
		96 Hours	1.934	0.350	.000
		96 Hours	1.111	0.350	.012
Arching body	Control	24 Hours	1.608	0.377	< .001
		48 Hours	1.274	0.377	.006
		96 Hours	0.997	0.377	.049
Charging	Control	48 Hours	3.821	1.096	.005
		96 Hours	3.765	1.096	.005
Total activity	Control	96 Hours	–34.267	11.152	.016
Component 1	Control	48 Hours	7.176	2.404	.020
Component 2	Control	24 Hours	–16.666	4.350	.002
		48 Hours	–19.277	4.350	< .001
		96 Hours	–20.499	4.350	< .001
Component 3	Control	48 Hours	–30.110	10.027	.019
		96 Hours	–35.666	10.027	.004

Note. Only the treatments in which a significant statistical difference was observed are shown.

Statistical analysis (one-way MANOVA, detoxification as inter-subject factor, type III sum of squares) revealed poisoning effects, mediated by detoxification from methylmercury, on the behaviors of floating, $F(89.769, 3) = 5.096$, $p = .003$; resting on the tank bottom, $F(63.361, 3) = 5.135$, $p = .003$; swimming with fins, $F(899.549, 3) = 8.04$, $p < .001$; undulations, $F(2121.513, 3) = 10.3087$, $p < .001$; shaking, $F(177.651, 3) = 4.702$, $p = .005$; opening the gills, $F(36.775, 3) = 11.099$, p,< .001; arching, $F(25.397, 3) = 6.767$, $p < .001$; charging, $F(188.922, 3) = 5.827$, $p = .001$; and total activity, $F(10972.043, 3) = 3.268$, $p = .026$. The MANOVA of the components of the factor analysis revealed effects on all of the components (Component 1: $F(583.483, 3) = 3.738$, $p = .015$; Component 2: $F(4916.596, 3) = 9.623$, $p < .001$; Component 3: $F(13318.386, 3) = 4.907$, $p = .004$).

The post-hoc tests carried out on the ANOVA indicated a detoxification-dependent effect from acute poisoning. Table 3 shows the delimited statistical differences from the post-hoc test (Tukey's HSD) for the recorded variables.

Discussion

The present results appear to indicate, for acute poisoning, a detoxification-dependent effect from methylmercury only for the behavior of opening the gill covers. When the detoxification times are compared with each other for the other analyzed variables, we observe an effect from detoxification from the substance. Given that the central control of the gill cover opening is different from the rest of the behaviors (Gorlick, 1989), it is possible that methylmercury differentially affects the elements of the display. Additionally, with the dose studied, we did not observe effects on the horizontal and vertical display behaviors, even though this effect is observed when these behaviors are taken conjointly (molar analysis). These data present a more pronounced effect from methylmercury on Component 2 (the motility component). In fact, we observed a decrease in the detoxification-dependent decrease in motility (see Figure 2), less pronounced when we observed Component 3 (display proper). These data indicate a detoxification-dependent effect on motility and for emotional elements of aggression, with a greater effect on motility than on the emotional state.

Experiment 2: Effects of chronic poisoning on the display in *Betta splendens*

Method

Subjects

The subjects ($n = 10$) were subjected to the same conditions as those in Experiment 1. Upon completion of the period of acclimation and adaptation to the dietary regime, the the fish were submitted to definition of their base line, as in Experiment 1. After this phase, they were subjected to chronic, trophic poisoning by ingestion of salt-water artemias (*Arthemia salina*), previously contaminated hydrically with methylmercury at a dosage of 16μg/l, offering a dose of 1 g of artemia for each 30 g of fish on alternating days of collection, totaling 15 doses over a 35-day period. The subjects weighed 5.1±0.6 mg.

Data collection was alternated with the poisoning, so as to discriminate the effect of each dose cumulatively on the behavior of the fish. The subjects' baseline display was acquired through observation of agonistic interactions, in separate aquariums, in daily sessions lasting 5 minutes each. The same behaviors were observed as in Experiment 1. The interactions were recorded, and the behaviors were transcribed using EthoLog software (Ottoni, 2000). Furthermore, the duration of the entirety of the display was also analyzed.

Results

The same variables analyzed in Experiment 1 were analyzed with chronic poisoning. Figures 4 and 5 show the means (± standard deviation) of the frequency of the occurrence of the behaviors.

The statistical analysis of the entire display (two-way MANOVA, poisoning factor with inter-subject factor, sum of squares type III) revealed effects from the poisoning on the behaviors of floating, $F(35.33, 6) = 2.98$, $p = .008$; breathing, $F(226.74, 6) = 2.83$, $p = .011$; swimming with pectoral fins, $F(277.60, 6) = 2.51$, $p = .023$; undulations, $F(1351.352, 6) = 3.208$, $p = .005$; opening the gill cover, $F(1315.039, 6) = 2.138$, $p = .051$; vertical display, $F(932.745, 6) = 2.503$, $p = .023$; arching, $F(169.699, 6) = 11.075$, $p < .001$; and retreat, $F(83.951, 6) = 9.906$, $p < .001$.

The post-test (Tukey's HSD) revealed statistically significant differences among the doses, as shown in the matrix of differences (see Table 4).

Analysis of the total activity (one-way ANOVA, poisoning dose as inter-subject factor, sum of squares type III) did not show statistically significant effects, $F(7284, 6) = 1.79$, $p = .102$.

When the data were analyzed as a function of the behaviors clustered in components (see Figure 5), we observed the effects of the dose on the three components (Component 1: $F(4640.308, 6) = 4.148$, $p = .001$; Component 2: $F(6108.585, 6) = 3.390$, $p = .003$; Component 3: $F(2600.979, 6) = 2.711$, $p = .015$. The post-test (Tukey's HSD) revealed statistically significant differences among the doses.

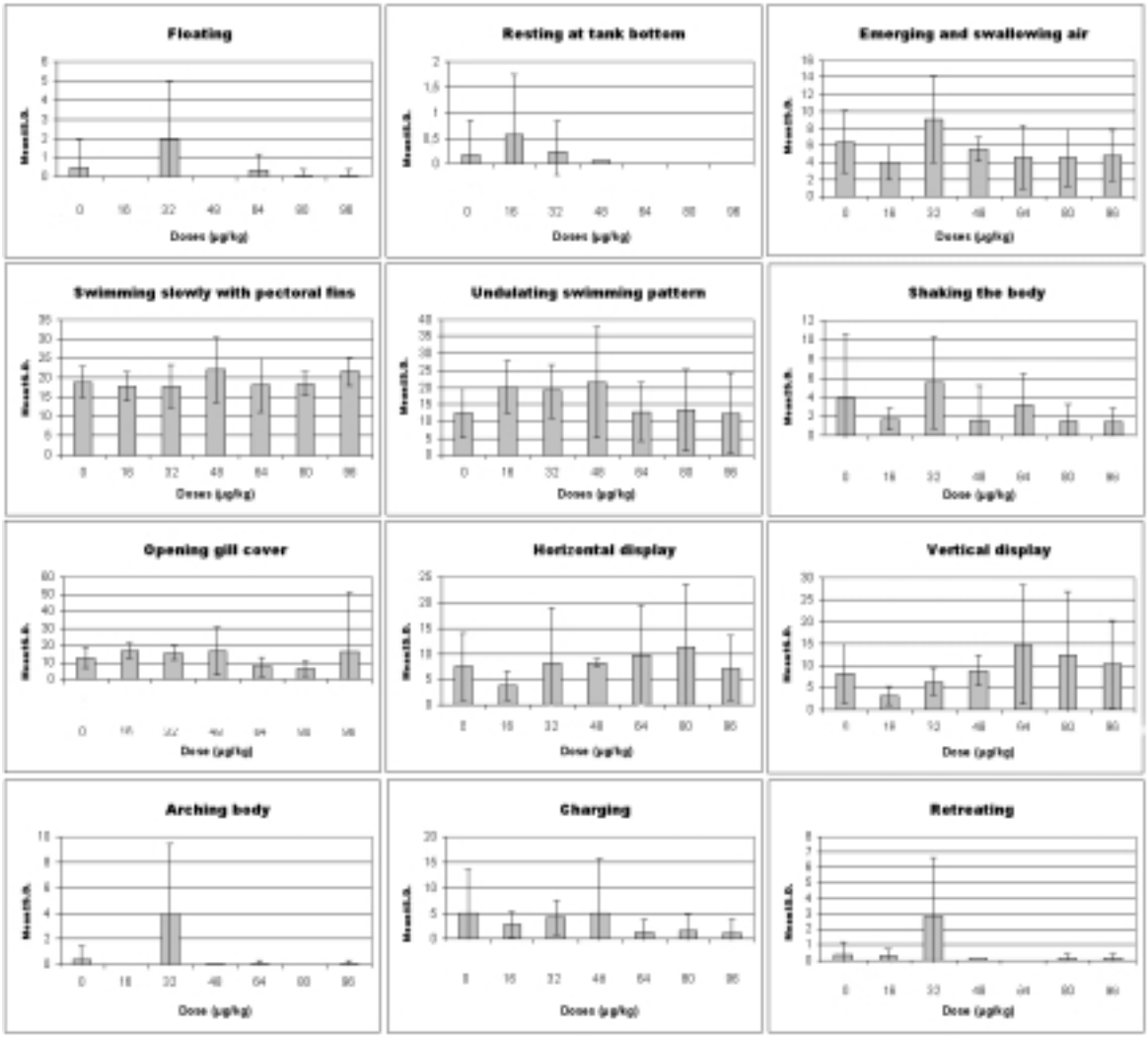

Figure 4. Frequencies (*M* ± *SD*) of the behaviors observed as a function of the dose applied in chronic poisoning. The behaviors are described in Table 1.

Table 4
Matrix of Differences among Doses, as a Function of Behavior

Dependent variable	Treatment	Treatment	Mean difference	Standard error	Significance
	0	32	–1.41	0.41	.01
	32	48	1.92	0.56	.01
Floating		80	1.84	0.56	.02
		96	1.84	0.56	.02
		32	–1.92	0.56	.01
Emerging and swallowing air	32	64	4.49	1.46	.03
		80	4.49	1.46	.03
Undulating swimming pattern	0	48	–8.94	2.52	.01
Vertical display	16	64	–11.75	3.94	.05
Retreating	16	32	–2.51	0.59	0

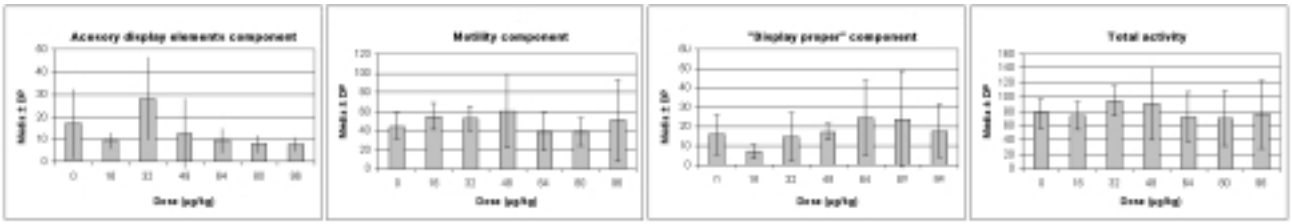

Figure 5. Frequencies (*M* ± *SD*) of the behaviors grouped by Components and by total activity in chronic poisoning, as a function of the dose used. The factor analysis is found in Table 2 and Figure 1.

Table 5
Matrix of Difference between Doses, as a Function of Behavior, Grouped in Components

	Dose (μg/kg)		Difference	Standard Error	*p*
Component 1	0	32	–11.04	3.97	.08
(Accessory display elements)	16	32	–18.42	5.47	.01
	32	48	–15.34	5.47	.07
		64	18.42	5.47	.01
		80	19.92	5.47	.00
		96	20.26	5.47	.00
Component 2 (Motility)	0	48	–16.44	5.22	.03
(Force)	48	64	22.17	7.07	.03
		80	22.17	7.07	.03
Component 3 (Display proper)	16	64	–17.42	5.16	.01
(Display "specifically")		80	–16.75	5.16	.02

Discussion

These results appear to indicate, for chronic poisoning, a detoxification-dependent effect from methylmercury on various emotional parameters in *Betta splendens*. The fact that only the motor elements exhibit statistically significant differences in relation to control shows that the effect of $HgCH_3$ (methylmercury), in these conditions, changes to a modulation of emotional elements, instead of, for example, motor, motivational, or cognitive elements. These results are compatible with the emotional effects of methylmercury on models of depression and anxiety in rats and fish, in other experiments conducted in our laboratory (unpublished data), and appear to emulate the emotional effect that is also found in poisoned human beings (cf. Hartman, 1995). The data also seem to indicate effects on the general tone of behavior, which may also be concurrent with metabolic alterations that are corollary to mercury poisoning (Castoldi, Coccini, Ceccatelli, & Manzo, 2001; Harris & Bodaly, 1998; Yasutake, Nakano, Miyamoto, & Eto, 1997), as well as the emotional effects. Some hypotheses may be proposed about the neural basis of such an effect. The neural basis of the components of aggression in *Betta splendens* may be dissociated with motor and motivational components (Gorlick, 1989; Marino-Netto & Sabbatini, 1983b), and, as judged by the effects of LSD on the display (Arbit, 1957)—as this drug exhibits a gross serotoninergic effect—it has a reasonable serotoninergic component (although further study is needed). As methylmercury appears to affect intracellular concentrations of calcium in neuroblastomas that express the $5\text{-}HT_3$ receptor (Hare & Atchison, 1995a) and the activity of monoamine-oxidase (Chakrabarti et al., 1998), it is possible that the emotional effect observed here, isolated from the motor effect, is due to these alterations in the serotoninergic dynamic. In zebra fish (*Danio rerio*), MAO expressed (Z-MAO) is sufficiently similar, in structural terms, to that encountered in mammals (Setini, Pierucci, Senatori, & Nicotra, 2005).

General Discussion

The present experiment sought to analyze the effects of acute and chronic mercury poisoning on aggressive behavior in *Betta splendens*. Unlike the effect observed in acute poisoning, we observed a more gradual and "fine" effect in chronic mercury poisoning. We observed a differential effect in various components of the display, of a dose-dependent

nature. Detoxification was not tested in chronic poisoning, as the behavioral tests were always conducted after 24 hours. On the other hand, acute intoxication shows a detoxification-dependent effect on more motor elements of the display. This result agrees with the literature (Royalty, Taylor, & Korol, 1987; Bernstssen et al., 2003; Daré et al., 2003; Roegge et al., 2004), and may indicate a dopaminergic modulation from methylmercury. In fact, we can find references in the literature to effects from dopaminergic drugs on aggression in the *Betta splendens* (Baenninger, 1968) and other teleosts (Munro, 1986). The dose and detoxification dependent effects of methylmercury on the behavioral categories analyzed may depend on the amount of time elapsed from the mercury poisoning, which alters specific systems differently in relation to the time (e.g., Belletti et al., 2002).

The most detailed analysis of the elements of the display, taken in ungrouped behaviors, allows observation of this differential effect of methylmercury on motility, on the one hand, and emotional behavior, on the other. When we analyze the topography of the response, instead of using a more global category, we see more clearly an emotional modulation of the agonistic behavior of the *Betta*. This effect of methylmercury corroborates other findings in our laboratory (unpublished data), where we could observe dose- and detoxification-dependent effects on the behavior of rats in the forced swim test (FST) and in the elevated plus-maze (see also Onishchenko et al., 2007), and of zebrafish in a dark/light preference model. These results reveal a possible effect on putative behavioral inhibition systems and behavioral approximation systems (e.g., McNaughton & Corr, 2004), connected to defense reactions in mammals, as these systems are controlled by monoaminergic systems. As the distribution of mono-amines in the central nervous system of fish is very similar to that observed in mammals (Adrio, Anadón, & Rodríguez-Moldes, 1999; Frankenhuis-van de Heuvel & Nieuwenhuys, 1984; Kapsimali, Vidal, Gonzalez, Dufour, & Vernier, 2000; Meek, Joosten, & Hafmans, 2004; Ritchie, Livingston, Hughes, McAdoo, & Leonard, 1983), indicating an evolutionary conservation of these systems, it is possible that the defense reactions are controlled by the same functions that appear in mammals. Thus, the emotional and motor alterations that were observed in this experiment indicate a possible effect of methylmercury on the biogenic amine systems that emotionally control defense behaviors.

In general, these results, along with the findings in the literature, present nested effects on various levels of the biological organization in fish (Weis, Smith, Zhou, Santiago-Bass, & Weis, 2001). At a neuro-chemical level, emotional effects can be interpreted from the viewpoint of the methylmercury effects on the monoamine oxidase system (Chakrabarti et al., 1998). In fact, whereas MAO-A has an effect on the serotoninergic system, MAO-A, as much as MAO-B, affect the central dopaminergic system (Westlund, Denney, Kochersperger, Rose, & Abell, 1985). Thus, alteration of the function of the MAO system could affect emotional and motor components of behavior. Given that the monoamine oxidase is also found in astroglia, where it primarily has an effect on the metabolism of serotonin (Fitzgerald, Kaplinsky, & Kimelberg, 1990), it is possible that the alteration of the monoamine oxidase system occurs in both types of cells, thus connecting the two neurotoxicological effects discussed in the literature. Meanwhile, the references in the literature about monoaminergic modulation of the display of the *Betta splendens* are different (cf. Arbit, 1957; Baenninger, 1968). All in all, it is likely that the behavioral effects, both motor and emotional, are due to an effect on the monoaminergic system of the poisoned subjects. As the display is essential for the behavioral ecology of the *Betta splendens* (Bronstein, 1980, 1981b), these behavioral alterations, at an organic level, can compromise the fitness of entire populations of contaminated animals. Given that methylmercury is bioaccumulative, this effect may also reverberate throughout the food chain.

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