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Bioaccumulation of Mercury by Tucunaré (*Cichla ocellaris*) from Tapajós River Region, Brazilian Amazon: A Field Dose-Response Approach

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Studies on the mercury (Hg) concentrations of the fish fauna and their potential impact to humans have been performed in a contaminated and a background area in the Tapajós river region. Due to Hg concentrations in the fish, and above all, the high rate of fish consumption by the local population (from 0.2 to 0.4 $Kg.day^{-1}$), the results pointed out to a potential health risk in both areas (Bidone et al, 1997; Castilhos et al, 1998). The transference factors of Hg through the food-chain involving amazonian ichtyofauna suggested that biomagnification may occur in both the contaminated and background areas (Castilhos & Bidone, 2000).

A lot of factors have been considered important in the bioaccumulation and/or biomagnification of Hg in fish. Among them, the Hg load-dependent factors in the aquatic environment, specially in those related to Hg in sediments and environmental conditions, like bioproduction (Häkanson, 1980; 1991); as well as local biota's physiological-dependent factors, like size, length, age and metabolic rate (Phillips, 1980; WHO, 1990); and also food-chain characteristics (Cabana et al, 1994). Hg levels in fish for spacial and/or temporal comparisons have been normalized by mean of Hg content in 1-Kg fish (as pike) (Johnels et al., 1967; Häkanson, 1991), or by using only fish of one year of age (Post et al, 1996), or by using a specific length (Scruton, et al., 1994), or by using a specific weight (Watras et al., 1998). We suggest using the length to infer upon the time of exposure of fish to Hg, which could be considered as indirect dose-normalizer. This assumes that the length increases along the time (hormonally controlled) (Zaret, 1980).

The Tucunaré (*Cichla ocellaris*) specie was chosen for many reasons. At the moment, there are few toxicokinetics studies from field or laboratory-controlled conditions about Hg in *Cichla ocellaris*, but this specie may be considered good bioindicator of Hg accumulation in the Amazonian ecosystem, specially because of its time-integration capacity. According to the reproductive strategies, carnivorous icthyophagous *Cichla ocellaris* could be classified as "in equilibrium" (Winemiller, 1989 cited in Ruffino & Isaac, 1995). The fish considered "in equilibrium" are the most sedentary and present a territorial behavior. Their density does not change strongly during the year. Spawning season is long and it is not necessarily at the beginning of the flood time. Their preferred habitat is lentic (slow moving) water. The influence of amazonian seasonality (well

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characterized by two hydrological periods: a low waterlevel and a high waterlevel period) on Hg accumulation in fish was studied and the results showed that carnivorous fish, including *Cichla ocellaris*, were not affected by seasonality Castilhos, 1999). Their fine taste and abundance in native habitat have made it an important commercial specie (Ruffino & Isaac, 1995).

The objectives of this work are : (i) to estimate the daily Hg uptake rate by *Cichla ocellaris* during growth up to the attainance of its maximum length; (ii) to establish and compare the dose-response relationship for Hg accumulation by *Cichla ocellaris* for a contaminated and a background area, and (iii) estimate the potential time of exposure necessary for Hg accumulation to reach $0.5\mu g.g^{-1}$, the concentration limit for human consumption adopted by many countries.

MATERIALS AND METHODS

Fish samples were collected from two areas, at the contaminated gold mining area and at a background area. The contaminated area is located in the Tapajós river between the cities of Jacareacanga and Itaituba (04°15'23"S-55°54'33"W), where the gold mining sites are distributed alongside the tributaries of the Tapajós river. Several authors have shown this area to be strongly contaminated by Hg from gold mining (Akagi, et al., 1994; Bidone et al., 1997). The mean annual temperature is 25 °C without great fluctuations. The background site is located in a fluvial lacustrine system near Santarém city (02°25'11"S-54°42'16"W), more than 250 Km downstream from the contaminated site. It is not as contaminated as the site influenced by the goldmining, but has the same basic environmental characteristics.

We sampled and analyzed 69 specimens of *Cichla ocellaris*; 41 specimens (ΔLt= 245-580mm) from the contaminated site and 28 specimens (ΔLt= 200-660mm) from the background site. Each specimen was weighed (Wt), and its length (Lt) was measured at the time of collection. The samples were put in polyethylene bags and frozen. Hg was analyzed in the fish muscle through Atomic Absorption Spectrophotometer (A-G/VARIAN MODEL) using a Vapor Generation Accessory-VGA (CVAAS). The samples were digested in sulfuric-nitric acid solution in the presence of vanadium pentoxide 0.1%; the oxidation completed by adding potassium permanganate 6% until the fixation of the violet color. Immediately before the determination, the excess of permanganate was reduced with hydroxylamine 50% (Campos, 1990). Reference standard IAEA-fish muscle tissue with a certified Hg concentration of $0.74\pm0.13\ \mu g.g^{-1}$ were also analyzed, giving a value of $0.73\pm0.08\ \mu g.g^{-1}$ (n=4).

The growth of fish is considered as an interaction between the specimen and the environment. Growth has many aspects. Growth in relation to age can be described by entirely empirical mathematical equations (From and Rasmussen, 1989). The relationship between length and age of fish can be expressed by the von Bertalanffy (1957) mathematical equation: $L_t = L_\infty\ [1-e^{-k\Delta T}]$, where, L_t = specific length taken into account; L_∞= asymptotic length or maximum length; k = correlation parameter ($year^{-1}$) or growth rate, ΔT = period of time necessary for the fish to attain the considered length; $\Delta T = t - t_0$; t = estimated age for considered length; to = the hypothetical age at which the fish is on the zero length.

If von Bertalanffy's equation is used, one could assume that there is a direct relationship between the weight and the cubic length. In other words, one will have to fix the value 3 for the angular regression coefficient (*b*), which correlates weight and length parameters. The value *b* comes from the growth equation W = aL^b where: Wt is weight; Lt is length and a and *b* are constants of the growth which directly express the relationship between log weight and log length (Le Cren, 1951). We found 2.9 for *Cichla ocellaris* from studied areas. This result has no difference from value 3 (Student's t-test; Zar, 1999). Consequently, *Cichla ocellaris* showed a relationship between weight and length in accordance with von Bertalanffy equation.

RESULTS AND DISCUSSION

The elimination of MeHg by fish is very slow relative to the uptake and the accumulation rates. Positive correlation between concentrations in muscle and both size, length and/or age for a given specie have been well documented (WHO, 1990). In this study significant linear correlation (Pearson's correlation) between Hg levels in muscles and length (0.67; $p<0.0001$; n=28) and weight (0,9; $p<0.006$; n=10) was found for *Cichla ocellaris* in the background area. The correlations for *Cichla ocellaris* from the contaminated area were not significant.

The asymptotic length of *Cichla ocellaris* specimens has been estimated at 710 mm (L_∞) (Ruffino & Isaac, 1995) and the time necessary for the fish to attain 50% of its maximum length is around one year (Fontenele, 1950 cited in Ruffino & Isaac, 1995; Zaret, 1980). Thus, following the Bertalanffy equation, the fish growth rates (k) corresponds to 0.6 $year^{-1}$. At one year of age, the males and females of *Cichla ocellaris* have no differences in growth rates; they are at their first maturity stage. After the fish is mature, size differences change considerably, with the male increasing relatively more than the female, which presumably converts food resources to egg production (Zaret, 1980). For this work, the collected specimens were not segregated as males or females.

The period of time necessary for the fish to attain 50% of its L_∞ can provide inference about the potential exposure time for Hg present in the environment. The estimated fish growth rate for *Cichla ocellaris*, can also be used to correlate potential ages to different intervals of length, i.e., different percentages of the asymptotic length (10%, 25%, 50%, 75%). These estimated ages were used in order to infer the equivalent potential time of exposure. Little information has been gathered on *Cichla* in their native ecosystems, including Tapajós River Basin. In South America, Jepsen et al. (1999) studied otolith macrostructure and field data to estimate age and growth in several species of *Cichla* and compare these estimatives with populations from different habitats. The hypothesis that fish from different systems would have different body condition and growth rates was not supported. Then, in the present work, it was assumed that fish growth is the same both in the contaminated area and in the reference site.

A linear relationship was observed between the period of the *Cichla ocellaris* time exposure related to $\%L_\infty$ and their Hg tissue levels for the background and contaminated areas are showed in Figure 1.

By comparing the daily uptake rates by *Cichla ocellaris,* which could be defined by the slope of straight lines resulting from linear relationship in Figure 1, one can conclude that the daily uptake rate of Hg by *Cichla ocellaris* from the contaminated area (0,19μg.Kg^{-1}.day^{-1}) is about 2 times higher than that from the background area (0,08 μg.Kg^{-1}.day^{-1}). It is notable that the slopes relating mercury concentration to age in fish, increase in a declining slope and increase in the degree of contamination, i.e., age dependence is more important in the more contaminated samples (Phillips, 1980). In addition, the initial Hg concentration, defined as the intersection points by straight line on the y-axis are almost one order of magnitude higher in the contaminated area (~300μg.Kg^{-1}) than for the background area (~50μg.Kg^{-1}). Therefore, the analysis of covariance, ANCOVA (Zar, 1999) showed that *Cichla ocellaris* from contaminated area had similar daily uptake rates but higher intercept ($p<0,05$) than those from background area. The estimated exposure time necessary for fish to accumulate 0.5μg.g^{-1} was calculated by using the straight line equations showed in the Figure 1. The results suggest that *Cichla ocellaris* from the contaminated area could attain this level in 2.5 years of exposure, whereas those from the background area could attain the same level after 14 years.

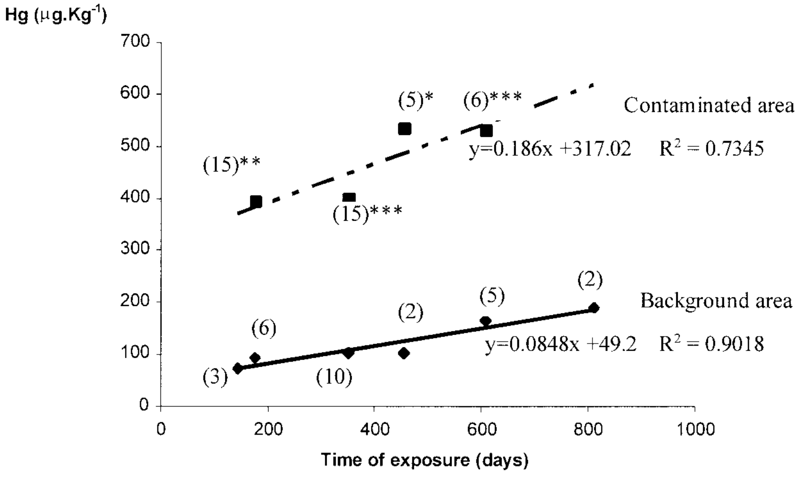

Figure 1. Linear relationship between Hg concentration and time of exposure estimated for *Cichla ocellaris* from background and contaminated areas in the Tapajós River Region; (n) is the correspondent number of specimens; and *$p<0.05$, **$p<0.005$ and ***$p<0.001$ (Mann-Whitney) are significant differences between areas.

If the individual variability, which is generally shown by fish toxic substances accumulation rates, is considered, those estimated differences would seem overpredicted or underpredicted. The dose-response relationship has the competence to absorb such individual variabilities. Responses are of two kinds: quantal and quantitative. In a quantal test, an organism either shows the response under study or does not show it. Thus, a certain percentage of test organisms will show the response within some stated conditions. In a quantitative or graded test, each organism responds to a variable degree. Quantal test are designed to estimate the concentration of a test material that affects 50% of the test organisms, the median effective dose (ED 50% or ED_{50}). One must choose the effect to be observed. The chosen effect was the Hg accumulated in muscles of *Cichla ocellaris* at least 100μg.Kg^{-1} for the background area and 300μg.Kg^{-1} for contaminated area. Then, this is a quantal rather than a graded response, since the specific effect is either present or absent. The D_{50} for accumulation of Hg by *Cichla ocellaris* (AD_{50}) indicate the time of exposure necessary to attain those tissue concentration levels by half of the exposed individuals.

Some methods are used to calculate D_{50}. Among them, there is a probit method (American Public Health Association, 1985; Ross & Gilman, 1985). The potential times of exposure were transformed in their logarithms and the frequency of responses were transformed in probit units. The results showed that half of *Cichla ocellaris* individuals exposed to a potential AD_{50} in the background area would accumulate at least 100μg.Kg^{-1}, whereas in the contaminated area, the expected response would be 300μg.Kg^{-1}. The graphic representation of the dose-response relationship for Hg accumulation by *Cichla ocellaris* from the contaminated area is shown in Figure 2. The number of specimens are the same showed in Figure 1.

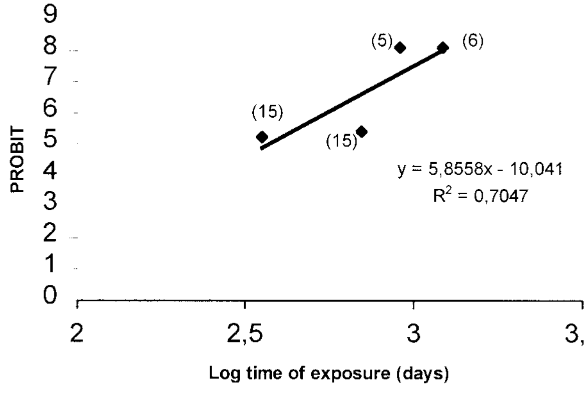

Figure 2. Graphic representation of dose-response relationship between Hg accumulation by *Cichla ocellaris* from the contaminated area; (n) is the correspondent number of specimens.

The determination of AD_{50} for each area estimates that close to 1.0 years of exposure, half of *Cichla ocellaris* exposed to a potential dose in the contaminated area could accumulate at least 300μg.Kg^{-1}. Whereas in the background area, half of *Cichla ocellaris* exposed to a potential dose in the background area could accumulate at least 100μg.Kg^{-1} after 1.3 years of exposure ($y = 4.5x-7.08$; $R^2 = 0.66$).

Regardless of the large uncertainties always present in estimates of fish age from its length, the specie-pollutant pairing is unique in terms of pollutant kinetics or flux. If we accept that exposure and response can be interrelated as: $t_{exposure} * C$ = constant (adapted from Dämgen & Grünhage, 1998); in which a certain response (constant) can be achieved from a time of exposure $t_{exposure}$ and a concentration of aquatic environment C; these concentrations will result as a potential dose: (i)for contaminated area: 370 (days) * C = 300μg.Kg^{-1} ; C ≅ 0.8μg.Kg^{-1}.day^{-1} and, (ii) for background area: 479 (days) * C = 100μg.Kg^{-1} ; C ≅

$0.2\mu g.Kg^{-1}.day^{-1}$. These Hg doses must be assumed that they come from both sources, by water and food. Considering previous results, *Cichla ocellaris*, from contaminated area takes 1.7 years to achieve $0.5\mu g.g^{-1}$, whereas from background area, takes close to 6.5 years. The predictions derived from dose-response associated with exposure-response are less overestimated than those derived from simple and direct relationship (Figure 1), but the magnitude of the increase of Hg dose, assumed as $\mu g.Kg^{-1}.day^{-1}$, in contaminated area compared with background area turns out higher than predicted by the slopes of linear relation showed in Figure 1. We believe that the difference between daily doses (~4.0 times), is significant and could be attributed to Hg load differences between the studied areas, and also, could be a consequence of a potential pollution source, the goldmining activity. If the fish contamination by Hg can be considered a risk, the risk-benefit analysis of goldmining activity will take into account the increase of the Hg dose to fish in the contaminated area relatively to the background area.

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