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Mercury Accumulation in a Brazilian Reservoir in Relation to Carbon Sources and Ecological Characteristics of the Fish Community



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Abstract

It has frequently been reported from temperate areas that concentrations of mercury in fish increase significantly, often by three to four times, in recently constructed hydroelectric reservoirs. The objective of this study was to determine the concentrations of mercury in fish in a tropical hydroelectric reservoir in the central-western parts of Brazil and relate these concentrations to carbon sources and ecological characteristics of the fish community. The mercury concentration varied widely between species and showed a trend that depended on diet and trophic position in the food web. A piscivorous species, *Salminus brasiliensis*, showed the highest mean mercury concentration, 1.50 µg/g wet weight, while an omnivorous species, *Brycon hilarii*, demonstrated the lowest mean concentration, 0.40 µg/g wet weight. The degree of mercury accumulation in fish was shown by means of a bioconcentration factor (concentration in biota/concentration in water) which ranged between 4.08 and 5.93 log units with an individual of the species *Brycon hilarii* exhibiting the lowest value and an individual of the species *Salminus brasiliensis* demonstrating the highest value. The within species concentration of mercury also varied widely and could be explained by differences in size for one species, *Serrasalmus marginatus*, which had a positive correlation between mercury concentration and length/weight. No relationship was found for the other species, which could be due to inadequate sampling sizes and length/weight ranges. The groups of primary producers contributing energy to the fish food web was determined by stable carbon isotope analysis ($\delta^{13}\text{C}$). The most important carbon sources in the reservoir seemed to be terrestrial C_3 plants, aquatic macrophytes and phytoplankton. The relationship between mercury concentration and carbon source was examined and found to be correlated, but the mercury concentration in different species of fish is probably better explained by differences in trophic position.

Resumo

Tem sido frequentemente publicado, que as concentrações de mercúrio em peixes de áreas temperadas aumentam significativamente de três a quatro vezes em barragens hidroelétricas recentemente construídas. O objetivo deste estudo foi a determinar a concentração de mercúrio em pescado num reservatório hidroelétrico tropical na região Centro-Oeste do Brasil e correlacionar aquelas concentrações às fontes de carbono e às características ecológicas da comunidade de peixe. A concentração de mercúrio variou muito entre espécies e mostrou uma inclinação dependente da dieta e posição trófica na cadeia alimentar. A espécie piscívora, *Salminus brasiliensis*, apresentou a maior concentração média de mercúrio, 1,50 µg/g por peso úmido, enquanto que a espécie onívora, *Brycon hilarii*, apresentou a menor concentração média, 0,40 µg/g por peso úmido. O grau de acumulação de mercúrio nos peixes foi mostrado por meio de um fator de bioconcentração (concentração na biota/concentração na água) que variou entre 4,08 e 5,93 unidades logarítmicas com relação aos indivíduos da espécie *Brycon hilarii*, que exibiu um valor médio menor e com os indivíduos da espécie *Salminus brasiliensis*, que apresentou o valor médio mais elevado. A concentração de mercúrio dentro da espécie também variou muito e foi possível explicar pela diferenças do tamanho para a espécie *Serrasalmus marginatus*, que teve correlação positiva significante entre concentração de mercúrio e comprimento/peso. Nenhuma relação foi encontrada para as outras espécies, o que pode ser devido aos numeros inadequados das amostras e da variação limitada do comprimento/peso. Os grupos de produtores primários contribuindo de energia para o cadeia alimentar dos peixes foi determinado pela análise do isótopo estável de carbono ($\delta^{13}\text{C}$). As fontes de carbono mais importantes do reservatório pareceram ser plantas terrestres do tipo C_3 , macrófitas aquáticas e fitoplâncton. A relação entre a concentração de mercúrio e a fonte de carbono foi examinada e demonstrou ser significante, mas a concentração de mercúrio nas diferentes espécies de peixe provavelmente explica-se melhor pelas diferenças na posição trófica.

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Introduction

Mercury in the Environment

Mercury (Hg) and mercurous compounds such as methylmercury (MeHg) are highly toxic and persistent pollutants (UNEP, 2002). Studies from various geographic areas indicate that a significant portion of humans throughout the world are exposed to levels of concern. Mercury has repeatedly been shown to cause severe neurological and behavioural disorders, especially in children exposed during pregnancy as fetuses (UNEP, 2002). Most people are primarily exposed through the diet, above all through consumption of fish which is an extremely valuable component of the human diet in many parts of the world. In 1991, a joint committee from FAO (The Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) revised guideline levels for mercury in fish aimed at human consumption. The recommended dietary limit was set at 0.5 µg MeHg/g wet weight for non-predatory fish and 1.0 µg MeHg/g wet weight for predatory fish (FAO/WHO, 1991). In the last decades, numerous studies have shown that a relationship exists between elevated levels of mercury in fish and inundation of land due to construction of hydroelectric power plants. Mercury concentrations have been reported to increase significantly, often by three to four times, in newly formed hydroelectric reservoirs (Bodaly *et al.*, 1984; Verdon *et al.*, 1991). In many cases, the recommended dietary limit has been exceeded.

In aquatic systems, mercury is converted to methylmercury which is taken up by biota and accumulated in the food chain, sometimes to levels that are many thousands of times greater than levels in the surrounding water (UNEP, 2002). A range of factors influence the bioavailability as well as the bioaccumulation of mercury in freshwater biota. Mercury bioavailability is determined by abiotic factors and often increased at low pH, high concentration of organic matter or low O₂ saturation (reviewed by Verta, 1984 & Tropp, 2000). Mercury bioaccumulation is influenced by physiological factors such as sex, age, size, growth rate or metabolic rate (Huckabee *et al.*, 1979) and ecological factors such as trophic position or food chain length (Kidd *et al.*, 1995; Cabana *et al.*, 1994).

Stable Isotope Analysis

Fish take up mercury primarily through the diet (Hall *et al.*, 1997). Information on diet and feeding interactions is therefore of utmost importance when assessing observed levels of mercury. Analyses of the relationship between the stable carbon isotopes ¹³C and ¹²C (δ¹³C) have been widely used to identify the primary producers that support production at higher trophic levels and to trace the flow of carbon in aquatic food webs (Forsberg *et al.*, 1993; Hecky & Hesslein, 1995; Hobson & Welch, 1992). The stable carbon isotopic composition of a primary producer is determined during photosynthesis. The δ¹³C-value of a primary producer remains relatively unchanged when passed on by consumers through the food chain, which means that the δ¹³C-value of an organism corresponds to the value of its major food source. The ratio only increases about 0.2 to 1.0‰ for each trophic transfer (DeNiro & Epstein, 1978; Peterson & Fry, 1987). The δ¹³C-value of a consumer reflects relatively long-term feeding as well as assimilated and not just ingested food (Peterson & Fry, 1987). Even the relative amounts of different food sources assimilated by a consumer may be estimated if the ¹³C/¹²C ratios of the food sources differentiate sufficiently (DeNiro & Epstein, 1978).

The variability in δ¹³C-values between different terrestrial primary producers is above all decided by the type of carboxylating enzyme involved in CO₂ fixation. Primary producers

fixing carbon dioxide through the Calvin cycle differ in $^{13}\text{C}/^{12}\text{C}$ ratios from primary producers incorporating carbon dioxide through the C_4 -dicarboxylic acid pathway (reviewed by O'Leary, 1981). Plants using the C_4 -dicarboxylic acid pathway are called C_4 plants whereas plants incorporating CO_2 through the Calvin cycle are called C_3 plants. The $^{13}\text{C}/^{12}\text{C}$ ratios of primary producers in freshwater environments such as periphyton (attached algae), phytoplankton and aquatic macrophytes are primarily determined by the $\delta^{13}\text{C}$ -values of the dissolved inorganic carbon present in the water, which can vary significantly contrary to the stable carbon isotopic composition of atmospheric CO_2 used by land plants (reviewed by Rounick & Winterbourn, 1986). (The $\delta^{13}\text{C}$ -intervals exhibited by different types of primary producers are presented in connection with the results of the stable isotope analysis to facilitate comparison.)

Aim of the Study

The objective of this study is to determine the degree of mercury accumulation in the fish community in a tropical hydroelectric reservoir and identify the groups of primary producers contributing energy to the fish food web. The effects of physiological and ecological factors on the accumulation of mercury by fish will be examined, with focus on possible causes of both between and within species variations. This research paper is part of a greater project with the aim to increase the knowledge of processes affecting the accumulation of mercury in fish communities in tropical hydroelectric reservoirs.

Background

Seventy kilometres northeast of Cuiabá, the capital of Mato Grosso state in the central-western parts of Brazil, a hydroelectric plant reservoir was completed in 1999 by the company AMP-Manso (Aproveitamento Multiplo de Manso). The hydroelectric reservoir, called Lago Manso, is supplied by two large rivers, Rio Manso and Rio Casca, which join prior to the dam. The reservoir covers an area of 427 km² and has a maximal depth of about 80 metres.

The increase in mercury concentrations in fish in newly constructed hydroelectric reservoirs has mainly been studied in temperate areas and surveys from tropical regions are limited. Studies from temperate areas have shown that both predatory and non-predatory fish react to impoundment with increased levels of mercury within two to three years (Bodaly *et al.*, 1984). Mercury concentrations in non-predatory species peak after two to five years whereas levels in predatory fish keep rising for five to nine years after impoundment (Bodaly *et al.*, 1984; Verdon *et al.*, 1991). Verdon *et al.* (1991) reviewed data from dams constructed 6-67 years ago and concluded that it could take 20-30 years before mercury concentrations in fish return to background levels observed before impoundment. The duration as well as the degree of increase in mercury concentrations is difficult to predict due to differences in the relative importance of controlling factors between different types of ecosystems and regions. Elevated mercury levels in fish in newly constructed reservoirs are believed to be caused by an increase in the mobility and bioavailability of mercury. Decomposition of newly inundated vegetation and the resulting increase in organic matter favour bacterial methylation of mercury, particularly in hypoxic waters (see Tropp 2000 and references therein). In a typical freshwater lake, more than 90% of the mercury that can be converted to methylmercury is located in the upper most five cm of bottom sediments (Verta, 1984). From the sediments, methylmercury may be transferred to the water column directly by diffusion or indirectly via trophic relationships by benthic organisms.

Two studies have previously been performed in the area, which has no known sources of mercury pollution. The first study in 1999 investigated background levels of total mercury in soil, sediment, and fish in Rio Manso and Rio Casca before completion of the reservoir. Concentrations in soil and sediment of aluminium, iron, manganese, carbon, nitrogen and sulphur were also determined (Tropp, 2000; Wolpher, 2000). The second study in 2002 continued to monitor levels of total mercury in soil, sediment and fish in the reservoir (Gröhn & Vikström, 2003). Fish caught in Rio Manso and Rio Casca before flooding the area had total mercury levels ranging between 0.073 and 0.756 µg/g wet weight with an average of 0.237 µg/g (Tropp, 2000; Wolpher, 2000). All the species examined were either carnivorous or piscivorous. Three years thereafter, fish caught in the reservoir had total mercury concentrations varying between 0.088 and 0.938 µg/g wet weight (Gröhn & Vikström, 2003). The species with the lowest concentration was an omnivore while the species with the highest concentration was a piscivore. Based on the results from these previous studies, Hylander *et al.* (2005) concluded that mercury levels in fish have increased since inundation. The results suggest that the bioavailability of mercury has increased significantly after impoundment, which is probably due to increased concentrations of dissolved organic carbon as well as reduced concentrations of dissolved oxygen.

Methods and materials

Study Area

The climate of the area is tropical with two distinct seasons, dry during April to August and hot and rainy during September to March (Hylander *et al.*, 2005). The landscape surrounding the reservoir is dominated by sandy hills covered by bush vegetation and some higher trees. Cattle-ranching and small-scale farming is common in the area.

The pH in the reservoir ranges between 6.9 and 7.5 while the pH downstream is 6.7 (Tuomola, 2005). The amount of dissolved organic carbon (DOC) in the reservoir varies between 2.3 and 6.2 mg/L (Tuomola, 2005), with most of the values centred on ~2.7 mg/L. The amount of DOC downstream is 2.9 mg/L. There is no clearly defined littoral zone due to the water level changes caused by the hydroelectric power plant. No emergent, floating-leaved or submerged macrophytes were found and just one species of a freely floating macrophyte was discovered (*Salvinia auriculata* Aublet). There are, however, some terrestrial species tolerant to flooding in the margins of the reservoir as well as many dead trees standing partially or totally submerged in the water.

Sample Collection

The field work was conducted during April and May 2005 in Lago Manso and downstream in Rio Manso. Terrestrial and aquatic plants were gathered in the dam and along the dam margins while leaves and seed cases from trees were collected in the flooded forest areas. All plant parts were stored in separate plastic bags. Periphyton was scraped off from submerged rocks and wood in the dam margins and kept in plastic jars. Plankton was collected in the pelagic zone with a 20 µm phytoplankton net and stored in plastic bottles. All samples containing algae were kept cold and out of direct sunlight.

The terrestrial and aquatic plants were identified by staff at EMBRAPA Pantanal and at the Central Herbarium, Universidade Federal de Mato Grosso (UFMT). The periphyton or phytoplankton was not investigated taxonomically. The collected species of plants included

two grasses *Brachiaria humidicola* (Rendle) Schweick and *Panicum maximum* Jacquin, one herb *Conocloa scoparioides* Benth, one freely floating aquatic plant *Salvinia auriculata* Aublet, and one unidentified tree from the genus *Mimosa*. *B. humidicola* is a stoloniferous perennial, which form dense, somewhat woody stands in valley grasslands in moist environments (FAO, 2005). *P. maximum* is a tufted perennial, which naturally inhabits moist grasslands and open woodlands (FAO, 2005). Both species come from Tropical Africa and are used for grazing in Brazil. The species *C. scoparioides* is a native erect herb growing in seasonally inundated areas (Maia *et al.*, 2000). *S. auriculata* is an aquatic fern, which flourishes in slow-moving nutrient-rich freshwater in tropical, sub-tropical or warm temperate areas of the world (Global Invasive Species Database, 2005).

Fish were caught, between the end of April and the beginning of May, 2005, in the reservoir close to sampling sites three and four (Figure 1) and downstream in Rio Manso by local fishermen. A total of 44 fish were captured, 21 in the dam and 23 downstream, and stored frozen in plastic bags. The fish were weighed (kg), measured for length (cm), sexed and identified by staff from Departamento de Botânica e Ecologia, UFMT. The fish species collected comprised two omnivores *Brycon hilarii* (Valenciennes 1850, locally named piraputanga) and *Piaractus mesopotamicus* (Holmberg 1887, locally named pacú), two carnivores *Serrasalmus spilopleura* (Kner 1858, locally named piranha) and *Serrasalmus marginatus* (Valenciennes 1836, locally named piranha) and three piscivorous species *Salminus brasiliensis* (Cuvier 1816, locally named dourado), *Pseudoplatystoma fasciatum* (Linnaeus 1766, locally named cachara) and *Pseudoplatystoma corruscans* (Spix & Agassiz 1829, locally named pintado).

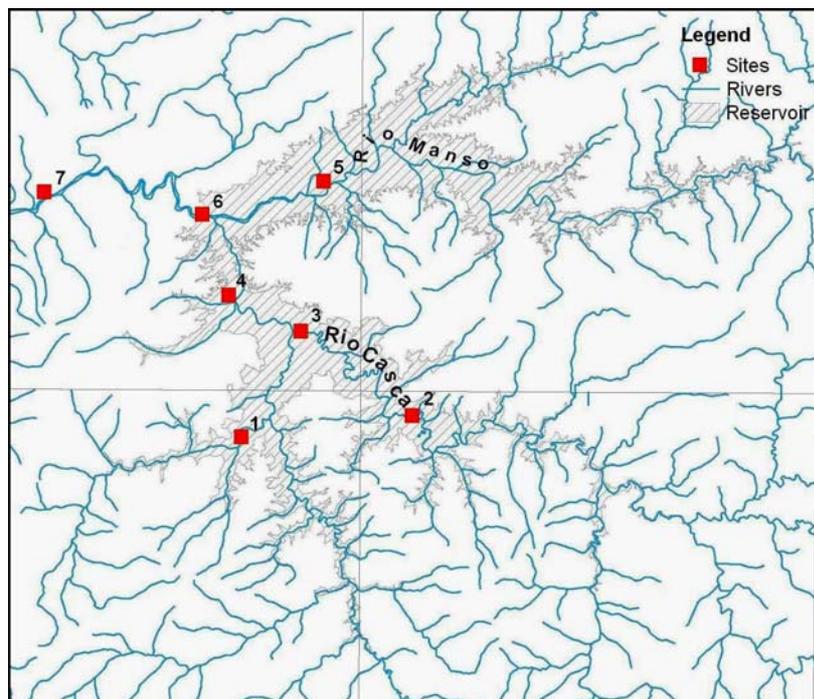


Figure 1. A map of the reservoir and the location of the different sampling sites. Number six is situated next to the hydroelectric power plant and number seven is located downstream in Rio Manso. (Published with the permission of Tuomola.)

Analysis of Total Mercury in Fish

Fish was kept frozen until preparation, when a sample of skinless fillet from the dorsal muscle tissue was cut off and chopped into small pieces with a scalpel. Following homogenization, samples were frozen until digestion. Three replicates of every sample, consisting of 2 g each, were digested in Erlenmeyer flasks. The samples were being kept in an ice bath during the whole preparation procedure unless otherwise stated. First of all, 2 mL of concentrated hydrogen peroxide (H_2O_2) was added in two applications (1+1 mL), after which the samples were put on ice for ten minutes. For further digestion, 15 mL of concentrated sulphuric acid (H_2SO_4) was added gently in two treatments (5+10 mL) followed by thirty minutes' incubation in a water bath at 60°C. When all tissue were dissolved, 5+10 mL of 5 weight % of potassium permanganate (KMnO_4) was added, after which the samples were kept on ice for sixty minutes. Next, the samples were treated with 40 mL of 5 weight % of potassium persulphate ($\text{K}_2\text{S}_2\text{O}_8$). After that, the samples were stored in a refrigerator, for no more than 72 hours, until analysed. Prior to analysis, approximately 1-2 mL of 12% hydroxyl ammonium chloride (HONH_3Cl) was added drop by drop until transparency of the sample was achieved, indicating that excess oxidizing agents had been reduced.

Total mercury concentrations were determined by cold vapour atomic absorption spectroscopy (CV-AAS) at Departamento de Química, UFMT. Ten selected samples were also run for comparison at Laboratório Radioisótopos, Universidade Federal do Rio de Janeiro (UFRJ). At both laboratories, the analyses were quality assured by analytical blanks and triplicated analyses of each fish in all sample batches, and by an internal reference sample from Laboratório Radioisótopos (AFPX-5130, 13.8 $\mu\text{g Hg/g}$ dry weight). The analyses run at Laboratório Radioisótopos were also verified by DORM-2 (4.64 \pm 0.26 $\mu\text{g Hg/g}$ dry weight), which is a certified reference material from the Institute for Environmental Chemistry, Ottawa, Canada. The two laboratories showed good consistency (coeff. = 0.92; intercept = 0; $r^2 = 0.98$; $p < 0.01$), except for three values regarded as outliers.

Analysis of nine samples of the internal reference at Departamento de Química resulted in a mean value showing 110.56% consistency with the established value, while the two references run at Laboratório Radioisótopos showed on average 93.92% consistency with the certified and established values. All analytical results were adjusted according to the error of percentage of the certified/established value. The samples run at Departamento de Química were adjusted by decreasing the obtained values of the samples with the percentage of error of the reference sample in that particular sample batch. The results of the analysis performed at Laboratório Radioisótopos were corrected by increasing the obtained values of the samples with the mean percentage of error of the internal reference sample and DORM-2. Total mercury concentrations given in this report were blank corrected, as well. All mercury concentrations are expressed as $\mu\text{g/g}$ wet weight. Methylmercury is the dominant mercury species in fish, but since approximately 95% of the mercury in fish muscle is present as monomethylmercury, it makes little difference whether total mercury or methylmercury is measured (Bloom, 1992).

Bioconcentration Factors

Bioconcentration factors (BCF) are a measure of the relationship between the mean mercury concentration in a particular biota and the concentration in water. The total concentration of mercury in water was determined in samples collected 20 cm beneath the surface, close to sampling site six in the reservoir (Figure 1). The samples were stored in scintillation bottles in a refrigerator at about 4°C until analysed. Prior to analysis, hydrochloric acid (HCl) was

added for conservation. The concentration of total mercury was determined in approximately 33 mL of water by oxidizing all mercury compounds to Hg(II) using bromine monochloride (BrCl), followed by reducing Hg(II) to Hg⁰ with stannous chloride (SnCl₂). The Hg⁰ was separated from the solution onto a gold amalgamation trap by flushing with N₂ and then conveyed to another gold-trap by means of thermal desorption followed by cold-vapor atomic fluorescence spectrometry (CVAFS). The detection limit for the analysis is 0.06 ng/L. (Based on: United States Environmental Protection Agency, 2001).

The analysis was performed at IVL Swedish Environmental Research Institute and verified by a commercially available mercury solution called NIST, Standard Reference Material no 1641 d (1.59 µg/g), and an independent OPR (Ongoing Precision Recovery), which was run several times during the day of analysis to ensure that the system worked properly. Quality was also assured by first analysing a small amount of the sample (0.1 mL), which did not contain any mercury, indicating that no contamination had occurred during handling. The mercury concentration given in this report is expressed as ng/L and µg/g.

Bioconcentration factors were calculated for each species according to the formula:

$$\text{BCF} = \log (C_{\text{Biota}}/C_{\text{Water}})$$

C_{Biota} is the mean mercury concentration in the biological sample in µg/g wet weight whereas C_{Water} is the concentration in the water column in µg/g (Watras *et al.*, 1998).

Stable Carbon Isotope Analysis

Plants were rinsed in tap water, 1 M hydrochloric acid (HCl) and deionised water in the order mentioned to remove contaminating mud and debris before being cut into small pieces. Periphyton was washed with deionised water and so was phytoplankton while being filtered through a 250 µm net to remove traces of zooplankton and debris. Samples of vascular plants of the same species as well as periphyton and phytoplankton collected from different locations in the dam were combined to form composite samples. Twenty grams of dorsal fish muscle tissue was removed from *B. hilarii*, *S. marginatus* and *S. brasiliensis* and homogenised with a scalpel. Plants, phytoplankton and periphyton were rapped in aluminium foil and fish homogenate was placed on glass plates and dried in an oven at 60°C until all water had evaporated. After drying, the samples were ground to a fine powder using a mortar and pestle and stored in 20 mL glass bottles, which were put in plastic bags containing silica gel to keep them dry. The samples were processed further in a mill before isotopic analyses were carried out at the Department of Soil Science, Swedish University of Agricultural Sciences, in Uppsala. Two replicates of every sample, consisting of 2000 µg each, were analysed in a Delta Plus Continuous Flow Massspectrometer (Thermoquest Finnigan). The analysis was verified by a reference gas, CO₂, calibrated against the National Institute of Standards & Technology's reference material called NBS18, 8543 with a certified δ¹³C-value of -5.04 ± 0.06‰. Analysis of five samples of the standard reference showed 99.44% consistency with the certified value.

Carbon isotope ratios are a measure of the amount of heavy to light isotope in a sample (Peterson & Fry, 1987). During photosynthesis primary producers favour the light isotope of carbon, ¹²C, which results in primary producers containing less ¹³C than the atmosphere. δ¹³C-values are measured as parts per thousand differences (‰) between the isotope ratio of a

sample and that of a defined international standard called Pee Dee belemnite carbonate (PDB). The $\delta^{13}\text{C}$ -value is calculated according to the formula:

$$\delta R = [(R_{\text{Sample}} - R_{\text{Standard}}) / R_{\text{Standard}}] * 1000$$

δR expresses the $\delta^{13}\text{C}$ -value while R_{Sample} and R_{Standard} are the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and PDB respectively (Rounick & Winterbourne, 1986). Usually, biological materials contain less ^{13}C in relation to PDB and therefore have negative $\delta^{13}\text{C}$ -values (Fry & Sherr, 1984). The more negative the $\delta^{13}\text{C}$ -value, the less ^{13}C or the more ^{12}C it contains. Thus, samples enriched in ^{13}C have less negative $\delta^{13}\text{C}$ -values while samples depleted in the ^{13}C -isotope have more negative $\delta^{13}\text{C}$ -values.

Results

Total Mercury Concentrations and Bioconcentration Factors

The results demonstrate clear evidence of biomagnification of mercury in Lago Manso. The concentration varies widely between species but a trend that depends on feeding strategy can be seen. The mean mercury concentration is highest in piscivorous fish, followed by carnivorous and omnivorous species (Table 1). The species showing the highest mean mercury concentration is *S. brasiliensis* while *B. hilarii* demonstrates the lowest mean concentration. Very few individuals of the species *P. fasciatum*, *P. corruscans* and *P. mesopotamicus* were caught, but they are shown for comparison. The within species concentrations of mercury also vary significantly. The species with the largest variation is *S. brasiliensis*.

Table 1. The mean mercury concentration ($\mu\text{g/g}$) of the fishes.

Species	N	Feeding Strategy	Mean Hg conc. ($\mu\text{g/g}$)	Hg conc. range ($\mu\text{g/g}$)	1st and 3rd quartile	Length interval (cm)	Weight interval (kg)
<i>S. brasiliensis</i>	10	Piscivore	1.50	0.65-2.82	1.04; 1.62	45.8-87.0	1.0-4.8 ^a
<i>S. marginatus</i>	7	Carnivore	1.11	0.29-2.00	0.64; 1.47	14.8-29.0 ^b	0.046-0.5 ^c
<i>S. spilopleura</i>	8	Carnivore	0.80	0.30-1.30	0.45; 1.03	13.0-26.6 ^d	0.05-0.4 ^e
<i>B. hilarii</i>	10	Omnivore	0.40	0.04-1.03	0.11; 0.53	28.4-47.2	0.29-2.7
<i>P. fasciatum</i>	2	Piscivore	1.12	1.09-1.15	-	76.0-87.0	-
<i>P. corruscans</i>	1	Piscivore	0.54	-	-	117.6	-
<i>P. mesopotamicus</i>	1	Omnivore	0.072	-	-	49.8	-

a. Six specimens weighed more than 5 kg, but their exact weight could not be decided because a scale managing these weights was not available.

b. Three specimens were not measured.

c. Three specimens were weighed with their stomachs missing, and their weight interval was 0.65-0.85 kg.

d. One specimen was not measured.

e. One specimen was weighed with its stomach missing, and its weight was 0.7 kg.

No regression analysis was conducted on the relationship between mercury concentration and sex due to the low number of males sampled. Of all specimens in the study, 79% were females.

The relationship between mercury concentration and length as well as weight was tested using regression analysis. *S. brasiliensis*, *S. spilopleura* and *B. hilarii* showed no correlation while regressions for *S. marginatus* yielded a positive relationship for both length (coeff. = 0.09; intercept = -0.97; $r^2 = 0.99$) (Figure 2), and weight (coeff. = 2.79; intercept = 0.20; $r^2 = 0.96$; $p < 0.01$) (Figure 3).

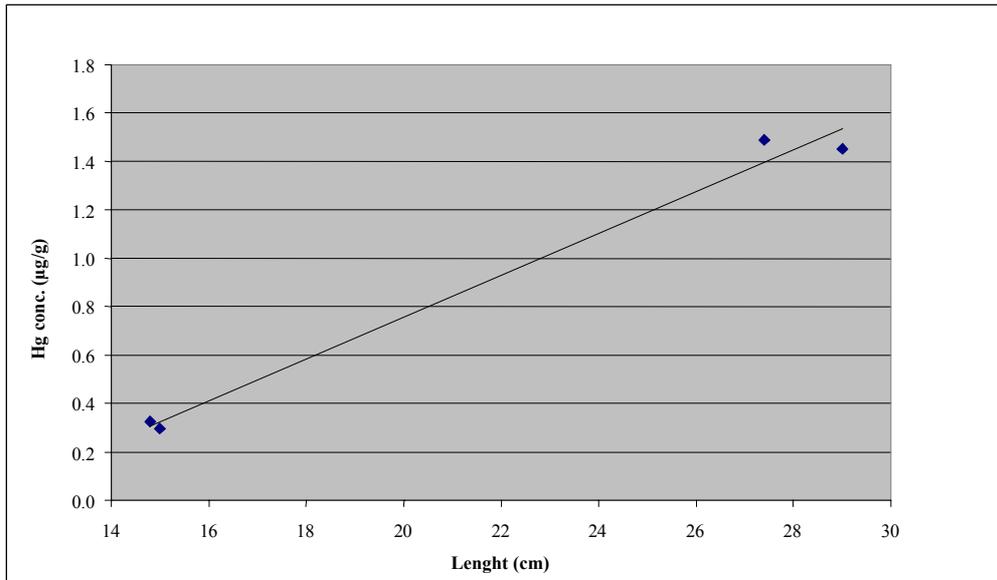


Figure 2. Mercury concentration ($\mu\text{g/g}$) versus length (cm) for *S. marginatus*.

The regression analysis between mercury concentration and weight, shown in Figure 3, is for the whole specimen, including the stomach. A regression analysis for specimens of *S. marginatus* weighed without their stomachs also showed a significant correlation (coeff. = 5.23; intercept = -2.44; $r^2 = 1.00$; $p < 0.01$).

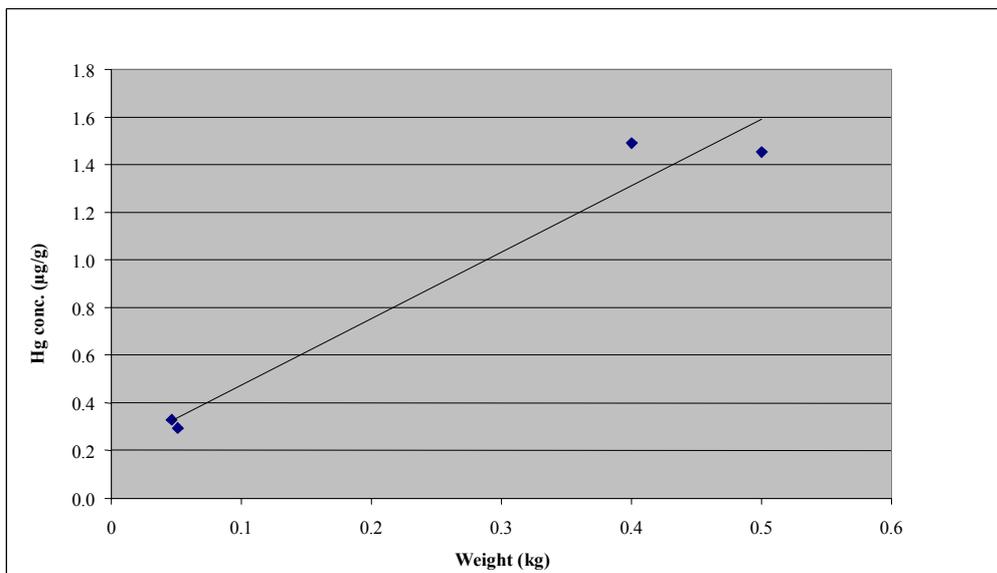


Figure 3. Mercury concentration ($\mu\text{g/g}$) versus weight (kg) for *S. marginatus*.

The concentration of total mercury in water is 3.3 ng/L ($3.3 \times 10^{-6} \mu\text{g/g}$) with an uncertainty in measurement of $\pm 0.19 \text{ ng/L}$ (95% confidence interval). The concentration in the water in $\mu\text{g/g}$ was used to determine the bioconcentration factor for each species (Table 2).

Table 2. The bioconcentration factors of the fishes.

Species	N	BCF	Range BCF
<i>Salminus brasiliensis</i>	10	5.66	5.29-5.93
<i>Serrasalmus marginatus</i>	7	5.53	4.95-5.78
<i>Serrasalmus spilopleura</i>	8	5.39	4.96-5.60
<i>Brycon hilarii</i>	10	5.08	4.08-5.50

The bioconcentration factors range between 4.08 and 5.93 log units with an individual of an omnivorous and a piscivorous species exhibiting the lowest and highest values, respectively.

Stable Carbon Isotope Ratios

The results of the $\delta^{13}\text{C}$ -analysis are shown in Table 3. According to a review by Dawson *et al.* (2002), $\delta^{13}\text{C}$ -values of terrestrial C_4 plants range from -11‰ to -15‰ while terrestrial C_3 plants lie between -20‰ to -35‰. However, there is no agreement on the subject. Differing values are commonly found in the literature. Carbon isotope values for terrestrial C_4 plants may range between -10‰ and -20‰ or -6‰ to -19‰ whereas C_3 plants may vary between -22‰ and -33‰ or -24‰ to -34‰ (Bender, 1971; Smith & Epstein, 1971). In any case, the results for the terrestrial plants show that *B. humidicola* and *P. maximum* fall well in the range of being classified as C_4 plants whereas *C. scoparioides* and *Mimosa* sp. are C_3 plants. The $\delta^{13}\text{C}$ -values of freshwater macrophytes and algae have been relatively little investigated (Rounick & Winterbourn, 1986). According to a review by France (1995a), periphyton from freshwater lakes exhibit $\delta^{13}\text{C}$ -values between -18‰ and -30‰, with most of the values centred on -22‰, which agrees with the value obtained for periphyton in this study. According to the same review, values for aquatic macrophytes range between -10‰ and -30‰, with one peak at -16‰ to -18‰ and a second peak at -28‰, which is consistent with the $\delta^{13}\text{C}$ -value for *S. auriculata*. The $^{13}\text{C}/^{12}\text{C}$ ratio for phytoplankton also corresponds with values found in the literature. Pooled samples of particulate organic matter (POM) and microplankton (<30 μm) from freshwater lakes exhibit $\delta^{13}\text{C}$ values within a range between -36‰ and -17‰ (del Giorgio & France, 1996).

Table 3. Stable carbon isotope values for plants, algae and fish.

	Species	Average $\delta^{13}\text{C}$ (‰)
Plants	<i>Brachiaria humidicola</i>	-11.68
	<i>Panicum maximum</i>	-11.80
	<i>Mimosa</i> sp.	-26.38 ^a
	<i>Conobea scoparioides</i> Benth.	-27.09
	<i>Salvinia auriculata</i> Aubl.	-27.59
Algae	Periphyton	-20.80
	Phytoplankton	-25.43
Fish	<i>Brycon hilarii</i>	-22.88
	<i>Salminus brasiliensis</i>	-27.13
	<i>Serrasalmus marginatus</i>	-28.28

a. The $\delta^{13}\text{C}$ -value of *Mimosa* sp is the mean value between seeds (-25.85‰) and leaves (-26.92‰).

Two of the terrestrial C_3 plants, *C. scoparioides* and *Mimosa* sp., as well as the aquatic macrophyte, *S. auriculata*, exhibit the most negative carbon isotope ratios of the primary producers. They are more ^{13}C depleted than phytoplankton, which in turn has a lower value than periphytic algae. The terrestrial C_4 plants, *B. humidicola* and *P. maximum*, are enriched in ^{13}C and therefore exhibit the least negative values of all the primary producers. The mean $\delta^{13}\text{C}$ -value for terrestrial C_3 plants is -26.62‰ whereas the mean value for the terrestrial C_4 plants is -11.74‰. The fishes *S. brasiliensis* and *S. marginatus* exhibit similar carbon isotope ratios while *B. hilarii* has a slightly higher value. *B. hilarii* has a $\delta^{13}\text{C}$ -value that lies in the mid range between phytoplankton and periphyton whereas *S. brasiliensis* and *S. marginatus* have values that correspond to the values of the terrestrial C_3 plant *C. scoparioides* and the aquatic macrophyte *S. auriculata*.

Mercury Concentration versus Carbon Source

The relationship between mercury concentration and carbon source ($\delta^{13}\text{C}$ -value) was determined by regression analysis for *B. hylarii*, *S. marginatus* and *S. brasiliensis* (Figure 4).

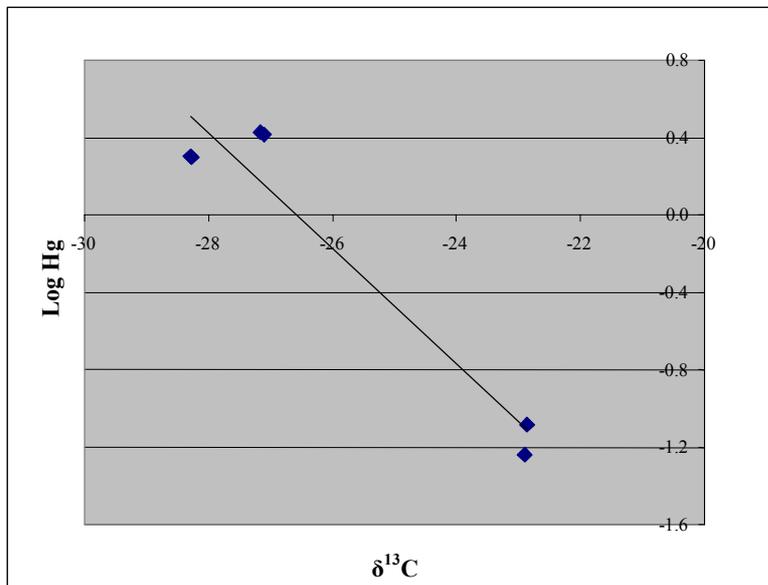


Figure 4. The relationship between logged mercury concentration and $\delta^{13}\text{C}$ (‰).

The result indicates that fish with less negative $\delta^{13}\text{C}$ -values have lower concentrations of mercury (coeff. = -0.30; intercept = -7.93; $r^2 = 0.92$).

Discussion

Differences in Mercury Concentrations within and between Species

The mercury levels in fish from Lago Manso are high in comparison to another hydroelectric reservoir in Brazil of similar age. In Balbina reservoir in the Amazon, the mercury concentrations in omnivorous and carnivorous species ranged from 0.02 to 1.1 $\mu\text{g/g}$ wet weight seven years after impoundment (Kehrig *et al.*, 1998), while the concentrations in Lago Manso range between 0.04 and 2.82 $\mu\text{g/g}$ wet weight five years after impoundment. The maximum permitted levels of mercury in non-predatory and predatory fish in Brazil correspond to the guidelines of FAO/WHO. In Balbina reservoir, only 14% of the specimens had a mercury concentration exceeding the lower dietary limit of 0.5 $\mu\text{g/g}$ wet weight. In Lago Manso, two species (*S. brasiliensis* and *S. marginatus*) have mercury levels well above the 1.0 $\mu\text{g/g}$ wet weight limit. One individual of *S. brasiliensis* has nearly three times the recommended concentration. Even though *S. spilopleura* and *B. hylarii* have a mean mercury concentration lower than the dietary limit for predatory and non-predatory species respectively, some individuals of these species exceed the limits. From a health perspective, the division between predatory and non-predatory fish is irrational since mercury in predatory fish is no less harmful to humans than mercury in non-predatory fish. It should also be noted that the limits are too high for subsistence populations, who base their food intake on fish (UN-FAO/WHO-JECFA, 2003).

The mean mercury concentrations vary widely between species in Lago Manso. *S. brasiliensis* exhibits the highest mean mercury concentration, which can be explained by its trophic position in the food web. Fish from top trophic levels in a food chain usually have higher

mercury concentrations than fish from lower trophic levels as a result of biomagnification (Kidd *et al.*, 1995). *S. brasiliensis* is piscivorous from a size of approximately 28 cm and onwards but younger individuals also consume insects, crustaceans and some grasses (Almeida *et al.*, 1997; Esteves & Pinto Lôbo, 2001). All individuals of *S. brasiliensis* in this study are larger than 28 cm. The two carnivorous species *S. marginatus* and *S. spilopleura* also show high mean mercury concentrations, which is probably also attributed to their trophic position. *S. spilopleura* exhibits a lower mean mercury concentration and a significantly lower maximum concentration than *S. marginatus*. This can be explained by differences in diet. *S. spilopleura* has a more omnivorous diet consisting of, in decreasing order of importance, scales, insects, plants, fins, snails, crabs, pieces of fish and whole fish, while *S. marginatus* eats fins and scales and to a lesser extent plants, pieces of fish, insects and shrimps (Sazima & Machado, 1990). Thus, *S. spilopleura* probably has a diet containing lower levels of mercury than *S. marginatus*. *B. hilarii* exhibits the lowest mean mercury concentration, which is in agreement with a predominantly herbivorous diet of fruits and seeds and smaller amounts of insects, crustaceans and molluscs (Sabino & Sazima, 1999; Wantzen *et al.*, 2002). Apart from diet and trophic level, differences in longevity, growth rate and other physiological and ecological factors can also lead to differences in mercury concentrations between species (Huckabee *et al.*, 1979). For example, Jackson (1991) has shown that two species belonging to the same trophic level in a hydroelectric reservoir in Canada varied in mercury concentrations due to differences in habitat. The species spending part of its time in the offshore regions had lower levels of mercury than the species inhabiting the inshore regions where the methylation of mercury in the sediments was higher. However, information about the physiology and ecology of the fishes included in this study is not sufficient to draw such conclusions.

The concentration of mercury also varies widely between individuals of the same species in this study, which is a common phenomenon. The lowest and highest mercury concentration within a population has been reported to vary by a factor of 10 or more (Meili, 1991). According to Huckabee *et al.* (1979) are age and size (length/weight) important variables determining levels of accumulation of mercury by fish. Length and weight are generally a function of age. Mercury concentrations have been reported to be positively correlated with size in numerous studies (Bowles *et al.*, 2001; Kidd *et al.*, 2003; Power *et al.*, 2002). In this study, the mercury concentration was found to be positively related to size for one species, *S. marginatus*. Elevated mercury concentrations with increasing age and size may reflect increases in prey size and changes in the diet composition as well as longer time of exposure (MacCrimmon *et al.*, 1983; Meili, 1991). Accumulation of mercury occurs throughout the whole life of a fish usually resulting in the oldest members in a population containing the highest concentrations (Mathers & Johansen, 1985). In this study, no relationship between mercury concentration and size could be found for three species, *S. brasiliensis*, *S. spilopleura* and *B. hilarii*, which is perhaps due to inadequate sampling size and size range. Another explanation could be that factors such as metabolic rate and sex determine the mercury concentration in these species. Some studies have shown that smaller fish have higher uptake rates of mercury due to higher metabolic rate than larger individuals of the same species (Huckabee *et al.*, 1979). Numerous studies have also shown that mercury concentrations are related to sex in some species, with one sex having a higher concentration than the other (Huckabee *et al.*, 1979).

The Bioconcentration Factors

The bioconcentration factors for fish from Lago Manso show similarities with values obtained in an uncontaminated tropical freshwater lake in Papua New Guinea, called Lake Murray. A comparison with other tropical hydroelectric reservoirs was not possible, due to the lack of studies. The bioconcentration factors for the carnivorous fishes *S. marginatus* and *S. spilopleura* fall in the range observed for piscivorous fishes in Lake Murray while the bioconcentration factor for the piscivore *S. brasiliensis* is approximately 0.1 to 0.2 log units higher than the values obtained for piscivorous species in Lake Murray (Bowles *et al.*, 2001). This may suggest that the bioaccumulation of mercury is somewhat higher in Lago Manso. However, it is risky to compare different water systems because of differences in controlling factors between different types of ecosystems and regions.

The Ultimate Carbon Sources of the Fishes

As mentioned previously, the $^{13}\text{C}/^{12}\text{C}$ ratios of primary producers remain relatively unchanged when passed on by consumers through the food chain, which mean that the $\delta^{13}\text{C}$ -value of an organism matches the value of its ultimate carbon source. If two food sources are used by an organism, the resulting carbon isotope ratio of the organism is an average of the two food sources. The $\delta^{13}\text{C}$ -value of *B. hilarii* does not correspond to any of the primary producers. Species of the genus *Brycon* are believed to consume mostly allochthonous food that has originated from the nearby forest (Goulding *et al.*, 1988). One explanation could therefore be that *B. hilarii* consumes a mixture of terrestrial C_3 and C_4 plants. The carbon isotope ratio of *S. brasiliensis* lies in the same range as terrestrial C_3 plants and aquatic macrophytes, which could imply that this fish has either of these groups of primary producers as ultimate carbon source. The average carbon isotope ratio of the C_3 plants (-26.62‰) is slightly higher than the value for *S. brasiliensis* (-27.13‰) but this may be due to the low number of C_3 plants sampled. The isotopic ratios of terrestrial C_3 plants are relatively fixed around -28‰ to -26‰ independent of the environment they are growing in, according to Hecky and Hesslein (1995). *S. marginatus* has the lowest $\delta^{13}\text{C}$ -value measured for all organisms in the study, which is not consistent with the notion that the ratio increases about 1‰ or less for each trophic transfer. A reason for this could be that an autotrophic carbon source has been overlooked in this study. Peterson *et al.* (1980) have suggested that chemosynthetic bacteria could be an important carbon source in some aquatic ecosystems, predominantly marine environments, distinguished by high concentrations of reduced inorganic compounds such as CH_4 and H_2S (Conway *et al.*, 1994).

A perhaps more likely explanation, that could apply both to *S. marginatus* and *S. brasiliensis*, is that the $\delta^{13}\text{C}$ -value obtained for phytoplankton is incorrect. Phytoplankton is difficult to separate from other living and non-living suspended material in water, and as a result particulate organic material (POM) and microplankton (<30 μm) are normally used as substitutes (del Giorgio & France, 1996). Unfortunately POM has been shown to consist not only of phytoplankton but also of terrestrial and littoral detritus, which are usually enriched in ^{13}C relative to phytoplankton and consequently have less negative $\delta^{13}\text{C}$ -values (France, 1995b; Hamilton & Lewis, 1992). Further support comes from a literature survey by del Giorgio and France (1996), who concluded that zooplankton was on average -2.59‰ depleted in ^{13}C in relation to POM and consequently had more negative $\delta^{13}\text{C}$ -values. This would not be the case if the bulk of POM consisted of phytoplankton (keep in mind that the $^{13}\text{C}/^{12}\text{C}$ ratio of a consumer is less negative than its food source due to fractionation during trophic transfer). If the average ^{13}C depletion percentage of -2.59‰ is used to estimate the real value of phytoplankton in the reservoir, a $\delta^{13}\text{C}$ -value of -28.01‰ for zooplankton ($[-25.42] + [-2.59]$)

and a value of -29.01‰ for phytoplankton are obtained, assuming an increase of 1‰ during trophic transfer. The approximated $\delta^{13}\text{C}$ -value for phytoplankton is in agreement with the values for *S. marginatus* (-28.28‰) and *S. brasiliensis* (-27.13‰) and allows for some isotopic fractionation during trophic transfer.

In conclusion, the results from the $\delta^{13}\text{C}$ -analysis imply that terrestrial C_4 plants are not a major carbon source for the fishes studied. Or else, the $\delta^{13}\text{C}$ -values of the fishes would have been less negative than observed. This result is consistent with other studies performed in South American floodplains (Araujo-Lima *et al.*, 1986; Forsberg *et al.*, 1993; Wantzen *et al.*, 2002). The results also indicate that periphyton is of minor importance as carbon source. This is not in agreement with studies in tropical, temperate and arctic lakes where periphyton have been shown to contribute significantly to the productivity of the fish community (Hecky & Hesslein, 1995).

The Relationship between Mercury Concentration and Carbon Source

Several studies have shown that there is a relationship between mercury concentration and carbon source (measured as $\delta^{13}\text{C}$). For example, a study in a sub-arctic lake revealed, by means of multiple regression analysis, that weight, trophic position and $\delta^{13}\text{C}$ were the most important explanatory variables of mercury variation among individuals of the fish community (Power *et al.*, 2002). The same result was obtained in a tropical lake where 74% of the variability of mercury was ascribed to these variables (Kidd *et al.*, 2003). The regression analysis in this study between mercury concentration and $\delta^{13}\text{C}$ implies that fish, which have an ultimate carbon source enriched in ^{13}C have lower concentrations of mercury than fish, which have an ultimate carbon source depleted in ^{13}C . However, the three species in the analysis do not employ the same feeding strategy. The observed trend is probably therefore better explained by the fact that the fishes belong to different trophic levels. If all species had been for instance piscivorous, one could have suggested that the differences in mercury concentration had something to do with the fishes having different ultimate carbon sources.

Future Research

To further deepen the understanding of mercury accumulation in Lago Manso, complementary studies on the physiology and ecology of the fishes as well as additional stable isotope analysis should be undertaken. The causes of the observed variations in mercury concentration between species could be determined by examining certain characteristics of the fishes such as longevity and growth rate. Huckabee *et al.* (1979) have suggested that differences in longevity and growth rate between species could be well as important factors explaining variations in mercury concentration as position in the food chain. Fish from top trophic levels generally have longer life spans leading to longer duration of exposure, than fish from lower levels in the food chain. Predatory fish usually also have slower growth rates than their prey. Faster growth rates cause growth dilution effects resulting in lower concentrations.

The ambiguities that arose in the $\delta^{13}\text{C}$ -analysis can be resolved by using a combination of different stable isotopes, for instance sulphur ($\delta^{34}\text{S}$) and nitrogen ($\delta^{15}\text{N}$) (Peterson *et al.*, 1985). Stable isotopes of sulphur can be used in the same way as stable isotopes of carbon to trace the flow of organic matter in the food web. Consumers are on average 0.2‰ enriched in their $\delta^{34}\text{S}$ -values compared to their food (reviewed by Peterson & Fry, 1987). One application of

$\delta^{34}\text{S}$ is to distinguish carbon sources with similar $\delta^{13}\text{C}$ -values from each other. Stable isotopes of nitrogen can be used to give a measure of the trophic position of an organism and provide information about feeding relationship in aquatic environments (Hobson & Welch, 1992; Minagawa & Wada, 1984). The $\delta^{15}\text{N}$ -value increases on average 3.2‰ from prey to predator (reviewed by Peterson & Fry, 1987) and the highest ratio should be found in the top trophic level, assuming that the original source of nitrogen for the organisms studied is the same (Minagawa & Wada, 1984). Coupled with contaminant analyses, $\delta^{15}\text{N}$ can also be used to determine the biomagnification potential of a contaminant and to trace the pathway of biocontaminants in aquatic food webs (Atwell *et al.*, 1998; Power *et al.*, 2002). The biomagnification potential is estimated by relating the contaminant concentration in an organism to its relative trophic position.

In brief, a combination of stable isotope analysis, contaminant analyses as well as increased knowledge of the ecology and physiology of the fishes studied can clarify the processes affecting bioaccumulation of mercury in individual fish species in the reservoir.

Conclusion

The mercury levels in fish from Lago Manso are well above the recommended dietary limit for mercury in fish for human consumption. The concentration varies widely between species, which could be related to differences in diet and trophic position. Mean mercury concentration is highest in piscivorous fish, followed by carnivorous and omnivorous species. The concentration also varies widely in individuals of the same species, which could be explained by differences in size (length/weight) for one species, *S. marginatus*. No relationship could be found between mercury concentration and size for *S. brasiliensis*, *S. spilopleura* and *B. hilarii*, which is probably due to small sampling sizes and narrow size ranges. Complementary studies on for example longevity and growth rate of the fishes could further deepen the understanding of the bioaccumulation of mercury in the reservoir. The carbon sources of *S. marginatus*, *S. brasiliensis* and *B. hilarii* are difficult to discern due to a small sampling size and large overlaps in $\delta^{13}\text{C}$ -values between different primary producers. The most important carbon sources seem to be terrestrial C_3 plants, aquatic macrophytes and phytoplankton, while terrestrial C_4 plants and periphyton appear to be of minor importance. Additional stable isotope analysis of the primary producers, especially the terrestrial C_3 plants, the aquatic macrophyte and the phytoplankton, could resolve the ambiguities. The relationship between mercury and carbon source was found to be correlated but the mercury concentration in fish is probably better explained by differences in trophic position.

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