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Sequential analysis of hair mercury levels in relation to fish diet of an Amazonian population, Brazil

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Abstract

Several studies in the Amazonian Basin have shown that riverine populations are exposed to methylmercury through fish consumption. It has been suggested that seasonal variations in hair mercury observed through sequential analyses may be related to the changes in fish species ingested by the local communities. The aim of the present study was to investigate the relationship between fish-eating practices and seasonal variation in mercury exposure. A group of 36 women from a village located on the banks of the Tapajós River, a major tributary of the Amazon, comprised the present study population. An interview-administered questionnaire was used to gather information on socio-demographic characteristics, fish-eating practices and other relevant information. The women also provided hair samples of at least 24 cm in length for mercury analysis. Hair total and inorganic mercury concentration was measured using a cold vapor atomic absorption analytical method. Trigonometric regression analysis was done to assess the seasonal variation of total mercury levels. Variations in inorganic mercury were examined by repeated measures analysis of variance, and analysis of contrast variable with a polynomial transformation. The results showed that hair mercury levels varied with the season. Higher levels were observed in months corresponding to the dry season, with lower levels in the rainy season. Herbivorous fish predominated the diet for 47.2% of the women during the dry season, but this rose to 72.2% during the rainy season. Those who reported eating fish daily had higher mercury levels in hair compared to those who only ate fish a few times per week. Retrospective mercury analyses, evaluated by the quantity of mercury present in each centimeter of hair, indicate that mean mercury level of the population decreased over the 2 years prior to the study. The percentage of inorganic mercury over the total mercury

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in hair increased towards the extremities of the hair strand. Higher percentages of inorganic mercury were found for the group who ate more fish (on a daily consumption basis). These results support the assumption that there are seasonal variations in methylmercury exposure and also a relationship between type of fish species consumed and the resulting hair mercury levels.

Keywords: Mercury; Hair; Amazon; Fish; Sequential analysis

1. Introduction

Over the past few years, there has been increasing evidence of mercury pollution in the Amazon Basin and high hair mercury levels among riverine communities, for whom fish constitute the dietary mainstay. Studies from different areas in the Brazilian Amazon indicate that hair mercury median values vary between 2 μg/g and 20 μg/g (Akagi et al., 1995; Barbosa et al., 1995, 1998; Malm et al., 1995; Boischio and Henshel, 1996; Lebel et al., 1996, 1997, 1998; Boischio and Cernichiari, 1998). Mercury, from gold-mining activities (Pfeiffer et al., 1993) and soil lixiviation (Roulet et al., 1999), is released into the aquatic ecosystem and enters the aquatic food chain, reaching humans through fish consumption. Recent studies have shown that neurotoxic effects are associated with long term exposure to mercury at low levels (Lebel et al., 1996, 1998; Dolbec et al., 2000).

Hair has been used in many studies as a bioindicator of mercury exposure for human populations (WHO, 1990). At the time of hair formation, mercury from the blood capillaries penetrates into the hair follicles. As hair growth is approximately 1 cm each month, mercury exposure over time is recapitulated in hair strands (WHO, 1990; Cernichiari et al., 1995); the mercury levels in hair closest to the scalp reflect the most recent exposure, while those farthest from the scalp are representative of previous blood concentrations. Sequential analyses of hair mercury have been useful for identifying seasonal variations over time in hair mercury content (Phelps et al., 1980; Akagi et al., 1995; Kehrig et al., 1997; Lebel et al., 1997). They have also been used to estimate fetal and newborn exposure during pregnancy and lactation (Cox et al., 1989; Cernichiari et al., 1995; Barbosa et al., 1998; Boischio and Cernichiari, 1998).

There are important seasonal variations in water levels in the Amazonian Basin (Goulding, 1980). The year is separated in two distinct seasons: the dry season (June to November) and the rainy season (November to June). Lebel et al. (1997), suggest that variations in hair mercury levels, observed for the village of Brasilia Legal on the Tapajós River, a major tributary of the Amazon, may be due to seasonal differences in bioavailibility of fish and differential consumption of piscivorous and herbivorous fish species. Knowledge of the relation between fish-eating practices and hair mercury levels is particularly important for adequate mitigation strategies. The objective of the present study was to examine variations in hair mercury levels with respect to fish-eating practices, in a second village on the Tapajós River.

2. Materials and methods

2.1. Population

This study was carried out in May, 1996, at the end of the rainy season, with the participation of the population of Cametá (3°17′08″ S, 55°06′03″ W), a village situated on the banks of the Tapajós river (Fig. 1).

During a first meeting with the villagers, carried out in the local schoolhouse, the general objectives of a study on the effects of fish consumption on mercury levels and human health were presented and they were invited to participate on a voluntary basis. A total of 98 individuals, aged between 12 and 79 years old responded

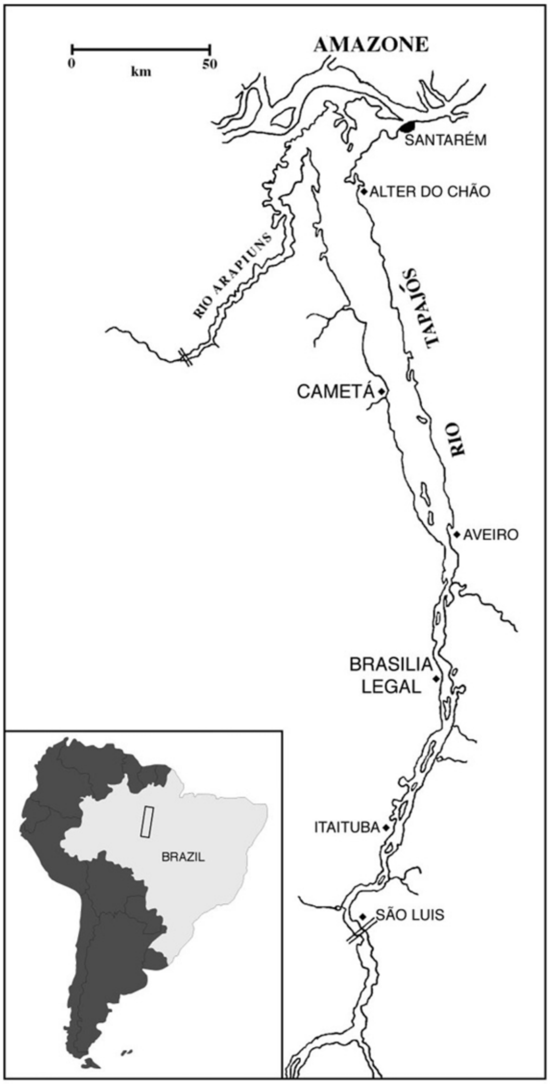

Fig. 1. Map of the study area, the lower Tapajós basin.

favorably. A subsample of 36 participants whose hair length was at least 24 cm, all women, provided hair samples for the present sequential analysis of hair mercury. The selection criteria for the present analyses, made on the basis of hair length, were used to obtain an estimation of the exposure to mercury over 2 years.

2.2. Questionnaire

Information concerning dietary habits were collected using an interview-administered questionnaire. These questions included fish consumption with an emphasis on fish species the most frequently consumed during high and low waters and over the entire year. Information concerning socio-demographics, smoking habits, alcohol drinking, medical and work history was also surveyed.

2.3. Hair collection and analysis

Hair from the occipital portion of the head was cut close to the scalp, placed in an identified plastic bag and stapled to prevent the shift of the hair strand. The hair samples were analyzed at the Laboratory of Indians and Northern Populations Health, Health Canada. Hair strands were cut into sequential centimeters, which were analyzed for total and inorganic mercury, using stannous chloride–cadmium chloride solution for the determination of total mercury and stannous chloride solution for the analysis of inorganic mercury, according to the method described by Farant et al. (1981). Hair mercury concentration was determined by cold vapor atomic absorption using a mercury UV monitor (model 1235, Laboratory Data Control, Riviera Beach, Florida). Detection limit was 0.5 μg/g. All the samples had total hair mercury level above this detection limit, but many samples had inorganic hair mercury below 0.5 μg/g. Measures below the detection level were set at 0.25 μg/g for the statistical analyses.

Precision and accuracy of mercury determination were ensured by the use of internal hair standards, provided by the Hair Mercury Interlaboratory Comparison Program, Health Canada, Ottawa, Canada.

2.4. Statistical analysis

The characteristics of the population and fish consumption habits were examined using descriptive statistics. Trigonometric regression analysis was applied to mean hair mercury levels of all strands. This allowed us to estimate the curve of best fit for the profile of mercury over time. Since this is a non-linear procedure, the confidence

Table 1
Socio-demographic characteristics (36 women)

	n	Mean ± S.D.	Median	(%)	Range
Age (years)	36	30.7 ± 14.1	28		12–68
Education (years)	35	4.4 ± 2.4	4		0–11
Alcohol consumption					
Drinker	20			55.6	
Ex-drinker	7			19.4	
Non-drinker	9			25.0	
Smoking habits					
Smoker	9			25.0	
Ex-smoker	6			16.7	
Non-smoker	21			58.3	
History of malaria	3			8.3	
History of parasitosis	16			44.4	
Born on the Tapajós river	27			75.0	

limits, which are asymptotic, were used to determine statistical significance. Non-parametric statistics were used to examine the relation between overall mean hair mercury levels and fish consumption parameters. The variation of inorganic mercury over time was assessed by repeated measures analysis of variance. In addition, analysis of variance of contrast variable with a polynomial transformation was performed to evaluate the linear trend of the percentage of inorganic mercury over time. Statistical analyses were performed using the SAS version 6.12 statistical package and JMPin 3.2.1 (SAS Institute Inc.).

3. Results

3.1. Population characteristics

Socio-demographic information on the population are presented in the Table 1. Since only women had 24 cm of hair, men are not represented in this subsample of the total population. Average hair total mercury level varied from 2.9 to 27.0 μg/g (median: 12.5 μg/g) and was not related to age, educational level, alcohol consumption, smoking habits or history of malaria or parasitosis. Hair total mercury was higher for those born on the Tapajós river comparatively to those born in other locations (median: 13.9 vs. 10.2 μg/g, respectively; Mann–Whitney $U' = 190$; $P = 0.01$).

3.2. Fish consumption

In response to the question 'What is the fish

Table 2
Frequency of reports for fish most frequently eaten during the year

Species common names (scientific names)	Feeding habits[a]	*n*	(%)
Caratinga (*Geophagus surinamensis*)	Herbivorous	23	63.9
Pescada (*Plagioscion* sp.)	Piscivorous	6	16.7
Jaraqui (*Semaprochilodus* sp.)	Detritivorous	2	5.6
Tucunaré (*Cichla* sp.)	Piscivorous	2	5.6
Filhote (*Brachyplatystoma filamentosum*)	Piscivorous	2	5.6
Charuto (*Hemiodus* sp.)	Detritivorous	1	2.8
Total		36	100

[a]The feeding habits were classified according to Ferreira et al. (1998) and Goulding (1980).

Table 3
Frequency of reports for fish species predominantly eaten during the high water period

Species common names (scientific names)	Feeding habits[a]	*n*	(%)
Caratinga (*Geophagus surinamensis*)	Herbivorous	22	61.1
Pescada (*Plagioscion* sp.)	Piscivorous	5	13.9
Tucunaré (*Cichla* sp.)	Piscivorous	3	8.3
Aracu (*Leporinus* sp.)	Herbivorous	3	8.3
Mapara (*Hypophthalmus* sp.)	Herbivorous	1	2.8
Tucunaré and caratinga (*Cichla* sp.) (*Geophagus surinamensis*)	Piscivorous and herbivorous	1	2.8
Pescada and caratinga (*Plagioscion* sp.) (*Geophagus surinamensis*)	Piscivorous and herbivorous	1	2.8
Total		36	100

[a]The feeding habits were classified according to Ferreira et al. (1998) and Goulding (1980).

Table 4
Frequency of reports for fish species predominantly eaten during the low water period

Species common names (scientific names)	Feeding habits[a]	*n*	(%)
Caratinga (*Geophagus surinamensis*)	Herbivorous	12	33.3
Tucunaré (*Cichla* sp.)	Piscivorous	11	30.6
Pescada (*Plagioscion* sp.)	Piscivorous	3	8.3
Aracu (*Leporinus* sp.)	Herbivorous	3	8.3
Cara bararua (*Uaru amphiacanthoides*)	Herbivorous	2	5.6
Sorubim (*Pseudoplatystoma fasciatum*)	Piscivorous	1	2.8
Jaraqui (*Semaprochilodus* sp.)	Detritivorous	1	2.8
Charuto (*Hemiodus* sp.)	Detritivorous	1	2.8
Other		2	5.6
Total		36	100

[a]The feeding habits were classified according to Ferreira et al. (1998) and Goulding (1980).

species you most frequently consumed during the year?', 63.9% of the women named a herbivorous fish (Table 2). The same question was asked for the high water and low water periods (Tables 3 and 4, respectively). For the high water period, 72.2% of the respondents reported herbivorous fish species, while for the low water period, it fell to 47.2%. Caratinga, a small herbivorous fish, was the most frequently consumed, independently of the period of the year.

3.3. Seasonal variations of mercury

Mean hair total mercury levels and the trigonometric regression curve are presented in Fig. 2. Results of the trigonometric regression indicate a sinusoidal relation with highest values at 9 and 22 cm, while lowest values are observed at approximately 2 and 14 cm. Considering that 1 cm represents approximately 1 month of hair growth, taking into account interindividual variations (Cernichiari et al., 1995), the highest concentrations of hair total mercury would correspond to the months of September 1995 and August 1994, while the lowest means concentrations would be in April 1996 and 1995. Since none of the confidence limits for the trigonometric regression

Table 5
Estimate parameters and asymptotic confidence limits for the trigonometric regression equation: $y = a + bx + c * \cos(d + ex)$[a]

Parameter	Estimate	Lower confidence limit	Upper confidence limit
a	17.27	16.74	17.80
b	−0.25	−0.29	−0.21
c	1.38	1.04	1.72
d	4.31	3.69	4.98
e	0.50	0.46	0.54

[a]The dependent variable corresponds to mercury and the independent variable is the centimeter of hair or the corresponding month.

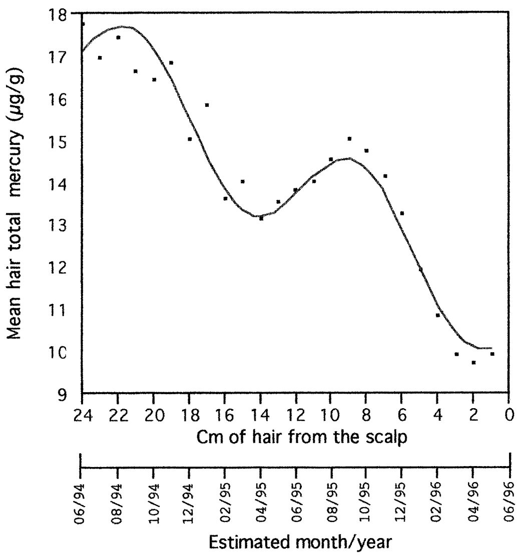

Fig. 2. Profile of mean hair total mercury levels for 24 cm. The estimated curve is weighted for standard deviation.

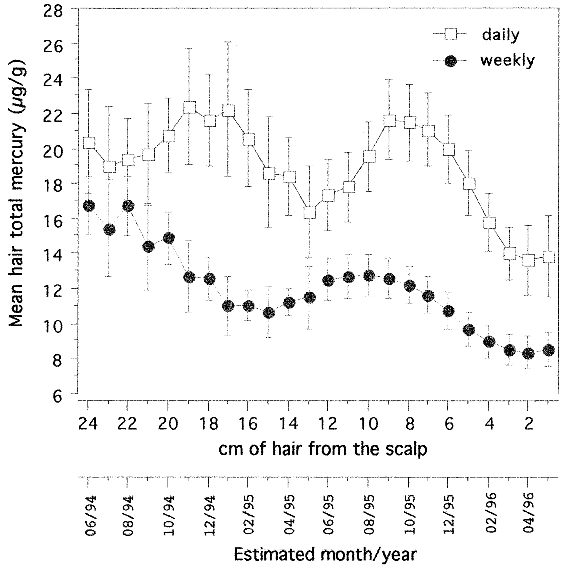

Fig. 3. Sequential representation of hair total mercury levels separated for daily fish consumers ($n = 10$) and those who ate fish a few times per week ($n = 26$). Means and standard errors are presented.

equation overlapped zero (Table 5), the non-linear estimation provides a good fit of the data (Seber and Wild, 1989).

The slope (b) of the regression equation indicates that overall, there was a decrease in total hair mercury over the 2-year period. Indeed, differences were significant between peak values observed in August 1994 and September 1995 (17.5 ± 8.3 μg/g and 15.1 ± 7.3 μg/g, respectively) (paired t-test: $t = -2.16$; $P = 0.04$), and between trough values in April 1995 and 1996 (13.2 ± 5.9 μg/g and 9.8 ± 5.7 μg/g, respectively) (paired t-test: $t = -4.59$; $P < 0.0001$).

Seasonal variations were still present when the mean hair mercury was calculated separately for those who reported eating fish daily and those who ate fish a few times per week (Fig. 3). However, for those with daily consumption, the mean level was higher for all centimeters. Comparison of mean hair total mercury levels from all centimeters was significantly higher among the daily fish eaters (18.6 ± 5.5 μg/g) as compared to those who ate fish a few times per week (11.8 ± 5.0 μg/g) (Mann–Whitney $U' = 215$; $P = 0.003$).

The profile of seasonal variations was also examined with respect to the fish species most frequently consumed throughout the year. Fig. 4 shows that mean hair mercury is higher for respondents who ate more piscivorous fish com-

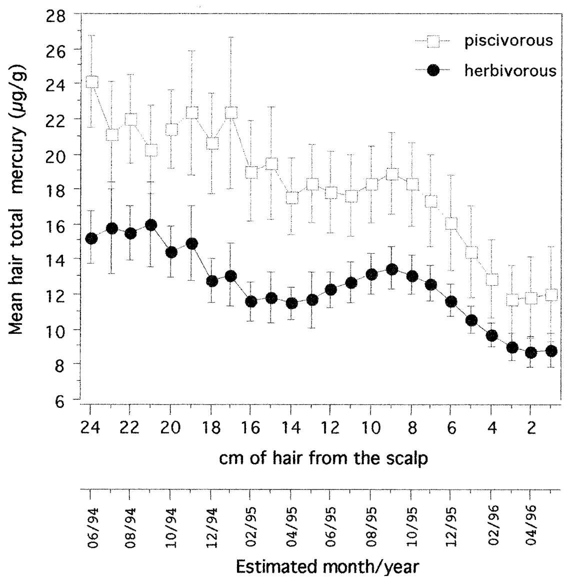

Fig. 4. Sequential representation of hair total mercury levels separated for predominantly piscivorous ($n = 10$) and predominantly herbivorous ($n = 23$) fish consumers. Means and standard errors are presented.

pared to those who predominantly ate herbivorous ones. Comparison of mean hair total mercury levels from all centimeters was significantly higher for those who ate predominantly piscivorous fish (17.3 ± 6.6 μg/g) as compared to those who ate more herbivorous fish (12.0 ± 4.2 μg/g) (Mann–Whitney $U' = 171$; $P = 0.03$).

All of the persons who were not born on the Tapajós River ($n = 9$) reported predominantly eating herbivorous fish throughout the year. When their average hair mercury levels were compared to those born on the Tapajós ($n = 14$), who likewise reported eating predominantly herbivorous fish throughout the year, there was still a difference in average hair mercury levels (10.2 vs. 13.7 μg/g; Mann–Whitney $U' = 94$; $P = 0.05$). The differences decreased over time and were highest 2 years ago (24 cm): median: 13.2 vs. 17.8 μg/g; Mann–Whitney $U' = 100$; $P = 0.02$; compared to 1 cm: median: 6.2 vs. 7.6 μg/g; Mann–Whitney $U' = 94$; $P = 0.05$.

3.4. Inorganic mercury

Since inorganic mercury was measured and organic mercury calculated as the difference between total and inorganic levels, the results for percentage of inorganic hair mercury values are presented here. Variations in the mean percentage of inorganic mercury along the centimeters of hair is illustrated in Fig. 5. The repeated measures analysis of variance indicates that the mean percentage of inorganic mercury differs for distinct centimeters of hair (Wilks' $\lambda = 0.19$; $F_{17,19} = 4.79$; $P < 0.001$). The analysis of variance of contrast variables with polynomial transformation reveals that the decrease percentage of inorganic mercury when approaching the scalp follows a statistical significant linear relation ($F = 27.49$; $P < 0.0001$), although there appears to be a little peak at approximately 11 cm.

It should be noted that the repeated analysis of variance was performed with estimated levels of inorganic mercury for values under the detection limit. When using only the values over the detection limit the same tendency was obtained, however, there were only nine individuals with no values below the detection limit, and thus the degrees of freedom were insufficient to perform the statistical analyses.

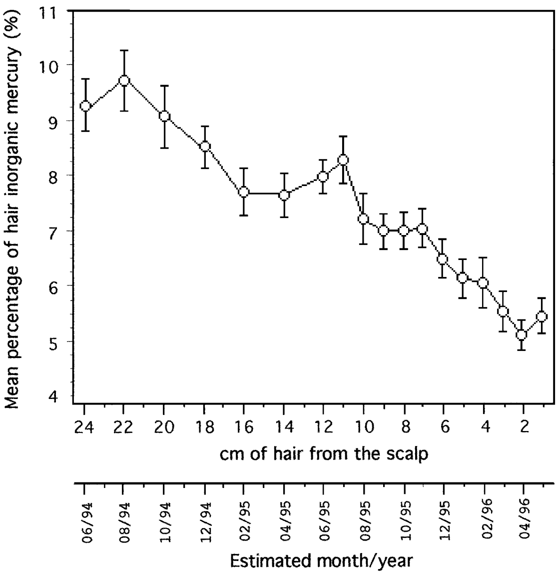

Fig. 5. Variations in the percentage of inorganic mercury in hair along 24 cm of hair strands. Means and standard errors are presented.

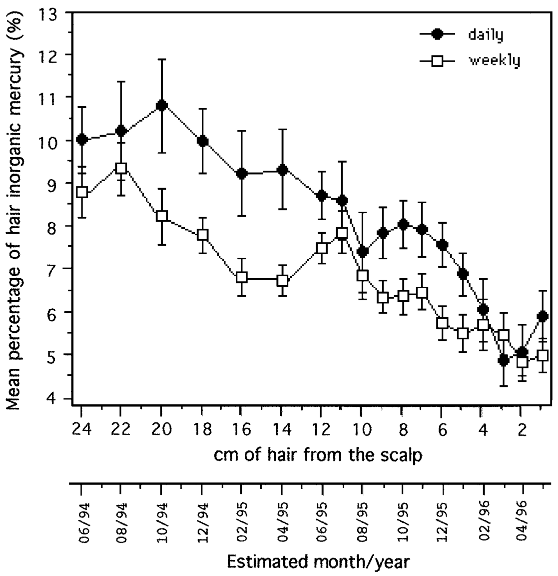

Fig. 6. Variation in the percentage of inorganic mercury in hair along 24 cm of hair strands. Means and standard errors are presented separately for daily consumers ($n = 10$) and those who ate fish a few times per week ($n = 25$).

While no differences were observed between those who reported eating predominantly piscivorous or herbivorous fish, those who ate fish daily had an overall higher percentage of inorganic mercury as compared to those who ate less fish ($8.1 \pm 1.7\%$ and $6.8 \pm 1.3\%$, respectively. Mann–Whitney $U' = 182$; $P = 0.04$). Fig. 6 shows the profile of the percentage of inorganic mercury over time for the daily fish eaters and the others.

The corollary of these analyses is that the proportion of organic mercury content of hair increased towards the scalp and that the proportion of methylmercury was less for the daily fish eaters, compared to those who ate fish a few times per week.

3.5. Inorganic mercury vs. total mercury

Overall, the proportion of inorganic mercury decreased over time, paralleling the decrease of hair total mercury over time. However, when the relation between the mean of total mercury of all centimeters and the mean of the percentage of inorganic mercury of all centimeters is tested, the regression equation is not significant ($P = 0.09$). For each centimeter examined individually, a significant relation was observed for some of the centimeters (Table 6).

Table 6
Parameters of linear regression between percentage of inorganic mercury and total mercury in hair for each centimeter

cm	β Coefficients	Correlation coefficient (r)	P value
1	0.112	0.35	0.04
2	0.140	0.46	< 0.01
3	0.061	0.14	0.44
4	0.055	0.11	0.54
5	0.094	0.29	0.10
6	0.142	0.47	< 0.01
7	0.115	0.37	0.03
8	0.129	0.42	0.01
9	0.133	0.48	< 0.01
10	0.063	0.15	0.39
11	0.015	0.04	0.83
12	−0.005	0.02	0.92
14	0.112	0.26	0.13
16	0.145	0.39	0.02
18	0.083	0.27	0.12
20	0.092	0.20	0.26
22	0.055	0.13	0.44
24	0.126	0.37	0.03

4. Discussion

The findings of the present study indicate clear seasonal variations in the levels of hair mercury, confirming previous studies done in the Amazonian Basin (Akagi et al., 1995; Kehrig et al., 1997; Lebel et al., 1997). Peak levels of mercury were observed during the dry season and trough levels during the rainy season, paralleling the differences in fish diet in the two seasons. Villagers reported eating more herbivorous fish species at the end of the rainy season (high water period) and piscivorous fish species at the end of the dry season (low water period), piscivorous fish species are known to contain more mercury than herbivorous ones (Barbosa et al., 1995; Malm et al., 1995; Porvari, 1995; Bidone et al., 1997; Lebel et al., 1997).

Seasonal variations were likewise present for those who ate predominantly piscivorous fish and those who ate predominantly herbivorous fish, with the former having significantly higher levels of hair mercury. Since subsistence fishing provided the dietary mainstay, everyone ate fish at least a few times per week; those who ate fish daily had higher levels than the others. The type and frequency of fish consumption are well reflected over time in sequential hair mercury levels, confirming the parallel between hair mercury and fish intake (Airey, 1983; Leino and Lodenius, 1995).

A very significant decrease in hair mercury levels over time was observed for this population. This could result from changes in fish-eating practices, however, fish is included in 62% of the meals (Dolbec et al., 2000). Other possibilities are decreasing environmental mercury levels around this village, decreasing methylation or changes in the bioavailibity of the different fish species in this ecosystem. It is interesting to note that fish captured in this area, at the time of the study had less mercury compared to fish of the same species and size, captured in other regions on the Tapajós (unpublished data), however, we do not know if this was always the case. The inter-annual variations in environmental conditions, such as the intensity of the rainy season, the rapidity of rising or falling water levels may greatly influence mercury methylation and fish life cycle. Future studies should be prospective, rather than retrospective and survey diet over the period that is covered by the hair strand analyses in order to better understand increasing or decreasing trends.

In this region of high biodiversity, the local ecosystem probably plays an important role in fish bioavailability and bioconcentration of mercury. In a previous study carried out in a village 100 km upstream, the seasonal variations were in the opposite direction, corresponding to differences in fish-eating practices; herbivorous species were more frequently consumed during the dry season and piscivorous species during the rainy season (Lebel et al., 1997). Moreover, in this other village, contrary to what was observed here, there was a tendency towards an increase in hair mercury levels over time (Lebel et al., 1997). Exposure studies should be accompanied by investigations into the local ecosystem in order to propose appropriate mitigation measures and avoid generalizations that may not be applicable.

In studies concerning fish-eating populations, the percentage of hair inorganic mercury has been estimated at less than 20% (Phelps et al., 1980; Barbosa et al., 1995; Cernichiari et al., 1995; Boischio and Cernichiari, 1998), which is similar to the proportions observed here. In the sequential analysis of the present study, the percentage of inorganic mercury increased towards the extremities of the hair strand. Phelps et al. (1980) reported no changes in the proportion of inorganic mercury over 24 cm using 3643 hair segments of 1 cm each. Since total mercury and hair inorganic mercury decreased over time (from extermity to scalp), we examined whether the percentage of inorganic mercury is a function of total mercury in hair. No relation was observed between total mercury and the percentage of inorganic mercury for the group as a whole, although the percentage of inorganic mercury was higher for the daily fish-eaters compared to those who consumed fish a few times per week. Some hypotheses could explain the decrease in inorganic mercury levels over time: First, the decrease in the proportion of inorganic mercury could be related to an increase in the relative methylmercury content in this fish resource. Second, with hair growth, methylmercury may be transformed into inorganic mercury. Clarkson (1997) has suggested that a small amount of methylmercury is broken down into inorganic when it enters the hair follicle. The transformation of methylmercury form into inorganic with the hair possibly continues after keratinization of the hair shaft. The higher proportion of inorganic mercury observed for the daily fish eaters compared to the others may result from an adaptive mechanism due to constant exposure, resulting in improved transformation and excretion of methylmercury.

The levels of hair mercury in this population of women were in the range of those observed in other studies in the Amazon Basin (Akagi et al., 1995; Barbosa et al., 1995, 1998; Malm et al., 1995; Boischio and Henshel, 1996; Lebel et al., 1996, 1997, 1998; Boischio and Cernichiari, 1998). The majority of these women are of childbearing age and the seasonal variations of exposure could result in high peaks during particularly vulnerable times during pregnancy, with consequences for fetal neurodevelopment. Indeed, the threshold for adverse neurological effects in the fetus is considered to be between 10 and 20 $\mu g/g$ of mercury in maternal hair (WHO, 1990). In the adult population (men and women of 15 years and over) from which this subsample of women was drawn, a dose–effect relationship was observed between diminished psychomotor performance and an increase in mean total mercury for the first 2 cm of hair (Dolbec et al., 2000). These effects on human health were in accordance with results obtained from other studies performed in the Amazonian Basin on adults (Lebel et al., 1996, 1998) and children (Grandjean et al., 1999) populations.

The biodiversity of the ecosystems in the Amazon coupled to the variations of this hydrological system, complexify the comprehension of methylmercury exposure. More work is needed to adequately describe the situation and assess the extent of the problem in order to propose realistic mitigation measures that will allow the population to benefit from fish consumption, while diminishing mercury intake.

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