

Zinc status of riverside populations of the rivers

Solimoes and Negro in the state of Amazonas, Brazil

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Abstract

Zinc nutrition has been shown to be critically limiting among urban populations of the central Amazon valley, but no information on the zinc status of rural populations has been published. Nutrition surveys were carried out on the river Solimoes and the river Negro in years 1976/7. Hair samples collected at that time were analysed for their zinc content, by Atomic Absorption Spectrophotometry. The mean hair zinc value of children aged under seven years from the river Negro (140 µg/g) was about one third lower than hair values from the river Solimoes (204 µg/g) with high statistical significance ($p < 0.001$). Hair zinc levels were influenced by a variety of non-dietary conditions in these riverine children populations, including anthropometric classifications and gastrointestinal parasites, and these are discussed. The significantly lower hair zinc levels in children on the river Negro than in children on the river Solimoes may be part of the reason why young child stunting rates are higher on the river Negro. The importance of zinc status for reproduction is also discussed, especially the evidence for relationships to still teratogenesis and still births, the latter being six times more common among mothers on the river Negro than among those on the Solimoes at that time. Although no indicators of maternal zinc status were measured, the findings of zinc deficiency in their young children support the hypothesis that these mothers were also suffering from zinc deficiency. It also suggests poor zinc status may be associated with the lower population densities found along the banks of the river Negro as compared to that of the river Solimoes. The situation seems unlikely to have improved in the forty years since these observations were made and may even be getting worse. Interventions are suggested that would allow this type of situation to be remedied.

Keywords: Hair zinc; young child stunting; stillbirth rates; antenatal multiple micronutrient supplementation.

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Introduction

Zinc was found to be one of the principal dietary inadequacies in the urban areas of the central Amazon valley in the late seventies and early eighties. Analysis of the data from a household expenditure survey conducted in Manaus in the mid-seventies, found that zinc was one of the most deficient nutrients among the household diets (Shrimpton, 1984). Further studies at that time, found that a third of low-income urban workers in Manaus had low serum zinc levels associated with inadequate dietary intakes (Shrimpton, et al, 1983). Zinc supplementation trials carried out among poor urban Amazonian mothers in the early eighties, showed positive effects on both zinc and vitamin A levels of their breast milk, as was published just recently (Shrimpton and Marinho 2018).

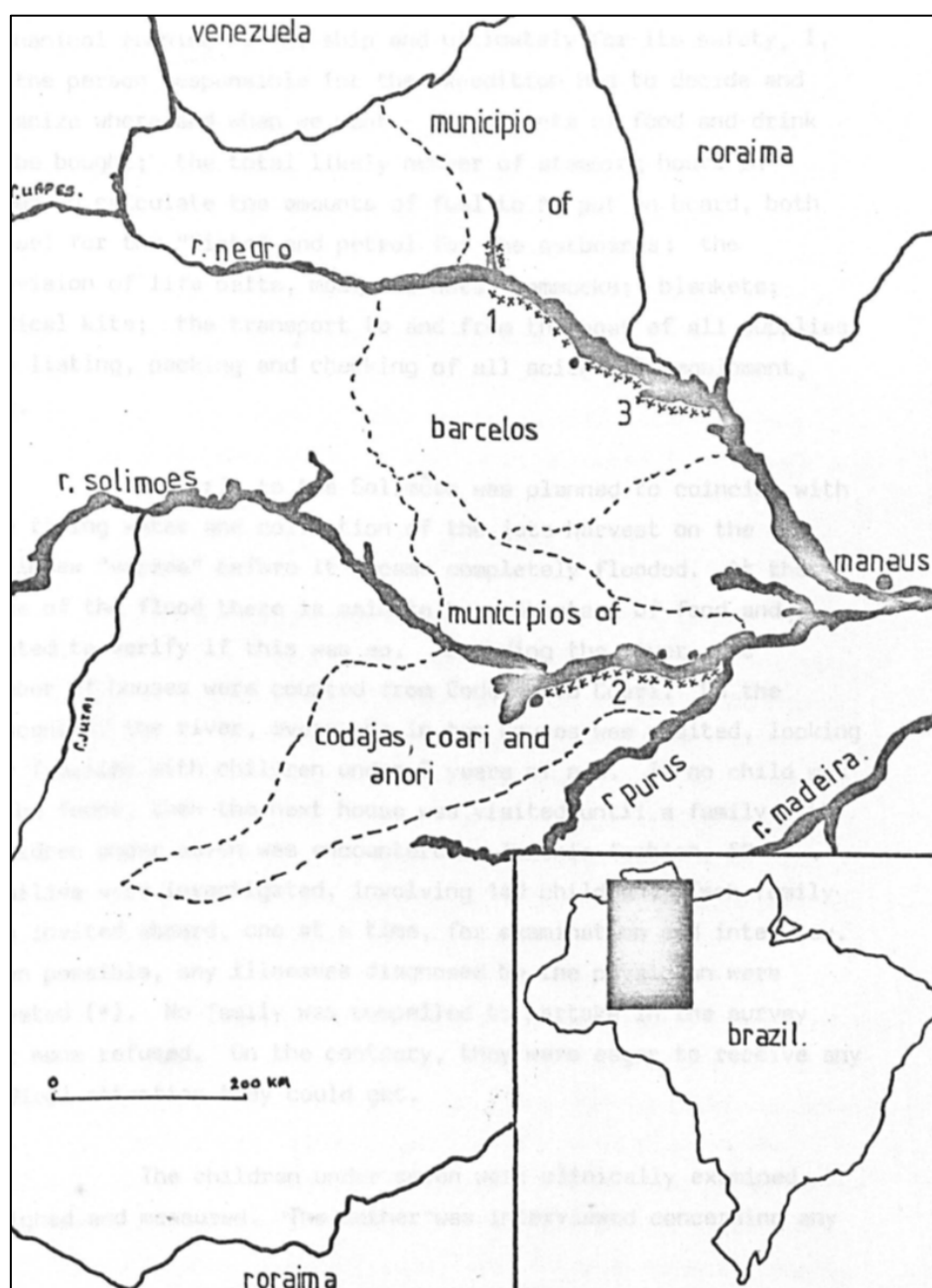
No measurements of zinc status have been reported for the rural populations of the Amazon region. While mild to moderate zinc deficiency is a common problem worldwide, difficulties of measuring zinc deficiency complicate estimates of the exact size of the problem (de Benoist et al, 2007). Young child stunting has been chosen as one possible indicator of zinc deficiency (King, et al 2016).

Stunting affected 51% of young children on the Solimoes, and 70% of those on the river Negro back in the seventies (Giugliano et al, 1981; Giugliano et al, 1984). The purpose of this article is to finally report on the zinc status of the populations of the river Negro and Solimoes back in the seventies and discuss how these results may still be relevant today. The hair zinc results reported here were included in a PhD thesis of the University of London (Shrimpton, 1980) but have not been published previously.

Materials and Methods

The material for this research was collected during three boat expeditions in the period from December 1976 to August 1977. The first expedition in December 1976 studied the rural populations living on the bank of the river Negro 100km above the town of Barcelos as far as the mouth of the river Preto, marked as area number 1 in Figure 1. The second expedition in April 1977 was to the river Solimoes between the towns of Codajas and Coari, marked as area number 2 in Figure 1. The third expedition in August 1977 was to the lower river Negro from the mouth of river Branco up to about 100 km above Barcelos, marked as area number 3 in Figure 1.

Figure 1. Location of the populations surveyed in the three expeditions



The second and third expeditions were nutrition surveys, and so more nutrition relevant information was collected on the families of the children studied than during the first expedition, the main purpose of which was to research the presence of infectious diseases. The results of the nutrition surveys carried out in areas 2 and 3 included anthropometry, clinical examinations, as well as assessments of family diet, household hygiene and maternal reproductive history, as has been reported previously (Giugliano et al, 1981; Giugliano et al, 1984). Anthropometric assessments methods were those of Jelliffe (1966) using the international growth standard of Stuart & Stevenson (1959).

Table 1. Characteristics of the areas studied

Characteristics	River Negro (Area 1)	River Solimoes (Area 2)	River Negro (Area 3)
Geographic location	From the town of Barcelos to the mouth of the River Preto (+/- 100kms)	From the mouth of the River Purus to the town of Coari (+/- 200kms)	From the mouth of the River Branco to the town of Barcelos (+/- 200kms)
Municipalities	Barcelos	Coari, Anor, Codajas	Barcelos
Estimated rural population*	16,400	43,700	16,400
Population density of municipalities (p/km ²)	0.10	0.44	0.10
Approximate number of families/km of river	0.3	2.9	0.3
Soils	Latosols	Alluvial deposits	Latosols
Main economic activities	Extractivism – collection of nuts, fibres, resins, and timber from the jungle.	Agricultural -plantations of malva and jute	Extractivism – collection of nuts, fibres, resins, and timber from the jungle.

* Source: Anuario Estadístico do Estado do Amazonas (1975).

For the area 1 expedition all households on the left bank were visited irrespective if they had young children or not and hair samples were collected from 35 children under seven years of age. For the area 2 expedition, the number of houses on the left bank were counted while ascending the river from Codajas to Coari, and then on the descent of the river, every tenth houses was visited, looking for families with children under 7 years of age. If no such child was found, then the next house was visited until a family with children under seven was encountered, and hair samples collected from 140 children under seven years of age. For the area 3 expedition on the river Negro, we visited all households found on the left bank going upriver, looking for those with children aged under seven years, and 121 hair samples were collected from them.

Hair samples were collected from children aged under seven years, always from the nape of the neck, and only the 3cm nearest to the scalp were kept for analysis. Hair samples were also stored in material that would not contribute zinc to the samples. The children in area 3 also had faecal samples collected by leaving a sample bottle with the family on the way up the river and then collecting them on the way back down again.

The faecal samples collected from 78 children (65%), were preserved in a liquid consisting of Methiolate, Iodine and formalin (MIF) and subsequently examined in the laboratory (Sapero and Lawless 1953).

Hair zinc levels were determined by Atomic Absorption Spectrophotometry (AAS) following procedures recommended by the manufacturers (Perkin Elmer, 1976). Hair samples were washed in a gentle 1.0% soap flake solution, rinsed 6 times with deionized water and then dried to constant weight in a 100°C oven in Erlenmeyer flasks. Ten millilitres of concentrated nitric acid were added to the flask and gently heated on a sand bath until clear. The acid was then evaporated off, without drying completely, and the contents of the flask made up to volume with a 10.0% nitric acid solution. After preparation of the samples of hair, the solutions were aspirated into a Perkin Elmer 403 AAS using a Bolling burner and a Deuterium Arc background corrector. Results were recorded on a flatbed recorder in the absorption mode at 4x amplification. Standards were prepared daily from stock solutions of 1 000 ppm zinc prepared from Merk "Tritosol" Atomic Absorption Standards.

To check zinc quantification, a National Bureau of Standard (NBS) reference bovine liver sample was also analysed by the dry ashing method. Nine replicates of the NBS standard typically gave a mean +/- SD of 122.5 +/- 4.3 mcg/g against an NBS value of 130.0 +10.0 mcg/g, i.e. an underestimation of 5.9%. An internal standard was also made from a mass of our own hair as suggested by Sorenson et al (1973). Samples of this standard were continuously included in the batches of hair samples being analysed. The coefficient of variation of 20 such samples over the period of a year was 12.8%. The main cause of variation was due to reagent blanks. It was always necessary to run 3 and average them.

Deficient hair zinc levels have been suggested to be those below 70 µg/g (Hambidge et al, 1972). Analysis of hair from 10 apparently healthy young Amazonian adults, from the higher socioeconomic strata of Manaus society, who were working at the Institute gave a mean value of 172.3 mcg/g, with a standard deviation of 32.2 µg/g. Three standard deviations below this mean gives a value of 66.7 µg/g, similar to Hambidge's lower limit of normality. Individuals with hair zinc levels below 70.0 µg/g were thus considered to be zinc deficient.

The hair zinc level results obtained were analysed using standard methods of statistical inference (Armitage, 1971). The significance of differences in mean values, by different groupings were evaluated using the t test. The significant differences in proportions considered deficient, were tested using the X² test, with continuity correction.

Results

The characteristics of the two areas studied are quite different as described in Table 1. The rural population of the river Solimoes area 2 was two and a half times as big as in the river Negro area 1, and the populations density of the municipalities four times greater. There were ten times as many families per kilometre of riverbank on the Solimoes as compared with the Negro. In both areas the houses were typically made of wood or straw and built on stilts to accommodate the annual rise and fall in the level of the river, which can be 10 to 15 meters. While the soils of the banks of the river Negro were largely infertile latosols, those of the river Solimoes were very fertile alluvial deposits. The families on the river Negro were largely involved in extracting produce

from the forests, while the families on the Solimoes practiced agriculture on the fertile flood plains called “varzeas”.

Some of the characteristics of the families included in the nutrition surveys of areas 2 and 3 are shown in Table 2. As these questions were not asked in Area 1, they cannot be included. On the river Solimoes 59 families were investigated, involving 140 children aged under seven years. On the river Negro (area 3) 60 families were investigated involving 121 children under seven years of age. The 140 children studied in area 2 were about 1% of the children under 7 in the rural areas of the municipalities of Anori, Coari and Codajas. The 120-child sample studied in area 3, represented 5% of the children under 7 years of age in the rural area of the municipality of Barcelos. Surprisingly perhaps, the maternal illiteracy rate was higher on the river Solimoes than on the river Negro, perhaps a result of the efforts of the nuns in Barcelos, as many of *ribeirinhos* (riverside dwellers) are at least nominally catholic. On both rivers over ninety percent of mothers gave birth at home, indicating how remote their rural locations really were.

Table 2. Characteristics of the populations studied

CHARACTERISTICS	RIVER SOLIMOES (AREA 2)	RIVER NEGRO (AREA 3)
General		
Number of families examined	59	60
Number of children aged <7yrs	140	121
Mothers mean age (yrs.)	28	31
Mother unable to read/write (%)	78	57
Home births (%)	94	90
Reproductive History		
Mothers pregnant (%)	21	17
Mean number of pregnancies	5.9	6.3
Pregnancies aborting (%)	6.2	6.8
Stillbirths (%)	1.3	8.8
Child deaths in 1 st year of life (per thousand live births)	76.4	96.1
Sanitary Conditions		
Boiled/filtered river water before drinking (%)	12	4
Destination of faeces (%):		
In river	2	3
In a hole	72	18
On the ground	24	79
Foods consumed by mothers in previous 24 hours (%)		
Cereals	66	27
Cassava flour	95	95
Fish	76	70
Game Meats	43	52
Chicken	22	0
Milk powder	26	12
Vegetables	47	18
Fruits	28	13
Sugar	92	92
Coffee	93	78

Mothers on the river Solimoes had more successful reproductive histories than mothers on the river Negro. Mothers were slightly older on the river Negro, where fewer were actually pregnant, and they had fewer pre-school aged children in their houses. The mean number of pregnancies per mother were very similar, as were the percentages of pregnancies aborting. But the still birth rate was 7 times higher on the River Negro than on the River Solimoes. The infant mortality rate was also 22% higher on the River Negro than the River Solimoes.

Sanitary conditions of the families were quite precarious on both rivers. All families obtained their drinking water from the river. Those that treated the water before drinking it were in a minority on both rivers, with the Solimoes doing best with 12% either filtering or boiling as compared to 4% on the Negro. The majority (83%) just sieved their drinking water on the Solimoes as opposed to 33% on the Negro.

There were differences in the foods consumed by the mothers in the previous 24 hours on each river. Diets on the river Negro seemed less varied, with less frequent consumption of cereals such as bread and rice, as well as less milk powder, fruits and vegetables. Fish consumption was similar, although the sorts of fish differed, and while game meat consumption was more common on the Negro, chicken was only consumed on the Solimoes. On both rivers almost all mothers consumed coffee and sugar. Mothers were also asked about whether earth eating was common, and just 7% of mothers on the river Negro and 3% of mothers from the river Solimoes said that their children did so.

Hair zinc results were only obtained for a total of 159 children aged under seven years of age from the rural areas of the rivers Solimoes and Negro. Of the 260 children aged under seven years that were measured and examined in the nutrition surveys of the rural areas of the lower river Negro and river Solimoes (areas 2 and 3), hair zinc results were obtained on 124 hair samples weighing more than 15mg. Hair samples from a further 35 children aged under seven years on the upper river Negro (area 1) were also successfully analysed. For the remaining children either an insufficient hair sample was obtained, or the sample was lost during the washing and acid digestion process.

As shown in Table 3, the mean hair zinc value of children aged under seven years from the river Negro (140 $\mu\text{g/g}$) was about one third lower than mean hair zinc values from the river Solimoes (204 $\mu\text{g/g}$) with high statistical significance ($p < 0.001$).

As shown in Table 4, the prevalence of zinc deficiency based on hair zinc level ($<70 \mu\text{g/g}$) was almost double among young children on the banks of the river Negro than on the banks of the river Solimoes. There was no significant difference in these prevalence's however, probably because of the small number of samples obtained in each group, and lack of consistency in the variability across the years of age.

Hair zinc levels appear to be influenced by a variety of conditions in these riverside child populations. As shown in Table 5, hair zinc values and anthropometric measurements were obtained for 68 children on the river Solimoes and 53 children on the lower river Negro (area 2), all (the entire sample of) pre-school children on the river Negro had a lower mean hair zinc value than all children on the river Solimoes ($p < .001$). Children on the river Solimoes who were not stunted and not wasted had almost the same mean value as the assumed normal mean hair zinc value (180 $\mu\text{g/g}$). On the river Negro, children who were not stunted and not wasted had a mean hair zinc value lower than children not stunted nor wasted on the river Solimoes, although the

Table 3. Hair zinc levels ($\mu\text{g/g}$) by age group in rural riverside children from the river Solimoes and River Negro in the State of Amazonas.

Age Group (months)	Rural River Solimoes			Rural River Negro			Significance of difference
	n	mean	standard deviation	n	mean	standard deviation	
0-11	6	191	73	6	152	162	N.S.
12-23	11	191	66	7	75	24	P < 0.001
24-47	16	166	72	18	136	102	N.S.
36-47	9	286	189	20	119	89	P < 0.02
48-59	15	182	97	21	156	82	N.S.
60-71	12	236	142	18	172	64	N.S.
All	69	204	116	90	140	85	P < 0.001

Table 4. Prevalence of zinc deficiency (Hair zinc < 70 $\mu\text{g/g}$) in children of the rural riverside populations of the state of Amazonas, by age group.

Age Group (months)	Rural River Solimoes		Rural River Negro		Significance of difference
	n	% Zinc Def.	n	% Zinc Def.	
0 -11	6	0	6	17	NS
12 - 23	11	9	7	43	NS
24 -35	16	13	18	28	NS
36 - 47	9	0	20	20	NS
48 - 59	15	7	21	10	NS
60 - 71	12	10	18	0	NS
All	69	7	90	17	NS

difference was not significant. Both stunted and wasted children on the river Solimoes had higher mean hair zinc values than the normal children, although the differences were not significant. The mean hair zinc value of wasted children was lower than that of normal children, but again the difference was not significant. On the river Negro the mean hair zinc value of wasted children was significantly less than that of stunted but not wasted children ($p < .001$). However, overall there does not appear to be a link between zinc levels and malnutrition.

Table 6 shows the hair zinc values in children from area 3 on the river Negro with and without different gastrointestinal parasites. Children with *Ascaris* and *Trichuris* had significantly higher mean hair zinc values than those without these parasites ($p < .01$ and $< .05$ respectively). Mean hair zinc values of children with hookworm were also higher than those without, but this was not significant. These parasite infections were affecting over 60% of the children studied.

Discussion

A recent systematic review confirmed that in healthy individuals, hair zinc is a reliable biomarker of zinc status (Lowe, Feteke, Decsi 2009). That the mean hair zinc level of preschool aged children on the river Negro was significantly lower than in such children on the river Solimoes, strongly suggests that zinc status is worse in rural riverside

Table 5. Differences in hair zinc levels by stunting and wasting classifications among rural Amazonian children from the River Negro and River Solimoes.

Location	Anthropometric classification	Hair zinc ($\mu\text{g/g}$)			Significantly different from
		N	mean	stand dev	
River Solimoes	1. Not stunted not wasted	22	178	89	10
	2. Stunted	44	215	124	
	3. Wasted	8	195	89	
	4. Stunted not wasted	38	218	129	
	5. All	68	202	114	
River Negro	6. Not stunted not wasted	6	120	81	9 8 5
	7. Stunted	46	149	97	
	8. Wasted	11	86	35	
	9. Stunted not wasted	42	158	100	
	10. All	53	148	95	

Table 6. Hair zinc level ($\mu\text{g/g}$) by gastrointestinal parasite infection in rural children from the River Negro, Amazonas.

G.I Parasite	Positive			Negative			Significant difference
	N	Mean	Standard Error	N	Mean	Standard Error	
Hookworm	25	163	17	14	128	31	N.S.
Ascaris	28	172	19	11	79	18	P < 0.01
Trichuris	29	166	19	10	107	19	P < 0.05

populations of the black water river Negro. That wasting was significantly associated with the low mean hair zinc levels on the river Negro but not the Solimoes, suggests that the process of wasting is associated with greater zinc deficiency in preschool children on the river Negro. Wasting in young children is commonly associated with diarrhoea and diarrhoea greatly increases zinc losses (Hambidge 1999). However, this was not the case in the River Solimoes sample.

Although stunting is considered a functional indicator of zinc status (King et al 2016), it must be recognized that the process of stunting is not the result of chronic processes ongoing throughout the preschool years, but is concentrated in the first one thousand days, from conception to two years of age (Shrimpton et al 2001). Deficient hair zinc levels ($<70 \mu\text{g/g}$) were more than twice as common among preschool children on the river Negro than the river Solimoes, although this difference was not significant. Zinc deficient hair was found more often in children 1-3 years old, but few younger than this were included in the analysis. This most probably reflects the difficulty of getting sufficient hair from young babies.

Our original method for hair zinc analysis used a sample weight of 40mg. However, difficulty was often encountered in collecting this quantity of hair. Babies' hair tended to be very fine and many mothers were reluctant to let it be cut. In older children it was common for boys to have close cropped hair and repeated cuts were needed at some sites. Using a homogenous mixture of our own finely cut up hair we tested the accuracy

and precision of the method and found that smaller sample weights tended to produce higher zinc values with less precision. Six replicates of 10, 20 and 40 mg hair samples were analysed for their zinc content and while the results obtained with 20mg were the same as 40mg samples, the 10mg hair samples gave a 10% higher mean with double the coefficient of variation. In order to be able to include the maximum number of hair zinc samples in the data analysis we included all results obtained on hair samples weighing 10mg or more. As neither the mean hair sample weights nor the distribution of hair sample weights on the lower river Negro and the Solimoes were significantly different (results not shown) we don't consider that this influenced the results obtained.

Hair zinc is still not widely accepted as a biomarker of zinc status, largely because of the lack of standardized methods for sampling and washing samples, and their analysis (King et al 2016). Fully aware of these problems, as already described by others (Dorea, Paine 1985), we avoided as many of the known sources of error as possible. Hair samples were always collected from the nape of the neck and only the 3cm nearest to the scalp kept for analysis. Washing and drying, as well as the digestion methods we used were all in line with modern recommendations (Dogan and Kaya 2010). Furthermore, atomic absorption spectrophotometry is still the most used technique for the analysis of elements such as zinc in mammalian cell materials with low concentration levels (Cerchiaro, Manieri and Bertuchi 2013).

That hair zinc levels were higher in children that were infected with *Ascaris lumbricoides* and *T. Trichuris* as compared to those without such gastrointestinal infections is unexpected. As zinc deficiency impairs immune responses against parasitic infections in animal models (Scott, Koski 2000) one might have expected lower hair zinc levels in parasitized children. Higher serum zinc levels have been observed in school children infected with soil transmitted helminths in Vietnam (Gier et al 2016), and attributed to an anti-inflammatory response, that leads to raised serum zinc levels. Whether these mechanisms could also be influencing hair zinc levels is something that should be investigated. It certainly suggests that while low hair zinc is a sign of zinc deficiency, high hair zinc levels do not necessarily mean zinc status is satisfactory.

The importance of enteroparasites in the Amazon cannot be denied, because the levels of infestation are so high, with an extensive geographic distribution and principally affecting low income populations groups. That eighty percent of families on the river Negro defecated in the forest while almost three quarters of families on the river Solimoes used a pit latrine does suggest that there may be differences in exposure to faeces across the two rivers. The situation on the river Negro doesn't seem to have improved as a recent survey in the city of Barcelos found almost 80% of those studied had at least one gastrointestinal parasite, with *Ascaris*, *Trichuris* and Hookworm being the most common (Gonçalves et al 2016). Studies in three capitals of the eastern Brazilian Amazon found *Ascaris* and *Trichuris* to be the most prevalent helminths affecting 70% of preschool children (Marinho 2000). Furthermore, these authors found that the absence of a latrine was the main risk factor for *Ascaris* and having earth or wooden floors the main risk factor for hookworm.

It is tempting to speculate that the poorer reproductive history found among mothers on the river Negro as compared to those on the river Solimoes might be related to a poorer zinc status. A large body of evidence supports the concept that human pregnancy outcome is significantly influenced by nutritional status of the mother, and that zinc deficiency in particular produces effects ranging from infertility and embryo/foetal death, to intrauterine growth retardation and teratogenesis (Uriu-Adams, Keen 2011).

Increased zinc intake has been shown to reduce the damage to DNA in white blood cells even without changing plasma zinc levels in Ethiopian women receiving supplements (Joray et al 2015) and in American men on a restricted zinc diet (Zyba et al 2017). While there is some evidence that zinc supplementation during pregnancy reduces preterm births (Ota et al, 2015), the difficulties of studying such interventions, both before and during early pregnancy, limit the availability of evidence that such interventions improve fertility and other foetal outcomes even though observational studies suggest this to be the case (Ramakrishnan et al 2012).

It is hard to make a definitive assessment of the adequacy of the diet on the two rivers from the food frequency surveys that were performed. With little consumption of cereals or pulses, and a high consumption of cassava flour, the main source of dietary zinc will be from animal protein sources. In these surveys, the consumption of game meat the day before was restricted to a quarter of the mothers on the river Negro, whereas two thirds had consumed fish. On the Solimoes three quarters of mothers had eaten fish and just under a half had consumed some form of meat, mostly chicken. Studies in Manaus have shown that for zinc requirements to be met in the typical Amazon diet based on cassava, the protein should have a zinc to energy ratio of at least 2.2mg/MJ, and the white meat of chicken for example doesn't meet that level (Shrimpton 1984). The most commonly consumed fish in Manaus were relatively poor in zinc (Rocha et al 1982), and zinc intake consequently low and linked to poor zinc status (Shrimpton et al 1983). Other more recent studies also confirm that Amazonian diets, largely based on fish and cassava flour, although adequate for energy and protein, are still likely to be insufficient for micronutrients such as zinc (Dufour et al 2016). Studies in the Peruvian Amazon found that the more diverse a diet was, using traditional nutrient dense local foods, the better the quality of the diets with regard to their micronutrient content (Roche et al 2007).

Earth eating or pica amongst children was reported by just 6% of mothers on the river Negro and 3% of mothers on the river Solimoes. According to health staff that we met on both rivers, geophagia was common, although something that mothers were loath to admit to. In Portuguese it is called a "*vicio*" or a vice, and something that children would be strongly discouraged from doing. Pica was found to be associated with zinc deficiency in the middle east (Prasad et al 1961), and a case of pica in the USA was reported to have been cured by zinc supplementation (Hambidge, Silverman 1973). Pica is now recognized to be significantly associated with increased risk for anaemia and low haemoglobin, haematocrit, and plasma zinc levels (Miao et al, 2015). Pica is something that was frequently described in early accounts of health conditions in the Amazon. Spix and von Martius (1976) said that in 1819 it was "common to all the Indian inhabitants", who "ate clay either with their fish or cassava flour, or as a desert".

The ethnic origins of the ribeirinhos include the original indigenous natives, plus the Portuguese and other Europeans attracted to the Amazon during the rubber boom, as well as slaves from Africa. All of these different races have now become homogenized in these "ribeirinho" populations and their common language is now Portuguese. Those on the Solimoes are more involved in agricultural activities and those on the Negro more in "extractivism", all of which exert a major influence on how they live their lives (Lira, Chaves 2016).

There is little evidence to suggest that conditions for the families on the rivers Negro and Solimoes have improved significantly in the four decades that have passed since these surveys were conducted. In the past, it is likely that the turtle and the manatee

were the animal meats that filled the role of satisfying zinc requirements in the rural Amazon dietaries. With both species now protected and/or rarely encountered, the pressure of hunting activities has increasingly been directed towards the terrestrial fauna of the Amazon. Bushmeat was still found recently to be a critically important source of zinc in urban/peri-urban inhabitants of the western Amazon on the border between Peru and Brazil (Sarti et al 2015). However, the tendency today as the populations of the Amazon become more and more domesticated and urbanized, is for bushmeat to be replaced by the consumption of chicken and frozen fish (Nardoto et al 2011; Van Vliet et al 2015), both of which are relatively poor zinc sources. Furthermore, obesity is increasing among the Ribeirinhos, as their diets change under the increasing influence of urban areas (Piperata 2007).

The scarcity of humans in the Amazon basin, rich in both fauna and flora, has long perplexed researchers. There is evidence of that prior to European conquest, there were large settled populations, especially in the fertile dark earths, living close to the banks of the Amazon (Clement et al 2015). However, the low chemical fertility of most Amazon soils has long been suggested as the major environmental factor limiting extensive colonization of the Amazon region (Batista 1963; Meggers 1971), principally due to the poor diets which in the past produced the shortest adults of all the regions of Brazil (Castro 1955). Some suggested it was the low availability of animal protein that limited the size, density and permanence of indigenous settlements (Gross 1975). Our studies suggest it might not be the protein, but the zinc often associated with it that could be the limiting factor. Studies in women from eight towns spread across the Brazilian Amazon have found low levels of zinc intake (Lehti 1989), as well as low levels of zinc in breast milk (Lehti 1990). Similarly, a study in three capitals of the Eastern Brazilian Amazon, verified that serum zinc levels were below normal in 48.4% of preschool children (Marinho 2000).

The nutrition intervention most commonly promoted globally for mothers during pregnancy is that of iron supplementation for anaemia, even though it is poorly implemented almost everywhere (Mason et al 2013). As the mothers in the Amazon are typically not only iron and vitamin A deficient (Neves et al 2019) but also zinc deficient, it would seem important that instead of the iron folate supplements that are currently provided in Brazil, multiple micronutrient supplements that also contain zinc should be taken during pregnancy (Haider, Bhutta 2017). These supplements should ideally be promoted as part of preparations for the mother getting pregnant, rather than waiting to be taken during pregnancy.

Conclusions

In studies conducted in 1976-7, zinc status of children from the river Negro was found to have been worse on the river Negro than on the river Solimoes. Hair zinc values of the children from the infertile “terra firme” regions of the river Negro were significantly lower than those from the fertile “varzea” regions of the river Solimoes.

Mothers on the river Negro had a poorer reproductive history than mothers on the river Solimoes. The still birth rate was 7 times higher among the mothers on the River Negro than in those on the River Solimoes, and the infant mortality rate was also 22% higher. While direct evidence of the zinc status of mothers was not obtained on any of these two rivers, zinc status of mothers in the river Negro region may not have been adequate, as their young children had a poor zinc status. These facts support the hypothesis that

poor zinc status may be associated with the lower population densities found along the banks of the river Negro as compared to that of the river Solimoes.

Since modern research suggests that zinc status has not improved in this region, direct interventions to improve zinc status before conception in Amazonian mothers would seem to be needed, and multiple micronutrient supplements exist that can be used for this purpose. This would not only correct any zinc deficiency, but also contribute to reducing the iron deficiency anaemia that most mothers in the region also suffer from.

References

- Armitage P 1971. *Statistical methods in medical research*. Oxford: Blackwell Scientific Publications.
- Batista D 1963. Da habitabilidade da Amazônia. *Cadernos da Amazônia*, INPA, 39p.
- Castro J 1955. *Geopolítica da Fome*. Rio de Janeiro: Livraria Editora Casa do Estudante do Brasil. 3a. Ed., 350p.
- Cerchiaro G, Manieri TM, Bertuchi FR 2013 Analytical methods for copper, zinc and iron quantification in mammalian cells. *Metallomics* 5:1336-1345.
- Clement C, Denevan W, Heckenberger M et al 2015. Amazonia: The rain forest is a human creation. *World Nutrition* 6(9-10):694-703.
- de Benoist B, Darnton-Hill I, Davidsson L, et al 2007. Conclusions of the Joint Report WHO/UNICEF/IAEA/IZINCG Interagency meeting on zinc status indicators. *Food and Nutrition Bulletin* 28: S480-48
- Dufour DL, Piperata BA, Murrieta RSS, et al 2016. Amazonian foods and implications for human biology. *Annals of Human Biology* 43(4): 330-348.
- Dogan S, Kaya FND 2010. Determination of zinc and lead in human hair by atomic absorption spectrophotometry after digestion with tetramethylammonium hydroxide and conventional methods. *Trace Elements and Electrolytes* 27(3):110-114.
- Dorea JG, Paine PA 1985. Hair zinc in children: its uses, limitations and relationship to plasma zinc and anthropometry. *Human Nutrition: Clinical Nutrition* 39(6):389-98.
- Giugliano R, Giugliano LG, Shrimpton R 1981. Estudos nutricionais das populações rurais da Amazônia: I. Várzea do Rio Solimões. *Acta Amazonica* 11(4):773-788.
- Giugliano R, Shrimpton R, Marinho HA, et al 1984. Estudos nutricionais das populações rurais da Amazônia II. Rio Negro. *Acta Amazonica* 14(3-4):427-449.
- Gonçalves AQ, Junqueira ACV, del Barrio PC, et al 2016. Prevalence of intestinal parasites and risk factors for specific and multiple helminth infections in a remote city of the Brazilian Amazon. *Revista da Sociedade Brasileira de Medicina Tropical* 49(1):119-124.
- Gross DR 1975. Protein capture and cultural development in the Amazon Basin. *American Anthropologist* 77:526-540.
- Haider BA, Bhutta ZA 2017. Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database of Systematic Reviews* Issue 4, Article No.: CD004905.
- Hambidge KM, Hambidge C, Jacobs M, Baum JD 1972. Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. *Pediatric Research* 6:868-874.
- Hambidge KM, Silverman A 1973. Pica with rapid improvement after dietary zinc supplementation. *Archives of Diseases of Childhood* 48:567-568

- Hambidge KM 1999. Zinc and Diarrhoea. *Acta Paediatrica Supplement* 381:82-6.
- Jelliffe DB 1966. The assessment of the nutritional status of the community. WHO Monograph series 53:1-235. Geneva: World Health Organization.
- Joray ML, Yu T-W, Ho E, et al 2015. Zinc supplementation reduced DNA breaks in Ethiopian women. *Nutrition Research* 35(1): 49-55.
- King JC, Brown KH, Gibson RS, Krebs NF, Lowe NM, Siekmann JH, Raiten DJ 2016. Biomarkers of Nutrition for Development (BOND)-Zinc Review. *J Nutr.* 146(Suppl):858S–885S.
- Lehti KK 1989. Iron, folic acid and zinc intakes and status of low socio-economic pregnant and lactating Amazonian women. *Eur J Clin Nutr.* 43(8):505-13.
- Lehti KK 1990. Breast milk folic acid and zinc concentrations of lactating, low socio-economic, Amazonian women and the effect of age and parity on the same two nutrients. *Eur J Clin Nutr.* 44(9):675-80.
- Lira T de M, Chaves MPSR 2016. Comunidades ribeirinhas na Amazônia: organização sociocultural e política. *INTERAÇÕES* 17(1)66-76.
- Lowe NM, Fekete K, Decsi T 2009. Methods of assessment of zinc status in humans: a systematic review. *Am J Clin Nutr.* 89(6):2040S-2051S.
- MacDonald RS 2000. The role of zinc in growth and cell proliferation. *Journal of Nutrition* 130:1500s–1508s.
- Marinho H 2000. Prevalência da Deficiência de Vitamina A em três Capitais da Amazônia Ocidental Brasileira. Tese de Doutorado. Universidade de São Paulo. 124 p.
- Megggers B 1971. Amazonia: man and culture in a counterfeit paradise. Chicago: Aldine-Atherton, 182p.
- Mason J, Martorell R, Saldanha L, et al 2013. Reduction of anaemia. *Lancet Glob Health* 1(1):e4-6.
- Miao D, Young SK, Golden CG 2015. A meta-analysis of pica and micronutrient status. *Am J Hum Biol.* 27(1):84–93.
- Nardoto GB, Murrieta RSS, Prates LEG, et al 2011. Frozen chicken for wild fish: Nutritional transition in the Brazilian Amazon Region determined by carbon and nitrogen stable isotope ratios in fingernails. *American Journal of Human Biology* 23:642-650.
- Neves PAR, Lourenço BH, Pincelli A, et al 2019. High prevalence of gestational night blindness and maternal anemia in a population-based survey of Brazilian Amazonian postpartum women. *PLoS One* 14(7):e0219203.
- Ota E, Mori R, Middleton P, et al 2015. Zinc supplementation for improving pregnancy and infant outcome. *Cochrane Database of Systematic Reviews Issue 2.* Art. No.: CD000230.
- Perkin E 1976. Analytical methods for atomic absorption spectroscopy. Norwalk, Connecticut: The Perkin Elmer Corporation.

- Piperata BA 2007. Nutritional status of Ribeirinhos in Brazil and the nutrition transition. *Am J Phys Anthropol.* 133:868-878
- Prasad AS, Halsted JA, Nadimi M 1961. Syndrome of iron deficiency anaemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *Am. J. Med.* 31:532-546.
- Ramakrishnan U, Grant F, Goldenberg T, et al 2012. Effect of women's nutrition before and during early pregnancy on maternal and infant outcomes: A systematic review. *Pediatric and Perinatal Epidemiology* 26 (Suppl. 1):285-301.
- Rocha YS, Aguiar JLP, Shrimpton R 1982. Aspectos nutritivos de alguns peixes da Amazônia. *Acta Amazonica* 12(3):787-794.
- Roche ML, Creed-Kanashiro HM, Tuesta I, Kuhnlein HV 2007. Traditional food diversity predicts dietary quality for the Awajún in the Peruvian Amazon. *Public Health Nutrition* 11(5): 457-465.
- Sapero JJ, Lawless DK 1953. The MIF stain-preservation technic for the identification of intestinal protozoa. *Am J Trop Med Hyg.* (4):613-9
- Sarti FMC, Adams C, Morsello N, et al 2015. Beyond protein intake: bushmeat as source of micronutrients in the Amazon. *Ecology and Society* 20(4):22.
- Scott ME, Koski GK 2000. Zinc deficiency impairs immune response against parasitic nematode infections at intestinal and systemic sites. *J. Nutrition.* 130 (5): 1412S-1420S.
- Shrimpton R 1980. Studies on Zinc Nutrition in the Amazon Valley. PhD Thesis. University of London. 326p.
- Shrimpton R, Franca TS, Rocha YS, et al 1983. "Estudos sobre o estado nutricional em relação ao zinco na Amazônia 1. Níveis de zinco no soro e ingestão de zinco em operários de Manaus, 1978" *Acta Amazonica* 13(1):73 94.
- Shrimpton R 1984. "Food and Nutrient intake by income in 1200 families of Manaus, Amazonas, Brazil". *Archivos Latinoam. Nutr.* 349(4):615 629.
- Shrimpton R, Victora CG, Onis M, et al 2001. The worldwide timing of growth faltering: implications for nutritional interventions. *Pediatrics* 107(5) e75.
- Shrimpton R, Marinho H 2018. The effects of zinc supplementation on zinc, retinol and carotene levels in lactating Amazonian women. *World Nutrition* 9(1): 4-21
- Sorenson JRJ, Melby EG, Nord, P.J, et al 1973. Interferences in the determination of metallic elements in human hair. *Archives of Environmental Health* 27:36-39
- Spix JB, von Martius CFP 1976. Viagem pelo Brasil, 1817-1820. Volume. 3. Tradução. Sao Paulo: L.F. Lahmeyer, Editora Melhoramentos. 335p.
- Stuart H.C, Stevenson SS (1959). In: Nelson WE. Ed. *Textbook of Paediatrics*. Philadelphia: Saunders.
- Uriu-Adams JY, Keen CL 2011. Zinc and Reproduction: Effects of zinc deficiency on prenatal and early postnatal development. *Birth Defects Research (Part B)* 89:313-325.

Van Vliet N, Quiceno-Mesa MP, Cruz-Antia D, et al 2015. From fish and bushmeat to chicken nuggets: the nutrition transition in a continuum from rural to urban settings in the Colombian Amazon region. *Ethnobiology and Conservation* 4(6): 1-12.

Zyba SJ, Shenvi SV, Killilea DW, et al 2017. A moderate increase in dietary zinc reduces the DNA strand breaks in leukocytes and alters plasma proteins without changing plasma zinc concentrations. *American Journal of Clinical Nutrition* 105:343-351.