NMR in Analysis of the Nutritional Value of Lipids from Muscles and Livers of Wild Amazonian Fishes with Different Eating Habits over Seasonal Variation

Banny Correia, Gilberto Gaspar Duarte Ortin, Maiara da Silva Santos, Raquel Susana Torrinhas, Natalia Cristina Mor, Adalberto Luis Val, Ljubica Tasic

Submitted date: 31/01/2020 • Posted date: 03/02/2020
Licence: CC BY-NC 4.0

Citation information: Correia, Banny; Ortin, Gilberto Gaspar Duarte; da Silva Santos, Maiara; Torrinhas, Raquel Susana; Mor, Natalia Cristina; Val, Adalberto Luis; et al. (2020): NMR in Analysis of the Nutritional Value of Lipids from Muscles and Livers of Wild Amazonian Fishes with Different Eating Habits over Seasonal Variation. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.11771388.v1

Lipid composition of the Amazonian fishes remains unexplored although fishes in general show very high nutritional potential. Endogenous and environmental factors can influence the lipid contents of fishes among which, in the Amazon River, seasonal dynamics influences stand out. Herein, nine most consumed fish species were analyzed and their lipid composition evaluated in terms of effects of tissue from where were extracted, season of the Amazon River and the fish eating habits. Higher amounts of lipids were obtained from livers than dorsal muscles in all studied species. Statistical analysis has shown that Amazonian fishes present different lipid profiles according to their eating habits, which mainly comprises saturated fatty acids to distinguish detritivorous livers, and linolenic acid, cholesterol, polar lipids for carnivorous and piscivorous fish muscles. Furthermore, in Amazonian fish, some very important lipids for human nutrition are present, such as omega 3 and 6 fatty acids whose availability depended on the tissue metabolism and fishes’ eating habit along the seasonal periods. For example, our findings indicate that the piscivorous fish C. monoculus presented higher levels of linoleic acid for liver than linolenic acid and the opposite occurred for muscles. The omega 6 and 3 fatty acids ratio was influenced by the season dynamic of the Amazon River and availability of food according with each specific eating habit, poiting mainly to the piscivorous fishes as the healthiest fish for human consumption.

File list (2)

ChemRix_Final.pdf (2.06 MiB) view on ChemRxiv  download file
JAFC_Final SI.pdf (0.99 MiB) view on ChemRxiv  download file
NMR in analysis of the nutritional value of lipids from muscles and livers of wild Amazonian fishes with different eating habits over seasonal variation

Banny Silva Barbosa Correia*1, Gilberto Gaspar Duarte Ortin1, Maiara da Silva Santos2, Raquel Susana Torrinhas3, Natalia Cristina Mor4, Adalberto Luis Val5, Ljubica Tasic*1.

1Institute of Chemistry, State University of Campinas, Rua José de Castro, s/n - Cidade Universitária, 13083-970, Campinas, Sao Paulo, Brazil
2Department of Organic Chemistry, Center for Exact and Technological Sciences, Federal University of Sao Carlos, Rodovia Washington Luis s/n Km 235, 13565-90, Sao Carlos, Sao Paulo, Brazil.
3Department of Gastroenterology, Surgical Division (LIM 35), Medical school, University of Sao Paulo, Av. Dr. Arnaldo, 455, Cerqueira César, 01246903, Sao Paulo, Sao Paulo, Brazil.
4Faculty of Pharmaceutical Sciences, State University of Campinas, Rua Cândido Portinari, 200, Cidade Universitária, 13083-87, Campinas, Sao Paulo, Brazil
5Department of Ecology, National Institute of Amazonian Research, Avenida André Araújo, 2936, Petrópolis, 69067-375, Manaus, Amazonas, Brazil

* Correspondence:
Ljubica Tasic ljubica@unicamp.br
Banny Silva Barbosa Correia banny.barbosa@gmail.com

Abstract: Lipid composition of the Amazonian fishes remains unexplored although fishes in general show very high nutritional potential. Endogenous and environmental factors can influence the lipid contents of fishes among which, in the Amazon River, seasonal dynamics influences stand out. Herein, nine most consumed fish species were analyzed and their lipid composition evaluated in terms of effects of tissue from where were extracted, season of the Amazon River and the fish eating habits. Higher amounts of lipids were obtained from livers than dorsal muscles in all studied species. Statistical
analysis has shown that Amazonian fishes present different lipid profiles according to their eating habits, which mainly comprises saturated fatty acids to distinguish detritivorous livers, and linolenic acid, cholesterol, polar lipids for carnivorous and piscivorous fish muscles. Furthermore, in Amazonian fish, some very important lipids for human nutrition are present, such as omega 3 and 6 fatty acids whose availability depended on the tissue metabolism and fishes’ eating habit along the seasonal periods. For example, our findings indicate that the piscivorous fish C. monoculus presented higher levels of linoleic acid for liver than linolenic acid and the opposite occurred for muscles. The omega 6 and 3 fatty acids ratio was influenced by the season dynamic of the Amazon River and availability of food according with each specific eating habit, poiting mainly to the piscivorous fishes as the healthiest fish for human consumption.

**Keywords**: lipids, Amazonian fish, omega 3 & 6, nutrition, nuclear magnetic resonance

**INTRODUCTION**

The consumption index of fishes by the Amazon inhabitants is one of the highest in the world. [1, 2, 3, 4] It is estimated as > 50 kg of fishes/person/year in the Manaus city and as 510-600 g/day in the countryside. [5] The top ten Amazonian fishes most consumed rankings are 1) Colossoma macropomum, 2) Semaprochilodus insignis, 3) Prochilodus nigricans, 4) Brycon amazonicus, 5) Cichla monoculus, 6) Mylossoma duriventre, 7) Triportheus elongate, 8) Plagioscion squamosissimus, 9) Piaractus brachypomus, and 10) Pseudoplatystoma tigrinum. The Hypophthalmus edentates could be added to this list as a peculiar taste dish mainly at Para State due to its fat amounts. [3, 1] Indeed, it is an interesting fact that there is a preference in consumption of a few fish species (100 species), regardless the fact that over 2,500 fish species live in the Amazon River. Also, Amazon inhabitants avoid to consume no scales fish, which are considered as very greasy and harmful to the health [6], in fact they recommend to eat piscivorous or carnivorous fishes when the person is ill. Therefore, they prefer big fat fishes to celebration occasions and some species are consumed with the entire inside content, organs and fish ova as Hypostomus affinis. [7]

Fishes meat is considered as a great source of essential fatty acids, such as docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) recommended to a human diet. [8, 9, 10] These polyunsaturated and very long chain fatty
Acids have been shown to have a positive impact on human health since they can prevent many diseases, including cardiovascular, neurological, autoimmune diseases, and cancer. [8, 11, 9]

Amazonian fishes’ diet can vary among living plants (herbivorous), plankton (planktvorous), debris (detritivorous), mud (iliophagous), blood (haematovorous), non-fish animals (carnivorous), fish (piscivorous) or both plants and animals (omnivorous). These feeding niches fluctuate depending on the seasonal dynamics of the river flooding (distributed in low, rising, high, and falling water periods), once the concentration of river's water favours specific predatory hunting and spatial distribution that imposes new biomes, new habitats, and consequent drastic changes in food sources availability. [12, 13, 14] It is why chemical composition of Amazonian fish may be significantly variable, once besides Amazon River Basin harbours the largest worldwide diversity of freshwater fish, including singular native species, the particular ecology of the area provides a varied nutrient source and a challenging environment that can influence the biochemical pattern of inhabiting fishes. [2]

The lipid composition of Amazonian fish, as well as freshwater fish in general, had been reported as fatty acids and cholesterol composition mainly represented by the amount of its bioactive polyunsaturated fatty acids (omega 3 and 6) per species fish and/or place, which could be from farm or wild environment. [15, 16, 17, 18, 19, 20, 21] The amounts of fatty acids found can be varied among the freshwater fish, however usually present the same fatty acids, which are those with 14-22 carbons, comprises saturated and unsaturated chains, presenting the C18 series the most variable in unsaturation possibilities. The total amount of the unsaturated fatty acids can be presented as higher than the saturated fatty acids sum in many cases [16, 22], however also the opposite can occur. [17] In the majority of papers, only dorsal muscles’ lipids were evaluated, although some works reported on other tissues’ lipids, such as liver, eyeball, and brain [20, 23, 24, 18].

While wild freshwater fish lipids reports are focused on the fatty acid profiles, the global lipidomes, which could be influenced by several factors, are poorly characterized. Also, many works showed and investigated DHA and EPA as the main bioactive polyunsaturated fatty acids in fish, however the precursor of these important fatty acids, which are the omega-3 linolenic acid for DHA, and EPA, and the omega-6 linoleic acid for arachidonic acid (AA, 20: 4n-6) [25, 26], are often set aside in the discussion even if those are more abundant than ones with the longer chains [16]. Nevertheless, ratio of
linoleic and linolenic acids in fish can indicate their potential to produce EPA, DHA and ARA, besides of suggesting how healthy is that food source since the balance between omega 6 and 3 must be less than 5:1 to be considered healthy. [27]

Thus, our aims were to discriminate the lipid differences of the fish species most consumed by Amazonian population according to their eating habits and to seasonal variation of Amazon River, and, also, determine linoleic and linolenic amounts in nine the most consumed fishes by Amazonians.

**MATERIALS AND METHODS**

**Sampling**

The top nine most consumed species of Amazonian fishes (*Colossoma macropomum, Triportheus elongate, Brycon amazonicus, Prochilodus nigricans, Semaprochilodus insignis, Pseudoplatystoma tigrinum, Cichla monoculus, Hypophthalmus edentates, and Plagioscion squamosissimus*) were collected (approved study protocol by Brazilian Institute of the Environment and Renewable Natural Resources-IBAMA, license No. 29837 and 39985) at the Catalao Lake using the floating base of Ecophysiology and Molecular Evolution Laboratory of National Institute of Amazonian Research (INPA) in six points disposed in Supplementary information: 1) S 03°10.570´ W 059°55.000´, 2) S 03°10.736´ W 059°54.180´, 3) S 03°09.967´ W 059°54.534´, 4) S 03°09.746´ W 059°54.488´, 5) S 03°10.030´ W 059°55.579´ and 6) S 03°10.437´ W 059°54.277´ (SI, see Fig.S1). Fishes were captured with sizes bigger than that corresponding to their respective sexual maturity during the Amazon flood (08-12 July) and drought (26-29 November) periods in 2013.

The recommended protocol for euthanasia, using ice and water at -4 °C, was performed according to national animal care regulations, which was approved by the Ethics Committee on Animal Experiments of INPA under registration N° 026/2015. Samples of muscle and liver were collected using individual scalpel blades and kept frozen at -20 °C and transferred to the State University of Campinas (UNICAMP) laboratory. The species were identified as described elsewhere [1] and grouped by their eating habits as (1) omnivorous, (2) detritivorous, (3) piscivorous, (4) planktivorous and (5) carnivorous. A scheme that illustrates the number of captured fish species and success of tissue obtaining are summarized at Supplementary Information (SI, see Fig.S2). In
summary, a total of 93 specimen of fishes were collected. From those 170 samples were obtained, comprising 93 muscles and 77 livers, in which 70 are from flood period and 100 from drought period.

**Lipid extraction**

The dorsal muscles and livers tissues were submitted to protocols for lipids extraction as described in literature. [28, 29, 30] Total lipids’ extracts were fractionated into three classes of lipids with different polarities as described elsewhere. [29, 30] We have extracted 224 lipid samples from 9 fish species (170 samples of total lipids from livers and muscles and 54 samples of lipids classes from muscles).

All lipid samples were stored at -80 °C until analysed.

**NMR analysis**

5 mg of lipid samples were dissolved in 600 µL of 99.8% deuterated chloroform (CDCl3; Cambridge Isotope Laboratories, Inc., USA) and transferred to NMR tubes (5 mm). 1H and 13C NMR analyses were conducted using a Bruker ADVANCE 600 MHz spectrometer equipped with a Triple Band Inverse (TBI) probe. Used conditions for qualitative and quantitative analysis were as those described previously. [30]

**Data processing**

The obtained 1H-NMR spectra (170) from total lipids were referenced to tetramethyl silane, aligned and had their phase corrected, then binned (0.04 ppm) using MestReNova38 (Mestrelab Research SI) and saved as the data matrix. Principal component analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were performed using MetaboAnalyst 3.0 platform (http://www.metaboanalyst.ca/faces/home.xhtml), applying the parameters used by Mor et al. [30]

The levels of linolenic and linoleic acids were calculated from the quantitative 1H-NMR data according to the method previously reported by others [31, 13], where the concentrations of fatty acids are expressed in molar percentages according to the equations 1 and 2.

**Equations**

\[ Ln \% = 100 \times \frac{A_{Ln}}{3A_0} \]

(Equation 1)
Where $A_{Lr}$ and $A_L$ are the areas of the bis-allylic proton peaks for linolenic ($\omega$-3) and linoleic ($\omega$-6) acids, respectively, and $A_G$ is the area of the proton peaks of glycelyl group.

**RESULTS AND DISCUSSION**

Lipids of wild Amazonian fishes over seasonal variation

The lipids amount extracted from dorsal muscle and liver of nine Amazonian fishes showed to be depended on the analysed tissue, where those from dorsal muscle showed 3–24 mg per gram of the muscle mass (exceptions were planktivorous fish), while total lipids extracted from liver were in the range of 12–65 mg per gram of liver mass (exceptions were planktivorous fish) (Fig. 1). Planktivorous fish showed very close values for lipids, independent from where they were obtained, 88–154 mg per gram of the muscle mass and 85 mg per gram of liver mass.

![Figure 1](image-url). Total lipids (mg/g of tissue) of Amazonian fishes with five different eating habits extracted from the dorsal muscle or liver at flood or drought period of Amazon River. P.s., *Plagioscion squamosissimus* (carnivorous); S.i., *Semaprochilodus insignis* and P.n., *Prochilodus nigricans* (detritivorous); B.a., *Brycon amazonicus*; T.e., *Triportheus elongatus* and C.ma. *Colossoma macropomum* (omnivorous); C. mo., *Cichla monoculus* and P.t., *Pseudoplatystoma tigrinum* (piscivorous); H.e., *Hypophthalmus edentates* (planktivorous).
The studied species of Amazonian fishes presented higher lipid amounts in livers than in muscles, irrespective of the seasonal period. This is coherent with the fact that liver is a highly metabolic organ. [32] However, the exception were two species *B. amazonicus* and *H. edentates*, which presented lower lipid amounts in livers than in muscles.

For the *H. edentates*, Carvalho et al. showed that this fish does not deposit fat, instead the lipids are diffused in the muscular tissue. [33] This fish as well the other studied Amazonian fish species experienced an important change in total lipid amounts of dorsal muscles and livers in response to seasonal dynamics of Amazon river (flood and drought) among the eating habits. Lipid amounts varied according to the eating habit groups, as follows (from the highest to the lowest levels): planktivorous > *B. amazonicus* (omnivorous) > detritivorous > omnivorous (except *B. amazonicus*) > piscivorous > carnivorous for muscles at flood period. At drought season the lipid amounts rankings from muscles presented: planktivorous > *P. nigricans* (detritivorous) > omnivorous > *S. insignis* (detritivorous) > carnivorous > piscivorous at drought season. For livers following the *C. macropomum* (omnivorous) > carnivorous > omnivorous (except *C. macropomum*) > detritivorous > piscivorous at flood season; planktivorous > omnivorous > piscivorous > carnivorous > detritivorous at drought period. It means that in general muscles presented planktivorous as the fattest fish, followed by detritivorous and omnivorous, and as the fish containing less fat, carnivorous and piscivorous took places. These findings are in line with previous studies reporting an influence of different feeding habits on the total lipid content in fishes and also agree with the popular knowledge. For example, *H. edentates* is a planktivorous fish and it used as a dish which tastes fat, while piscivorous or carnivorous fishes are preferred and recommend to ill people because those are considered no greasy and good to the health. [18, 7]

An interesting fact is that lipids from carnivorous and piscivorous fish did not suffer an apparent influence of season dynamics and the food availability did not affect total lipids contents. However, distribution of the lipids (types) in studied seasons is an issue to debate. Liver and muscle also may contain different proportions of lipids when classified per their polarities. The lipid class profiles response to the seasonal dynamic in dorsal muscles for the studied Amazonian fishes are illustrated in Figure 2. The higher amounts of phospholipids were found in the dorsal muscle of piscivorous fishes at flood period when compared with samples from the drought period, while the opposite pattern was found for carnivorous specie. The opposite was observed for neutral lipids. Only
piscivorous and the *P. nigricans* (detritivorous) had their phospholipids increased in flood periods, all the other fish species had the neutral lipids increased in flood periods instead of phospholipids. For instance, the abundance of food sources for piscivorous habits occurs during the drought period, enabling fat storage as neutral lipids. [34]

![Figure 2](image)

**Figure 2.** Lipid classes (mg/g of oil) of Amazonian fishes with five different eating habits extracted from the dorsal muscle at A) flood or B) drought period of Amazon River. P.s., *Plagioscion squamosissimus* (carnivorous); S.i., *Semaprochilodus insignis* and P.n., *Prochilodus nigricans* (detritivorous); B.a., *Brycon amazonicus*; T.e., *Triportheus elongatus* and C.ma. *Colossoma macropomum* (omnivorous); C. mo., *Cichla monoculus* and P.t., *Pseudoplatystoma tigrinum* (piscivorous); H.e., *Hypophthalmus edentates* (planktivorous).

Although differences between periods may explain the seasonal availability of food sources, but they don’t explain detritivorous species lipids distribution once this type of fish shows quite variable diet. In this way, a distinct seasonal difference in muscle lipid class amount was observed between the two detritivorous fishes: *S. insignis* presented an increase of neutral lipid content balanced with the decrease of phospholipids in the flood period compared to drought period, while *P. nigricans* showed the opposite pattern. These findings may be explained by another fact, as the distinct seasonal migratory habits could be the causes of differences in lipids distribution. The *S. insignis* migrates for dispersion in rising water periods and *P. nigricans* in falling water periods. High amounts of neutral lipids may be required for fish migration, as their energy source. It was reported that dispersal migration of *S. insignis* is characterized by the accumulation of lipids (neutral lipids) while foraging in the flooded forest. [35] Also, it is noteworthy to state that even within a trophic category, there is enough diversity in the diet among the Amazonian fishes, or that it is difficult to classify them by eating habits. The two species *S. insignis*
and *P. nigricans* are considered as detritivorous, but they also consume genipap fruit at flood period. [36]

**Wild Amazonian fishes’ lipids identification**

Typical $^1$H NMR spectra for lipids extracted from the fish with characteristic eating habits are shown in Figure S3. Lipids were assigned according to reported NMR data. [37, 30, 38, 39] The assigned peaks are displayed in Table 1. [40].

**Table 1. Assignment of the main resonances in the $^1$H-NMR spectra of lipids isolated from the Amazonian fishes**

Legend: $^a$ linoleic acid (L), $^b$ linolenic acid (Ln), $^c$ Glycerol (G)

| Number | $^1$H Chemical shifts (ppm) | Assignments |
|--------|-----------------------------|--------------|
| 1      | 0.75-1.00                   | Terminal methyl protons -CH$_3$ |
| 2      | 0.93-1.02                   | -C$_3$H$_7$ protons of linolenyl chain (ω-3) |
| 3      | 1.20-1.50                   | Methylene protons of aliphatic chains -(CH$_2$)$_n$ |
| 4      | 1.50-1.75                   | β-methylene protons of the carbonyl-OOC-CH$_2$- |
| 5      | 1.95-2.10                   | Methylene protons in the α-position of double bonds –CH$_2$-CH=CH- |
| 6      | 2.20-2.50                   | Methylene protons in the carbonyl α-position –OOC-CH$_2$- |
| 7      | 2.70-2.84                   | CH$_2$-bis-allylic protons of polyunsaturated fatty acid (PUFA) chains |
| 8      | 2.80-2.90                   | Divinyl methylene protons =HC-CH$_2$-CH= |
| 9      | 2.79 $^a$                  | Divinyl methylene protons =HC-CH$_2$-CH= of linoleyl chain (ω-6) |
| 10     | 2.82 $^b$                  | Divinyl methylene protons =HC-CH$_2$-CH= of linolenyl chain (ω-3) |
| 11     | 3.44-3.59                  | CH of cholesterol relative to the C-3 proton |
| 12     | 4.10-4.30 $^c$             | Sn-1 and Sn-3 protons of glycerol -CH$_2$-OCOR |
| 13     | 5.25-5.50                  | Sn-2 protons of glycerol >CH-O-COR |
| 14     | 5.27-5.38                  | Protons of double bonds with conformation Z cis -CH=HC- |
| 15     | 5.30-5.40                  | CH of cholesterol relative to the C-6 proton of vinyl |
| 16     | 3.40-3.60                  | Heteroatom proton –OH |
| 17     | 3.65-3.75                  | Hexoses protons on α-carbon to the heteroatom |
|       | 3.88                       | Methine proton at C4 of galactose |
| 18     | 4.00-4.30                  | Protons on α-carbon to the heteroatom |
| 19     | 4.10-4.40                  | Protons on α-carbon to the heteroatom (OH) and β to the amine -O-CH$_2$-CH$_2$-N'(CH$_3$)$_3$ |
| 20     | 5.00                       | Anomeric carbon protons of galactose |
| 21     | 5.20-5.40                  | Amine protons –HN(CH$_3$)$_2$ |
| 22     | 3.10-3.20                  | Methylene protons α to the heteroatom –CH$_2$-OH |
| 23     | 3.20-3.40                  | Methyl protons of charged nitrogen N(CH$_3$)$_3$+ |
| 24     | 3.50-3.85                  | Methylene protons α to a charged nitrogen CH$_2$-N'(CH$_3$)$_3$ |
| 25     | 3.90-4.40                  | Methylene protons α to the heteroatom connected to phosphorus CH$_2$-O-P |
By grouping of fishes according to the influences of tissue metabolism, seasonal dynamics of the Amazon River and eating habits (Fig. 3), we were able to see and determine endogenous lipid patterns and their correlation with ecological behaviours. The PLS-DA using the $^1$H NMR spectral data as a matrix showed that the tissue metabolism (Fig. 3A) influenced the lipid profile of the studied Amazonian fishes more than the seasonal periods (Fig. 3B). This is very obvious when we correlate the studied seasonal periods with each tissue (Fig. 3C). In fact, we expected a big difference between the tissues as their specific functions can lead to different lipid composition.

![Figure 2](image.png)

**Figure 2.** PCA score charts constructed from $^1$H-NMR data of lipids isolated from Amazonian fishes versus A) tissue (liver or muscle); B) seasonal period (drought or flood); C) tissue and seasonal period; D) eating habit (carnivorous, detritivorous, omnivorous, piscivorous or planktivorous).

However, the seasonal periods had an important role in distinguish the lipid profile of the studied Amazonian fishes (Fig. 3B), showing that the chemical composition of Amazonian fishes may depend on the seasonal dynamics, which correlates with the type and amount of available food in Amazon river. [18] Also, the lipid profiles of samples
under study were important for separating groups of fishes with different eating habits (Fig. 3D) despite of the studied seasonal periods for each tissue.

The most pronounced lipid profiles differences can be seen in their variable importance projection (VIP) in Figure S4, which revealed general fatty acids, linoleic and linolenic acids besides cholesterol and/or polar lipids (phosphocholine, sphingomyelin or saccharolipids) as having higher intensity in muscle samples than liver. These differences are assigned, as described in Table 1, by the following NMR signals: 1.24, 5.34, 2.81 and 2.77 ppm (Fig. S4A). When we observe the season from which the fish samples came from, the flood period presented general fatty acids, while the drought period showed cholesterol and choline based lipids as the main difference between the seasons (Fig. S4B). These results are confirmed by the features for muscle in both season periods with cholesterol or polar lipids (phosphocholine, sphingomyelin or saccharolipids), and cholesterol or polar lipids for drought period independent from the tissue type (Fig. S4C). However, the lipid influence of the eating habits without considering the environmental factors does not present useful information to distinguish the samples, once the only general fatty acids was observed (Fig. S4D).

Separation of season sample type, i.e. drought or flood regardless of tissue factor and considering different eating habits, have found some singularity to the eating habits comparison (see Fig. S5). Detritivorous habit from both season periods had their lipid profile similar between them, as also omnivorous habits. Carnivorous and piscivorous presented similar lipid profile, further means carnivorous from drought period was similar to piscivorous from flood period and carnivorous from flood period was found to be similar to piscivorous from drought period. This fact perfectly explains by the food preferences being uniformly between each other. Planktivorous fish distinguish from the others responding similar to omnivorous when caught in flood period, which is the period that omnivorous have their food as seed and fruit more available, and similar to piscivorous when both were caught in drought period once again which is the period that piscivorous have their preference food most available (other fishes). [3]

Influences on Lipids composition and distribution in Amazonian fishes

Many factors can influence on the fish lipid profile such as season, tissue, food and eating behaviour (Fig. 4). As related in literature the season has an effect on the lipids profile of fish. [41] In the drought period livers were separated in distinct groups in which only planktivorous fish presented slight similarity to those from omnivorous habit (Fig.
4A), while muscle from this period had similarity between omnivorous and detritivorous (Fig. 4B). At the flood period, the liver presented distinct groups (Fig. 4C), and the muscles showed similarity between planktivorous and omnivorous again as at drought period, and between piscivorous and carnivorous (Fig. 4D).

Figure 3 PLS-DA charts constructed from $^1$H-NMR data of lipids isolated from Amazonian fishes versus eating habits showing score of A) liver from drought period; B) muscle from drought period; C) liver from flood period; D) muscle from flood period.

The top three features of eating habits samples by each sample type related to the Figure 4 is showed in Figure 5. These results revealed that at drought periods general fatty acids and saturated fatty acids were found in livers (Fig. 5A), with more intensity of response of saturated fatty acids for detritivorous fish, while muscles showed besides of general fatty acids, the linolenic acid and cholesterol or polar lipids (phosphocholine, sphingomyelin or saccharolipids) with higher concentration for carnivorous and piscivorous habit (Fig. 5B). At flood periods general fatty acids were found in both tissues, livers and muscles as important lipids that distinguish between eating habits (Fig.
5C, D) with higher intensity for piscivorous fish. Additionally, saturated fatty acids presented higher contribution to planktivorous fish for muscle.
Figure 5. Top 3 feature graphical summary (mean ± SD) obtained for the lipids of Amazonian fishes versus eating habits showing A) liver from drought period; B) muscle from drought period; C) liver from flood period; D) muscle from flood period.
Therefore, our data suggest that lipid composition of Amazon fishes can vary because of the fish feeding habits (mainly in quality), according to the biosynthetic tissue capacity (liver or muscle) to attend the fish individual needs (mainly in amount), which may be influenced by Amazon River seasonal changes. Linoleic and linolenic acids appeared at muscle tissue being linolenic acid particularly important in drought period in carnivorous and piscivorous habits which may be presented comprised in phospholipids (phosphocholine, sphingomyelin or saccharolipids) as indicated by polar lipids signals. These habits as mentioned before have their preference food most available at drought period which are animals and other fishes. [3] Although polyunsaturated fatty acids are synthesized by phytoplankton, which are consumed by fish, molluscs and crustaceans (food chain) fishes which consume other fish and/or animals (that are already rich in omega 3 lipids) must be observed in Amazon river as a unique source of bioactive lipids at drought period. [42] Also, it is not possible to discard that unique lipids may be enrolled in fish adaptation to the challenges imposed by low oxygen availability in this studied season (drought period), for instance. The saturated fatty acids found in muscles of planktivorous fish at flood period seems to be point to production of omega 3 and 6 lipids once they (saturated fatty acids) or other functional molecules (dietary carbohydrates, cholesterol, insulin, testosterone and oestrogen) may do the role of desaturase enzyme activators; while inhibitors of polyunsaturated fatty acids include the dietary polyunsaturated fatty acids itself. [43]

Changes in linolenic and linoleic acids amounts

Amounts of the omega-3 linolenic acid and the omega-6 linoleic acid for all lipid samples are presented in Figure 6 as well the samples’ omega-3 and omega-6 ratios based on amounts of these acids.
Figure 6. Changes in the fatty acid compositions (percentage of linolenic and linoleic acids) of A) liver collected in drought period; B) liver collected in flood period; C) muscle collected in drought period; B) muscle collected in flood period; of Amazonian fish species classified as its eating habit.

H.e., Hypophthalmus edentates (planktivorous); P.t., Pseudoplatystoma tigrinum and C. mo., Cichla monoculus (piscivorous); B.a., Brycon amazonicus; T.e., Triportheus elongatus and C.ma. Colossoma macropomum (omnivorous); P.n., Prochilodus nigricans and S.i., Semaprochilodus insignis (detritivorous); P.s., Plagioscion squamosissimus (carnivorous); Ln, linolenic acid; L, linoleic acid; ω6/ω3, omega-6 to omega-3 ratio.

The omega-3 linolenic acid is the precursor for docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), and the omega-6 linoleic acid is the precursor of arachidonic acid (AA, 20:4n-6). These two polyunsaturated fatty acid (PUFA) families have been the target of many studies in recent years since they are biomolecules involved in the regulation of organism functions and present in many food such as fish. [25] High omega-3 PUFAs consumption is associated with benefits for the prevention or treatment of several diseases, mainly those enrolling in chronic inflammation; while high omega-6 consumption has shown debatable opposite effects. [44]

Many good nutritional habits are based on use of the two serving portion per week of 250–500 mg of EPA+DHA that are consider healthy. [45].
Lipids isolated from muscles from Amazonian planktivorous and omnivorous fishes presented coherent amounts of linoleic acid that were similar to other planktivorous and omnivorous species, respectively, near 6% to planktivorous and 4% to omnivorous as compared to Vasconi et al. work. [16] However, the livers’ lipids showed higher amounts (near 8% to planktivorous, 6% to omnivorous or 10% for B. amazonicus in flood season) [16], while linolenic amounts were lower even in liver samples [16], although similar species had lower contents of these as reported in other works [19, 46]. As well, the Amazonian carnivorous fish showed higher amounts of linoleic acid than other carnivorous species, such as those from South African Cape hake. [17]

Therefore, even rich in linoleic acid, the lipidome of the studied Amazonian fishes can be recognized as potentially great nutritional sources. Even potentially detrimental, linoleic acid is also the precursor of lipoxins (lipid mediators with a relevant role on the resolution of inflammation) and an adequate omega-6/omega-3 ratio can be required for proper immune response. [47] Importance of the omega-6/omega-3 ratio in the diet has been addressed in human nutrition and is considered as healthy when ratio is ≤ 5:1. [27]

The species Plagioscion squamosissimus, Prochilodus nigricans, Semaprochilodus insigni, Cichla monoculus and Pseudoplatystoma tigrinum presented omega-6/omega-3 ratios lower than 5 in at least one of the evaluated conditions, tissue (liver or muscle) and/or period of the year (drought or flood). It means that the fattest fish according to the popular knowledge of taste which are C. macropomum, T. elongates, B. amazonicus (omnivorous) and the H. edentates (planktivorous) had presented levels of omega 6 and 3 ratio unsatisfactory to be considered the healthiest fishes, while the Amazonian fish within carnivorous, piscivorous and detritivorous eating habits can be considered great sources of balance between omega-6 and 3 fatty acids and thus healthy food sources at specific seasonal period.

The P. nigricans species (detritivorous fish) has an ideal omega-6/omega-3 ratio only when the liver is evaluated as regardless of the season. Thus, as fish consumption is based mostly on the muscle tissue, in fact it is not recommended to eat this fish as a health source, unless liver-based food can be explored. However, the other detritivorous fish studied here is S. insignis, have shown optimal results for lipids from liver and muscle at drought season, point to a health fish for this period. The difference between the two detritivorous species was already explained by the distinct seasonal migratory habits, and they can be supported by the agreement with the above mentioned S. insignis’s, an increase of phospholipids content balanced with the decrease of neutral lipids at drought.
period [35]. It is possible to relate these findings with eating habits, since this fish usually have more food abundance due to the increase of the flooded area, which contributes to the accumulation of energy reserves in the form of triglycerides rich in omega-6, during the flooding period. Thus, during the drought season the accumulation ends up being lower from omega-6 fatty acids, which contributes to the reduction of omega-6/omega-3 ratio. Furthermore, species with ideal omega-6/omega-3 ratios in both tissues and periods of the year are *C. monoculus* and *P. tigrinum* (piscivorous fish), which means they are great fishes for consumption at any time of the year, as they often accumulate omega-3 fatty acids in large amounts in their tissues to balance the omega 6 amounts. While the carnivorous fish evaluated, the *P. squamosissimus* specie presents an ideal omega-6/omega-3 ratio at any time of the year only when the animal's muscle is evaluated, being considered an excellent source of omega-3 lipids for human consumption, since the human consumption is based on the muscle tissue. Indeed, once more the popular knowledge showed itself accurate since Amazonian population recommends piscivorous and carnivorous fish as a healthy food source to ill people.

Our data indicate the Amazonian’s fish as beneficial lipid source once corroborates with the idea of that diets enriched in fish oil omega-3 is good for health. [48] This is based on omega 6 and 3 ratio but also based on the fact that fatty Amazon fish can change the lipid metabolism effectively lowering plasma Low Density Lipoprotein (LDL) and increasing High Density Lipoprotein (HDL). [49] While when we interpreted in light of the crosstalk between the ecological environment and fish physiology, its fact that species sharing the same food habits can have similar substrates for the endogenous synthesis of lipids, these seem to be used to attend the specific fish metabolism. [50] Amazon fishes developed different adaptations to survive in the challenging environment of the region, such as migratory habits and mechanisms to deal with hypoxia exposure. [51, 52] Therefore, species may synthetize different lipids in response to seasonal changes according to the individual adaptations. At conjunct, this explains why fishes within the same eating habit show a similar lipid profile (building blocks), but this is sensitive to seasonal changes in terms of organ concentration (where lipid synthesis or storage is occurring) and consequent lipids composition (as a consequence of target organ metabolism), regardless of the eating habit.

Thus, our findings suggest that Amazonian fishes have a lipidome with a high nutritional potential to benefit human health, which can vary according to tissue origin (mainly in amount) and is generally sensitive to the eating habits of the species (mainly
in quality) and individually sensitive to seasonal changes. The observed high amounts of omega-3 with a significant contribution of omega-6 (mainly linoleic acid) reflect an ideal balance between these fatty acids, which associated with omega-6/omega-3 ratio < 5:1 might be a perfect combination to promote human health. This is a relevant finding, considering that local population consumes high amounts of almost all parts of these fishes (including muscle, head, eyeball, liver, and ova) and that these are acquired in a low cost, favoring the worldwide consumption.

SUPPORTING INFORMATION DESCRIPTION

Mapping of fish capture; Flowchart of tissue obtained from the number of exemplars captured; Chart showing contents of lipids with different polarities of Amazonian fishes; PLS-DA data on variable importance in projection (VIP) obtained for lipids from Amazonian fishes; PLS-DA VIP of Amazonian fishes in heatmap format.

FUNDING SOURCES

FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the scholarships given to BSBC (PhD, Process Nº. 2013/14707-9) and NCM (Scientific Initiation, Process Nº. 2014/11258-1) is kindly acknowledged.

REFERENCES

[1] Santos, G. M.; Ferreira, E. J. G.; Zuanon, J. A. S. Peixes Comerciais de Manaus. 2nd edition; INPA: Manaus, Brazil, 2009; 144 p.
[2] Lobón-Cervia, J., Hess, L. L., Melack, J. M.; Araujo-Lima, C. A. R. M. The importance of forest cover for fish richness and abundance on the Amazon floodplain. Hydrobiologia. 2015, 750, 245–255.
[3] Barthem, R. B.; Fabré, N. N. Biologia e diversidade dos recursos pesqueiros da Amazônia. In: A pesca e os recursos pesqueiros na Amazônia Brasileira, 1st edition; Ruffino, M. L., Ed.; Ibama/Provárzea: Manaus, Brazil, 2004; 268 pp.
[4] Junk, W. J.; Soares, M. G. Freshwater Fish Habitats in Amazonia: State of Knowledge, Management, and Protection. Aquat. Ecosyst. Health. 2001, 4, 437-451.
[5] Murrieta, R. S.; Dufour, D. L. Fish and farinha: protein and energy consumption in Amazonian rural communities on Tuqui island, Brazil. Ecol. Food Nutr. 2004, 43, 231-255.

[6] Begossi, A.; Hanazaki, N.; Ramos, R. M. Food Chain And The Reasons For Fish Food Taboos Among Amazonian And Atlantic Forest Fishers (Brazil). Ecol. Appl. 2004, 14, 1334–1343.

[7] Silva, A. L. Comida de gente: preferências e tabus alimentares entre os ribeirinhos do Médio Rio Negro (Amazonas, Brasil). Rev. Antropol. 2007, 50, 125-179.

[8] Cheng, K.; Wagner, L.; Moazzami, A.A.; Gomez-Requeni, P.; Vestergren, AL. S.; Brannas, E.; Pickova, J.; Trattner, S. Decontaminated fishmeal and fish oil from the Baltic Sea are promising feed sources for Arctic char (Salvelinus alpinus L.)—studies of flesh lipid quality and metabolic profile. Eur. J. Lipid Sci. Technol. 2016, 118, 862–873.

[9] Simopoulos, A. P. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. 2008, 233, 674–688.

[10] World Health Organization. Healthy diet. World Health Organization, Geneva, 2015.

[11] Lunn, J.; Theobald, H. E. The health effects of dietary unsaturated fatty acids. Nutr. Bull. 2006, 31, 178–224.

[12] Costa, I. D.; Petry, A. C.; Mazzoni, R. Responses of fish assemblages to subtle elevations in headwater streams in southwestern Amazonia. Hydrobiologia. 2018, 809, 175-184.

[13] Val, A. L.; Almeida-Val, V. M. F. de. Fishes of the amazon and their environmentphysiological and biochemical aspects. Springer:New York, 1995; 107-160.

[14] Hurd, L. E.; Sousa, R. G. C.; Siqueira-Souza, F. K.; Cooper, G. J.; Kahn, J. R.; Freitas, C. E. C. Amazon floodplain fish communities: Habitat connectivity and conservation in a rapidly deteriorating environment. Biol. Conserv. 2016, 195, 118–127.

[15] Wang, D. H. ; Jackson, J. R.; Twining, C.; Rudstam, L. G.; Zollweg-Horan, E.; Kraft, C. E.; Lawrence, P.; Kothapalli, K.; Wang Z.; Brenna, J. T. Saturated branched chain, normal odd-carbon-numbered, and n-3 (omega-3) polyunsaturated fatty acids in freshwater fish in the northeastern United States. J. Agric. Food Chem. 2016, 64, 7512-7519.

[16] Vasconi, M.; Caprino, F.; Bellagamba, F.; Busetto, M. L.; Bernardi, C.; Puzzi, C.; Moretti, V. M. Fatty Acid Composition of Freshwater Wild Fish in Subalpine Lakes: A Comparative Study. Lipids. 2015, 50, 283-302.

[17] Swanepoel, H.; Lues, J. F. R.; Venter, P. The Contribution Of Fatty Acids To The Composition Of The Total Lipids In Juvenile Cape Composition Of The Total Lipids In Juvenile Cape Hake Fillet. JNGS. 2017, 14, 247.

[18] Inhamuns, A. J.; Franco, M. R. B.; Batista, W. S. Seasonal variations in total fatty acid composition of muscles and eye sockets of tucunáre (Cichla sp.) from the Brazilian Amazon area. Food Chem. 2009, 117, 272–275.

[19] Rodrigues, B. L.; Canto, A. C. V. C. S.; Costa, M. P.; Silva, F. A.; Marsico, E. T.; Conte-Junior, C. A. Fatty acid profiles of five farmed Brazilian freshwater fish species from different families. PlosOne. 2017, 12, e0178898.
[20] Petenuci, M. E.; Santos, V. J.; Gualda, V. J.; Lopes, A. P.; Schneider, V. A.; Santos Jr, O. O.; Visentainer, J. V. Fatty acid composition and nutritional profiles of Brycon spp. from central Amazonia by different methods of quantification. *J. Food Sci. Technol.* 2019, 56, 1551-1558.

[21] Citil, O. B.; Kalyoncu, L.; Kahraman, O. Fatty Acid Composition of the Muscle Lipids of Five Fish Species in Isikli and Karacaören Dam Lake, Turkey. *Veterinary Medicine International*, 2014, 5p.

[22] Rude, N. P.; Trushenski, J. T.; Whitledge, G. W. Fatty acid profiles are biomarkers of fish habitat use in a river-floodplain ecosystem. *Hydrobiologia* 2016, 773, 63–75.

[23] Almeida, N. M.; Franco, M. R. B. Fatty acid composition of total lipids, neutral lipids and phospholipids in wild and farmed matrinxã (Brycon cephalus) in the Brazilian amazon area. *J. Sci. Food Agric.* 2007, 87, 2596-2603.

[24] Almeida, N. M.; Visentainer, J. V.; Franco, M. R. B. Composition of total, neutral and phospholipids in wild and farmed tambaqui (Colossoma macropomum) in the Brazilian amazon area. *J. Sci. Food Agric.* 2008, 88, 739–1747.

[25] Kaur, N.; Chugh, V.; Gupta, A. K. Essential fatty acids as functional components of foods - a review. *J. Food Sci. Technol.* 2014, 51, 2289-22303.

[26] Waitzberg, D. L.; Torrinhas, R. S. Fish oil lipid emulsions and immune response? what clinicians need to know. *Nutr. Clin. Pract.* 2009, 24, 487-99.

[27] Simopoulos, A. P. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* 2002, 56, 365-379.

[28] Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 1959, 37, 911-917.

[29] Johnston, J. J.; Ghanbari, H. A.; Wheeler, W. B.; Kirk, J. R. Characterization of shrimp lipids. *J. Food Sci.* 1983, 48, 33-35.

[30] Mor, N. C.; Correia, B. S. B.; Val, A. L.; Tasic, L. A Protocol for Fish Lipid Analysis Using Nuclear Magnetic Resonance Spectroscopy. *J. Braz. Chem. Soc.* 2019, 1-11.

[31] Jiang, X.; Huang, R.; Wu, S.; Wang, Q.; Zhang, Z. Correlations between 1H NMR and conventional methods for evaluating soybean oil deterioration during deep frying. *J. Food Meas. Charact.* 2018, 12, 1420–1426.

[32] Gurr, M. I. *Lipids in nutrition and health: a reappraisal*. Copyright:England, 2009; p.13, 37, 119-124.

[33] Carvalho, F. M. Composição química e reprodução do mapará (Hypophthalmus edentatus Spix, 1829) do lago do Castanho, Amazonas. *Acta Amaz.* 1980, 2, 379-389.

[34] Duarte, C.; Souza, V. S.; Nunes, C. O. Variação nictemeral na composição da ictiofauna no lago Catalão (confluência dos rios Solimões e Negro). *Amazon Sci.* 2012, 1, 18-27.

[35] Arrington, D. A.; Davidson, B. K.; Winemiller, K. O.; Layman, C. A. Influence of life history and seasonal hydrology on lipid storage in three neotropical fish species. *J. Fish Biol.* 2006, 5, 1347-1361.
[36] Braga, T. M. P.; Rebêlo, G. H. Conhecimento tradicional dos pescadores o baixo Rio Juruá: Aspectos relacionados aos hábitos alimentares dos peixes da região. *Interciência* **2014**, *9*, 659-665.

[37] Popescu, R.; Costinel, D.; Dinca, O. R.; Marinescu, A.; Stefanescu, I.; Ionete, R. E. Discrimination of vegetable oils using NMR spectroscopy and chemometrics. *Food Control* **2015**, *48*, 84-90.

[38] Li, J.; Vosegaard, T.; Guo, Z. Applications of nuclear magnetic resonance in lipid analyses: An emerging powerful tool for lipidomics studies. *Prog. Lipid Res.* **2017**, *68*, 37-56.

[39] Erikson, U.; Standal, I. B.; Aursand, I. G.; Veliyulin, E.; Aursand, M. Use of NMR in fish processing optimization: a review of recent progress. *Magn. Reson. Chem.* **2012**, *50*, 471–480.

[40] Nuzzo, G.; Gallo, C.; d’Ippolito, G.; Cutignano, A.; Sardo, A.; Fontana, A. Composition and Quantitation of Microalgal Lipids by ERETIC 1H NMR Method. *Mar. Drugs* **2013**, *11*, 3742-37.

[41] Kaçar, S.; Başhan, M.; Oymak, S. A. Effect of season on the fatty acid profile of total lipids, phospholipids and triacylglycerols in *Mastacembelus mastacembelus* (Atatürk Dam Lake, Turkey). *Grasas Aceites* **2018**, *69*, e242.

[42] Netleton, J. A. Omega-3 Fatty Acids and Health. In: *Omega-3 Fatty Acids and Health*. 1st edition; Springer: Boston, MA, 1995; pp 64-76.

[43] Ferreri, C.; Chatgilialoglu, C. Role of fatty acid-based functional lipidomics in the development of molecular diagnostic tools. *Expert Rev. Mol. Mol.* **2012**, *12*, 767–780.

[44] Calder, P. C. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim Biophys Acta.* **2015**, *1851*, 469-84.

[45] Flock, M. R.; Harris, W. F.; Kris-Etherton, P. M.; Kris-Etherton, P. M. Long-chain omega-3 fatty acids: time to establish a dietary reference intake. *Nutr. Rev.* **2013**, *10*, 692-707.

[46] Lu, H.; Hong, H.; Luo, Y. The seasonal fatty acids composition of different tissues of farmed common carp (*Cyprinus carpio*). *J. Food Sci. Technol.* **2016**, *1*, 11-19.

[47] Serhan, C. N.; Yacoubian, S.; Yang, R. Anti-inflammatory and proresolving lipid mediators. *Annu. Rev. Pathol.* **2008**, *3*, 279-312.

[48] Silva, V.; Barazzoni, R.; Singer, P. Biomarkers of fish oil omega-3 polyunsaturated fatty acids intake in humans. *Nutr. Clin. Pract.* **2014**, *29*, 63-71.

[49] Souza, F. C. A.; Garcia, N. P.; Sales, R. S. A.; Aguiar, J. P. L.; Duncan, W. L. P.; Carvalho, R. P. Effect of fatty Amazon fish consumption on lipid metabolism. *Rev. Nutr.* **2014**, *27*, 97-105.

[50] Muro, E.; Atilla-Gokcumen, G. E.; Eggert, U. S. Lipids in cell biology: how can we understand them better? *Mol. Biol. Cell.* **2014**, *25*, 1819–1823.

[51] MacCormarck, T. J.; Lewis, J. M.; Almeida-Val, V. M. F.; Driedzie, W. R. Carbohydrate management anaerobic metabolism, and adenosine levels in the armoured catfish, *Liposarcus pardalis* (Castelnau), during hypoxia. *J. Exp. Zool.* **2006**, *305A*, 363-375.

[52] Sloman, K. A.; Baker, D.; Winberg, S.; Wilson, R. W. Are there physiological correlates of dominance in natural trout population? *Anim. Behav.* **2008**, *76*, 1279-1287.
Supplementary information

NMR in analysis of the nutritional value of lipids from muscles and livers of wild Amazonian fishes with different eating habits over seasonal variation

Banny Silva Barbosa Correia¹, Gilberto Gaspar Duarte Ortin¹, Maiara da Silva Santos², Raquel Susana Torrinhas³, Natalia Cristina Mor⁴, Adalberto Luis Val⁵, Ljubica Tasic∗,¹.

¹Institute of Chemistry, State University of Campinas, Rua José de Castro, s/n - Cidade Universitária, 13083-970, Campinas, Sao Paulo, Brazil
²Department of Organic Chemistry, Center for Exact and Technological Sciences, Federal University of Sao Carlos, Rodovia Washington Luis s/n Km 235, 13565-90, Sao Carlos, Sao Paulo, Brazil.
³Department of Gastroenterology, Surgical Division (LIM 35), Medical school, University of Sao Paulo, Av. Dr. Arnaldo, 455, Cerqueira César, 01246903, Sao Paulo, Sao Paulo, Brazil.
⁴Faculty of Pharmaceutical Sciences, State University of Campinas, Rua Cândido Portinari, 200, Cidade Universitária, 13083-87, Campinas, Sao Paulo, Brazil
⁵Department of Ecology, National Institute of Amazonian Research, Avenida André Araújo, 2936, Petrópolis, 69067-375, Manaus, Amazonas, Brazil

∗Correspondence: ljubica@unicamp.br
Figure S1. A) Map showing the spots where fish capture occurred along the Amazon Region (Catalao lake): 1) S 03°10.570´ W 059°55.000´, 2) S 03°10.736´ W 059°54.180´, 3) S 03°09.967´ W 059°54.534´, 4) S 03°09.746´ W 059°54.488´, 5) S 03°10.030´ W 059°55.579´, 6) S 03°10.437´ W 059°54.277´. B) Specimen of the 9 Fish species collected at 2013.

Figure S2. Scheme of tissues used in analyses of lipids. Total of 93 specimen of fishes with five different eating habits were evaluated in which 170 samples (93 muscles and 77 livers) were collected. C.ma. *Colossoma macropomum*; T.e., *Triportheus elongatus*; B.a., *Brycon amazonicus*; P.n., *Prochilodus nigricans*; S.i., *Semaprochilodus insignis*; P.t., *Pseudoplatystoma tigrinum*; C. mo., *Cichla monoculus*; H.e., *Hypophthalmus edentatus*; P.s., *Plagioscion squamosissimus*. 
Figure S3. $^1\text{H}$ NMR spectrum of the total lipids extracted from the *T. elongates* fish given as representative for analysed Amazonian fishes. Peaks were numbered according to Table 1.
Figure S4. PLS-DA data on variable importance in projection (VIP) obtained for $^1$H-NMR lipids’ data of Amazonian fishes versus A) tissue (liver or muscle); B) seasonal period (drought or flood); C) tissue and seasonal period; D) eating habit (carnivorous, detritivorous, omnivorous, piscivorous or planktivorous).
Figure S5. PLS-DA charts obtained for $^1$H-NMR lipids data *versus* eating habits showing season period (drought or flood) in heatmap of top 15 PLS-DA VIP.
