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Evaluation of the Amazon River Seasonal Influences on Glycerophospholipids in Wild Fish

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Glycerophospholipids (GP) are important lipids of direct relevance to health and disease, and in marine fish, they are often enriched in omega-3 polyunsaturated fatty acids (PUFA) due to marine microorganisms consumption. It has been speculated that the different diets and habitats to which Amazonian fish are exposed can be ascribed to different lipid compositions, differently than those that occur in marine fish. Here, the GP in Amazonian fish is investigated to achieve nutritional value. Using mass spectrometry and multivariate statistical analysis, we investigated the GP composition of muscles and livers from nine species of wild Amazonian fish. GP profiles mainly comprised a diversity of phosphatidylcholines and varied according to eating habits, the season of capture, and tissue metabolism. In this sense, our results may help in the future aquaculture as a potential source of lipid-based nutrient supplementation to supply food to eradicate global hunger.

Keywords: fish lipids, mass spectrometry, glycerophospholipids, liver, muscle

Introduction

Lipids exhibit several biological functions related to energy reserves and play an important role in the formation of the cell membrane.[1] Facing this, lipid-based nutrient supplementation (LNS) has been used as one of the strategies to prevent or alleviate micronutrient deficiency among children who live in low- and middle-income countries.[2]

Hunger is a global concern that the United Nations Organization aims to eradicate by 2030 as stated in its Sustainable Development Goal 2-Zero Hunger.[3] A potential source of LNS and a sustainable strategy that aims to supply global food is aquaculture,[4] where new fish species are introduced in the aquatic system aiming for new food compositions and market products.[5] Fish are abundant in the essential omega-3 (n-3) polyunsaturated fatty acids (PUFA) which are bonded in triacylglycerol or glycerophospholipid (GP).[6]

It comes to attention that GPs participate in several endogenous metabolic and signaling functions. Due to its hydrophilic and hydrophobic properties, GPs are essential to maintain the bilayer structure and homeostasis of cell membranes and to enable the vascular delivery of lipids.[7,8] GPs are characterized by a phosphate group at *sn*-3 of glycerol, with fatty acyl chains connected by ester bonds to *sn*-1 and *sn*-2 positions. The phosphate group binds various chemical groups, stratifying GP into different categories. Chemically, GPs are represented by several main classes, such as phosphatidylcholine (PC), -serine (PS), -ethanolamine (PE), and -inositol (PI). [9-11]

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PCs are essential for human brain function, irrespective of their n-3 content.[12-15] In addition, choline disassociated from GP also reflects on gut microbiome metabolites, and phosphatidylcholine intakes are associated with a lower risk of developing Type 2 diabetes.[15] PE supplementation in mice was related to improved health in hepatic steatosis conditions.[16]

An interesting natural aquatic system with unknown fish GP compositions that could inspire future aquaculture is the Amazon River Basin which contains a significant diversity of freshwater fish. The ecology of the area provides a challenging environment since the availability of food sources for their fish is strongly influenced by the dynamics of the river (ranging from drought to flood water periods).[1,17-19] In a recent study using nuclear magnetic resonance (NMR) spectroscopy,[20] we discovered that the GP class is one of the lipid classes that are dependent on the tissue metabolism and eating habits of the fishes along with seasonal periods of the Amazon River. Thus, it is valuable to investigate the GP profile of Amazonian fish and its production mechanisms related to the environment.

Herein, we describe and characterize the GP molecular profiles of nine species of wild Amazonian fish with distinct eating habits through the mass spectrometry (MS) technique using two metabolically distinct tissue samples (muscle and liver) in different seasons (drought and flood).

Experimental

Samples

Total lipids were extracted using the Bligh and Dyer method[21,22] from liver and muscle samples (0.05 g) of nine species of wild Amazonian fish and kept frozen at −80 °C until the MS analysis. The extraction based on the Bligh and Dyer method was done using a mixture of methanol (LabSynth, Diadema, Brazil), chloroform (LabSynth, Diadema, Brazil), and Milli-Q water in a 2:1:1 (v/v/v) ratio, which was added to the tissue and crushed. For the crush process, a handheld electric mixer was used for muscles and a mortar and pistil were used for livers which were covered with liquid nitrogen. After filtering the material using the Büchner funnel, the filtered liquid sample was centrifuged for 20 min, 1,694 × g at 25 °C. The hydroalcoholic phase was removed and the chloroform phase was reserved. To the hydroalcoholic phase, chloroform was added in a proportion of 1:1 (v/v), and the process of liquid/liquid extraction was repeated. The chloroform phases were jointed and the hydroalcoholic phase was discarded. Three fish (biological replicates) from each species were captured during periods of flood and drought, from six different geographical coordinates at the Catalão Lake (Iranduba, Amazonas State, Brazil) disposed in Figure S1 of Supplementary Information (SI) section: (1) 03°10.570'S 059°55.000'W, (2) 03°10.736'S 059°54.180' W, (3) 03°09.967'S 059°54.534'W, (4) 03°09.746'S 059°54.488'W, (5) 03°10.030'S 059°55.579'W and (6) 03°10.437'S 059°54.277'W. Approved study protocol by Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA), license No. 29837 and 39985 at the Catalão Lake used the floating base of Ecophysiology and Molecular Evolution Laboratory of National Institute of Amazonian Research (INPA). The fishes were collected with sizes superior to those of their respective sexual maturity, regardless of gender. The length of fish was measured using a ruler, and the ones bigger than the size (cm) indicated for the species as correct to their plain sexual maturity were kept. Those fish were put into iceboxes with water for 1 h. The fish were euthanized according to national animal care regulations and were approved by the Ethics Committee on Animal Experiments of INPA under registration No. 026/2015. The fish were cut from the anus to the mouth with surgical scissors and livers and muscles were removed using a scalpel and forceps. The fish were washed prior to handling, a sheet of filter paper was maintained and exchanged for each specimen in the fish handling table, distilled water and 70% alcohol were used in the handling of the scalpel, and the scalpel blade was changed for each sample. An ice box was kept for the manipulation of the samples. Livers and muscles were transferred to the sterile Falcon tubes and stored in the freezer at −20 °C. The frozen tubes were transported always on ice from the floating base of the Ecophysiology and Molecular Evolution Laboratory at Catalão Lake to its respective Laboratory in Manaus and then transported by airplane to the Laboratory of Biological Chemistry at the State University of Campinas where the samples were kept in the freezer at −20 °C.

The GP profile of each species was assessed individually, and its feeding habit was recorded:[23] (*i*) omnivorous: *Colossoma macropomum* (Cuvier, 1816), *Triportheus elongatus* (Garman, 1890), *Brycon amazonicus* (Agassiz, 1829); (*ii*) detritivorous: *Prochilodus nigricans* (Agassiz, 1829), *Semaprochilodus insignis* (Agassiz, 1829); (*iii*) piscivorous: *Pseudoplatystoma tigrinum* (Agassiz, 1829), *Cichla monoculus* (Agassiz, 1831); (*iv*) planktivorous: *Hypophthalmus edentatus* (Agassiz, 1829); and (*v*) carnivorous: *Plagioscion squamosissimus* (Heckel, 1840). Thus, the GP profile was assessed in relation to the season of capture and feeding habit, to determine the impact of ecological factors and tissue metabolism.

A total of 100 lipid extracts were analyzed, as described in Table 1. We were unable to obtain adequate amounts of lipids from some tissue samples of *P. squamosissimus* (muscle, n = 1; liver, n = 1) and *B. amazonicus* (muscle, n = 3; liver, n = 3) species, which were collected during only the flood and drought seasons, respectively.

Analysis of the glycerophospholipid profile of the Amazonian fish by flow injection/electrospray ionization (ESI)/mass spectrometry (MS)

Individual analysis of muscle and liver GP profiles from the nine species of Amazonian fish was first performed using tandem mass spectrometry (MS/MS) infusing lipid extracts at 10 μg mL^{-1} in methanol (Fisher Scientific, Leicestershire, England). Neutral Loss Scan (NLS) and Precursor Ion Scan (PIS) (*m/z* 600-1,000) were applied.[24,25] Specifically, NLS of 87 Da was used for PS in negative ion mode, PIS of *m/z* 184 was applied for PC in positive mode, and PIS of *m/z* 196 and 241 Da were used for PE and PI, respectively in negative ion mode. MS was performed on 6500 Q-Trap (Sciex, Toronto, Canada), using electrospray ionization (ESI). MS spectra were acquired using the following setting for negative ion mode (–eV): 650 to 950 Da at 200 Da s^{-1}, −50 eV declustering potential (DP), −10 eV electron potential (EP), −42 eV collision energy (CE), −13 eV cell exit potential (CXP).[26,27] The same parameters were applied in positive ion mode (+eV). Mass resolution for the work was 0.7+/−0.1 FWHM (full width at half-maximum). The intensity/signal strength was taken from Analyst Software version 1.5. Each sample was first run as a PREC scan and then to see the relative levels of the others, they were directly infused into the MS before analysis of signal strength by the Analyst software. MultiQuant was applied for MS data extraction. Peaks of at least three times signal to noise from PC, PE, PE, and PI were chosen for further characterization. Putative assignments, based on headgroup and *m/z*, were made using tables of molecular and product ions LIPID MAPS and Murphy.[28] PA (phosphatidic acid) and PG (phosphatidylglycerol) were not included in the analysis since we did not have a validated method for them when the study was conducted.

Glycerophospholipid analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS)

Liquid chromatography tandem MS (LC-MS/MS) was applied to confirm putative identifications obtained using multiple reaction monitoring (MRM) and enhanced product ion (EPI) analysis to assign fragments of selective GP as side chains, as described below.

Lipid extracts (100 μg mL^{-1}) were separated using reverse-phase LC (Phenomenex Luna 3 μm C18 150 × 2 mm) with a gradient of 50-100% B from 0-10 min followed by 30 min at 100% B (A, methanol/acetonitrile/water, 1 mmol L^{-1} ammonium acetate, 60:20:20, v/v/v; B, methanol, 1 mmol L^{-1} ammonium acetate) with a flow rate of 200 μL min^{-1}.[26,27] LC-MS grade solvents for HPLC analysis was purchased from VWR chemicals. Enhanced product ion spectra were obtained using independent data acquisition criteria, at the apex of the product ion transition peak with MS settings as above. No internal standard was used once samples were extracted in Brazil, in advance of being sent to Cardiff. We acknowledge that the absence of standards does not allow us to follow the efficiency of GP extraction and may be that some lipids are extracted with a greater efficiency than others. This limits comparisons to a brief chemometric analysis rather than full quantitation of lipid amounts, in which relative abundances were the data used and thus we acknowledge the limitations of this approach.

Table 1. Number of lipid samples extracted from wild Amazonian fish and analyzed individually by mass spectrometry, according to fish feeding habit, species, tissue origin, and season of capture

Species	Eating habit	Muscle		Liver	
		Flood	Drought	Flood	Drought
Triportheus elongatus	omnivorous	3	3	3	3
Colossoma macropomum	omnivorous	3	3	3	3
Brycon amazonicus	omnivorous	3	0	3	0
Semaprochilodus insignis	detritivorous	3	3	3	3
Prochilodus nigricans	detritivorous	3	3	3	3
Pseudoplatystoma tigrinum	piscivorous	3	3	3	3
Cichla monoculus	piscivorous	3	3	3	3
Hypophthalmus edentatus	planktivorous	3	3	3	3
Plagioscion squamosissimus	carnivorous	2	3	2	3

Chemometrics analysis of the influence of ecological factors on the GP profile of Amazonian fish

Because it is qualitative data analysis, in order to assess the influence of eating habits during different seasons and tissue sources on the GP profile, MS data were exported into a data matrix and analyzed by hierarchical cluster analysis, using MetaboAnalyst 5.0 online platform.[29,30] The relative abundance of a total of 22 lipids was used as the input matrix. These hierarchical cluster analyses were performed using features autoscaling normalization (mean-centered and divided by the standard deviation for each variable) for data pre-processing, no sample normalization, and no data transformation. Euclidean distance was employed as the similarity measure, and Ward's linkage (clustering to minimize the sum of squares of any two clusters) as the clustering algorithms. Hierarchical clustering is performed with the "hclust" function in package stat. Clustering results are shown in the form of a dendrogram combined with a heatmap.

Results

The GP profiles of Amazonian fish

In our analyses, all four main GP classes (PC, PS, PE, PI) were identified in the Amazonian fish samples. A typical MS spectrum for PC, obtained in lipid extracts from the liver of a wild Amazonian fish, is shown in Figure 1.

PIS and NLS scanning were used to identify the GP molecular species, with an example shown in Figure 1a for PC. Next, MS/MS was used to assign the fatty acyl side chains shown in Figure 1b for PC 36:4. Here, this lipid was assigned based on the following: loss of a methyl group (*m/z* 766.4); loss of ketenes from *sn*-1 and *sn*-2 (RCH=C=O) (*m/z* 480.6); loss of *sn*-2 or *sn*-1 RCOOH

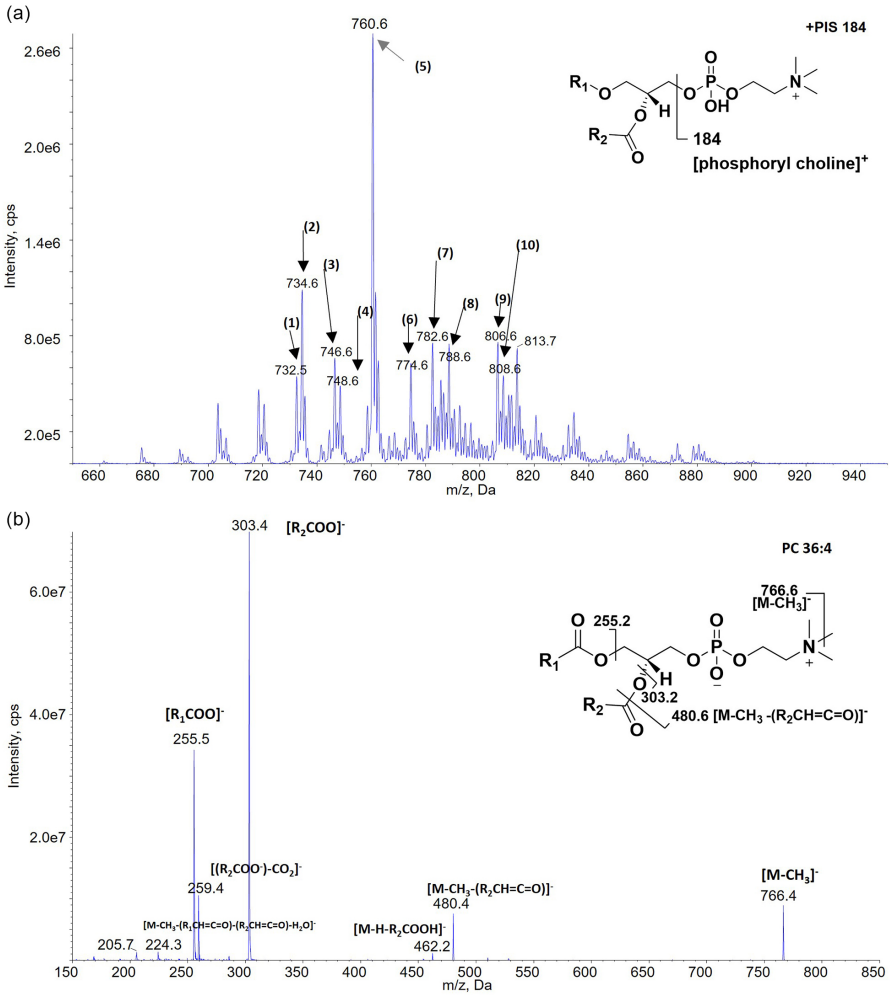

Figure 1. Example of mass spectra obtained for GP in liver lipid extracts from an Amazonian fish. (a) Precursor Ion Scan (PIS) MS/MS of PC (+eV) using product ion *m/z* 184, with corresponding putative assignments. (b) Product ion spectrum of ions from precursor *m/z* 782.6 Da showing fatty acyl chains at *m/z* 255.5 and 303.4, in lipids extracted from the liver of *P. squamosissimus* during the drought period. Lipids: (1) PC 32:1; (2) PC 32:0; (3) PC O-34:1; (4) PC 34:3; (5) PC 34:1; (6) PC O-36:4; (7) PC 36:4; (8) PC 36:1; (9) PC 38:6; (10) PC 38:5.

(ester) group (*m/z* 462.3; of CO_2 from *sn*-2 $RCOO^-$ ion, when *sn*-2 is PUFA (*m/z* 259.3); as well as, the loss of H_2O (water) from glycerol-3-phosphate choline ion (*m/z* 224.1) and the ions *sn*-2 $RCOO^-$ (*m/z* 303.2) and *sn*-1 $RCOO^-$ (*m/z* 255.2). The corresponding chromatogram for this mass spectrum together with other chromatograms related to PC side chains identified is described in Figure S2 (SI section). Examples of PS, PE, and PI mass spectra and chromatograms are shown in Figures S3-S7 (SI section). Interestingly, the precursor scanning for PE lipids showed very low levels of this lipid class in all samples (Figure S5). However, putative assignments have been made here to allow a general comparison between other lipid classes. Due to the complex nature of the samples, ions from other lipids with the same precursor mass can sometimes be visible, however, all ions from the lipid of interest were expected to be prominent and strongly visible in spectra. Through this approach and considering that fish can synthesize *de novo* from acetate, the even-chain, saturated fatty acids, 22 total molecular GP were assigned: 10 PC, 5 PE, 2 PI, and 5 PS, with 6 fatty acyl side chains (Table 2).

The putative notation of compounds was done using an identification of level-2 in accordance with the Metabolomics Standards Initiative (MSI), this means that the notation was based on spectral similarity of experimental mass spectra in comparison to data available in databases.[32] Total fatty acyl (FAs) chains were from 32 to 40 carbons as their putative assignment is shown in Table 2. Furthermore, side chain experiments (MRM) showed that FA were composed mainly of PUFA, but also included some saturated fatty acids (at least in one of the two side chains) in those lipids in which the MRM were evaluated.

GP from the Amazonian fish studied here were mostly diacyl forms. Moreover, the plasmalogen side chain was found for choline and ethanolamine classes. It was not possible to distinguish plasmalogen chain from ether chain, because it is not known the location of double bond in the *sn*-1. Therefore, herein we used both abbreviations in the notation.

GP fatty acyl composition showed length and unsaturation differences in number and position of the double bonds, and, also, double bond oxidation. PC molecular species were more abundant in number than other GP classes in our lipid samples, while PI showed less species diversity.

Specific GPs from fish samples were fragmented and characterized by LC-MS/MS. Two PC, two PS, and two PI from the Amazonian fish were selected for complete characterization by LC-MS/MS using product ion scan analysis. PE was not chosen due to its high signal/noise in the full spectrum. Again, we highligh that the assignments shown in Table 2, are putative unless specified. It was noted that GP from Amazonian fish contain the PUFAs docosahexaenoic acid (DHA, n-3; FA 22:6) (Figures S4, S7b) which is an omega 3 PUFA (Figure S8), and arachidonic acid (AA, n-6; FA 20:4) (Figures S2b, S7a) which is an omega 6 PUFA (Figure S8).

Table 2. Putative assignments for phospholipids classes from liver and muscle samples of wild Amazonian fish based on MS/MS analysis numbered in agreement with spectra figures, containing the assignment for the side chains by multiple reaction monitoring (MRM) and enhanced product ion (EPI) analysis of two PC, two PS, and two PI

$[M + H]^+$	Species level	$[M - H]^-$	Species level
732.6	PB 32:1 (1)[a]	788.6	PS 36:1 (11)[a]
734.6	PB 32:0 (2)[a]	810.5	PS 38:4 (12)[a]
746.6	PB O-34:1 (PB P-34:0)(3)[a]	834.6	PS 40:6 (18:0_22:6)(13)[b]
756.6	PB 34:3 (4)[a]	836.6	PS 40:5 (14)[a]
760.7	PB 34:1 (16:0_18:1)(5)[b]	838.4	PS 40:4 (15)[a]
774.7	PB O-36:1 (PB P-36:0)(6)[a]	686.4	PE 32:2 (16)[a]
782.6	PB 36:4 (16:0_20:4)(7)[b]	710.4	PE 34:4 (17)[a]
788.7	PB 36:1 (8)[a]	746.8	PE 36:0 (PE P-38:6)(18)[a]
806.6	PB 38:6 (9)[a]	790.8	PE 40:6 (19)[a]
808.6	PB 38:5 (10)[a]	792.6	PE 40:5 (20)[a]
		885.4	PI 38:4 (18:0_20:4)(21)[b]
		909.6	PI 40:6 (18:0_22:6)(22)[b]

[a]Putative assignment; [b]characterized by product ion MS. PC: glycerophosphocholines; PE: glycerophosphoethanolamines; PI: glycerophosphoinositols; PS: glycerophosphoserines; O-: for an ether phospholipid; P-: for a "plasmalogen". Separator "_": *sn*-position of acyl/alkyl constituents is not known. Order of constituent presentation as described for glycerolipids. The notation of lipids is in accordance with the literature.[31]

Fish eating habits influenced by the dynamic of the Amazon River according to their GP profile

We analyzed GP by chemometric methods (hierarchical cluster analysis) to observe the influence of eating behavior, tissue of origin, and season of capture, as presented in Figures 2 and S9 (SI section). Higher chemical normalized relative abundance was found for GP from the liver than GP from muscle (Figure S9a), and from GP from flood than from drought periods (Figure S9b). This means that the differences in livers from the flood period were the most important for chemical differences among fish when compared to other seasonal and tissue influences as shown in Figures 2a-2b. When eating habits were evaluated planktivorous showed the lower GP chemical diversity (Figures 2c-2d).

In order to acknowledge the eating habits with the highest chemical diversity presented in each tissue by season, we compared eating habits in each tissue-season combination (Figure 3). GP clustered livers relative to both seasons (Figures 3a-3b), with piscivorous being distinct, clustering with carnivorous and detritivorous in drought period, while clustering with omnivorous and planktivorous in flood season. PE, PI and PS normalized relative abundance in piscivorous collected during the drought period (Figure 3b) were mainly responsible for this finding. Regarding the muscles (Figures 3c-3d), while piscivorous continue to cluster with omnivorous fish in flood season, during the drought season it was highlighted as the eating habit with less similarity with the others eating habits including PC 38:5, PC 34:3, and PC 38:6 to the GP list responsible for its differentiation (Figure 3d).

Lipids from carnivorous and detritivorous fish were clustered together and distinguished from omnivorous fish (livers and muscles during flood and drought periods).

When GP was analyzed by the percentage difference of amounts (relative abundance) in fish livers captured in flood *versus* drought periods, fish comprehending all eating habits presented PC changes, while PE, PS and PI had minimum or no changes to all eating habits (Figure 4).

Thus, despite carnivorous and detritivorous fish appear to behave like each other, when the percentage difference (delta) between 100% stacked relative abundance of GP in flood *versus* drought is analyzed a new pattern appears. PC 34:1 (16:0_18:1) showed to be the highest relative amount comprehending the GP composition among eating habits. Omnivorous and detritivorous showed the highest

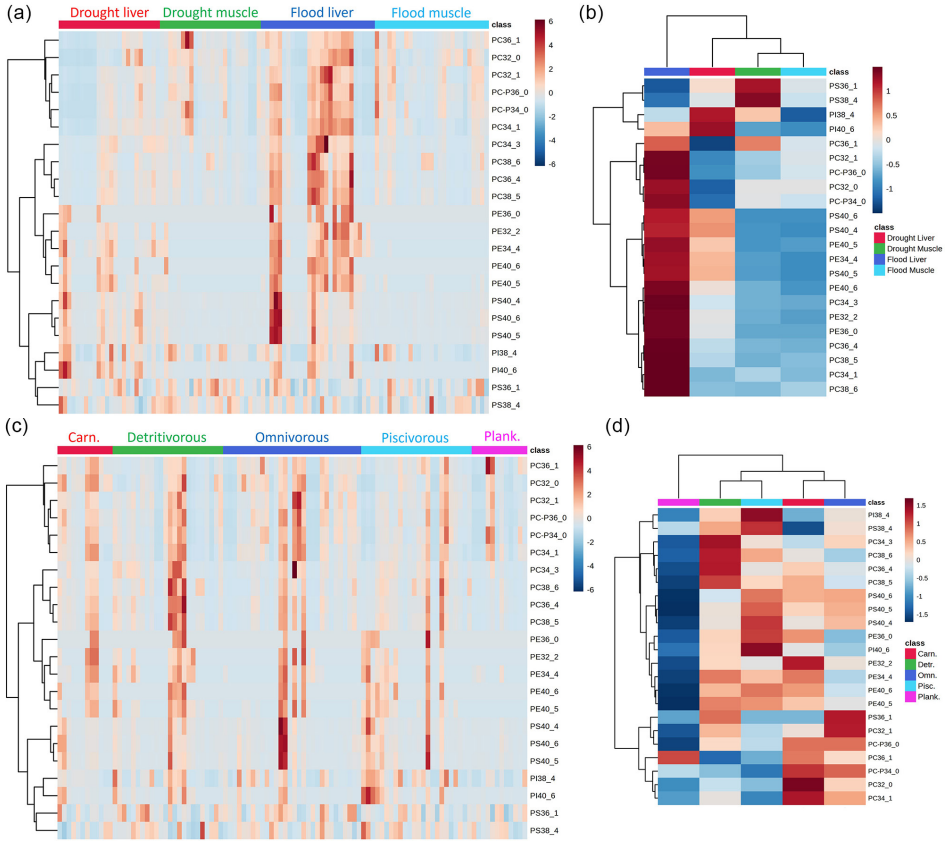

Figure 2. Heatmaps of GP from muscles and livers of Amazonian fish comparing: (a) season and tissues as individual samples; (b) as group averages; (c) eating habits as individual samples; (d) and as group averages. Carn.: Carnivorous, Detr.: Detritivorous, On.: Omnivorous, Pisc.: Piscivorous, Plank.: Planktivorous.

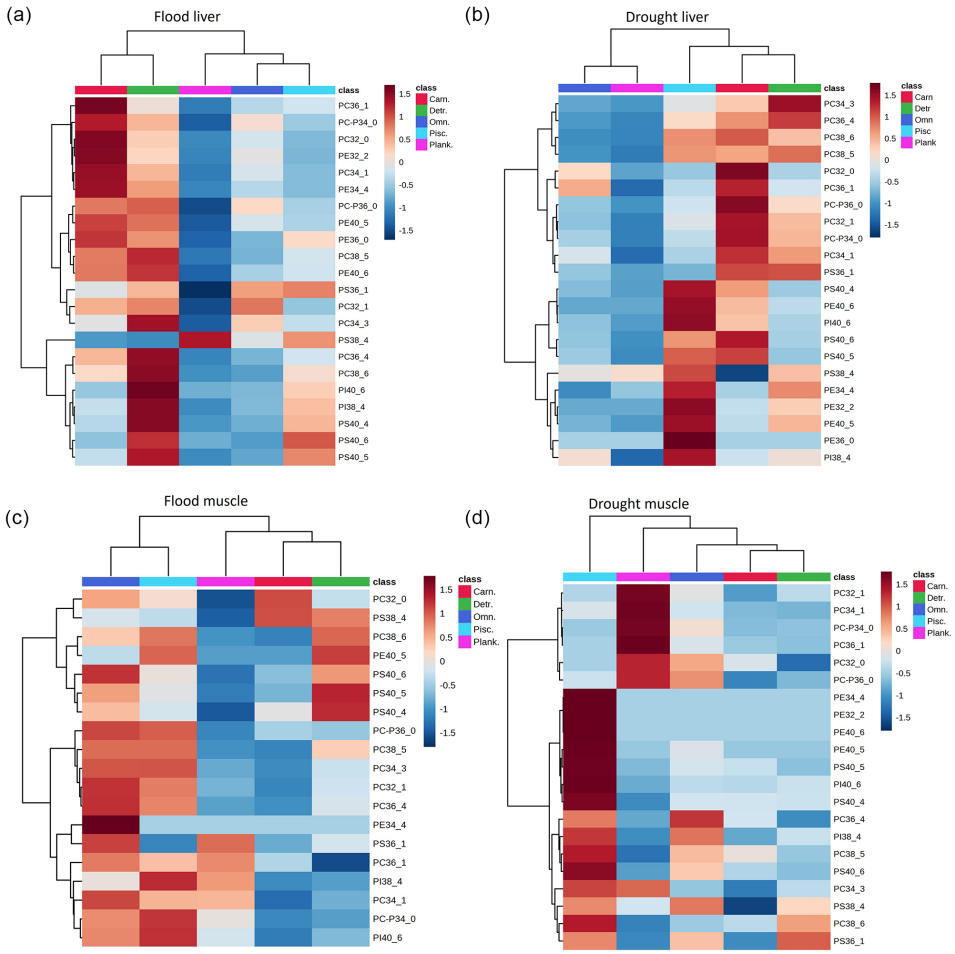

Figure 3. Heatmap of GP of Amazonian fish comparing eating habits from livers of Amazonian fish during the flood period (a); from livers of Amazonian fish during the drought period (b); from muscles of Amazonian fish during the flood period (c); and from muscles of Amazonian fish during the drought period (d). Carn.: Carnivorous, Detr.: Detritivorous, On.: Omnivorous, Pisc.: Piscivorous, Plank.: Planktivorous.

changes with relative amount of shorter PC (PC 34:1) decreasing approximately 11 and 5%, respectively, and with longer PC relative amount (PC 36:4 for omnivorous, PC 38:6 for detritivorous) increasing 8 and 6%, respectively, during flood season. Piscivorous, planktivorous, and carnivorous had the opposite behavior with longer PC relative amount (PC 38:5 for piscivorous, planktivorous, and PC 38:6 for carnivorous) decreasing and shorter PC relative amount (PC 34:1) increasing.

Discussion

The total number of GP molecular species (n = 22) was significantly lower than previously reported in seawater fish,[33] but like that reported in freshwater fish using similar methods.[34] Two previous studies[19,35] assessing the fatty acid content of other Amazonian fish have reported the presence of omega 3 and 6 PUFAs.

Herein, freshwater wild Amazonian fish were found to be rich in GP PUFAs. Sea fish have widely recognized sources of PUFAs, for instance, salmon and trout have high amounts of DHA and eicosapentaenoic acid (EPA).[36] PUFA comprised in GP have been shown to be reduced or absent in some brain diseases and to prevent intestinal dysfunction for example, DHA (n-3) comprised in PC.[37] Also, EPA was related to restoring glucose and lipid metabolism in case of metabolic disorders that report a malfunction in lipid metabolism, with mitochondrial respiratory chain and tricarboxylic acid cycle in muscles, alleviating physical fatigue, and being anti-atherosclerosis.[38-40] Furthermore, fish oil supplementation might help promote the cardiometabolic benefits by attenuating the proinflammatory reactions in hypertension.[41,42] Thus, it has been suggested that supplementary PUFAs from fish oil could help reduce neurodegenerative and cardiac diseases, although this remains to be conclusively shown.[43-45]

Studies[12-14] have suggested that PC regardless of FA composition can alleviate senescence and improve cognitive function. It is suggested that PC stimulates acetylcholine synthesis, an essential neurotransmitter for

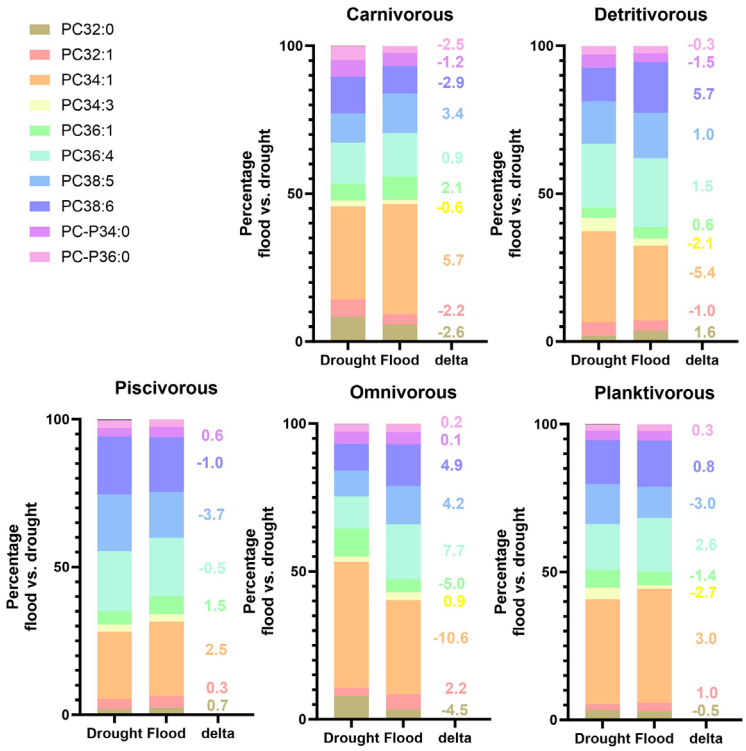

Figure 4. Distribution showing the percentage difference (delta) between 100% stacked relative abundance of GP in flood *versus* drought. GP containing less than 0.1% are not shown.

memory function that can decrease with aging.[13] In humans, depleted levels of phospholipids and GP are associated with several neurological disorders.[46-48] It is not known whether plasmalogens can pass through the blood-brain barrier, but it was reported that treatment with plasmalogens improves cognitive performance in a subgroup of mild Alzheimer's disease (AD) patients.[49,50] PE also plays important roles in human health it is mostly found in nervous system tissues (45% of the total phospholipids) it helps in human blood clot formation as it works together with PS to increase the rate of thrombin production.[51]

It was previously reported that the general chemical composition of Amazonian fish may show seasonal variation and seems also to be influenced by the species, stage of maturity, and other factors.[17] Indeed, in our study, fishes showed distinguished lipids in drought and flood periods. Also, PC and some PE were differently biosynthesized among fish of different eating habits, which indicates that lipid content in fish is influenced by its eating, which again is in accordance with the literature.[20]

The Amazonian fishes' diet can vary among consuming the living plants (herbivorous), plankton (planktivorous), debris (detritivorous), mud (iliophagous), blood (haematovorous), fish and non-fish animals (carnivorous), exclusively fish (piscivorous) or both plants and animals (omnivorous). The sensitivity of GP to seasonal capture may be caused by the water dynamics of the Amazon River, which influences the type and amount of available food.[17] For instance, food availability is higher for carnivorous and piscivorous fish in drought than in flood, where there is less water in which its prey can hide. The opposite occurs for omnivorous fish that have the highest abundance of food in the flood period when the environment is composed of flood forests and fruits that fall in the river.[22,52] Our findings indicated that specific GP classes are influenced by the season in different eating habits. Fish livers during the flood were revealed to reach the greater change, especially carnivorous and detritivorous. However, changes in muscles were also observed, with piscivorous fish achieving GP diversity during drought season. Piscivorous, carnivorous, and planktivorous fish show a decrease of longer PC, and an increase of shorter PC in livers during flood season compared with drought, while detritivorous and omnivorous present the opposite pattern. It differs from what occurs to neutral lipids, since in the period of food source abundance for carnivorous and piscivorous fish (drought period) all the neutral lipids are stored fat.[20] The finding of longer PC with a high number of unsaturation in their fatty acid chain during drought season in piscivorous fish may be related to PUFA elongation produced after consumption of planktivorous fish.[36]

The detritivorous fish were represented herein by *S. insignis* and *P. nigricans*. Detritivorous present a nutritional diversity in their diets. These fish species

commonly feed on detritus, organic matter, algae, and periphyton.[53,54] The two species *S. insignis* and *P. nigricans* also consume genipap fruit in the flood period which could explain the similar pattern of percentage difference (delta) between GP in flood *versus* drought.

The biochemical composition of the lipids in Amazonian fish of different species is sensitive to environmental and physiological factors, which makes it difficult to find the best nutritional composition for human benefit.[17] However, despite this, Amazonian fish is an important part of a varied and healthy diet for people living in this region. It is worth mentioning that the relevant results presented here were in the liver and not in the muscle. Although Amazonians consume a lot of fish, it is not very common to find liver fish in a human diet even among the Amazonian population, with the exception of the fish *Hypostomus affinis* which liver is consumed together with muscle. However, it is worth considering that the liver is a highly lipogenic organ.[55] Thus, PC found in the liver of our wild Amazonian fish indicates that this lipid class is necessary for fish physiology. Indeed, in fish, PC has been previously shown to be associated with improved growth performance.[56]

Furthermore, by profiling GP, our study contributes to building a new perspective of the future aquaculture in which Amazonian fish can be explored or can inspire the management of other species with having mimicked Amazonian diet in order to deliver LNS based on liver products to global food.

Conclusions

The investigated nine wild Amazonian fish appeared to be richer in PC when compared to other GP classes. In this sense, the role of the lipids that were identified in this study is interesting for consumption and human health, since these compounds benefit cognitive and neurological functions. Further MS analysis ensuring the characterization of each lipid and incorporating lipid standards during extraction could help quantify these lipid changes in the samples described. Nonetheless, the putative assignments and proposed changes represent an interesting comparison between species and may influence future aquaculture procedures and dietary intake. In addition, the GP of those fish was influenced by external factors studied here: food habit, the season of capture, and tissue source. These pieces of information can be later explored to produce LNS to help with micronutrient deficiency among children who live in low- and middle-income countries.

Final conclusion is that the best time to get nutritional GP from Amazonian fish regardless of their eating habit is during the flood. However, this information is more reliable for the liver than for muscles. Consuming livers from carnivorous and detritivorous fish collected in any season (flood and drought) can benefit people through their GP diversity. GP from fish muscles is particularly beneficial nutritionally from piscivorous fish during the drought season with their higher relative amount of longer PC.

Supplementary Information

Supplementary information (map of collection points; examples of PC, PS, PE, and PI mass spectra and chromatograms; examples of GP chemical structures; and heatmaps) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

BSBC performed sample preparation, and MS analysis, designed the study, and wrote the manuscript; CPT performed MS analysis and wrote the manuscript; BSBC, and CPT, processed and analyzed the data; JGMP and RST participated in the manuscript writing the manuscript; ALV, and LT conceived and designed the study and corrected the text. All authors read and approved the manuscript.

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