Authors' response

(1) comments from Referees

The manuscript is well written, has a proper and logical layout and makes a valid and valuable scientific contribution. The text can, however, benefit from minor editing (see notes below under editing). The authors investigated a very complex river system draining an even more complex geological setting. Sr isotope analyses is a good method for investigating such a system as it is mainly determined by the weathering of up-stream geology which is not expected to vary much within a 10 or even 50 years period. Please see Jordaan, L.J., Wepener, W. and Huizenga, J.M. (2016). The strontium isotope distribution in water and fish within C1 BGD Interactive comment Printer-friendly version Discussion paper major South African catchments. Water SA, 42(2), 213-224. In this case, very similar data were obtained in a smaller and more controlled river system, but it confirms the underlying assumptions made by the authors for the Amazon system. Data gathering can be much expanded over a multi-year sampling period to include seasonal variation if a further study is ever undertaken.

(2) author's response

We fully agree that it is important to monitor river seasonal variation of Sr isotope to improve the Amazon system knowledge. The Hybam project has been providing pioneer monitoring and characterization of the Amazon system, including monthly water sampling for more than 10 years at 15 sites (Santos et al. 2015). This data set constitutes a baseline and background that has benefited several studies related to fishery sciences (migration pathways, fish stock localization, or like in our manuscript commercial traceability) as well as to the evolution of the basin. These data clearly highlight the Sr isotopic composition contrast among major Amazon sub-basins as well as seasonal isotopic fluctuations related to differential erosion sources (see Santos et al. 2015). Due to the logistic complexity of water sampling in the Amazon system, it is difficult and expensive to expand the monitoring although it is surely an important issue. We hope that our work to provide a new application of this knowledge, thus reinforcing the argument to improve such a monitoring program. Our finds are in agreement with Jordaan et al. (2016), which will be included in our revised manuscript. In the Amazon basin, Pouilly et al. (2014) established the correlation between Sr isotopic ratio in water and in fish, as provided by Jordaan et al. (2016) in South Africa.

(1) comments from Referees

Using C isotopes is a good approach to this problem. It has however several more factors influencing isotope fractionation than Sr isotopes and should be used within the constraints of the technique, as the authors rightfully did. Data can be much expanded if further study is ever undertaken.

(2) author's response

As state in our conclusions, the C isotope results are still preliminary and need further understanding. Nevertheless, they are promising as a commercial traceability tool that can be improved based on trophic marker studies.

(1) comments from Referees

The problem of obtaining fish samples with a known origin is not unique to this study and it is recommended that fish obtained from markets be treated with caution.

(2) author's response

We agree with this remark.

(1) comments from Referees

The value of this work lies in the fact that it can be extended to solve more than one issue. The biggest being the illegal use of protected natural fish populations. The techniques will work for other fish species as well and it will provide data as to the sediment load and erotional patterns of such large rivers.

(2) author's response

We thank the reviewer for these positive points of view, which we share!

(1) comments from Referees #2

I have reviewed this ms and find it to deserve publication after a few revisions are made. The ms tests the general idea that biochemical tags can be used to identify the origin of harvested individuals of arapaima in various regions of the Amazon, and this can be used to improve the management of this economically important but overexploited fish. While the authors have done an apparent good job in analyzing data, I feel like the true contribution of this ms is not reflected in the text. The introduction generally sets out the research question clearly, but there are important issues that were not considered and would help sharpen it and increase the value of this research. For instance, about 3/4 of the introduction is devoted to describing the use of biochemical tags to trace the origin of fish worldwide, and Amazon is introduced only after that. When the subject of the Amazon is introduced, a key idea that is missing here is spatial heterogeneity in the chemistry of river waters. That heterogeneity is what allows the authors to test the main hypothesis, yet it is not described here, not even briefly. The hypothesis only makes sense IF there is spatial heterogeneity in chemistry, so this needs to be established in the introduction (and could be expanded in methods).

(2) author's response

We thank the referee for this important comment. We have included a better description of the existing contrasts in Sr isotopic composition among the different sub-basins of the Amazon, to provide a clear background for the reader (page 2 lines 28-30)

(1) comments from Referees

Also, the study focuses on arapaima and its conservation. But while a lot of space in the introduction is devoted to reviewing the use of chemical markers for sustainable fisheries management in general terms (paragraphs 1-3), there is almost no mention of details about the conservation measures that currently are bringing arapaima back from overexploitation. Given the paper focuses on arapaima, that seems to need attention. In particular, some 500 fishing communities in the State of Amazonas in Brazil (alone, and more now in Para State) are setting fishing quotas and selling their "sustainable" fish to the market while fulfilling strict government limits. Each individual fish harvested under this management system receives a unique, government-issued, identifying a tag that buyers can use to know where the fish came from, where and when it was harvested. But many such tags are illegally re-used to allow the "legal" sale of unsustainably harvested fish. This presents a major management problem for arapaima that the study in question can help solve because its results can potentially be used to 'trace' back the origin of the fish and hence determine if the origin of the fish matches the tag. This study should link its results to such major ongoing management initiative for arapaima.

(2) author's response

We thank the referee for this important comment and for the details. We have addressed this issue in the introduction section of the revised manuscript as it constitutes an important justification of the study (page 3 lines 8-15). Since 1989 Arapaima stocks are recovering, indicating that the adaptive management of Arapaima fisheries has been a success and may hopefully became a positive example of synergetic social and political actions in the region. However, as stated by the referee the situation is not completely controlled and we hope the development of such tools of commercial traceability would help to improve further the situation.

(1) comments from Referees

Finally, the hypothesis of the study only makes sense IF arapaima is not highly migratory and move between and among river systems with different water chemistries. As such, known data on the general migratory behavior of arapaima should be determined 'before' the hypothesis for the hypothesis to make sense. When this is done, typical habitat and food sources (some of which are presented in methods), should also be presented here, to provide context for the hypothesis. To

implement such changes, I suggest shortening the first 3 paragraphs and expanding the remainder of the introduction.

(2) author's response

We agree with the reviewer comments and suggestion about the introduction. We have revised it and included a description of arapaima habitat and trophic relations (page 3 lines 21-27).

(1) comments from Referees

Fig 1 needs to be edited so the font is readable at half page width size; currently, the most important information cannot be read for having font too small. A lot of space on the sides is used to show regions of no interest. The fig could be "zoomed in" to the area of interest. As for the analysis, there is a major mismatch in geographical precision of the otolith data. The fish otoliths were collected from fishermen residing in sites (Table 1). Each of the 'sites' mentioned, such as Itacoatiara, Manaus, or Mamiraua ¸A reserves are, in fact, enormous regions, each of which encompasses several different habitats, each of which can have varied water chemistries. For instance, the Mamiraua Reserve is flooded by some five or more major river tributaries and includes surrounding areas influenced by blackwater ria lakes. This variability in regional water chemistry is not matched by the literature data used for each "site" It also constitutes a limitation of the analytical approach undertaken. More detail on fish capture location will be introduced Could this lack of specificity in the otolith origin data help explain part of the unexplained variance in the analyses? I would seem so. As such, the "match" between the otolith and water chemistry data should be presented and discussed in methods, as well as in the discussion. It does not invalidate the analysis, but it adds more nuance and probably helps explain its results.

(2) author's response

We have improved Fig. 1 by zooming and increasing the fonts. We have also emphasized the lack of precise information about fish capture location, which is part of the fragility of the actual system of traceability (page 5, lines 1-10). We agree that this issue, which may be related to the unexplained variance of the analysis and have added another row in Table. 1 about the specimen origin. This topic was further addressed in the discussion sections.

(1) comments from Referees

Discussion: Line 10: are there movement studies showing arapaima do not migrate long distances? If so, this is the place to cite them (again, after introducing them in the intro)

(2) author's response

We have cited previous studies about arapaima migration (page 14, lines 9-10).

(1) comments from Referees

Lines 12-14: this is where a well-developed discussion of the potential for lack of geographical specificity in the otolith data to influence the results could go.

(2) author's response

We have stated that the lack of precise information of fish origin have limited the approach and are one of the main causes of incongruity in the analysis (page 14, lines 10; page 18 line 19-21)

(1) comments from Referees

In general, the text of the discussion is sound. But I find it to be too long and unclear at times, so I suggest condensing it and revising it for clarity. What is really missing is linking the results to their application, following the idea suggested above.

(2) author's response

We have revised the text in order to make it clearer.

(1) comments from Referees

Spp is not italicized Line 25: use 'developed' instead of 'satisfying'

(2) author's response

We have made the changes suggested by the reviewer. The authors strongly valued all the suggestions made that are important contributions to improve the quality of the work.

We strongly valued all the suggestions made that are important contributions to improve the quality of the work.

(3) Editions

- Over all the documented spp was formatted as non-italic
- The in text citation and references was reviewed.
- The authors' filiation was reviewed
- Page 1 line 25 replaced satisfying with developed
- The introduction section was restructured according to the Referee 2 suggestions. the review of biogeochemical tag was shortened and a better description of the method assumption was provided as well as a contextualization of actual system of management and traceability of Arapaima spp.
- Page 2 line 10: replaced "certificate" with "characterize";
- page 2 lines 28-30: We have included a better description of the existing contrast in Sr isotopic composition among the different sub-basins of the Amazon, to provide a clear background for the reader;
- page 3 lines 8-15: We have addressed this issue in the introduction section of the revised manuscript as it constitutes an important justification of the study;
- page 3 lines 21-27: We have revised it and included a description of arapaima habitat and trophic relations;
- Page 4 line 5: replaced with: "The Amazon basin represents a dynamic and heterogeneous ecosystem extending over more the 45% of the surface area of South America.";
- Page 4 line 8: replaced with "These habitats are therefore some of the most biodiverse in the world, particularly in regard to the Amazonian freshwater fish fauna which is under pressure of degradation by dams, buildings, mining, land cover and global climate change"
- Page 4 line 12: replaced: "geologic" with "geological";
- page 5 Fig1-zoomed and the font was increased
- page 5, lines 1-10: We have also emphasized the lack of precise information about fish capture location, which is part of the fragility of the actual system of traceability;
- page 5-6 we added a column in table 1 with the informed origin of the fishes and reviewed the Mean Water 87Sr/86Sr
- Page 7 line 9: replaced "Inter laboratorian" with "Inter-laboratory";
- Page 8 line 10: replaced "repitability" with "repeatability";
- Page 8 Figure 2: replaced "87/86Sr" with "87Sr/86Sr" (numbers in superscript) and use this notation consistent throughout the entire document;
- Page 9 line 2: replace "The slice preparations were drilled per 6.8 mm interval" with "The slice preparations were drilled with intervals of 6.8 mm"
- Page 9 line 10: replaced "Also a test t was applied" with "A t-test was also applied"
- Page 11 Figure 4: replaced "87/86Sr" with "87Sr/86Sr" (numbers in superscript) and use this notation consistent throughout the entire document. Inserted "87Sr/86Sr" (numbers in superscript) for part (b) of the figure also;
- Page 11 line 8: replaced "interindividual" with "inter-individual";
- Page 12 Figure 5: replaced "87/86Sr" with "87Sr/86Sr" (numbers in superscript) and used this notation consistent throughout the entire document. Insert "87Sr/86Sr" (numbers in superscript) for Central Amazon part of the figure also;
- Page 13 Figure 6: replaced "87Sr/86Sr" with "87Sr/86Sr" (numbers in superscript);

- Page 13 line14: replace “(sample origin in a row, predicted origin in the column)” with “(sample origin rows, predicted origin columns)”;
- Page 14 line 1: replace “(sample origin in a row, predicted origin in the column)” with “(sample origin rows, predicted origin columns)”;
- page 14, lines 9-10: We have cited previous studies about arapaima migration;
- Page 14 line 18: changed: “The role of food is more controversial as revealed by Sturrock et al. (2012) that reviewed significant or nonsignificant food up taking processes on the Sr isotopic composition of fish.” Per “the role of food in the strontium otolith uptake is debated, as revealed by Sturrock et al. (2012) that reviewed the significance of food up taking processes on the Sr isotopic composition of fish”
- page 14, lines 10; page 18 line 19-21: We have stated that the lack of precise information of fish origin have limited the approach and are one of the main causes of uncertainty.
- Page 15 line 21: confirmed: “backwater” throughout the document.

(4) Marked-up manuscript version

Commercial traceability of *Arapaima* spp. fisheries in the Amazon Basin: can biogeochemical tags be useful?

Formatado: Fonte: Não Itálico

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Abstract. The development of analytical tools to determine the origin of fish is useful to better understand patterns of habitat use and to monitor, manage and control fisheries, including certification of food origin. The application of isotopic procedures to study fish calcified structures (scales, vertebrae, and otoliths) may provide robust information about the fish geographic origin and environmental living conditions. In this study, we used Sr and C isotopic markers recorded in otoliths of wild and farmed commercialized pirarucu (*Arapaima* spp.) to evaluate their prediction potential to trace the fish origin. Wild and farm fish specimens, as well as food used for feeding pirarucu in captivity, were collected from different sites. Isotope analyses of otoliths performed by IRMS ($\delta^{13}C$) and LAfs-MC-ICPMS ($^{87}Sr/^{86}Sr$) were compared to the isotopic composition of water and of the food given to the fish in the farms. Wild fish specimens that lived in environments with

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the largest fluctuation of river water Sr isotope ratios over time presented the largest Sr isotope variations in otoliths. A quadratic discriminant analysis on otolith isotopic composition provided 58% of correct classification for fish production (wild and farmed) and 76% of correct classification for the fish region. Classification accuracy for region varied between 100% and 29% for the Madeira and the lower Amazon fishes, respectively. Overall, this preliminary trial is not yet fully ~~satisfying~~developed to be applied as a commercial traceability tool. However, given the importance of *Arapaima* spp. for food security and the generation of economic resources for millions of people in the Amazon basin, further analyses are needed to increase the discrimination performance of these biogeographical tags.

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1 Introduction

Food production is becoming increasingly associated with sustainable practices ensuring environmental preservation goals. Food origin and production conditions have become important issues in a national and international trade to attest of ~~good practices. For instance, consumers want to know whether fish being consumed belongs to an endangered or vulnerable species and whether they have grown in natural or farmed conditions (Pracheil et al. 2014; Kim et al. 2015; Baffi and Trincherini 2016). In order to address some of these questions, and further improve wild and farming fish management, recent studies have used biogeochemical tracers to better understand fish population dynamic, their ecological strategies and stock origin (Thresher 1999; Kennedy et al. 2005; Rojas et al. 2007; Kerr and Campana 2013; Pracheil et al. 2014; Brennan and Schindler 2017). These tools have also been used to control national and international fish trade (Pracheil et al. 2014) and to identify the geographical origin and conditions under which fish have been raised (Bell et al. 2007; Rojas et al. 2007; Barnett-Johnson et al. 2008; Turchini et al. 2009). A sustainable management is important to ensure a~~adequate practices. For instance, consumers want to know whether fish being consumed belongs to an endangered or vulnerable species and whether they have grown in natural or farmed conditions (Baffi and Trincherini, 2016; Kim et al., 2015; Pracheil et al., 2014). In order to address some of these questions, and further improve wild and farming fish management, recent studies have used biogeochemical tracers to better understand fish population dynamic, their ecological strategies and stock origin (Brennan and Schindler, 2017; Kennedy et al., 2005; Kerr and Campana, 2013; Pracheil et al., 2014; Rojas et al., 2007; Thresher, 1999). These tools have also been used to control national and international fish trade (Pracheil et al., 2014) and to identify the geographical origin and conditions under which fish have been raised (Barnett-Johnson et al., 2008; Bell et al., 2007; Rojas et al., 2007; Turchini et al., 2009). A sustainable community-based management is important to ensure secure and fair food production, thus valorizing the local economy, respecting the ecosystems functioning, and maintaining ecosystem services.

~~Determination of fish geographic origin by stable isotopic analyses has been investigated by different authors aiming to characterize food origin, manage fisheries and fishes stocks, build knowledge of species life history, identify critical habitats for conservation, and avoid overexploitation of fish stocks (Pouilly et al. 2014; Pracheil et al. 2014; Hegg et al. 2015; Garcez et al. 2015; Baffi and Trincherini 2016; Duponchelle et al. 2016; Hauser 2018). For example, Turchini et al. (2009) discriminated the geographic origin of Australian Murray Cod (*Maccullochella peelii peelii*) using isotopic signatures of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$. In another study, Bell et al. (2007) analyzed~~

~~δ^{13}C and δ^{18}O in the fat meat of sea bass (*Dicentrarchus labrax*) to distinguish farmed versus wild fish. Rojas et al. (2007) analyzed δ^{13}C and δ^{15}N in *Sparus aurata* muscle from four Mediterranean countries to distinguish wild specimens from farmed ones. Besides soft tissues, isotopic information can also be extracted from fish calcified structures (scale, otolith or vertebrae) in order to preserve fish integrity for the commercial use and eventually reconstruct fish life history (Campana 1999; Pouilly et al. 2014; Pracheil et al. 2014).~~

~~Strontium isotopes have been used as origin tracer of food products because of their robust response in terms of origin authenticity and fraud detection for vegetables, drinks, milk derivatives, meat and fishes (Fortunato et al. 2004; Barnett-Johnson et al. 2008; Rummel et al. 2010; Di Paola-Naranjo et al. 2011; Trincherini et al. 2014; Baffi and Trincherini 2016). Fish otoliths, or ear bones, are calcified structures that grow continually and record ambient condition along fish's lives, from hatching to death (Campana 1999). Since Sr isotopes in otolith are not reabsorbed and do not fractionate during biological uptake, the isotopic ratio of this element is a robust geographic and trophic marker (Kennedy et al. 2000; Kerr and Campana 2013; Pouilly et al. 2014). Most studies using Sr isotopes in fish otoliths were performed on marine and freshwater ecosystems of temperate regions (Kennedy et al. 1997, 2000, 2002; Gillanders 2002; Woodhead et al. 2005; Comyns B. H. 2008). Only a few studies have focused on fish migration and living conditions in tropical river systems (Walther et al. 2011; Pouilly et al. 2014; Hegg et al. 2015; Garcez et al. 2015; Sousa et al. 2016; Duponchelle et al. 2016; Hauser 2018).~~

~~The Amazon basin has the largest rainforest on the planet and constitutes a complex system of rivers, lakes, and wetlands (Oliveira 1996). The region is also known to support a large diversity of fish species, many of which play an important economic role in the region, such as the *Arapaima* spp., known as one of the largest freshwater fish genus (Queiroz 2000; Hrbek et al. 2007; Stone 2007). The four described species (*A. agassizii, A. mapae, A. leptosoma,* and *A. gigas)* of this genus are endemic to the Amazon basin, where they are popularly called Pirarucu or Paiche (Arantes et al. 2010; Stewart 2013b, a). This genus is socially, economically and ecologically important in the region because it constitutes one of the main food sources for the local community, providing important economic resources on a local and regional scales (farming, fishing, trading). Due to overexploitation, *Arapaima spp.* have been classified as vulnerable by CITES and fishing is subject to legal restriction, such as seasonal fishing prohibition, the minimum size of capture and, most important, *Arapaima's* commercialization is restricted to fish proceeding from management areas or aquaculture farms (Feio and Mendes 2017). Paradoxically, *Arapaima spp.* are considered an exotic invasive species in the Upper Madeira watershed in Bolivia and Peru, after being introduced in the region in the 70' (Queiroz 2000; Arantes et al. 2010; Van Damme et al. 2011; Miranda-Chumacero et al. 2012; Araripe et al. 2013; Figueiredo 2013).~~

Determination of fish geographic origin by stable isotopic analyses has been investigated by different authors aiming to characterize food origin, manage fisheries and fishes stocks, build knowledge of species life history, identify critical habitats for conservation, and avoid overexploitation of fish stocks (Baffi and Trincherini, 2016; Duponchelle et al., 2016; Garcez et al., 2015; Hauser, 2018; Hegg et al., 2015; Jordaan et al., 2016; Pouilly et al., 2014; Pracheil et al., 2014). The methodological assumptions to discriminate fish stocks are based on (i) the isotopic heterogeneity among stocks and (ii) the low mobilization rates in the analyzed tissue (Kerr and Campana, 2013). Isotopic information can be extracted from fish calcified structures (scale, otolith

or vertebrae) in order to preserve fish integrity for the commercial use and eventually reconstruct fish life history (Campana, 1999; Pouilly et al., 2014; Pracheil et al., 2014). Strontium isotopes have been used as origin tracer of food products because of their robust response in terms of origin authenticity and fraud detection (Baffi and Trincherini, 2016). On the other hand, carbon isotopes have been used to distinguish farmed and wild fishes according to feeding patterns (Rojas et al., 2007; Turchini et al., 2009). Fish otoliths, or ear bones, are calcified structures that grow continually and record ambient condition along fish's lives, from hatching to death (Campana 1999). Since Sr isotopes in otolith are not reabsorbed and do not fractionate during biological uptake, the isotopic ratio of this element is a robust geographic marker (Kennedy et al., 2000; Kerr and Campana, 2013; Pouilly et al., 2014). Most studies using Sr isotopes in fish otoliths were performed on marine and freshwater ecosystems of temperate regions (Comyns B. H., 2008; Gillanders, 2002; Kennedy et al., 1997, 2000, 2002; Woodhead et al., 2005). Only a few studies have focused on fish migration and living conditions in tropical river systems (Duponchelle et al., 2016; Garcez et al., 2015; Hauser, 2018; Hegg et al., 2015; Pouilly et al., 2014; Sousa et al., 2016; Walther et al., 2011).

The Amazon basin has the largest rainforest on the planet and constitutes a complex system of rivers, lakes, and wetlands (Oliveira, 1996). It has a variety of different river waters (white, black, and clear) that drains complex and heterogeneous geologic formations (Santos et al., 2015). Because these rivers drain rocks with different origins and ages, their waters present contrasting Sr isotopic compositions, thus providing an adequate scenario for the application of Sr isotopes as a geographic tracer (Duponchelle et al., 2016; Hauser, 2018; Pouilly et al., 2014; Santos et al., 2015). The region is also known to support a large diversity of fish species, many of which play an important economic role in the region, such as the *Arapaima* spp., known as one of the largest freshwater fish genus (Hrbek et al., 2007; Queiroz, 2000; Stone, 2007). The four described species (*A. agassizii, A. mapae, A. leptosoma,* and *A. gigas)* of this genus are endemic to the Amazon basin, where they are popularly called Pirarucu or Paiche (Arantes et al., 2010; Stewart, 2013a, 2013b). This genus is social, economically and ecologically important in the region because it constitutes one of the main food sources for the local community, providing important economic resources on a local and regional scales (farming, fishing, trading). Due to overexploitation, *Arapaima* spp. have been classified as vulnerable by CITES and fishing is subject to legal restriction, such as seasonal fishing prohibition, the minimum size of capture and, most important, *Arapaima*'s commercialization is restricted to fish proceeding from management areas or aquaculture farms (Feio and Mendes, 2017). Paradoxically, *Arapaima* spp. are considered as exotic invasive species in the Upper Madeira watershed in Bolivia and Peru, after being introduced in the region in the 70'(Van Damme et al., 2011; Figueiredo, 2013; Miranda-Chumacero et al., 2012).

Synergic actions initiated in 1989 and involving communitarian lake management, governmental conservation policies, ONG's projects, and aquaculture production of *Arapaima* have allowed the recovery of overexploited stocks (Figueiredo, 2013; McGrath et al., 2015). For instance, more than 500 groups have permission for fishing *Arapaima* under an annual quota stipulated by the Federal Agency (IBAMA), which have limited resources to monitor, control and regulate fishery stocks (McGrath et al., 2015). Even though the "sustainable fishery" is certified with tags in order to allow traceability, these tags are easily frauded and illegally reused. In this

context, the development of an isotopic tag to track back the original precedence of *Arapaima* fishery could reinforce the actual system of traceability and combat illegal exploitation of *Arapaima* stocks.

Some aspects of *Arapaima* spp. biology qualify this group as a model for isotopic certification. This genus is described as sedentary, although individuals migrate locally from lateral lakes to rivers during flooding pulses in order to complete their life cycle (Arantes et al., 2010; Araripe et al., 2013; Castello, 2008). Also, because this genus is air breath dependent, individuals come out the surface to breath regularly and local fishermen can estimate populations size and realize the certification of the communitarian management. These characteristics lead to a spatial distinction between stocks of the major Amazonian regions and allow to use biogeochemical tags as a tracer of origin. Moreover, trophic patterns of *Arapaima* vary among populations and over the ontogeny from omnivorous (Watson et al. 2013) to carnivorous or piscivorous, predominantly based on C3 sources (Carvalho et al., 2018; Domingues et al., 2006; Queiroz, 2000). Because of this variation on feeding sources (Carvalho et al., 2018; Castello, 2008), a common strategy used in aquaculture and strongly recommended by governmental manuals of *Arapaima* farming is the nutritional training with three rations during different life stages (Ono and Kehdi, 2013). As a reference, $\delta^{13}C$ values of the food used on farming activity vary between -19.3‰ and -14.9‰, thus corresponding to a diet based mostly on C4 macrophytes, corn, and soya beans.

In this study, we hypothesize that farmed and wild fishes of different sub-basin would present different $\delta^{13}C$ and $^{87}Sr/^{86}Sr$ values, depending on their food sources ($\delta^{13}C$) and geographical origins ($^{87}Sr/^{86}Sr$). Hence, the main objective is to test whether $\delta^{13}C$ and $^{87}Sr/^{86}Sr$ measured on fish otoliths can be used as biogeochemical tags of the geographic origin and provenance (wild vs. farmed) of *Arapaima* specimens and, consequently, evaluate if these isotopes can be used as a traceability tool. We analyzed Sr and C isotopic composition of *Arapaima*'s otoliths from farmed and wild fishes proceeding from four different Amazonian regions (Madeira, Solimões, Central Amazon, Lower Amazon). Sr and C isotope data were analyzed across transect in *Arapaima*'s otoliths in order to identify habitat change or geographic mobility during the fish lifetime. These data were also compared to the C and Sr isotopic composition of fish food supplied by the farmers.

2. Material and methods

2.1 Study area

The Amazon basin represents a dynamic and heterogeneous ecosystem extending over more the 45% of the surface area of South America. The Amazon River and its huge network of tributaries drain different geological formations ~~(Gibbs 1967; Stallard 1980; Stallard and Edmond 1983; Gaillardet et al. 1997; Santos et al. 2015)~~(Gaillardet et al., 1997; Gibbs, 1967; Santos et al., 2015; Stallard, 1980; Stallard and Edmond, 1983) covered by primary forests, chaparral savannas, floodplains, and swamps. These habitats are therefore some of the most biodiverse in the world, particularly in regard to the Amazonian freshwater fish fauna, which are under pressure of degradation by dams building, mining, land cover and global climate change (Anderson et al., 2018; Carnicer et al., 2015; Castello et al., 2013; Castello and Macedo, 2016; Finer and Jenkins, 2012; ~~Castello~~Forsberg et al~~. 2013; Carnicer~~., 2017; Latrubesse et al~~. 2015; Castello and Macedo~~

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2016., 2017; Lees et al., 2016; Winemiller et al., 2016; Forsberg et al. 2017; Latrubesse et al. 2017; Anderson et al. 2018).

The Amazon basin is a geomorphological depression located between two old and stable geologic regions: the Guiana shield at the North; and the Brazilian shield at the South. While the Andes Mountain chain limits the western border of the basin, cratonic terrains and the Atlantic Ocean limit its eastern border. Owing to its complex geological history, rivers of the Amazon basin drain rocks with a wide range of Sr isotope compositions (Santos et al., 2015). For example, the Madeira River and Negro River drain old rock formations, such as Precambrian and Ordovician rocks that imprint a strong radiogenic Sr isotope signature in their waters (respectively 0.7168 ± 0.0007 and 0.7318 ± 0.0074, Santos et al., 2015). In contrast, the Solimões river drains younger formations as well as carbonate rocks, so that their water is characterized by less radiogenic Sr isotope ratios (0.7091 ± 0.0002, Gaillardet et al., 1997; Santos et al., 2015). Because of this heterogeneity, Sr isotopes in Amazon river waters may be used as a robust biogeographic marker for aquatic fauna (Pouilly et al. 2014; Hegg et al. 2015; Garcez et al. 2015; Sousa et al. 2016; Duponchelle et al. 2016; Hauser 2018).

The carbon isotopic composition of an organism depends primarily on the isotopic composition of the primary producer that constitutes the beginning of the trophic chain it feeds on. In particular, plants present two main photosynthetic pathways, C3 and C4, which produce a two-contrasted $\delta^{13}C$ range of values (-32‰ to -24‰ for C3 plants; -14‰ to -9‰ for C4 plants; De Niro & Epstein, 1978). In general, wild Amazon fishes feed dominantly on a trophic chain derived from C3 carbon source (Araujo-Lima et al. 1986; Forsberg et al. 1993; Jepsen and Winemiller 2007; Marshall et al. 2008; Watson et al. 2013; Mortillaro et al. 2015). Feeding habits of Arapaima vary among populations from omnivorous (Watson et al. 2013) to carnivorous or piscivorous (Queiroz 2000; Domingues et al. 2006; Carvalho et al. 2018). Because of this variation on feeding sources (Castello 2008; Carvalho et al. 2018), a common strategy, strongly recommended by governmental manuals of Arapaima farming, is the nutritional training with three rations during different life stages (Ono and Kehdi 2013). These rations are generally based on soya bean (C3), corn (C4), and macrophytes (C3 or C4).

AThe Amazon basin is a geomorphological depression located between two old and stable geological regions: the Guiana shield at the North; and the Brazilian shield at the South. While the Andes Mountain chain limits the western border of the basin, cratonic terrains and the Atlantic Ocean limit its eastern border. Owing to its complex geological history, rivers of the Amazon basin drain rocks with a wide range of Sr isotope compositions (Santos et al., 2015). For example, the Madeira River and Negro River drain old rock formations, such as Precambrian and Ordovician rocks that imprint a strong radiogenic Sr isotope signature in their waters (respectively 0.7168 ± 0.0007 and 0.7318 ± 0.0074, Santos et al., 2015). In contrast, the Solimões river drains younger formations as well as carbonate rocks, so that their water is characterized by less radiogenic Sr isotope ratios (0.7091 ± 0.0002, Gaillardet et al., 1997; Santos et al., 2015). Because of this heterogeneity, Sr isotopes in Amazon river waters may be used as a robust biogeographic marker for aquatic fauna (Duponchelle et al., 2016; Garcez et al., 2015; Hauser, 2018; Hegg et al., 2015; Pouilly et al., 2014; Sousa et al., 2016).

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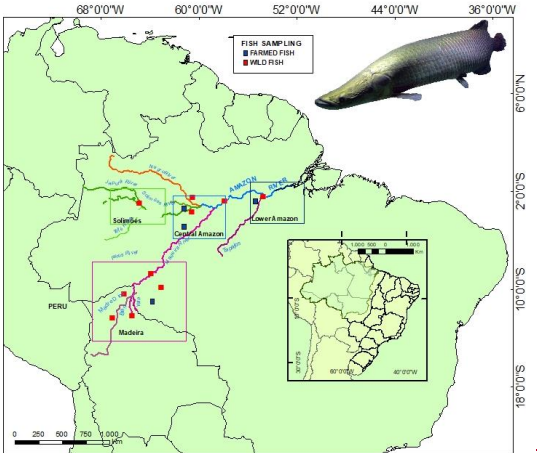
2.2 Fish otolith sampling ~~of fish otoliths and of farming food~~

Thirty-eight otolith samples of *Arapaima* spp. were collected in different sites of four main regions (Figure 1): 22 were obtained from professional fishermen for a commercial purpose; 6 were obtained from farmers, and 10 others were collected in Manaus and Santarém markets (Table 1). The sagittae otoliths of each specimen were extracted by head dissection after capture. Afterward, they were washed, dried, and kept ~~under cool conditions until laboratory analyses.~~until laboratory analyses. None of the fishes had precise location of capture, and the informed origin in four main regions were (Figure 1): Solimões (Mamirauá Reserve at the Solimões river), Madeira (Yata, Beni and Madre de Dios Rivers and Ariquemes farm), Central Amazon (Manacapuru farm, at Solimoes River; Itacoatiara, at Amazon River; Novo Airao at Negro River) and Lower Amazon (Santarem, Amazon river). These regions have a complex drainage system that may include different sources of water with different Sr isotopic compositions in each site (Table 1). For example, Mamiraua Reserve in Solimoes region is located at the confluence of five major rivers, which waters range from white (lower $^{87}Sr/^{86}Sr$ values) to black water type (higher $^{87}Sr/^{86}Sr$ values). Similarly, Itacoatiara is located at the confluence zone of the Amazon and Madeira rivers, which also have quite distinct Sr isotopic compositions. Therefore, the lack of precise information of collection and the variability in regional water chemistry may not be exactly matched by literature data and may be important to explain the unexpected variance of fish otolith data.



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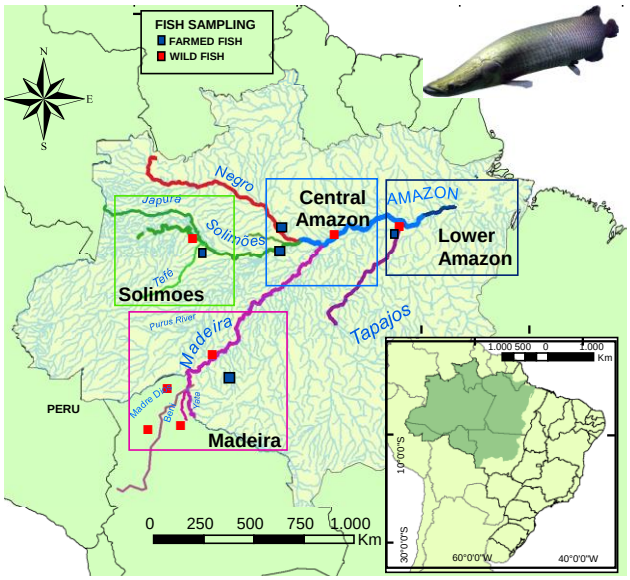

Figure 1. Map of the Amazon basin showing the regions and sampling sites for *Arapaima* ~~*gigas*~~spp. Wild collect sites are represented by red squares, farm collect sites by blue squares. The Amazon River (Lower Amazon and Central Amazon) is colored blue, the Solimões green, the Madeira purple, and the Negro ~~orange.~~ red.

~~**Table 1. $\delta^{13}C$ (mean ±SD) and $^{87}Sr/^{86}Sr$ (mean ±SD) measured in sagittae otolith for 38 wild and farmed *Arapaima gigas* proceeding from five Amazonian regions with correct classification and correspondent water $^{87}Sr/^{86}Sr$ reviewed from literature. [1]Palmer and Edmond (1992), [2]Gaillardet et al. (1997), [3]Queiroz et al. (2009), [4]Pouilly et al. (2014), [5]Santos et al. (2015) and [6]Duponchelle et al. (2016)**~~

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Specimen code	~~Origin~~ Region	~~Geographic region~~Fish origin	Site of collection	Informed origin~~Otolith $^{87}Sr/^{86}Sr$~~	Mean Water $^{87}Sr/^{86}Sr$	Otolith $^{87}Sr/^{86}Sr$	QDA prediction ~~provenience~~	Otolith $\delta^{13}C$
COO	~~Farming~~	Farming	Farming	Manacapuru ~~farming~~	~~0.71543~~ 0.7095~~7~~091±0.0008[2,3]0003[5,6]	0.71543~~Negro~~	Central Am	-18.6
I1	~~Wild~~	Wild	Fisherman	Itacoatiara ~~fishers~~	~~0.71029~~ 0.7111 ±0.0004[2,5,6]	0.71029	Central	-18.3

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											Amazon Am	
I2	Wild	Central Amazon	Wild	Fisherman	Itacoatiara fishers	0.7267	0.7111 ±0.0004[2,6]	0.71267			Central Amazon Am	-15.4
I3	Wild	Central Amazon	Wild	Fisherman	Itacoatiara fishers	0.7020	0.7111 ±0.0004[2,6]	0.71020			Central Amazon Am	-20.0
I4	Wild	Central Amazon	Wild	Fisherman	Itacoatiara fishers	0.7041	0.7111 ±0.0004[2,6]	0.71041			Central Amazon Am	-19.9
NA1	Farming	Central Amazon	Farming	Manaus market	Novo Airao 0.70997		0.71117300 ±0.0004[2]0138[2,6]	0.70997			Solimões	-25.2
NA2	Farming	Central Amazon	Farming	Manaus market	Novo Airao		0.7300 ±0.0138[2,]	0.70977	0.7111 ±0.0004[2,6]		Solimões	-25.2
NA3	Farming	Central Amazon	Farming	Manaus market	Novo Airao		0.7300 ±0.0138[2,]	0.70926	0.7111 ±0.0004[2,6]		Solimões	-26.0
NA4	Farming	Central Amazon	Farming	Manaus market	Novo Airao		0.7300 ±0.0138[2,]	0.70982	0.7111 ±0.0004[2,6]		Solimões	-24.1
FMn1	Farming	Solimões	Farming	Manaus market	Tefé 0.70896		0.7095±0.0008 [2,3,6]	0.70896			Solimões	-25.1
FMn2	Farming	Solimões	Farming	Manaus market	Tefé 0.70894		0.7095±0.0008 [2,3,6]	0.70894			Solimões	-24.1
FMn3	Farming	Solimões	Farming	Manaus market	Tefé 0.70894		0.7095±0.0008 [2,3,6]	0.70894			Solimões	-23.2
M1	Wild	Solimões	Wild	Fisherman	Mamirauá Reserve	0.70904	0.7095±0.0008 [2,3,6]	0.70904			Solimões	-25.6
M2	Wild	Solimões	Wild	Fisherman	Mamirauá Reserve	0.70932	0.7095±0.0008 [2,3,6]	0.70932			Solimões	-25.4

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M3	~~Wild~~	Solimões	Wild	Fisherman	Mamiauá Reserve	~~0.70963~~	0.7095±0.0008 [2,3,6]	0.70963		Solimões	-24.8
M4	~~Wild~~	Solimões	Wild	Fisherman	Mamiauá Reserve	~~0.70941~~	0.7095±0.0008 [2,3,6]	0.70941		Solimões	-25.1
M5	~~Wild~~	Solimões	Wild	Fisherman	Mamiauá Reserve	~~0.70944~~	0.7095±0.0008 [2,3,6]	0.70944		Solimões	-25.1
CS1	~~Farming~~	Lower Amazon	Farming~~Santarém Market~~	Santarem market~~0.71382~~	Santarem		0.7112±0.0004 [2,3,6]	0.71382		Lower ~~Amazon~~Am	-13.7
CS2	~~Farming~~	Lower Amazon	Farming~~Santarém Market~~	Santarem market~~0.71291~~	Santarem		0.7112±0.0004 [2,3]	0.71291		Lower ~~Amazon~~Am	-15.6
CS5	~~Farming~~	Lower Amazon	~~Santarém Market~~Farming	antarem arket~~0.71479~~	Santarem		0.7112±0.0004 [2,3,6]	0.71479		Lower ~~Amazon~~Am	-14.0
S1	~~Wild~~	Lower Amazon	Wild~~Santarém fishers~~	Fisherman~~0.70868~~	Santarem		0.7112±0.0004 [2,3,6]	0.70868		Solimões	-22.9
S3	~~Wild~~	Lower	Wild~~Santa~~	Fisherme	Santarem		0.7112±0.0004	0.70873		Solim	-
S4	~~Wild~~	Lower Amazon	Wild~~Santarém fishers~~	Fisherman~~0.70868~~	Santarem		0.7112±0.0004 [2,3,6]	0.70868		Solimões	-28.4
S5	~~Wild~~	Lower Amazon	Wild~~Santarém fishers~~	Fisherman~~0.70865~~	Santarem		0.7112±0.0004 [2,3,6]	0.70865		Solimões	-25.6
R1		Madeira	Farming	~~Madeira~~Farming	Arquimes ~~farming~~	~~0.72245~~	0.7188±0.0012 [1,5,6]	0.72245		Madeira	-8.7
R2		Madeira	Farming	~~Madeira~~Farming	Arquimes ~~farming~~	~~0.71479~~	0.7188±0.0012 [1,5,6]	0.71479		Madeira	-8.3
R3		Madeira	Farming	~~Madeira~~Farming	Arquimes ~~farming~~	~~0.71479~~	0.7188±0.0012 [1,5,6]	0.71479		Madeira	-4.8

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R4		Madeira	Farming	~~Madeira~~Farming	Arique mes ~~farming~~	~~0.71479~~	0.7188±0.0012 [1,5,6]	0.71479			Madeira	-8.6
R5		Madeira	Farming	Farming ~~Madeira~~	Arique mes ~~farming~~	~~0.71479~~	0.7188±0.0012 [1,5,6]	0.71479			Madeira	-8.2
YA-1	~~Wild~~	Madeira	Wild	Fisherman	Yata ~~fishers~~ river	~~0.72854~~	0.7245±0.0018 [4,6]	0.72854			Madeira	-24.8
YA-12	~~Wild~~	Madeira	Wild	Fisherman	Yata ~~fishers~~ river	~~0.72848~~	0.7245±0.0018 [4,6]	0.72848			Madeira	-26.6
YA-13	~~Wild~~	Madeira	Wild	Fisherman	Yata ~~fishers~~ river	~~0.72885~~	0.7245±0.0018 [4,6]	0.72885			Madeira	-28.1
FL-1	~~Wild~~	Madeira	Wild~~Madre de Dios fishers~~	Fisherman	Beni river		0.7184±0.0011 [1,6]	0.72468	~~0.7100±0.0004 [1,6]~~		Madeira	-27.6
FL-2	~~Wild~~	Madeira	Wild~~Madre de Dios fishers~~	Fisherman	Beni river		0.7184±0.0011 [1,6]	0.72143	~~0.7100±0.0004 [1,6]~~		Madeira	-27.0
FL-20	~~Wild~~	Madeira	Wild~~Madre de Dios fishers~~	Fisherman	Beni river		0.7184±0.0011 [1,6]	0.72232	~~0.7100±0.0004 [1,6]~~		Madeira	-24.8
LV-6	~~Wild~~	Madeira	Wild	Fisherman	Madre de Dios ~~fishers~~	~~0.71441~~	0.~~7100~~7119±0.0004 [1,6]	0.71441			Madeira	-24.7
LV-18	~~Wild~~	Madeira	Wild	Fisherman	Madre de Dios ~~fishers~~	~~0.71479~~	0.~~7100~~7119±0.0004 [1,6]	0.71479			Madeira	-23.7
LV-20	~~Wild~~	Madeira	Wild	Fisherman	Madre de Dios ~~fishers~~	~~0.72232~~	0.~~7100~~7119±0.0004 [1,6]	0.72232			Madeira	-23.8

~~Additionally, three samples of farmed fish food commonly used to feed Arapaima were obtained from farmers. The three food types used to feed the different fishes life's stages were collected,~~

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~~presenting different grain sizes: 0.8 to 2.5 mm to feed fingerlings (0+ fish), 4 mm to feed subadults (1+ and 2+ fish) and 10 mm to feed adults.~~

2.3 Samples Preparation and Analytical Methods

The otoliths were sonicated in distilled water, dried and mounted in Araldite epoxy resin at MALBEC laboratory in Montpellier University (France). Afterward, they were transversally cut with a low-speed saw (Isomed Buehler, Düsseldorf, German, 2009) to obtain a dorso-ventral slice including the otolith core. The slices were then fine-polished until the core could be seen, sonicated in distilled water and mounted on glass using crystal bond glue. Sr isotope analyses were performed at the *Laboratoire de Chimie Analytique Bio-inorganique et Environnement* (LCABIE) from the *Institut Pluridisciplinaire de Recherche sur l'Environnement et les Matériaux* (IPREM), *Université de Pau et des Pays de l'Adour* and in the laboratory PSO-IFREMER (Pole Spectrometrie Océan, Brest), France. Inter-laboratory cross-calibration was performed to confirm the ~~reatability~~repeatability and comparability of the analysis, (~~For more details~~ see Hauser, 2018 for details). The isotope ratios were measured using ~~a~~an fs- La-MC-ICPMS -following the procedure detailed by ~~(Claverie et al. 2009; Tabouret et al. 2010)~~(Claverie et al., 2009; Tabouret et al., 2010). Laser ablation conditions were 500 Hz, 20 µJ pulse energy until the depth limit ablation (<30 µm), the beam spot size of 10 µm, and velocity 5 µm/s. The Sr isotope ratios were obtained by transects (200 µm width, see Tabouret et al. 2010) along the major otolith axes, i.e. perpendicular to growth otolith lines. The laser ablated material was carried with He gas to a double torch chamber in which the laser aerosol was mixed with a 2% HNO_3 solution before introduction into the plasma ~~(Barats et al. 2007)~~(Barats et al., 2007). These conditions were adjusted to obtain the maximal plasma sensibility and stability. Interferent ^{87}Rb signal was monitored by ^{85}Rb, and $^{87}Sr/^{86}Sr$ was corrected following ~~Barnett-Johnson et al. (2010)~~Barnett-Johnson et al. (2010) procedure. Similarly, ^{83}Kr was measured to control ^{84}Kr and ^{86}Kr impact in ^{84}Sr and ^{88}Sr values, respectively. Finally, the ratio $^{86}Sr/^{88}Sr$ was used to correct $^{87}Sr/^{88}Sr$ and mass bias using the exponential law ~~(Walther and Thorrold 2008).~~(Walther and Thorrold, 2008). Internal pattern $^{87}Sr/^{86}Sr$ ratio (NIESS 22, certificated by the National Institute of Japan Environmental Studies) was analyzed ~~in~~at the beginning and end of each session of analysis to check the ~~reliability~~repeatability of the $^{87}Sr/^{86}Sr$ measures.

Complete transects from core to edge were performed on 10 wild otolith samples, all of which presented a flat $^{87}Sr/^{86}Sr$ ratio pattern along the transect. For the remaining samples, the transect was performed only on the final 1/3 part of the otoliths, which records the environmental condition during the last life period of the adult fish (Figure 2). The results presented hereafter corresponding to the final part of the otolith for all the individuals.

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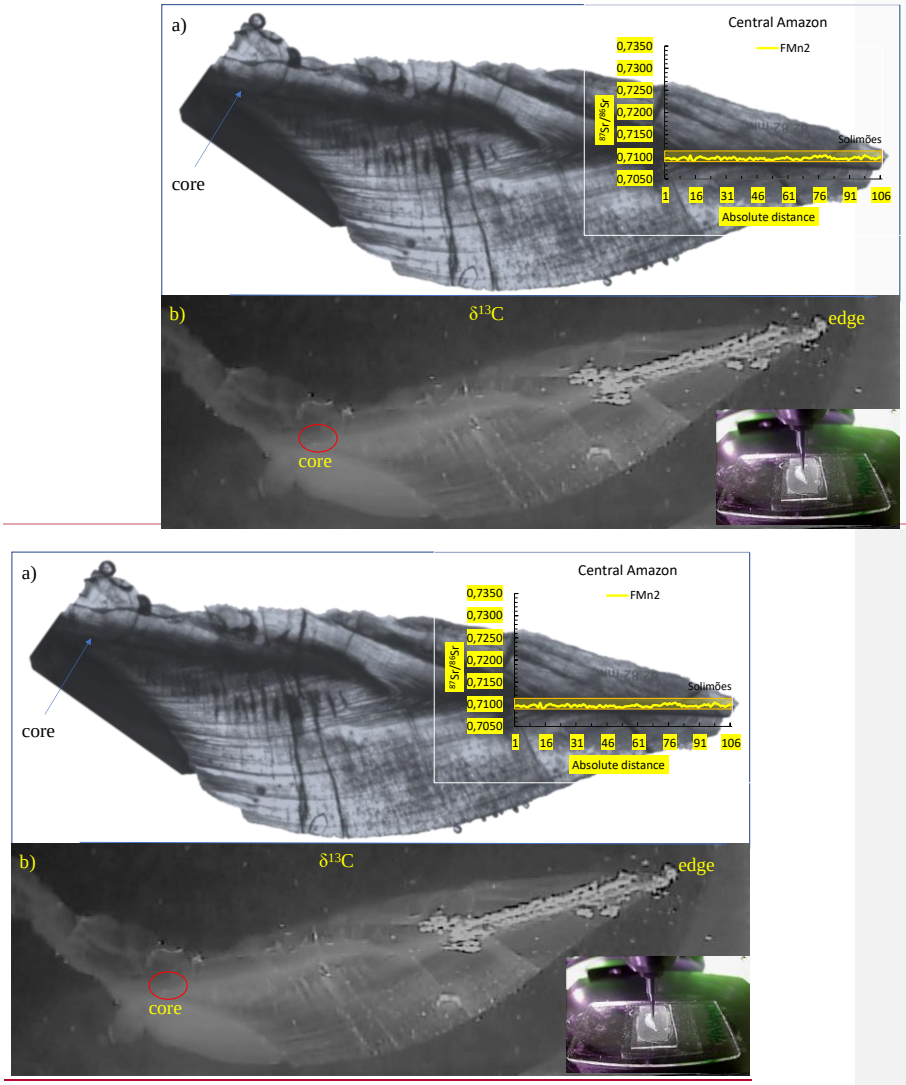

Figure 2. Photography of an Arapaima's otolith slice preparation. a) with the corresponding $^{87}Sr/^{86}Sr$ isotopic profile over the raster, which corresponds to the final part of a core to edge transect representing the last period of life of the individual before its capture, analyzed by laser ablation. The yellow rectangle illustrates the range of $^{87}Sr/^{86}Sr$ values of the Solimoes waters in the Central and Lower Amazon. b) Illustration of the micro-drilling sampling performed in the same transect in order to analyze $\delta^{13}C$ signatures.

To obtain dietary information based on carbon isotopes, the same last 1/3 portion of each otolith was micro-drilled on the same slice preparations of otolith used to gather the Sr isotope data. The slice preparations were drilled ~~a~~with intervals of 6.8 mm ~~interval~~ using a New Wave Microdrill at the Universidade de Brasília. The drilled carbonate powder samples were placed directly into vials for isotope analysis. Carbon isotopes were measured using a Delta Plus V Thermo-Fisher mass spectrometer connected to a Finnigan GasBench II .at *the Laboratório de Isótopos Estáveis (LAIS), Instituto de Geociências Rede de Estudos Geocronológicos, Geodinâmicos e Ambientais (GEOCHRONOS), ~~University~~Universidade de Brasília, Brazil.* The results were validated against reference standards NBS 18 and 19 (respectively $\delta^{13}C$= -5.0‰ and 1.9‰).

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~~2~~2.4 Statistical Analysis

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ANOVA was applied to test $^{87}Sr/^{86}Sr$ and the $\delta^{13}C$ mean difference in otoliths among 1) wild fish proceeding from the four regions, and 2) farmed fishes from the four regions. A t-test ~~t~~ also was applied to ~~test~~evaluate the mean difference between all wild vs. farmed fishes. To evaluate the use of $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ as a predictive tracer of fish origin (farmed or wild) and sub-basin/region of capture (Upper Amazon: Madeira and Solimões, Central Amazon, Lower Amazon), a quadratic discriminant analysis (QDA) ~~(Anderson et al. 2010; Li et al. 2016)~~(Anderson et al., 2010; Li et al., 2016) was carried out using a cross-validation by Jackknifed (leave one out) predictions procedure. All the statistical analyses were performed in R freeware (http://www.r-project.org/).

3. Results

3.1 Otolith Sr isotopic composition

Significant differences were observed (Figure 3) between 1) mean $^{87}Sr/^{86}Sr$ of wild fishes from the four sampled regions (ANOVA, F=18,397, p< 0,01); 2), mean $^{87}Sr/^{86}Sr$ of farmed fishes from the four sampled regions (ANOVA, F=5.614, p=0.0161), and 3) mean $^{87}Sr/^{86}Sr$ of wild vs. farmed of the same region (t = -3.764, df = 31.805, p-value p< 0,01).

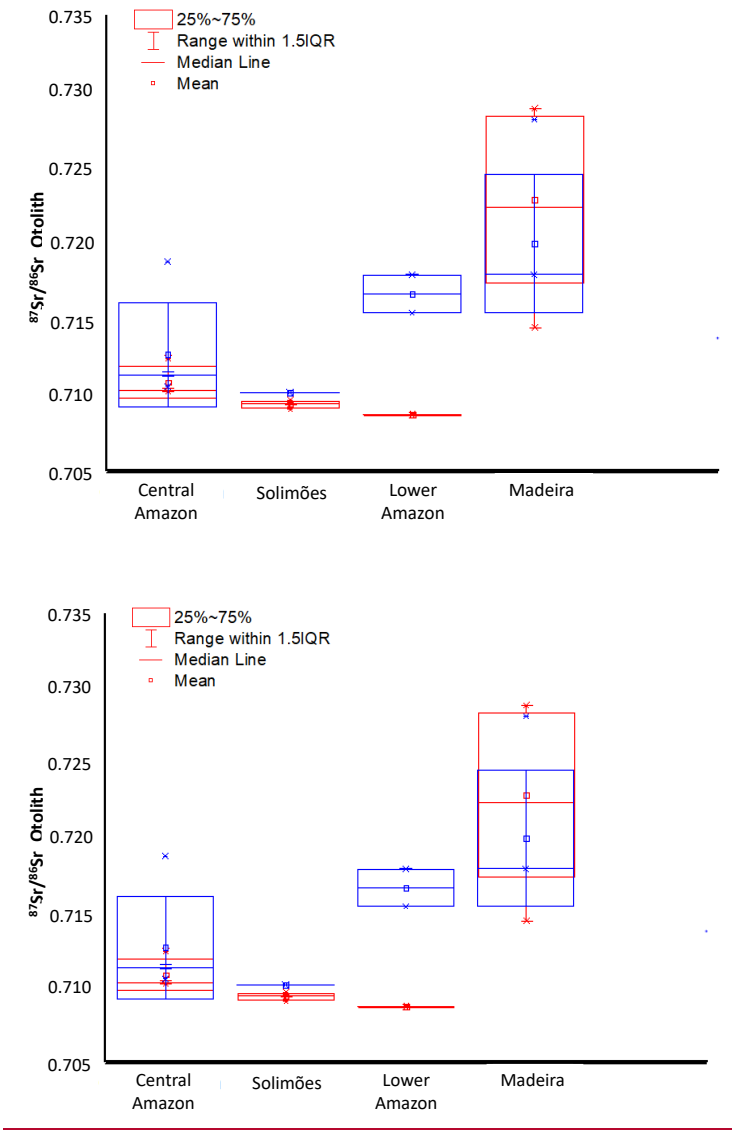

Figure 3. Boxplot of otolith $^{87}Sr/^{86}Sr$ ratio of wild (red) and farmed (blue) *Arapaima* spp. from four Amazonian regions.

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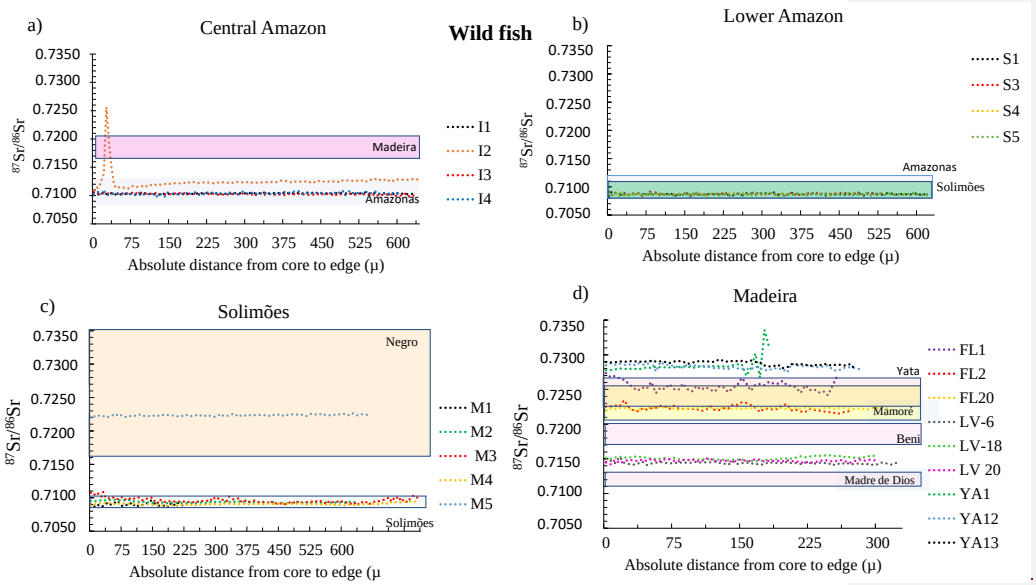
Except for two specimens, the wild fishes presented a narrow range of $^{87}Sr/^{86}Sr$ across the otoliths (Figure 4) and their average $^{87}Sr/^{86}Sr$ values are comparable to $^{87}Sr/^{86}Sr$ of the river waters

in which they were living (Table 1). The first exception is a fish from Central Amazon-Itacoatiara (I2) that exhibited $^{87}Sr/^{86}Sr$ similar to other individuals from the same site but also displayed a peak of $^{87}Sr/^{86}Sr$ value up to 0.7259 (Figure 4a). The second exception is a specimen from the Solimões-Mamirauá area (M2) that presented higher $^{87}Sr/^{86}Sr$ values (0.7223 +/- 0.0001) in comparison to the river values (0.7090-0.7100) and to other individuals from the same site (0.709-0.7110, Figure 4c).



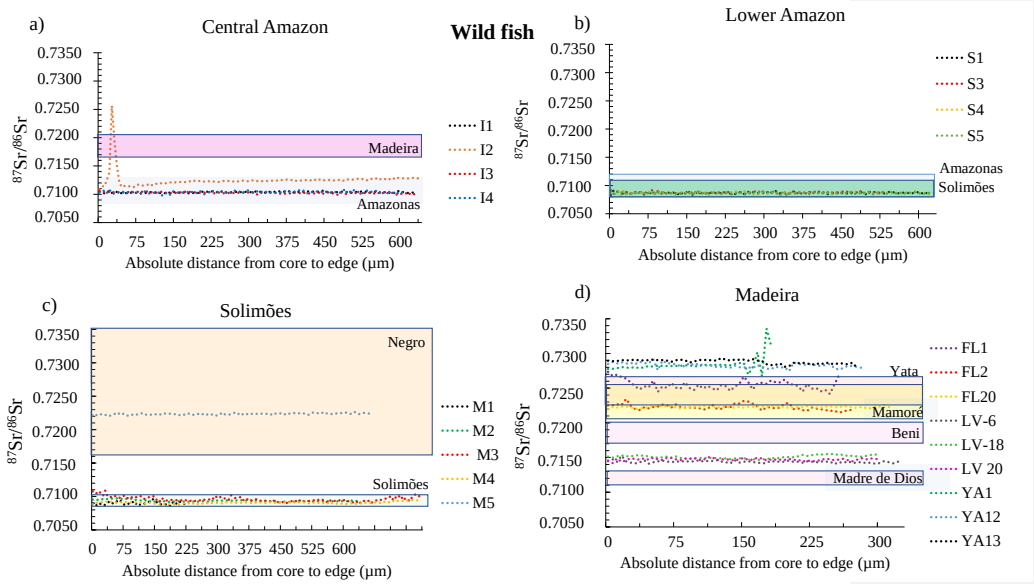


Figure 4. Variation of $^{87}Sr/^{86}Sr$ measured by LAfs-MC-ICPMS on wild fish otoliths core - edge transects. Only the final part of the transect (approximately 1/3) is represented. -Individual fishes were grouped by geographic region: a) Central Amazon; b) Madeira c) Lower Amazon and d) Solimões. The range of $^{87}Sr/^{86}Sr$ of river water dissolved matter for each geographic region is indicated by a colored rectangle (based on literature and additional analyses, see appendix 1)

The data also revealed differences in the variability of $^{87}Sr/^{86}Sr$ values among specimens from the same region (Figure 3). Individuals from Solimões, Central Amazon (0.7090 to 0.7096) and ~~lower~~Lower Amazon (0.7086 to 0.7087) presented a low inter-individual variation in comparison to individuals from the Upper Madeira (0.7144 to 0.7288) region.

A clear relationship also existed between the average $^{87}Sr/^{86}Sr$ values in otolith of farming fish and in local river water. Farmed fishes also generally showed a flat profile of $^{87}Sr/^{86}Sr$ values along the otolith (Figure 5), except for three individuals. One of the fishes collected directly with farmers at Manaus (COO) presented initial $^{87}Sr/^{86}Sr$ values similar to the Negro River waters. In contrast, $^{87}Sr/^{86}Sr$ values of the other specimens of the same region presented isotope ratios in the range of Solimões River waters during all their life (Figure 5a). One specimen from the ~~lower~~Lower Amazon – Santarém area (CS1) presented important fluctuations of $^{87}Sr/^{86}Sr$ values across the otolith, corresponding to values in the range of Tapajos river, although the two other specimens collected in the same area (CS2, CS3) presented flat profiles with values intermediate between the Amazon and Tapajos river waters (Figure 5b). Finally, one of the farmed specimens of the Madeira River (R1) also showed a fluctuating $^{87}Sr/^{86}Sr$ profile (Figure 5c), although the other four fishes showed a flat and completely overlapping profile.

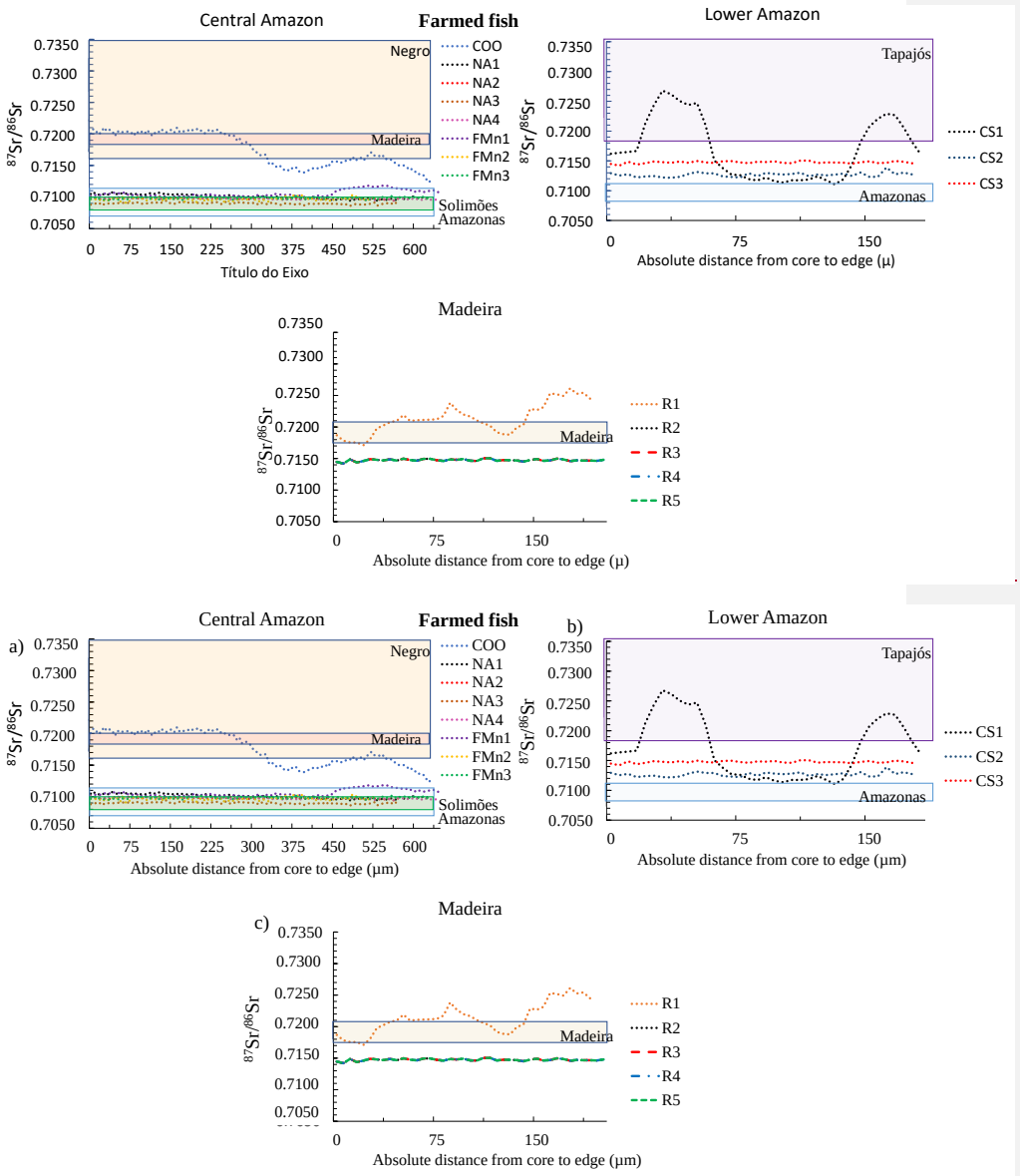


Figure 5. Variation of $^{87}Sr/^{86}Sr$ measured by LAfs-MC-ICPMS on farmed fish otoliths core - edge transects. Only the final part of the transect (approximately 1/3) is represented. Individual fishes were grouped by geographic region: a) Central Amazon (Manaus Market); b) Lower Amazon (Santarém Market) c) Madeira (~~Rondônia~~Ariquemes farm). Note that individuals R2, R3, R4, and R5 present the same $^{87}Sr/^{86}Sr$ profile. The range of $^{87}Sr/^{86}Sr$ of river water dissolved matter for each geographic region is indicated by a colored rectangle (based on literature and additional analyses, see appendix 1).

3.2 Food and Otolith Carbon isotopic composition

The $\delta^{13}C$ values of otoliths were significantly different among wild and farming specimens (ANOVA, F=124.44, p< 0,01). Most samples of wild fish present $\delta^{13}C$ consistent with C3 sources~~,~~ (mean -28.9 ± 1.2‰). The exceptions ~~are~~were all samples from Itacoatiara (Central Amazon), which display a mean $\delta^{13}C$ ~~values (-~~value of -18.4‰±1.8~~),~~ that fall ~~in~~ between the C3 and C4 signatures (Figure 6). In contrast, otoliths of farmed fish ~~present~~presented a wide range of $\delta^{13}C$ (mean -17.1‰ ± 7.7, min = 26.0‰, max = 4.8‰). The otoliths of the fishes from the Madeira region farms ~~present averaged~~presented a mean $\delta^{13}C$ value of -8.5‰ ± 0.1, indicating a strong contribution of C4 plants in their feeding source. Farmed fish from the market of Santarém (Lower Amazon) presented a mean $\delta^{13}C$ value of -14.4‰ ± 0.8 in their otoliths, thus revealing a contribution of both C3 and C4 plants in their feeding source. Otoliths of farmed fish from the market of Manaus (Central Amazon) presented a lower mean $\delta^{13}C$ value (-24.7‰ ± 0.8), indicating a main C3 feeding source.

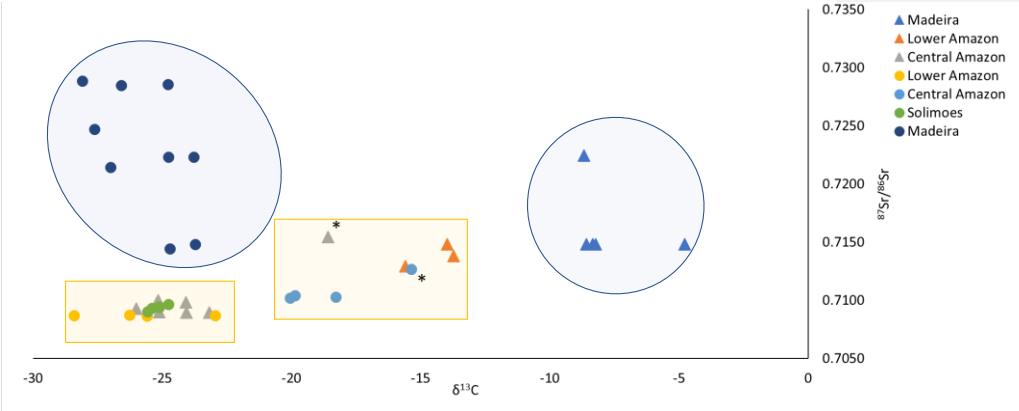


Figure 6. Biplot of mean $\delta^{13}C$ and $^{87}Sr/^{86}Sr$ for ~~41~~38 wild (circle) and farmed (triangle) *Arapaima* otoliths from ~~five~~four geographic regions. Large blue circles represent farmed and wild fish from the Madeira, and yellow squares wild and farmed fishes from ~~Low~~Lower Amazon, Central Amazon, and Solimões. The only two samples that are out of their group are tagged with *.

~~3.2 As a reference, $\delta^{13}C$ values of the food used on farming activity varies between -19.3‰ and -14.9‰, thus corresponding to a diet based mostly on C4 macrophytes, corn, and soya beans.~~

3.3 QDA Discriminant Analysis

The $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ isotopes biplot shows that all otolith samples fall within four main groups (Figure 6). The carbon isotopic composition combined with the average $^{87}Sr/^{86}Sr$ ratio of the fish otolith allows to partially distinguish the different fish origin (farmed or wild) as well as their geographical region.

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Wild fishes presented more negative $\delta^{13}C$ values (mean -24.3‰ ± 3.2) corresponding to a diet more influenced by C3 macrophytes carbon source, whereas farmed fishes presented less negative $\delta^{13}C$ values (mean -17.1‰ ± 7.7), corresponding to a higher influence of C4 carbon source. However, farmed fishes presented a higher variability indicating different sources of food depending on the ~~region or~~ farm. This variability led to ~~a~~ low predictability of fish origin (58% of correct classification, Table 2).

Table 2 Confusion matrix of fish origin classification by QDA (sample origin rows, predicted origin columns).

	~~farm~~Farm	~~wild~~Wild	~~correct~~Correct prediction
~~farm~~Farm	8	8	0.50
~~wild~~Wild	8	14	0.64
~~total~~Total correct prediction			0.58

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On the contrary, QDA analysis gave a higher score of correct classification of fish's region (76%, Table 3). This percentage varied between sub-basins, from 100% (Madeira) to 29% (Lower Amazon).

Table 3 Confusion matrix of fish region classification by QDA (sample origin rows, predicted origin columns). CA = Central Amazon; LA = Lower Amazon; MA = Madeira Basin; SO = Solimoes Basin

	CA	LA	MA	SO	~~correct~~Correct prediction
CA	10	1	1	0	0.83
LA	4	2	1	0	0.29
MA	0	0	14	0	1.00
SO	2	0	0	3	0.60
~~total~~Total correct prediction					0.76

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~~**(1) Discussion**~~

~~The data presented showed major differences in otolith's C and Sr isotopic composition among the studied populations of Arapaima. In general, the $^{87}Sr/^{86}Sr$ measured in otoliths of wild specimens were similar to the $^{87}Sr/^{86}Sr$ reported in the dissolved fraction of the river water in~~

which they were caught. Furthermore, the pattern of variation of the $^{87}Sr/^{86}Sr$ ratio along the life of these individuals was mostly flat, indicating that studied *Arapaima* predominantly stays in the water with the same chemical composition. However, some variations were recorded and may either correspond to the temporal natural variation of water composition, or to movements between the river and adjacent lagoons, which have been shown to have higher $^{87}Sr/^{86}Sr$ values than the river (Pouilly et al. 2014).

On the other hand, most farmed fishes also presented a flat profile, but some specimens presented abrupt variations of $^{87}Sr/^{86}Sr$ values along the otolith. These variations may be produced by changes in the water isotopic composition, due for example to a transfer of the fish to another pond, or to a modification of the water source filling the pond. However, the variation could also be a consequence of food change. Most strontium-otolith studies indicated a role of ambient water in the control of strontium uptake. Controversially, the role of food in the strontium otolith uptake is debated, as revealed by Sturrock et al. (2012) that reviewed the significance of food up taking processes on the Sr isotopic composition of fish. For example, Kennedy et al. (2000) suggested that food consumption in adult hatchery-reared salmon is preponderant in the uptake of strontium, although Walther and Thorrold (2006) indicated that water chemistry is the dominant factor for marine fishes. Finally, recent advances highlighted that physiological factors may also contribute to Sr control in the otolith, because these elements are transferred into the blood plasma *via* branchial or intestinal uptake, before reaching the endolymph fluid, and finally the otolith (Payan et al. 2004; Sturrock et al. 2014). Although the importance of food in strontium uptake is not clear, we may speculate that in natural condition strontium composition of fish and food sources are in equilibrium with river water, so that fish and water are directly correlated, with no significant relative contribution of food in the uptake. In artificial condition, however, strontium composition of exogenous food source could be different from water and may result in a gap between fish and water strontium composition corresponding to the relative importance of food in the uptake.

a. Isotope record of wild Arapaima

The relationship between Sr isotope ratios in water and fish otoliths or scales has revealed to be a robust tool to study fish migration and geographical origin of population in the Amazon basin (Pouilly et al. 2014; Hegg et al. 2015; Garcez et al. 2015; Sousa et al. 2016; Duponchelle et al. 2016; Hauser 2018). The $^{87}Sr/^{86}Sr$ values of wild fishes from the Lower and Central Amazon and from the Upper Madeira and Solimões rivers presented differences according to the regions (Figures 3 and 4). Fishes from Lower and Central Amazon presented the narrowest variation of $^{87}Sr/^{86}Sr$ values during their life. These values were around 0.7100, which agrees with the reported values for this river waters (Santos et al. 2015). One fish presented a peak of the high $^{87}Sr/^{86}Sr$ value of >0.7250 (Figure 4a), which could correspond to a period during which this fish has lived in a habitat with water $^{87}Sr/^{86}Sr$ values close to the Madeira River waters or some granitic shield tributaries. Because of the sharpness of the peak, it could also be interpreted as an irregularity in the otolith. In contrast, fishes from the Madeira region, including from the Upper watersheds of the Beni, Mamoré, Yata, and Madre de Dios rivers, presented a higher degree of variability in $^{87}Sr/^{86}Sr$ values (Figure 4d, from 0.7150 to 0.7350). Fishes from this region also presented a higher $^{87}Sr/^{86}Sr$ variation across each otolith profile when compared to fishes from other sites, which is consistent with the natural seasonal variation of these older geological regions. In the Madeira

~~waters and its Upper tributaries, Santos et al (2015) observed dissolved $^{87}Sr/^{86}Sr$ data with a high seasonal variation, owing to the nature of rocks being eroded during the rainy and dry seasons. The data presented here suggest that fish otolith also record these seasonal variations.~~

~~The wild fishes from the Solimões River were caught in the Mamirauá Reserve. This reserve does not correspond to the main channel of the Solimões channel, but to lateral lakes that developed in a mixing zone with other tributaries, some of which may be of black waters, that generally present $^{87}Sr/^{86}Sr$ similar to that reported for the Negro river (Santos et al. 2015)~~

4. Discussion

The data presented showed major differences in otolith's C and Sr isotopic composition among the studied populations of *Arapaima*. In general, the $^{87}Sr/^{86}Sr$ measured in otoliths of wild specimens were similar to the $^{87}Sr/^{86}Sr$ reported in the dissolved fraction of the river water in which they were caught. Furthermore, the pattern of variation of the $^{87}Sr/^{86}Sr$ ratio along the life of these individuals was mostly flat, indicating that studied *Arapaima* predominantly stayed in water with the same chemical composition (Araripe et al., 2013; Castello, 2004, 2008; Hermann et al., 2016; Núñez-Rodríguez et al., 2015; Queiroz, 2000; Viana et al., 2007). However, some variations were recorded and are related to the lack of precise information of the fishery site and the intricate mosaic of the Amazon water chemistry. They may either correspond to the seasonal variation of water composition, or to movements between the river and adjacent lagoons, which have been shown to have higher $^{87}Sr/^{86}Sr$ values than the river (Pouilly et al. 2014).

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The wild fishes from the Solimões River were caught in the Mamirauá Reserve. This reserve does not correspond to the main channel of the Solimões channel, but to lateral lakes that developed in a mixing zone with other tributaries, some of which may be of black waters, that generally present $^{87}Sr/^{86}Sr$ similar to that reported for the Negro river (Santos et al., 2015). One of the five fishes analyzed presented higher $^{87}Sr/^{86}Sr$ values (>0.720), suggesting it may have lived part of its life in such a black water tributary.

Besides this regional variation, the data presented revealed that in general individual fish lived in waters with a limited range of $^{87}Sr/^{86}Sr$ values, suggesting a resident behavior. The pattern of $^{87}Sr/^{86}Sr$ along the otolith from the Madeira fishes presented higher variations. This could either result from movements between habitats with contrasted $^{87}Sr/^{86}Sr$ water signatures (e.g. adjacent lakes and lagoon, as shown by Pouilly et al. 2014 for the Beni River) or from the integration of the important natural seasonal variations of $^{87}Sr/^{86}Sr$ signature in the Madeira waters described by Santos et al. (2015).

Previous studies also concluded to a resident behaviour of *Arapaima* species ~~(Queiroz 2000; Castello 2004, 2008; Viana et al. 2007; Araripe et al. 2013; Hermann et al. 2016), including a study of individual behaviour of restocked and wild Arapaima using radio telemetry (Núñez-Rodríguez et al. 2015). A flat $^{87}Sr/^{86}Sr$ profile along the otolith does not directly implicate an absence of movement. Indeed, if a fish moves across two habitats presenting the same isotopic signature, the movement would not be revealed by otolith microchemistry analyses. On the other hand, Castello (2008) demonstrated lateral migration of Pirarucu between the Solimões River and the floodplain during water pulse in the Mamirauá reserve with other observation methods. Based on our results, we can conclude that studied Arapaima didn't show movements across contrasted habitat (like for example white vs. black water systems, Santos et al. 2015). We cannot, however,~~

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~~exclude lateral movements across habitats with similar water signatures. We argue that the $^{87}Sr/^{86}Sr$ variations observed in each otolith can be a combination of (1) small changes in the isotopic composition of water due to the hydrological seasonal cycle and/or (2) a lateral migration. An example is one fish from the Mamiraua reserve (M3), which showed ripples that might be interpreted as lateral movements between the Solimões white waters and adjacent lagoons or lakes with slightly higher signature (see Pouilly et al. 2014). Due to the weakness of the pattern and the absence of $^{87}Sr/^{86}Sr$ seasonal data from lakes and rivers in the Amazon basin, a~~(Araripe et al., 2013; Castello, 2004, 2008; Hermann et al., 2016; Queiroz, 2000; Viana et al., 2007), including a study of individual behaviour of restocked and wild *Arapaima* using radio telemetry (Núñez-Rodríguez et al., 2015). A flat $^{87}Sr/^{86}Sr$ profile along the otolith does not directly implicate an absence of movement. Indeed, if a fish moves across two habitats presenting the same isotopic signature, the movement would not be revealed by otolith microchemistry analyses. On the other hand, Castello (2008) demonstrated lateral migration of Pirarucu between the Solimões River and the floodplain during water pulse in the Mamirauá reserve with other observation methods. Based on our results, we can conclude that studied *Arapaima* didn't show movements across contrasted habitat (like for example white vs. black water systems, Santos et al. 2015). We cannot, however, exclude lateral movements across habitats with similar water signatures. We argue that the $^{87}Sr/^{86}Sr$ variations observed in each otolith can be a combination of (1) small changes in the isotopic composition of water due to diverse tributary sources in the hydrological seasonal cycle and/or (2) a lateral migration. As an example, the M3 fish from the Mamiraua showed ripples that might be interpreted as lateral movements between the Solimões white waters and adjacent lagoons or lakes with slightly higher signature (see Pouilly et al. 2014). Due to the weakness of the pattern and the absence of $^{87}Sr/^{86}Sr$ seasonal data from lakes and rivers in the Amazon basin, more detail studies would be necessary to confirm one or the other hypothesis of movement behavior, which are probably complementary.

Strontium and carbon isotopes in fish otolith record different parameters during ~~the~~ specimen life. As $^{87}Sr/^{86}Sr$ could be used as a robust fish geographical indicator, even in small scales (Pouilly et al., 2014), carbon isotopes composition ($\delta^{13}C$) are related to the feeding source of the fish.

Most wild fishes analyzed presented $\delta^{13}C$ values between -24‰ and -30‰ (Figure 6). Hence, wild *Arapaima* in this study had $\delta^{13}C$ mostly derived from C3 plants (-28.9 ± 1.2‰), as also observed in previous studies ~~(Forsberg et al. 1993; Domingues et al. 2006; Watson et al. 2013)~~(Domingues et al., 2006; Forsberg et al., 1993; Watson et al., 2013). However, a higher contribution of C4 plants could be observed in some specimens, in particular, fishes from the Central Amazon region (Itacoatiara site) that presented more positive $\delta^{13}C$ values (-15.4‰ and -20.1‰). Forsberg et al. (1993) showed that C3 may account for 82.4% to 97.5% of the fish diet in the Amazon basin. Nonetheless, it is worth mentioning that these studies analyzed muscle tissue and there may exist isotopic differences between carbon in muscle tissue and in bone structures because of physiologic pathway incorporation. Although this fractionation is not known in Amazonian fishes, available data from Atlantic cod indicate that $\delta^{13}C$ value can be 15.9‰ higher in otoliths than in body tissues ~~(Radtke et al. 1996)~~(Radtke et al., 1996). If this same fractionation were applied to the otoliths of *Arapaima*, the carbon source would have an unlikely value of -44.8‰. This fractionation difference is likely lower for Amazonian fish as also suggested by the

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carbon isotopic composition of other calcified tissues, like scales, reported by Domingues et al. (2006). They showed that these calcified tissues have $\delta^{13}C$ values between -18.0‰ and -29.2‰, which are in the same range as samples from the present study. The more positive $\delta^{13}C$ values observed in fishes from Itacoatiara in Central Amazon may reflect environmental heterogeneity related to water types (white, black and clear), channels formations in dry season, and other hydrologic seasonality related to the Flood Pulse Concept ~~(Junk et al. 1989; Domingues et al. 2006; Oliveira et al. 2006)~~(Domingues et al., 2006; Junk et al., 1989; Oliveira et al., 2006). Moreover, these isotopic values may be related to seasonal resource availability, such as *Schizodon fasciatus* that presents major digestibility of C4 macrophytes in the varzea areas (Forsberg et al~~.~~., 1993; ~~Oliveira et al. 2006;~~ Mortillaro et al~~.~~., 2015; Oliveira et al., 2006).

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~~3.4~~4.2 Isotopic variations in farmed fish otolith

In general, farmed fishes also presented a flat $^{87}Sr/^{86}Sr$ profile, except for three fishes (CS1, FMn2, and R1) that showed a larger $^{87}Sr/^{86}Sr$ profile variation when compared with wild fishes from the same region (Figure 5). These variations could be related to ~~the diversified water and~~diverse water's pond conditions in which they have been raised and ~~also~~/or to abrupt changes in water type, such as seasonal pond transfer. We argue that these fishes were probably used as breeders and that the changes in Sr isotope ratio indicate that they were transferred between different ponds with different Sr isotope compositions. Indeed, fish farming manuals indicate that changing the breeders from one pond to another is an important strategy to increase reproduction (Ono & Kehdi, 2013; SEBRAE, 2010). On the other hand, four fish (R2, R3, R4, and R5) of the Madeira farm had the exact same Sr isotopic profile, suggesting they have lived the last part of their lives in the same common pond.

Compared to wild fish, farmed fish also showed a higher variation of $\delta^{13}C$, thus indicating more diversified food sources. ~~Fishes from the Madeira (Ariquemes farm) presented less negative $\delta^{13}C$ values (-4.8‰ - -8.7‰), which could be related to C4-based food (DeNiro and Epstein 1978; Sant'Ana et al. 2010), probably made of corn. Farmed fishes from the Lower Amazon (Santarém farms) presented intermediate values (-13.7‰ - -15.6‰) and those from the central Amazon (Manaus farms) had more negative values (-23.2‰ - -26.0‰) more related to C3-based food or food web.~~Fishes from the Madeira (Ariquemes farm) presented less negative $\delta^{13}C$ values (from -8.7‰ to -4.8‰), which could be related to C4-based food (DeNiro and Epstein, 1978; Sant'Ana et al., 2010), probably containing a large proportion of corn. Farmed fishes from the Lower Amazon (Santarém farms) presented intermediate values (from -15.6‰ to -13.7‰) and those from the central Amazon (Manaus farms) had more negative values (from -26.0‰ to -23.2‰) more related to C3-based food. Therefore, we conclude that our hypothesis of an artificial alimentation based on C4 plants is not always verified, and that food farming seems to depend from local or regional production or from feeding strategies used by the farm.

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~~3.5~~4.3 Combining $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ signatures

We aimed at verifying if the combination of Sr and C isotopes may be a powerful tool to distinguish between farmed and wild specimens from different Amazonian regions. The quality of

information concerning fish origin is an important parameter for sustainable fish commercialization. The absence of precise fishing site and the heterogeneity of the water system may bring uncertainties to the data but does not compromise the scope of this study and our conclusions. This is also a sensitive question ~~when there is a restriction of the~~ considering that commercialization of ~~fishes proceeding~~*Arapaima* is only allowed from farming or management areas. In this sense, the isotope tool ~~used~~applied in this study ~~would~~can be ~~useful, pending~~ improved ~~precision and performance, as it is independent of any information provided~~to better control its commerce in the actual system of traceability by ~~fishers or sellers~~tracing back the origin of fish and combat the illegal reuse of tags on illicit fisheries.

The QDA analyses presented in our study gave the proportion of correct indication of the origin of fishes (production method: farm or wild, geographic regions). The results showed a good but not sufficient enough (>75%) proportion of correct classification of the geographic origin (mainly based on $^{87}Sr/^{86}Sr$ values). This percentage is downgraded by the overlaps of $^{87}Sr/^{86}Sr$ values of some regions (Solimoes, Central and Lower Amazon). The lack of contrast in $^{87}Sr/^{86}Sr$ between Lower Amazon, Central Amazon, and Solimões regions leads to a higher confusion: four fishes from Lower Amazon (on a total of 7) and two fishes from Solimões (on a total of 5) were misclassified in Central Amazon region, most of them prevenient from farmed sources. On the contrary, it is upgraded by some clear contrasts existing in different Amazonian sub-basins, such as the Madeira, but we can also indicate the Tapajos or Negro rivers that also presented specific values ~~(Santos et al., 2015; review in Hauser, 2018)~~(Santos et al., 2015; review in Hauser, 2018).

On the other hand, results showed low predictability (58%) of fish origin (farmed or wild). This is mainly due to the variety of food sources used to feed the farmed fishes. We hypothesized that farms used food based on a mixture of C3 and C4 plants (soya bean, corn) but some farms apparently used food based on C3 plants, generating confusion with the food of wild fishes. On the other hand, all fishes sold in Manaus marked as farmed fishes presented C3-based $\delta^{13}C$ signatures. This could mean that these supposedly farmed-fish actually came from wild provenance, which is illegal and contributes to the over exploration of this natural resource. False information on the fish provenance would also hamper the precision of our approach.

~~However, as~~As a preliminary intent, the method gave some interesting results that emphasize the potential of such analyses to obtain a performing tool. In only a few cases the $^{87}Sr/^{86}Sr$ values recorded in wild fish otoliths were not in agreement with the water $^{87}Sr/^{86}Sr$ of the reported origin. For instance, the $^{87}Sr/^{86}Sr$ values of wild *Arapaima* obtain from Santarém market, lower Amazon, were similar to those observed in the Solimões River (CS3). Hence, it is possible that these wild specimens were caught in the Solimões River (e.g. Mamirauá Reserve) and not in the Santarém area as reported by the fish seller. Nonetheless, because of the scarcity of water $^{87}Sr/^{86}Sr$ baseline in this area, a Santarém origin cannot be completely ruled out.

Some farmed fishes may also have $^{87}Sr/^{86}Sr$ that is not in agreement with the expected values of the reported origin. For example, farmed fishes from the lower Amazon (Santarém) were probably raised in a pond filled with water from both the Amazon and Tapajós River. Thus, the farming conditions are likely to interfere with the two tracers used in this study.

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4.5. Conclusion

The expected differences of $\delta^{13}C$ between farmed and wild fishes (related to artificial vs. natural food sources) could not be confirmed, owing mainly to the C4 macrophyte contribution to the natural alimentation of wild fishes and to the use of C3-based food sources for farmed fishes. False information on the fish provenance in markets may also have contributed to decreasing the precision of the approach and market sampling should be avoided in future studies. Another weakness of our approach is to the $^{87}Sr/^{86}Sr$ overlapping among Amazon sub-basins and the lack of a more extensive $^{87}Sr/^{86}Sr$ water baseline. Hence, this preliminary result is not yet fully sufficient to be applied as a commercial traceability tool and further analyses are needed to increase the discrimination performance because millions of people rely on *Arapaima* spp. to subsistence and income. Nonetheless, these initial results encourage a more detailed seasonal $^{87}Sr/^{86}Sr$ water sampling in lakes and rivers in all the four regions analyzed, and especially in the Madeira and in the Mamirauá reserve, in order to refine the spatial water base and consequently to understand the causes of the otolith profile variation in wild *Arapaima* spp. They also suggest further axes of the investigation, such as controlled physiological experiment to clarify the sources (water and food) for $^{87}Sr/^{86}Sr$ otolith assimilation pathway in farmed conditions and investigating the actual importance of C4 macrophyte influence to both farmed and wild *Arapaima* according to seasons.

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Appendix A. Water sampling and respective dissolved $^{87}Sr/^{86}Sr$.

Local sampling	Latitude	Longitude	$^{87}Sr/^{86}Sr$
Manacapuru	3°17.383'S	60°37.914'W	0.7091+/-1
Itacoatiara	2°48.115'S	57°56.085'W	0.71018+/-1
Novo Airão	2° 39.688'S	60° 53.032'W	0.7091+/-1
Mamirauá	2° 58.164'S	64° 53.911'W	0.7104+/-1
Tefé lake/ Solimões/ Mamirauá	3°20.815'S	64°42.826'W	0.71053+/-1
Tucuxi lake /Japurá/Mamirauá	2°49.491'S	65°00.818'W	0.70860+/-1
Japurá/Mamirauá	2°52.614'S	64°55184W	0.70874+/-1
Japurá mix with Aranapu/ Mamirauá	2°14.897S	65°11.149'W	0.70993+/-2
Aranapu/Mamirauá	2°22.730'S	65°15.426'W	0.70857+/-1
Solimões/Mamirauá	2°14.897S	65°11.147'W	0.70858+/-1
Arapaima lake/ Solimões	2°59.016S	64°55.193'W	0.7088+/-2
Santarém	2° 24.212'S	54° 44.149'W	0.71114+/-1
Santarém	2°23.663'ST	54°43.468'W	0.71073+/-1
Porto Velho	8° 42.619'S	63° 55.425'W	0.7168+/-1
Ariquemes	9° 53.682'S	63° 3.977'W	0.72961+/-1

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Beni	11°01.276'	66°06.462'W	0.71903+/-1
Beni+ Madre Dios	10°59.191'S	66°03.440'W	0.71310+/-1
Mamoré	14°52.982S'	65°01.963'W	0.72135+/-1

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Author contribution

In this work, Marc Pouilly and Roberto V. Santos designed the overall project ideas; Roberto V. Santos and Luciana A. Pereira developed the sampling plan. In the project execution, Luciana A Pereira collected samples from Central and Lower Amazon and Fernando Carvajal and Marilia Hauser sampled in Bolivia and Madeira. Then, the sample preparation, $\delta^{13}C$ otolith analysis, and $^{87}Sr/^{86}Sr$ water analysis were made by Luciana A. Pereira with the supervision of Roberto V. Santos while, the $^{87}Sr/^{86}Sr$ otolith analysis was performed by Marilia Hauser, Christophe Pecheyran and Sylvain Bérail with the supervision of Marc Pouilly and Fabrice Duponchelle. Later, Marc ~~Poully~~Pouilly and Luciana A. Pereira ~~developed the~~performed statistical and data analysis. Finally, Luciana A. Pereira prepared the manuscript with the contributions from all coauthors.

Competing interests

The authors declare that they have no conflict of interest.

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