Artigo Original *DOI:10.5902/2179-460X18431*

Ciência e Natura, Santa Maria v.38 n.1, 2016, Jan.- Abr. p. 24 – 31
Revista do Centro de Ciências Naturais e Exatas - UFSM
ISSN impressa: 0100-8307 ISSN on-line: 2179-460X

CIÊNCIAeNATURA

Isotopic ecology ($\delta^{13}C$ and $\delta^{15}N$) of fish farming species: possible consequences of diet change and habitat variation

Ecologia isotópica ($\delta^{13}C$ e $\delta^{15}N$) de espécies de piscicultura: possíveis consequências da mudança de dieta e variação de habitat

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Abstract

This study aims to assess changes of the isotopic signal - $\delta^{13}C$ and $\delta^{15}N$ - of muscle tissue among fish species of natural and breeding environments as a way to understand the relationship between habitat, diet and trophic composition of these watersports animals. Thus, we used four different species of fishes, being the peacock bass and tuna from different natural environments and the salmon and tilapia of breeding environments or fattening farms. From this, excepting the species of tuna the values of $\delta^{13}C$ of tilapia was significantly larger than others, while for the values of $\delta^{15}N$ the species of tilapia were significantly lower. Furthermore, the hatchery species of tilapia and salmon considerably had the lower variability. These results together suggest a significant difference in isotopic signal between species of hatchery and natural environments, reflecting different diets and composition of food chains according to the source environment of species.

Keywords: *Diet fish. Source environments. Food chains*

Resumo

Este estudo tem como objetivo avaliar as mudanças do sinal isotópico - $\delta^{13}C$ e $\delta^{15}N$ - do tecido muscular entre espécies de peixes de ambientes naturais e de criação como uma forma de entender a relação entre habitat, dieta e composição trófica destes animais aquáticos. Assim, foram utilizadas quatro espécies diferentes de peixes, sendo o tucunaré e o atum de diferentes ambientes naturais e o salmão e a tilápia de ambientes de criação ou engorda. A partir disso, excetuando as espécies de atum os valores de $\delta^{13}C$ de tilápia foram significativamente maiores do que os outros, enquanto que para os valores de $\delta^{15}N$ as espécies de tilápia foram significativamente menores. Além disso, as espécies de piscicultura de tilápia e salmão tiveram consideravelmente a menor variabilidade. Estes resultados em conjunto sugerem uma diferença significativa no sinal isotópico entre as espécies de piscicultura e ambientes naturais, refletindo as dietas diferentes e a composição das cadeias alimentares de acordo com o ambiente de origem das espécies.

Palavras-chave: *Dieta de peixe. Ambiente de origem. Cadeias alimentares*

Recebido: 16/06/2015 Aceito: 10/12/2015

1 Introduction

One study conducted in Brazil showed that only 2% of freshwater fishes consumed are native species, whereas the remaining 98% are exotic species created in ponds and tanks (WORLDPAPER, 1999). The problem of this habit of consumption and aquaculture is that the changes in environment and fish diet can reduce the size of food chains and, consecutively, depletion the nutrients available for the natural development of individuals (POLLAN, 2008).

From an ecological perspective we tend to evaluate the environment of a species in terms of factors such as habitat, predator / prey and resources available, which gives an idea of the food chain. According to this point of view the most critical element to an ecological species is the type of food available, the food chain which it belongs (LOWE-MCCONNELL, 1999; POLLAN, 2008).

So, it is noteworthy that in the natural habitat the basis of fish food chain is composed by numerous organisms, including algae and plankton that provide them with a series of macro and micronutrients such as vitamins A, B complex, folic acid, vitamin C, and especially the omega-3 fatty acids, that has proven to help prevent cardiovascular disease (DAVIGLUS et. al, 1997; KUBITZA, 2003; LEE, LIP, 2003, HIBBELN et al., 2006; POLLAN, 2008).

Thus, since in natural environments fish diets usually are much richer and more diversified, becomes at least more complex to simulate the ecologic conditions needed to natural develop of individuals. In this regard, some studies show that if the conditions of aquaculture are not well controlled there is a greater probability of incidence of parasitic diseases in tanks (FIGUEIRA, CECCARELLI, 1991; KUBTIZA, KUBTIZA, 1999; KUBITZA, 2000; MARTINS et al. 2000; LEMOS et al., 2006).

However, as the objective is to evaluate the isotopic variations of $\delta^{13}C$ and $\delta^{15}N$ between fish from natural environments and captive as a way to infer possible differences in diet and trophic structure, we emphasize that the nutritional aspect by itself will not be analyzed. Therefore, it is noted that the combined use of stable isotopes ^{13}C and ^{15}N it is useful to investigate both the type of diet, whether it comes from a C3 or C4 photosynthetic type, and to characterize the trophic chain of organisms (HOBSON, 1999; MANETTA et al., 2003; MARTINELLI et al., 2009).

Stable isotopes are atomic species of the same chemical element with the same number of protons but different numbers of neutrons in the atomic nucleus. They are stable because it does not decay by emission of energy over time; so there is no change in mass, unlike what happens with radioactive isotopes (MARTINELLI, et al., 2009).

Differences in the ratio of carbon isotopes (^{13}C /^{12}C) and nitrogen (^{15}N /^{14}N) occur naturally. Varying from a predictable way to be incorporated by plants and animals, the isotopic signal integrated in the tissues allows inferring what types of food were consumed (MARTINELLI et al., 2009).

Plants with the C4 photosynthetic pathway have $\delta^{13}C$ values varying from -11 to -13‰, while those with C3 photosynthetic type has a characteristic carbon isotopic composition varying from -25 to -29‰ (FARQHUAR et al., 1989). The most important carbon sources for freshwater fish also have a C3-like origin, since they usually feed seeds and fruits from C3 plants and phytoplankton (OLIVEIRA et al., 2006). Regarding nitrogen isotopic composition, the $\delta^{15}N$ values increase approximately 3‰ per trophic level (MARTINELLI et al., 2009). So, fishes with more complex food chain (omnivorous species like Peacock bass) will have elevated values of $\delta^{15}N$ (OLIVEIRA et al., 2006).

Therefore, considering that in natural environments there is a greater diversity of food items for fish, mainly of plankton and algae, organisms predominantly of C3 photosynthetic system, is expected that: 1) the fish hatchery and fattening farms have larger isotopic values of $\delta^{13}C$ and smaller of $\delta^{15}N$ due to standardization of diet in maize ration (C4), principally and 2) consecutively, reduction of isotopic variability due to the homogenization in aquaculture environments.

2 Material and methods

2.1 Field sites and sampling

For the sampling of natural environments and hatcheries were chosen four fish species considered among the most consumed by Brazilians, in general, purchased in local markets of the cities of Piracicaba-SP (*Sarotherodos niloticus*, Tilapia and *Salmo salar* - Salmon), Santos - SP (*Thunnus alalunga* - Tuna) and Santarém - PA (*Cichla monoculus* - Peacock Bass).

Each individual was collected for isotopic analysis of a piece of muscle near the base of insertion of the dorsal fin of each individual, for a total of one hundred and five (N = 105), being twenty-six of Salmon (n = 26), nineteen of Tilapia (n = 19) and thirty of species of natural environment, Peacock Bass and Tuna (n = 30).

Its noteworthy that the isotopic analyses were not applied with the adipose tissue integrates into the muscle of fishes. Futures works should focuses on this more complex approach to reveal the integrated signal isotopic of muscle that was adipose cells intercalates with muscle fibers.

2.2 Stable isotope ratio analysis

Samples were then dried overnight at 65^{o} C, and then were cut into between one and four sections depending on the sample size to be weighed (1-2 mg) in tin capsules to be submitted the isotopic analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). All values recorded used the δ notation in units per million (‰) with reference to the international standard of Pee Dee Belamite for carbon and atmospheric air for nitrogen (MARTINELLI et al., 2009). Determining the delta value samples of fishes was possible:

1) by means of $\delta^{13}C$ estimate the relative proportion of the diet which is derived from plants C3 and C4 from the equation isotopic mass balance or isotopic dilution equation (FRY, 2006):

$$\%C_4 = (\delta^{13}C_{peixe} - \delta^{13}C_{C3\ food})/(\delta^{13}C_{C4\ food} - \delta^{13}C_{C3\ food}) \quad (1)$$

where % C4 is the relative proportion of C4 sources in the muscle and $\delta 13\ C_{C3\ food}$ is the average isotopic composition of C3-based food obtained in a survey administered by Nardoto et al. (2006) on food items of Brazil (-26.1‰). Likewise, $\delta 13\ C_{C4\ food}$ is the average isotopic composition of exclusively C4 based food obtained in a similar survey by the same authors (-11.2‰).

2) and of $\delta^{15}N$ infer if the fish diet, according to the source environment, has become less complex in terms of trophic chains involved (MANETTA et al., 2003).

The carbon isotope ratio ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) of fish samples was determined using a Delta Plus mass spectrometer for isotope ratios (FINNIGAN-MAT, California, USA) coupled to an elemental analyzer (Carla model 1110 Erba, Milan, Italy) (NARDOTO et al., 2006).

2.3 Statistical analysis

The assumptions of normality and homogeneity of variances were verified by residual analysis and the tests of Shapiro-Wilk and Box-Cox, respectively.

Then, a Kruskal-Wallis nonparametric test for several independent samples was carried out to verify possible differences for $\delta^{13}C$ and $\delta^{15}N$ among four fish species. Subsequently, a Student-Newman-Keuls posttest was used to explain any significant differences detected by Kruskal-Wallis.

The statistical analyses were performed using the software's SAS 9.2 and and Statistica, version 9 for Windows (STATSOFT, INC. 2009) for graphical analysis. Differences at the < 0.01 level were reported as significant.

3 Results

Despite the variability both in carbon and nitrogen stable isotope values among species, four clear groups stood out in a plot of $\delta^{13}C$ versus δ^{15} N in muscle tissues (Figure. 1). Residual analysis and the tests of Shapiro-Wilk and Box-Cox confirmed the need of a non-parametric test, since residuals appeared non-normal and with heterogeneous variance.

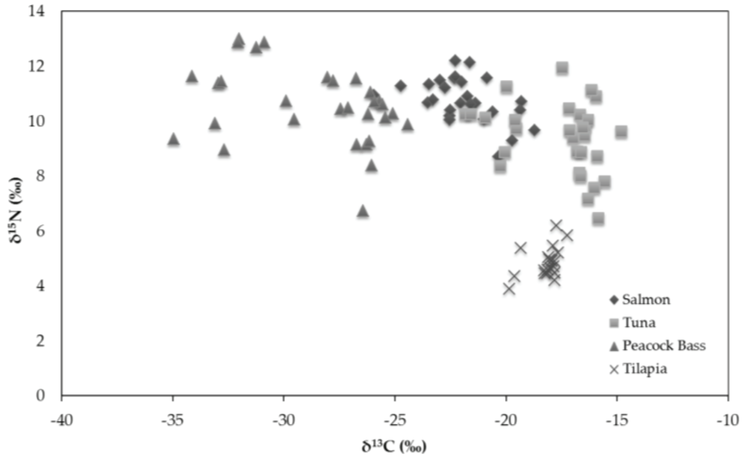

Figure 1. Distribution of $\delta^{13}C$ and $\delta^{15}N$ values of fishes muscle tissues: Peacock Bass, Salmon, Tuna and Tilapia.

There was a significant increase ($p < 0.01$) of $\delta^{13}C$ of muscle from the species of Peacock bass compared to the Salmon and these to Tuna and Tilapia, respectively (Figure 1). In other words, the species of tuna and tilapia have similar isotopic pattern of $\delta^{13}C$. However, this change in the carbon isotopic composition into three groups was followed by a significant decrease ($p < 0.01$) of $\delta^{15}N$ muscle values from Peacock bass, Salmon and Tuna compared to the Tilapia (Figure 1).

Therefore, to nitrogen isotopic composition the species of Tuna and Tilapia was significantly different. So, the average δ^{15} N for the species of Tilapia was significantly lower than Tuna (table), possibly due to the increase of corn feed consumption in breeding tanks. Although the δ^{15} N of Peacock bass and Salmon wasn't significantly different towards one another was greater than Tuna (Table)

Table. Mean values of $\delta^{13}C$ and $\delta^{15}N$ (μ ± S.D.) of fishes (muscle) tissues with the number of individuals sampled (N). Different letters in a column are statistically different ($P < 0.01$).

Species	**Common name**	**$\delta^{13}C$**	**$\delta^{15}N$**	**N**
Sarotherodos niloticus	Tilapia	-18,2 ± 0,64 a	4,8 ± 0,56 a	19
Salmo salar	Salmon	-21,7 ± 1,45 b	10,7 ± 0,81 c	26
Thunnus alalunga	Tuna	-17,5 ± 1,94 a	9,4 ± 1,25 b	30
Cichla monoculus	Peacock bass	-28,6 ± 3,18 c	10,5 ± 1,4 c	30

Beyond the significant average differences of $\delta^{13}C$ values between fish species, another evident difference was observed in the $\delta^{13}C$ variability (spread), with the species of tilapia and salmon clearly more grouped than others (Figure 1). This isotopic pattern distribution probably derived from the homogeneous and controlled food creation in the tanks and fattening farms of these species, respectively.

Additionally, in the lack of isotopic fish studies if we assume the results of Nardoto et al. (2006) as a baseline we can estimate the relative proportion of C3 and C4 sources (plants and seaweeds) in the diet of fish species, using the muscle $\delta^{13}C$ values as a proxy related to bulk dietary inputs (NARDOTO et al. 2006). Then we used the following isotopic dilution equation.

Therefore, assuming no fractionation between the carbon stable isotopic composition of muscle and diet (NARDOTO et al., 2006 and 2011) this approach allow us to estimate the proportion of C4 sources present in the muscle of fishes: approximately only 13% of the carbon intake by Peacock bass had a C4 origin with a little increase around 29% to Salmon, 53% to Tilapia and 58% to Tunas species sampled.

4 Discussion

According to extensive information available in the literature, the muscle isotope data of fishes can be interpreted in quantitative way based on the fact that animal's tissues offer an isotopic signature that is systematically related to bulk diet. Thus, from the $\delta^{13}C$ and $\delta^{15}N$ values were evaluated aspects more directly related to diet and food chain, respectively.

Considering the relationship between change in habitat and diet the results suggest that the homogenization of diet in creating environments indeed is expressed in the isotopic signal. In this sense, it is observed that the species created under conditions of greater control (Tilapia and Salmon), tanks and fattening farms, respectively, were the ones with the smaller isotopic variability (Table). The standardization of food graphically is translated by a relatively clustered distribution of these species in relation to those of natural environments (Figure 1).

The issue of diet homogenization in fattening farms and tanks it is evident once that the species with greater control over creation of the present study (Tilapia) showed significantly higher values of $\delta^{13}C$. In addition, species of Tilapia also showed significantly lower levels of $\delta^{15}N$, being soon the species with the food chain simplest of all, probably resulting from the standard food (ration) of corn (C4) and soybean (C3) in breeding tanks.

Therefore, the isotopic signal in favor of a C4 source of hatchery species can be explained mainly by excessive use of corn ration in the diet (POLLAN, 2007:26). According to Hayashi et al. (1999), in farms generally the diet of omnivorous fish is composed of 90% or more by vegetables sub products, mainly corn and soybean bran.

Additionally, except the species of Tuna, the significantly higher values of $\delta^{13}C$ of species of Tilapia and Salmon in relation to the species of Peacock Bass and Tambaqui (see OLIVEIRA et al., 2006) shown that the hatchery species are markedly feeding over C4 plants than the natural environments (Table). In this way, considering the assumption of homogeneity of the diet in shelters, a factor that deserves attention regarding the fish ecology is that normally in natural environments C4 plants are not part of the menu.

Oliveira et al., (2006) studied the seasonality of food sources for the Amazonian Tambaqui points out that most food items in an omnivorous diet of these fish comes from a source C3. Hamilton et al. (1992), in a study performed in the Orinoco River in Venezuela, reinforce that the species of fish and aquatic invertebrate biota hardly depend on the C4 plants for food.

Already Forsberg et al. (1993), in a study of fish species in Central Amazonia showed that 82.4 to 97.5% of the primary source of carbon derived from C3 plants. Therefore, these and other studies show that in natural environment C4 plants usually has little influence in the diet of fish (MANETTA et al., 2003; VAZ et al., 1999).

However, an interesting exception to the rule was the species of tuna. Despite being an oceanic species and active predator of small crustaceans and plankton, as evidenced by the high value of $\delta^{15}N$, some important item in their diet comes from a source C4, considering the equation isotopic dilution approximately 58% of their food is from C4. Probably some phytoplankton or algae of signal C4.

Thus, from the present results it was noted that depending of the source environment there is considerable variation in the composition of diets and food webs of fish species. Consecutively, these results show the influence of omnivorous diet in fish isotopic signal and the character of top chain of salmon, tuna and peacock bass, which corresponds with the Amazonian Tambaqui reported by Oliveira et al., (2006).

In natural environments fishes choose foods that best supply their nutritional needs and preferences, balancing their diets through a wide range of items from several different trophic levels (KUBITZA, 2003; MANETTA et al., 2003). In this sense, some studies note that in natural environments the species tend to show great plasticity concerning the choice of food items (WIMBERGER, 1992; ABELHA et al., 2001).

In this sense, it is quite possible that the low plasticity of food items due to lack of choice option in hatchery environments is one of the factors responsible for the low variability of the isotopic species of Tilapia and Salmon (Figure 1) and low value of $\delta^{15}N$ of Tilapia (Table). However, considering the ecology of species of Tilapia, this low value of $\delta^{15}N$ may denote its adaptive flexibility of foraging behavior (Dill, 1983). One example of this adaptive adjustment is that in some cases of infestation by algae and macrophytes can even be used as biological control of plagues (ZAMBINONI-FILHO, 2005).

In this regard, Manetta et al. (2003) argues that low values of $\delta^{15}N$ of certain species of fish are due to the small feeding plasticity. Another possible consequence of lower plasticity in tanks and fattening farms may explain the low variability of both species of Tilapia and Salmon, with significantly more clustered distribution than others, Peacock Bass and Tuna.

The low plasticity of food items in diet of fish hatchery can generate ecological and nutritional unexpected. In this sense, it is worth pointing the possible increase of parasitism in cultivation environments (KUBTIZA, KUBTIZA, 1999; MARTINS et al. 2000) and the reduction of macro and micro nutrients available for last human consumption (POLLAN, 2008). Additionally, the large amount of nutrients in ponds can lead to the development of cyanophytes algae and fungus actinomycetes and, consecutively, the occurrence of unpleasant taste and smells in the meat of fishes (BIATO, 2005).

Hamilton et al. (1992), revealed that the microalgae - organisms predominantly C3 - are the main energy source for many aquatic animals, including fishes. Thus, once the fishes originally get the fatty acids of algae, the main nutrient to combat humans cardiovascular diseases, the change of environment and diet can indeed result in serious nutritional damages to final consumers (POLLAN, 2008).

However, although the nutritional requirements of most cultivated species be known the control of farming conditions must be done well for not rely on parasitic problems and, especially, in mineral and vitamin disarrangement for the species. In the case of tilapia, Kubitza (1999) points out that the intensification of cultivation of species in Brazil, in systems where the availability of natural food is limited, increased the incidence of nutritional disorders due to inadequate vitamin and mineral enrichment of feeds.

Nevertheless, considering the high current demand for fish consumption it is worth to emphasize that this study does not ignore the value and necessity of fish farming practices, but makes an alert to the possible ecological and nutritional consequences of simple change of habitat. So from the above and the isotopic results we ponders that the change in habitat and diet of fishes can lead to problems for human consumption if the final farming practices are not rigorous and quite well drawn up.

5 Conclusion

So, although our data are not sufficient to distinguish the specific food items and the detailed composition of the fish food chain, the isotopic patterns encountered suggest: 1) a subtle association between diet and environment of origin and 2) successively, that the change of diet and decreased variability of food items available in shelters can significantly alter the isotopic patterns, principally of $\delta^{13}C$ - in relation to fishes from natural environment, which can cause problems to final human consumption, according to some literatures.

From this, future studies using the same fish species to investigate the isotopic variation between natural environment and hatchery will be valuable for a more accurate control of scientific treatments and to understanding the consequences of habitat changes in the trophic ecology of fishes.

Acknowledgements

Thanks the grant from the Brazilian research agencies FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) that through the project n° 2011/08686-3 enabled the development of this work.

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