The influence of fish cage culture on $\delta^{13}C$ and $\delta^{15}N$ of filter-feeding Bivalvia (Mollusca)

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Abstract

The objective of this study was to evaluate the effect of *Oreochromis niloticus* cage culture promoted variations in the $\delta^{13}C$ and $\delta^{15}N$ in *Corbicula fluminea* (Mollusca; Bivalvia) and in the sediment of an aquatic food web. Samples were taken before and after net cage installation in the Rosana Reservoir (Paranapanema River, PR-SP). Samples of specimens of the bivalve filterer *C. fluminea* and samples of sediment were collected using a modified Petersen grab. All samples were dried in an oven (60 °C) for 72 hours, macerated to obtain homogenous fine powders and sent for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic value analysis in a mass spectrometer. There were significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values of the invertebrate *C. fluminea* between the beginning and the end of the experiment. There were no differences between the $\delta^{13}C$ and $\delta^{15}N$ values of sediment. These results indicate that the installation of fish cage culture promoted impacts in the isotopic composition of the aquatic food web organisms, which could exert influence over the native species and the ecosystem.

Keywords: Corbicula fluminea, sediment, stable isotopes.

A influência dos tanques rede sobre o $\delta^{13}C$ e $\delta^{15}N$ de uma espécie filtradora Bivalvia (Mollusca)

Resumo

O objetivo deste estudo foi avaliar o efeito do cultivo de *Oreochromis niloticus* em tanques rede sobre os valores de $\delta^{13}C$ e $\delta^{15}N$ em tecidos *Corbicula fluminea* (Mollusca; Bivalvia) e no sedimento da cadeia alimentar aquática. As amostragens foram realizadas em períodos prévios e posteriores a instalação de tanques redes no reservatório de Rosana (Rio Paranapanema, PR-SP). As amostras de espécimes de bivalves filtradores exóticos e as de sedimento foram coletadas utilizando uma versão modificada do pegador tipo Petersen. Todas as amostras foram secas em estufa (60 °C) durante 72 horas, maceradas até obtenção de pó fino e homogêneo e enviadas para determinação do carbono (^{13}C) e do nitrogênio (^{15}N) em espectrofotômetro de massa. Houve diferenças significativas nos valores $\delta^{13}C$ e $\delta^{15}N$ do invertebrado *C. fluminea* entre o início e o fim do experimento. Entretanto, não foram evidenciadas diferenças nos valores $\delta^{13}C$ e $\delta^{15}N$ presente no sedimento. Estes resultados demonstram que a instalação dos tanques rede promoveram impactos na composição isotópica do invertebrado, que podem influenciar as espécies nativas e o ecossistema.

Palavras-chave: Corbicula fluminea, sedimento, isótopos estáveis.

1. Introduction

In recent years, some reservoirs have been used for fish farming using net cages. This activity has been growing on a large scale in Brazil (Agostinho *et al.*, 2007). However, several studies have shown that the installation of fish cage cultures promotes changes, of varying intensity, to the environment due to the eutrophication processes that they generate (Tundisi and Henry, 1986; Agostinho *et al.*, 2007), causing variations in the quantity and availability of food in the ecosystem (Strictar-Pereira *et al.*, 2010). Cage cultures can to promote intensification of nutrient input, from both uneaten feed and the release of faeces and ammonia excretion (Troell and Berg 1997), leading to proliferation of algae in adjacent areas and, therefore, changes in various communities, such as zoo-

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plankton (Dias *et al.*, 2012) and zoobenthos (Guo and Li, 2003).

Corbicula fluminea (Müller, 1774) (Mollusca; Bivalvia) is an exotic species from Southeast Asia. This invertebrate species was chosen because it is a filter-feeding mollusc that strongly influences the aquatic ecosystem, since it obtains its energy directly from primary producers, transferring it to other links of the trophic chain, including some species of fishes, aquatic birds and mammals (McMahon, 2000).

The use of stable isotopes in ecological studies is an important tool for tracing the path of organic matter, and its variation, through food webs (Vander Zanden *et al.*, 2003, Schmidt *et al.*, 2007). The carbon stable isotope ratio ($\delta^{13}C$) of an organism reflects the small enrichments that occur along the food chain (from 0.2 to 1‰ for each trophic level), and is used to identify the carbon sources of the consumers (De Niro and Epstein, 1981). The nitrogen ($\delta^{15}N$) stable isotope ratio undergoes an enrichment of about 3-4‰ between the tissues of the prey and predator, reflecting the preferential excretion of the lighter isotope during metabolisation and providing a means of measuring consumer trophic position (Minagawa and Wada, 1984).

Fish cage cultures could influence the energy sources and affect the trophic structures of their ecosystems through the addition of organic waste. Within this context, the objective of this study was to assess whether the fish cage culture promoted variations in the $\delta^{13}C$ and $\delta^{15}N$ on the filter-feeding species Corbicula fluminea (Mollusca, Bivalvia) and in the sediment of an aquatic food web.

2. Material and Methods

2.1. Area of study

This study was conducted along with the experimental cage production of tilapia *Oreochromis niloticus* (Linnaeus, 1758) in the Corvo streams (22º38'19'' S and 52º47'16'' W), in the Rosana Reservoir, low Paranapanema River, one of the main tributaries from the left margin of the Paraná River (Figure 1). Corvo streams show the margins with grasses and initial stages of reforestation, have banks of aquatic macrophytes and are greatly affected by wind. The abiotic factors that had the greatest influence during the experiment were nitrate, organic matter, ammonia and total nitrogen.

Fifteen net cages were used in the experimental design, each one 2 x 2 x 1.7 m, resulting in a volume of 6 m^3. The average stocking density of the *Oreochromis niloticus* was 150 fish per m^{-3}. The initial amount of fish food was estimated at 10% of the total biomass of the net cage and was established by the technique of providing *ad libidum*. This was regulated by observations of the consumption of all the food in the first hour after offered. Fishes were fed twice a day at 10:00 and 16:00 h, with commercial extruded fish food containing crude soybean protein, in which the isotopic values of carbon and nitrogen, previously analysed, were -19.9‰ ± 0.3 and 4.5 ± 0.1‰, respectively.

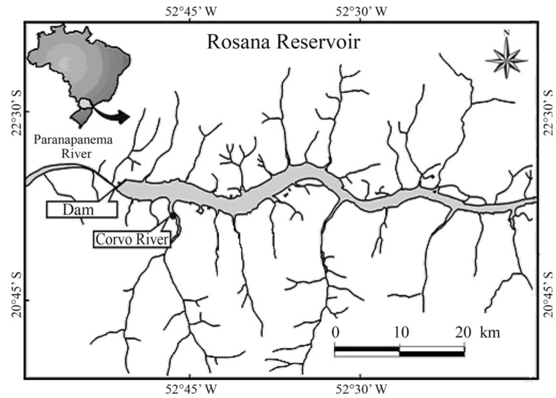

Figure 1 - Map of the Rosana Reservoir, indicating the location of the net cages in the Corvo River (•).

Invertebrate samples and sediment were taken in April 2006, before the beginning of the experiment and in the end of the experiment in August 2006. Ten samples of specimens of filter-feeding bivalve *Corbicula fluminea* (Müller, 1774) were taken in each period from the sediment below the cages using a modified Petersen grab (0.018 m^2 area); samples were collected below the culture cages. The bodies were separated from the valves in the laboratory. Three samples of sediment were also taken using a modified Petersen grab.

Invertebrate samples and sediment were dried in an oven (60 °C) for 72 hours, macerated to obtain homogeneous fine powders and were sent to the Center for Stable Isotopes (CIE), at the State University Paulista (UNESP) in Botucatu, for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic value analysis using a mass spectrometer.

2.2. Data analysis

To test possible differences in the values of $\delta^{13}C$ and $\delta^{15}N$ at the beginning and the end of the farming period, the isotope values were submitted to a Mann-Whitney non parametric test for independent samples. Statistica for Windows 7.1 (Statsoft Inc. 2005) was used to carry out the analysis. The sediment could not be tested due to the low number of samples.

3. Results

The Mann-Whitney test evidenced that there were significant differences in the $\delta^{13}C$ values of the invertebrate *C. fluminea* ($Z = -2.39$; $p < 0.016$) between the beginning and the end of the experiment, with enrichments in $\delta^{13}C$ values. There were no differences between the $\delta^{13}C$ values of sediment (Figure 2).

Corbicula fluminea also showed significant variability in the $\delta^{15}N$ values between the two sampling periods ($Z = 2.39$; $p < 0.016$), increasing from 9.3 to 11.7‰,

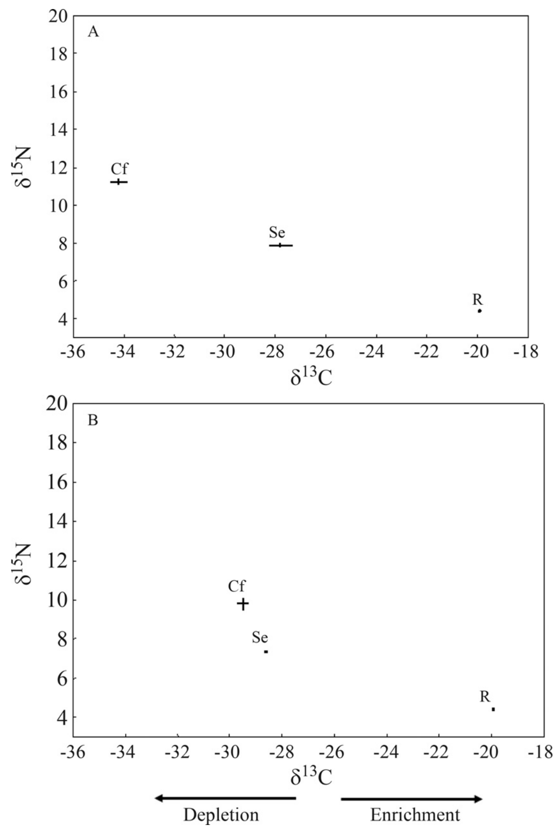

Figure 2 - Bi-plot showing the mean values and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ in April (A) and August (B). Where Cf = *C. fluminea*; Se = Sediment, R = Fish food.

while the sediment did not present differences for this element.

4. Discussion

This study identified changes in the $\delta^{13}C$ values of the organisms between the beginning and end of the experiment period: environmental changes due to the installation of net cages (Penczak *et al.*, 1982). Considering that this invertebrate *C. fluminea* is filter-feeding and that phytoplankton show the most depleted values among the producers (Araujo-Lima *et al.*, 1986; Manetta *et al.*, 2003, Lopes and Benedito-Cecilio, 2002), it is assumed that the negative isotopic values of *C. fluminea* at the beginning of the experiment were related to the use of phytoplankton as a source of energy. However, at the end of the experiment, it was found that *C. fluminea* showed high isotopic variation, with significant enrichment in $\delta^{13}C$ values (~6‰). This change in the isotopic compositions could be related to the influence of allochthonous material in the trophic web in the river and with the soybean content of the commercial fish food as responsible for the enrichment in $\delta^{13}C$ values.

The sediment, in turn, consists of organic matter derived from C_3 (perifiton and macrophytes) and C_4 plants. However, in this study, the $\delta^{13}C$ isotopic compositions of sediment showed no variations between the two periods of study. Thus, although there is a constant input of enriched carbon isotope, due to the fish food, the absence of changes in the isotopic compositions sediment could be related with the current of the river that must have taken the feed or diluted it, not interfering in the sediment isotope values.

With respect to the $\delta^{15}N$ values, it was found that the specie *C. fluminea* had depleted final values compared to those found at the beginning of the experiment. It can be assumed that the producers of the ecosystem assimilated much lighter isotope nitrogen from the fish food. Producers that assimilate nitrate show more enriched $\delta^{15}N$ values than those that use NH_4 (Pennock *et al.*, 1996). This is because nitrate is exposed to fractionation for less time (Robinson, 2001). Furthermore, the fish cage culture changes the water conditions due to the input of nutrients, which raises the levels of certain compounds, such as NH_4 (Beveridge, 1987; Troell and Berg, 1997). High levels of NH_4 can alter the isotopic values of producers, such as C_3 plants. This, in turn, could alter the isotopic values of the other taxonomic groups, as was observed in this study. According to Grey *et al.* (2001), changes in the isotopic compositions of organisms may also be related to seasonal changes in diet and the input of allochthonous sources to the system. These factors affect the determination of the extent of isotopic fractionation and, consequently, the isotopic variability of the organisms.

As for sediment, no differences were found between the $\delta^{15}N$ isotopic compositions for the two periods of study, although a tendency of depletion was observed. This lack of variation, as mentioned above for $\delta^{13}C$, may be due to the great influence of allochthonous material.

Thus, it can be concluded that the input of organic material and nutrient in areas of caging aquaculture, produce variations in the river food web indirectly, through fish food, promoting differences in the carbon and nitrogen isotopic compositions of *C. fluminea.* In this sense, for the expansion of aquaculture, it is important to ensure healthy freshwater ecosystems, and the fish cultivation in net cages in reservoirs should be subject of suitable management, permanent monitoring and more studies that evaluate impacts of net cages (Borges *et al.*, 2010).

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