STABLE ISOTOPE EVALUATION OF TERRESTRIAL AND AQUATIC SOURCES OF CARBON IN A BRAZILIAN SEMI - ARID STREAM

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INTRODUCTION

Stable isotope ratios offer an effective natural tracer for energy and nutrient flows studies in ecosystems. The stable isotope method is based on the fact that certain isotopes fractionate in predictable ways as elements travel through the food web (Fry, 1991).

The nitrogen isotope relative abundance (&948;15N) is used to determine feeding and other trophic relationships among animals and plants, once there is significant change of &948;15N values between organism and diet (about 2 to 4 extperthousand). Since there is consistent enrichment through the food web, 15N isotopes can be used to evaluate a consumer's trophic level (Deniro & Epstein, 1981). The &948;13C values are used to determine the sources of energy, because the 13C content of an organism reflects the 13C contents of its diet with little or no change.

In numerous studies on food web relationships, the relative ratios of &948;13C and &948;15N have been used as indicators of food source to resolve ambiguities in sources of energy and energy flows (Fry, 1991; Creach *et al.*, 997). Furthermore, when combined with gut contents data, stable isotopes are an important tool in assessing the potential role of unidentifiable, undetectable or unquantifiable prey items in the diets of consumers (Johannsson *et al.*, 001), as well as the origins of organic matter found in digestive tracts and the role played by bacteria in the diet of macro consumers (Creach *et al.*, 997).

Once carbon is fixed by a producer the ratio of 13C to 12C remains relatively constant as it passes through the food chain (enrichment of about 0.8 to 1 extperthousand per trophic level). Thus, when there are relatively few, isotopically distinct, source groups, it is often possible to determine the primary producer carbon source(s) (as well as if it is terrestrial or aquatic) of an animal directly from its carbon isotope ratio (Forsberg *et al.*, 993). Therefore, stable isotope analysis can provide an estimation of the mean level of autochthonous and allochthonous sources of organic carbon actually assimilated by a given species.

OBJECTIVES

The aims of this study are (1) to describe stable isotope ratios (&948;13C and &948;15N) of available terrestrial and aquatic sources of carbon to consumers in an intermittent stream of the Brazilian semi - arid region and (2) to evaluate the distinctiveness of source isotope signatures and, therefore, their value in describing the food web structure in Brazilian semi - arid streams.

MATERIAL AND METHODS

3.1 - Study area and design

The present study was performed in the Seridó stream, located in the Seridó/Borborema area , in southern Rio Grande do Norte (RN)/northern Paraíba (PB), between the cities of Patos and Caicó. The study area is classified as being of extreme biological importance and was identified as a priority area for biodiversity conservation in the Caatinga biome by Silva *et al.*, (2003). <p/ >

The average annual temperature is 30.7^{0}C, with the maximum monthly averages in October (31.0^{0}C) and the minimum averages in February (29.3^{0}C). Precipitation is concentrated between January and April, with 350 to 800 mm per annum and an annual average of 600 mm (Amorim *et al.*, 005). Altitude in Seridó reaches between 100 and 800 m. The Seridó area is drained mostly by the Piranhas - Açu River basin, with the Seridó stream as the main affluent. Aquatic systems in the study area are rich in habitat elements (littoral grass, aquatic macrophytes, overhanging and submerged vegetation and submerged wood debris) and in substrate composition (mud, sand and cobbles). Average water temperature, dissolved oxygen and secchi depths for aquatic systems in the study area are 29.5 ±2.9 ºC, 5.8 ±2.0 mg/l and 47.2 ±19.5 cm, respectively (see Medeiros *et al.*, 008 for further detail).

A reach of approximately 500m in the middle Seridó stream (at the locality of Sitio Riacho da Serra, Caicó - RN) was sampled in four occasions during the 2007 hydrological cycle: April and July (wet season), and October and January 2008 (dry season).

3.2 - Collection of primary sources

Major primary sources of terrestrial and aquatic organic carbon and nitrogen were collected from different areas in the study stream reach. Fallen leaves from major riparian trees (riparian vegetation) were collected by hand from the margins, and leaves of C4 grass were clipped from marginal vegetation. Benthic detritus was collected with dip and hand nets and wet - sieved into fine (250 &956;m to 1 mm) and coarse (>1 mm to 1 cm) particulate organic matter fractions (FPOM and CPOM, respectively).

Algae samples (filamentous algae) were taken from the shallow littoral margins both directly off the bottom surface and from submerged wood or rocks, and washed in the field to remove any associated organic debris. Aquatic plants, were collected by hand from the margins. The aquatic plants collected included submerged species (*Myriophyllum* spp.) and rooted floating species (*Ludwigia* spp. and *Nymphaea* sp.). Whenever possible at least three replicate samples of each potential source were collected. All samples were tagged, placed in 70% ethanol (Sarakinos *et al.*, 002) and taken to the Laboratório de Zoologia (Universidade Estadual da Paraíba, Campus I) for processing. In the laboratory, primary sources were rinsed in distilled water and, in the case of algae samples, any remaining organic debris was removed. All samples were oven - dried at 80&176;C for 36 to 48 hours and then ground by hand to a powder - like consistency with mortar and pestle.

Dried samples were sent to CENA (Centro de Energia Nuclear na Agricultura/USP Campus Piracicaba) where they were oxidized at high temperature and the resultant CO2 and N2 were analyzed for percentage C, N and stable isotope ratios with a continuous - flow isotope - ratio mass spectrometer (Finnigan Delta Plus). Ratios of 13C/12C and 15N/14N were expressed as the relative per million (extperthousand) difference between the sample and the conventional standards (PeeDee Belemnite carbonate and N2 in air), where:

, X =13C or 15N and R =13C/12C or 15N/14N.

Measurement precision was approximately 0.1 extperthousand for 13C/12C and 0.3 extperthousand for 15N/14N.

3.3 - Data analysis

Data were first described using the &948; values ranges and averages (±SD) of sources and, when necessary, for each sampling occasion. Significance of differences of &948;13C and &948;15N between sources and sampling occasions were tested using simple ANOVA (Zar, 1999), followed by post - hoc pairwise comparisons (Tukey's HSD test, at a significance level of 0.05). Samples were considered enriched or depleted if their ratios departed from standards in a positive (i.e. more heavy isotopes) or negative direction, respectively. No correction for fractionation was applied to the data at the present study.

RESULTS AND DISCUSSION

Important patterns of variation in &948;13C and &948;15N were observed for the terrestrial and aquatic sources sampled in the Seridó stream. Algal &948;13C was quite variable, ranging from –14.1 to –27.2 extperthousand . The submerged aquatic plant Myriophyllum sp. also presented some variability in &948;13C values with a range between –17.2 and –23.1 extperthousand . The remaining sources showed little variation with &948;13C SD below 1.2 extperthousand and &948;15N SD below 1.9 extperthousand .

Evaluation of temporal patterns of &948;13C and &948;15N (across sampling occasions) of these variable sources showed that algae were progressively more depleted in 13C with time (&948;13C averages were –16.1 ±2.8 extperthousand ; –19.7 ±6.6 extperthousand ; –20.3 ±3.8 extperthousand and –22.8 ±1.1 extperthousand , respectively for April, July, October and January). A similar pattern was observed for algal &948;15N, with averages of 4.9 ±1.7 extperthousand ; 4.1 ±1.6 extperthousand ; 4.4 ±1.1 extperthousand and 2.3 ±1.1 extperthousand (respectively for April, July, October and January). Interestingly, variations were greater within sampling occasions than between them, since the overall averages were –20.1 ±4.3 extperthousand for algae &948;13C and 3.8 ±1.5 extperthousand for algal &948;15N, with SD similar to or greater than SD of sampling occasions. Despite that, ANOVA showed no significant differences across sampling occasions for algal &948;13C (ANOVA, df=3,7; F=1.0; p=0.44) and &948;15N (ANOVA, df=3,7; F=1.9; p=0.21).

Temporal differences were also not significant for <Myriophyllum sp. values of &948;13C (ANOVA, df=3,13; F=0.85; p=0.48) and &948;15N (ANOVA, df=3,13; F=3.3; p=0.05), which presented an overall average of –19.9 ±1.5 extperthousand and 4.2 ±1.9 extperthousand , respectively.

The extremes of flooding and drought are known as the main events structuring communities of primary producers in Brazilian semi - arid streams (Maltchik *et al.*, 999; Pedro *et al.*, 006). In the present study, the low temporal variability in isotope values for some of the primary sources of carbon to the food web is likely to be the result of relatively stable flow conditions in the study site, associated to little temporal variation in the habitat structure and similar proportions of major producers over time, such as aquatic macrophytes, marginal grass and algae (Silva, 2008).

Variation in average &948;13C between sources was significant (ANOVA, df=6,66; F=69.3; p <0.05), with C4 grass being significantly enriched (–11.1 ±0.3 extperthousand) compared to all other sources (Tukey post - hoc p <0.01). Algae (–20.1 ±4.3 extperthousand) and Myriophyllum sp. (–19.9 ±1.5 extperthousand) &948;13C values were also significantly enriched (Tukey post - hoc p <0.01), but not significantly different from each other (Tukey post - hoc p=1.00). Such 13C - enriched carbon isotope values of algae and the submerged plant *Myriophyllum* sp., may be a consequence of aquatic carbon limitations. Studies have reported that small temporary pools may experience marked fluctuations in CO2 concentrations, being considerably depleted in CO2 when densely vegetated (Keeley & Busch, 1984). In such cases, aquatic plants may take up HCO^{3-} , which has less negative &948;13C (Keeley, 1991). That may be the case in the present study, where the stream reach studied was densely colonized by submerged aquatic macrophytes.

The remaining sources were consistently 13C - depleted, with values ranging between –23.7 and –29.7 extperthousand . Riparian vegetation leaves showed the lowest average among the terrestrial sources (–26.7 ±1.2 extperthousand). Other possible terrestrial source was POM (coarse and fine particulate organic matter samples were pooled in the present study). This source was considerably 13C - depleted with an average –25.4 ±1.2 extperthousand . Even though, studies have shown POM to be slightly enriched in comparison with riparian vegetation (Bunn *et al.*, 003), suggesting a potential contribution of a more 13C - enriched algal carbon to POM, in the present study, riparian vegetation tree leaves and POM showed no significant differences in &948;13C values (Tukey post - hoc p=0.80). Such similarities in &948;13C values indicate that tree leaves are a much greater contributor to the POM carbon available for consumers than algal carbon.

The low overall variability in &948;13C values of these terrestrial sources highlights the similar riparian vegetation characteristics in the river reach during the study period. Furthermore, the absence of contribution of the highly variable algal carbon to riparian vegetation leaves is also reinforced by the low variability of both riparian tree leaves and POM.

Isotope values of rooted floating vegetation were consistently 13C - depleted, with *Nymphaea* sp. &948;13C values ranging from –23.8 to –26.7 extperthousand (–25.2 ±0.96 extperthousand) and *Ludwigia* sp. &948;13C values ranging from –25.9 to –28.1 extperthousand (–27.1 ±0.73 extperthousand). These aquatic sources were not significantly different from each other (Tukey post - hoc p=0.40). In fact, &948;13C values of riparian tree leaves, POM and the rooted floating plants were not significantly different (Tukey post - hoc p $>$0.40). Furthermore, all these sources showed very little temporal variation. Eve though, *Nymphaea* sp. and *Ludwigia* sp. were not very abundant aquatic macrophytes in the study site (Silva, 2008), these sources may be contributing to the POM in the sediment.

In contrast with the relatively high variability in carbon isotope values, &948;15N showed little change across sampling occasions and sources, with an overall average of 3.8 ±1.7 extperthousand . Nevertheless, ANOVA revealed some significant differences (ANOVA, df=6,66; F=3.6; p $<$0.05). Tukey post - hoc comparisons showed that the only significant differences occurred between Ludwigia sp. and both riparian tree leaves (p=0.01) and C4 grass (p=0.02). Ludwigia sp. had the lowest &948;15N values (2.5 ±1.1 extperthousand). *Nymphaea* sp. was also 15N - depleted (3.1 ±1.0 extperthousand) in comparison with the other sources, even though such difference was not significant (Tukey post - hoc p $>$0.06).

CONCLUSION

Stable isotope studies require sources with different isotope signatures in order to be able to separate carbon from terrestrial and aquatic origins entering the food web. The present study showed that, for a group of sources, carbon stable isotopes are different. However some terrestrial and aquatic sources had fairly similar carbon isotope values, being unable to discriminate carbon origin. Isotope values of nitrogen, despite being similar among sources, showed important differences across some terrestrial and aquatic sources. Therefore, a dual isotope approach would be necessary to evaluate the sources of organic carbon in food web studies in Brazilian semi - arid streams.

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