Trophic relationships in an estuarine environment: A quantitative fatty acid analysis signature approach

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ABSTRACT

In order to better understand the functioning of aquatic environments, it is necessary to obtain accurate diet estimations in food webs. Their description should incorporate information about energy flow and the relative importance of trophic pathways. Fatty acids have been extensively used in qualitative studies on trophic relationships in food webs. Recently a new method to estimate quantitatively single predator diet has been developed. In this study, a model of aquatic food web through quantitative fatty acid signature analysis was generated to identify the trophic interactions among the species in the Rocha Lagoon. The biological sampling over two consecutive annual periods was comprehensive enough to identify all functional groups in the aquatic food web (except birds and mammals). *Heleobia australis* seemed to play a central role in this estuarine ecosystem. As both, a grazer and a prey to several other species, probably *H. australis* is transferring a great amount of energy to upper trophic levels. Most of the species at Rocha Lagoon have a wide range of prey items in their diet reflecting a complex food web, which is characteristic of extremely dynamic environment as estuarine ecosystems. QFASA is a model in tracing and quantitative estimate trophic pathways among species in an estuarine food web. The results obtained in the present work are a valuable contribution in the understanding of trophic relationships in Rocha Lagoon.

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1. Introduction

Estuaries are among the most productive natural habitats in the world, and their elevated productivity is maintained by high levels of nutrients in both sediment and water column. These transition zones between land and sea can provide unique ecosystems services ranging from trapping contaminant in their sediments to providing nursery areas for marine fish and feeding grounds for migratory birds (McLusky and Elliot, 2004).

Since Platt and Denman (1978) stated that the structure of aquatic communities of species resulted from trophic interactions, several works have been focused at the ecosystem level, emphasizing the central role of food web ecology to understand how aquatic systems function (Pasquaud et al., 2007). Despite this, quantitative diet composition at species level are among the least well known and the most uncertain information in most ecosystems (Kavanagh et al., 2004), especially in estuarine and coastal areas where modeling approaches have shown that food web ecology is complex and variable and that trophic spectrum are quite wide (Monaco and Ulanowicz, 1997; Rybarczyk and Elkain, 2003; Giménez et al., 2006).

Several techniques have been used in food web ecology to study trophic relationships such as stomach/gut content analysis, stable isotopes ratios and biochemical markers (fatty acids and sterols) (Elliott and Hemingway, 2002). Since techniques based on stomach content are the ones that have been the most widely used, the majority of the knowledge generated to date derived from this method. However, estimates of diets with this approach present several biases (Hyslop, 1980; Bowen, 2000) in addition to representing only snapshots of recent meals and may therefore not be

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reliable indicators of long-term diet (Iverson, 2009). More recently, fatty acids and stable isotopes have been used to more specifically identify food web relationships and the strength of interactions among dominant taxa in the estuarine environment (Kharlamenko et al., 2001; Ramos et al., 2003; Persic et al., 2004; Abed-Navandi and Dworschak, 2005; Alfaro et al., 2006; Torres-Ruiz et al., 2007; Alikunhi et al., 2010; Dubois et al., 2014; Prado et al., 2014). Both approaches have advantages and disadvantages, and probably the combination of them constitutes a powerful tool (Alfaro et al., 2006).

Stable isotope ratios of carbon and nitrogen are commonly used to provide information on the trophic position and the contribution of food source of the organisms in a food web. Although useful in addressing a variety of ecological questions, stable isotopes of carbon and nitrogen typically cannot provide quantitative estimates of species composition of diets when more than three species are consumed (Bowen and Iverson, 2013).

Fatty acids have been extensively used in qualitative studies on trophic relationships in food webs (Napolitano, 1999; Iverson et al., 2002; Dalsgaard et al., 2003; Alfaro et al., 2006; Budge et al., 2006, 2007; Rossi et al., 2008; Iverson, 2009; Kelly and Scheibling, 2012). Fatty acids are carbon-rich compounds that are widespread in organisms, and they are relatively easy to metabolize when consumed as part of the animal's diet. Furthermore, their biological specificity, and the fact that some of them (essential fatty acids) are transferred from primary producers to higher trophic levels without major changes, make fatty acids suitable for use as biomarkers (Parrish et al., 2000). Iverson et al. (2004) developed a new method that quantitatively estimates long term predator's diet using fatty acids signatures: Quantitative Fatty Acid Signature Analysis (QFASA).

QFASA method was firstly designed to infer top predator mammal diets. It is a statistical model developed to quantitatively estimate predator diets by means of fatty acids signatures among the predator and its potential preys. The technique involves the combination of prey's FA signatures that most closely resembles the predator's FA stores to thereby infer its diet (Iverson et al., 2004). Up to now, this methodology has been applied in several organisms, like birds and fish, with a good reliability in its estimations (Young et al., 2010; Wang et al., 2010; Budge et al., 2012; Magnone et al., 2015).

Rocha Lagoon is one of the main brackish lagoon ecosystem across the Atlantic ocean in the Uruguayan coastline. It constitutes the most studied and best known coastal lagoon in Uruguay (Sommaruga and Conde, 1990; Fabiano et al., 1998; Vizziano et al., 2002; Norbis and Galli, 2004; Aubriot et al., 2005; Giménez et al., 2006; Rodriguez-Graña et al., 2008; Milessi et al., 2010). As the majority of estuarine coastal environments, it serves as a nursery and sheltering area for migrating birds and fishes (Mianzan et al., 2001; McLusky and Elliot, 2004; Alfaro et al., 2006). Today, this ecosystem is part of a national park located within a biosphere reserve and belongs to a protected area.

Recently, the food web of Rocha Lagoon was studied using stable isotopes analyses (Rodriguez-Graña et al., 2008) and Milessi et al. (2010) contrasted the results obtained by the stable isotope approach to the mass-balance trophic modeling by comparing estimated trophic level assigned to several relevant species with the two methods.

The aim of the present study was to generate a model for the aquatic food web through quantitative fatty acid signature analysis to identify the trophic interactions among the species in the Rocha Lagoon and to contrast the results with previous studies in the same Lagoon using stable isotopes and mass-balance trophic modeling, focusing on those species where energy flow is passing through the system.

2. Materials and methods

2.1. Study area

Rocha Lagoon (Fig. 1) is a brackish, shallow, and microtidal coastal lagoon (mean depth = 0.6 m, area = 72 km^2) located on the Atlantic coast of South America (34°38′S, 54°17′W) (Fig. 1) (Sommaruga and Conde, 1990). At irregular intervals of time, a connection with the ocean opens through a restricted inlet in the southernmost region of the lagoon, producing a north–south salinity gradient (Conde et al., 2000).

2.2. Biological sampling

Biological samples were collected from April 2008 to October 2010 at Rocha Lagoon, at four locations (north, south, mouth, and central zone). In this study, we use the term phytoplankton to name the fraction ranging from 5 μm to 50 μm obtained filtering water. Phytoplankton was collected in plastic bottles, filtering the water, previously filtered 50 μm with a vacuum pump trough 5 μm paper filter. Zooplankton refers to those animals which were trapped pumping water between 60 μm and 1 mm mesh. Zooplankton samples were collected with motorized water pumps, filtrating water between 1 mm and 60 μm mesh. Benthic animals were collected using seine nets, corers, dredges and manual collections. Fish were collected using gill and seine nets. Sediments were collected using corers.

Benthic animals and fish (except for *Heteromastus similis*) were counted, measured and weighted (total length with 1 mm precision and wet weight with 0.0001 g precision), and when possible identified at the lowest possible taxonomic level.

2.3. Biochemical analyses

All samples for biochemical procedures were stored at −20 °C until analysis. Lipid extraction and quantification was made in duplicate according to Folch et al. (1957). All organisms were analyzed as a whole prey, as eaten in nature. Organisms and sediment were freeze-dried and homogenized prior to lipid extraction. FA methyl esters (FAMEs) of total lipids were obtained by transesterification with H_2SO_4 in methanol solution (Christie, 1982). FAMEs were separated using a gas chromatograph (Hewlett Packard 5890) equipped with a flame ionization detector and a Supelcowax fused silica capillary column (30 m 0.32 mm ID, Supelco, USA) using nitrogen as carrier gas. Samples were injected in split mode at 250 °C. FA were identified by comparing retention times of methyl esters standards (Supelco) and by reference to a well characterized fish oil (Salhi and Bessonart, 2013). FA data were expressed as the mass percentage of total FA ± sd.

2.4. Fatty acid database

The main fatty acid database with all dietary fatty acid profiles of all species/group recognized at Rocha Lagoon in this study was constructed using the average of each and considering 37 fatty acids which covered 97.3 ± 1.3%, as the mass percentage of total fatty acids. The model was run considering each of the items as a predator, with the exception of phytoplankton and sediment. Each time the model was run, a new matrix from the main database was constructed as follows: the first column was always the predator, and subsequent columns were the potential preys for this predator (based in the bibliography for each species and taking into account biological aspects for each functional trophic group). The matrix included also the prey's lipid contents expressed in wet weight, to finally express the results corrected by wet weight lipids. Due to the