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Diet estimation of *Paralichthys orbignyanus* in a coastal lagoon via quantitative fatty acid signature analysis

Larisa Magnone [a,*], Martin Bessonart [a,b], Martín Rocamora [c], Juan Gadea [a], María Salhi [a]

[a] Laboratorio de Recursos Naturales, Instituto de Ecología y Ciencias Ambientales, Facultad de Ciencias, Universidad de la República, Iguá 4225, CP 11400 Montevideo, Uruguay
[b] Estación Experimental de Cultivos Marinos y Acuicultura, Dirección Nacional de Recursos Acuáticos (DINARA) Ministerio de Ganadería Agricultura y Pesca (MGAP), Parque Nacional Cabo Polonio s/n, Uruguay
[c] Departamento de Procesamiento de Señales, Instituto de Ingeniería Eléctrica, Facultad de Ingeniería, Universidad de la República, Julio Herrera y Reissig 565, CP 11300 Montevideo, Uruguay

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ABSTRACT

Quantitative fatty acid analysis (QFASA) is a statistical model designed to quantitatively estimate predator diets using fatty acid (FA) signatures among the predator and its potential prey. QFASA estimated the diet of a migratory flatfish *Paralichthys orbignyanus* over its fattening stage in the Rocha lagoon (a semi-closed estuary) where all prey available to this top predator species are well known. A 20-week controlled feeding trial obtained calibration coefficients (CC) for *P. orbignyanus* fed two types of prey (silverside and menhaden). Several subsets of FA were tested in order to elucidate which is the most suitable for applying QFASA to this species. QFASA was applied to all CC and FA subsets to validate the model. The model predicts better the consumed diet with silverside CC than with menhaden CC. The subset which best adjusts the diet over the validation process, includes approximately 34% of total FA, containing mainly dietary FA. The diet estimation in nature for *P. orbignyanus* varied according to whether the model is applied with or without CC. When the diet was estimated without CC, results were similar to those based on stomach content analysis (reported in previous studies); it fed mainly on silverside (~88%), but also some minor soft-body species that are only evident using this kind of methodology (QFASA). When the diet was estimated with silverside CC, a higher presence of silverside (~97%) was observed. These results seem to indicate a tendency to overestimate the presence of the item used as prey for CC calculations.

1. Introduction

Continental shelves and their associated estuaries are among the most productive ecosystems in the world (Day et al., 1981), where major fishery resources, including flatfish (Munroe, 2005) are located. Estuaries constitute areas used by several species as permanent or transitory habitats for reproduction, migration, feeding and nursery (Elliott and Hemingway, 2002). Establishing and quantifying trophic relationships between the species of an ecosystem is of primary importance to understand the ecosystem functioning (Connan et al., 2007).

Paralichthyidae flatfishes constitute important commercial and recreational fisheries throughout the Atlantic, from the deep Arctic to the coasts of southern Africa and South America (Díaz de Astarloa, 2002). They are the most productive demersal fisheries in the world from the commercial point of view and Paralichthyidae flatfishes are by far the most valuable fish per unit weight landed. (Díaz de Astarloa and Munroe, 1998). In Uruguay, there are three species of flatfishes, but only one (*Paralichthys orbignyanus*) inhabits estuaries. This flatfish occurs from Río de Janeiro, Brazil, to San Matías Gulf, Argentina (Fabré and Díaz de Astarloa, 1996). It is categorized as a eurihaline and euritherm species (López Cazorla, 2005) and in summer it is captured mainly in coastal areas (Lopez Cazorla, 1987). *P. orbignyanus*, like other North Atlantic flatfishes, is a catadromous fish spawning in marine water, but its juveniles migrate towards coastal lagoons (Bergman et al., 1988; Koutsikopoulos and Lacroix, 1992; Whitfield, 1998) and fatten there (Robaldo, 2003).

Across the Atlantic shoreline of Uruguay there are several coastal lagoons and stream mouths where *P. orbignyanus* is found from juvenile to adult stages throughout the year (Rivera Prisco et al., 2001). The Rocha Lagoon is a sand flat coastal lagoon that, as an estuarine environment, serves as a nursery and sheltering area for migrating birds and fish (Mianzan et al., 2001). In recent years, increasing eutrophication of the lagoon has been observed (Aubriot et al., 2005) related to the main activities of the land use: extensive cattle raising and agriculture. Today, this ecosystem belongs to a conservation area where

Abbreviations: CC, calibration coefficients; DHA, docosahexaenoic acid; EFA, essential fatty acids; EPA, eicosapentaenoic acid; FA, fatty acids; FAMEs, fatty acid methyl esters; HIS, hepatosomatic index; HUFA, high unsaturated fatty acids; KL, Kullback–Liebler; MUFA, monounsaturated fatty acids; PER, predator energy reservoir; PUFA, polyunsaturated fatty acids; QFASA, quantitative fatty acid signature analysis; SAFA, saturated fatty acids.

* Corresponding author.
E-mail addresses: larisa@fcien.edu.uy (L. Magnone), martinb@fcien.edu.uy (M. Bessonart), rocamora@fing.edu.uy (M. Rocamora), juanluisgadea@gmail.com (J. Gadea), msalhi@fcien.edu.uy (M. Salhi).

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P. orbignyanus – which has been reported as a top predator (Norbis and Galli, 2004; Rodriguez-Graña et al., 2008) – represents a high proportion of the captures by local fishermen.

Top predators play an important role in determining the structure and functioning of ecosystems (Bowen, 1997). The dynamics of predator–prey relationships, the structure of food webs, and the foraging behavior of individuals are key factors to understand the functioning of these types of areas (Pimm et al., 1991; Schoener, 1971; Sih et al., 1998), which is crucial for their management (King et al., 1995).

The common way to address the study of trophic relationships is by producing accurate estimates of the diet of predators. Currently, the diet of *P. orbignyanus* has been estimated using the classic method of stomach content analysis (Norbis and Galli, 2004). Although often used for determining diets, such estimates can be biased since soft-bodied prey are rapidly digested whereas prey with hard parts can be overestimated (Bowen, 2000). In addition, this estimate provides only a snapshot of the last meal of an animal. For these reasons, methods to assess the feeding habits based on fatty acid (FA) signatures seem to be a promising alternative. They can provide new insight into the long-term diet of species taking advantage of FA as trophic markers. Moreover, the detection of soft-bodied prey can be improved and the sampling process can be undertaken while keeping the predator alive.

Fatty acids have been extensively used in qualitative studies about trophic relationships in food webs (Dalsgaard et al., 2003) based on the demonstrated influence of dietary FA on predator fat stores (Colby et al., 1993; Kanazawa et al., 1979; Kirsch et al., 1998; Raclot et al., 1998; Rouvinen and Kiiskinen, 1989). Specifically the concept of individual lipid biomarkers has been focused on mainly in the linkage between organisms at lower levels of the food webs (Falk-Petersen et al., 2002; John and Lund, 1996; Leveill et al., 1997; Mansour et al., 1999). Recently, Iverson et al. (2004) have developed a new method to quantitatively estimate top predators' long-term diet using fatty acid signatures (quantitative fatty acid signature analysis, QFASA). The technique involves the use of a statistical model to determine the combination of prey FA signatures that most closely resembles the predator FA stores to infer its diet. The predator differential metabolism of FA is taken into account by introducing calibration coefficients (CC) in the model, which are obtained from controlled feeding experiments. These experiments not only provide correction factors that allow a more accurate quantitative estimation, but they also provide a rigorous validation of the method. The determination of how long these experiments have to last to truly reflect the diet in the predator fat storage tissue is critical (Budge et al., 2006). Several studies have been conducted to determine calibration coefficients for birds and mammals (Iverson et al., 2007; Nordstrom et al., 2008; Rosen and Tollit, 2012; Wang et al., 2010; Williams et al., 2009) but, in regard to fish, only Atlantic salmon has been studied (Budge et al., 2011, 2012). A careful selection of the predator fat store tissue to use in QFASA has been shown to be of crucial importance (Budge et al., 2006; Iverson, 2009). The adipose tissue is usually selected in vertebrates as it should experience a rapid turnover in response to dietary lipid intake. Fish, despite being vertebrates, have their lipid stores in muscle with skin, viscera or liver, and it is well known that the fatty acid composition of these tissues in fish largely resembles the fatty acid composition of the diet (Ackman, 1980; Jobling, 1993; Shearer, 1994).

Although qualitative FA techniques have been used to infer foraging ecology in fish (Elsdon, 2010; Stowasser et al., 2009; Young et al., 2010), to this date, to our knowledge, quantitative analysis has not been performed or validated in this group of vertebrates. The QFASA method was designed and assessed for upper trophic level endothermic vertebrates (Iverson et al., 2007; Nordstrom et al., 2008; Thiemann et al., 2008; Tucker et al., 2009; Wang et al., 2010), but it has not yet been applied to lower vertebrates (Iverson, 2009).

The aim of this work was to obtain a quantitative estimation of the diet of a flatfish (*P. orbignyanus*) in an estuarine coastal lagoon by applying the QFASA. Additionally, we aimed to determine calibration coefficients and validate the model for this species under controlled experimental conditions.

2. Materials and methods

2.1. Sampling site and sample database

Field sampling for wild prey and predator fish was conducted in Rocha Lagoon, Uruguay (Fig. 1). Over the validation process of the model, fish were housed and managed in the Experimental Institute of Marine Aquaculture of DINARA (Department of Rocha, Uruguay).

2.2. Site

Rocha lagoon 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) (Sommaruga and Conde, 1990), included in a protected area of MaB-UNESCO. At irregular intervals of time, a connection with the ocean opens through a restricted inlet in the southernmost region of the lagoon, allowing the migration of many species, including *P. orbignyanus*, and producing a north–south salinity gradient (Conde et al., 2000).

2.3. Wild P. orbignyanus

A total of 33 adult *P. orbignyanus* (23 females and 10 males) obtained at Rocha Lagoon with the help of local fishermen from April 2008 to October 2010, were measured and weighed (44.0 ± 8.1 cm and 1.2 ± 0.5 kg) and sampled for lipid and fatty acid analysis. Samples were obtained from gonads, liver and a piece of upper dorsal muscle with skin (sampled together to include subdermal lipids) and stored at −20 °C. The livers of the fish were also weighed to obtain the hepatosomatic index (HSI), calculated as: [liver weight (g) / fish weight (g)] × 100.

2.4. Potential prey

Based on available information about the items cited as prey for *P. orbignyanus* according to Rivera Prisco et al. (2001), Norbis and Galli (2004), López Cazorla (2005) and Rodriguez-Graña et al. (2008), a comprehensive sampling of the cited prey and non-cited potential prey of *P. orbignyanus* was carried out. Prey samples were collected from April 2008 to October 2010 at Rocha lagoon using gill nets, seine nets, corer samples, dredge samples and manual collections. A total of 17 dietary items were collected and identified to the lowest possible taxonomic level.

Prey, with the exception of *Heteromastus similis*, were counted, measured and weighed (total length with 1 mm precision and wet weight with 0.0001 g precision). As *P. orbignyanus* displays cannibalism, juveniles of this species could be considered as a prey item. However, this option was not considered in order to avoid artificial noise in the diet estimation, due to the resemblance between this prey and the predator FA profile.

2.5. Lipids and FA analysis

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). To generate the predator profile, lipids were extracted from freeze-dried and homogenized dorsal muscle with skin. In the case of potential prey, whole organisms were freeze-dried and homogenized prior to lipid extraction. FA methyl esters (FAMEs) of total lipids of all samples were methylated by transesterification with H_2SO_4 in methanol solution (Christie, 1982). FAMEs were separated using gas chromatography (Hewlett Packard 5890) equipped with a flame ionization detector, a Supelcowax fused silica capillary column (30 m 0.32 mm ID, Supelco, USA) and nitrogen as a carrier gas. Samples