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# Trophic links and nutritional condition of fish early life stages in a temperate estuary

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#### ABSTRACT

The physiological and nutritional condition of fish larvae affect their survival and thus, the success of estuaries as nursery areas. Fatty acid composition has been useful to determine fish nutritional condition, as well as trophic relationships in marine organisms. The present study analyses the fatty acid (FA) composition of fish larvae during spring and summer in the Mondego estuary, Portugal. FA composition, trophic markers (FATM) and fish nutritional condition was analysed for Gobiidae and Sardina pilchardus larvae and the relationships with the local environment evaluated. Results showed that both taxa differed mainly in the stearic acid (C18:0) and eicosapentaenoic acid (EPA) content, with important amounts in Gobiidae and S. pilchardus, respectively. Gobiidae larvae presenting high nutritional condition and omnivore FATM. Fatty acid composition seems to be related with their natural habitat selection and food availability, while fish larvae nutritional condition also showed a strong link with the water temperature and presence of potential predators. This study suggests that FA composition can be a useful tool in assessing planktonic trophic relationships and in identifying species natural habitat

#### 1. Introduction

Estuaries are among the most productive ecosystems in the world, providing a vital function for fish as nursery areas. The success of an estuary as a nursery area will depend on its capacity of producing adult recruits, enhancing fish growth and survival mainly during the larval and juvenile stages (Able et al., 2006; Beck et al., 2001). The nutritional condition of a fish represents an expression of both the biological and physical factors that acted on the individual, in the period prior to the assessment. The study of nutritional condition enables the evaluation of fish larvae physiological state and it is believed to be a useful method for the estimation of fish recruitment (Suthers, 1988).

Fulton's condition index (FCI) is extensively used in fisheries research as a morphometric condition index and provides a useful tool to examine overall growth and relate fish condition with environmental forcing (De Raedemaecker et al., 2012; Suthers, 1998; Vasconcelos et al., 2009). According to this index, heavier fish for a given length are in better condition. In addition, lipid components have also been used as a measure of the nutritional and physiological condition of fish (e.g. Amara et al., 2007; Costalago et al., 2011; Teodósio et al., 2017). The

increase in some fatty acids (FA) has been related with increased larval growth and with prey quality (Paulsen et al., 2014a, 2014b), as well as, with the improved ecological performance of fish larvae by enhancing their swimming ability (Perez and Fuiman, 2015; Silva et al., 2015). The large demand for fatty acids in the larval diet at these critical early stages makes these FAs crucial for larval growth and survival (Tocher, 2010).

Fatty acids show variations among functional/taxonomic groups and are transferred along the food web, being useful biomarkers for identification of trophic interactions (Dalsgaard et al., 2003). Fatty acid trophic markers (FATM) have been used in marine ecosystems to study predator–prey relationships and energy transfer (Dalsgaard et al., 2003; Falk-Petersen et al., 2004). Some well-known FATM are, for example, C20:5 (n-3) for diatoms; C22:6 (n-3) and C18 polyunsaturated fatty acids (PUFA) for dinoflagellates, and C20:1 (n-9) and C22:1 (n-11) monounsaturated fatty acids (MUFA) for Calanus copepods. Also, the ratio PUFA/SFA (saturated fatty acids) and DHA/EPA (docosahexaenoic acid to eicosapentaenoic acid, C22:6n-3/C20:5n-3) are indicative of carnivory and reflect the proportion of dinoflagellates and diatoms, as DHA is dominant in dinoflagellates (Dalsgaard et al., 2003).

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Gobiidae fishes are important inhabitants of lagoons, coastal areas and estuaries of the Atlantic and Mediterranean regions (Arruda et al., 1993; Jaquet and Raffaelli, 1989; Leitão et al., 2006). They represent a relevant role in the trophic webs as intermediate predators and prey for upper trophic levels (Leitão et al., 2006; Arruda et al., 1993). Their larvae are frequent in the planktonic compartment of temperate estuarine areas such as the Mondego estuary (Portugal), which can represent up to 80% of fish larval communities (Primo et al., 2011). Although not so numerous within estuaries, the European sardine Sardina pilchardus is also quite abundant in nearby coastal areas. With 21000 tonnes caught in 2015, the European sardine is the main target of purseseine fisheries in Portugal and Spain, which have shown drastic reductions of annual catches (ICES, 2016). Hence, changes in environmental conditions (i.e. oceanographic conditions, prey availability) affecting early life stages could be significant for the species recruitment and commercial exploitation.

The aim of this study was to characterize the seasonal variation of fatty acid composition in fish larvae present in the estuarine trophic web of the Mondego estuary. The specific goals include (1) infer about the estuarine nursery capacity for fish larvae by comparing FA composition and condition of species with different nursery habitats, (2) to use FATM to determine fish larvae food preferences, and (3) to identify potential environmental forces affecting larval condition and survival throughout the study area.

#### 2. Material and methods

#### 2.1. Study area

The Mondego Estuary (40°08′N, 8°50′W) is situated in a warm temperate region at the west cost of Portugal (Fig. 1). With an area of about 8.6 km², it is an intertidal and shallow system composed by two arms (north and south) divided by the Murraceira Island. The two branches exhibit different hydrographic characteristics: the north branch presents a low residence time (< 1 day), is deeper (4–8 m during high tide) and since it constitutes the main navigation channel, suffer from regular dredging activity; the south branch is shallower (2–4 m deep, during high tide) and has higher residence time (2–8 days). A detailed description of the system can be found inMarques et al., 2006. The biological characteristics of the ecosystem are mostly controlled by meteorology, hydrodynamics and by river run-off (Marques et al., 2014).

#### 2.2. Sample collection

The study was performed during spring and summer of 2014-2016, in order to cover the peak of larval abundance in the area (Primo et al., 2011). Sampling was performed monthly (April/May/June/July/August 14, June/July/August 15, April/May 16), in two stations in the estuary, one in the north (M) and other in the south arm (S1) (Fig. 1). Fish larvae were collected by horizontal hauls at 1 m below the surface, using a 500 µm mesh bongo net (mouth diameter: 0.5 m). At least five replicates of 5 min tow length (2 knots) were performed at each sampling site, to assure enough number of larvae for the subsequent analysis. After each replicate, samples were collected and kept under dry ice until arriving at the laboratory, where they were stored frozen at -80 °C. At the laboratory, each sample was thawed and sorted. Fish larvae were identified, weighted (wet) and measured to the lower 0.1 mm (standard length - SL), as quickly as possible, then the muscle sample (tail) was separated from the head and gut, dried at 60 °C during 48 h and stored in the drying oven until analysis. Gobiidae and Sardina pilchardus were the most abundant larvae present in the samples and hence, those were chosen for the subsequent analysis, representing estuarine resident and marine migrant species, respectively. Gobiidae larvae were grouped together to avoid material degradation due to the challenging and time consuming species identification of this family.

At each sampling site, microplankton and mesozooplankton samples were simultaneously collected by 3min horizontal hauls (2 knots) using a 64  $\mu m$  mesh bongo net (mouth diameter: 0.3 m) and a 200  $\mu m$  mesh bongo net (mouth diameter: 0.5 m), respectively, both equipped with a Hydro-bios flowmeter to calculate the filtered volume (average 20 m<sup>3</sup>). Organisms were immediately fixed with 4% buffered formalin and later transferred to 70% ethanol. Samples with very high numbers of individuals were sub-sampled as necessary by using a Folsom plankton splitter. For microzooplankton samples, organisms were counted and classified in 3 main groups (Copepodites, Cop1; Ciliate, Cil; and Dinoflagellate, Dino). For mesozooplankton samples, copepods and gelatinous organisms were counted (Cop2\_N, Jelly\_N) and separated into small previously weighed aluminum capsules, placed in heat resistant acrylic multiwell trays and dried at 60 °C for at least 24 h. After that, the aluminum capsules were weighted again and the biomass determined (Cop2\_B, Jelly\_B). Mesozooplankton biomass was expressed as mg  $\,\mathrm{m}^{-3}$  and organisms' abundance was expressed as ind  $\,\mathrm{m}^{-3}$ . In parallel with the plankton tows, water temperature (T) and salinity (Sal) were recorded at each sampling point using a WTW Cond 330i

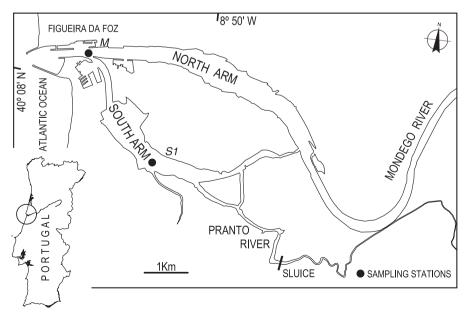


Fig. 1. Location of the sampling stations (M, S1) in the Mondego estuary.

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