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Are tidal lagoons ecologically relevant to larval recruitment of small pelagic fish? An approach using nutritional condition and growth rate

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ABSTRACT

There are numerous studies dealing with larvae of Small Pelagic Fish (SPF), but only a few have actually addressed advanced larval phases. Temperate coastal lagoons are particularly understudied, due to the absence of standard method to capture advanced larval fish in these near shore shallow habitats. Accordingly, this study aims to describe abundances, nutritional condition and in situ growth of post-flexion (SPF) from the Ria Formosa, a tidal coastal lagoon in southern Portugal. The nutritional condition and in situ growth were determined through cohort analysis and standardized RNA:DNA ratio (sRD), complemented with feeding incidence (gut content) and fatty acids (FAs, trophic biomarkers) of post-flexion larvae sampled sequentially with light traps, from spring 2005 to summer 2006. Simultaneously, environmental parameters such as water temperature, salinity, dissolved oxygen and chlorophyll *a* were measured. Post-larvae of SPF were captured through the year in important numbers. The dominant species were *Sardina pilchardus* (50.7%), *Engraulis encrasicolus* and *Atherina presbyter* (11.4%). These results are distinct from those based on adult/juvenile surveys or early planktonic phases in the Ria Formosa where clupeiformes were occasionally reported. Sardines were captured mainly in winter, spring and early summer and anchovies mainly during summer and autumn. Sand smelt, a resident species, was present throughout the year. In the early summer, the three species were present and during this period some diet overlapping occurred, the feeding incidence of the clupeiformes was very low, but atherinids always exhibited full guts reflecting the different gut morphology or indicating different life-cycle strategies. The bulk of the diet was mollusks, crustaceans and appendicularians, for sand smelts, sardines and anchovies, respectively. The results of FA analyses showed some contribution of phyto-plankton to SPF in the area. All SPF exhibited higher condition (sRD) and growth rates in summer, which are explained by the adequate temperature and higher planktonic productivity. This is especially relevant because the successful development of postflexion larvae in these nursery areas largely determines the successful recruitment to adult fish populations.

1. Introduction

Small Pelagic Fishes (SPF) include a diverse group of mainly planktivorous organisms that share surface waters, usually above the continental shelf. The SPF can be defined as the clupeiformes (Engraulidae, Clupeidae), scads (Carangidae), mackerels (Scombridae), and sand smelt (Atherinidae).

Off the Iberian Peninsula, high concentrations of SPF species (mainly *Sardina pilchardus* (Walbaum, 1792), *Engraulis encrasicolus* (Linnaeus, 1758)) are found. Despite the large interannual landing variation of these fisheries on the Iberian Peninsula, there are few studies about late larval phases and juveniles (Garcia et al., 2005; Drake et al., 2007; Costalago et al., 2011), and production and recruitment dynamics are usually studied indirectly, e.g., using acoustic surveys (Tugores et al., 2010).

Nevertheless, routine acoustic fisheries or ichyoplanktonic cruises did not include near shore ecosystems, but early stages of SPF may dominate very productive shallower areas, in some cases classified as protected areas. In fact, coastal lagoons worldwide are recognized as important nursery habitats for early stages of invertebrates (Chícharo and Chícharo, 2000, 2001a,b) and fishes (Elliott and Hemingway, 2002). Nevertheless, some SPF species, in larval

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phases, are not described as users of these areas, and few studies describe late larval condition and growth of SPF in such variable habitats. Indices of feeding conditions, such as diet (Garrido et al., 2008; Morote et al., 2010), and FA content (Rossi et al., 2006) and indices of growth, such as nucleic acids (Buckley et al., 2008), are especially useful in these areas because of their ability to integrate interspecific, temporal and spatial patterns of life and feeding history.

The ratio of tissue RNA to DNA is a widely used index of recent growth and nutritional condition in larval and juvenile fish (Buckley et al., 2004). The amount of RNA in a cell varies in proportion to protein synthesis, whereas DNA concentrations remain fairly constant in a somatic cell, even during starvation. Thus, the RNA:DNA ratio is an indicator of the protein-synthesizing potential of a cell. Fast-growing individuals tend to be more prepared to face adverse conditions, such as predation, and take advantage of good environmental conditions, such as temperature and food availability usually characteristic of nursery areas. More research is needed to fully understand the use of interim nursery areas critical to coastal fish survival worldwide, especially because these transitional habitats are being altered or lost due to anthropogenic impacts (Wasserman and Strydom, 2011).

Moreover, to understand recruitment variability, it is necessary to study the underlying physical processes and the complex food web processes that sustain fish in nursery areas during their whole planktonic period, not only the early phases (López-Sanz et al., 2011) or juveniles phases (Woodland et al., 2012).

In fact, it has been shown that the magnitude of recruitment might be linked to the abundance of advanced larval phases and not to that of the early larvae (Leggett and DeBlois, 1994). Besides deterministic recruitment, it seems most recruitment is indeterminate, because of the large number of factors that can interact and cause variability during the early phases. Chaos theory has an important concept to offer to ecology; an apparently irrelevant factor or an apparently irrelevant change in a relevant factor can have an important impact on the history of a complex system, i.e., a system regulated by many factors (Boero et al., 2004). Because of the observed variability of the egg and early phases of SPF and the difficulty of sampling late larvae because of their increased ability to evade plankton nets, abundance, production and recruitment dynamics are usually studied indirectly by projecting the results of oceanographic studies (mainly linking early larval phase distribution and probability of survival) to local circulation and environmental conditions. Therefore, to reduce the number of factors that can interact and cause imprecise recruitment estimates, a key task must be to focus on late larvae ecology. In fact, notochord flexion appears as a milestone in fish ontogeny involving rapid development of specific morphological and physiological characteristics (e.g., caudal fin, Somarakis and Nikolioudakis, 2010).

We hypothesized that the presence of postflexion larvae of SPF in tidal coastal lagoons would not be accidental and that individuals would exhibit a high condition and growth rate. The present study aimed to determine the relative abundance of postflexion SPF species in a temperate tidal coastal lagoon (the Ria Formosa) and to identify relevant environmental and biological processes at postflexion phase. This was done by analyzing larval fitness through RNA:DNA ratio (sRD) and their weight-specific growth rate (based on cohort analysis and derived from nucleic acids), complemented with diet analysis (gut content and FAs as biomarkers).

2. Methods

2.1. Study site

This study was carried out in the western part of the Ria Formosa .The Ria, located in the northwest of the Bay of Cadiz, is a large (170 km^2) tidal coastal lagoon extending along the eastern part of the south coast of Algarve (Fig. 1). The Ria is protected from the Atlantic Ocean by multi-inlet barrier islands forming a sand dune cordon of ~80 km between Ancão to the west, and Guadiana estuary near the border with Spain. The average depth is less than 3 m, with 14% of the lagoon surface permanently submersed (subtidal channels). The Ria Formosa is under the influence of a Mediterranean climate with humid moderate winters (rainfall concentrated between November and February), and hot, dry summers. There are few freshwater sources flowing into the lagoon, most of which are dry during summer. This shallow lagoon is well mixed vertically (Barbosa, 2010).

2.2. Sampling strategy and laboratory processing

Between May 2005 and September 2006, on a weekly basis in spring/summer and autumn and twice a month during winter, SPF larvae were collected with a light trap composed of an acrylic pipe 25 cm in diameter and 50 cm high on a base consisting of a PVC box containing a light. The trap was used at different zones in Ancão Inlet (for details see Chícharo et al., 2009). Before the use of the light trap, water temperature, salinity and dissolved oxygen were measured with a multiparametric probe (YSI Professional). In situ chlorophyll *a* was determined with a fluorometer (10 AU Turner). When a high abundance (<20 larvae) was captured, several samples were taken over the following days to estimate growth. After 40–60 min of sampling, the captured larvae were frozen in liquid nitrogen.

In the laboratory, larvae were thawed, identified according to Ré (1999) and measured to the nearest 0.1 mm under a dissecting microscope equipped with an ocular micrometer. Only post-flexion larvae were selected for the study, taking into consideration the observation of the flexion of notochord, which occurs at standard lengths of 11–12.5 mm for *Sardina pilchardus* and 9–10 mm for *Engraulis encrasicolus* and *Atherina presbyter* (Ré, 1999; Santos et al., 2007). The larvae were freeze-dried, then individually dry homogenized in microtubes, with two QIAGEN 3 mm Tungsten Carbide Beads, in a shaking mill (Retsch MM 300). Next, the total weight was determined and 1–5 mg of sample was stored at −80 °C for later nucleic acid and FA determinations.

2.3. Gut analysis and fatty acids

Fish larvae of the three studied species, co-occurring during June, all postflexion and larger than 20 mm, were analyzed for gut content and FAs. These analyses were carried out on 45 intact anchovy larvae (*Engraulis encrasicolus*), 36 intact sardine larvae (*Sardina pilchardus*) and 12 sand smelt (*Atherina presbyter*) selected from the light-trap samples in which they were most abundant and hence deemed the most representative. The entire gut from each specimen was removed using a fine needle and placed in a drop of 50% glycerin–distilled water on a glass slide. Prey organisms were teased out for identification, enumeration, and measurement. Food particles in the gut were identified to the lowest taxon possible. After gut removal, larval bodies were processed for FA as described in previous section.

The determination of FA profiles was based on the experimental procedure of Lepage and Roy (1986) modified by Cohen et al. (1988). FA methyl esters were analyzed in a CP 3800 Varian gas chromatograph equipped with an autosampler and fitted with a flame ionization detector. The separation was carried out with helium as the carrier gas in a DB-Wax Polyethylene Glycol column (30 m × 0.25 mm id) programmed to start at 180 °C for 5 min, then heat at a rate of 4 °C min^{-1} for 10 min and maintained at 220 °C for 25 min, with detection conducted at 250 °C using a split injector