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Coupling suitable prey field to in situ fish larval condition and abundance in a subtropical estuary

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ABSTRACT

Survival of fish larvae is influenced by the suitability of the prey field and its variability in time and space. Relationships among food quality, quantity and recruitment have been explored in temperate ecosystems where spawning and secondary production are strongly seasonal, but for subtropical estuaries the mechanisms responsible for larval survival remain poorly identified. This study evaluated the nutritional condition (feeding incidence and AARS activity) and abundance of a multi-specific assemblage of fish larvae from a subtropical estuary in South America (Solís Grande, Uruguay) during the fish reproductive season; and related both variables to prey abundance, composition, size and fatty acids content. The larval assemblage was composed of 13 species belonging to different functional groups and composition varied seasonally. Contrary to expectations larval condition did not match an increase in prey quality. Food availability was high throughout the study period, although significant changes existed in the size and taxonomic structure of the prey assemblage. The temporal succession of complementary factors - temperature, prey composition, abundance and quality - promoted a wide window of opportunity for larvae, where quality seemed to have compensated quantity. Such combination of factors could allow an extended larval survival along the spawning season. These findings underline the importance of a better understanding of subtropical estuaries as nursery areas.

1. Introduction

Spawning in natural fish populations is largely controlled by environmental conditions (Wootton and Smith, 2015). The timing of the annual plankton production cycle determines the overlap between first feeding larvae and their prey, and is considered a core driver of larval fish survival (Hjort - Cushing critical period and match-mismatch hypotheses; Hjort, 1914; Cushing, 1969). Fish spawning is strongly seasonal at high latitudes where plankton production cycles are most marked, while it is more extended in tropical regions where plankton production is nearly continuous throughout the year (Robertson, 2013). The match-mismatch hypothesis has been tested in temperate systems like the North Atlantic, Baltic and North Seas, and supportive evidence was found for several species such as herring, cod, haddock and sprat (Cushing, 1990; Fortier et al., 1995; Beaugrand et al., 2003; Platt et al., 2003; Voss et al., 2006). However, in mid latitude subtropical environments with smoother seasonality, this hypothesis has been scarcely evaluated (e.g. Chícharo, 1998; Chícharo et al., 2003).

Prey suitability for larval survival is linked to traits such as size and biochemical composition (Paulsen et al., 2014a; Pepin et al., 2015). These traits depend on the taxonomic structure of the plankton assemblage. The diet of fish larvae typically shifts during ontogeny from phytoplankton or nauplii during first-feeding stages to larger prey such as adult copepods and cladocerans during older larval stages (Pepin and Penney, 2000; Robert et al., 2011; Llopiz, 2013). Fatty acids - in particular the essential highly unsaturated forms (HUFA) - are also important for larval development and survival (Sargent et al., 1999; Izquierdo et al., 2000; Koussoroplis et al., 2011). They are acquired only through diet and this is related to the taxonomic composition and seasonal succession of phytoplankton (Arts et al., 2009) and zooplankton (Veloza et al., 2006).

The nutritional status of fish larvae is related to growth and

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survival probabilities, and can be assessed through biochemical indices (e.g. RNA:DNA ratio, metabolic enzymes, triacylglycerol/cholesterol ratio) (Clemmesen, 1994; Catalán et al., 2007; Costalago et al., 2015). These indices are highly sensitive and estimate short time responses in feeding regime. However, they tend to be time consuming, require large sample sizes and may be valid only for given larval stages. A decade ago, the aminoacyl-tRNA synthetases enzyme activity method (AARS) was first applied as an index of growth rate in freshwater and marine crustaceans like cladocerans (Yebra and Hernández-León, 2004), copepods (Yebra et al., 2005, 2011; Herrera et al., 2012), euphausiids and recently for Atlantic herring larvae (Herrera, 2014). The aminoacyl-tRNA synthetases catalyze the first step of protein synthesis and their specific activity correlates with larval growth rates under laboratory and field conditions (Herrera, 2014).

Estuaries play an important role in the life cycle of many fishes. Estuarine fish fauna includes estuarine resident species and marine spawners with larvae and juveniles depending on, or inhabiting these ecosystems (Potter et al., 2015). Despite high environmental variability in estuaries (e.g. salinity, turbidity) it is assumed that the costs are compensated by enhanced recruitment (Day et al., 2013). In temperate and subtropical estuaries, larval and juvenile fish benefit from the high productivity and rich food supply, turbid waters provide refuge against predators, and favourable temperature conditions allow high growth rates (Able, 2005; Strydom, 2015). In subtropical estuaries, the higher trophic status compared to open marine waters and a more extended productive season may reduce the need for a close match between spawning and planktonic production, providing a wider temporal window for fish spawning (Bye, 2000; Acha and Macchi, 2000; Wootton and Smith, 2015). Extended spawning is actually common in subtropical estuarine and marine coastal areas and may typically extend over six months, from early spring to early autumn (September through March in the southern hemisphere, Acha and Macchi, 2000; Vizziano et al., 2002). Fish larvae peak in spring, summer and early autumn and are least abundant in winter (Whitfield, 1989; Strydom, 2015).

Despite the relevance of seasonal variability in food resources upon larval success, few studies have addressed the link between zooplankton availability and quality with larval survival. These studies focused on the relationship between zooplankton availability and/or zooplankton HUFA composition and larval condition mainly for temperate species (e.g. sardine, Baltic sprat, Atlantic herring, south Atlantic hake; Voss et al., 2006; Diaz et al., 2014; Paulsen et al., 2014a; Temperoni and Viñas, 2013). However, few cases have dealt with this issue in subtropical species (Veloza, 2005). Moreover, according to Pepin and Penney (1997), interspecific studies under identical sampling and processing protocols are needed in order to define general patterns in larval feeding ecology. Therefore, the present study aimed to determine the temporal variability in larval nutritional condition and abundance during the reproductive season in a subtropical estuary - the Solís Grande Estuary (SGE) - and explore its link with suitable prey availability in terms of abundance, composition, size and fatty acid contents. Larval condition was derived from two indices: feeding incidence and the aminoacyl-tRNA synthetases enzymatic activity. The working hypothesis was that in SGE, optimal feeding success and rapid somatic growth of fish larvae result from a combined supply of sufficient amounts of high quality food, e.g., prey of proper size and biochemical composition. Given that prey abundance is high during most of the reproductive season (Froneman, 2001; Murrel and Lores, 2004) larval nutritional condition is mainly driven by the biochemistry of prey. According to this hypothesis, larval abundance and condition in the SGE during the reproductive season should be higher in periods when prey quality is better in terms of fatty acids content.

2. Materials and methods

2.1. Study area

Within the subtropical south-west Atlantic Ocean along the Uruguayan coast in South America, several estuaries run into the Río de la Plata river (RdlP). These estuaries support recreational and artisanal-scale commercial fisheries, and earlier studies highlighted the importance of these ecosystems as nursery areas for valuable fish species such as sciaenids and sardines (Acuña et al., 2010; Machado et al., 2011; Gurdek et al., 2016). The present study was conducted in the Solís Grande Estuary (SGE; 34° 45′59.4″ S, 55° 24′29.8″ W; Fig. 1A), a 90 km long river with a mean annual flow of 14.5 m^3s^{-1} where brackish waters entering from RdlP reach approx. 10 km upstream (Gómez-Erache et al., 2000). Winds and runoff are the main forces in estuarine hydrodynamics while tides <40 cm have little influence (Nagy et al., 2002). Salinity in the lower SGE estuary varies between 2 and 30 according to winds and runoff, and temperature ranges between 10 and 25 °C depending on the season. Zooplankton community is dominated by copepods (*Acartia tonsa, Paracalanus* sp. and *Oithona* spp.) but chaetognaths (*Sagitta friderici*), mysids (*Neomysis americana*), cirriped nauplii and cypris larvae (*Balanus improvisus*) are also common (Gómez-Erache et al., 2000; Calliari et al., 2001). The ichthyofaunal community is represented typically by estuarine residents and marine-estuarine opportunist, (e.g. *Odontesthes* sp., *Platanichthys platana*, *Mugil liza* and *Micropogonias furnieri)* (Acuña et al., 2010; Gurdek et al., 2016).

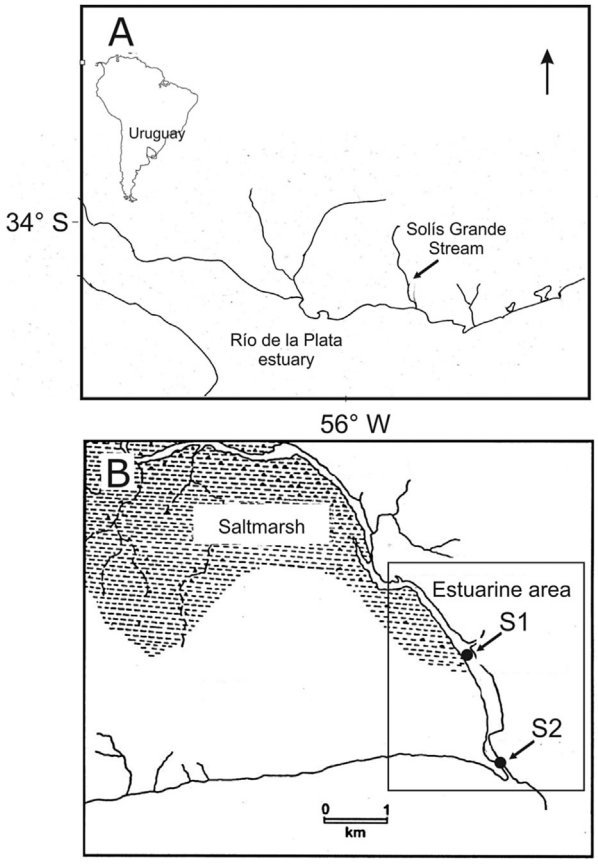

Fig. 1. Location of Solís Grande Estuary (black arrow) on the southeastern coast of South America (A). Sampling sites (S1 and S2) are indicated (B).

2.2. Field collections

Environmental variables and plankton were sampled during the main fish reproductive season (Acha and Macchi, 2000; Vizziano et al., 2002). Sampling was performed in three distinct periods between September 2009 and April 2010, corresponding to austral spring (September), summer (January) and autumn (March). Within each period, samples were taken on four consecutive occasions every second day at two stations (S1 and S2) located 1.5 km apart in the lower SGE (Fig. 1B). This sampling strategy was directed to capture intra-seasonal variability which may be important in this ecosystem (Gómez-Erache et al., 2000; Calliari et al., 2001). At each station, temperature (°C), dissolved oxygen (mg L^{-1}) and salinity were measured at surface and near the bottom (YSI Proplus), and PAR profiles (Li-Cor LI-250/2π collector) were registered to determine the light vertical diffuse attenuation coefficient K_d (m^{-1}) following Kirk (1994). Sampling site mean depth (±SD) was 2.0 ± 1.3 m during the whole study.

Ichthyoplankton was collected at each site during daylight hours using two approaches: subsurface sampling was performed with a 55 cm mouth diameter (ca. 0.24 m^2) conic plankton net, and near bottom waters were sampled using a 0.9 m^2 epibenthic sled specifically designed for plankton sampling in shallow systems (La Bolle et al., 1985). Each sampler was fitted with a 500 μm plankton mesh net and a flowmeter (General Oceanic®) to estimate larval abundance. Sampling was conducted during 3–5 min and sampling effort totalled 48 tows. For both gears, one sample was preserved in 4% buffered formaldehyde for identification and counting of fish larvae, and for gut content analyses. A second sample was collected for enzymatic nutritional condition analyses (AARS activity). For that, larvae were immediately picked and sorted over ice upon collection, quickly measured to the nearest 1 mm with an ocular micrometer, individually placed in 2 mL criovials and instant-frozen in liquid nitrogen. Vials were stored at −80 °C until analysed within one month.

Prey availability was estimated by: i) chlorophyll-a as a proxy of phytoplankton biomass, ii) microzooplankton abundance, iii) mesozooplankton abundance and HUFA content, and iv) HUFA content in particulate organic matter (POM) as a proxy of microzooplankton. Chlorophyll-a and microzooplankton were sampled with 5 L Hydrobios® bottles at subsurface and close to the bottom. A volume of water between 50 and 300 mL was filtered through GF/F filters, which were quickly frozen for later estimation of chlorophyll-a. Microzooplankton samples were concentrated on a 23 μm sieve and then rinsed into wide mouth flasks containing 4% buffered formaldehyde. Mesozooplankton was sampled in duplicate by oblique tows with a 40 cm mouth diameter plankton net fitted with a 117 μm mesh and a flowmeter (Hydrobios®). Tows lasted for 3 min. One replicate was preserved in 4% buffered formaldehyde for identification and counting. The second was immediately concentrated on a 100 μm sieve, placed in 10 mL cryovials and instant-frozen in liquid nitrogen for later fatty acids extraction and analyses. A volume of water between 0.5 and 1 L was filtered through pre combusted GF/F filters, placed in 2 mL cryovials and instant-frozen in liquid nitrogen for later fatty acids extraction and analyses of POM.

2.3. Laboratory procedures

In the laboratory, ichthyoplankton in formaldehyde preserved samples was sorted, taxonomically identified (e.g. Cassia and García de la Rosa, 1993; Bonecker and Castro, 2006) and counted, and abundance expressed as individuals 100 m^{-3}. Notochord length (NL), standard length (SL) and body depth (BD) of preserved larvae were measured to the nearest 0.1 mm using a stereoscopic microscope and classified into yolk sac, preflexion, flexion and postflexion stages. For gut content analysis, the entire digestive tract from each larva was dissected under high magnification microscope. Prey items in the gut were identified, counted and measured to the nearest 0.5 μm using an inverted microscope. Composition of the diet was summarized as frequency of occurrence (%FO) and percent in number (%N) of prey items in preflexion and postflexion larval stages (Hyslop, 1980). The product of these 2 factors yields an index of relative dietary importance referred to as IRI (%) (Pinkas et al., 1971). Selectivity for a given prey was estimated by comparing the frequency of a prey in the gut with its frequency in the plankton (in the collection day) using Chesson's selectivity index α (Chesson, 1978):

$$\alpha j = (d,/p,)(Cd,/p,), \text{ for } i = 1,\ldots, n$$

where n = number of prey items considered for a given fish species; d and p = frequencies of prey j in the diet and in the plankton respectively; d and p = the same frequencies for the ith prey. This index ranges from 0 to 1 and the threshold for a positive selectivity is 1/n.

Microzooplankton and mesozooplankton were identified (e.g. Balech, 1988; Boltovskoy, 1999; Foissner et al., 1999) and counted, and abundances expressed as individuals L^{-1} and individuals m^{-3}, respectively. For microzooplankton, aliquots corresponding to between 2 and 10 mL (depending on abundance) were allowed to settle in Utermöhl chambers and observed at magnification of 200–1000×. At least 100 individuals were counted in each sample. Mesozooplankton was identified to the highest possible taxonomic separation and counted under low magnification microscope. A number of individuals of each dominating group were measured (usually approx. 20 per taxon and sample) in order to estimate individual biomass as organic carbon using empirical allometric equations from the literature. For copepods (mostly *A. tonsa* and *Paracalanus* sp.) conversion equations were taken from Berggreen et al. (1988) and Davis (1984). For nauplii and copepod eggs, biomass was also calculated from individual measures using the conversion equation of the most abundant species in the corresponding sample.

Prey quality was determined using the percentage of essential fatty acid content in POM (as a proxy of microzooplankton, Tiselius et al., 2012) and in dominant mesozooplankton groups: copepods (selected species), cladocerans (selected species) and cirripeds nauplii. For fatty acids analyses in mesozooplankton, frozen sieves were allowed to thaw under room temperature and a number of individuals of target species were selected under a dissecting microscope. The number of individuals varied according to size of species, and ranged between 50 and 200 per replicate sample. Two to three replicates were taken for each selected species in all samples. Total lipids were extracted by homogenizing the samples in a mixture of chloroform:methanol:water (2:2:1 by volume) and stored at −20 °C (Folch et al., 1957). Lipid samples were transesterified with boron trifluoride (BF_3) and hexane, and heated for 15 min at 100 °C (Metcalfe and Schmitz, 1961). The fatty acid methyl esters (FAMEs) were analysed according to Chu and Ozkizilcik (1995) using gas/liquid chromatography and identified by comparing their retention times with known standards (Sigma, Supelco, Bellefonte, USA) and confirmed with GC/MS. The fatty acid C23:0 was used as an internal standard for quantification.

Larval condition and feeding success were assessed by two approaches: feeding incidence (%FI), defined as the percentage of larvae with prey in the guts (Pepin et al., 2015), and AARS activity following Yebra et al. (2006). For the AARS method, each larva was homogenized in 1 mL Tris–HCl buffer (pH 7.8) in an ice bath (0 °C). After centrifugation (10 min at 0 °C, 1000×*g*), 150 μl of the

supernatant was added to 50 μl of pyrophosphate reagent (PPi, Sigma P7275) in a 96-well microplate. The decrease in absorbance at 340 nm was followed for 10 min at 37 °C in a spectrophotometer microplate reader (Varioskan Flash, Thermo®). The release of PPi from the AARS-catalyzed reaction is coupled to NADH oxidation as detailed in Chang et al. (1984). The NADH oxidation rate measured at 340 nm (dA min^{-1}) was converted to PPi release rate according to Yebra et al. (2006) and adapted to our experimental conditions:

$$\text{nmol PPi } h^{-1}\ mL^{-1} = (\text{dA } min^{-1} * 10^3 * 60) * \text{Vrm} * 2.52 * 2)^{-1}$$

where Vrm is the volume of the reaction mixture in milliliters, 2.52 is the millimolar absorptivity of NADH at 340 nm in a flat bottom microplate (200 μl per well), and 2 is the number of moles of β-NADH oxidized per mole of PPi consumed.

The activity of the aminoacyl-tRNA synthetases was estimated as the rate of NADH oxidation (thus PPi release) indicated by the initial slope of the absorbance-time profile. Enzyme activity was normalized by protein concentration in the corresponding sample, and the specific aminoacyl-tRNA activity (AARS-sp, nmol PPi h^{-1} mg $prot^{-1}$) was taken as a proxy of growth. Protein concentration in the supernatant was determined according to the method of Bradford (1976) using bovine serum albumin as standard.

AARS-sp activity in our study represents the growth status for wild individuals under in situ conditions and since no experimental validations were performed for each species, our results are expressed as relative values. As a consequence, AARS-sp activity was applied to establish in which period larval condition was comparatively better or worse, but we did not establish a specific level of AARS as an indicator of high or low nutritional condition.

2.4. Data analyses

To evaluate an optimal period for larval survival, differences in environmental conditions, food availability and quality, larval abundance, feeding incidence and AARS-sp activity amongst periods were tested with ANOVA. Normality and homogeneity of variance were analysed with Shapiro-Wilk and Levene tests, respectively. If the assumptions were not met, a non-parametric Kruskal-Wallis test (K-W) was applied instead. Post-hoc Mann-Whitney test (M-W) was used to determine differences between periods. Abiotic and biological data from daily sampled stations were pooled and considered as replicates within each period. To evaluate effects of environmental conditions on larval abundance, feeding incidence and specific enzyme activity, Pearson correlations were applied as a first exploratory analysis. In order to further explore the effects of prey properties on AARS-sp activity, a Principal Component Analysis (PCA) was performed on log-transformed datasets: food quantity (eggs, nauplii and copepods abundance and biomass), food quality (essential fatty acids content in POM and copepods), larval abundances and specific enzyme activity.

3. Results

3.1. Physical environment

Seasonal variation was observed in most environmental properties measured (Table 1). Temperature varied from 14.0 ± 1.3 °C (mean ± SD) in spring to 24.1 ± 1.2 °C in summer (M-W; $p < 0.01$; $N = 49$). Salinity was highly variable within each period and no seasonal trends were found (range: 1.7–27.8; K-W, $p > 0.05$, $N = 50$). Oxygen levels indicated near saturation in spring and summer (9.8 ± 0.9 and 7.2 ± 1.8 mg L^{-1}, respectively) but lowest values were registered in autumn (6.7 ± 1.0; M-W, $p < 0.01$, $N = 49$).

Table 1
Environmental conditions during the study at Solís Grande estuary. Mean ± sd seasonal temperature (T, °C), salinity, dissolved oxygen (DO, mg L^{-1}), light extinction coefficient (Kd, m^{-1}) and total chlorophyll-a (Chl, mg m^{-3}). The number of averaged samples is in parenthesis.

	Spring	Summer	Autumn
T	14.0 ± 1.3 (18)	24.1 ± 1.2 (15)	22.3 ± 0.8 (16)
S	11.6 ± 8.6 (18)	12.3 ± 3.7 (16)	11.7 ± 3.9 (16)
DO	9.8 ± 0.9 (18)	7.2 ± 1.8 (15)	6.7 ± 1.0 (16)
K_d	0.38 ± 0.3 (7)	0.7 ± 0.2 (8)	0.7 ± 0.2 (8)
Chl	1.7 ± 1.5 (14)	6.1 ± 3.8 (16)	4.3 ± 3.4 (16)

Light extinction coefficient K_d did not change along the periods (range: 0.006–0.980 m^{-1}, ^{K}-W, $p > 0.01$, $N = 23$) while chlorophyll-a was lower in spring (1.7 ± 1.5) (K-W, $p < 0.05$, $N = 46$; M-W, $p < 0.05$, $N = 24$).

3.2. Ichthyoplankton composition, abundance, and prey selection

A total of 48 ichthyoplankton samples were collected during this study. Larval abundance (Fig. 2) ranged between 2.0 ± 0.9 (spring) and 11 ± 5.8 (autumn) ind 100 m^{-3} and seasonal differences were found between spring and autumn (highest in autumn, M-W; $p < 0.05$; $N = 46$). Thirteen species were identified and their occurrence and abundance exhibited seasonal changes. Dominant species were *Platanichthys platana* and *Brevoortia aurea* in spring,

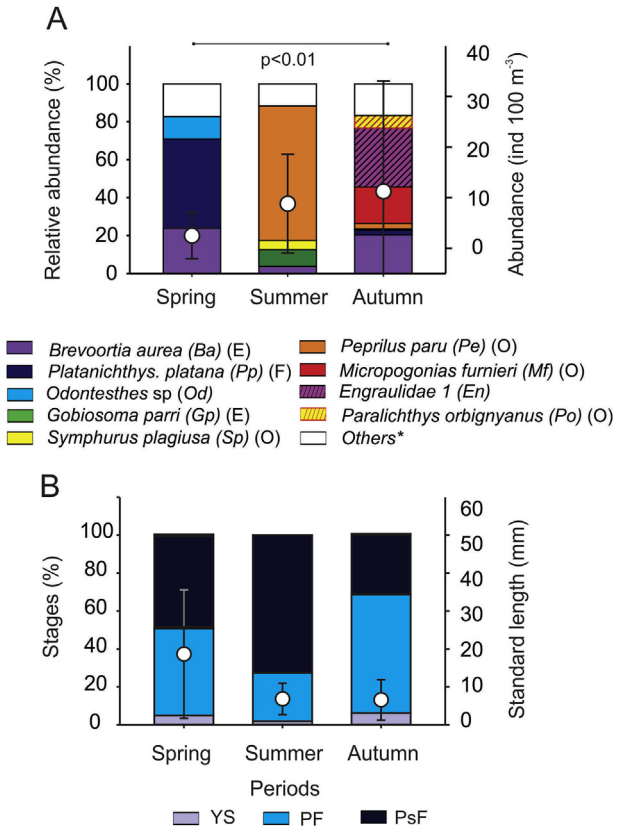

Fig. 2. Ichthyoplankton collected during the study at Solís Grande Estuary. Mean ± SD seasonal abundances and species composition (A). Acronyms and life cycle category (according to Potter et al., 2015) are in parenthesis. E: estuarine, F: freshwater, O: marine-estuarine opportunist. Others* included *Anchoa marinii, Lycengraulis grossidens, Hypleurochilus fissicornis, Jenynsia* sp. and *Elops smithi*. Larval developmental stages and mean ± SD standard length (B). YS: Yolk sac, PF: Preflexion, PsF: Postflexion larval stages. Horizontal bars: statistical differences in larval abundance, p: p-value.

Peprilus paru in summer and *Micropogonias furnieri* and non-identified engraulids (Engraulidae 1) in autumn. Freshwater species were more abundant in spring (47.2%, *P. platana*), marine-estuarine opportunist in summer (84.4%, *P. paru, Symphurus plagiusa, Hypleurochilus fissicornis, Lycengraulis grossidens*) and estuarine in autumn (39.5% *M. furnieri, B. aurea*). Average larval standard length ranged between 6.6 ± 5.3 (autumn) and 18.7 ± 17.0 mm (spring). In spring and autumn most larvae were in yolk sac and preflexion stages, whereas in summer most were in postflexion stage (Fig. 2).

Stomach contents of 142 fish larvae were examined, corresponding approx. 80% of total larvae collected belonging to the species *P. paru, Paralichthys orbignyanus, L. grossidens, S. plagiusa, Gobiosoma parri, M. furnieri, B. aurea,* and *P. platana*. Adult copepods and their developmental stages, and invertebrate eggs (presumably copepod eggs) constituted the bulk of the diet (Table 2). The number of prey in the guts ranged from 0 to 29. The relative importance index showed differences between periods. Preflexion larvae fed mainly on copepod nauplii, except in summer when adult copepods were more important. Postflexion larvae fed on adult copepods in all periods. Species-specific diet composition data are provided in the electronic supplement (Table A1, electronic appendix). Width of consumed prey ranged between 7.5 and 200 μm (Fig. 3A) and varied seasonally. In summer, larvae fed on a higher prey width (120 ± 44 μm) than in autumn (91 ± 25 μm), followed by spring (62 ± 33 μm) (M-W, $p < 0.001$, $N = 130$ and $p < 0.001$, $N = 95$, respectively). Morphometric relationships between body length and body depth with prey width followed different patterns: while larval BL did not correlate with prey size (Fig. 3B), BD was positively correlated (Pearson, $r = 0.65$, $p < 0.001$) (Fig. 3C). It was not possible to perform morphometric species-specific analyses due to the low number of individuals collected for single species.

Selectivity was assessed based on 17 identified potential prey items in the environment, with a selection threshold $1/n = 0.05$ (Table A1, electronic appendix). Despite high prey availability, larvae exhibited strong selection, and ontogenic and seasonal changes in prey selectivity were found. During the whole study, all postflexion stages fed on copepods adults or juveniles ($\alpha = 1$), mainly on *Acartia tonsa* and *Paracalanus* sp. During spring and autumn, preflexion larvae fed on copepod nauplii ($\alpha = 0.4$) and invertebrate eggs ($\alpha = 0.4$), but in summer they fed heavily on adult copepods ($\alpha = 1$) including small-sized *Euterpina acutifrons* and *Paracalanus* sp. (Table 2).

3.3. Prey environment

Average microzooplankton density at SGE was 9540 ± 3300 ind L^{-1} and seasonal differences were detected (Fig. 4A). Summer was characterised by higher densities (21225 ± 28733 ind L^{-1}) than spring (3220 ± 4110 ind L^{-1}) and autumn (5390 ± 5670 ind L^{-1}) (M-W, $p = 0.008$, $N = 20$ and $p = 0.03$, $N = 20$, respectively). Regarding taxonomic composition, dinoflagellates (e.g. *Ceratium* sp.) followed by ciliates (naked and with lorica) were most abundant. A cautionary note refers to the use of neutral formaldehyde as fixative for microzooplankton, which may work best for micro-crustaceans (i.e., copepod eggs and nauplii, small cladocerans, other) but may result in lower abundance estimates for taxa like ciliates (Gifford and Carson, 2000). Consequently, the total amount of ciliates could have been even higher than the figures here reported.

Mesozooplankton abundances ranged between 80 and 37300 ind m^{-3} and no statistical differences were found between periods (K-W, $p > 0.05$, $N = 24$) (Fig. 4B). Widely distributed estuarine mesozooplankton species were the most abundant, such as copepods *Acartia tonsa* and *Paracalanus parvus*. Moreover, cirripeds nauplii and cladocerans (*Evadne* sp. and *Podon* sp.) reached high

Table 2
Prey composition and Chesson alpha selectivity index of larvae collected at Solís Grande Estuary sorted by developmental stage and season. Diet composition is expressed as percent frequency of occurrence (% FO) in larval guts, percent of the total number (% N) of items in the diet, and the index of relative dietary importance % FO x % N. The Chesson alpha index (α) is given for prey categories with $\alpha > 1/n$. Positive selectivity prey ($\alpha > 0.05$) are in bold. N: number of larvae examined. -: not applicable.

Prey items	Spring					Summer					Autumn				
	%FO	%N	%IRI	α	N	%FO	%N	%IRI	α	N	%FO	%N	%IRI	α	N
Preflexion					14					15					20
Euterpina acutifrons						13	148	5	-						
Acartia tonsa						27	296	10	-		5	3	0.5	-	
Paracalanus sp.						33	833	29	-		5	3	0.5	–	
Copepods	21.4	9.1	3.2	**0.13**		80	81	96	**1.00**		40	37	38.9	0.02	
Copepod nauplii	78.6	69.7	90.4	**0.42**		7	19	1	<0.01		40	57	67.0	<0.01	
Cirriped nauplii						7	19	1	<0.01						
Dinoflagellates						7	19	1	<0.01						
Tintinnids	14.3	9.1	2.1	0.02							5	3	0.5	<0.01	
Invertebrate eggs	21.4	12.1	4.3	**0.43**		20	222	8	<0.01		5	3	0.5	**0.98**	
Ostracods															
Mysids															
Polychaetes															
Forams															
Postflexion					6					26					11
Euterpina acutifrons						4.0	2.0	0.0	-		9.0	3.0	1.0	–	
Acartia tonsa	67.0	70.0	71.8	–		19.0	19.0	7.0	-		27.0	26.0	25.7	–	
Paracalanus sp.	17.0	10.0	2.6	–		27.0	13.0	7.0	-						
Copepods	83.0	100	100	**1.00**		81.0	100	100	**1.00**		64.0	79.0	95.5	**1.00**	
Copepod nauplii															
Cirriped nauplii															
Dinoflagellates															
Tintinnids															
Invertebrate eggs															
Ostracods											18.0	6.0	3.8	–	
Mysids											9.0	12.0	3.8	–	
Polychaetes											9.0	3.0	1.0	–	
Forams															

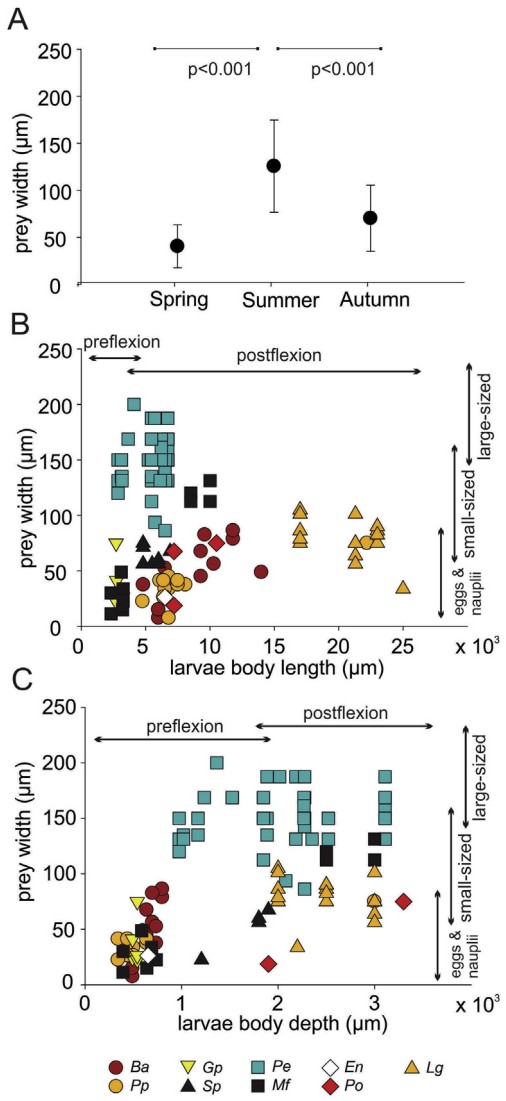

Fig. 3. Ichthyoplankton and prey size. Changes in prey width during the season (A). Prey width and larvae body length sorted by developmental stage (B). Prey width and larvae body depth sorted by developmental stage (C). Lg: *Lycengraulis grossidens*. See species acronyms in Fig. 2.

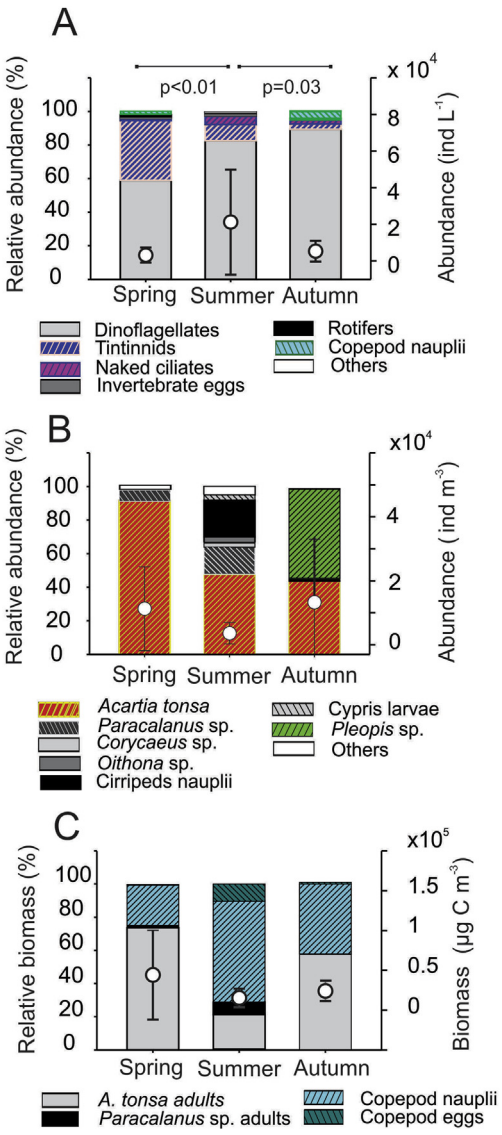

Fig. 4. Zooplankton collected during the study at Solís Grande Estuary. Mean ± SD and relative microzooplankton abundances (A). Others included amoeboids and loricated ciliates. Mean ± SD and relative mesozooplankton abundances (B). Others included *Euterpina acutifrons*, *Oncaea* sp., *Temora* sp., and ostracods. Mean ± SD and relative copepod biomass (C). Horizontal bars: statistical differences in abundances, p: p-value.

abundances in summer and autumn, respectively.

No seasonal differences in copepod biomass were found (all stages and species considered; K-W, $p > 0.05$, $N = 24$) (Fig. 4C). However, the biomass contributed by smaller items (eggs and nauplii) was higher in summer and autumn than spring (2150 ± 1682 µg C m^{-3}) (M-W, $p < 0.05$, $N = 20$). In turn, biomass of juveniles and adults was higher in spring and autumn than in summer (M-W, $p < 0.05$, $N = 16$, in both cases). *A. tonsa* was the single species that contributed the most to copepod biomass.

The fatty acids composition of prey varied seasonally, among size fractions and/or taxa considered. The %HUFA in POM was higher in summer (19.2 ± 12.1) than in autumn (5.8 ± 3.4) and spring (3.4 ± 2.3) (M-W, $p < 0.05$ in all cases) (Table 3). Regarding specific HUFA, %ARA was highest in summer (0.3 ± 0.5) (M-W, $p < 0.05$, $N = 15$) and DHA/EPA ratio was higher in summer (24.4 ± 58.3) and lower in autumn (0.7 ± 0.2) (M-W, $p < 0.004$, $N = 15$). For the mesozooplankton, the fatty acid content in *A. tonsa* was analysed only in summer and autumn. The %HUFA was higher in autumn (14.4 ± 5.4) than in summer (10.0 ± 2.3) (M-W, $p = 0.02$, $N = 13$) (Table 3). %ARA was higher in summer (0.6 ± 0.6) than in autumn (<0.01) M-W, $p = 0.004$, $N = 13$ but no significant differences in DHA/EPA were found between both periods (2.0–6.8) (M-W, $p > 0.05$, $N = 13$). Only two samples could be analysed for cirripeds nauplii (corresponding to summer) and for cladocerans (summer and autumn) due to the low densities found in the frozen samples, and consequently no statistical analyses were carried out for those taxa. Cirripeds nauplii had low %HUFA (7.6 ± 3.3), low % ARA (0.3 ± 0.2) and similar amounts of DHA/EPA (0.9 ± 0.6) (Table 3). In cladocerans, the %HUFA was similar in both periods (14.6 and 13.3), %ARA was <0.01, and DHA/EPA ratio evidenced high EPA content in summer (0.01) but more even values in autumn (0.7) (Table 3).

3.4. Larval condition

Feeding incidence for all fish larvae analysed ($N = 142$) ranged

Table 3
Fatty acids composition (%) during the study at Solís Grande Estuary. Mean ± sd particulate organic matter (POM) and zooplankton fatty acids. SFA: Saturated fatty acids, MFA monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, HUFA: highly unsaturated fatty acids. ARA: arachidonic (20:4ω-6), DHA: docosahexaenoic acid (22:6ω-3), EPA: eicosapentaenoic acid (20:5ω-3). The total number of averaged samples is in parenthesis.

		Spring	Summer	Autumn
POM (22)	Σ SFA	78.0 ± 9.5	66.9 ± 12.2	80.1 ± 4.9
	Σ MUFA	14.2 ± 4.2	11.1 ± 1.8	11.6 ± 1.5
	Σ PUFA	4.5 ± 3.5	3.0 ± 1.1	2.7 ± 0.7
	Σ HUFA	3.4 ± 2.3	19.2 ± 12.1	5.8 ± 3.4
	ARA	0.0 ± 0.0	0.3 ± 0.5	0.0 ± 0.1
	EPA	1.1 ± 0.4	4.6 ± 4.2	3.0 ± 1.9
	DHA	1.3 ± 1.2	12.0 ± 8.8	2.2 ± 1.4
	DHA/EPA	1.1 ± 0.5	24.4 ± 58.3	0.7 ± 0.2
Acartia tonsa (11)	Σ SFA		78.9 ± 7.4	70.0 ± 7.0
	Σ MUFA		6.7 ± 2.9	11.3 ± 7.3
	Σ PUFA		3.9 ± 3.0	3.9 ± 1.9
	Σ HUFA		10.0 ± 2.3	14.4 ± 5.4
	ARA		0.6 ± 0.6	0.0 ± 0.0
	EPA		2.2 ± 0.7	4.2 ± 2.1
	DHA		6.6 ± 2.1	8.7 ± 3.8
	DHA/EPA		6.8 ± 8.7	1.9 ± 0.6
Paracalanus sp. (1)	Σ SFA		88.0 ± 2.8	
	Σ MUFA		4.6 ± 1.6	
	Σ PUFA		1.0 ± 0.0	
	Σ HUFA		6.0 ± 4.5	
	ARA		1.9 ± 0.4	
	EPA		1.2 ± 1.1	
	DHA		2.7 ± 3.8	
	DHA/EPA		1.3	
Cirripeds nauplii (2)	Σ SFA		73.0 ± 5.5	
	Σ MUFA		15.2 ± 1.2	
	Σ PUFA		3.5 ± 1.2	
	Σ HUFA		7.6 ± 3.3	
	ARA		0.3 ± 0.2	
	EPA		4.0 ± 2.3	
	DHA		3.2 ± 2.0	
	DHA/EPA		0.9 ± 0.6	
Evadne sp. (1)	Σ SFA		38.7	
	Σ MUFA		29.4	
	Σ PUFA		17.0	
	Σ HUFA		14.6	
	ARA		0.0	
	EPA		14.0	
	DHA		0.2	
	DHA/EPA		0.01	
Podon sp. (1)	Σ SFA			74.8 ± 1.8
	Σ MUFA			8.3 ± 0.1
	Σ PUFA			3.3 ± 1.2
	Σ HUFA			13.2 ± 3.2
	ARA			0.0 ± 0.0
	EPA			6.8 ± 2.4
	DHA			4.3 ± 0.5
	DHA/EPA			0.7 ± 0.2

Table 4
Larval nutritional condition at Solís Grande Estuary. Mean ± sd larval feeding incidence (%) sorted by developmental stage. Mean ± sd size-corrected aminoacyl tRNA synthetases specific activity (AARS-sc) sorted by species. The number of averaged individuals is in parenthesis.

	Spring	Summer	Autumn
Feeding incidence			
Total larvae	83.3 (24)	73.2 (56)	45.2 (62)
Preflexion	77.8 (18)	65.2 (23)	23.4 (47)
Postflexion	100 (6)	78.8 (33)	73.3 (15)
AARS-sc activity			
Engraulidae 1	2.1 (1)		0.3 ± 0.1 (2)
Platanichthys platana	1.8 ± 1.8 (10)		0.3 (1)
Odontesthes sp.	3.2 ± 0.5 (2)		
Brevoortia aurea		0.3 ± 0.3	
Micropogonias furnieri		1.7 (1)	
Peprilus paru		2.5 ± 3.1 (16)	1.8 (1)
Hypleurochilus fissicornis			5.6 (1)
Paralichthys orbignyanus			1.0 ± 1.2 (4)

between 23.4 and 100.0% and exhibited seasonal variability (Table 4). In spring, FI was higher (83.3%) than in autumn (45.2%) (M-W, $p = 0.03$, $N = 7$, Table 4) in spite of contrasting eggs and nauplii availability during those periods. Preflexion larvae exhibited highest FI in spring, while postflexion larvae showed high FI during the whole study (73.3–100%).

Specific enzyme activity was assessed individually for 42 larvae between 3.1 and 52.0 mm body length, most in postflexion stage (86%). Larvae analysed belonged to the following species: *Platanichthys platana*, *Brevoortia aurea*, *Odontesthes* sp., *Peprilus paru, Micropogonias furnieri, Paralichthys orbignyanus, Hypleurochilus fissicornis* and Engraulidae 1. Protein content per individual ranged between 0.02 and 9.64 mg and showed a positive correlation with larval length (Spearman, $r = 0.51$; $p < 0.001$, $N = 42$) (Fig. 5A). Larval length and the natural logarithm of specific enzyme activity showed a negative correlation (Pearson, $r = -0.35$; $p = 0.02$, $N = 42$) (Fig. 5B), an expected relationship according to the metabolic rate theory (Gillooly et al., 2001). Therefore, the effect of larval

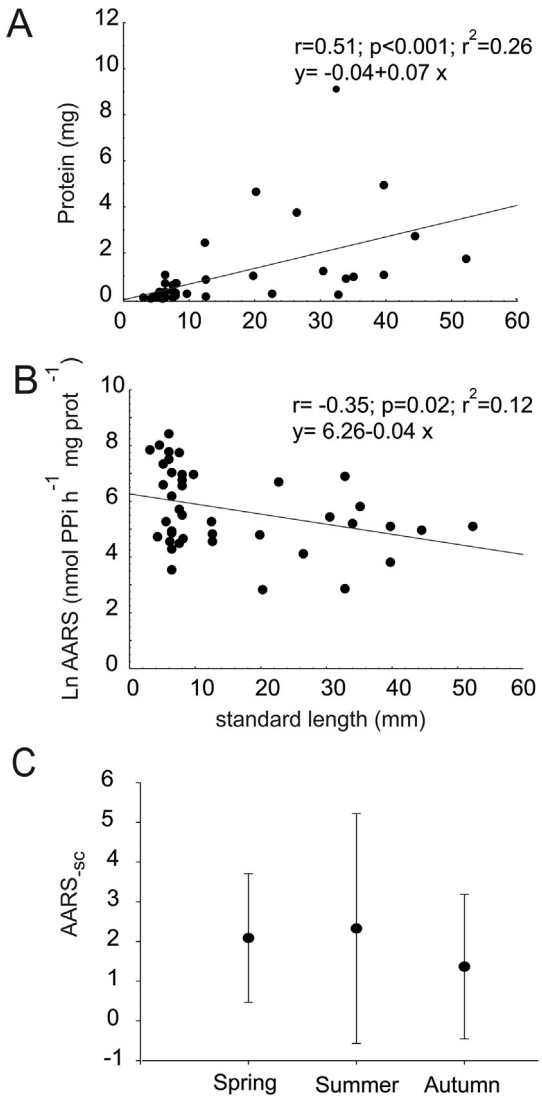

Fig. 5. Protein content and nutritional condition based on specific enzymatic activity in fish larvae. Total body protein vs. larval body length (A). Aminoacyl t-RNA synthetases specific activity (AARS) vs. larval body length (B). Mean ± SD size-corrected aminoacyl t-RNA synthetases specific activity (AARS-sc) for each period (C).

length was removed from the data by using the residuals of the linear regression in all further analyses (Gillanders and Kingsford, 2003; Vasconcelos et al., 2009) and was expressed as size-corrected AARS (AARS-sc, dimensionless). Although AARS-sc was slightly higher in summer, no statistical differences were found among periods (K-W, $p = 0.4$, $N = 42$) (Fig. 5C, Table 4).

3.5. Larval abundance and condition, suitable food and physical environment

Larval abundance showed a positive correlation with chlorophyll-a (Pearson; $r = 0.48$; $p < 0.01$, $N = 43$) and temperature (Pearson, $r = 0.32$; $p = 0.02$, $N = 46$) whereas no correlation was found between condition indices (FI and AARS-sc) and food quantity (copepod biomass) or quality (% fatty acids) (FI: Pearson, $r < 0.5$; $p > 0.05$, $N = 18$, AARS-sc: Pearson; $r < 0.3$; $p > 0.05$, $N = 41$). The first two components of the PCA explained 73.7% of total variation in the data matrix (Fig. 6). In the first axis (variance explained = 52%) AARS-sc correlated positively with %HUFA

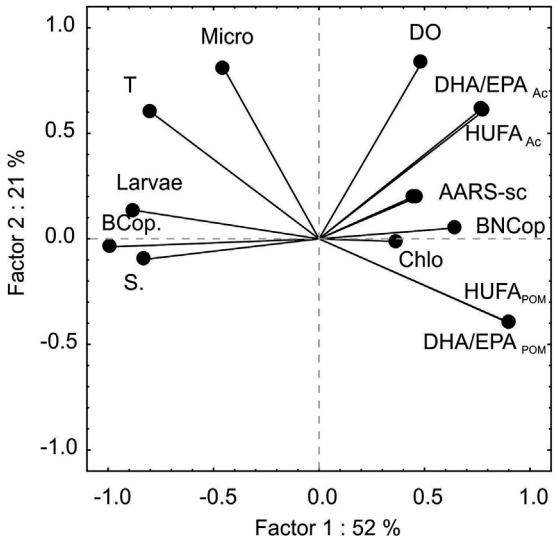

Fig. 6. Principal Component Analysis (PCA). Abiotic and biotic variables were included. S: salinity, DO: dissolved oxygen, T: temperature, Chlo: chlorophyll-a, BCop: adult copepods biomass, BNCop: copepod nauplii biomass, Micro: microzooplankton abundances, DHA/EPA_{AC}: *Acartia tonsa* DHA/EPA, $HUFA_{AC}$: *Acartia tonsa* %HUFA, $HUFA_{POM}$: Particulate organic matter %HUFA, DHA/EPA_{POM}: Particulate organic matter DHA/EPA, larvae: larval abundances, AARS-sc: size-corrected aminoacyl t-RNA synthetases specific activity.

(r = 0.90) and DHA/EPA in POM (r = 0.90) and copepods nauplii biomass (r = 0.65). In contrast, specific enzyme activity was negatively correlated with copepod biomass (r = −0.99), fish larval abundance (r = −0.88) and salinity (r = −0.82). In turn, AARS-sc had low loading factor on the second axis (variance explained = 21%).

4. Discussion

This study evaluated the nutritional condition and abundance of a multi-specific natural assemblage of fish larvae from the subtropical Solís Grande Estuary in relation with food suitability in terms of abundance, size and fatty acids composition during the reproductive season. Larvae from several species and different estuarine functional groups alternated their occurrence and dominance along the season and overall abundance exhibited a positive correlation with temperature and chlorophyll-a. Despite fluctuations in the abundance of micro and mesozooplankton, prey availability was high throughout the season. Also, major variability was observed not only in the size and taxonomic structure of the prey assemblage, but also in its biochemical quality in terms of fatty acids composition. Contrary to expectations derived from the working hypothesis there was not a given period where an increase in larval condition clearly matched an increase in prey quality. On the contrary, results suggest that the temporal succession of complementary factors - temperature and abundance of prey of proper size and quality - varying at different time scales along the reproductive season might provide a wide window of opportunity for larvae, where quality seems to balance quantity (Fig. 7). This situation resulted in successful feeding along the season, and in specific growth indices that did not differ among species and/or periods.

4.1. Temporal variability in environmental properties and food availability

Environmental variables - temperature, salinity, dissolved oxygen and chlorophyll-a were in accordance with ranges observed

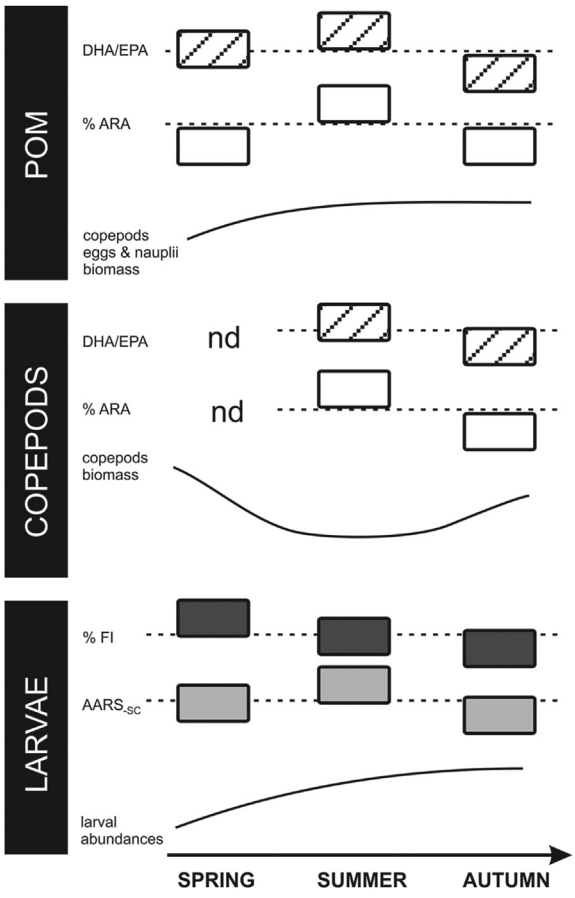

Fig. 7. Summary scheme. Relative differences in prey quality (DHA/EPA and %ARA) and availability (biomass), larval abundances and nutritional condition (%FI and AARS-sc) among periods. %ARA: arachidonic acid (20:4ω-6), DHA/EPA: docosahexaenoic acid (22:6ω-3) and eicosapentaenoic acid (20:5ω-3) ratio. %FI: feeding incidence, AARS-sc: aminoacyl t-RNA synthetases activity, nd: no data available.

previously at SGE (Gómez-Erache et al., 2000; Calliari et al., 2001; Giménez et al., 2014) and in other South American subtropical estuaries (Conde et al., 1999; Bonilla et al., 2005; Calliari et al., 2009; Acuña et al., 2010). These are suitable conditions for spawning and larval development of several fish species that inhabit subtropical areas within the South West Atlantic like white-mouth croaker *M. furnieri*, menhaden *B. aurea*, and silverside *Odonthestes* sp. (Phonlor and Cousin, 1997; Acha and Macchi, 2000; Vizziano et al., 2002; Berasategui et al., 2004). Both micro and mesozooplankton evidenced fluctuations in their taxonomic composition and abundance during the sampled periods. For instance, dominance of adults of calanoid *A. tonsa* in early spring was followed in summer by a numerical increase of invertebrate eggs, nauplii and small copepods; in turn, cladocerans dominated in autumn. Copepod biomass showed seasonal differences between stages and taxa but interestingly, such variability was not mirrored by feeding selection by fish larvae (see below). Furthermore, the estuarine nature of SGE allowed the alternation of marine, estuarine and freshwater zooplankton of proper size as prey for fish larvae, which were present at high concentrations similar to other estuarine ecosystems (Eskinazi-Sant'anna and Bjonberg, 2006; Calliari et al., 2009). Fluctuations in salinity allowed for the occurrence of species with different optimal ranges. For instance, *A. tonsa* is a common estuarine species with high biomass and abundances at intermediate salinities (Calliari et al., 2006), while *Paracalanus* sp. is a marine species with high abundance, biomass and production rates at salinities over 30 (Uye and Shibuno, 1992).

The HUFA content of microzooplankton (based on POM) and mesozooplankton was on average 9.5 and by 12.7% of total fatty acids, respectively. These values are similar to other subtropical estuaries also used as fish nurseries (Veloza, 2005). At SGE, fatty acids and zooplankton abundances varied seasonally and among groups. For instance, POM exhibited highest %HUFA and DHA in summer, coinciding with an increase in the abundance of heterotrophic dinoflagellates within the microzooplankton. *Acartia tonsa* presented higher HUFA and DHA in autumn than in summer, and may have represented a proper food option for larvae during that period. Also, copepods exhibited higher percentages of HUFA and DHA/EPA than cirriped nauplii and cladocerans. Inter-specific differences in fatty acids contents among those taxa have been reported previously (Kainz et al., 2004; Gonçalves et al., 2012; Tiselius et al., 2012; Leu et al., 2013) and attributed to phylogenetic differences (Person and Vrede, 2006). Copepods were positively selected most of the time and specifically *A. tonsa* was actively consumed, suggesting that SGE represents a high quality nursery for fish larvae.

4.2. Prey selectivity, larval abundance and condition

Larval abundance increased from spring through autumn. Detailed analysis of abundance, size and stage structure of the larval assemblage (Fig. 2) suggests that the few larvae present in spring represented a heterogeneous mixture of newly produced individuals (i.e., in yolk-sac stage) and older larvae likely born before the current reproductive season. During summer and autumn average larval size was much smaller (average size decreased from nearly 20 to about 6 mm) and homogeneous. But it was during autumn when highest abundance matched small size and dominance of pre-flexion stages. Such evidence suggests that the main larval pulse occurred near the end of the reproductive season.

Despite the potentially wide range of food items in the SGE estuary, fish larvae selected copepods, in accordance with studies in other ecosystems including estuaries of subtropical and temperate latitudes (Pepin and Penney, 2000; Rodríguez-Graña et al., 2005; Robert et al., 2011; Llopiz, 2013; Temperoni and Viñas, 2013). Selectivity upon copepods was related with two main factors. Firstly, copepods and their development stages were found in all periods, frequently in very high abundances. Ontogenic changes in food selection is a well known fact in fish larvae (e.g. Pepin and Penney, 1997; Sabatés and Saiz, 2000; González-Queiroz and Anadón, 2001; Robert et al., 2011), and during the study, preflexion larvae fed on smaller preys as copepods eggs and nauplii, while postflexion larvae tended to switch to adults of small-sized copepod species (e.g. *Paracalanus* sp., *Euterpina acutifrons*) or large-sized species (*A. tonsa*). That switch was more evident for *P. paru*, *M. furnieri*, *B. aurea* and *P. orbignyanus* probably due to deep changes in their morphology during early development (Cassia and García de la Rosa, 1993; Bonecker and Castro, 2006). Secondly, as mentioned above, copepods had higher DHA/EPA in comparison to other coexisting zooplankton groups. Previous studies have shown that many fish larvae species improve their growth and survival by feeding on prey with higher DHA/EPA content (Salhi et al., 1997; Bessonart et al., 1999; Cutts et al., 2006). Therefore, positive selectivity on copepods by fish larvae could arise from a preference for food of relatively high nutritional quality. It is also important to highlight that in summer, preflexion larvae of *G. parri, S. plagiusa* and *P. paru,* consumed small adult copepods such as *Paracalanus* sp. and *E. acutifrons* despite their low abundances with respect to higher abundances of copepods eggs and nauplii (see Table A1 in the electronic appendix). Copepods may increase their fatty acids

content along their life-span (Evjemo et al., 2003; Kattner and Hagen, 2009), meaning that adults could represent better prey in terms of quality than early stages. A positive selection towards adults could thus explain why in summer larvae preferred these prey in contrast to more abundant items of proper size and easier to capture like eggs and nauplii.

Larval assemblage exhibited an average trophic incidence >70%, reflecting high feeding success, particularly in spring and summer. Feeding failure was found in recently hatched larvae (e.g. *G. parri* and *Engraulidae* 1). For some species, such as *M. furnieri*, results agree with values estimated previously for other estuaries in the same region (Vera, 2011); for other species, FI were higher even for those taxa with a tendency to regurgitation during sampling as clupeiforms (e.g. *B. aurea, P. platana;* Vera, 2011; Llopiz, 2013). In the case of *P. platana, G. parri* and *P. paru,* FI here reported represent the first records. Highest FI - considering also preflexion larvae - occurred in spring, coinciding with highest abundances of calanoid copepods. Altogether, FI suggested that despite strong changes in relative and absolute abundance of prey items, larvae at SGE were actively feeding and there was no indication of food limitation at any time during the reproductive season. These results partially agree with condition derived from the enzymatic index. During the present study it was possible to estimate nutritional condition based on the AARS method in a wild larval fish assemblage. Current results cannot be directly compared with those reported by Herrera (2014) though, since that study was performed on a single species (*Clupea harengus)* under laboratory conditions. Furthermore, Herrera (2014) did not apply body size correction for AARS activity. Current results showed no seasonal trends in larval condition based on the enzymatic index. Instead, AARS-sc activity exhibited wide variability along the study season. Lack of differences among periods may arise from a systematic high feeding success and the combination of quantity, quality and diversity of prey over the whole spawning season. At SGE, different descriptors of the food environment that drive growth (prey quantity, diversity and biochemical composition) varied asynchronously. For example, in summer lower copepod biomass co-occurred with a wider size spectrum, higher taxonomic richness and higher DHA content in *A. tonsa.* Recently, Paulsen et al. (2014a, 2014b) showed the importance of food quality in the natural environment, particularly based on DHA, to enhance nutritional condition and growth in Atlantic herring larvae during periods of low food.

In the present study, high intra-seasonal variability in specific AARS-sc activity may have arisen, at least in part, from the joint consideration of different larval stages and species in order to obtain estimations at the assemblage level. However, average AARS-sc levels were rather similar for the three periods, suggesting that the lack of significant differences was due to an actual pattern of similar instantaneous growth rates among periods rather than to high variability promoted by the pooling of species. Also, it is of note that current AARS-sc results indicate the existence of important variability among individuals of the same species, which contributed to variability within periods. A clear case was the larvae of *P. paru* collected in summer, which showed very different AARS-sc levels in some cases. That observation, derived from the application of a novel strategy to infer short-term growth and condition in fish larvae, is in line with earlier observations derived from otolith microstructure analyses (Pepin, 2004; Pepin et al., 2015).

Larvae in poor feeding environments develop more variable daily growth rates than those in richer food conditions (Pepin, 2004). However, Pepin et al. (2015) showed that fast growing individuals during the larval period seem to be those characterised by more variable daily growth rates, and presumably also more variable feeding success. Chance encounters of small-scale patches of high food density would be an ultimate driver that allows the emergence of individuals with exceptionally high growth rates. Hence, strong variability in instantaneous growth rates amongst larvae within an assemblage may well be the expected scenario.

The relationship between growth indices and lipids may be not straight-forward. Recently, Peters et al. (2015) reported a negative relationship between growth and condition indices measured in fish larvae as RNA:DNA ratio and total lipids, respectively. In early larvae lipids are mostly inherited (as yolk), used to fuel growth during the first days, and tend to diminish during development. In young larvae, the strong pressure to grow as fast as possible implies that energetic gains via feeding are mostly derived into growth, rather than into accumulation of new reserves. In that sense, growth and lipid accumulation can be seen as competing processes within the same organism. Instead, in the present study a positive relationship was found between larval condition (AARS-sc) and fatty acids, but a fundamental difference compared to Peters et al. (2015) is that here fatty acids were measured in the prey assemblage, as indicators of food quality. The positive association likely reflects faster growth under better food conditions. A temporal succession of complementary factors along the reproductive season promoting similarly favourable growth conditions could imply that larvae hatched at different periods may have similar chances to recruit. In subtropical and tropical regions, factors that control the extension of spawning period are partially relaxed (Bye, 2000; Sumpter, 1990; Fromentin and Fonteneau, 2001; Wootton and Smith, 2015), opposite to strongly seasonal high latitude ecosystems where productive cycles restrict reproduction to one narrow period each year. In this subtropical region, most coastal species are batch spawners, i.e. they have several spawning events along a reproductive season that extends over more than six months (Acha and Macchi, 2000; Acuña et al., 2000; Vizziano et al., 2002; Macchi et al., 2003). Multiple spawning events work as a 'bet-hedging strategy' (Ripa et al., 2010) and fits well within a scenario where environmental trophic conditions are similarly favourable during a long period, as suggested here.

5. Conclusions

Here we highlight the role of copepods in fish larval nutrition in natural environments: they were strongly selected and presented high biochemical quality in comparison with other co-occurring prey groups. Selected species included *A. tonsa* but also other small-sized species such as *Paracalanus* sp., and *Euterpina* sp. Seasonal shifts in plankton community, as well as estuarine hydrodynamics (e.g. salinity variability) favoured the occurrence of a variety of prey sizes and species. The abundance, size and stage structure of the larval assemblage suggested that even if the reproductive season was rather extended, the main larval pulse occurred at the end of the season. No optimal period was identified for larval growth in SGE estuary within the reproductive season in relation to quantity and quality of food, but a compensation of both during the season. Such compensation could enhance the possibility of larval survival along the extended spawning period. These findings underline the importance of a better understanding of the functioning of subtropical estuaries as nursery areas important for fish and planktivorous organisms such as crustaceans and other marine invertebrates.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2016.12.021.

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