



Evaluating environmental forcing on nutritional condition of *Engraulis anchoita* larvae in a productive area of the Southwestern Atlantic Ocean

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ABSTRACT

The main goal of this study was to examine the nutritional condition of *Engraulis anchoita* larvae during the austral spring-summer seasons of 2010/2011 and 2012/2013 in highly productive waters in the southwestern Atlantic Ocean called El Rincón. RNA/DNA (RDs) index and derived index of Growth performance (Gpf) were used. Significant differences were observed in average RDs indices for 2010/2011 (5.27 ± 3.26) and 2012/2013 (0.81 ± 0.44). A difference in the years was also observed in Growth performance with higher values in 2010/2011 (2.45 ± 1.83) compared to 2012/2013 (-0.03 ± 0.25). Anchovy larvae captured in 2010/2011 were in good condition ($Gpf > 1$). During 2012/2013, extremely high larval densities were observed although Gpf values were very low indicating poor larval condition. Chlorophyll dynamics differed between sampled periods, with higher maximum values during spring 2012 as compared to 2010. Concerning chlorophyll concentrations showed a sharp decline in the values for the rest of the 2012/2013 season. We propose that the low larval growth and condition observed during 2012/2013 may have resulted from a decrease in chlorophyll concentrations together with very large larval densities which enhanced the competition for food within the larval population. Our results reinforce the idea that satellite ocean color products can be valuable tools for understanding variability in ecosystem dynamics and its effects on reproductive success in fish.

1. Introduction

The Argentine anchovy, *Engraulis anchoita* (Hubbs and Marini, 1935) is the most important pelagic fishery of the Southwest Atlantic Ocean in terms of biomass (Pájaro et al., 2009). There is wide interest in the study of this species due to its key role in the pelagic ecosystem, acting as the link between zooplankton and numerous species of commercial importance (Hansen, 2004; Sánchez and Ciechomski, 1995). *E. anchoita* spawns between 34 and 48°S, where two populations are distinguished, a northern and a southern one separated at approximately 41°S (Hansen, 2004).

This study focuses on the northern population, which finds suitable reproductive conditions in the area mainly due to the stability of the water column and high food availability (Sánchez and Ciechomski,

1995). Adults are found mostly in coastal waters (shallower than 50 m) where massive spawning occurs during the austral spring (Pájaro, 1998; Sánchez and Ciechomski, 1995). Anchovy eggs and larvae are present throughout the year with a main peak of abundance in the austral spring and summer (Sánchez, 1995). The patterns of abundance and spatial distribution of early developmental stages of this species are influenced by the oceanographic conditions (Brandhorst and Castello, 1971; Pájaro et al., 2011). Maximum larval densities are usually observed in proximity of the 50 m isobath within the area of “El Rincón” (Pájaro et al., 2008). According to Bakun’s fundamental triad statement (Bakun, 1996), this area exhibits adequate conditions for an increase in larval survival and recruitment, taking into consideration retention processes, water column stability and trophic enrichment that characterize this area (Auaud and Martos, 2012; Pájaro et al., 2008; Viñas

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et al., 2013).

The large fluctuations often observed in pelagic fish biomass have been widely studied in fisheries biology and it is well known that diverse factors can affect the abundance of fish stocks. Several hypotheses have tried to explain mechanisms driving year-class strength during the early life of marine fish. The “match–mismatch” hypothesis (Cushing, 1990), an extension of Hjort’s “critical period” hypothesis, proposed that the temporal overlap between the spring plankton bloom and larval production is critical for larval survival and subsequent recruitment. It is widely accepted that main causes of larval mortality are predation and starvation (Bailey and Houde, 1989), and although predation mortality is difficult to quantify, starvation mortality can be estimated from the assessment of the nutritional condition of the larvae. This approach allows the evaluation of the individual’s physiological state, which in turn is a reflection of the environmental conditions in which the larvae were found. There are several indices for the assessment of the nutritional condition in fish larvae, with the RNA/DNA index (RD) being the most widely used (Chícharo and Chícharo, 2008). This method is based on the determination of the concentrations of nucleic acids and allows inferring functions related to tissue formation and the physiological condition of individuals (Ferron and Leggett, 1994). Since nucleic acids contents differ between larval tissues, reporting the analyzed tissue to calculate RD becomes crucial (Olivar et al., 2009). In the present study trunk muscle tissues were employed.

Here we propose the existence of a link between bloom progression and anchovy larvae nutritional condition. RNA/DNA index was employed as an indicator of the nutritional condition of anchovy larvae captured in the area of El Rincón during the austral spring-summer seasons of 2010/2011 and 2012/2013. Surface chlorophyll concentrations were examined as a predictor of food availability for larval *E. anchoita*. RNA/DNA index and derived growth indices were thus expected to be positively related to food availability in this nursery area. In addition, the abundance and distribution of eggs and larvae was examined in relation to the main oceanographic features of the study area.

2. Materials and methods

2.1. Study area

El Rincón (between 39 and 41°S, depth < 40 m) is a frontal area where fresh waters from the Negro and Colorado rivers meet high salinity waters of the San Matías Gulf advected from the south. Advection of continental shelf waters characterized by lower temperatures and intermediate salinities is also observed (Guerrero and Piola, 1997; Martos et al., 2004). The interaction between river discharge, high salinity waters from San Matías Gulf, and mid-shelf waters generate two adjacent frontal systems: a more coastal one where dilute waters meet saltier coastal waters and another system where high salinity waters meet mid-shelf waters (Pájaro et al., 2011). The second frontal system known as the Mid Shelf Front (MSF), is established during the austral spring (21 September–20 December) and summer (21 December–20 March) close to the 50 m isobath along the shelf of the Buenos Aires province separating vertically homogeneous coastal waters from stratified mid-shelf waters (Romero et al., 2006). El Rincón represents a favorable area for early development and survival of fish larvae. High nutrient concentrations that result in high phytoplankton and zooplankton concentrations serve as the main food for many species of ichthyoplankton (Viñas et al., 2002).

2.2. Sampling

Samples were collected during two research cruises conducted by the National Institute of Fisheries Research and Development (INIDEP) in the area of El Rincón during the austral spring and summer in two consecutive years 2010/2011 (S1) and 2012/2013 (S2) (S = survey).

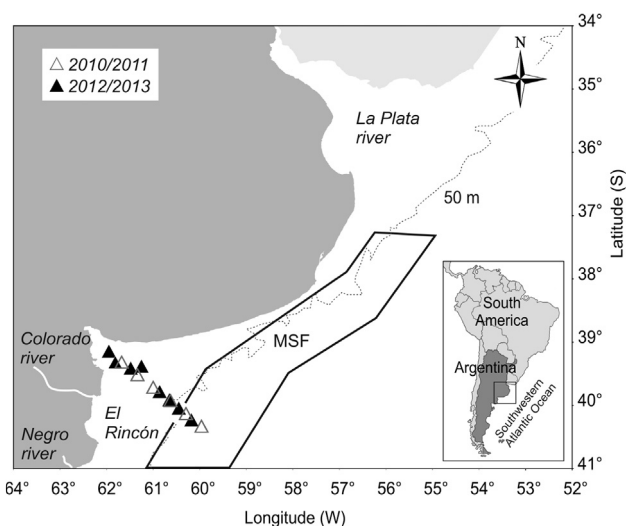


Fig. 1. Position of studied stations during 2010/2011 and 2012/2013 seasons. In gray are shown samplings performed in 2010/2011 and in black those in 2012/2013. The black polygon represents the Mid-Shelf Front (MSF) subregion.

Six and 8 stations were sampled in S1 and S2, respectively, along a 200 km section perpendicular to the coast in waters of the coastal zone between 6 and 70 m depth (Fig. 1).

During S1 (9–10 February 2011) ichthyoplankton was collected with a multinet at three strata: surface, thermocline (when it was detected) and near bottom. During S2 (7–8 December 2012) a Bongo net was employed. In both cases samplers were equipped with flowmeters in order to estimate the volume sampled. The different samplers were not intercalibrated; instead, anchovy eggs and larvae densities were arranged into broad density classes. At stations where the presence of anchovy larvae was observed, random individuals were selected for the analysis of nutritional condition and frozen in liquid nitrogen. Station and depth of provenance were recorded. The rest of the samples were fixed in a solution of 5% formalin in seawater. Additionally, vertical profiles of temperature, salinity, and fluorescence were recorded at each station using a Seabird SBE19 CTD profiler.

2.3. Chlorophyll estimation

Surface chlorophyll concentrations (mg/m^3) from MODIS onboard Aqua were examined as a proxy for the abundance of phytoplankton during the spring and summer. All available high-resolution ($\sim 1 \text{ km}/\text{pixel}$) level 2 data were processed with the standard flags and the empirical algorithm OC3M (O’Reilly et al., 2000), binned and mapped to a 2 km/pixel spatial resolution. Reprocessing version 2013.1.1 was used. Chlorophyll concentrations < 0.02 and > 30 mg/m^3 were excluded from all analyses. Previous *in situ* values of $\sim 19 \text{ mg}/\text{m}^3$ have been reported for continental shelf and shelf-break locations in our study area (e.g., Almandoz et al., 2007; Bianchi et al., 2009; Garcia et al., 2008). Thus, to prevent overestimations we limited our results to values < 30 mg/m^3 . All data were weighed equally and 5-day and monthly composites were generated for each spring-summer season analyzed (October–February 2010/2011 and 2012/2013). Data are distributed by the Ocean Biology Processing Group (OBPG) at NASA’s Goddard Space Flight Center.

Previous studies revealed that the spring phytoplankton bloom frequently occurred offshore of the 50 m isobath following the bathymetry south of 35°S. To examine the variability in the dynamics of the spring phytoplankton bloom, average chlorophyll concentrations for each 5-day period between October and February were calculated for the Mid-Shelf Front (MSF) subregion defined between 37°30’S and 41°S (black polygon in Fig. 1). Although spawning in *E. anchoita* extends

north of the MSF subregion into the Rio de la Plata estuary (Pájaro et al., 2008, 2011), accurate estimation of chlorophyll concentrations from ocean color is challenging in estuarine waters where high concentrations of suspended matter result in the overestimation of pigment densities. For this reason, estuarine waters north of 37 °S were excluded from the analyses; however, the conditions in MSF are assumed to be a good indicator of those in the northern section of the reproductive area of *E. anchoita* (Marrari et al., 2013).

2.4. Laboratory analyses

The ichthyoplankton in all samples were separated from the rest of the plankton and the total number of eggs and fish larvae was recorded. Results were expressed as individuals per cubic meter of sea water filtered.

Larvae were thawed in the laboratory, photographed, and measured. Standard length (SL) was measured to the nearest 0.01 μm using a Carl Zeiss stereoscope equipped with Axio Vision software. No shrinkage corrections were made because only larvae from a restricted size range were employed. We assigned to each larva a developmental stage: preflexion (< 8 mm of SL), and flexion (between 8 and 12.9 mm of SL) analyzing the notochord flexion stage and hypural development under the binocular microscope. The head was dissected according to suggestions made by Olivar et al. (2009). Muscular trunks were placed individually in Eppendorf tubes and freeze dried during 24 h. Larvae were weighed using a Sartorius microbalance to the nearest microgram and stored at -80 °C until analyses were carried out (N = 167). The minimum mass from which reliable relationships were obtained RD was 33 μg. For the assessment of the nutritional condition of the anchovy larvae, the concentration of nucleic acids per larva was quantified following the protocol described by Caldaroni et al. (2001) with modifications made by Diaz and Pájaro (2012). The protocol involves mechanical and chemical homogenization of each larva and subsequent fluorescence-photometric measurements using ethidium bromide (EB) as a specific nucleic acid fluorochrome dye. Fluorescence was measured on a Perkin Elmer spectrofluorometer (excitation: 360 nm, emission: 590 nm). Total nucleic acid concentrations were first determined and then samples were incubated with ribonuclease A. The fluorescence due to total RNA, mainly ribosomal, was calculated as the difference between total fluorescence (RNA and DNA) and the fluorescence measured after ribonuclease treatment, which is assumed to be due to DNA (Clemmesen, 1988). Since we followed a standard protocol based on the use of EB and detergent (Belchier et al., 2004; Caldaroni et al., 2001, 2006), which has been previously used in similar species and stages, we assume that residual fluorescence was insignificant and did not add DNase. Estimates of the DNA and RNA content of each larva were obtained from calibration curves established using standard DNA and RNA in the appropriate range of concentrations. We measured endogenous fluorescence (before EB addition) from the first set of samples, which resulted negligible; therefore it was not considered in calculations of nucleic acids concentrations.

Once the content of larval DNA and RNA were obtained, RD was calculated. The RD values were standardized (RDs) according to the procedure described by Caldaroni et al. (2006) using 2.4 as the reference value of the slope of the calibration curves of the patterns of DNA and RNA ultrapure. To make statistical comparisons RD standardized index values were used. Thus, RDs values are comparable with other bibliographic data. The average ratio of the slopes of DNA and RNA standard curves was 3.30 ± 0.21 .

Growth rate (G) was estimated with the best-fit meta-analysis model RNA/DNA_s - T - G estimated by Buckley et al. (2008).

$$G = 0.145 \times \text{RDs} + 0.0044 \times T \times \text{RDs} - 0.078 \quad (1)$$

where G is the instantaneous growth rate, RDs the standardized RNA/DNA index, and T the mean temperature at 10 m depth.

To estimate the RDs threshold level for the growth of anchovy

larvae, the turning point from positive G to negative G was calculated, followed by back-calculating the related RDs of this turning point. This RDs threshold (i.e. RDs value at G = 0) was reported as critical RDs (cRDs).

Growth performance (Gpf), the quotient of the observed growth rate and the growth rate achieved by larvae under optimal feeding and environmental conditions (G_{max}), provides an objective measure of larval condition (Buckley et al., 2008). Due to the lack of a G_{max} model for *Engraulis anchoita*, larval growth rates were compared to a reference growth rate (G_{ref}). G_{ref} was calculated according to Houde and Zastrow (1993) who published a multi-species model based on 80 marine and estuarine fish species:

$$G_{ref} = 0.0106 \times T - 0.0203 \quad (2)$$

G estimated using this equation is < G_{max}, since this relationship was established using larvae with different nutritional condition. Growth rate and nutritional condition strongly depend on larval size or developmental stage, thus, this was considered in the analyses.

In order to test if RDs or G were significantly different between years an ANCOVA was performed using larval length as covariate. Also, a two-way ANOVA was performed with “year” and “developmental stage” as factors. INFOSTAT® statistical software was used for all statistical analyses.

Correlation between RDs and water temperature or salinity was studied performing Pearson's correlation tests.

3. Results

3.1. Environmental features

In general, environmental conditions were similar between the seasons analyzed in the study area. During the 2010/2011, the temperature profile showed a thermally homogeneous area in the more coastal stations and stratified deeper waters with a marked thermocline at about 35 m depth (Fig. 2a). The three typical oceanographic regimes previously described for the area (Carreto et al., 1995) were observed during both seasons in the salinity and temperature sections along the sampled transect (Fig. 2a and b): an estuarine area (from the coast to 60–80 km) with temperatures above 20 °C, an intermediate regime with high salinities (33.95–34.05) and intermediate temperatures (16–20 °C), and a mid-shelf area with slightly more dilute waters (< 33.85) and temperatures below 20 °C that decreased offshore to less than 12 °C.

During the 2012/2013 season, a thermally homogeneous area near the coast and a stratified area in deeper waters were also observed with a well-defined thermocline at ~35 m depth. The horizontal surface temperature gradient showed values of 20 °C in the coastal area decreasing to approximately 16 °C towards deeper waters. A more abrupt change in temperature is evident at the deepest stations (Fig. 2c). Low salinities near the coast with values of ~33.2 were observed, increasing along the transect to values greater than 33.7 in the most offshore stations. The presence of three water masses was also observed during this season, with coastal waters with salinities below 33.7, high salinity waters between 80 and 120 km offshore (~33.8), and intermediate values in the deepest waters (Fig. 2d).

Monthly chlorophyll concentrations varied widely between 34 and 41 °S and also showed interannual differences in 2010/2011 and 2012/2013. The highest concentrations occurred in frontal areas such as the shelf-break front, MSF in the vicinity of the 50 m isobath, the Rio de la Plata estuarine front, and coastal waters of El Rincon (Fig. 3). Minimum values were generally observed in oceanic waters of the Malvinas and Brazil currents and in coastal areas shallower than 50 m between 37 and 41 °S. Differences were observed between average chlorophyll concentrations at MSF during 2010/2011 (mean Oct-Feb $1.03 \pm 0.19 \text{ mg/m}^3$) and 2012/2013 (mean Oct-Feb $1.68 \pm 0.19 \text{ mg/m}^3$) ($F = 5.93$, $P = 0.0179$), and chlorophyll dynamics differed

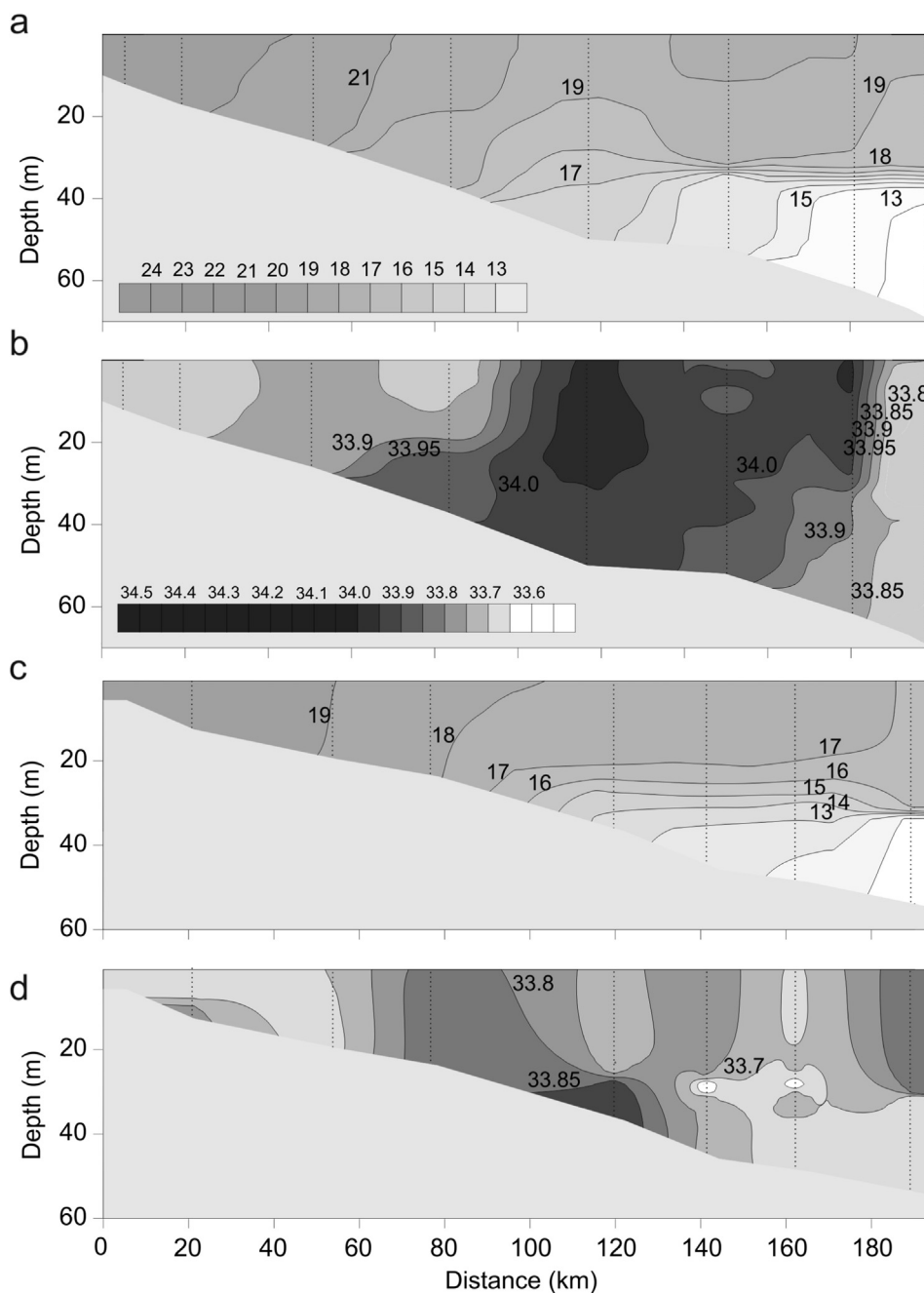


Fig. 2. Vertical profiles of temperature during 2010/2011 (a) and 2012/2013 cruises (c) and salinity profiles corresponding to 2010/2011 (b) and 2012/2013 (d) cruises, obtained at El Rincón area using a CTD.

(Table 1, Figs. 3 and 4).

During the spring 2012/2013 the chlorophyll maximum occurred earlier in the season (October–November 2012) and chlorophyll concentrations were larger than those observed during 2010/2011; however there was a drastic decrease during December 2012, resulting in similar values for the rest of the season to those observed during the previous cruise (Fig. 4). Higher overall chlorophyll concentrations were observed during spring 2012/2013 (Sep–Dec), although values were similar between years during austral summer (Jan–Feb).

In addition, during 2012/2013 the phytoplankton bloom was longer-lived (125 days) than during 2010/2011 (65 days).

3.2. Distribution and abundance of anchovy

The highest anchovy eggs and larvae densities were registered in the

MSF subregion during both cruises. A mean value per station was calculated in order to perform comparisons between cruises (Fig. 5). During 2010/2011, maximum densities of 3.00 eggs/m³ and 3.08 larvae/m³ were observed, while mean values were 0.94 eggs/m³ and 1.16 larvae/m³ (Table 1). During 2012/2013, the highest density of anchovy eggs was recorded on the most offshore station, with 64 eggs/m³. The distribution of anchovy larvae showed an increasing trend towards deeper waters, reaching a maximum density of 8.8 larvae/m³ at the same station. Mean densities were 8.06 eggs/m³ and 1.18 larvae/m³ during this season (Table 1).

3.3. Nutritional status

The analysis of the larval size distribution at each station showed the highest larval abundance, the widest range of sizes and the largest

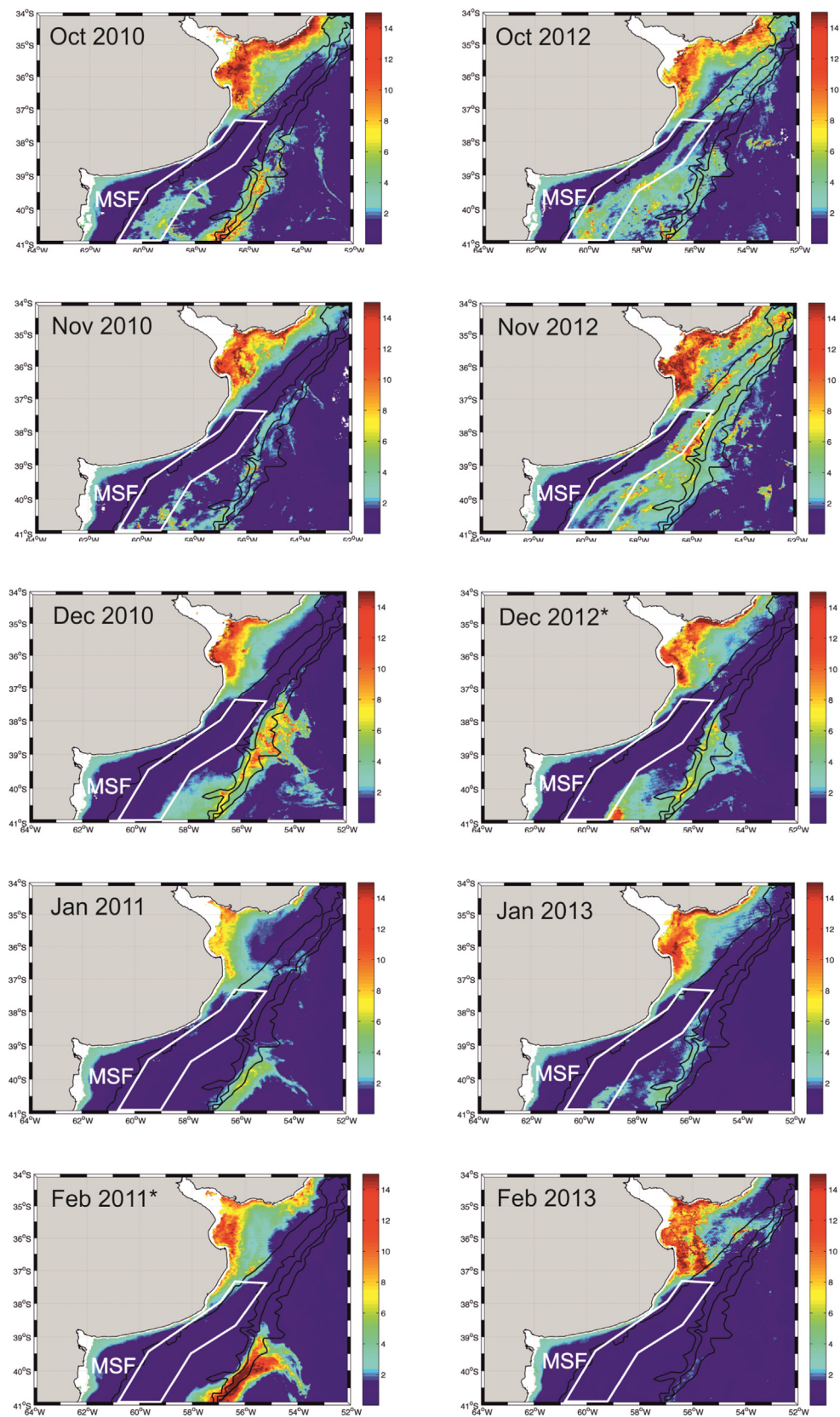


Fig. 3. Distribution of monthly chlorophyll *a* concentration (Chl mg/m^3), obtained from MODIS Aqua, 2×2 km, during October 2010–February 2011 and October 2012–February 2013. Asterisk (*) indicates sampling period.

Table 1

Mean values (\pm S.D.) of *Engraulis anchoita* eggs and larvae (individuals/m³), larval standard length (SL in millimeters), Temperature at 10 m (°C), Chlorophyll (mg/m³), critical RNA/DNA standardized index (cRDs), growth rate (G), reference growth rate (G_{ref}), standardized RNA/DNA index (RDs) and growth performance (Gpf) for larvae in the El Rincón area during 2010/2011 and 2012/2013 seasons.

	2010/2011	2012/2013
Eggs (ind./m ³)	0.940 \pm 1.452	8.061 \pm 22.631
Larvae (ind./m ³)	1.158 \pm 1.239	1.177 \pm 3.092
SL (mm)	7.635 \pm 1.210	8.208 \pm 1.774
Temperature (°C)	18.396 \pm 2.562	17.325 \pm 0.372
Chlorophyll (mg/m ³)	1.028 \pm 0.196	1.680 \pm 0.195
cRDs	0.817	0.859
G	0.430 \pm 0.303	-0.004 \pm 0.040
G _{ref}	0.175 \pm 0.027	0.163 \pm 0.004
RDs	5.272 \pm 3.257	0.810 \pm 0.444
Gpf	2.453 \pm 1.827	-0.028 \pm 0.249

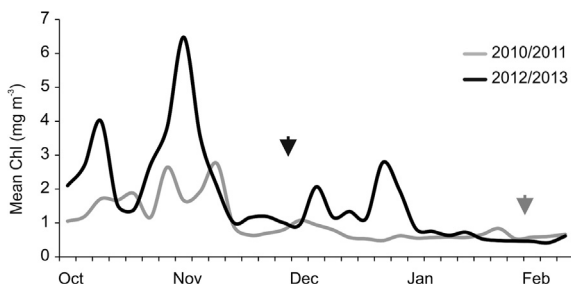


Fig. 4. Mean Chlorophyll a concentration (Chl mg/m³) obtained from MODIS Aqua, 2 \times 2 km, 5 days temporal resolution, during the austral spring-summer 2010/2011 and 2012/2013 seasons for Mid-Shelf Front (MSF) subregion. Arrows indicate sampling periods, gray arrow corresponds to 2010/2011 and black arrow to 2012/2013.

larvae in the deeper offshore stations. During 2010/2011, larvae ranged between 4 and 11 mm SL, while during 2012/2013, slightly larger sizes were observed (5–12 mm SL). No significant differences were found between larval size range between the sampled seasons (Mann Whitney U test: $W = 1602, P = 0.1855$). Since RDs is size dependent, an ANCOVA was performed using larval length as the covariate to assess RDs differences between cruises. Significant differences were observed in average RDs indices for both seasons. The average RDs in 2010/2011 was 5.27 ± 3.26 ($n = 87$) with a median of 4.82, whereas in 2012/2013 a mean RDs of 0.81 ± 0.44 ($n = 25$) and a median of 0.69 were observed (Fig. 6, Tables 1 and 2a).

The cRDs for growth was calculated with Eq. (1), assuming a growth rate equal to zero ($G = 0$) and using the mean temperature at 10 m. Mean 10 m temperature was significantly different between the sampling seasons (18.40 ± 2.56 °C for 2010/2011 and 17.32 ± 0.37 °C for 2012/2013, $p < 0.001$). However, no correlation was observed between RDs and temperature at 10 m (Pearson correlation, $P > 0.01$). Similarly, no correlation was observed between RDs and salinity at 10 m (Pearson correlation, $P > 0.01$). The calculated cRDs was 0.82 for 2010/2011 and 0.86 for 2012/2013 (Table 1). Only 10% of the larvae analyzed had RDs below the cRDs in 2010/2011, while 60% were below cRDs during 2012/2013.

During 2010/2011, no significant differences were observed in RDs for larvae collected at different depths ($F = 1.79, P = 0.1236$). An average RDs was calculated for each sampling station with no significant differences observed in 2010/2011 ($F = 1.40, P = 0.1842$) or in 2012/2013 ($F = 0.58, P = 0.5661$).

Growth performance (Gpf) also differed between sampled periods and was 2.45 ± 1.83 for 2010/2011 and -0.03 ± 0.25 for 2012/2013 (Tables 1 and 2b).

Finally, a two-way ANOVA was performed with year and

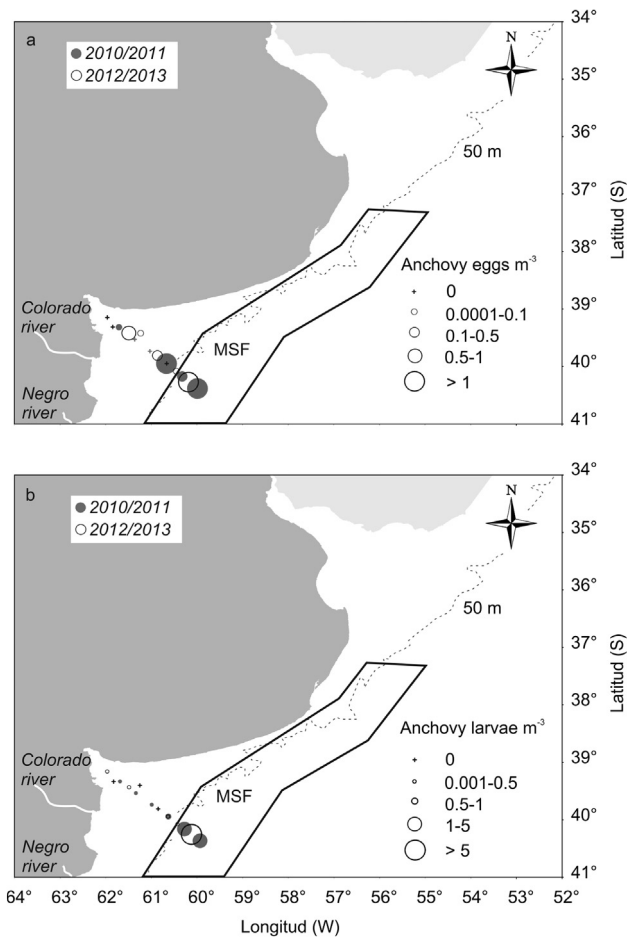


Fig. 5. Distribution and abundance of *Engraulis anchoita* eggs (a) and larvae (b) expressed as individuals/m³ in the studied area. Reference circles are proportional to individuals' densities. The black polygon represents the Mid-Shelf Front (MSF) subregion.

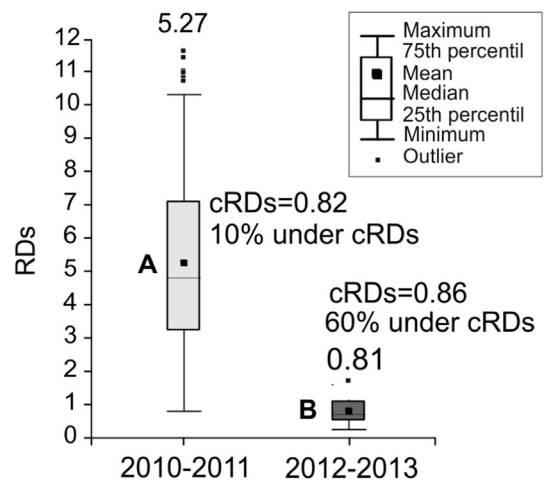


Fig. 6. Boxplot diagram of the RNA/DNA standardized (RDs) values obtained for *Engraulis anchoita* larvae in the El Rincón area during 2010/2011 and 2012/2013 seasons. Mean RDs values are indicated above bars. Critical RDs (cRDs) obtained from Eq. (1) assuming a growth rate equal to zero ($G = 0$) and using mean 10 m temperatures for each cruise was reported. Percentages of larvae below cRDs are indicated. Different capital letters indicate significant differences ($P < 0.001$) among sampled seasons.

Table 2

ANCOVA performed to compare mean RDs (a) and Gpf (b) for *Engraulis anchoita* larvae in the El Rincón area during 2010/2011 and 2012/2013 seasons, standard length (SL) was employed as covariate.

(a)						
RDs	SS	df	MS	F	p-value	Coef
Model	409.71	2	204.86	24.97	< 0.0001	
Year	408.04	1	408.04	49.74	< 0.0001	
SL	23.10	1	23.10	2.82	0.0962	0.34
Error	894.25	109	8.20			
Total	1303.96	111				

(b)						
Gpf	SS	df	MS	F	p-value	Coef
Model	126.85	2	63.43	24.56	< 0.0001	
Year	126.29	1	126.29	48.91	< 0.0001	
SL	7.33	1	7.33	2.84	0.0949	0.19
Error	281.47	109	2.58			
Total	408.32	111				

developmental stage as factors. This analysis showed an increasing trend of RDs and Gpf from preflexion to flexion stages. No significant differences were observed between stages for larvae collected during the same season; however, strong differences were observed between years when comparing the same developmental stage (Fig. 7 and Table 3). It is also worth mentioning that during 2012/2013, mean Gpf values for preflexion and flexion larvae were below 1, indicating a poor condition (Fig. 7b).

4. Discussion

The RNA/DNA index represents the most widely used biochemical indicator of fish larvae nutritional condition (Chícharo and Chícharo, 2008). Usually, larvae showing higher RD are in better condition in comparison to larvae with lower values (for example, Clemmesen, 1994; Robinson and Ware, 1988). However, the critical RD for larval survival must be determined experimentally. The critical RD values for fish larvae available in the literature are usually between 1.0 and 3.0 (Ferron and Leggett, 1994) depending on temperature, larval size, and other factors. Critical RD values experimentally obtained for species of clupeoids varied between 1.0 and 1.3 (Chícharo, 1997; Clemmesen, 1994; Kimura et al., 2000; Yandi and Altinok, 2018). In this study, RDs obtained for larvae collected in 2012/2013 season were below one

Table 3

Two-way ANOVA performed to test differences in RDs (a) and Gpf (b) obtained for *Engraulis anchoita* larvae in the El Rincón area during 2010/2011 and 2012/2013 seasons, cruise “year” and “development stage” were employed as factors.

(a)						
RDs	SS	Df	MS	F	p-value	
Model	408.88	3	136.29	16.45	< 0.0001	
Year	403.84	1	403.84	48.73	< 0.0001	
Stage	7.77	1	7.77	0.94	0.3351	
Interaction	3.20	1	3.20	0.39	0.5355	
Error	895.08	108	8.29			
Total	1303.96	111				

(b)						
Gpf	SS	Df	MS	F	p-value	
Model	126.60	3	42.20	16.18	< 0.0001	
Year	125.03	1	125.03	47.93	< 0.0001	
Stage	2.54	1	2.54	0.97	0.3264	
Interaction	0.97	1	0.97	0.37	0.5433	
Error	281.72	108	2.61			
Total	408.32	111				

(RD < 1), with an average of 0.81 ± 0.44 , indicating that larvae were in an unfavorable nutritional condition. Since there is no experimental information for *Engraulis anchoita* larvae a critical RDs value should be determined using larvae under different feeding treatments in future studies.

We calculated a critical RDs for the growth of anchovy larvae (RDs when G = 0) and larval growth performance (ratio of the observed growth rate and the theoretical growth rate under optimal conditions) in order to provide measurements of larval condition. A Gpf below one (Gpf < 1) indicates that larval condition is below the optimum. During 2012/2013 60% of the larvae analyzed had RDs below the cRDs and a mean Gpf below one. These results were unexpected considering the high productivity and elevated chlorophyll concentrations often observed in El Rincón, an area known to be favorable for fish larvae development and survival (Acha et al., 2004). In contrast, larvae collected during 2010/2011 showed average RDs of 5.27 ± 3.26 , indicating a considerably better condition than in 2012/2013. Diaz et al. (2011) reported similar results from anchovy larvae collected during October 2006, which showed average RDs values of 4.91 ± 1.57 and a good nutritional status, as could be expected for an area of abundant food for ichthyoplankton.

Previous studies in El Rincón area have also reported the presence of

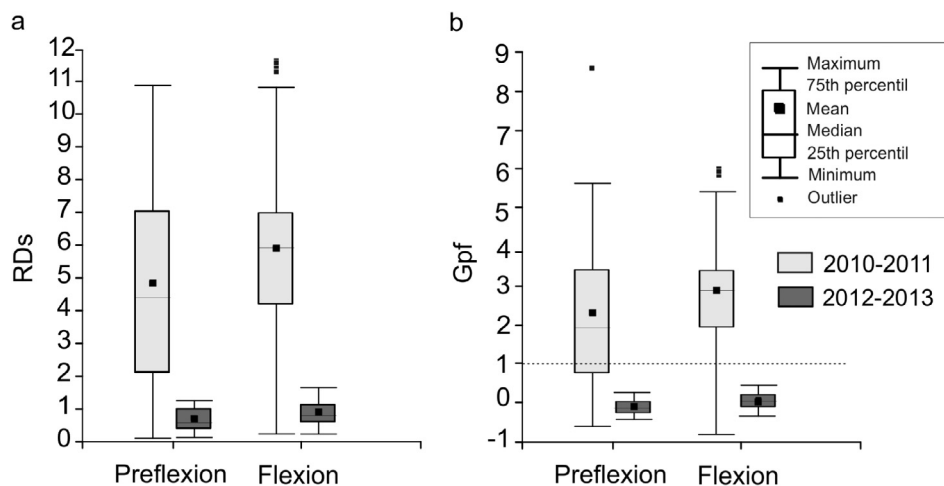


Fig. 7. Boxplot diagram of the RNA/DNA standardized (RDs) (a) and Growth performance (Gpf) (b) values obtained for preflexion and flexion *Engraulis anchoita* larvae in the El Rincón area during 2010/2011 and 2012/2013 seasons. Dotted line indicates the Gpf = 1.

individuals in poor nutritional condition (Diaz et al., 2009), which was explained in terms of density-dependent mechanisms which might have been operating as a result of the high density of anchovy larvae present. Limited food resources could lead to intra-specific competition for food due to the high abundance of individuals, which would explain poor larval nutritional conditions under a favorable food environment. Density-dependent mechanisms could explain the poor larval condition observed in 2012/2013 considering the high abundance of larvae observed. According to Beverton (1995) species that tend to concentrate spatially during vulnerable life stages are more likely to be affected by density-dependent forces, as is the case of anchovy larvae that tend to aggregate in patches. It is expected that an area with high primary and secondary productivity, as the case of El Rincón, is a suitable site for the survival of larvae due to the availability of food. However, a balance between larvae dispersal and high food availability is needed (McGurk, 1986).

El Rincón is considered a productive area in terms of chlorophyll concentrations and the occurrence of elevated densities of zooplankton and fish (Acha et al., 2004; Viñas et al., 2002). The circulation in the area is not yet fully understood, but a gyre-like pattern has been detected, which would favor the retention of fish larvae and their prey (Piola and Rivas, 1997). Viñas et al. (2013) found that El Rincón area, in regards to mesozooplankton composition, is included within a stable “coastal faunistic area”, characterized by the dominance of small copepods (such as *Paracalanus parvus* and species from the genus *Oithona*). These species reproduce throughout the year with a constant production of eggs and nauplii, the main prey item of first-feeding larvae of *E. anchoita* (Sato et al., 2011). The spatio-temporal co-occurrence of anchovy eggs and larvae and high densities of small copepods has been observed previously in El Rincón area (Marrari et al., 2004, 2013; Viñas et al., 2013), but there are no previous records of anchovy larvae condition and potential food abundance.

Marrari et al. (2013) found high inter-annual variability in spring chlorophyll concentrations, which are mainly influenced by changes in water temperature and vertical stratification, and in turn control nutrient supply to the surface. Platt et al. (2003), combining the use of remote-sensing satellite data with information regarding haddock recruitment, showed that the survival of the larval fish depends on the timing of the local spring bloom of phytoplankton. Marrari et al. (2013) also stated that the variability observed in *E. anchoita* recruitment was explained in terms of chlorophyll dynamics, not only the timing of the spring bloom of phytoplankton as well as the fish spawning, but also the magnitude and duration of the spring bloom. These authors assumed that this was due to fluctuations in the availability of adequate food for the larvae.

A tight coupling between chlorophyll concentration at MSF and small copepods abundance was found by Marrari et al. (2013) and this fact allowed them to suggest that satellite-retrieved chlorophyll data might represent a good predictor of food availability for larval *E. anchoita* on the Buenos Aires province shelf. We agree with this idea since the observed differences in larval condition between 2010/2011 and 2012/2013 might be due to variations in food availability as a consequence of the variation in the intra/inter-specific competition. A spatio-temporal decoupling between the peaks of chlorophyll concentration, zooplankton abundance, and anchovy larvae might have led to the low larval nutritional condition observed in 2012/2013.

Kristiansen et al. (2011) stated that a key factor for enhancing survival is the duration of the overlap between larval and prey abundance. During 2010/2011 chlorophyll concentrations were moderate and relatively constant providing adequate conditions for zooplankton growth for a longer period of time. Thus, the prolonged overlap between larval anchovy and prey abundance during 2010/2011 season might explain the high RDs index observed. On the other hand, the combination of high larval densities and scarce adequate food for a longer part of the season likely enhanced competition between the larvae, resulting in poor larval conditions during 2012/2013 season.

Supporting Marrari et al. (2013) findings, our results reinforce the idea that satellite ocean color products can be valuable tools for understanding variability in ecosystem dynamics and its effects on reproductive success in fish.

Temperature and food availability are usually correlated with fish larvae growth rates (Buckley et al., 1999). Temperature governs the rate of chemical processes, sets the pace for metabolic needs and digestive processes, as well as the growth rate (Fry, 1971) and should be taken into account in any study of growth and mortality. RNA concentrations and RD indices can be examined directly without corrections for temperature only within a very limited range of temperatures (in the order of 2 °C) (Buckley et al., 1999). The RD indices are used to estimate growth rates but models require the recording temperature (Buckley, 1984). These Growth-Temperature-RD models have shown that for a certain link RD the growth rate increases by 1% with an increase of 1 °C. Malzahn et al. (2003) also observed that increases in temperature result in significant increases in growth rate of *Coregonus oxyrinchus* larvae. They also have found a significant response in the concentrations of nucleic acids, but indicated that the effect of temperature on the RD is not as marked as that on growth rate. We found no correlation between RDs and water temperature. Mean surface temperature recorded during 2010/2011 was 1.1 °C higher than that in 2012/2013 (18.40 and 17.3 °C, respectively) but this difference does not seem to explain the interannual differences observed in RDs. Supporting these results, Diaz et al. (2011) observed RDs values of 4.91 ± 1.57 for this species in the same area with a mean temperature at 5 m depth of 16.7 °C, even lower than values observed during 2012/2013.

Recent studies have shown that freshwater input may affect negatively early life stages of marine fishes, altering dispersion patterns, feeding habits and condition (Landaeta et al., 2012, 2018). During 2012/2013, freshwater input was more important than during the previous sampling. However, no correlation between RDs and salinity was found in this study.

Díaz et al. (2008) analyzed the biochemical composition and condition of *Engraulis encrasicolus* finding that RNA/DNA ratios increased from larvae in preflexion to larvae in postflexion, indicating that larger larvae are better able to synthesize protein than the smaller ones. In this study there was no correlation between the size of the larvae and the RD index, probably because the size range used here was between 5 and 12 mm, which corresponds to preflexion and flexion larvae. Future studies should include more advanced stages of development to better understand the behavior of the RD index development advances.

Several studies have pointed out that predation is a cause of larval death as important as starvation (Ferron and Leggett, 1994; Hunter, 1982; Purcell, 1985; Skajaa et al., 2004). Jellyfish could act as predators or competitors of fish larvae depending on the availability of other prey in the environment (Alvarino, 1985; Purcell, 1985, 1990; Purcell and Arai, 2001). During 2012/2013, we observed an important presence of jellyfish (mainly ctenophores) in the samples but unfortunately their abundance was not estimated. Even though it has not been shown for our study area, it is possible that jellyfish could have been competing with anchovy larvae for food where adequate preys were not available. Diaz et al. (2016) examined a set of parameters that could potentially drive variability in anchovy larvae condition (RDs index) using generalized linear models, and noting that the abundance of copepod nauplii and the presence gelatinous plankton were significant variables and had a positive correlation between nauplii abundances and larval condition. On the other hand, the presence of gelatinous plankton showed a negative relationship with the nutritional condition of larvae of *E. anchoita*. In this way, larval condition might be influenced by several fluctuating variables in the environment such as prey density and quality, predator density, among others variables which were not herein studied.

We did not observe significant differences in RDs of larvae obtained from different depths, but in general larvae found deeper showed a

better nutritional status. The number of samples analyzed might have been insufficient to detect a vertical gradient in the RDs index. Other studies demonstrated that larval nutritional condition varies with depth and concluded that larvae in better condition are capable of crossing the thermocline in stratified environments (Catalán et al., 2011; Grønkjær et al., 1997).

There is evidence that zooplankton dominates the diet of *E. anchoita* larvae and that phytoplankton is also included (Viñas et al., 2002). Although the phytoplankton bloom may not be sufficient to ensure high survival, it could be a necessary condition. Additional studies of trophic position of anchovy larvae together with the relation between larval condition and prey abundance or quality could be reinforcing our findings in near future. The main goal of this study was to assess anchovy survival using information on their nutritional status in relation to chlorophyll dynamics.

Continuing with this analysis would allow us to obtain some longer time series of larval nutritional condition data. This tending to the identification of patterns of variability as well as extreme events that control recruitment variability of the species. We stress the importance of continuing with periodic monitoring of larval nutritional status, especially for commercially exploited species such as *Engraulis anchoita*.

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