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RNA/DNA and derived condition indices for anchovy and hake larvae as relevant information for comprehensive fisheries management

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Summary: The nutritional condition of anchovy and Argentine hake larvae in the Northern Patagonian Frontal System (NPFS) area was studied in the austral spring of 2018. We hypothesized that this area provides adequate features for larval growth and survival. The RNA/DNA index (RD) and its derived index of growth performance were employed. A critical RD value for starvation was calculated. The percentage of individuals under starvation and in optimal growth conditions was calculated. Because the period of study was the beginning of the hake spawning period, a limited number of larvae of this species were collected. The RD index showed a significant increase throughout larval ontogeny for anchovy larvae, being 1.84±1.39 (N=739) and 2.77±1.50 (N=220) in the pre-flexion and flexion stages respectively. These values were significantly higher at stations close to the NPFS and at the upper level of the water column. No differences were observed throughout the day. The area inside the NPFS showed a lower proportion of starved anchovy and a higher proportion of individuals in optimal growth, standing as a favourable nursing area. For hake larvae, the average RD was 1.64±0.55 (N=15). The great sensitivity of the RD index makes it a powerful tool for assessing the probability of larval survival and posterior recruitment into fisheries and allowing the identification of favourable rearing areas for these important species for fisheries.

Keywords: nutritional condition; RNA/DNA index; ichthyoplankton; *Engraulis anchoita*; *Merluccius hubbsi*; North Patagonian Frontal System.

ARN/ADN e índices de condición derivados de larvas de anchoíta y merluza como información relevante para la gestión integral de las pesquerías

Resumen: Se estudió el estado nutricional de larvas de anchoíta y merluza argentina en la zona del Sistema Frontal Norpatagónico (SFNP) (primavera austral 2018). Nuestra hipótesis fue que esta área proporciona características adecuadas para el crecimiento y la supervivencia de las larvas. Se empleó el índice de ARN/ADN (RD) y su índice derivado denominado *performance* de crecimiento. Se calculó un valor crítico de RD para la inanición. Se determinó el porcentaje de individuos en inanición y en condiciones óptimas de crecimiento. Debido a que el período de estudio fue al inicio del período de desove de la merluza, se recolectó un número limitado de larvas de esta especie. El índice RDs mostró un aumento significativo a lo largo de la ontogenia para las larvas de anchoíta; siendo 1,84±1,39 (N=739) y 2,77±1,50 (N=220) en preflexión y flexión respectivamente. Estos valores fueron significativamente más altos en las estaciones cercanas a SFNP y en el nivel superior de la columna de agua. No se observaron diferencias a lo largo del día. El área al interior del SFNP presentó una menor proporción de anchoíta en inanición y una mayor proporción de individuos en óptimo crecimiento, destacándose como un área favorable para la crianza. Para las larvas de merluza, el RD promedio fue de 1,64±0,55 (N=15). La gran sensibilidad del índice RDs lo convierte en una poderosa herramienta para evaluar la probabilidad de supervivencia de las larvas y posterior reclutamiento en las pesquerías; permitiendo la identificación de áreas de cría favorables para estas especies con relevancia pesquera.

Palabras clave: condición nutricional; índice ARN/ADN; ictioplancton; *Engraulis anchoita*; *Merluccius hubbsi*; Sistema Frontal Norpatagónico.

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INTRODUCTION

The Argentine anchovy, *Engraulis anchoita* (Hubbs and Marini, 1935), represents the pelagic resource with the highest biomass in the southwest Atlantic Ocean, with a total catch of approximately 9000 t reported in 2021 (Ministry of Agroindustry 2022). Its high densities and wide distribution make it the most ecologically important fish in the Argentine Sea. It plays a key role in the food web, sustaining several species of commercial value within this region (Angelescu 1982, Hansen 2004). Two populations of anchovy are separated by the 41°S parallel (Sánchez and Ciechomski 1995). The spawning and breeding areas of this species are related to upwelling regions, estuarine, tidal and shelf break fronts characteristic of the Argentine continental shelf. The reproductive activity of the Patagonian stock, distributed south of 41°S, begins in November and shows maximum spawning in December (Sánchez and Ciechomski 1995). The Argentine hake *Merluccius hubbsi* is one of the most important fishing resources for the Argentine bottom trawling fleet, with a total catch of approximately 287000 t reported in 2021 (Ministry of Agroindustry 2022). It inhabits the waters of the southwest Atlantic Ocean between Cabo Frio in Brazil (22°S) and southern Argentina (55°S), at depths between 50 and 500 m (Cousseau and Perrota 1998). There are two main fishing stocks in Argentina, north, and south of 41°S. Owing to the increase in fishing pressure in recent decades, a decrease in spawning biomass has been observed in this species, as well as variations in the age structure of the parental stock and the location of spawning schools (Macchi et al. 2005, 2021). The reproductive activity of the southern or Patagonian stock, which is the one with the highest population abundance, occurs on the North Patagonian shelf mainly between November and April and peaks in January (Ehrlich 1998, Macchi et al. 2004, Macchi et al. 2007).

As in other fish species, variability in the recruitment of both anchovy and hake is affected by processes that operate on different spatial and temporal scales. This variability depends on physical and thermodynamic factors that determine survival during the early stages of life (Houde 2008). The study of larval nutritional condition allows us to evaluate the individual physiological state, which at the same time reflects the environmental context to which the larvae have been exposed (Chícharo and Chícharo 2008). It is also a useful instrument for determining favourable breeding areas (Diaz and Pájaro 2012). Various nutritional condition indices have been used to estimate mortality due to starvation in marine fish larvae (Buckley 1984, Clemmesen 1994). The RNA/DNA ratio (RD) stands as one of the best indicators of the nutritional status of various marine organisms (Clemmesen 1994, Folkvord et al. 1996) and is currently the biochemical index most widely used as an indicator of the nutritional condition of fish larvae (Chícharo and Chícharo 2008). The RD ratio varies with age, developmental stage and size under different environmental conditions (Bulow 1970). It has also been shown to respond to changes in the concentration of available prey (McGurk et al. 1992, Chícharo and Chícharo 1995) among other factors. The monitoring of the larval state in situ over time could be a useful tool for determining favourable breeding areas for the species and for developing a time series to assess the effects of climate change in these areas. Detecting these favourable areas and periods for larval survival makes a valuable contribution to the comprehensive management of a population subjected to fishing exploitation (Viladrich et al. 2016).

The northern Patagonia region is hydrographically characterized by the existence of a tidal front, the Northern Patagonian Frontal System or NPFS (Guerrero et al. 1997, Martos and Sánchez 1997, Sabatini and Martos 2002). This system is characterized by the formation of a seasonal thermocline, particularly during the austral summer, which gives rise to a homogeneous coastal zone and an increasing stratification towards the offshore zone. Figure 1 depicts three typical vertical temperature profiles observed in this area during summer: coastal homogeneous stations (Fig. 1D-F), frontal stratified stations (Fig. 1G) and stratified offshore stations (Fig. 1H-I). The dynamic that characterizes the frontal system causes a high availability of nutrients that are mainly due to upwelling and concentration and favour primary and secondary productivity (Bakun and Parrish 1991, Bakun 1997), generating major phytoplankton blooms (Carreto and Benavídez 1990) and large aggregations of copepods (Derisio et al. 2014, Temperoni et al. 2014, Temperoni and Viñas 2015), in addition to a great diversity of other zooplanktonic organisms (Mianzan and Guerrero 2000, Schiariti 2008, Schiariti et al. 2015). The water circulation in this area also favours the retention of the first stages of life of both fish and invertebrates (Álvarez Colombo et al. 2011). The NPFS is therefore a propitious area for the reproduction and breeding of many species during spring and summer, including important fishing resources such as the Patagonian stocks of hake and anchovy (Hansen et al. 2001, Macchi et al. 2004, Pájaro et al. 2005). Given that the survival during the first phases of life of these species is affected by the existence of the NPFS, the spatial and temporal coincidence of these organisms with this tidal front during the larval stage could be one of the most important factors that explain the observed variability in recruitment. Because of the importance of the NPFS for the conservation and management of major fishery resources and the aforementioned hydrographic characteristics, part of this area is currently under study for the implementation of a new marine protected area (MPA). For this reason, it is essential to carry out a comprehensive analysis of the NPFS, covering the environmental and biological aspects of this region.

In December 2018, a research survey was carried out to analyse the oceanographic and biological conditions in the NPFS region within the project "Strengthening the Management and Protection of Coastal Biodiversity Marine in Key Ecological Areas and the Application of the Ecosystem Approach to Fisheries" (GEF-FAO). Within the framework of this survey, our main objective was to evaluate the nutritional condition

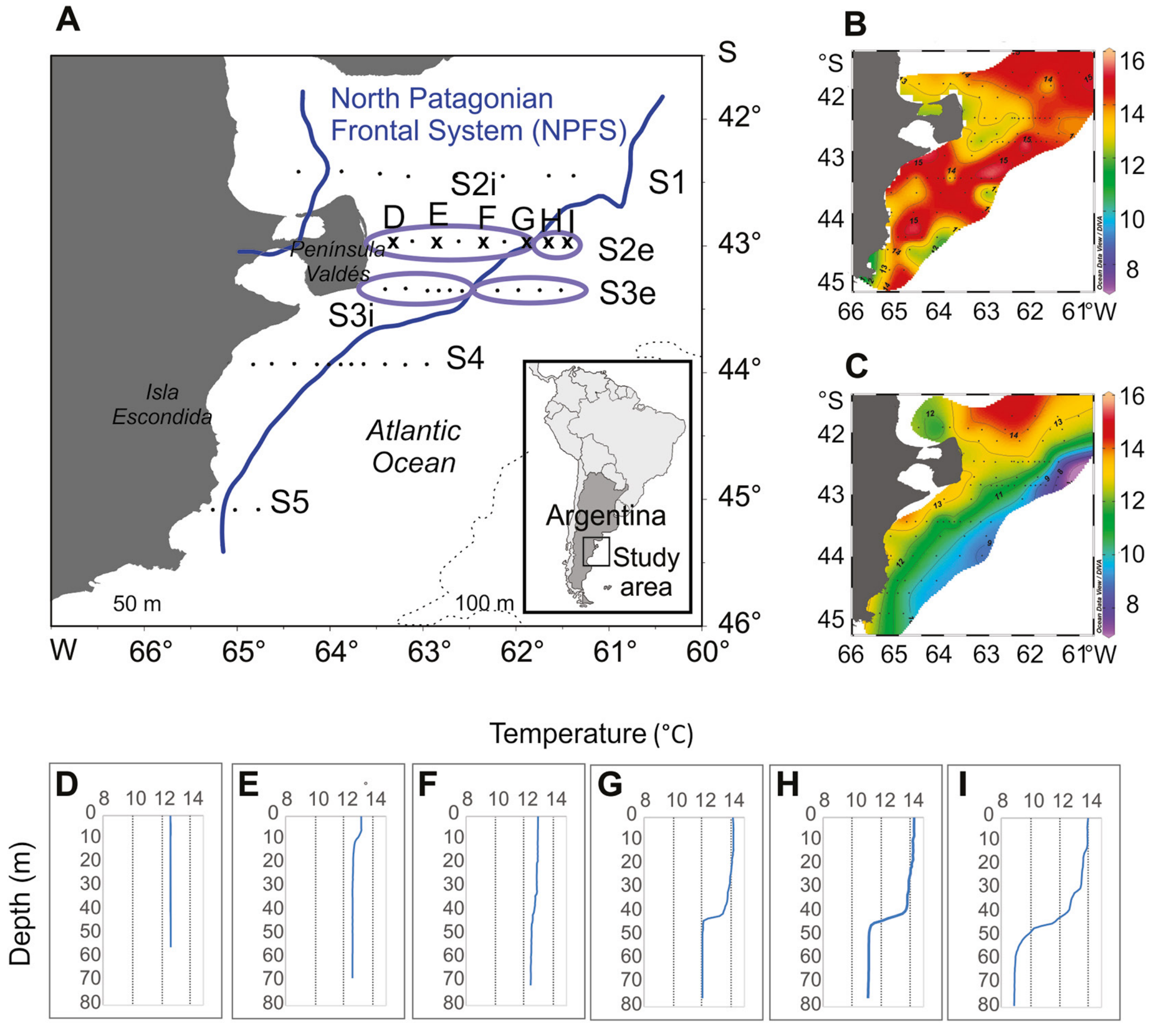

Fig. 1. – Spatial distribution of the sampling stations. (A) Five transects or oceanographic sections (S1-S5) were made. The schematic position of the Northern Patagonian Frontal System (NPFS) during December 2018 is indicated in blue (calculated according to Simpson 1981; by Martos and collaborators, INIDEP Physical Oceanography Office). For the analysis, the stations of sections S2 and S3 were grouped in relation to the position of the NPFS: the stations in the homogeneous zone of the front or internal zone (S2i, S3i) and the stations of the stratified offshore or external zone (S2e, S3e). (B) Horizontal isolines of temperature (ºC) at the surface and (C) at the bottom are indicated. The vertical gradients of temperature (ºC) with respect to depth (m) are indicated in the stations with an "x" in Section 2 (S2), (D-I) from coast to offshore stations. Data obtained from the BaRDO-INIDEP regional oceanographic database. Dotted lines indicate 50 and 100 m isobaths.

of *E. anchoita* and *M. hubbsi* larvae during the beginning of the austral summer in the northern Patagonia region and to analyse the spatial variation of this parameter. Because the sampling was performed early in the spawning period for hake, a small number of larvae of this species were collected. Thus, the study is mainly based on anchovy larvae, with some additional information on the nutritional condition of initial larval stages of hake. We hypothesized that the presence of the NPFS provides adequate features for larval growth and survival evidenced in a better nutritional condition of anchovy and hake larvae than that observed in the offshore area. To test this hypothesis, we assessed the RD index and a derived index of growth performance. The proportion of individuals in starvation and in optimal growth conditions was estimated. Larval condition was mapped in the study area to determine the existence of favourable areas for growth and larval survival in relation to the NPFS.

MATERIALS AND METHODS

Sample collection

The samples were taken during a research survey carried out on board the vessel *Victor Angelescu* of the National Institute for Fisheries and Development (INIDEP) in the northern Patagonia area between 4 and 16 December 2018 (Fig. 1). At each station, temperature and salinity measurements were made using the CTD Seabird Electronics system. The data collected by the staff of the Physical Oceanography Office of INIDEP are part of the BaRDO database. A stratified plankton sampling was performed with the HydroBios

Model Midi Multinet (0.5×0.5 m), equipped with three opening and closing nets (300 µm pore size) and soft collectors. Oblique trawls were carried out at different levels in the water column. The towing speed during the ascent was maintained between 2 and 3 knots, with a duration that varied between 5 and 7 minutes per sampling strata. When the station was in the homogeneous area the nets were operated covering strata of equal width, while in the frontal and stratified regions the nets were operated above, on and below the thermocline. Five transects or oceanographic sections were made (Fig. 1: S1-S5). The stations of sections S2 and S3 were grouped for the analysis in relation to the position of the frontal system: the stations in the homogeneous or internal zone (Fig. 1: S2i, S3i) and the stations of the stratified offshore or external zone (Fig. 1: S2e, S3e). Larvae were not found in section S5.

Once the plankton samples (N=126) were obtained, they were inspected on board to detect and separate anchovy and hake larvae. These were extracted from the sample and placed in labelled cryotubes and then stored in an ultrafreezer (–80°C) for studies of nutritional condition and growth. A representative larval sample was taken at each sampling station with a maximum of 100 larvae in those where anchovy larvae were very abundant, including all sizes present in the entire sample. In total, 1045 anchovy larvae were collected from 29 stations, and 16 hake larvae were obtained from four hauls. The rest of the plankton sample was fixed in 5% formalin in seawater to be later analysed under a binocular stereoscope in the INIDEP laboratories.

Sample processing

Anchovy and hake larvae were identified and classified under a Carl Zeiss stereoscopic microscope equipped with the Axio Vision software. According to Betti et al. (2009) and Alheit et al. (1991), a developmental stage was assigned to each larva: (a) pre-flexion, (b) flexion and (c) post-flexion. Before the determination of nucleic acid content, the larvae were photographed and the standard length of each larva was measured. The head and digestive tract were dissected according to Olivar et al. (2009). The heads were preserved in 96% ethyl alcohol for later age studies, and the digestive tubes in 5% formalin for stomach content studies. The muscle trunks were individually lyophilized and weighed to the microgram using a Sartorius microbalance.

The protocol used for the analysis of nutritional condition was the one described by Caldarone et al. (2001), partially modified by Diaz and Pájaro (2012) to maximize the detection of nucleic acids in a 1 mL volume instead of microplates. This method is based on the quantification of total nucleic acids (TNA) by spectroluminescence, RNA digestion by adding a specific enzyme (RNase) and subsequent determination of the resulting DNA fluorescence. Thus, the fluorescence due to RNA is determined by difference: [RNA] = [TNA] - [DNA]. The DNase step was not performed in the protocol because residual fluorescence was negligible. The concentrations of nucleic acids corresponding to the fluorescences obtained were determined by comparing with a calibration curve obtained from a series of standards of known concentrations of ultrapure DNA and RNA. The results were expressed as µg DNA/mg dry weight (DW), µg RNA/mg DW, and RD. The minimum mass from which reliable RD ratios were obtained was 33 µg DW. Reported RD indices correspond to individual muscle trunks. Of the total hake and anchovy larvae processed, 15 and 959 reliable determinations were respectively obtained.

Data analysis

The RD values obtained in this study were standardized (RDs) according to Caldarone et al. (2006), using 2.4 as the reference value for the slope of the calibration curves of ultrapure DNA and RNA standards. This procedure allows direct comparison with other published RDs results, avoiding inter-laboratory differences caused by analytical protocols. The average value for the slope of the calibration curves obtained in the present study was 2.19 (±0.71 SD).

Growth rate (G) was estimated for each larva using the RDs–T–G model developed by Buckley et al. (2008). For hake larvae, the model developed by Buckley for gadiform fish was used, and the general multi-specific model was used to determine the instantaneous growth of anchovy larvae according to the following expressions:

$$G=0.0254xRDs+0.0037xTxRDs-0.0873 \text{ (hake)} \quad (1)$$
$$G=0.0145xRDs+0.0044xTxRDs-0.0780 \text{ (anchovy)} \quad (2)$$

where G is the instantaneous growth rate and T is the temperature measured at the sampling site, which corresponds to the average of the stratum in which the larvae were collected.

A critical value of the RD index was determined assuming null larval growth (CRD), that is G=0. The percentage of larvae below the CRD was calculated, and it was assumed that these specimens were in starvation (% starvation, Fig. 2A).

In addition, the growth performance (Gpf) was determined as a derived condition index. This index represents the quotient of the observed G and a reference growth (Gref) rate achieved by a larva under optimal environmental and feeding conditions. Due to the lack of a Gref for the studied species, larval growth rates were compared with a Gref that was calculated according to Houde and Zastrow (1993), who established a multi-specific model based on 80 marine and estuarine species:

$$Gref=0.0106xT-0.0203 \quad (3)$$

Larvae with Gpf higher than or equal to 1 were assumed to be in optimal growth condition. The percentage of larvae with Gpf higher than 1 (optimal %, Fig. 2B) was calculated. A Student t-test was used to compare mean RD values of pre-flexion and flexion anchovy larvae. ANOVA was used to compare mean RD values of each size class of anchovy larvae. Analyses

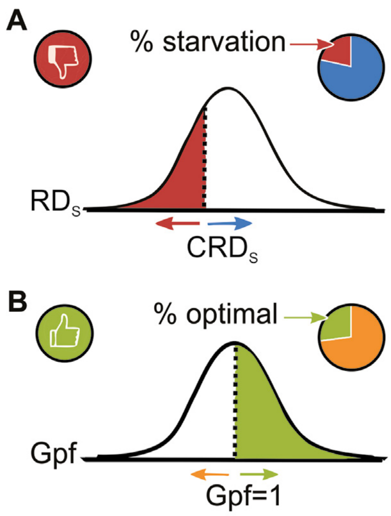

Fig. 2. – Theoretical distribution for (A) RNA/DNA index (RD) and (B) growth Pperformance (Gpf) values for fish larvae. (A) The curve corresponding to RD shows a critical value (CRD) used to detect starving larvae: above this value, the larvae are not in starvation (white colour) and below it they are in starvation (red colour). (B) The Gpf curve represents the growth performance being the quotient between the observed growth rate and the larval growth rate under optimal environmental and feeding conditions. Values greater than 1 (green colour) indicate more than optimal growth, and values less than 1 (white colour) indicate less than optimal growth. Modified from Alves et al. (2022).

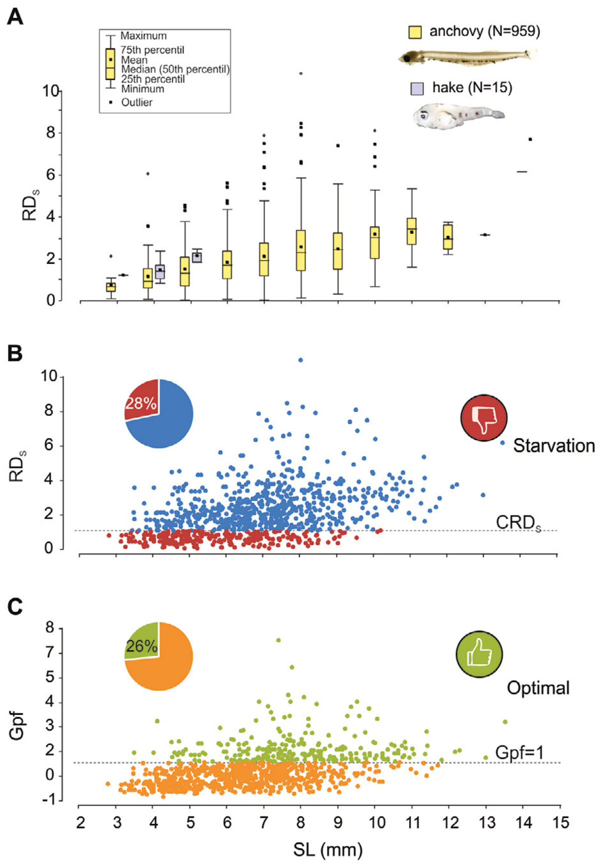

Fig. 3. – (A) Box plot of the standardized RD index as a function of standard length (SL, mm) class for larvae of anchovy *Engraulis anchoita* and hake *Merluccius hubbsi*. Dispersion graph of the (B) standardized RNA/DNA index (RD) and (C) growth performance (Gpf) as a function of the SL of larvae of anchovy *Engraulis anchoita*. The dotted line in (B) indicates the critical value of the RD index (CRD), the individuals in starvation (RD<CRD) are indicated in red, and the individuals in good nutritional condition are indicated in blue. The dotted line in (C) indicates optimal growth performance (Gpf=1); the individuals in optimal growth (Gpf>1) are indicated in green, and the individuals that showed less than optimal growth are shown in orange.

were performed to evaluate variability in the position of the frontal system (calculated according to Simpson (1981), see Fig. 1), time of the day and depth where the larvae were collected. The mean values of RD and Gpf obtained for the larvae were compared by ANCOVA, using the standard length of the specimens as a co-variable. When significant differences were found, a Tukey test was performed. The results are then expressed according to oceanographic section (S1-S4), oceanographic section grouping the internal and external stations in relation to the position of the tidal front (S1, S2i, S2e, S3i, S3e, S4), time of day (M, morning; A, afternoon; N, night), and depth (B, bottom; T, thermocline; S, surface).

RESULTS

A large number of the anchovy larvae obtained (N=959) were classified in pre-flexion (SL<8 mm) and in flexion (8-12.9 mm) and a small proportion in post-flexion (SL>12.9 mm). The RD index showed a significant increase throughout the anchovy larval ontogeny, with an average value of 1.84±1.39 (N=739) and 2.77±1.50 (N=220) in pre-flexion and flexion, respectively (*t*-test, T (N= 957) = 8.54; p<0.0001). A small number of *Merluccius hubbsi* larvae were collected (N=15) and classified in pre-flexion (SL<6.49 mm), and the RD obtained was 1.64±0.55.

The mean values of the RD index for size class showed a positive trend in both species. For anchovy larvae, the mean RD by size class also showed an increase towards the post-flexion stage (Fig. 3A), but no significant differences were observed when the mean values were compared by size class, except for the class 14 mm (ANOVA, F (11, 947) = 15.47; p<0.0001); however, only one larva of this size class was collected.

The scatterplot of the RD indices as a function of the standard length (SL, mm) of the anchovy larvae shows the proportion of individuals below the critical RD index (28%), that is, in starvation (Fig. 3B), and the proportion of individuals with a growth performance (Gpf) above one (26%), that is, in optimal growth (Fig. 3C).

Significant differences were observed in the mean RD values in the oceanographic sections for anchovy larvae. A co-variance analysis was performed using the SL of the specimens as a co-variable (Fig. 4, Table 1), and differences were observed when the stations were grouped according to the position of the NPFS. The larvae from the internal stations of the frontal system showed higher condition indices than the larvae from the external stations. (Fig. 4 and 5A-B, Table 2). A better nutritional condition was observed in the larvae collected in the upper stratum (surface), but no differences were observed at the different times of the day (Fig. 4, Table 3).

Table 1. – Analysis of variance of mean values of the standardized RNA/DNA index (RD) for *Engraulis anchoita* larvae collected in different oceanographic sections (S1-S4) in December 2018. Standard length was used as a co-variable (ANCOVA). SS, Sum of quares; MS, Mean squares

	SS	df	MS	F	p-value	Coeff.
Model	339.14	4	84.78	47.25	<0.0001	
Section	43.31	3	14.44	8.04	<0.0001	
SL (mm)	251.78	1	251.8	140.32	<0.0001	0.30
Error	1711.81	954	1.79			
Total	2050.95	958				

Table 2. – Analysis of variance to compare the mean values of the standardized RNA/DNA index (RD) between the oceanographic sections zone grouping the internal and external stations in relation to the position of the tidal front (S1, S2i, S2e, S3i, S3e, S4) for *Engraulis anchoita* larvae collected in December 2018. Standard length was used as a co-variable (ANCOVA).

	SS	df	MS	F	p-value	Coeff.
Model	349.92	6	58.32	32.64	<0.0001	
Zone	54.09	5	10.82	6.05	<0.0001	
SL (mm)	162.79	1	162.79	91.11	<0.0001	0.27
Error	1701.03	952	1.79			
Total	2050.95	958				

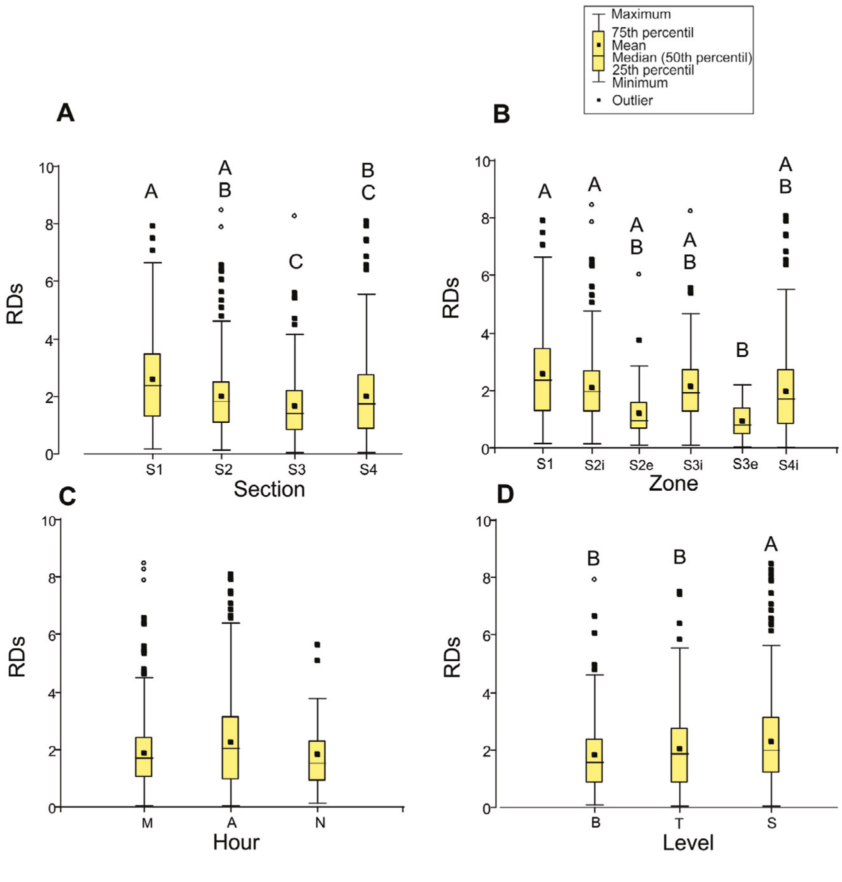

Fig. 4. – Box plot of the standardized RNA/DNA index (RD) for larvae of the anchovy *Engraulis anchoita*, according to (A) oceanographic section (S1-S4), (B) oceanographic section grouping the internal and external stations in relation to the position of the tidal front (S1, S2i, S2e, S3i, S3e, S4), (C) time of day (M, morning; A, afternoon; N, night), (D) depth (B, bottom; T, thermocline; S, surface). Different letters on the bars indicate significant differences in Tukey comparisons.

Table 3. – Two-way analysis of variance to compare the mean values of the standardized RNA/DNA index between time of day and depth for *Engraulis anchoita* larvae collected in December 2018. Standard length was used as a covariate (ANCOVA).

	SS	df	MS	F	p-value	Coeff.
Model	343.61	5	68.72	38.36	<0.0001	
Depth	37.19	2	18.60	10.38	<0.0001	
Time of day	38.01	2	19.01	10.61	<0.0001	
SL (mm)	268.40	1	268.40	149.82	<0.0001	0.30
Error	1707.34	953	1.79			
Total	2050.95	959				

Finally, the percentage of larvae in starvation (RDs<CRDs) and the percentage of specimens in optimal growth (Gpf>1) were mapped in both species. A higher percentage of anchovy larvae in starvation was observed at the stations outside the position of the NPFS, and a higher percentage of larvae in optimal growth was detected at the internal stations (Fig. 5C-F, Table 4). It was observed that between 18% and 32% of anchovy larvae were in starvation and between 24% and 44% above optimal growth inside the NPFS. At offshore stations between 59% and 66% of the anchovy larvae were in starvation and the percentage above optimal growth was low (0% to 6%). Of the hake larvae, it was observed that 25% were in starvation and 12.5% above optimal growth.

Table 4. – Percentage of *Engraulis anchoita* larvae in starvation (RD<CRD) and above optimal growth (Gpf>1) in each oceanographic section (S) grouping the internal (i) and external (e) stations in relation to the position of the tidal front (S1, S2i, S2e, S3i, S3e, S4).

Section	n	% starvation	% optimal
S1	209	22	44
S2i	262	19	24
S2e	50	66	6
S3i	110	18	25
S3e	75	59	0
S4i	255	32	27

DISCUSSION

Little information is available on the nutritional condition of the anchovy southern stock, so the results presented here are highly important. Somewhat lower RD values were observed than those previously reported in the study area (Diaz et al. 2016, Do Souto et al. 2019), and a large percentage of anchovy larvae were recorded below the critical value of the RD index in the zone external to the NPFS. The internal area of the NPFS showed a lower proportion of individuals under starvation and a higher incidence of individuals showing optimal growth.

Because the survey research was carried out during the beginning of the reproductive period of the Argentine hake, the number of hake larvae analysed was very low (Macchi et al. 2004), and was also reflected in the small size of the larvae. However, the RD values obtained for the nutritional condition of this species were similar to those recorded in previous studies, as were the percentages of hake larvae in starvation and in optimal growth conditions (Diaz et al. 2014, Cohen et al. 2021).

Various studies have inferred the importance of the NPFS as a nursery area for the southern anchovy stock (Bakun and Parrish 1991, Diaz et al. 2016, Do Souto et al. 2018), with better feeding conditions for anchovy and hake larvae in the area associated with the NPFS (Viñas and Ramírez 1996, Temperoni et al. 2014). Our results support this idea of better feeding conditions towards the NPFS.

Within the analysed area, the internal zone to the position of the tidal front seems to respond to the "Bakun triad" hypothesis (Bakun and Parrish 1991) in that the frontal structure would guarantee the stability of the water column as a result of vertical stratification, nutrient enrichment and retention of spawning products within a favourable habitat. It is evident that frontal systems play a fundamental role in the ecological processes of the ocean because they allow a high primary production, they are suitable areas for the reproduction and feeding of many nektonic species, they offer a suitable breeding environment for the feeding of the early stages of fish development, and they act as retention zones (Acha et al. 2004). NPFS could also be an area with a high concentration of prey, predators and competitors of anchovy and hake eggs and larvae (Mianzan and Guerrero 2000, Álvarez-Colombo et al. 2003, Diaz et al. 2016, 2020).

Regarding daily variations in nucleic acid content, the available information is not consistent: some authors have found differences in the RD ratio throughout the day, and others have observed no detectable pattern (Buckley et al. 1999). For example, Rooker and Holt (1996) while studying croaker larvae, found a marked daily pattern in the RD values. These authors observed that the RD values were higher during the day and lower at night and suggested that this phenomenon was due to differences in metabolic rates throughout the day, food requirements or food digestion. In this study, we observed an increase during the afternoon hours, but no statistically significant differences were observed during the morning and night. Previous studies have reported that *E. anchoita* larvae feed during daylight hours (Viñas and Ramírez 1996), which could

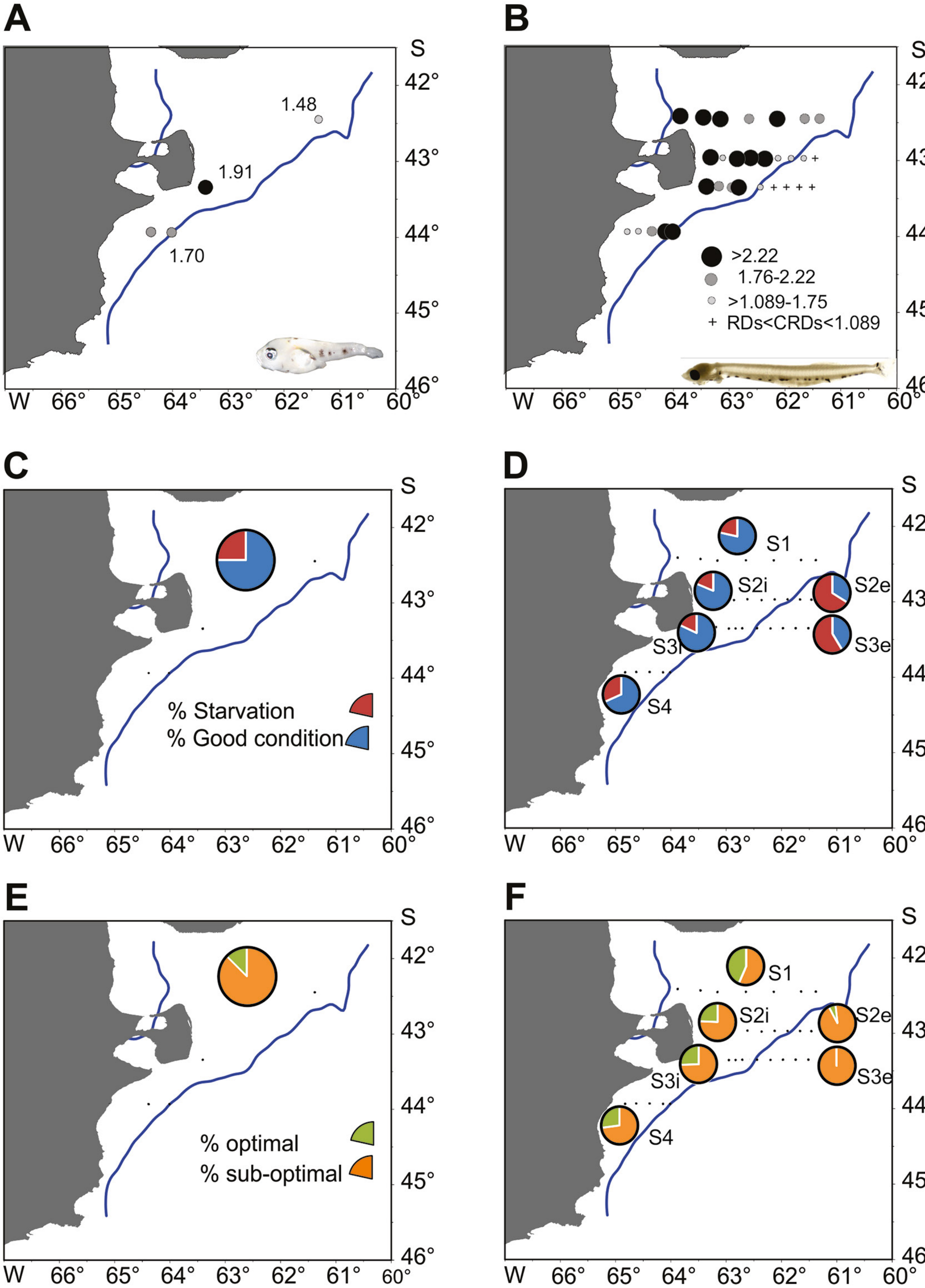

Fig. 5. –Average standardized RNA/DNA index (RD) obtained for each station studied for larvae of the hake *Merluccius hubbsi* (A) and the anchovy *Engraulis anchoita* (B). The size of the circles is proportional to the RD values obtained. Spatial distribution of the percentages of individuals below the critical standardized RNA/DNA index (CRD), i.e. in starvation, in red for (C) *Merluccius hubbsi* larvae and (D) *Engraulis anchoita* larvae and the percentage of individuals above the optimal Gpf (Gpf>1) in green, that is, in sub-optimal condition, for (E) hake *Merluccius hubbsi* larvae and (F) anchovy *Engraulis anchoita* larvae. Dots in (C) to (F) indicate the stations where the fish larvae were caught. S1, S2i, S2e, S3i, S3e, S4: oceanographic sections grouping the internal and external stations in relation to the position of the tidal front (blue line).

explain the increase in the RD values during the afternoon hours.

There is also conflicting evidence in the literature about the variability observed in the nutritional condition of larvae in the water column. Grønkjær et al. (1997) found that protein growth rates of *Gadus morhua* larvae were significantly higher for all age groups of larvae in the upper layers. In contrast, Dänhardt et al. (2007), studying different indicators of nutritional condition in *Sprattus sprattus* larvae, did not find a better nutritional condition in the superficial layers, although this was not the case in all the condition indicators used. Palomera (1991) observed that *E. encrasicolus* larvae mainly occurred above the level of the thermocline, and the highest abundances were recorded in the first 10 m of the water column. The thermocline could function as an upper or lower barrier for larval distribution, favouring the permanence in superficial layers with greater availability of prey associated with the depth of the thermocline (Smith and Suthers 1999). In our work, we found differences in anchovy larvae condition at different levels with respect to the vertical stratification of the water column. This fact should be considered in the future in designing the collection of samples and making comparisons of larvae condition. However, it is not easy to make comparisons between different studies because these aspects are very dependent on variables such as the zone, period, prey availability and species.

Although the anchovy is currently a low-exploitation species, it has a key position in the food web, regulating the systems towards lower and higher levels (Do Souto et al. 2018). Changes in the annual abundances and mean lengths of this species have recently been recorded, so studying its life traits during its early ontogeny is important for understanding the variability of its recruitment (Orlando et al. 2019). Currently, with a continuous increase in fishing effort, many of the main resources are exploited to the limit of their possibilities. The impact of fishing on ecosystems leads to a decline in commercial and non-commercial species. There is a clear need for an integrated vision for the correct management of fishing resources, including both commercial and non-commercial species in the analysis (Coll and Palomera 2007, Pauly 2009). Therefore, the measures adopted must consider integral care of the ecosystem, including zonation of the oceans and the generation of new MPAs (Pauly 2009).

Previous studies have shown that the application of MPAs has positively influenced the nutritional condition of fish species of commercial interest (Viladrich et al. 2016). The RD index and its derivatives are highly sensitive, which has made it possible to map the larval condition and establish favourable areas in relation to the NPFS. Studying nutritional condition allows us to estimate the probabilities of survival of the organisms and their potential recruitment to fisheries. The results presented here are relevant as a baseline for future studies that consider the evolution of the larval condition of these two species and evaluate the potential of areas as MPAs. The RD index is a simple and useful tool that provides complementary information to that provided by indicators of diversity and abundance usually used in strategies aimed at ensuring the conservation and comprehensive management of these fishery resources in the Argentine Sea.

In conclusion, the results presented herein are of great importance because little information is available on the nutritional condition of *E. anchoita* larvae from the southern stock. In this species, lower RD values were observed than those previously recorded in the study area. Nevertheless, as was hypothesized, the larvae collected in the areas influenced by the NPFS showed a good nutritional condition. The area inside the NPFS showed a lower proportion of individuals in starvation and a higher proportion of individuals in optimal growth, whereas the area outside the NPFS showed a high percentage of anchovy larvae below the critical value of the RD index. It was also observed that the hake RD values and the percentages of larvae in starvation at the beginning of the spawning period in the austral summer of 2018 were similar to those recorded in previous studies. Finally, the RD ratio and its derived index are highly sensitive, making it possible to "map" the larval condition and establish the most favourable areas for survival during the early phases of the life cycle.

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