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Larval condition of *Merluccius hubbsi* (Marini, 1933) in the northern Patagonian spawning ground

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ABSTRACT

Argentinean hake, *Merluccius hubbsi*, is one of the most important fishery resources of the South-western Atlantic Ocean, but its spawning biomass decreased in the last fifteen years, mainly due to the increased fishing pressure. It is widely accepted that to understand recruitment variability is necessary to study the factors that determine survival of early stages of development. Nutritional condition indexes have been widely used to determine starvation in wild larvae. In the present investigation, condition of *M. hubbsi* larvae was estimated employing field collected material by means of RNA/DNA index. This is the first attempt to use this index in this species. The change in the RNA/DNA index showed a clear ontogenetic pattern: increasing from preflexion to postflexion stages, showing an apparent decrease at the end of postflexion stage and a conspicuous increase once transformation stage was achieved. This pattern could be indicating that the transition between postflexion and transformation stages might represent a critical phase along larval development. RNA/DNA index also showed significant differences between areas from the spawning grounds characterized by different chlorophyll a concentration and abundance of potential prey. The study of nutritional condition represents a useful tool for identifying favorable nursery areas, providing valuable information for a comprehensive management of a population subject to overfishing.

1. Introduction

Argentinean hake, *Merluccius hubbsi*, is one of the most important fishery resources of the Argentine Sea, with annual catches between 170,000 and 370,000 t since 2000 (Sánchez et al., 2012). It inhabits the southwestern Atlantic Ocean waters from Cabo Frío in Brazil (22° S) to southern Argentina (55° S) at depths ranging from 50 to 500 m (Cousseau and Perrota, 1998). There are two fishing stocks, north and south of 41° S. Southern or Patagonian stock, which was the subject of this study, is the most important fishing resource, accounting for 85% of the total estimated biomass for this species. During the last years of the 90s decade the spawning biomass of both stocks dramatically decreased, which was mainly attributed to increased fishing pressure during previous years (Aubone et al., 2000). Lately the Patagonian stock also started to show changes in fish density, sex composition, size structure and spawning location. Historically, the reproductive activity of this group took place near Isla Escondida (43°30′ – 44° S and 65° W), mainly between November and April with a peak in December and January (Ehrlich, 1998; Macchi et al., 2004, 2007). From 1998, studies showed variations in the reproductive pattern of this species, suggesting the movement of reproductive adults offshore toward deeper waters and a decrease in the density of the shoals (Ehrlich et al., 2000; Macchi et al., 2010).

The hydrography of the Patagonian shelf is characterized by the formation of a frontal system, produced as a result of the dynamics of tides (average position of the tidal front indicated in Fig. 1a). This tidal front separates homogeneous coastal waters from stratified waters (Glorioso, 1987). This frontal structure is not permanent and its formation occurs during the austral spring and summer.

Tidal fronts are characterized by high availability of nitrate typical from areas of high biological productivity caused by phytoplankton blooms and large aggregations of copepods (Ramírez et al., 1990). This scenario creates a variety of suitable habitats for adult fish spawning and nursery areas for their eggs and larvae (Sánchez and Ciechomski, 1995). Bakun and Parrish (1991) characterized Patagonian tidal frontal area as a successful spawning region. Thus, hake traditional spawning area would fulfill with Bakun's "fundamental triad" principles (Bakun, 1996): surface layers enrichment due to the rise of deep rich in nutrients waters, the concentration

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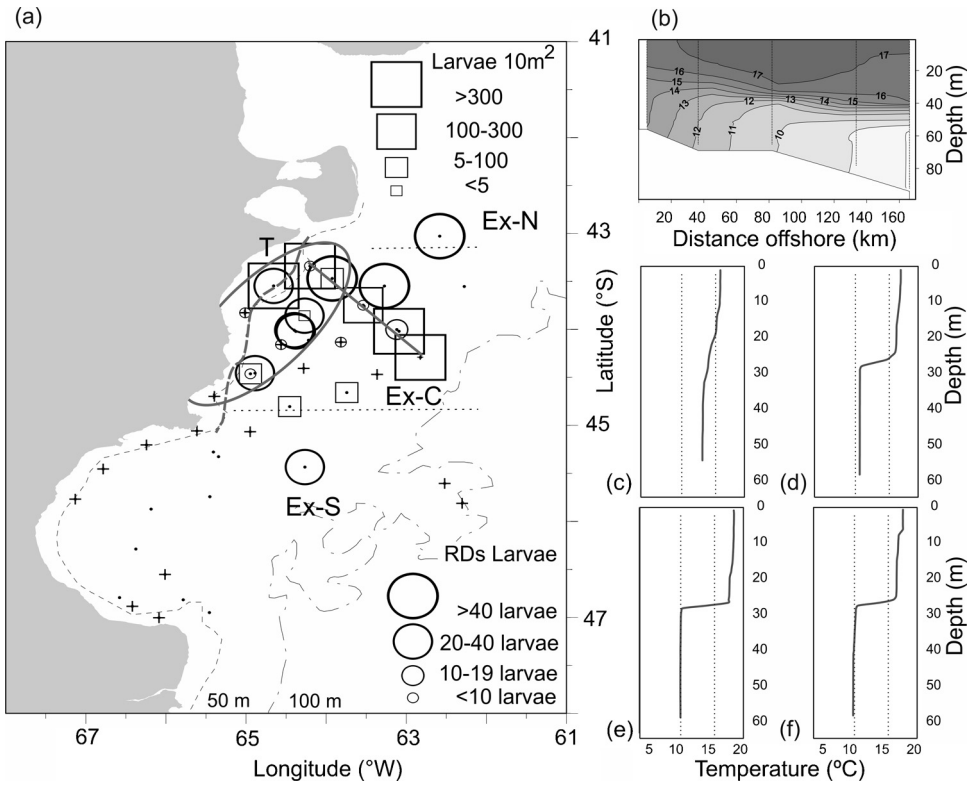

Fig. 1. Study area and larvae analyzed. (a) Encircled area indicates the schematic delimitation of the spawning grounds (from Álvarez Colombo et al., 2011). Gray dashed line shows the average position of the tidal front (critical Simpson parameter $\varphi = 40\,J\,m^{-3}$). Points indicate position of sampling stations, crosses indicate sampling stations without hake larvae, circles indicate larval densities and squares indicate larvae employed for RNA/DNA analysis. (b) Typical horizontal section (solid gray line in Fig. 1a) of temperature (°C) at the spawning ground during summer showing the well-mixed and stratified zones of the tidal front. Typical vertical section of temperature (°C) during summer in: (c) coastal stations of traditional spawning area, (d) offshore stations of traditional spawning area, (e) external northern area and (f) external southern area. T: traditional spawning area, Ex-N: external northern area, Ex-C: external central area, Ex-S: external southern area.

of planktonic organisms that are potential prey for larvae and the retention of eggs and larvae in favorable areas. The combination of these processes ensures survival of fish early life stages.

The observed changes in hake spawning pattern causes that hatching takes place in sites with different environmental features to those found close to frontal zones, and probably less favorable for larval growth and survival. The questions arising from the above are: What happens to larvae that hatch away from high-productivity traditional spawning areas? Will they have the same survival chances? Will they be more affected by starvation?

Nutritional condition indexes have been widely used to determine the importance of starvation in larvae caught in the ocean (Buckley, 1984; Clemmesen, 1994; O'Connell, 1980; Robinson and Ware, 1988; Theilacker, 1986). Different criteria have been developed to assess nutritional condition of fish larvae based on the differences that starvation produces in body form, condition factor, chemical cell constituents and histological integrity. These methodologies were extensively reviewed by Ferron and Leggett (1994). They divided condition indices into three main categories according to the main organization levels: organism, tissular and cellular. These indices operate at different time scales. Typically, the higher the organization level of the index, the longer it takes to respond to an environmental change. Morphometric techniques or lipid content determination allows to assess physiological changes on a weekly scale, growth rates (RNA/DNA, otolith growth, cell cycle studies) or indices of starvation (histological indexes such as intestinal epithelial cells height) give information about changes on a scale of days and rates of feeding (e.g. stomach fullness or trypsin activity) on a scale of hours. Many studies suggest that RNA/DNA index is one of the best indicators of the nutritional status of various marine organisms (e.g. Bailey et al., 1995; Clemmesen, 1994; Folkvord et al., 1996) and is currently the most widely used biochemical indicator of nutritional condition of fish larvae (Caldarone et al., 2003; Clemmesen, 1994; Grote et al., 2012; Malzahn et al., 2007; Meyer et al., 2012; Paulsen et al., 2013). The RNA/DNA index is an ecophysiological indicator that provides a measure of the capacity for synthesis of cells and usually correlates with the nutritional status of individuals (Buckley et al., 1999; Ferron and Leggett, 1994). The theoretical principle of using RNA/DNA ratio is based on the fact that DNA content is virtually constant in somatic cells, so that tissue concentrations reflect the number of cells and is independent of nutritional status, while the amount of cellular RNA, mainly ribosomal RNA (rRNA) available in the tissues is directly proportional to the level of protein synthesis (Clemmesen, 1994). RNA/DNA ratio varies with age, stage of development, size of the individual and changing environmental conditions (Bulow, 1970). It has also proved to be susceptible to environmental changes that affect the physiology of organisms, such as a low concentration of prey (Chícharo and Chícharo, 1995; McGurk et al., 1992). Thus, the organisms in good condition usually have higher values of the ratio RNA/DNA than those which are in a poor condition (e.g. Clemmesen, 1994; Robinson and Ware, 1988).

Due to the overfishing that hake has suffered in recent years, studies on reproduction and recruitment of this species have been intensified. There is detailed information on spatial and temporal distribution of the species, breeding and rearing, egg production and reproductive parameters (e.g. Macchi et al., 2004, 2006, 2007, 2010; Pájaro et al., 2005; Renzi et al., 2000, 2002). However, up to now, no research has been conducted on the nutritional condition of larvae. Thus, this study represents the first approach to this issue and the first time RNA/DNA determinations are made employing this species.

Table 1
General information about samplings and material employed from the different studied areas to determine nutritional condition of *Merluccius hubbsi* by means of RNA/DNA index.

Date	Plankton net	Time	Lat. (° S)	Long. (° W)	Depth (m)	Area	*N*	Temp. (°C)
02 Feb 2011	Bongo/RMT	21:40	44.46	64.95	76	T	28	11.84
07 Feb 2011	Bongo	6:25	43.04	62.56	75	Ex-C	2	09.55
07 Feb 2011	RMT	23:47	44.13	63.81	75	Ex-S	25	10.55
08 Feb 2011	Bongo	6:15	44.11	64.23	55	T	1	13.18
08 Feb 2011	Bongo	11:20	43.82	65.02	73	T	4	11.94
08 Feb 2011	EBS/RMT	12:50	44.00	64.41	70	T	34	11.94
08 Feb 2011	Bongo	16:30	44.15	64.58	67	T	11	11.94
08 Feb 2011	Bongo	19:50	43.84	64.28	46	T	14	15.49
09 Feb 2011	Bongo	0:45	43.55	64.66	56	T	6	14.62
09 Feb 2011	Bongo	2:27	43.33	64.23	69	T	25	11.67
09 Feb 2011	Bongo	5:28	43.47	63.92	69	Ex-C	7	10.08
09 Feb 2011	EBS	11:39	43.75	63.55	84	Ex-C	16	08.97
09 Feb 2011	RMT	21:45	43.99	63.12	68	Ex-C	51	10.36
10 Feb 2011	EBS	12:18	43.54	63.28	76	Ex-N	70	10.82

N, larvae employed for nutritional condition analyses; Temp, mean temperature registered under the thermocline (°C); RMT, Rectangular Midwater Trawl; EBS, epibenthic sampler; T, traditional spawning area; Ex-N, external northern area; Ex-C, external central area; Ex-S, external southern area.

The information obtained from this study will determine whether all areas of the nursery grounds are equally favorable for larval survival, and thus, will provide tools to explain the variations in recruitment and may be used for the management of this species under severe fishing pressure.

2. Materials and methods

2.1. Sampling and larval source

Larvae were collected during a research survey carried out in northern Patagonian coasts during January–February 2011, within the spawning peak of *M. hubbsi* southern stock. Samples were taken employing a 300 μm mesh Bongo net performing oblique tows from about two meters from bottom to surface. Additionally samples were taken according to the acoustics records with a 500 μm mesh Rectangular Midwater Trawl (RMT) and when the acoustic signal was close to the bottom a 1000 μm mesh epibenthic sampler (EBS) was used (Álvarez Colombo et al., 2011). Oceanographic data were obtained using a SBE 19 Seacat CTD profiler at each station. Moreover, information about chlorophyll *a* concentration, quantified from surface water samples, and abundance of the different copepod developmental stages, estimated from plankton samples collected with a 220 μm mesh minibongo net was analyzed in relation to the spatial variation of larval condition. Zooplankton samples were collected performing oblique tows from about two meters from bottom to surface.

Larvae for condition analyses were sorted out on board and frozen in liquid nitrogen. Standard length (SL) was measured to the nearest 0.01 μm using a Carl Zeiss stereoscope equipped with Axio Vision software. A developmental stage was assigned according to Betti et al. (2009). Head and gut (including the adjacent organs, liver and pancreas) were separated from the rest of the body by means of a scalpel in order to prevent the influence of viscera and brain tissues on RNA/DNA index among developmental stages (Olivar et al., 2009). This procedure was performed on ice to avoid nucleic acids degradation. Larvae were freeze dried and stored at −80 °C until analyses were carried out ($N = 275$). See Table 1 for information about samplings. Individual larval trunk was weighed to the nearest μg employing a Sartorius microbalance. From larval length distribution and length-age relationship obtained by Brown et al. (2004) for hake larvae collected in January, we assume that the age range of larvae herein employed was between two weeks (4 mm) and more than two month from hatching (11 mm).

2.2. Analytical protocol

Analysis of RNA and DNA contents was performed by a modification of the protocol published by Caldarone et al. (2001) and Caldarone (2005) for fish larvae. The main modification was related to the use of 1 ml of assay sample instead of microplate. 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 (type II-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 A treatment, which is assumed to be due to DNA. Concentrations of nucleic acids were determined by running standard curves of DNA and RNA with EB every day, with known concentrations of calf thymus DNA and yeast RNA, in the appropriate range of concentrations. The average ratio of the slopes of DNA and RNA standard curves was 2.50 ± 0.36.

All RNA/DNA values obtained from our analyses were standardized (RDs) according to the procedure described in Caldarone et al. (2006) using 2.4 as the reference slope-ratio value. RDs values were used in all the statistical analyses.

Growth rate (G) was estimated employing the best-fit meta-analysis model RDs – $T-G$ obtained by Buckley et al. (2008), developed for other Gadiformes (cod and haddock):

$$G = 0.0254 \times \mathrm{RDs} + 0.0037 \times T \times \mathrm{RDs} - 0.0873 \quad (1)$$

where G is the instantaneous growth rate, RDs the standardized RNA/DNA index, and T the median in situ temperature below the thermocline since this depth strata is where hake larvae are mainly found (Álvarez Colombo et al., 2011).

To estimate the RDs threshold level for the growth of hake larvae, the turning point from positive G to negative G was calculated, followed by back-calculating the related RDs of this turning point.

Growth performance (Gpf), the quotient of the observed growth rate and the growth rate achieved by larva 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 *M. hubbsi*, 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 species:

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

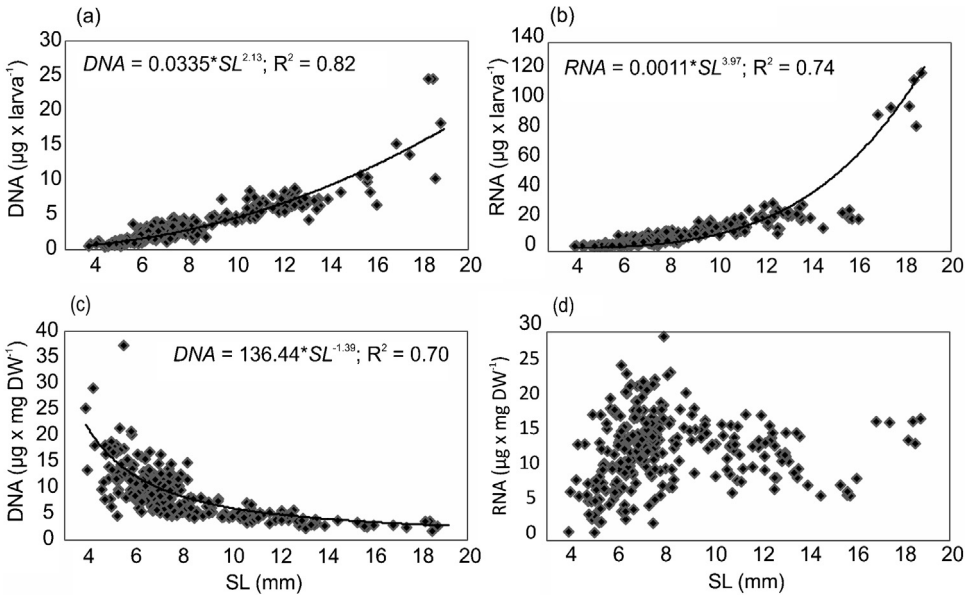

Fig. 2. Nucleic acids content in relation to *Merluccius hubbsi* larval standard length (SL), (a) DNA µg × larva^{-1}, (b) RNA µg × larva^{-1}, (c) µg DNA × mg DW^{-1} and (d) µg RNA × mg DW^{-1}.

2.3. Statistical analysis

Differences among four defined areas within the spawning grounds were determined through ANOVA followed by Tukey's Honestly Significant Difference test. Due to the rather reduced number of samples, data did not meet ANOVA assumptions of normal distribution and homogeneous variances, but it is generally agreed that the ANOVA is robust against violations of these assumptions. Nevertheless, complementary, we performed a non-parametric Kruskal–Wallis test followed by multiple comparisons and results were identical to those obtained employing ANOVA. The traditional spawning area was defined according to Álvarez Colombo et al. (2011), and three external additional areas were defined: northern, central and southern (see Fig. 1).

3. Results

3.1. Hydrography

A clear separation between the coastal area, characterized by well-mixed waters (Fig. 1c), and the offshore stratified region was observed within traditional spawning area. A sharp thermocline of varying thickness at 30–40 m depth separated the upper and bottom waters in most of the sampled stations: offshore stations of traditional spawning area and external central area (Fig. 1d), as well as external northern (Fig. 1e) and southern (Fig. 1f) areas.

3.2. Ontogenetic changes in nucleic acid content and RD index

Ontogenetic changes in RNA and DNA contents per larva and dry weight unit are shown in Fig. 2. A positive relationship was observed between larval nucleic acid content and SL (Fig. 2a). On the other hand, DNA content per dry weight unit (µg × mg DW^{-1}) showed a reduction with larval size but RNA per dry weight did not show a clear pattern with SL (Fig. 2b).

The RDs ratio increased slightly from preflexion to postflexion stages (4–8 mm SL), maintaining a steady level within postflexion stage. At the end of the latter developmental stage (15–16 mm SL) the ratio first showed a slight decrease and then a significant increment until transformation stage (Fig. 3). Mean RDs values resulted significantly different between developmental stages ($F_{(3,271)} = 113.34$; $P < 0.001$), with a mean and standard deviation of 0.86 ± 0.53 for preflexion larvae stage, 1.54 ± 0.66 for flexion stage, 2.51 ± 1.05 for postflexion and 6.62 ± 2.01 for transformation stage individuals. Besides, no correlation was found between RD and temperature for any of the analyzed developmental stages (Pearson's correlation coefficients: −0.19, −0.10, 0.13 and −0.98 for preflexion, flexion, postflexion and transformation stages respectively, $P > 0.01$ in all the correlations).

3.3. RD index within nursery grounds

Since RDs index showed an ontogenetic pattern, comparisons between different areas of nursery grounds were made within a certain developmental stage. No differences were found between mean RDs obtained for preflexion larvae from different areas. Maximum RDs mean values for flexion, postflexion and transformation individuals were observed within external central area followed by traditional spawning area. The lowest RDs values were observed for preflexion, flexion and postflexion larvae from northern area (Fig. 4 and Table 2). The lowest growth rate and growth performance values were observed for flexion and postflexion larvae from northern area (Table 2).

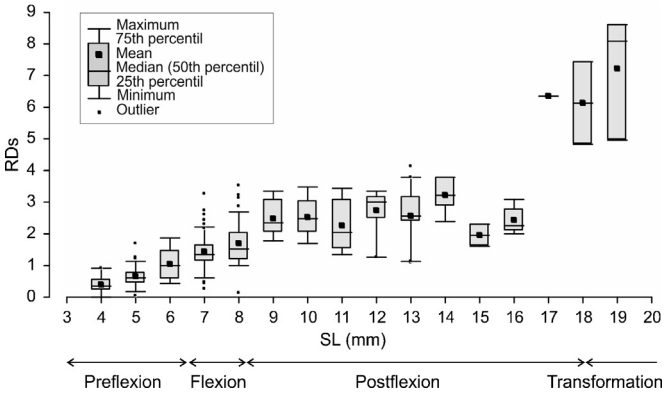

Fig. 3. Box-plot diagram of standardized RNA/DNA ratio (RDs) obtained for each size class of *Merluccius hubbsi* larvae. Developmental stages are indicated.

Table 2
Mean and standard deviation of standardized RNA/DNA ratio values (RDs), growth rate (G) and growth performance (Gpf) for each developmental stage of *Merluccius hubbsi* larvae.

Stage/area	Parameter	T	Ex-N	Ex-C	Ex-S	Differences between areas
Preflexion	RDs	0.84 ± 0.57	0.75 ± 0.44	1.20 ± 0.49	1.14 ± 0.37	$P > 0.01$
	G	−0.02 ± 0.04	−0.03 ± 0.03	−0.01 ± 0.03	−0.01 ± 0.02	$P > 0.01$
	Gpf	−0.03 ± 0.04	−0.04 ± 0.03	−0.01 ± 0.03	−0.01 ± 0.02	$P > 0.01$
	N	51	23	6	5	
Flexion	RDs	1.26 ± 0.37	1.24 ± 0.48	1.96 ± 0.71	1.74 ± 0.49	$P < 0.001$
	G	0.01 ± 0.03	−0.001 ± 0.03	0.04 ± 0.05	0.03 ± 0.03	$P < 0.001$
	Gpf	0.002 ± 0.03	−0.01 ± 0.03	0.03 ± 0.05	0.03 ± 0.03	$P < 0.001$
	N	18	39	37	4	
Postflexion	RDs	2.68 ± 1.01	1.55 ± 0.40	2.68 ± 1.22	2.14 ± 0.62	$P < 0.05$
	G	0.10 ± 0.07	0.02 ± 0.03	0.08 ± 0.07	0.05 ± 0.04	$P < 0.05$
	Gpf	0.10 ± 0.07	0.01 ± 0.03	0.08 ± 0.08	0.05 ± 0.04	$P < 0.05$
	N	36	6	30	16	
Transformation	RDs	4.89 ± 0.09	–	8.34 ± 0.38	–	$P < 0.001$
	G	0.25 ± 0.004	–	0.42 ± 0.02	–	$P < 0.001$
	Gpf	0.25 ± 0.005	–	0.44 ± 0.02	–	$P < 0.001$
	N	2		2		

T, traditional spawning area; Ex-N, external northern area; Ex-C, external central area; Ex-S, external southern area.
Differences among areas within a developmental stage, after one way ANOVA followed by a posteriori Tukey's test are indicated.

The RDs threshold level for growth was calculated for each larva employing Eq. (1) assuming a growth rate equal to zero ($G = 0$) and using the registered temperatures at the station where each larva was caught. Temperature ranged between 9 and 15 °C and the RDs threshold from 1.49 and 1.08.

A RDs threshold for growth was calculated for each area employing the mean temperature registered. It was 1.198 for the traditional spawning area, 1.334 in the external northern area, 1.421 in the external central area and 1.355 in the external southern area. Individual larva RDs in each area are showed in Fig. 5 and threshold for growth is indicated. The external northern area and traditional spawning area had higher proportion of larvae below threshold value, being 58% and 45% respectively. The external central and southern area showed only 24% of larvae below threshold value. Larvae with RDs indexes below threshold for growth were mainly preflexion and flexion individuals smaller than 8 mm SL. When we compared RDs obtained employing only larvae smaller than 8 mm SL the external northern area and traditional spawning area had higher proportion of larvae below threshold value, being 66% in both areas. The external central and southern area showed only 37% and 55% of larvae below threshold value respectively.

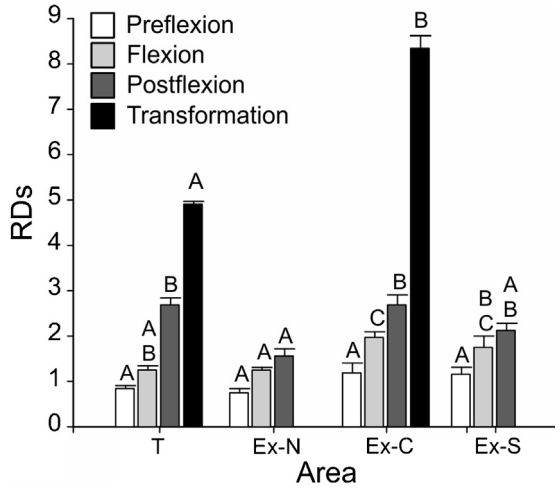

Fig. 4. Mean standardized RNA/DNA ratio (RDs) in relation to *Merluccius hubbsi* larvae developmental stages from analyzed areas, T: traditional spawning area, Ex-N: external northern area, Ex-C: external central area, Ex-S: external southern area. Different capital letters indicate significant differences ($P < 0.001$) among areas within a developmental stage, according to a posteriori Tukey's test.

4. Discussion

4.1. General considerations

Many studies have shown evidence that the RNA/DNA ratio is one of the best indicators of the nutritional condition and growth of several marine organisms (e.g. Bailey et al., 1995; Clemmesen, 1994; Folkvord et al., 1996). To date, it is the most widely used biochemical index to determine larval condition, especially for clupeids larvae since they are abundant and easily obtained. Nevertheless, for the many different hake species around the world, there is only one work available on this issue, i.e. for the southeast Atlantic Ocean, *Merluccius capensis* and *Merluccius paradoxus*, employing individuals younger than one month old (Grote et al., 2012). In the present study we analyzed RDs changes along the entire early ontogeny of *M. hubbsi* (larval stages and transformation) and compared different areas within the species nursery grounds.

Moreover, the use of the standardization methods established by Caldarone et al. (2006) and Buckley et al. (2008) for RD indexes allows direct comparisons with results from other studies, regardless of the nucleic acids quantification protocol employed, fact that has contributed to make this technique a powerful tool.

4.2. Ontogenetic changes in nucleic acid content and RD index

Nucleic acids contents per larval weight unit showed a reduction in DNA concentrations ($\mu g \times mg\,DW^{-1}$) related to size increment. This is due to the fact that DNA content, the primary carrier of genetic information, is stable within the somatic cells of a species and when cellular size increases, DNA amount stays stable (hypertrophy). On the other hand RNA concentrations ($\mu g \times mg\,DW^{-1}$) did not show a clear pattern with the increment in size of the specimens. Since the amount of RNA is directly involved in protein synthesis, this is related to larval condition, varing with age, life-stage, organism size, disease state and changing environmental conditions (Bulow, 1970). The change in the RD index showed a clear ontogenetic pattern: increasing from preflexion to postflexion stages, showing an apparent decrease at the end of postflexion stage and a conspicuous increase once transformation stage was achieved. This pattern could be indicating that the transition

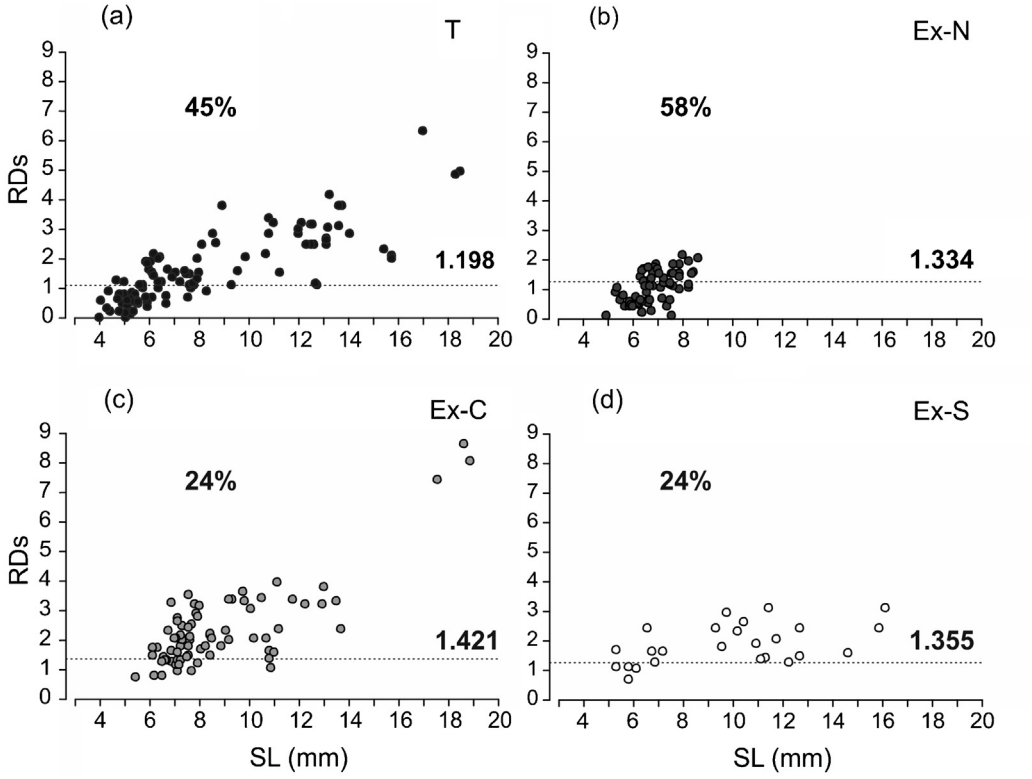

Fig. 5. Standardized RNA/DNA ratio (RDs) in relation to *Merluccius hubbsi* larvae standard length (SL) from analyzed areas, (a) traditional spawning area (T), (b) external northern area (Ex-N), (c) external central area (Ex-C) and (d) external southern area (Ex-S). Dashed line indicates the threshold RDs level for growth at mean temperature below thermocline obtained in each area. Percentage of larvae below RDs threshold level for growth is indicated in each area.

between postflexion and transformation stages might represent a critical phase along larval development. This is an issue that needs to be further validated with a larger number of samples, since only a small amount of late postflexion larvae and transforming specimens were available in the present study.

The transformation stage is characterized by important changes in the overall shape and structural features involving loss of larval characters and the acquisition adult ones (Moser, 1996). This transition between larvae and juvenile is accompanied by behavioral and habitat changes (Maynou et al., 2006). In demersal species such as hake, transformation individuals leave the pelagic habitat and begin the settlement process. These habitat switch also involve changes in biotic (i.e. available prey, aggregation indices, presence of new predators) and abiotic aspects (i.e. temperature, light, oxygen). Thus, adapting to the new environment (new diet, new predators and different physical conditions) implies a huge energetic cost, and makes this stage a particularly vulnerable one (Balon, 1985; Watanabe and Saito, 1997). Several authors pointed out the importance of the mortality during metamorphosis from larval to juvenile stages and its possible effect on recruitment variability (Bradford, 1992; Cushing, 1996; Myers and Cadigan, 1993; Thorisson, 1994).

Grote et al. (2012) found a similar pattern in the trend of the RDs index along larval development, since they obtained also an increment between preflexion and postflexion stages for larvae of *M. paradoxus* and *M. capensis*. However, there is no data available of any other species of hake with which to compare over the second month of life. On the other hand, RDs indexes herein obtained for *M. hubbsi* resulted somewhat lower than those showed by Grote et al. (2012) for pre-flexion larvae, but values obtained for flexion and postflexion larvae were within the range observed for *M. paradoxus* and *M. capensis*, and even higher if the provenance of the larvae is distinguished.

According to our results, Gwak and Tanaka (2001) observed a reduction in the RNA/DNA index of *Paralichthys olivaceus* larvae after larval settlement. This result was attributed to the energy cost required by morpho-physiological changes at this developmental stage and the starvation period suffered during settlement. This period of high mortality was characterized as a second critical period, following yolk absorption, in the ontogeny of larval *P. olivaceus* (Gwak and Tanaka, 2001; Gwak et al., 2003). Additionally, Tanaka et al. (2007) observed an important increase in RNA/DNA index toward the end of metamorphosis of *Thunnus orientalis* larvae, in coincidence with feeding switch from small prey (mainly mesozooplankton) to large prey (mainly fish larvae). Thus, the noticeable RDs increase here observed during the transformation stage may be explained by the feeding switch from mesozooplankton to macrozooplankton preys (mainly euphausiids and mysids) as was reported for *M. hubbsi* (Moriondo et al., 2001).

4.3. RD index within nursery grounds

Excepting for a few coastal stations within tradition spawning area, all sampled stations of Patagonian hake stock nursery grounds resulted similar in terms of hydrographical features. The transition zone between the homogeneous and stratified sectors of the frontal system was located in the inner shelf, with the well-mixed region confined to a narrow band near the coastline. Therefore, most of the habitat of hake larvae corresponded to the stratified offshore waters.

Larvae collected in the external northern area showed significantly lower condition indexes in comparison to those from the other areas studied. Probably these specimens have been transported to this area, but had little chance to survive given the low RDs values recorded and the high proportion of individuals below growth threshold. In other hake species it has been reported that

Table 3
Mean and standard deviation of temperature below thermocline (Temp, °C), surface chlorophyll *a* (Chl *a*, mg × m^{-3}), copepod nauplii density, small copepods (<1 mm CL) density, medium copepods (1–2 mm CL) density, big copepods (>2 mm CL) density (individuals × m^{-3}), obtained in each area.

Zona	Temp. (°C)	Chl *a*[a]	Nauplii[b]	Small cop.[b]	Medium cop.[b]	Big cop.[b]
T	12.83 ± 1.47	2.03 ± 1.31	3797 ± 3254	5029 ± 2741	1513 ± 816	301 ± 123
Ex-N	10.82 ± 0.02	0.32	–	–	–	–
Ex-C	9.74 ± 0.61	1.22 ± 0.34	580 ± 536	10,851 ± 209	6828 ± 1618	522 ± 112
Ex-S	10.55 ± 0.11	1.07 ± 0.43	606	8700	3515	1391

T, traditional spawning area; Ex-N, external northern area; Ex-C, external central area; Ex-S, external southern area.
[a] Unpublished data, A.D. Cucchi Colleoni, pers. comm.
[b] Unpublished data, M.D. Viñas and B. Temperoni, pers. comm.

larvae transported offshore can be trapped in an unfavorable environment and eventually die (Álvarez et al., 2001; Hollowed and Bailey, 1989). The circulation in the study area is characterized by a flow in two layers, wherein the upper layer moves toward the northeast while the lower layer moves much more slowly in the opposite direction (Palma et al., 2008). Álvarez Colombo et al. (2011) have shown that retention of hake larvae in northern Patagonia and the location of the main settlement site can be explained by the coupling of behavior and circulation patterns. Larvae of sizes below 4 mm SL, (less than seven days after hatching), concentrate at thermocline depth, a layer with little movement, which helps to minimize dispersion (Álvarez Colombo et al., 2011). The thermocline layer also concentrates eggs and larval stages of copepods (Viñas and Santos, 2000), thus including calanoid copepodites smaller than 2 mm that are hake preferred prey items (Temperoni and Viñas, 2013). Hake larvae in advanced developmental stages with functional swim bladders are able to perform diurnal vertical migrations, remaining near the bottom during the day and ascending to the depth of the thermocline at night (Álvarez Colombo et al., 2011). However, since the retention process is not perfect, some advective losses are expected. The presence of larvae in poor condition in northern external zone could be due to the fact that larvae in deficient nutritional condition are usually in upper layers of the water column due to the lack of buoyancy control (Neilson et al., 1986; Sclafani et al., 1993) and have lower swimming ability (Faría et al., 2011) than healthy larvae, and therefore are less able to perform daily vertical migrations and to remain in the spawning area. Thus, those larvae showing a bad physiological condition are more likely to be in the upper layer of water that flows in northeast direction and shipped away from traditional spawning grounds.

In addition, Macchi et al. (2010) detected an expansion of the *M. hubbsi* spawning activity into deeper waters and reported the presence of a breeding group of hake near the 100 m isobath, which has increased in abundance from 2006. Taking into account the circulation patterns described for northern Patagonia and the fact that offshore high levels of recently settled juvenile hake have been occasionally detected (Machinandiarena et al., 2006), the presence of larvae of this species in the external southern area could be a result of these external spawnings reported in recent years. Since individuals from this last area showed moderate condition indices, it could be considered that they will recruit to the stock in these southernmost breeding areas. Thus, according to the condition indices obtained, the larvae originating from the spawning at the external southern areas, or which had been transported there, would be more likely to survive than those that have been transported to the northern area.

The combination of enrichment, concentration and retention processes postulated by Bakun's "fundamental triad" in hake breeding area would ensure the survival of early life stages of this species. Biological production is enhanced by physical and biological processes coupled in front of tides. In the present study most hake larvae were found in the stratified offshore waters, that were separated from the homogeneous coastal strip by a frontal region (indicated in Fig. 1), located in the inner shelf (Sabatini and Martos, 2002). Several studies carried out in this same research area, have reported high concentrations of chlorophyll *a* and microzooplankton in relation to the frontal system (Sabatini and Martos, 2002; Viñas and Ramírez, 1996; Viñas and Santos, 2000). The presence of a marked thermocline (see Fig. 1b and c) favors organisms' aggregations ensuring high concentrations of plankton prey for larvae. Thus, Feeding on highly concentrated prey patches makes larval feeding efficient since prey searching costs are diminished. Moreover, the high chlorophyll *a* values registered at the thermocline level probably favor the occurrence of high densities of herbivorous calanoid copepods which are the preferred prey for hake larvae (Temperoni and Viñas, 2013). The traditional spawning area showed the highest chlorophyll *a* concentrations and copepod nauplii densities (Table 3). Also small copepods (<1 mm CL) were abundant there. The external central area showed intermediate chlorophyll *a* concentrations but higher densities of medium copepods (1–2 mm CL). The external southern area showed lower chlorophyll *a* concentrations but the highest density of big copepods (>2 mm CL). Even though no data about copepod abundance was available for external northern area, it could be assumed that this area was less productive than the others since the lowest chlorophyll *a* concentration was recorded there. These data support the idea that the traditional spawning area and the external central area are more favorable for hake larvae survival and growth. This could explain the higher RDs observed since the high concentrations of prey available allows greater larval feeding efficiency, since energy costs associated with foraging would be markedly reduced.

RDs indexes determined in this study showed an important variability and were not correlated with temperature. Buckley et al. (2008) studying a multi-specific model based on temperature and RNA/DNA indexes, found a lack of correlation between these two variables when reared larvae were in a well-fed situation. The high concentration of prey that occurs in the thermocline stratum may also explain the lack of correlation between RDs indexes and temperature in our study. Future combined studies of abundances of potential prey, RDs indexes and stomach contents will allow establishing whether the lack of correlation between RDs indexes and temperatures observed here is consequence to the fact that food actually was not a limiting factor. Moreover, the lack of correlation with the temperature may indicate that other factors might be influencing larval condition such as maternal effects or quality of the food eaten (Field et al., 2008; Grote et al., 2012; St. John et al., 2001).

Unlike what was observed by Grote et al. (2012) for *M. paradoxus* and *M. capensis* larvae, in this study we found that a significant proportion of larvae of *M. hubbsi* were below the RDs index threshold for growth. In particular, when distinguishing between different areas of the spawning grounds, the external northern area had a marked proportion of individuals below this threshold, giving evidence of starvation. Since larvae showing a poor nutritional condition would be more vulnerable to predation, the discrepancies between these studies could be due to dissimilar degrees of predation in different ecosystems.

To estimate the incidence of starvation in the ocean is needed to set laboratory calibrations and determine the critical RNA/DNA value that indicates that larvae are starving. The critical values of the RNA/DNA index available in the literature are typically between 1.0 and 3.0 (Ferron and Leggett, 1994) depending on temperature and other factors. Since, there is no information for hake larvae, this calibration should be determined using larvae reared in aquarium and subjected to different feeding treatments in future studies.

The spatial pattern of Argentine hake larval distribution in consecutive years showed that the bulk of larvae are mainly found round the traditional spawning area, suggesting the existence of intense retention mechanisms for larvae (Álvarez Colombo et al., 2011). However, significant inter-annual variability has been observed which may contribute, at least partially, to fluctuations in the recruitment of this fishing stock. Given the complexity of the circulation in the spawning grounds and the biophysical coupling for the distribution of hake early developmental stages demonstrated by Álvarez Colombo et al. (2011), it is required to study the spatial variation of annual spawning, larval abundance and condition, as well as the abundance of juveniles in the following year. This would improve the understanding of the impact of the observed changes in the distribution pattern of hake spawning on the recruitment of Patagonian stock of this species.

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