TROPHIC POSITION AND NUTRITIONAL CONDITION OF THE ANCHOVY ENGRAULIS ANCHOITA LARVAE IN THE CABO FRIO REGION, BRAZIL

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ABSTRACT. – In order to verify the trophic position and condition of Engraulis anchoita larvae, this study applied stable isotope technique and condition indexes. The area comprises the continental shelf off the Cabo Frio (from 40 to 120 m depth), where a seasonal upwelling of the South Atlantic Central Water (SACW) occurs in summer. Larvae were sampled in winter 2001 and in summer 2002. Stable isotopes of natural carbon (\(\delta^{13}C\)) and nitrogen (\(\delta^{15}N\)) were measured in larval pools separated by length whereas RNA/DNA, protein and histology were performed for individual larvae. The values of \(\delta^{13}C\) for larvae of different developmental phases were similar in winter and summer. Big larvae had a \(\delta^{15}N\)-enrichment of almost 3‰, in relation to the small ones, showing an increase of trophic level in summer. Despite the variation of RNA/DNA, protein values and histological classification, the tendency of better condition larvae in summer was clear. This variation is related to scenarios of SACW upwelling over the shelf. Those findings agree with the suspended particulate matter and zooplankton isotope values in winter that suggested a more oligotrophic situation than in summer. These results indicate that nutrient enhancement is coupled to better Argentine anchovy larval condition.

INTRODUCTION

Small pelagic fish represent a key role in the oceans, as they are the link between producers, small consumers and the highest trophic levels (Costalago 2015). Regime shifts can alter plankton communities and the variability in feeding environment is responsible for larvae growth rate variations and population fluctuations (Yasue et al. 2014).

Key processes involved in feeding of fish larvae are not completely understood due to the wide range of development patterns and feeding habits in teleosts (Ronnestad et al. 2013). Most studies on the trophic ecology of fish larvae have been based on the direct analysis of stomach content (Uehara et al. 2005). The main items often found in the stomach contents of small pelagic fish larvae are copepod nauplii, copepodids, small copepods, cocolithophorids, flagellates, ciliates, and invertebrate eggs, indicating that the larvae present a variety of diets, including particles of different sizes (Morote et al. 2010, Ara et al. 2011). Phytoplankton, composed of size classes smaller than 200 μm, can be consumed by microherbivores or directly by the larvae of fish including Clupeiformes as anchovies and sardines (Vasconcelos et al. 1998, Kurtz & Matsuura 2001). The main characteristic of this kind of study is the taxonomic accuracy relative to prey identification. On the other hand, it also introduces methodological errors because it cannot identify the prey items that are really assimilated, and its results are limited to a very short period (Vander Zanden et al. 1998), reflecting transient oceanographic situations. On the Brazilian coast, the knowledge on the feeding of small pelagic fish larvae is poor and based on the stomach content analysis (Freire 1995, Pedreira 1997, Kurtz & Matsuura 2001).

The stable carbon and nitrogen isotopic analysis have been used as tools for several purposes in the aquatic system, especially for trophic studies (Fry 2006). The primary producers have different carbon signatures that can be followed, since they show little change between trophic levels, enabling the understanding of the source of organic matter. The stable isotopes of nitrogen may be useful for identifying the trophic level. The isotopic signatures of consumers reflect the isotopic composition of their diet, with an enrichment of carbon (\(^{13}\text{C}/^{12}\text{C}\) or \(\delta^{13}\text{C}\)) and nitrogen (\(^{15}\text{N}/^{14}\text{N}\) or \(\delta^{15}\text{N}\)) heavy isotopes of about 1 to 2 %e for \(\delta^{13}\text{C}\) and 3 to 5 %e for \(\delta^{15}\text{N}\) (Minagawa & Wada 1984, Lindsay et al. 1998, Fry 2006). The heavier isotopes remain in the body and the lighter ones are disposed in different ways: nitrogen is excreted as ammonia and carbon in the respiration in the case of fish larvae and copepods (Lindsay op. cit.).

There are few studies on stable isotopes and larvae of small pelagic fish (Bode et al. 2003, Pepin & Dower 2007, Costalago et al. 2012, Yasue et al. 2014). It can be partially explained by the difficulty to get the amount of fish tissue necessary for the analysis (Uehara et al. 2005).

Engraulis anchoita Hubbs & Marini, 1935 has an important role in coastal ecosystems in Southwestern Atlantic both as secondary consumer in pelagic food chains and to fishery in Argentina and Uruguay (Cas-
In Brazilian coast, Madureira et al. (2009) showed that Argentine anchovy could be exploited in a sustainable way. They occur between 20°S (Vitória, ES, Brazil) and 48°S (Gulf San Jorge, Argentina) (Castello 2007), spawning almost throughout the whole year, with a peak in between late spring and the beginning of summer in Southeastern Brazilian Bight (Matsuura et al. 1992, Spach 1992). Therefore, larvae live under different oceanographic scenarios, including areas with advection and retention mechanisms, which greatly affect their condition and recruitment (Bakun & Parrish 1991, Lopes et al. 2006). A favorable habitat involves a compromise between adequate temperature and high production of food organisms (Bakun & Parrish op. cit.).

Condition of Argentine anchovy larvae has been accessed by RNA/DNA ratios, histology and morphometric methods in the north area of Argentina and southern Brazil, showing better condition in Argentinean waters in comparison to Brazilian ones, and in mixing areas of frontal systems in comparison to estuarine or shelf areas (Clemmesen et al. 1997, Sieg 1998, Diaz et al. 2011).

Costalago (2015) emphasizes the importance of knowledge about the early stages of the life cycle of small pelagic fishes concerning feeding behavior and oceanographic factors that affect their population.

The aim of this study was to verify ontogenetic changes on the trophic position of *Engraulis anchoita* larvae, based on stable isotopes of carbon and nitrogen, and relate them with the organic material source. Besides this, to relate the nutrient enhancement by upwelling processes with better condition of Argentine anchovy larvae.

### MATERIAL AND METHODS

**Study area:** The study area is situated over the inner-middle continental shelf off Cabo Frio, Rio de Janeiro State (~22°58.5’S, 42°03.3’W and ~23°04.5’S, 42°00.9’W, respectively, Fig. 1), in the Southeastern Brazilian Bight. This area is characterized by a relatively narrow shelf (ca 50 km in length), where the 50- and 100-m depth lines are close to the shore (less than 3 miles), and shows an abrupt change in coastline direction (from NE-SW in the northern portion to E-W in the southern portion). In general, the continental shelf, down to the 50 m isobath, exhibits a predominance of oligotrophic warm waters with low salinity and is influenced by a continental flux, referred to as Coastal Water (CW). High-nutrient colder water, with salinity < 36.4 – the South Atlantic Central Water (SACW) – originates at the Sub-Tropical Convergence at ~30° S and flows near the bottom of the shelf break around the 200 m isobath. The oligotrophic warm and salty Tropical Water (TW) (T > 20 °C, salinity > 36.4) is also transported by the Brazil Current (BC) but in the upper layer (Castro & Miranda 1998, Katsuragawa et al. 2014). This is an area influenced by the mesoscale eddies from BC and seasonal upwelling of SACW (Silveira et al. 2000).

Upwelling events are activated whenever winds are favorable and are not restricted to summer months (Matsuura 1986).

At the Cabo Frio region, hydrographic studies from winter show that the SACW thermal front is retreated to the outer shelf (Silveira et al. 2000). A high temperature and low salinity in the inner shelf indicate the presence of CW within the coastal zone. On the other hand, during summer the thermal front is located on the bottom of the inner shelf, showing a temperature of ~14 °C in Cabo Frio, characterizing SACW (Katsuragawa et al. 2006, Lopes et al. 2006). CW is found in the upper layer of water over the inner shelf, and oligotrophic conditions prevailed (Kampel 2003, Sumida et al. 2005). In summer 2002, downwelling and

![Fig. 1. – Sampling areas on the Cabo Frio continental shelf. Inner shelf: 40 m depth, outer shelf: 100 m.](image-url)
upwelling conditions occurred in the inner shelf. In upwelling, values of NO$_3^-$ were higher than 3.56 µM in surface and higher than 8.14 µM at 40 m depth. In contrast, during downwelling, values were around 0.34 µM in the surface (Guenther et al. 2008). More details related to the study area can be found in Corbisier et al. (2014).

Sample collection: Sampling was done during the austral winter 2001 (July) (W1) and the austral summer 2002 (January) (S2), on the inner shelf, up to 40 m local depth, and on the outer shelf, more than 95 m local depth and beyond, on board the RV Prof. W. Besnard of the University of São Paulo (Fig. 1). Before biological sampling, CTD casts were performed at each station.

Sampling and processing methods for suspended particulate matter (SPM) and zooplankton can be found in Corbisier et al. (2014).

Fish larvae were collected on oblique trawls with a bongo net (300 µm mesh) to sample the water column. Net towing (2014). matter (SPM) and zooplankton can be found in Corbisier biological sampling, CTD casts were performed at each station.

Fish larvae were collected on oblique trawls with a bongo net (300 µm mesh) to sample the water column. Net towing duration for bongo nets was 5 to 8 min, depending on the local depth. Immediately after towing, if Argentine anchovy larvae were present (Fig. 2) the nets were opened in a bucket, where ice cubs were added. The larvae were sorted on board, under naked eye, till a maximum time of 5 min after the end of the tow, and stored in liquid nitrogen. Details can be found at Dias et al. (2004). During sorting procedure, bongo nets were towed again and the plankton sampled were stored in buffered 4 % formaldehyde-seawater solution.

Stable isotopic analysis: Suspended particulate matter (SPM) and zooplankton values of δ$^{13}$C and δ$^{15}$N were obtained from Corbisier et al. (2014), since the sampling was done in the same seasons and areas. Technical difficulties prevent to isolate a significant amount of phytoplankton material without the microzooplankton or particulate organic material. The values used herein refer more to the SPM than the phytoplankton itself, and include heterotrophic organisms of the microzooplankton. However, it is assumed that the SPM, including phytoplankton, is the first trophic level, which varies geographically and seasonally. For the zooplankton averages in summer, the isotopic values of salps were not considered, then they were slight enriched than those found in Corbisier et al. (2014).

After unfreeze and rinsed in distilled water, anchovy larvae were checked for damages in the gut or in the body, measured (standard length – SL mm) and the digestive tract was completely removed.

Anchovy larvae were pooled, by size, in four classes ranging from smaller than 5.0 mm to longer than 15.0 mm, due to a minimum dry weight of larvae. The developmental stages to E. anchoita, based on Castelo & Castelo (2003) are: pre-flexion < 8 mm; flexion 8.1 to 13 mm; and post-flexion > 13.1 mm.

All samples were freeze-dried at –60 ºC for 24 h. Each sample was ground into a fine powder using a mortar and a pestle and stored dried in plastic 1.5 ml tubes appropriately labeled and placed in a desiccator containing silica gel. Powdered samples were weighed to 2.0 mg dry weight for larvae and packed in tin capsules for isotopic analyses. The number of larvae in each sample depended on their size class.

Stable isotopes of natural carbon (δ$^{13}$C) and nitrogen (δ$^{15}$N) were measured using a continuous flow isotope mass spectrometer (Europa Hydra 20/20) coupled with an elemental analyzer at the Stable Isotope Facility of the University of California, Davis, CA, US. The standard reference material was Pee Dee Bellemite (PDB) for δ$^{13}$C and atmospheric nitrogen (N$_2$) for δ$^{15}$N. An internal standard was included after every 12th sample, and the analytical precision based on the obtained standard deviation was 0.10 for carbon and 0.20 for nitrogen.

Stable isotope ratios are conventionally presented as the deviation from a standard material in parts per thousand (%), as follows:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 10^3,$$
$$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 10^3,$$

where $R = ^{13}C/^{12}C$ or $^{15}N/^{14}N$.

The trophic level (TL) of anchovy larvae was estimated using the formula:

$$TL = \left[\left(\delta^{15}N_{larsa} - \delta^{15}N_{zooplankton}\right)/3.4\right] + 2,$$

where 3.4 ‰ is the assumed $^{15}N$ enrichment factor between one trophic level and the next (Vander Zanden et al. 1998; Post 2002).

Condition: Anchovy larvae for condition analysis were obtained from two sources: larvae from liquid nitrogen for RNA-DNA ratio and protein estimations, and larvae from formaldehyde for histological analysis.

RNA and DNA per larvae from 171 specimens (85 from winter 2001 and 86 from summer 2002) were determined by analytical procedures developed by Clemmesen (1993) and described in Moksness et al. (2000).

From the remaining supernatant of RNA and DNA analysis in both cruises (winter 2001 and summer 2002), it was estimated the total content of soluble protein per larva, using the method developed by Bradford (1976), with “Coomassie Brilliant Blue G250”. The method is fairly accurate, detecting microquantities. The dyed compound was read in spectrophotometer, with maximum transmission in the region of 595 nm.

Histological procedures followed recommendations from Sieg (1998), and criteria used for investigation of larval condition were the presence of glycogen and lipid vacuoles in the hepatocytes and its nuclei organization, and the presence and amount of zymogen in the pancreas mainly (Table I). It is important to mention that bad condition does not mean starvation (point of no return), once there is no calibration data to compare with field caught larvae to establish this. Forty-two anchovy larvae (18 from winter and 24 from summer) were measured, dehydrated in alcohol series, diafanized in xylol and embedded...
in paraffin. Longitudinal/sagittal sections of 4 μm were mounted and dyed using PAS (Periodic Acid Schiff) or H-E (Harris hematoxylin-Eosin).

Statistical analysis: ANOVA tests were used to verify the differences in RNA/DNA rate among sizes in winter and summer. Post hoc comparisons were performed with a Tukey test.

RESULTS

Hydrography

Argentine anchovy larvae were captured in two oceanographic situations. In winter 2001 they were caught in areas where mixed waters prevailed in the water column. The continuous distribution of temperature in different depths and the absence of the SACW signal, with interactions of CW and TW, were noted (Fig. 3). During the summer, the larvae were found in areas with the presence of a thermocline and the observation of a front below 30 m depth. The thermal signature of low temperatures indicated the SACW presence in the deeper layers of the water column (Fig. 3).

Isotopic analysis

Isotopic results refer to 45 larvae collected on the 2001 winter cruise and 51 larvae on the 2002 summer cruise. The number and size of the processed larvae reflect the occurrence in the field and, therefore, could not be standardized. In summer, it was possible to obtain four size classes and only two in winter (Table II).

Isotopic ratios showed seasonal variations, with different combinations of δ¹³C and δ¹⁵N. The values of δ¹³C for larvae of different size classes were similar, varying between −18.20 and −18.91 ‰ in winter and −18.60 and −18.92 ‰ in summer. δ¹⁵N mean values ranged from 9.92 to 10.58 ‰ in winter, and from 7.45 to 10.23 ‰ in summer (Fig. 4).
The larvae presented similar $\delta^{15}$N values in winter. On the other hand, in summer, the pre-flexion larvae had lower values than the post-flexion larvae, which showed an increase of trophic level (Table II). The average values for the larger larvae in winter and summer were very close, with the overlapping of their standard deviations (Fig. 4).

Regarding the potential food sources, the mean $\delta^{13}$C values of SPM ranged from $-22.32$ to $-21.06$ ‰ and the $\delta^{15}$N values from $8.55$ to $6.52$ ‰ for winter and summer, respectively, off Cabo Frio (Fig. 4A, C). Zooplankton was enriched in terms of $13$C in relation to SPM. The $\delta^{13}$C mean values for zooplankton ranged from $-20.15$ to $-19.54$ ‰, while the $\delta^{15}$N values ranged from $7.09$ to $8.46$ ‰. $\delta^{15}$N values for zooplankton were higher in winter than in summer, as well as $\delta^{13}$C values.

**Nutritional condition**

In general, values of RNA/DNA of *E. anchoita* larvae showed a clear increase with the length of the larvae, suggesting ontogenetic patterns of better condition in bigger larvae. On the other hand, the mean values of the RNA-DNA ratio varied for larvae collected in different oceanographic conditions (Fig. 5). In the winter of 2001 the maximum average values do not exceed 4.5; instead, the larvae collected in the summer of 2002 had a maximum average value reaching 7.5. The greatest differences were found for larvae bigger than 15 mm SL (post-flexion), which already have better swimming performance. Larvae smaller than 15 mm presented similar range of mean values of RNA/DNA in both seasons.

Grouping individual values of RNA/DNA ratio by size (bigger and smaller than 15 mm) led to the conclusion that larvae bigger than 15 mm SL from summer cruise had significantly higher ratios ($F = 28.94, p < 0.0001$), representing the group with better condition (Fig. 6). On the other hand, the other three groups were similar in mean RNA/DNA ratios, although small larvae from the winter cruise presented lower values and small larvae from summer were comparable to big larvae from winter.

The number of larvae examined for protein analysis was 62 in winter and 71 in summer, since the amount of supernatant of homogenate was restricted by the need to repeat the RNA-DNA analyses for each larva.
Argentine anchovy larvae from the first cruise (winter 2001) presented values below 10,000 ng larva⁻¹ in lengths up to 20 mm; mean values of protein per larva in specimens of 21 and 24 mm were close to 20,000 ng larva⁻¹ (Fig. 7). On the second cruise (summer 2002) an increased amount of protein per larva above 10,000 ng was noted, including anchovy larvae with smaller lengths. For larger class sizes, standard deviations were larger.

In general, even taken into account that there is a natural increase of protein during growth, all summer larvae and the bigger ones from winter presented higher amounts of protein, suggesting better conditions.

Histological analysis of *E. anchoita* larvae collected off the Cabo Frio in the winter of 2001 and the summer of 2002 led to identification of tissues such as the intestine, liver, pancreas, cartilage, notochord and body muscles, among others, in different situations (Fig. 8). Despite the low number of larvae analyzed in both periods, there was a considerable variability in the cytoplasm of hepatocytes according to presence of uniform or irregular, dispersed or homogeneous granules. Larvae collected in the winter presented intracellular spaces of considerable size due to depletion of lipids and glycogen in the hepatocytes (Fig. 8A, B), associated with a vacuolated pancreas and huge gaps between muscle fibers. On the other hand, the presence of pink cytoplasm in the hepatocytes of larvae from summer indicates high acidophilia and storage of glycogen granules: the presence of large amounts of lipid and glycogen in the cytoplasm of hepatocytes reveals good nutritional condition (Fig. 8C, D). Glycogen is recognized by a PAS-positive affinity, whereas the lipids are observed as empty vacuoles after treatment and tissue preparation.

In general, anchovy larvae liver showed parenchyma with little connective tissue and a prismatic epithelium. In cross sections, the hepatocytes were arranged as tubules or cords, forming cell plates in direct contact with the sinusoid (Fig. 8A, C). The liver parenchyma was generally homogeneous and hepatocytes have polygonal shapes,
with nuclei, visible nucleoli and basophilic and lipidic inclusions.

It was possible to verify the presence of pancreatic zymogen (Fig. 8C) as an accessory indicator to classify the general condition of the larvae.

Based on the criteria used, it was found that 10 larvae collected in the winter of 2001 (56 %), presented poor nutritional status, with a depletion of glycogen and lipid reserves. But in the summer of 2002, 11 larvae of the 24 subjects examined presented a better nutritional condition (46 %) (Table III).

**Discussion**

This study focused on the northernmost area of the *E. anchoita* distribution, which is influenced by upwelling processes that enhance production in the continental shelf. In spite of inhabiting waters that vary in temperature (16 to 28 °C, Nakatani 1982, Castello & Castello 2003), the Cabo Frio region. Note the little acidophilia (PAS negative reaction) and vacuolated cells (A and B) in the liver; typical PAS positive reaction = acidophilia (C and D). L = liver; P = pancreas; M = muscle; G = gut. PAS, A and C = 80x; B and D = 400x.

Oceanographic features showed that during the winter of 2001, the South Atlantic Central Water thermal front was retreated to the outer shelf, and the water column was well-mixed; but during the summer of 2002 the thermal front was located on the bottom layer of the inner shelf. These physical seasonal processes are known attributes of the Cabo Frio region (Castro & Miranda 1998, Silveira et al. 2000, Coelho-Souza et al. 2012), related to the nutrient availability in the eutrophic zone that enhances phytoplankton production and growth. The link between hydrographical features of onshore intrusion of SACW over the inner continental shelf, the abundance and recruitment of sardine larvae in Southeastern Brazilian Bight, and even with landings of adult sardines was described by Matsuura (1996, 1998), based on a time series of 17 years of ichthyoplankton studies. Feeding success of fish larvae depends on growth and availability of food items (Rønnestad et al. 2013). At Cabo Frio region, the coastal upwelling or the penetration of SACW generates higher abundance and distribution of phyto- and zooplankton (Valentin 2001).

The δ¹³C and δ¹⁵N values recorded for SPM were similar to those found in the coastal shelf zone in the subtropical sector of the southeastern coast of Brazil, under the seasonal intrusion of SACW (~21.7 to ~20.5 ‰ and 6.9 to 9.6 ‰, respectively) (Matsuura & Wada 1994). The SPM isotopic values reflected the immediate processes that occurred in the water column (Corbisier et al. 2014). Despite the constant presence of SACW over the bottom of the shelf, upwelling events occur at different periods (Kampel 2003, Sumida et al. 2005), and the stable isotopic measurements were not continuous, so it will not reflect these events. Consequently, they are presented only as an indication of SPM isotopic values on the shelf.

The δ¹³C mean values for zooplankton were slightly higher than those found in the coastal shelf zone of the Ubatuba region (~21.9 to ~20.0 ‰) during autumn, while the δ¹⁵N values were within the range observed under those conditions (7.0 to 9.5 ‰) (Matsuura & Wada 1994). The temporal variation was not clearly related to oceanographic conditions; nevertheless, it indicated that some temporal variation could have occurred in the larvae’s source of organic matter. Both δ¹³C and δ¹⁵N values for zooplankton in the winter of 2001 suggested a more oligotrophic condition than in the summer of 2002: both δ¹³C and δ¹⁵N values were higher in the winter than in the summer (Corbisier et al. 2014).

Several factors can affect the isotopic composition to infer trophic relationships of fish larvae (Pepin & Dower 2007). Although Costalago et al. (2012) showed that there is an enrichment of δ¹³C from late larvae to juveniles in *E. encrasicolus* due to different diets, this fact was not observed in this study. The big larvae had lower δ¹³C values than the small ones. In the summer it could be linked to the zooplankton δ¹³C values, which were lower than in the winter, probably reflecting new production and dominance of diatoms in the phytoplankton, due to the advection of nutrients to the euphotic zone (Corbisier et al. 2014).

Our evaluation of δ¹⁵N larvae isotope results showed an increase in trophic level from the smallest Argentine anchovy larvae (pre-flexion) to the biggest ones (post-

**Table III.** Classification of nutritional condition of *E. anchoita* larvae from the Cabo Frio region by histological criteria.

<table>
<thead>
<tr>
<th>Cruises</th>
<th>Number analyzed</th>
<th>Good</th>
<th>Condition intermediate</th>
<th>Bad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter 2001</td>
<td>18</td>
<td>2</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Summer 2002</td>
<td>24</td>
<td>11</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>
flexion) in the summer, with an enrichment of 2.9 ‰, and oceanographic conditions characterized by the influence of upwelling. The zooplankton δ15N values represent a varied composition, and the big larvae could be more selective, preying on items more 15N-enriched. Changes in δ15N of fish larvae could be due to selective feeding or size-selectivity feeding (Lindsay et al. 1998). The δ15N values of capelin larvae, a pelagic feeder (Mallotus villosus), increased with larval length, indicating that they may prey on predatory zooplankton (Pepin & Dower 2007).

In our study, the difference of δ15N values between the same size larvae from winter or summer were not evident. Variations in isotopic composition in fish larvae can be also attributable to growth rather than turnover of existing tissue (Bosley et al. 2002, Pepin & Dower 2007), reflecting an accumulation of new material, and not a concentration of isotopes in existing tissue (Pepin & Dower op. cit.).

Despite the wide variation of RNA/DNA values, the trend of better larvae captured in the summer is clear and variability is related to typical summer scenarios when SACW penetrates on the inner shelf, and promoting a better feeding environment. Moreover, the presence of larvae in precarious conditions was not detected in both seasons. Short-term food deprivation as a possible source of mortality of E. anchoita was described by Clemmesen et al. (1997). But our results showed that seasonal differences in condition of larvae can be related to diet composition during oceanographic events, since both δ13C and δ15N values for zooplankton in the summer of 2002 suggested a more meso- to eutrophic situation of the water column. Pepin & Dower (2007) showed that the relative shift of trophic status of the larval species were consistent with stomach content data, and with the lower or higher trophic status of the preys.

The condition indexes (RNA/DNA, protein content and histology) did not detect larvae starving or in a severely bad condition, due to both the big larvae analysed (Clemmesen et al. 1997, Sieg 1998) and probably to predation pressure (Diaz et al. 2011).

The RNA-DNA ratio provides an assessment of the condition of larvae on a larger scale of time (days). Since there is no experimental data on values considered as limiting to starvation for anchovy larvae, the results were analyzed under the relative point of view of their condition in different hydrographic situations.

The mean values RNA/DNA ratio varied in the different oceanographic configurations, mainly for the bigger larvae (> 15 mm). Unlike known results on the Brazilian sardine (Dias et al. 2004), the values of the RNA/DNA ratio for Argentine anchovy were high, with the next maximum of 10, similar to those found by Dias (1995) in the southeast coast, and by Clemmesen et al. (1997), but higher than those described by Diaz et al. (2011). Considering same size ranges, average values in the summer were higher than those in the winter.

The mean amount of protein per anchovy larvae on the winter of 2001 cruise was lower and this may be due mainly to limited food resources. Leonarduzzi et al. (2007) discuss that the greater E. anchoita larval growth in the Brazilian ecosystem, in comparison to Argentinian coast, could not be associated with productivity but to higher temperature since the poverty of the system. In fact, differences in temperature in both oceanographic situations could result in larvae with same length but with lower protein content in the winter larvae.

Histological indexes have been developed for different species of Engraulis (O’Connell 1976, Sieg 1998, Theilacker & Watanabe 1989). Food deprivation can be characterized by changes, reducing the amount of cellular inclusions such as vacuoles in the mucosa of the intestine or glycogen in the liver; remobilization of cellular material, decomposition and decrease in the cytoplasm volume (wilting); atrophy of the cells, loss of contact between cells; and disruption of epithelia (O’Connell op. cit., Sieg op. cit.).

As a consequence of hydrographical situations and different planktonic communities, it was easy to detect larvae in good or poor condition by histological criteria. The extremely low number of Argentine anchovy analyzed through the histological technique does not allow conclusive issues, and the results provide indication of differences, strengthening those obtained from other techniques.

Our results showed that isotopic enrichment among Argentine anchovy larvae living under different hydrographic situations are difficult to understand since the variations of prey availability and composition can result in different trophic levels of larvae of same size ranges. Additionally, it was not possible to get SPM and zooplankton stable isotopic data of the different prey groups, preventing a more accurate interpretation. Growth mortality hypothesis predicts that faster-growing fish larvae present higher survival chances due to the increased feeding spectrum and swimming performance (Webber 2011). Variations in availability of different food items cause changes in the content, but from a certain size, fish larvae can perform selective capture of preys, which are fewer or reduced in the environment (Lehtiniemi et al. 2007). Thus, diversification of prey has, as consequence, carbon from different sources.

There is a difference in the trophic level of the smaller larvae in the summer of 2002. In general, during the summer larvae were in better condition, evaluated by the applied indicators in comparison to the winter 2001. Such differences can be attributed to distinct seasonal oceanographic conditions and phyto- and zooplankton communities and the feeding ability of the larvae. The condition indexes increase during ontogenetic development, but they also show fast response to modifications in feeding strategies. Besides, their temporal responses are different: RNA/DNA is suited to be used as growth index, whereas
protein content is a storage index and histology is a starvation indicator (Suthers 1997).

This study used, for the first time, stable isotopic analysis and three different tools to evaluate the condition of Engraulis anchoita for the same set of larvae. Despite the low number of larvae analyzed, the results of the better condition of Argentine anchovy larvae are related to the nutrient enhancement by upwelling processes. The environmental characteristics of the Cabo Frio region influence the feeding dynamics and, therefore, the condition of the larvae.

On the southeastern coast of Brazil, E. anchoita shares the hydrographical environment and spawning habitats with Brazilian sardine (Sardinella brasiliensis), including the area of Cabo Frio. This sharing of habitats is common to several regional populations of Engraulis and the different species of sardines in subtropical and temperate regions (Bakun & Parrish, 1991). Costalago et al. (2012) reported differences in trophic level between the late-larvae of E. encrasicolus and Sardina pilchardus in the Gulf of Lions, NW in the Mediterranean. To understand overlapping of niches and the trophic structure of the pelagic communities in the region of coastal upwelling, it is necessary to clarify isotopic values of the preys as well as to perform dietary complementary studies.

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