ORIGINAL RESEARCH

Trophic position and feeding sources of *Engraulis anchoita* larvae from the Buenos Aires stock, southwestern Atlantic Ocean

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ABSTRACT. The existing literature has classified *Engraulis anchoita* larvae as exclusively zooplanktophagous, with copepod eggs and nauplii larvae as their main prey. However, there is evidence that other plankton components that have not been identified by intestinal content analysis may be a part of their diet. The objective of this work was to explore trophic positions and main food sources of *E. anchoita* larvae by analyzing stable nitrogen and carbon isotopes ($\delta^{15}N$ and $\delta^{13}C$) with respect to other plankton components (particulate organic material, calanoid copepods, and chaetognaths) at a fixed sampling station close to the coast of the Province of Buenos Aires, Argentina, southwest Atlantic. Samples were collected at different times of the year between the end of 2016 and the beginning of 2018. By averaging $\delta^{15}N$ and $\delta^{13}C$ values of all surveys, it was found that the anchovy larvae in their three development stages coincided with the same trophic position as chaetognaths, suggesting that their diets overlap. When surveys carried out in autumn were analyzed separately, trophic levels of anchovy larvae differed in a staggered manner among their stages, being situated between the positions of copepods and chaetognaths. Compared to other stages of development, anchovy larvae in the preflexion stage consumed a higher proportion of particulate organic material rather than small copepods. These findings suggest that anchovy larvae may consume a variety of food items and shift their trophic position in response to environmental conditions.

Key words: Argentine anchovy, ichthyoplankton, stable isotopes, food web.

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Posición trófica y fuentes de alimentación de larvas del *stock* bonaerense de *Engraulis anchoita* en el Océano Atlántico Sudoccidental

RESUMEN. Las larvas de *Engraulis anchoita* han sido tipificadas por la bibliografía preexistente como exclusivamente zooplanctófagas, siendo los huevos y larvas nauplii de copépodos sus principales presas. Aun así, existen evidencias de que otros componentes del plancton podrían formar parte de su dieta, y que no han podido ser detectados a través del análisis basado en contenidos intestinales. El objetivo de este trabajo fue realizar un análisis exploratorio de la posición trófica y principales fuentes de alimentación de las larvas de *E. anchoita* a través del análisis de isótopos estables de nitrógeno y carbono ($\delta^{15}N$ y $\delta^{13}C$) respecto de otros componentes del plancton (material orgánico particulado, copépodos calanoideos y quetognatos) en una estación fija de muestreo próxima a la

costa de la Provincia de Buenos Aires, Argentina, Atlántico Sudoccidental. La recolección de las muestras se realizó en distintas estaciones del año entre fines de 2016 y comienzo de 2018. Al realizar un promedio de los valores de $\delta^{15}N$ y $\delta^{13}C$ durante dicho periodo, las larvas de anchoíta en sus tres estadios de desarrollo tuvieron posiciones tróficas similares a los quetognatos, lo que indicaría que estos organismos consumen recursos alimentarios similares. Al analizar las campañas realizadas en otoño en forma separada, las posiciones tróficas de las larvas de anchoíta difirieron en forma escalonada entre sus estadios, y se ubicaron entre las posiciones de los copépodos y de los quetognatos. Las larvas de anchoíta en preflexión se alimentaron en mayor proporción del material orgánico particulado que de copépodos pequeños respecto al resto de los estadios de desarrollo. Con estos resultados se puede inferir que las larvas de anchoíta podrían alimentarse de diferentes ítems presa y variar su posición trófica dependiendo las condiciones ambientales.

Palabras clave: Anchoíta, ictioplancton, isótopos estables, trama trófica.

INTRODUCTION

The ability to position and compare individual species in food webs is critical to understanding energy flow and ecological relationships. In fish, the definition of habits and trophic levels has long been based on direct quantification of gut contents (Hynes 1950; Hyslop 1980). In the case of fish larvae, several studies have detected through these techniques that some larvae feed mainly on copepods (Last 1978; Pepin and Penney 2000; Catalán et al. 2010), while others have a more diverse diet including several other planktonic organisms (Dickmann et al. 2007; Pepin and Dower 2007; Malzahn and Boersma 2009). Studies of nutrient assimilation in fish larvae using stable isotope techniques (Peterson and Fry 1987; Kling et al. 1992; France 1995; Vander Zanden et al. 1999) present difficulties due to the similar isotopic pattern of larvae and their prey. However, several studies have successfully used stable isotope analysis to describe the trophodynamics of zooplankton in marine environments. Seasonal changes in the composition and availability of primary producers have been studied, which are thought to cause trophic plasticity of zooplankton organisms, observable in changes in the trophic position of secondary consumers (Schmidt et al. 2003; Søreide et al. 2006; Tamelander et al. 2006, 2008; Petursdottir et al. 2008; El-Sabaawi et al. 2009).

Trophic position can be defined as a continuous measure of an organism's position relative to the transfer of energy from the bottom to the top of a food web (Levine 1980). The assignment of trophic position within complex aquatic food webs has been facilitated in recent decades by the use of stable isotope ratios present in organism tissues (Kling et al. 1992; Post 2002). Stable isotopes have the potential to simultaneously capture complex interactions, including trophic omnivory, and allow tracking the flow of energy or mass through ecological communities (Peterson and Fry 1987; Kling et al. 1992; Cabana and Rasmussen 1996). The $\delta^{15}N$ of a consumer is typically enriched by 3-4‰ relative to its diet (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987) while the carbon isotope ratio ($\delta^{13}C$) changes on the order of 1‰ in aquatic ecosystems (Fry and Sherr 1984) and can be influenced by species, food type, and feeding level (Webb et al. 1998; Focken 2001; Oelbermann and Scheu 2002). Heavy isotope enrichment between predators and prey is called trophic discrimination and its value is consistent across all aquatic ecosystems when averaged over a large number of trophic pathways (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001; Post 2002).

Engraulis anchoita is one of the most ecologically important pelagic fish species in the southwestern Atlantic (Angelescu 1982; Hansen et al. 2001; Bakun 2006) since it transfers energy from plankton to larger predatory fish, seabirds and marine mammals in the food web with a wasp-waist regulation system. This species is distributed

from the subtropical waters of Cabo Frio (22° S) to the subantarctic waters of Patagonia (47° S). *Engraulis anchoita* is common and abundant in southern Brazil, where it has not been exploited to any great extent (Buratti et al. 2020). Although it is not currently an intensively exploited species in Argentina and Uruguay, it has significant potential economic relevance due to its high biomass. The Buenos Aires stock is the largest and where the fishery is mainly concentrated. Annual catches of anchovy have remained well below the total allowable catch limit and is currently considered an underexploited species (Orlando et al. 2019; Ciancio et al. 2020). For this reason, *E. anchoita* is a model species for the study of environmental effects on small pelagic fishes in temperate waters, which is particularly important in the current context of climate change (Edwards and Richardson 2004). Literature based on gut content analysis indicates that *E. anchoita* larvae up to approximately 38 mm standard length feed almost exclusively on zooplankton, with their gut contents containing mainly eggs and immature stages of copepods (Ciechomski 1966; Ciechomski and Weiss 1974; Viñas and Ramírez 1996; Sabatini 2004; Sato et al. 2011a). The prey size of *E. anchoita* larvae is less than 500 μm, and typically smaller than 300 μm (Viñas and Ramírez 1996; Sato et al. 2011a; Spinelli et al. 2012). However, given the anatomy of the digestive tract, it has been observed that the trophic incidence is usually low for clupeiform fish since the dietary components are expelled from the digestive tract when larvae are captured due to the pressure exerted by the nets (Sato et al. 2011a). There is also evidence that *E. anchoita* larvae do not feed solely on copepods and their early stages. Through stable isotope research, Do Souto et al. (2025) found that postflexion stage *E. anchoita* larvae would have consumed organisms from the particulate organic material (POM), primarily diatoms, under specific oceanographic conditions. In that work, anchovy larvae shared the isotopic composition of $\delta^{15}N$ and $\delta^{13}C$ of carnivorous organisms (chaetognaths) under certain conditions, and the isotopic composition of calanoid copepods in others. Likewise, studies in which the nutritional condition of anchovy larvae was analyzed based on the RNA/DNA ratio indicated a positive relationship with the abundance of nauplii in the environment (Diaz et al. 2016; Do Souto et al. 2019a). Even so, these authors indicated that much of the variability recorded in this index could not be explained solely by this variable and could be due to the consumption of other prey that were not considered.

In the present work the objective was to perform a seasonal exploratory analysis of the trophic position of *E. anchoita* larvae with respect to groups of planktonic organisms present all year round and previously described in the literature of the area as carnivores (chaetognaths) and herbivores (calanoid copepods < 1 mm in length) (Daponte et al. 2004; Sato et al. 2011b; Viñas et al. 2013). The isotopic composition of organisms smaller than 67 μm that make up the POM was also considered. Additionally, the source of potential prey for different stages of *E. anchoita* larvae was evaluated in the season when the greatest amount of material was obtained (autumn). It is proposed as a hypothesis that the feeding of *E. anchoita* larvae varies according to the season and stage of development. It is specifically proposed that *E. anchoita* larvae would present different trophic positions among stages and consume different proportions of copepods and organisms that constitute the POM.

MATERIALS AND METHODS

Study area and sampling collection

Samples were collected at the Estación Permanente de Estudios Ambientales (EPEA), located at 38° 28′ S and 57° 41′ W (southwestern Atlantic) on the 50 m isobath, 27 NM from the city of Mar del Plata, Province of Buenos Aires (Argentina), aboard the motorsailer Dr. Bernardo Houssay (Ar-

gentine Naval Prefecture), and the research vessel Dr. L. Holmberg (INIDEP), between 2016 and 2018 (Figure 1). A total of six research surveys were carried out, each associated with a season of the year: summer, autumn, winter and spring (Table 1).

To capture copepods, oblique trawls were conducted in each survey using a MiniBongo net with meshes of 67 µm and 200 µm pore size and 18 cm mouth diameter. Trawls were conducted from near the bottom to the surface. The navigation speed was approximately 2 kts during the trawling of the net. Samples were filtered on board with a 67 µm sieve and then the two collectors were fixed together in 96% ethyl alcohol. Samples of ichthyoplankton and chaetognaths were collected with a 300 µm pore size mesh and 60 cm mouth diameter Bongo net, in oblique casts similar to the MiniBongo, at a navigation speed of 2.5 to 3 kts. Samples from one of the Bongo net collectors were filtered on board and fixed in 96% ethyl alcohol. Plankton samples collected by both nets were preserved in alcohol in order to maintain the material in a condition that would facilitate the separation and identification of the species on land. Lipids may be extracted from the tissue as a result of this preservation. However, it has been demonstrated to have little effect on the $\delta^{15}N$ and $\delta^{13}C$ values of fish tissue (Arrington and Winemiller 2002) and zooplankton organisms (Syväranta et al. 2008). To guarantee that the results obtained for organisms in each season were comparable, the same fixative was used in all of the material investigated in this work. Since it was impossible to replicate determinations for every case due to the limited amount of material available, no lipid correction or acidification procedure was applied to any of the samples examined in order to maintain $\delta^{15}N$ and $\delta^{13}C$ values unaltered. In every survey, water samples were collected at a depth of 5 m using a Niskin rosette bottle equipped with a Seabird 911 CTD to determine POM. Water samples were kept refrigerated until processing on land.

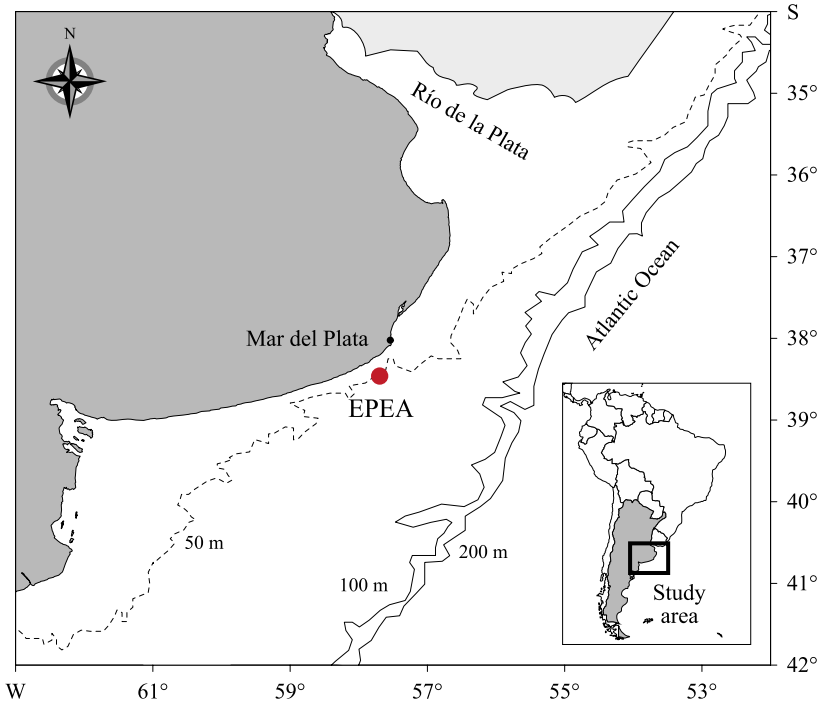

Figure 1. Location of the EPEA (Estación Permanente de Estudios Ambientales, 38° 28′ S-57° 41′ W) study site.

Table 1. Details of surveys carried out at the Estación Permanente de Estudios Ambientales (EPEA). Survey code, date, season and samples obtained from *Engraulis anchoita*, chaetognaths, calanoid copepods (< 1 mm total length) and particulate organic material (POM) are indicated. The number of organisms used to reach the mass required for the analyses (n), number of replicates in which the material was stored (1C, 2C, …×C), and dry weight (mg) of the material in each capsule are indicated. For anchovy larvae, the maximum standard length (SL) value is indicated. For POM, the volume (l) of water collected at 5 m depth and subsequently filtered is indicated.

Survey	Date	Season	*Engraulis anchoita*	Zooplankton	POM
AH0516	2016 Jan 9	Winter	Preflexion (n = 122; 1C = 1.09 mg); SL < 6 mm	Chaetognaths (n = 87; 1C = 1.25 mg; 2C = 1.123 mg)	4.2
AH0217	2017 Apr 5	Autumn	Preflexion (n = 82; 1C = 0.98 mg; 2C = 0.83 mg); SL < 7.9 mm Postflexion (n = 1; 1C = 1.03 mg; 2C = 0.85 mg); SL = 17 mm	Chaetognaths (n = 52; 1C = 1.23 mg; 2C = 1.01 mg) Copepods (n > 100; 1C = 1.03 mg; 2C = 1.10 mg)	5
AH0317	2017 May 24	Autumn	Preflexion (n = 18; 1C = 0.97 mg); SL < 7.9 mm Flexion (n = 36; 1C = 1.00 mg; 2C = 1.07 mg; 3C = 1.05 mg; 4C = 0.97 mg); SL < 11 mm Postflexion (n = 5; 1C = 1.02 mg; 2C = 1.19 mg; 3C = 1.03 mg; 4C = 0.69 mg; 5C = 0.90 mg; 6C = 1.26 mg; 7C = 1.11 mg); SL < 17 mm	Chaetognaths (n = 52; 1C = 1.09 mg; 2C = 1.14 mg) Copepods (n > 100; 1C = 0.50 mg)	4
AH0417	2017 Aug 18	Winter	Flexion (n = 1; 1C = 0.26 mg) Preflexion (n = 47; 1C = 0.68 mg); SL < 6 mm	Chaetognaths (n = 50; 1C = 1.15 mg) Copepods (n > 100; 1C = 0.82 mg)	3.5
AH0817	2017 Mar 11	Spring	Preflexion (n = 120; 1C = 0.98 mg); SL < 6 mm	Chaetognaths (n = 24; 1C = 0.58 mg) Copepods (n > 100; 1C = 0.79 mg)	5
EH0118	2018 Jul 2	Summer	Preflexion (n = 78; 1C = 1.15 mg); SL < 6 mm	Chaetognaths (n = 19; 1C = 0.25 mg) Copepods (n > 100; 1C = 0.63 mg)	5

Sample processing

In the laboratory, *E. anchoita* larvae were separated, photographed and their standard length (SL) was measured to the micrometer with a Zeiss dissecting microscope using Axio-Vision software. They were assigned to a developmental stage according to the criteria established by Alheit et al. (1991): preflexion, flexion and postflexion. The larvae grouped by stage were dried in an oven set at 60 °C for 72 h. They were encapsulated in tin capsules (5 × 9 mm) after being weighed until reaching 1 mg of total dry weight per sample (in cases where there was less material, a minimum of 0.25 mg was encapsulated).

Adult calanoid copepods and copepodites smaller than 1 mm in body length, and chaetognaths ranging from 5 to 11 mm in body length were separated in the lab. Organisms separated by groups were dried at 60 °C for 72 h in a stove. They were then weighed until reaching approximately 1 mg of total dry weight of the sample and encapsulated in tin capsules (5 × 9 mm), which were stored in a 96-well cell culture microplate.

For the isotopic analysis of POM, the water from each survey was filtered through GF/F glass fiber filters with a pore size of 0.7 μm that were previously muffled at 500 °C for 90 min. Depending on the amount of suspended material, as indicated by the clogging of the filter, 3.5 to 5 l of water were filtered for each sample. Filters were then dried in an oven at 60 °C for 72 h. After drying, portions of filters with organic material were removed with a low-pressure clamp and encapsulated in tin capsules (9 × 10 mm), while parts with no organic material were discarded. All capsules were stored individually in 96-well culture microplates for later shipping and analysis of stable isotope.

Determination of $\delta^{13}C$ and $\delta^{15}N$

Encapsulated samples stored in 96-well cell culture microplates were sent to the UC Davis Stable Isotope Facility (Davis, California, 95616, United States of America) for $\delta^{13}C$ and $\delta^{15}N$ determinations. Animal tissue samples (zooplankton and ichthyoplankton) were analyzed using an automated nitrogen and carbon PDZ Europa Gas-Solid-Liquid Elemental Analyzer, connected to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon). Particulate organic carbon samples were analyzed using an Elementar Vario EL Cube or Micro Cube analyzer connected to an Isoprime VisION IRMS (Elementar UK Ltd, Cheadle, UK) or a PDZ Europa 20-20 (Sercon). During analysis, samples were interspersed with several replicates of at least four reference materials. Final $\delta^{13}C$ and $\delta^{15}N$ values were expressed relative to the international standards VPDB (Vienna Pee Dee Belemnite) and Air for carbon and nitrogen, respectively (Sharp 2017).

Data analysis

Data analysis and graphics were performed using R software (R Core Team 2020). The relationships between $\delta^{13}C$ and $\delta^{15}N$ were analyzed through BiPlot-type graphics, considering organisms, surveys and seasons in which they were collected. To estimate the trophic position (TP) of organisms captured in autumn, the model of Post (2002) was used:

$$TP = \lambda + (\delta^{15}N_{secundary\ consumer} - \delta^{15}N_{base})/\Delta_n$$

where: λ = trophic position of samples used to calculate the value of $\delta^{15}N_{base}$. In this case λ =1 was used for primary producers (base = POM); $\delta^{15}N_{secondary\ consumer}$ = was measured directly; Δ_n = enrichment in $\delta^{15}N$ per trophic level = 3‰ (Peterson and Fry 1987).

In turn, the probable composition of the diet of *E. anchoita* larvae captured in autumn (n =17) was calculated by solving a mixing model with two potential prey: calanoid copepods < 1 mm and POM. Following the methodology indicated by Smith et al. (2013), the outright of the model was verified using the routine modified by Funes et al. (2018) and Do Souto et al. (2025), with 1,500 iterations.

Then, using the Bayesian mixing model MixSIAR (R package MixSIAR, Stock et al. 2018) the distribution of probable contributions to the diet of each prey was calculated (3 chains, each with $1e^{+05}$ iterations, with the first 50,000 discarded and then 1 in 50 retained) and the possible influence of the fixed factor 'developmental stage' (preflexion, flexion and postflexion) was considered. To assess the effect of the developmental stage, the null model was compared with the model considering developmental stages using the 'leave-one-out' (Loo) criterion (Do Souto et al. 2025). In the absence of specific trophic enrichment factors (TEF) for *E. anchoita* larvae, general values of $\Delta\delta^{15}N = 3‰$ and $\Delta\delta^{13}C = 1‰$ were used. The uncertainty around the TEFs was set at 0.3‰ (Galván et al. 2012) to avoid high uncertainty.

RESULTS

$\delta^{13}C$ and $\delta^{15}N$

When analyzing mean values and standard deviations of $\delta^{15}N$ and $\delta^{13}C$ for all the surveys (Figure 2), an alignment of zooplanktonic organisms was observed (copepods: -18.65 ± 0.88 $\delta^{13}C$/ 12.37 ± 1.56 $\delta^{15}N$; anchovy larvae in preflexion: -17.62 ± 0.81 $\delta^{13}C$/ 14.49 ± 1.14 $\delta^{15}N$; anchovy larvae in flexion: -17.23 ± 0.31 $\delta^{13}C$/ 14.23 ± 0.17 $\delta^{15}N$; anchovy larvae in postflexion: -17.39 ± 0.18 $\delta^{13}C$/ 14.71 ± 0.41 $\delta^{15}N$; chaetognaths: -17.29 ± 0.86 $\delta^{13}C$/ 14.88 ±1.11 $\delta^{15}N$) and POM (-21.70 ± 1.74 $\delta^{13}C$/ 8.65 ± 0.82 $\delta^{15}N$). In this case, a trophic overlap was evident between the three stages of *E. anchoita* larvae analyzed (preflexion, flexion and postflexion) and the chaetognaths, located at the right and upper end of the biplot graph. Calanoid copepods were below these organisms. The POM was found at the base of the graph with the largest deviation of $\delta^{13}C$.

The number of samples for $\delta^{13}C$ and $\delta^{15}N$ values was not sufficient for a statistical analysis per season, since only one survey in spring and one survey during summer were carried out (Figure 3). The presence of different anchovy stages varied with the season. Autumn was the time of the year that allowed obtaining preflexion larvae with the longest standard length and larvae at more advanced developmental stage (flexion and postflexion) (Table 1).

The POM showed the lowest $\delta^{15}N$ values compared to the rest of organisms in all seasons, while $\delta^{13}C$ values were more variable compared to the rest of organisms. The $\delta^{13}C$ values of POM deviated from the expected alignment with respect to the rest of the organisms, particularly in the case of winter and spring campaigns. On the other hand, certain differences were observed in the relative position of anchovy larvae with respect to copepods and chaetognaths based on their $\delta^{15}N$. More trophic similarity was evident during autumn when the position of anchovy larvae in all their stages was closer to that of copepods, particularly for those in the preflexion stage. The highest $\delta^{15}N$ values for anchovy larvae were recorded in the winter season.

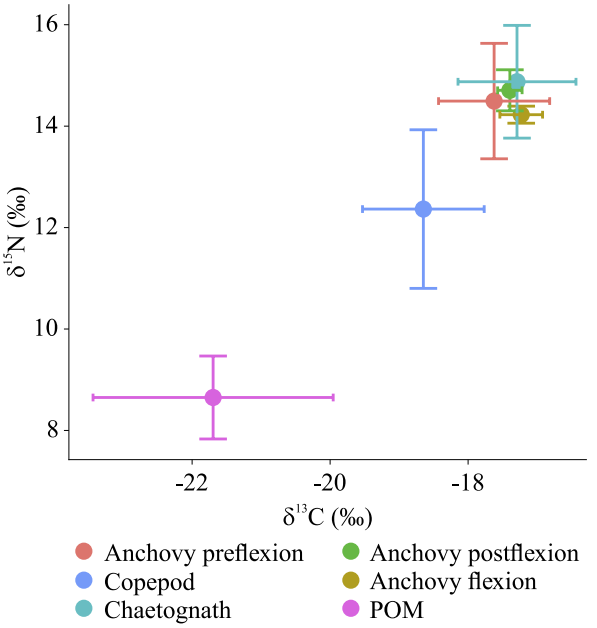

Figure 2. Mean values and standard deviations of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) from the six surveys analyzed. POM: particulate organic matter.

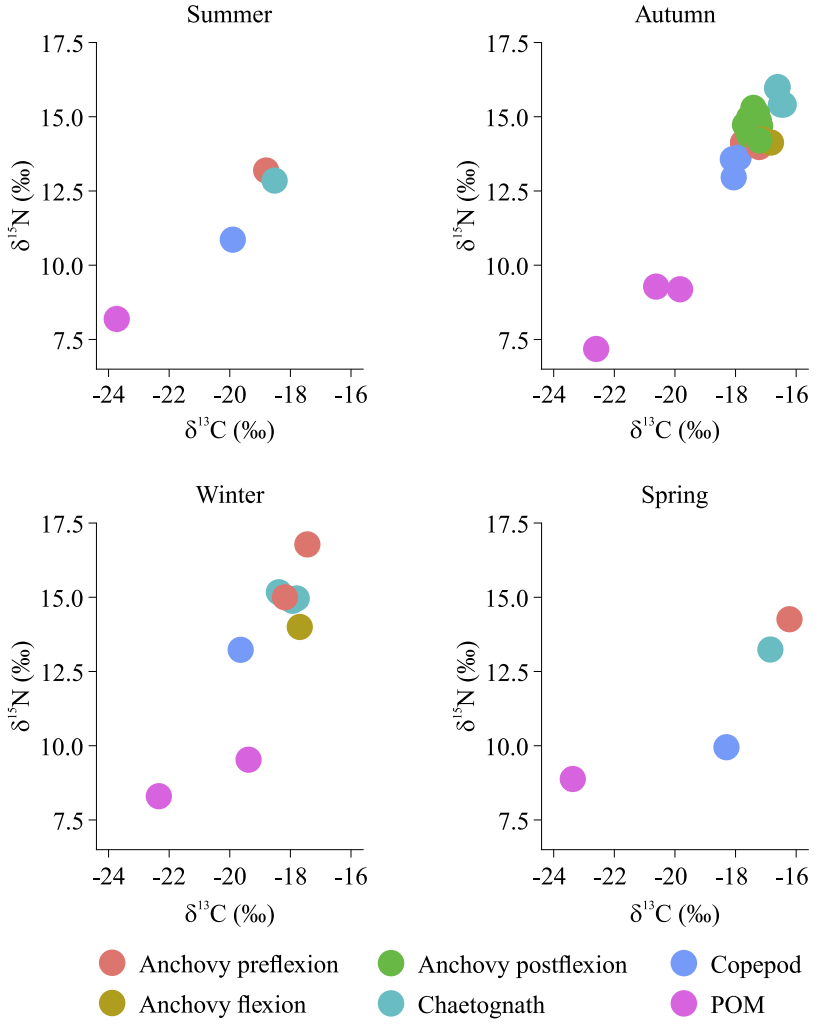

Figure 3. Mean values and standard deviations of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) from the analyzed surveys grouped by season (spring, summer, autumn and winter). POM: particulate organic material.

Trophic positions (TPs) and contribution of potential prey

Upon analyzing surveys conducted in autumn, which presented anchovy larvae in their three developmental stages (Figure 4), it was observed that TPs of larvae displayed a staggered trend between their stages, and were situated between the positions of copepods and chaetognaths (TP of calanoid copepods: 2.61 ± 0.12; TP of anchovy in preflexion: 2.84 ± 0.03; TP of anchovy in flexion: 2.91 ± 0.04; TP of anchovy in postflexion: 3.05 ± 0.14; TP of chaetognaths: 3.38 ± 0.11).

Regarding prey contribution (Figure 5), model selection according to Loo's criterion (Looic model considering stage = -9.6; Looic null model = 0.9) indicated that the developmental stage was a significant covariate for estimating dietary contributions during autumn. In all three stages, calanoid copepods were the main food item (proportion > 0.5). However, preflexion stage larvae fed on a higher proportion of POM organisms (0.46 ± 0.082; 95%

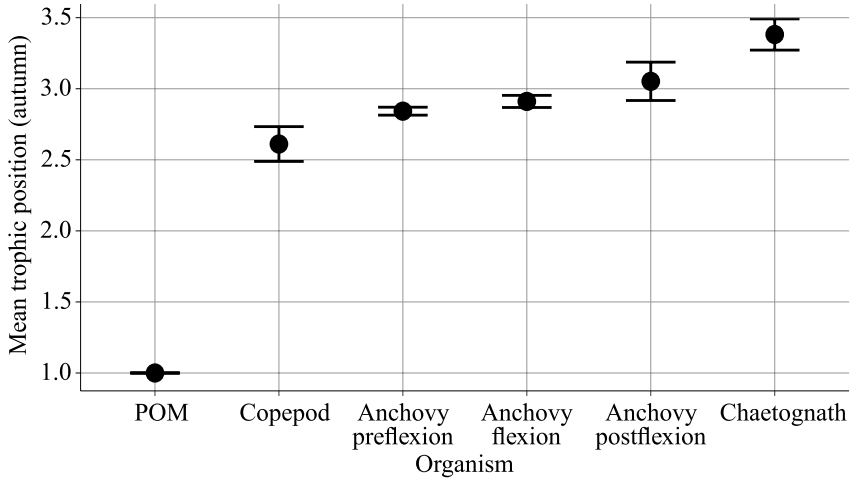

Figure 4. Average trophic positions of the organisms analyzed during the autumn season: chaetognaths, anchovy larvae at preflexion, flexion and postflexion stages, and calanoid copepods. Particulate organic material (POM) was used as a baseline for calculations.

CI 0.33-0.65) than flexion (0.36 ± 0.074: 95% CI 0.23-0.53) and postflexion (0.30 ± 0.058; 95% CI 0.20-0.43) stages.

DISCUSSION

Results obtained by analyzing average $\delta^{15}N$ and $\delta^{13}C$ values of organisms grouped during the entire study period coincide with studies of gut contents of anchovy larvae (Viñas and Ramírez 1996; Sato et al. 2011a). In their different stages of development and throughout the year, anchovy would be situated in a trophic position similar to that of chaetognaths, which are characterized as zooplankton predators mainly feeding on copepods < 1 mm (Sato et al. 2011b). The trophic position of anchovy with respect to that of chaetognaths and calanoid copepods, however, showed some variability in terms of trend in the results discriminated for autumn. During this season, the trophic position of anchovy and calanoid copepods seems to be closer to each other and further away from that of chaetognaths. One possible interpretation of these results would be a change in feeding habits of the larvae during the autumn, when they prey on smaller organisms that copepods also consume.

Seasonal variation in the planktonic community of the EPEA is a process that has been widely studied for years (Leonarduzzi et al. 2021; Viñas et al. 2021). Between 2000 and 2001, multiple studies were carried out considering the monthly composition of zooplankton, phytoplankton, chlorophyll-*a* and gut contents of both chaetognaths and *E. anchoita* larvae (Lutz et al. 2006; Leonarduzzi et al. 2010; Sato et al. 2011a, 2011b; Viñas et al. 2013). Based on these studies, the months of the year could be grouped into three periods: cold season (winter-spring, 10-17 °C), warm season (summer, 17-21 °C) and transitional season (autumn, 19-13 °C). Highest abundances of small copepods were recorded in summer coinciding with minimum concentrations of chlorophyll-*a*. Surface chlorophyll-*a*, microphytoplankton (with primary producer representation higher than in summer) and calanoid copepod abundance showed their maximum values during the cold period (winter-spring). The transition period (autumn) was characterized by intermediate values of both surface and bottom temperatures, with an intermediate surface chlorophyll-*a* concentration

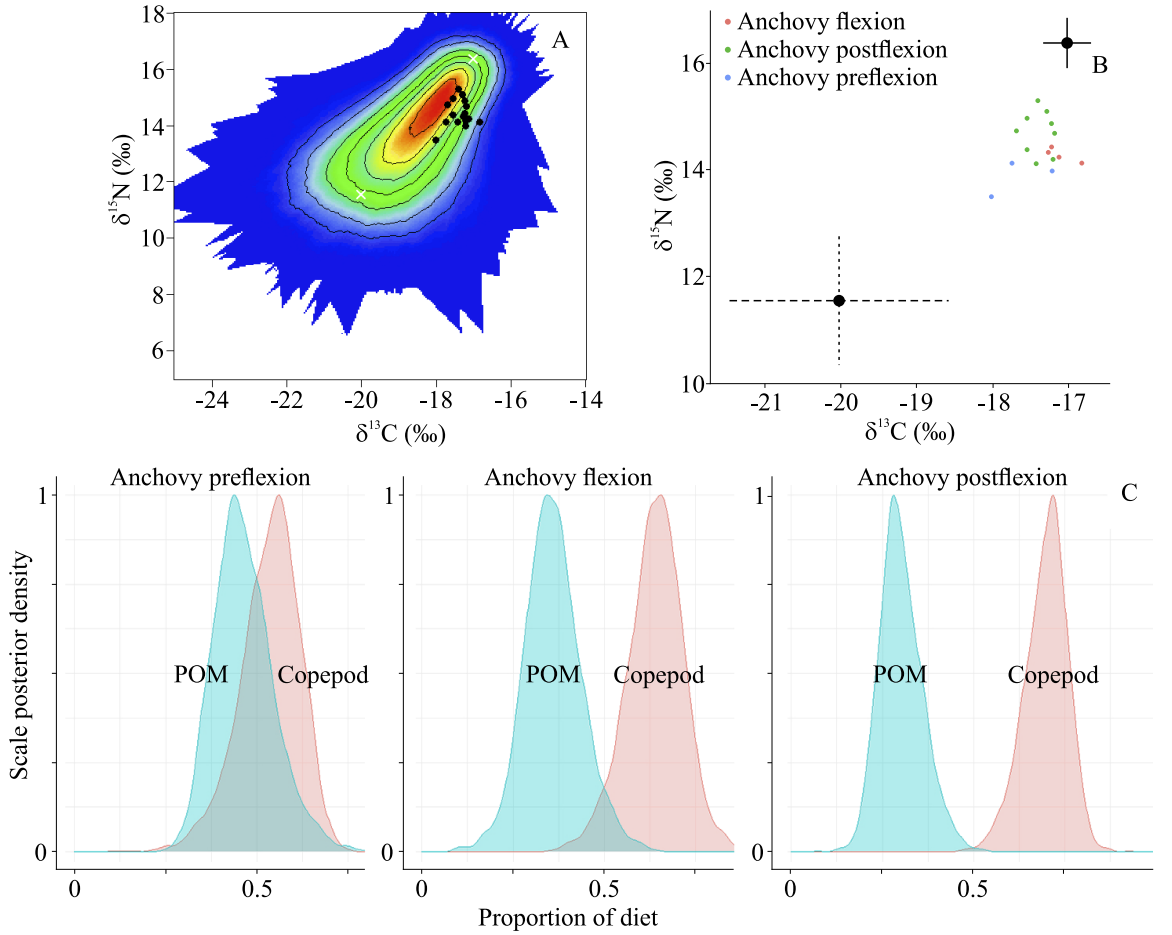

Figure 5. A) Monte Carlo simulation of the mixing region for a model with two stable isotopes and potential sources (calanoid copepods < 1 mm and particulate organic matter −POM) created using the procedure described by Smith et al. (2013). A) Trophic enrichment factors and errors were $\delta^{15}N$: 3.0 ± 0.3 SD; $\delta^{13}C$: 1.0 ± 0.3 SD. B) Biplot of corrected $\delta^{15}N$ and $\delta^{13}C$ values for consumers (*Engraulis anchoita* larvae by developmental stages) and potential prey (calanoid copepods < 1 mm and POM) created with MixSIAR. Error bars represent 95% confidence intervals and incorporate error in prey isotopic signal and trophic enrichment factors ($\delta^{15}N$: 3.4 ± 0.3 SD; $\delta^{13}C$: 1.0 ± 0.3 SD). C) Posterior distributions of the probability of prey item contribution (calanoid copepods < 1 mm and POM) for each developmental stage of *Engraulis anchoita* (preflexion, flexion, and postflexion) in the autumn season.

and decreasing values of microphytoplankton. Over the annual cycle, copepods < 1 mm in size accounted for 98% of the average total copepod abundance, outnumbering medium-sized (1 to 2 mm) and large-sized (> 2 mm) copepods by two orders of magnitude. In autumn, 79% of copepod abundance and 64% of biomass corresponded to calanoid copepods < 1 mm. Biomass was dominated by calanoids 1 > mm in size during winter and early spring. The situation significantly changed in the summer, when copepods < 1 mm (calanoids, cyclopoids, and harpacticoids) dominated the copepod population in terms of biomass and abundance (Viñas et al. 2013).

Sato et al. (2011b) described the diet of the dominant chaetognath species (*Sagitta friderici*) in the EPEA during 2000 and 2001, which, although very abundant throughout the year, exhibited its highest

density during the summer. These authors analyzed stomach contents and found mainly copepodites and eggs of calanoid and cyclopoid copepod species. Although no seasonal differences were found, they suggested that chaetognaths might compete for food with *E. anchoita* larvae. On the other hand, Sato et al. (2011a) observed that the highest feeding incidence of *E. anchoita* larvae during the annual cycle of the EPEA occurred in spring. Most of the larvae, mainly in the preflexion stage of development, fed essentially on the first stages of copepods, both calanoids and cyclopoids. In turn, their highest growth rates were also recorded in spring (Leonarduzzi et al. 2010). However, it was striking that the maximum abundance of *E. anchoita* larvae and their highest growth rates (in spring) did not coincide with the highest abundances of copepods < 1 mm recorded in summer.

Based on all the above, it can be concluded that there is a seasonal change in the composition of both phytoplankton and zooplankton in the EPEA, which is typical of a temperate-cold sea. Similarly, the present work found that the relative trophic position between anchovy, copepods and chaetognaths could present variability over the period analyzed. Do Souto et al. (2019b) observed that larvae of *E. anchoita* in the EPEA presented maximum values of nutritional condition and growth rates in spring, although high values of both indices were also detected during autumn. On the other hand, using the same techniques as in the present work, Do Souto et al. (2025) found that relative positions of postflexion anchovy larvae from the Buenos Aires stock changed significantly with regard to calanoid copepods and chaetognaths for the same time of year but under different oceanographic conditions. Considering this, it is clear that the feeding of *E. anchoita* larvae is adjusted to the availability of planktonic organisms in the environment, being able to consume both microzooplankton prey and smaller ones.

Regarding ontogeny, results obtained by analyzing different stages of *E. anchoita* in the autumn season suggest that preflexion larvae would feed a higher percentage of smaller organisms than larger flexion and postflexion larvae. Do Souto et al. (2025) did not find differences in the contribution of prey to the diet between larval development stages in *E. anchoita*, but found differences attributed to the environment. It is possible that both processes occur at the same time, being masked from each other depending on the degree of food availability, and the difference in size among larvae in different stages. There are further factors that may contribute to the observed diversity in the trophic position of anchovy larvae, such as a maternal effect on the isotopic signal of larval tissue in preflexion phases of smaller sizes. At this stage, the tissue of fish larvae reflects two factors: isotopes of their diets and isotopes of the parents (Uriarte et al. 2016). Based on diet-switch feeding experiments, Tanaka et al. (2016) showed that the ratios of $\delta^{13}C$ and $\delta^{15}N$ in eggs of the Japanese anchovy (*E. japonicus*) closely follow the isotope ratios of their parents' food. In this way, preflexion *E. anchoita* larvae could present a mixture effect of maternal isotopic composition and that of their diet, a phenomenon that would be 'diluted' throughout their ontogenic development. This could explain why $\delta^{15}N$ and $\delta^{13}C$ values of preflexion larvae were elevated in relation to the rest of organisms during winter and spring, since their standard lengths were less than 6 mm in those seasons.

Another condition that could generate an enrichment in heavy isotopes at all stages of larval development would be starvation. Do Souto et al. (2019b) observed that the nutritional condition and growth of *E. anchoita* larvae in the EPEA between 2008 and 2016 were lower during the winter. In a scenario where food is scarcer, organisms tend to consume their own energy reserves. It has been observed that $\delta^{15}N$ and $\delta^{13}C$ values obtained from starved animals usually increase because they mainly use light isotopes in catabolism and they are not restored by not feeding (Gannes et al. 1997; Doi et al. 2007). If the seasonal pattern observed by these authors is maintained for the following years (2016-2018), anchovy larvae collected during the

winter could present high $\delta^{15}N$ values because of both the maternal effect and an enrichment in heavy isotopes as a consequence of a poor diet. Dias et al. (2016) analyzed the trophic position of anchovy larvae off the coast of southern Brazil by measuring $\delta^{15}N$ and $\delta^{13}C$ values and determining nutritional status. In that study, the authors found differences in the trophic position of different developmental stages between summer and winter. During winter, larvae with lower SL had higher $\delta^{15}N$ and $\delta^{13}C$ values than larvae with higher SL. Conversely, in summer, the smallest larval stages had the lowest $\delta^{15}N$ and $\delta^{13}C$ values, and these values increased as larvae underwent ontogeny. This process was attributed to an oceanographic change specific to the study area, leading to an enrichment of waters in the warmer season. In winter, the larvae analyzed presented a poorer nutritional condition than in summer. Similar to the findings presented here, seasonality implied a shift in trophic position; in that scenario, poor winter feeding may also have an impact on the isotopic composition of the larvae. Finally, it is important to highlight that variability associated with the rest of the groups examined, such as calanoid copepods and chaetognaths with respect to their average trophic positions, cannot be ruled out. Future studies would require a more exhaustive analysis of the taxonomic composition and sizes of both copepods within the order Calanoida and chaetognaths.

Edwards and Richardson (2004) indicated that temperate marine environments are particularly vulnerable to climate change due to their dependence on the synchronization between the development of different organisms, from phytoplankton to secondary producers. According to these authors, there is evidence that a mismatch between various trophic levels is already occurring in the world as a result of a shift in seasonal cycles. In a context of global change, there is increasing interest in continuing this type of studies in order to gain a thorough understanding of the mechanisms governing fish life before the recruitment, especially those constituting economically valuable resources.

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Statement of use of artificial intelligence tools

No artificial intelligence tool was used in the preparation and/or writing of this manuscript.

Author contributions

Marina Do Souto: research; formal analysis and writing of the original manuscript. Fabiana Capitanio: funding acquisition; supervision and writing of the manuscript (review-editing). David E. Galván: data curation; supervision and manuscript writing (review-editing). Gustavo J. Macchi: supervision; project management and manuscript writing (review-editing). Marina V. Diaz: conceptualization; supervision and writing of the manuscript (review-editing).

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