




Shifts in the seasonal trophic ecology of larvae and juveniles of European hake (*Merluccius merluccius*): From the Galician upwelling system (NW Spain)

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Funding information

This study was made possible by the financial support of projects CRAMER of the Spanish Ministerio de Economía y Competitividad (CTM2010-21856-C03) and ECOPREGA (10MMA602021PR) of the Galician Government, Xunta de Galicia.

Abstract

The trophic ecology of European hake (*Merluccius merluccius*) larvae and early-juveniles from the Iberian shelf off Galicia (NW Spain) are assessed by means of stable isotopes (SIA) and nutritional condition in conjunction with hydrobiological variations observed during the winter and summer season of 2012. Hake early-juveniles (18–47 mm SL) showed higher $\delta^{15}\text{N}$ than larvae (3–11 mm SL) together with the microzooplankton (55–200 μm) and mesozooplankton (>200 μm) during both seasons. Low $\delta^{15}\text{N}$ values and high variability in both zooplankton and hake larvae were found during winter coinciding with an unusually strong upwelling event. Inversely, high $\delta^{15}\text{N}$ content in zooplankton in summer suggests strong nitrogen reutilization. However, hake larvae sampled in summer had a higher RNA:DNA ratio in line with higher Fulton condition factors than those sampled in winter. Higher $\delta^{13}\text{C}$ values for microzooplankton and mesozooplankton and hake larvae in summer compared to late winter point to prey availability differences. The relationship between nitrogen and carbon stable isotopes versus size or weight suggests an ontogenetic shift in the diet of hake larvae. Early-juveniles had a lower isotopic niche width compared to larvae in both late winter and summer, indicating a trophic specialization related to changes following settlement process from planktonic life to demersal habitat. Higher trophic specialization was observed in summer, which recorded a narrower isotopic niche and higher trophic position estimations.

KEYWORDS

European hake larvae, oceanographic conditions, stable isotope analysis, trophic ecology, isotopic niche width, NW Spain

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1 | INTRODUCTION

European hake (*Merluccius merluccius*) is a demersal species inhabiting both the Atlantic and Mediterranean waters. It is one of the most valuable and heavily exploited demersal species by western European fisheries (Casey & Pereira, 1995). The southern hake stock spreads along the northern and western coasts of the Iberian Peninsula and are distributed over a wide bathymetric range, from 50 to 600 m (Álvarez et al., 2004). The NW Galician shelf/slope region is one of the most important southern hake stock fishing and recruitment grounds. The NW Iberian Peninsula is at the northern edge of the Eastern Boundary Upwelling System extending from $\sim 10^\circ$ to $\sim 44^\circ$ N. This region is characterized by a seasonal pattern linked to the large-scale climatology of the North Atlantic Ocean. During late winter through summer, northerly winds prevail, favoring the upwelling of cooler and nutrient-rich subsurface oceanic water that form an equatorward surface current over the shelf. Inversely, during autumn through early winter, southerly winds induce coastal downwelling while the surface currents take a poleward direction (Álvarez-Salgado et al., 2000). This seasonal pattern favors high primary production (e.g., Bode et al., 1996) and affects hake recruitment in nearby regions (Sanchez & Gil, 2000).

European hake is a benthic-pelagic dweller that spawns in deep waters with a protracted spawning season, practically spawning throughout the year with three recognized peaks: One during winter-spring, another in summer, and occasionally during autumn (Domínguez-Petit & Saborido-Rey, 2010). In the area of study, the maximum larval concentrations are found at 60 and 80 m depth in the water column, independent of time of day (Rodríguez et al., 2015a). Hake larvae settle to the bottom when pelvic fins are completely developed at approximately 11–16 mm in length (Palomera et al., 2005). Benthic settlement is completed at depths over 200 m when the juveniles are approximately 2 months of age (Morales-Nin & Moranta, 2004).

Seasonal variations of the trophic sources can significantly impact growth variability during larval development, thereby imparting its effect on survival rates during ontogeny by starvation and/or predatory pressures (Anderson, 1988; Pepin et al., 2014). Although the larval fish diet is highly dependent on the abundance and diversity of prey encountered (García et al., 2003), the larvae of most marine fish selectively feed on specific prey species (see Pepin et al., 2014; Shiroza et al., 2021). While hake larvae and small juveniles feed on small crustaceans (euphausiacea, decapods, and misidaceans) ontogenetic development and larval growth in the early life of hake is driven by the abundance and quality of trophic resources (Itembu et al., 2012), and as they increase in size, piscivory bears more importance in their diet (Cartes et al., 2004).

Early larval stages of hake are planktivorous, mainly preying upon copepodites, copepods, and nauplii, preferentially of the calanoid species (Morote et al., 2011; Temperoni & Viñas, 2013). Copepods tend to be the main grazers in the Artabrian Gulf waters (Bode et al., 2003). Upon reaching early-juveniles, their diet shifts towards crustaceans (e.g., euphausiids) and eventually shift to be piscivorous (7–30 cm;

Mahe et al., 2007) preying mainly on small pelagics and horse mackerel. Morote et al. (2011) reported that *M. merluccius* larvae had clear preferences for adult calanoid copepods even when foraging under low light conditions in the Mediterranean Sea, independent of the size range (2–9 mm) analyzed. Similarly, other hake species from different regions have also shown feeding specificity by feeding on copepod eggs, nauplii, copepodites and adults calanoid copepod (*M. productus* from the Pacific Ocean, Sumida & Moser, 1980; *M. novaezelandiae* from Tasmanian Sea, Murdoch, 1990; *M. gayi* from Pacific Ocean, Balbontín et al., 1997; *M. merluccius* from Mediterranean Sea, Morote et al., 2011; *M. hubbsi* from Atlantic Ocean, Temperoni & Viñas, 2013).

Stable isotope analysis (SIA) and RNA:DNA ratios can provide complementary information on the trophic ecology of early life stages of hake. Typically, dietary studies examine stomach content analysis and offer a glimpse of an individual's recently ingested prey, which in reality can be highly variable. Alternatively, first, SIA of nitrogen and carbon has been used to determine the trophic positions (TPs) of marine fishes and their potential nutrient sources (e.g., Bode et al., 2018; Laiz-Carrión et al., 2015) because heavy nitrogen isotopes accumulate with each trophic transfer while carbon isotopes show comparatively less accumulation (Post, 2002). The SIA approach can improve and complement conventional means of dietary analysis because stable isotopes reflect time-integrated dietary records and present a perspective on trophic dynamics, which involves a longer period than the analysis of stomach contents. It is also a more appropriate method for testing hypotheses on developmental changes in sources of nutrition as it provides information on assimilated food, rather than ingested food that can be identified and quantified by other techniques. In addition, SIA can be used to analyze trophodynamics, in particular, the trophic niche represented by projecting carbon and nitrogen isotope abundance measured in different individuals or species (Layman et al., 2012). Spatial and temporal variations in the resources available for the same or different species or whole communities and the degree of overlap between different trophic niches has been estimated by SIA in fish early life stages (Laiz-Carrión et al., 2019) revealing trophic interactions, nutrient partitioning, and resource use.

Second, the larval nutritional condition can be estimated from the RNA:DNA ratio, an index of cellular protein synthesis and an indicator of nutritional condition and starvation of fish larvae (Buckley, 1984). RNA:DNA ratios indicate changes in growth potential and nutritional status over short periods (2 days) (Caldarone et al., 2006) and have been proven to be a reliable indicator of larval growth potential in South African hakes, *M. paradoxus* and *M. capensis* (Grote et al., 2012) and in Argentine hake, *M. hubbsi* (Cohen et al., 2021). For a predatory species such as the European hake, the successful larval transition to recruitment survival relies on several factors, including environmental variables mainly driven by upwelling events and related hydrographic circulation that eventually influence the composition and abundance of trophic resources (Sanchez & Gil, 2000) and trophic interactions whereby competing for or sharing trophic resources during the larval and juvenile stages (Botto et al., 2019). Trophic variability is

particularly essential bearing in mind that hake has a protracted spawning season, practically spawning throughout the year, thus requiring high energy intake from trophic resources for egg production (Dominguez-Petit & Saborido-Rey, 2010) to assure the viability of hake offspring. The objective of this study was to describe the trophic ecology of larvae and juveniles of European hake subject to seasonal variations of the hydrographic features that characterize the Galician upwelling ecosystem using SIA as an ideal tool to describe food web interactions (Bode et al., 2007). As upwelling events drive the production of the study region, it is of paramount importance to understand the underlying trophodynamics determining early life growth variability of hake, which may influence recruitment success.

2 | MATERIAL AND METHODS

2.1 | Hake and zooplankton sampling

Two oceanographic surveys were carried out onboard the *R/V Cornide de Saavedra* in (NW Galicia). The first survey was sampled during late winter (CRAMER 0312) from February 29 to March 12, 2012, and the second during summer (CRAMER 0712) from July 17 to 29, 2012.

Hake larvae were collected from 92 plankton sampling stations distributed over a series of gridded transects perpendicular to the coastline (Figure 1). Stations within transects were 4 nm apart, and the distance between transects was 8 nm. At each regular grid station, a SeaBird 25 CTD (Conductivity Temperature Depth) cast was carried out to a maximum depth bottom allowable, 200 m or to 5 m above the bottom at shallower stations and 400 m at selected stations.

Daily upwelling index (UI) values were obtained from the Spanish Institute of Oceanography (www.indicedeafloresamiento.ieo.es) using a geostrophic wind in a cell centered at 44°N, 9°W was calculated. Positive values of the UI corresponded to upwelling, while negative values corresponded to downwelling (González-Nuevo et al., 2014).

A Multinet (MTN) with 50-cm mouth opening and a mesh of 200 μm was used to carry out oblique tows to 5 different depth strata (0, 20, 40, 60, 100, and a maximum depth of 200 m or 10 m above the bottom at shallower stations) (Rodríguez et al., 2015a). A depth sensor (Scanmar) was used to monitor depth. Samples were sorted on board with stereomicroscopes which took size-calibrated pictures of each larval hake. To supplement our standard sampling grid, additional adaptive plankton sampling was conducted to acquire additional hake larvae.

The nets used for the adaptive sampling were a Bongo (60-cm mouth opening with 500- μm mesh) in the first CRAMER 0312 survey, whereas during the CRAMER 0712, a squared mouth Bongo (90-cm mouth opening with 500- μm mesh) was used. Two sets of larvae were separately stored, one in liquid nitrogen for SIA and another in RNAlater for nutritional conditions (RNA:DNA ratio).

A CalVET plankton net (53- μm mesh) was used to collect vertical plankton tows. Two parallel samples of zooplankton were collected, one sample was preserved in formalin (4%) for further analyses, the other sample was first sieved for collecting microzooplankton (55–200 μm) and mesozooplankton (>200 μm) and then frozen (–20° C) for laboratory analyses. A total of 46 alternate stations were selected for both microzooplankton and mesozooplankton for winter and summer biomasses estimation (Table 1) and SIA analyses.

Early-juveniles were collected in late winter and summer during daylight hours at a bottom depth range of 70–260 m, using two

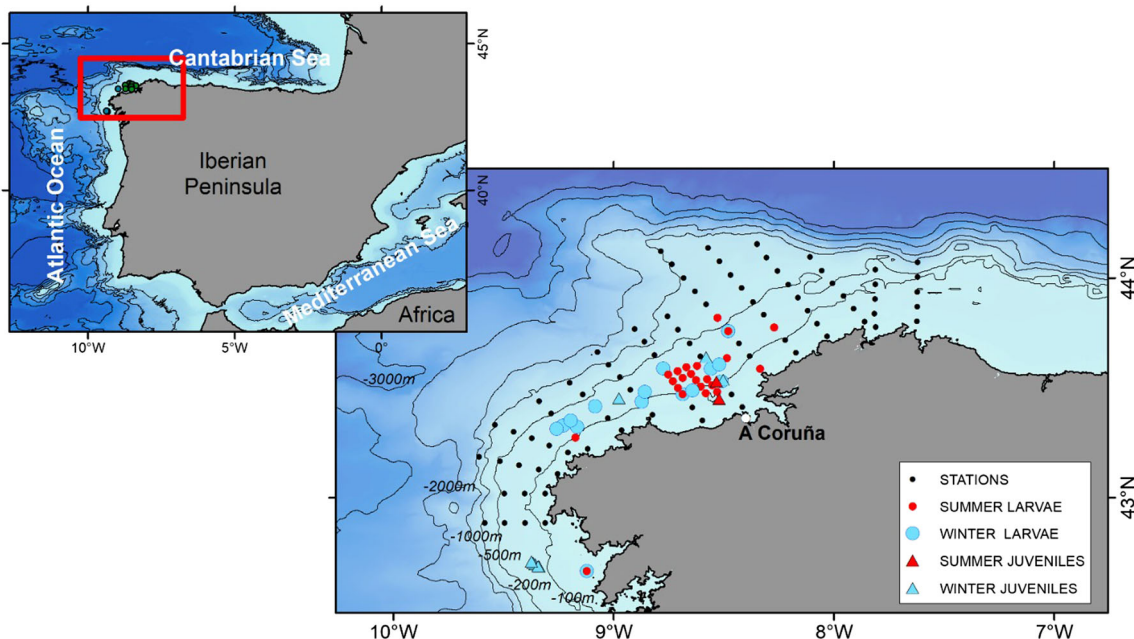


FIGURE 1 Location of the study area. Sampling stations in Galician waters (NW Spain) are shown in black. Stations where larvae (circles) and juveniles (triangles), from both winter and summer, were selected are indicated in red and blue respectively.

TABLE 1 Microzooplankton (55–200 micron) and mesozooplankton (>200 micron) variables (biomass [mg/m³], δ¹⁵N [‰], and δ¹³C [‰]) and results of Mann–Whitney *U* test nonparametric analyses (*p*) for winter and summer

	Microzooplankton			Mesozooplankton		
	Winter	Summer	MW- <i>U</i> test	Winter	Summer	MW- <i>U</i> test
Biomass (mg/m ³)	6.60 ± 0.56	7.93 ± 0.31	NS	18.70 ± 0.95	23.79 ± 0.53	NS
δ ¹⁵ N (‰)	1.24 ± 0.09	6.09 ± 0.05	**	1.28 ± 0.05	6.89 ± 0.02	**
δ ¹³ C (‰)	−20.23 ± 0.13	−17.53 ± 0.07	**	−21.23 ± 0.11	−19.77 ± 0.06	**

Note: NS: nonsignificant, ***p* < 0.01. Values represent means (mean ± SE) of 46 microzooplankton and mesozooplankton for both winter and summer surveys.

TABLE 2 Morphometric variables (SL and DW), Fulton's condition factor (*K*), C:N ratio, nitrogen and carbon isotopic signatures, trophic position (TP), and of larval and early-juvenile hake seasonal cohorts

	Larvae			Early-juvenile		
	Winter N = 35	Summer N = 129	ANOVA	Winter N = 65	Summer N = 6	ANOVA
SL (mm)	6.01 ± 0.22	6.31 ± 0.12	NS	27.14 ± 0.73	42.69 ± 2.39	**
DW (mg)	0.36 ± 0.13	1.06 ± 0.07	**	41 ± 3.40	114.01 ± 11.19	**
<i>K</i> (g mm ^{−3})	0.13 ± 0.02	0.36 ± 0.01	**	0.19 ± 0.01	0.15 ± 0.01	**
C:N ratio	4.19 ± 0.07	4.06 ± 0.04	NS	3.60 ± 0.01	3.38 ± 0.06	**
δ ¹⁵ N (‰)	4.02 ± 0.13	9.11 ± 0.07	**	6.38 ± 0.03	9.74 ± 0.13	**
δ ¹³ C (‰)	−20.50 ± 0.14	−18.56 ± 0.07	**	−17.94 ± 0.05	−18.66 ± 0.17	**
TP	-	-	-	3.26 ± 0.02	3.66 ± 0.07	**
Nucleic Acid analyses	N = 49	N = 38				
SL (mm)	5.96 ± 0.16	6.40 ± 0.18	NS	-	-	
DW (mg)	0.36 ± 0.13	1.06 ± 0.07	NS	-	-	
RNA:DNA	1.80 ± 0.04	2.19 ± 0.05	**	-	-	

Note: Larvae analyzed for nucleic Acid analyses are show in lower panel. ANOVA analyses (mean ± SE and *p*) between winter and summer cruises. NS: nonsignificant, **p* < 0.05, ***p* < 0.01.

different trawls. A pelagic trawl (Massé et al., 2018) was employed to collect hake individuals <5 cm (total length, TL), while a bottom trawl (ICES, 2017) was employed for sizes >5 cm TL. The early-juveniles were photographed and measured on board (standard length, SL, mm) with a precision of 1 mm and then stored frozen at −20° C for further analysis in the laboratory.

2.2 | Stable isotope analysis

A first set of 169 larvae and 71 early-juveniles were selected for SIA (see Table 2). The subsequent handling and treatment of larvae are described in Laiz-Carrión et al. (2011) and Quintanilla et al. (2015). Briefly, body length was measured as SL using the image analysis software *ImageJ* (National Institutes of Health USA; <https://imagej.nih.gov/ij/download.html>). Subsequently, larvae were dehydrated in a freeze dryer for 24 h. Larval dry weight (DW, mg) was weighed on a precision microbalance (0.01 mg), and stomach contents were removed. In addition, zooplankton samples were lyophilized for 48 h.

Every dehydrated hake sample, along with fractions of microzooplankton and mesozooplankton samples for each selected station in both study seasons were packed in tin capsules (0.03 ml) for SIA. Stable isotope ratios of nitrogen (δ¹⁵N) and carbon (δ¹³C) were measured using an isotope-ratio mass spectrometer (Thermo-Finnigan Deltaplus; www.thermoscientific.com) coupled to an elemental analyzer (FlashEA1112 Thermo-Finnigan) at the Instrumental Unit of Analysis of the University of A Coruña (Spain). Ratios of ¹⁵N:¹⁴N and ¹³C:¹²C were expressed in conventional delta notation (δ), relative to the international standard atmospheric air (N₂) and Pee-Dee Belemnite (PDB), respectively (Coplen, 2011). The analyses precision for δ¹⁵N_{bulk} and δ¹³C_{bulk} was 0.12‰ and 0.14‰, respectively, based on the standard deviation of internal references (repeatability of duplicates).

Carbon isotopic values in lipids have more negative δ¹³C. Due to the variability of lipid content in biological tissues, previous chemical extraction of lipids or a mathematical correction method for δ¹³C is required (Logan et al., 2008). A species-specific correction of the δ¹³C values for lipid content in hake was applied to hake larvae and

juveniles, based on bulk C:N ratio for a separate subset of juveniles *M. merluccius* before and after lipid extraction, according to the model proposed by Uriarte et al. (2013), following Equation (1). Hereafter, $\delta^{13}\text{C}$ will refer to lipid corrected values.

$$\delta^{13}\text{C} = -1.37 + 1.08 * \delta^{13}\text{C}_u + 1.14 * \text{C:N} \quad (1)$$

where $\delta^{13}\text{C}$ and $\delta^{13}\text{C}_u$ are the corrected and uncorrected carbon isotopic signatures, respectively.

A mathematical correction of $\delta^{13}\text{C}$ values for lipid content was performed for zooplankton size fractions. An average of the four equations is proposed by Logan et al. (2008) for marine invertebrate parameters for microzooplankton and mesozooplankton size fractions. The lipid correction models were applied to estimate a mean correction value of 0.99 ‰ (SD = 0.13) and 0.61 ‰ (SD = 0.19) for microzooplankton and mesozooplankton, respectively, following Laiz-Carrión et al. (2013, 2015).

2.3 | Isotopic niche width and overlap

Isotopic niche widths for larvae and early-juveniles in both late winter and summer seasons were compared using standard ellipse areas with a sample size correction (SEAc) (Jackson et al., 2012). The standard ellipse is the bivariate equivalent of standard deviation. The standard ellipse area is calculated using the variance and covariance of $\delta^{13}\text{C}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ values, encompassing 40% of the data for each group (Laiz-Carrión et al., 2019; Varela et al., 2019). While SEAc values allow a comparison of isotopic niche breadth, comparison in the overlap of these ellipses quantifies the overlap in isotopic niche space between two groups (Jackson et al., 2012).

Further, Bayesian inference was used to create credible intervals around the Bayesian standard ellipse areas (SEAB). The isotope data are then used to form likelihood values, which are then combined with the priors to form posterior distributions. A set of 4000 estimates of the SEA is calculated from these distributions to provide the mode of the SEAB areas and credible intervals. Isotopic niche widths and overlap analyses were conducted with the R package SIBER (Stable Isotope Bayesian Ellipses in R) using R 3.3.0 (<http://www.R-project.org> 2012; Jackson et al., 2011).

2.4 | TP estimations

Tps for each early-juvenile were estimated using the model applied previously in fish larvae (Laiz-Carrión et al., 2015, 2019) and postlarvae (Quintanilla et al., 2015, 2020) (Equation 2):

$$\text{TP} = \text{TP}_{\text{basal}} + (\delta^{15}\text{N}_{\text{hake}} - \delta^{15}\text{N}_{\text{micro}}) / \Delta^{15}\text{N} \quad (2)$$

where $\delta^{15}\text{N}_{\text{hake}}$ is the isotopic signature for each hake larva or early-juvenile and $\delta^{15}\text{N}_{\text{micro}}$ is the isotopic value of microzooplankton fraction from each station where hake was collected, respectively. A basal

TP (TP_{basal}) of 1.5 was assumed for microzooplankton, as proposed by Bode et al. (2007). The nitrogen isotopic discrimination factors ($\Delta^{15}\text{N}$) were calculated for each larva following the *all fish tissue* model proposed by Caut et al. (2009) (Equation 3):

$$\Delta^{15}\text{N} = -0.3268 * \delta^{15}\text{N}_{\text{hake}} + 4.907 \quad (3)$$

2.5 | Nucleic acid analyses

A second set of 87 RNAlater preserved larvae were selected for nucleic acid analyses: 49 from late winter and 38 from summer surveys, within a common size range (see Table 2) and no significant SL differences (ANOVA, $F_{1, 85} = 3.35$, $p > 0.05$). Larval size shrinkage caused by RNAlater preservation was corrected according to the equation proposed by Rodríguez-Fernández et al., 2021. RNA:DNA ratios were measured using the whole specimen. Larvae were homogenized and centrifuged, and two supernatant aliquots were taken, one to measure total nucleic acids (RNA + DNA) and another for the DNA content ($\mu\text{g fish}^{-1}$). For the DNA measurement, samples were incubated with RNase A (type I-AS, Sigma-Aldrich) at 37°C for 30 min. The difference between total nucleic acids fluorescence and DNA fluorescence was corrected to determine the RNA fluorescence as suggested by Caldarone et al. (2006) assuming for DNA, a ratio of 2.4 RNA content ($\mu\text{g fish}^{-1}$). Nucleic acids fluorescence was determined fluorometrically using a Perking-Elmer LS-5 (excitation: 327 nm and emission: 614 nm) by adding 200- μl ethidium bromide buffer solution (0.1 mg ml⁻¹). DNA and RNA content was estimated through calibrated standards curves of calf thymus DNA (Sigma-Aldrich) and baker's yeasts RNA (Sigma-Aldrich), respectively.

2.6 | Statistical analysis

One-way analysis of variance (ANOVA; homogenous variance) was used to assess differences in morphometry (SL and DW), $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) values between seasons for both larvae and early-juvenile hake. Mann-Whitney *U* test nonparametric analyses were used to test for differences in the zooplankton size-fractionated biomass (mg) and their isotopic signature, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰), between seasons (late winter and summer). Significance tests for RNA:DNA larval nutritional ratio and Fulton's condition factor ($K = 10^3 \text{ DW SL}^{-3}$) differences between larval groups were tested with ANCOVA, with DW or SL as covariates, respectively. Analyses to test the normality and to verify the homogeneity of variances were executed beforehand. Logarithmic transformations of the data were made when necessary to fulfill the ANOVA conditions, but data are shown in their decimal values for clarity. Post-hoc comparisons were made using a Tukey test. These results were generated using the Statistica 7.1 Statsoft software package at the significance level $p < 0.05$.

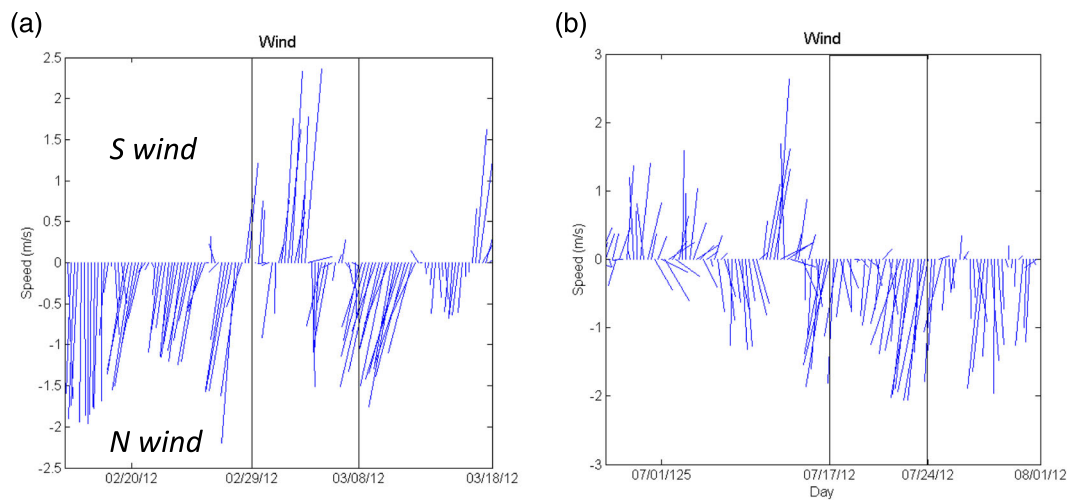


FIGURE 2 Wind direction and speed every 6 h. Data Buoy State Ports located in Cabo Vilano (Spain). The two oceanographic surveys are framed inside box (a) late winter (b) summer.

3 | RESULTS

3.1 | Comparative oceanographic seasonal scenarios

The hydrographic structure along the sampled sections changed accordingly to the wind regime, the coastal orientation, and the season. The wind regime during the late winter survey was characterized by alternating pulses of southerly winds that induce downwelling, with northerly winds favorable to upwelling. However, during the previous month to the winter survey, N winds blew continuously, which is atypical for this time of year (Figure 2a). During summer, N winds blew throughout the survey, with increased intensity toward the end of the survey. In the preceding days, N wind pulses alternated with S wind pulses (Figure 2b).

The wind conditions resulted in an average UI in the 3 months preceding the winter survey (from December 13, 2011, to March 13, 2012) of $-78.14 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$, while for the 3 months preceding the summer survey (April 29, 2012, to July 29, 2012) at $-310.80 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$. These UI values are far from the 1967–2012 historic seasonal average values, -813.7 and $227.2 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$, respectively (Figure 3).

In winter, the upper water column was completely mixed, reaching at least 200 m depth in the slope area (Figure 4a). The differences between mean temperature and salinity at 10 and 200 m depths were 0.25°C and 0.03, respectively. Mean temperature values were 12.64°C (10 m) and 12.54°C (100 m), and mean salinity values were 35.787 (10 m) and 35.782 (100 m). Overall, surface temperature and salinity in the southern stations are slightly higher than the northern stations.

In summer, the upper 60 m of the water column was stratified (Figure 4b). Mean temperature values were 17.75°C (10 m) and 13.00°C (100 m). Mean salinity values were 35.720 (10 m) and

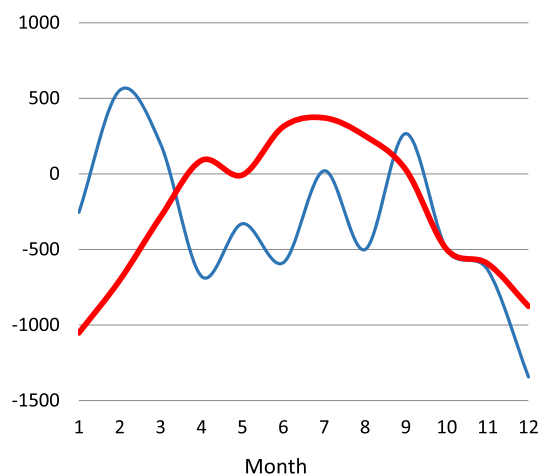


FIGURE 3 Monthly Upwelling Index in 2012 (blue line) and 1967–2012 historic average (red line). Values calculated in a cell centered at 44°N , 9°W . Positive values of the upwelling index correspond to upwelling, while negative values correspond to downwelling.

35.801 (100 m). The presence of a cross-shelf gradient in surface temperature, with lower values close to the coast was in consonance with the upwelling event (Figure 4b).

Despite the contrasting hydrographic features between surveys, the mean microzooplankton and mesozooplankton biomass showed no significant differences (Table 1). However, the isotopic signatures of $\delta^{15}\text{N}$ (‰) and $\delta^{13}\text{C}$ (‰) for both zooplankton size fractions were lower in late winter compared to summer (Table 1).

Furthermore, the spatial distribution of both microzooplankton and mesozooplankton biomass distribution and their carbon and nitrogen isotopic signatures varied between surveys (Figures S1–S4).

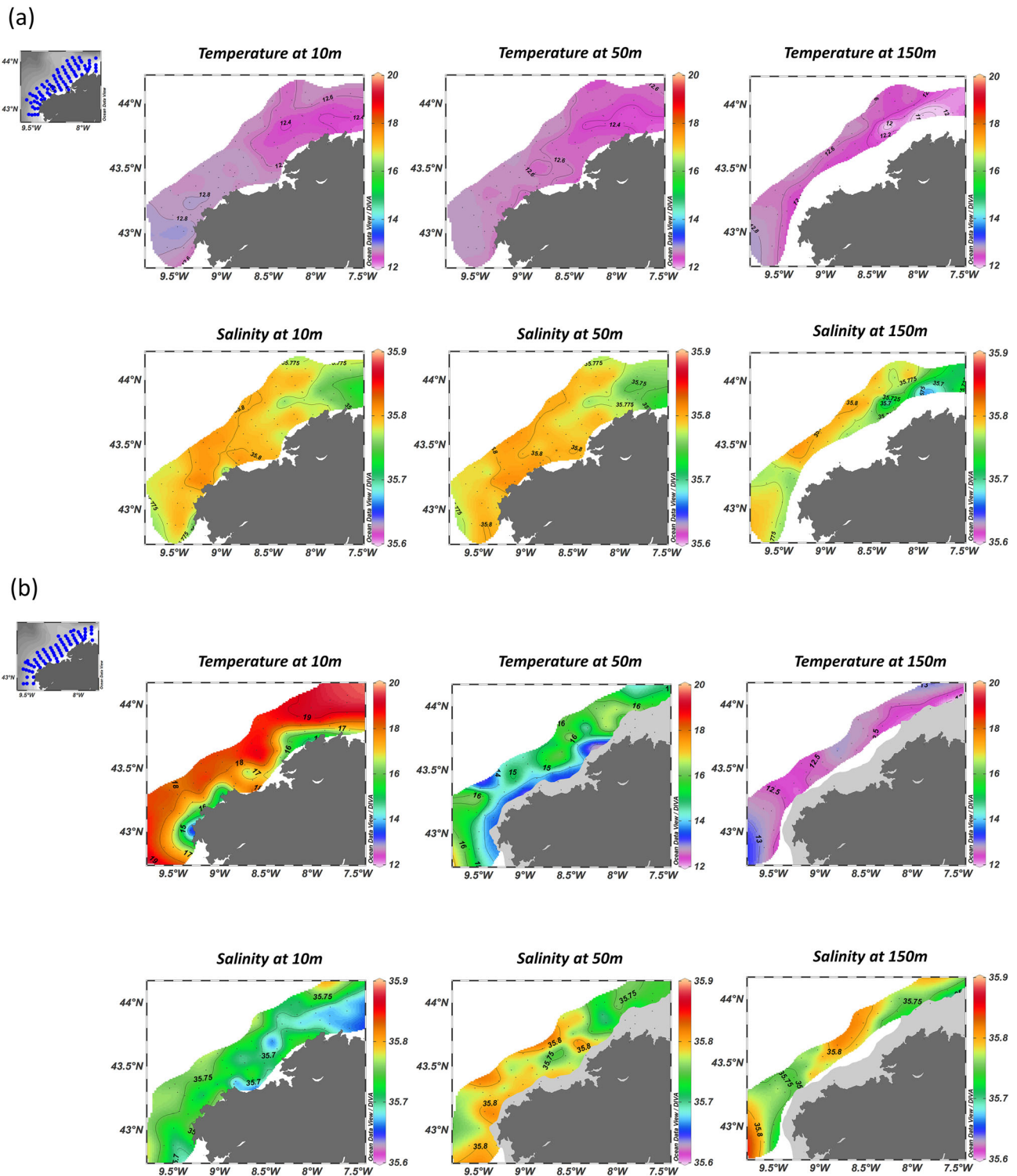


FIGURE 4 Temperature ($^{\circ}\text{C}$) and salinity at 10, 50, and 150 m in the study area for both (a) early winter and (b) summer sampling periods

3.2 | Larval and early-juvenile seasonal cohorts: Morphometric features comparison

The hake larvae were of 4.1–8.4 mm in winter and between 3.0 and 10.8 mm in summer. No significant differences between seasons were observed in size (SL) (ANOVA, $F_{1, 162} = 1.39, p > 0.05$) (Table 2).

However, the SL-DW relationship (Figure 5a), showed significant differences between seasons, whereby higher relative DW was observed for larvae captured during summer (ANCOVA $F_{1, 161} = 54.33, p > 0.01$, with SL as covariate). These larvae also corresponded to higher Fulton's K condition factors (ANOVA, $F_{1, 162} = 101.26, p > 0.05$). Early-juveniles presented different size

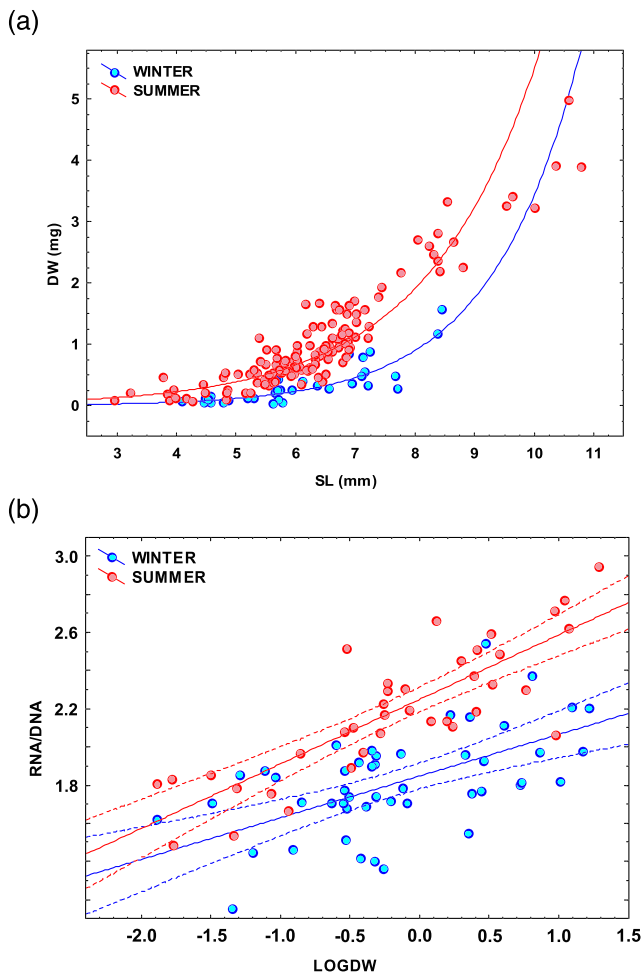


FIGURE 5 (a) Relationship between standard length (SL) and dry weight (DW) in *Merluccius merluccius* larvae for early winter (blue, $DW = 0.004^{[0.669 * SL]}$) and summer (red, $DW = 0.028^{[0.528 * SL]}$) populations. (b) Larval RNA:DNA and log transformed dry weight (LOGDW) relationship for winter (blue) and summer (red) cohorts. Solid dotted lines represent significant relationships ($p < 0.01$) and 0.95 confidence regression bands.

ranges between seasons. The size ranges analyzed for the winter and summer surveys were from 18.4 to 47.0 mm and 40.1 to 45.5 mm, respectively. Significantly higher SL (ANOVA, $F_{1, 69} = 38.67$, $p > 0.01$) and DW (ANOVA, $F_{1, 69} = 38.50$, $p > 0.01$) correspond to summer hake juveniles (Table 2).

3.3 | Larval nutritional factor (RNA:DNA)

Hake larvae were between 4 and 10 mm (Table 2) and had no differences in size (SL) between the two seasons (ANOVA, $F_{1, 85} = 3.35$, $p > 0.05$). Larvae captured during summer had higher RNA:DNA ratios (ANCOVA, $F_{1, 84} = 31.93$, $p > 0.01$) (Table 2) than larvae sampled in winter (SL as covariate). RNA:DNA ratios were positively correlated with logarithmic transformations of both SL and DW (Figure 5b).

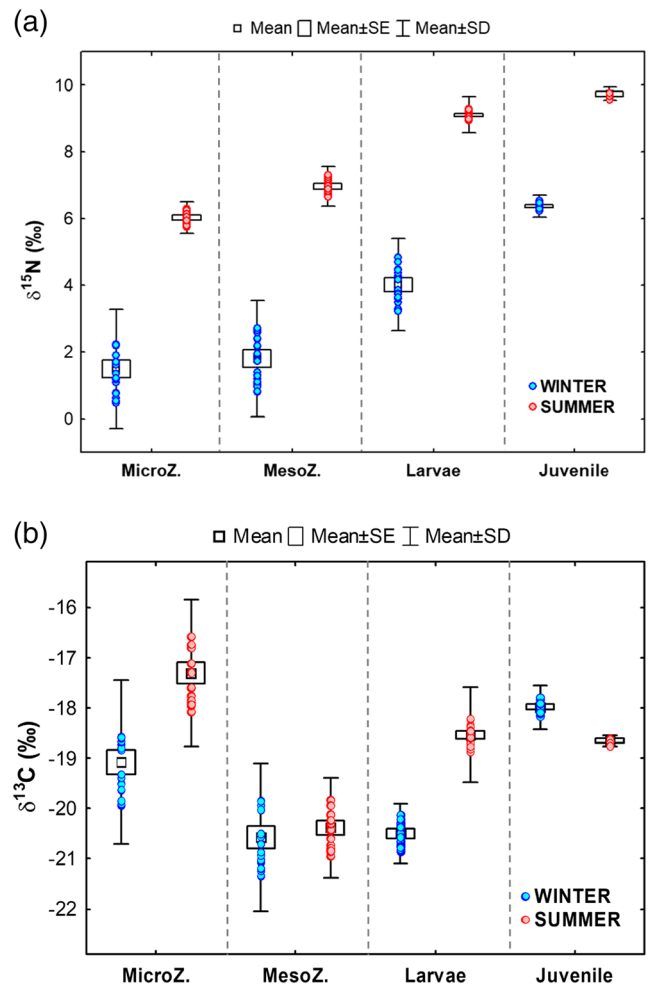


FIGURE 6 Boxplots and raw data of (a) nitrogen isotopic signature ($\delta^{15}N$) and (b) carbon isotopic signature ($\delta^{13}C$) in microzooplankton (55–200 μm), mesozooplankton (200–2000 μm), and hake-larvae and hake-early juvenile in the study area for both winter (blue) and summer (red) 2012

3.4 | Seasonal trophic pathways

$\delta^{15}N$ values increased from zooplankton fractions up to hake juveniles (Figure 6a), with significant differences between all analyzed groups (Tables 1 and 2) except for microzooplankton and mesozooplankton size fractions during the winter survey (Table 1). Moreover, mean $\delta^{15}N$ for all groups increased significantly in summer. (Figure 6a, Table 1). Figure S5 shows the overall isotopic values analyzed in this study, including microzooplankton, mesozooplankton, larvae for winter (Figure S5A), and summer (Figure S5B) and for early-juveniles for winter (Figure S5C) and summer (Figure S5D).

Microzooplankton and hake larvae also showed greater $\delta^{13}C$ values during the summer, while the mesozooplankton fraction and hake juveniles were not significantly different between seasons (Figure 6b). Hake juveniles sampled in late winter showed greater individual dispersion of $\delta^{13}C$ values between specimens (Table 2), perhaps

due to the small number of juveniles ($n = 6$) sampled in summer. In winter, mean $\delta^{13}\text{C}$ for juveniles was significantly higher than larvae, while in summer these differences disappeared.

3.5 | Ontogenetic isotopic shifts

Individual $\delta^{15}\text{N}$ values of hake larvae had a significantly negative linear relationship with SL during both seasons (Figure 7a), although the linear slope of the summer $\delta^{15}\text{N}$ signatures was less pronounced. Inversely, $\delta^{13}\text{C}$ showed a significant positive linear relationship with SL (Figure 7b) for both seasons in which the linear trend was higher during summer.

No relationships between $\delta^{15}\text{N}$ with SL has been observed in both late winter or summer early-juveniles, whereas $\delta^{13}\text{C}$ showed a significant negative linear relationship with SL in winter (Figure 7c).

3.6 | Larval and early-juveniles isotopic niche widths

The representation of standard ellipse areas corrected for sample size (SEAc , $\%o^2$) and by the Bayesian standard ellipse areas (SEA_B) (Figure 8a,b) show that the isotopic niche of hake larvae and juveniles sampled during late winter has a wider niche compared to those from summer. Furthermore, hake juveniles of both seasons showed a narrower trophic niche than larvae. In addition, no isotopic niche overlap was observed between hake larvae and juveniles during late winter, whereas a trophic niche overlap of 1.21% was observed between larvae and early-juveniles during summer.

3.7 | TP of early-juveniles

The TP estimated for early-juveniles showed higher TP values for those juveniles from the summer survey (3.66 ± 0.08) in comparison to late winter (3.26 ± 0.02) (ANOVA, $F_{1, 69} = 22.56$, $p > 0.01$) or by using SL and DW as covariates (ANCOVA, $F_{1, 68} = 14.87$, $p > 0.01$) (ANCOVA, $F_{1, 68} = 13.61$, $p > 0.01$), respectively.

4 | DISCUSSION

This study analyzed the European hake's trophodynamics in two different oceanographic scenarios where upwelling and nonupwelling conditions occurred during the late winter and summer of 2012, respectively. We observed strong seasonal gradients of zooplankton biomass that imparted divergent effects on the trophic pathways influencing the early life stages of hake during 2012 (see Figures S1–S4). In late winter, we observed an increasing north–south zooplankton gradient with increased nearshore values, while during summer, a

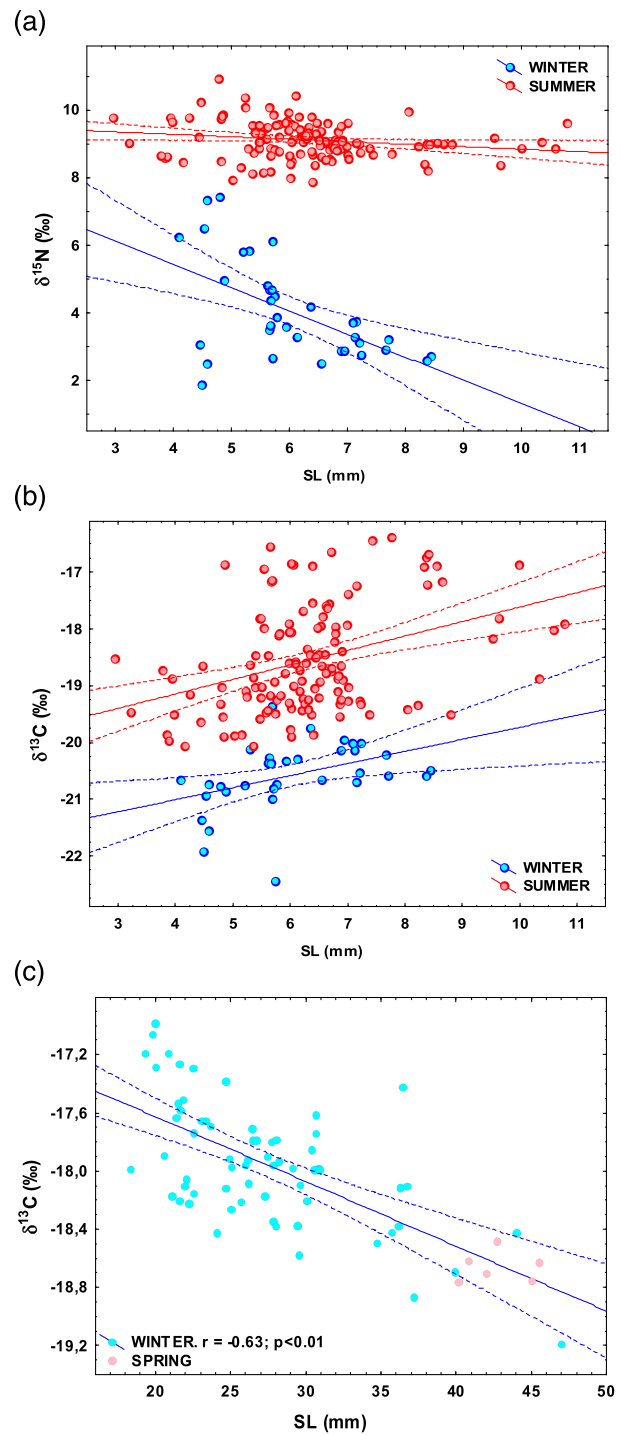


FIGURE 7 Linear relationships of Standard length, mm (SL) vs $\delta^{15}\text{N}$ (a) and $\delta^{13}\text{C}$ (b) for winter (blue) and summer (red) larval hake. Winter: $\delta^{15}\text{N} = 8.179 - 0.686 * \text{SL}$; $r = -0.55$; $p < 0.01$ and summer: $\delta^{15}\text{N} = 9.576 - 0.07 * \text{SL}$; $r = -0.19$; $p < 0.05$; winter: $\delta^{13}\text{C} = -21.861 + 0.213 * \text{SL}$; $r = 0.42$; $p < 0.01$ and summer $\delta^{13}\text{C} = -20.166 + 0.255 * \text{SL}$; $r = 0.37$; $p < 0.01$. (c) Linear relationships of SL vs. $\delta^{13}\text{C}$ for winter (blue) hake early-juveniles. $\delta^{13}\text{C} = -16.735 - 0.045 * \text{SL}$; $r = -0.63$; $p < 0.01$. Summer (red) hake early-juveniles are also represented. Dotted lines represent 0.95 confidence regression band.

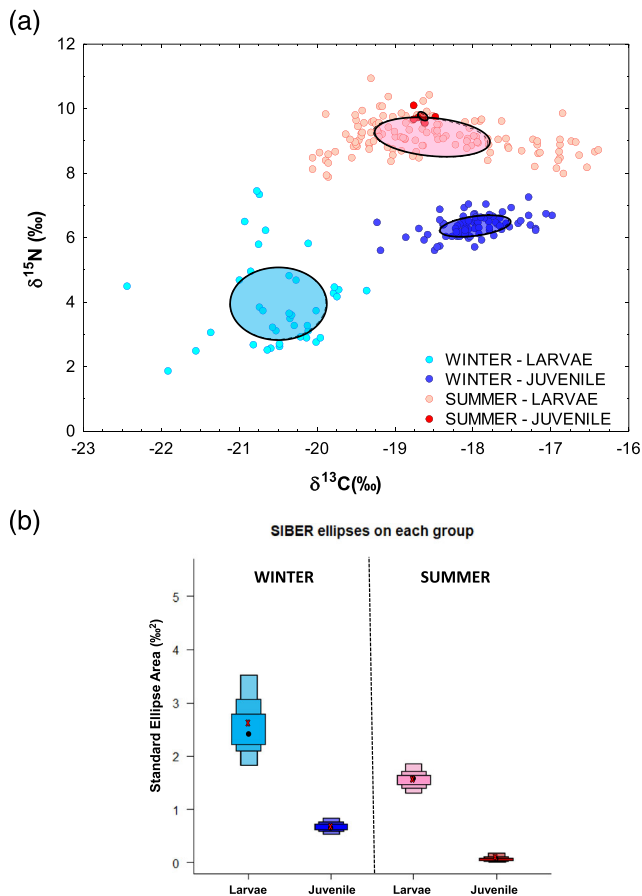


FIGURE 8 (a) Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope scatter biplots of *Merluccius merluccius* larvae (light) and early-juvenile (dark) for winter (blue) and summer (red) cohorts. Ellipses represent isotopic niche width for each group. (b) Trophic niche width as the sample size-corrected Bayesian standard ellipse area (SEA_B). Density plot shows the confidence intervals of the SEA_B, corresponding to the mean SEA_B and 95%, 75%, and 50% credible intervals in the four groups analyzed.

mesoscale anticyclonic structure centered in the study area concentrated the spatial zooplankton distribution. This spatial pattern also applies to hake's larval distribution. While in late winter hake larvae were distributed alongshore over the study area (Rodríguez et al., 2015b), larvae were more concentrated within the boundaries of an anticyclonic eddy in summer (García-Fernández et al., 2021).

4.1 | Seasonal oceanographic scenarios affect plankton and hake early life stages food web

The upwelling season in Galician waters generally occurs from early spring (April) and maximum UI values are reached in summer (July) (Álvarez et al., 2012), whereas during our study (2012), upwelling started unusually early, during the month of February. Upwelling influences the seasonal cycle of phytoplankton and planktonic grazers, which is dominated by copepods (Bode et al., 2003), pivotal in hake's larval diet (Morote et al., 2011). UI in late winter was atypically high

(Rodríguez et al., 2015a), whereas the summer UI values were unusually low (Figure 3).

From the upwelling seasonality perspective, the later winter of 2012 constituted an anomaly. Contrarily, no upwelling was observed during the sampled period in the summer survey of 2012. These contrasting upwelling versus nonupwelling processes could determine the dissimilarities in the nutrient origin and available food sources observed in each cruise. The combined effect of both winter mixing and the contribution of deeper waters due to upwelling contribute to fertilizing the surface, especially along the coast, providing a surplus of nutrients when compared with the nonupwelling conditions observed in summer, where we found a nutrient impoverishment of water masses. Such contrasting productive features led to the establishment of a specific food web structure for each cruise.

These food web differentiations may explain the seasonal differences in the relationships between RNA:DNA ratios and the Fulton factor with SL and DW. Both indices were higher for hake summer larvae. Greater size and weight (DW by SL) and higher RNA:DNA ratios may indicate differences in larval growth between cruises, and can ultimately affect early life survival rates, and thereby recruitment success (Grote et al., 2012). Summer larval condition ratios indicated better nutritional conditions when compared to winter larvae. The RNA:DNA ratio provides information on the feeding environment of the larvae within a time frame of days before sampling; thus, higher RNA:DNA ratios can be interpreted as higher recent growth capacity in summer larvae and can be useful to examine the survival processes (Bergeron, 1997) linked to predation avoidance, including cannibalism (Ocampo et al., 2011). The increase in RNA:DNA with DW for both winter and summer larvae suggests that bigger larvae had a better nutritional condition and only survivors with the optimal nutritional condition were found (Diaz et al., 2014). However, further, studies on the seasonal variability that also track daily growth are necessary to understand the growth and condition relationships. Nevertheless, there may be trade-off mechanisms whereby less abundant hake larvae that may be more concentrated and have greater survival than hake larvae under optimum trophic conditions that are widely spread over the shelf. It can be hypothesized that compensatory mechanisms may occur, whereby less abundant hake larvae may be more successful at reaching recruitment size by actively selecting more suitable prey, and benefiting from anticyclonic eddies, favoring retention and subsequent greater settlement rates of hake juveniles.

This study reveals marked temporal differences in the planktonic food web structure in NW-Galician waters. The high $\delta^{15}\text{N}$ values in summer for both zooplankton fractions and hake early life stages can be interpreted due to the decrease in inorganic nutrients after phytoplankton blooms. High $\delta^{15}\text{N}$ in plankton can be expected because low discrimination of nitrogen isotopes by phytoplankton is associated with low concentrations of nutrients (Needoba et al., 2004), as observed in transitional nonupwelling periods in the study area (Bode & Álvarez-Ossorio, 2004). In contrast, the low $\delta^{15}\text{N}$ values measured in the winter are consistent with higher nutrient concentrations and higher isotopic fractionation during phytoplankton uptake. These differential isotopic fractionation patterns translate into consistent

differences across the food web, as reported for seston and plankton (Bode et al., 2020) and also for hake larvae and juveniles in this study.

In late winter, the Iberian Poleward Current acted as a barrier and prevented the offshore transport of planktonic production. In consequence, the late winter hake larvae were distributed across the shelf of the study area (Rodríguez et al., 2015b). During the summer survey, an anticyclonic eddy located in the center of NW-Galician waters concentrated hake larvae in a defined spot (García-Fernández et al., 2021), located over the settlement area of juveniles that constitute the spawning and recruitment grounds of hake in the region (Izquierdo et al., 2021). Although an episodic upwelling event occurred during the sampling period, it was not sufficiently strong to break the stratification of the water column. The lack of differences in $\delta^{13}\text{C}$ between winter hake larvae and mesozooplankton suggests that both rely on similar carbon sources. In contrast, summer hake larvae showed large differences with both microzooplankton and mesozooplankton size fractions suggesting the use of different carbon sources. Inversely, early-juveniles collected in winter had higher $\delta^{13}\text{C}$ values than early summer juveniles, possibly related to the winter's broader distribution, as opposed to summer which concentrated juveniles in its settlement site. These differences could also be due to the larger sizes of the juveniles collected in summer. Álvarez et al. (2004) found that smaller hake larvae (<8 mm total length) were distributed around the spawning area over the shelf break, whereas larger larvae (>8 mm total length) appeared in shallower waters over the inner part of the continental shelf. Accordingly, Kacher and Amara (2005) found that more age-0 European hake were distributed inshore than in the areas of larger larval densities. Offshore drift of larvae to the open ocean has a negative impact on recruitment over the north and west Iberian Peninsula coast (Sanchez & Gil, 2000), whereas hydrographic meso-scales features, such as anticyclonic eddies, favor larval settlement and the formation of hake nursery grounds (Izquierdo et al., 2021), especially in winter when higher $\delta^{13}\text{C}$ signatures were observed in early-juveniles compared with larvae (Table 1).

4.2 | Trophic niches of hake early life stages

The availability of trophic resources can determine the segregation or overlap of the trophic niches of hake larvae and juveniles. During late winter when trophic resources were abundant, larval and hake trophic niches are separated, whereas in summer, their corresponding trophic niches overlapped possibly consequent with the scarce amount of prey available. A reasonable assumption of hake's early life trophodynamics is that during unlimited nitrogen supply (e.g., upwelling) conditions, they have passive trophic feeding behavior, as opposed to strong nitrogen limitation conditions, where hake larvae actively select prey, thus showing a specialist diet intake. Supporting this hypothesis, similar diet behavioral changes have also been observed in the Gulf of Mexico bluefin tuna larvae that preferred cladocerans (Shiroza et al., 2021). The early life trophodynamics of hake are prone to strong diet shifts. This is specially marked in the strong diet shift that hake larvae undergo when transitioning towards juveniles, when more

specialized predatory habits narrow down their trophic niche and can be observed by the narrower trophic niches of juveniles in late winter and summer compared to the trophic niches of larvae. However, despite similar sampling effort, the lower number of samples from the summer survey (6), than in late winter (65), should be noted as precautionary. The results of this study support the classic niche theory indicating that dietary generalists will be prevalent in regions where prey are scarce and competitors are few. Inversely, in highly biodiverse regions, a greater number of dietary specialists occur, as potential prey and competitors are found in abundance (Mac Arthur, 1969).

4.3 | Early life diet shifts and TP

A higher degree of trophic specialization is observed in summer, which recorded a narrower isotopic niche and higher TP's (see Figure 8). Furthermore, a species' TP, that is, their position on the food chain, maybe nonlinearly related to its dietary niche width, as omnivores consume greater diversity of prey types than herbivores or carnivores (Hayden et al., 2019). The differences in the estimated standard ellipses area (Figure 8b) suggest a more stenophagous or specialist trophic behavior in juveniles than in larvae, which show a wider isotopic niche and a more euriphagous or diverse diet.

During development great resilience to marked diet changes, changing from a passive behavior of prey selection and consuming available food resources in a productive environment to a more specialist behavior by actively selecting prey under limited food availability. This is particularly evident in the transition to early-juveniles, where our results reveal that the width of the isotopic niche is mainly reduced due to the greater feeding specialization. Thus, hake's trophic behavior varies in function of the availability and type of available trophic resources together with its ontogenetic development.

With growth, larval $\delta^{15}\text{N}$ linearly decreases with SL, and contrarily, $\delta^{13}\text{C}$ increases with SL for both winter and summer (Figure 7a, b). These ontogenetic dynamics have also been observed in hake preflexion larvae under laboratory-controlled experimental conditions (16° C) after 24 days post-hatch (Morales, 2016). Considering transgenerational transmission, Uriarte et al. (2016) demonstrated that stable isotopes were maternally transmitted to fish offspring, reporting that eggs and preflexion larvae of bluefin tuna corresponded to the N and C isotopic signatures of adult females. Therefore, the pattern of gradual decrease of maternal $\delta^{15}\text{N}$ signatures was observed during preflexion stages, as also observed in bluefin tuna reared larvae (Uriarte et al., 2016) and wild tuna larvae (García et al., 2017). Given that the majority of the hake larvae sampled in both surveys belonged to preflexion stages, the importance of maternal transmission of stable isotopes for hake cannot be stressed enough as it may be crucial for larval survival by the transfer of nutritional conditions to offspring (Pérez & Fuiman, 2015), as well as for developing growth potential (Green & McCormick, 2005).

Furthermore, summer hake juveniles have similar ranges of $\delta^{13}\text{C}$ signatures as larvae, which to some degree overlap. Such trophic overlap is not observed in larvae and juveniles from late winter. Thus, it

may be inferred that late winter larvae have a more oceanic-based trophic source (upwelling). The larvae from summer were sampled in an anticyclonic gyre located over the hake nursery grounds, which could influence the trophic overlap between larvae and juveniles during this season. Regarding $\delta^{13}\text{C}$ ontogenetic dynamics, the positive relationship between $\delta^{13}\text{C}$ and SL observed in late winter and summer hake larvae (Figure 7b) can be attributed to the origin of carbon sources.

The benthic primary producers $\delta^{13}\text{C}$ signatures are generally higher than pelagic signatures to be reflected in consumer $\delta^{13}\text{C}$ values (France, 1995). Therefore, $\delta^{13}\text{C}$ signatures are valuable to distinguish between benthic and pelagic food sources, particularly for fishes that transit from pelagic to benthic habitats (e.g., larvae and early-juveniles).

Assuming that TP₃ and TP₄ represent complete zooplanktivory and piscivory, respectively (Iitembu et al., 2012), our hake juveniles' TP estimations of 3.26 and 3.66 during late winter and summer are between 26% and 66% piscivorous, respectively. These results are as expected considering that higher average SL's were observed in summer in comparison to late winter. Our results agree with previous studies that estimated size-specific trophic levels of 3.2 for the smallest sizes (9–15 cm) and 3.7 in medium sizes (19–36 cm) (Le Loc'h & Hily, 2005). Our results, although considering smaller size ranges, indicate that early-juvenile *M. merluccius* may be considered third-level consumers, showing diet shifts from carbon sources with more positive $\delta^{13}\text{C}$ in smaller individuals and with more negative carbon sources $\delta^{13}\text{C}$ in larger juveniles (Figure 7c).

Settlement is one of the major changes during the early life history of demersal fishes (Able et al., 2006). During settlement, late larvae or early-juveniles move from pelagic to demersal habitat, with subsequent changes in their foraging habits (Secor, 2015). This diet change from pelagic to demersal environment can explain differences in nutrient source use because the diet of larger juveniles will have benthic trophic sources. This is the case of summer juveniles, where we only had large individuals compared to late winter, where these had more negative $\delta^{13}\text{C}$ values (see Figure 7c). Furthermore, we cannot exclude that upon attaining a piscivorous feeding behavior, larger hake juveniles may cannibalize on smaller specimens (Botto et al., 2019) possibly due to density-dependence processes and spatial and temporal overlap among larval size classes. Thus, the differences observed can be attributed to settlement processes undergone by the species where larger hakes feed on deeper prey sources that render lower $\delta^{13}\text{C}$ values (France, 1995).

In conclusion, the most prominent feature distinguishing both winter and summer spawning seasons is the productivity during each sampling period resulting in differential nutrient uptake from food web structure and thereby influencing the early life trophodynamics of hake. During late winter, the important nutrient input consequent with upwelling is responsible for the low $\delta^{15}\text{N}$ signatures throughout the food web structure, from baseline zooplankton sources to hake juveniles. An opposite situation occurs in summer, in which $\delta^{15}\text{N}$ signatures are significantly higher throughout the food web. The different trophic pathways observed during each season indicate shifts in

feeding behavior of hake larvae whereby larvae from an enriched environment as during late winter have a less selective feeding behavior possibly caused by the abundance of feeding resources, and possibly less prey diversity, reflected in their higher trophic niche. Contrarily, larvae during the less productive summer season showed a more selective behavior, with lower trophic niche, specially made evident in the transition to juvenile stages in which larvae and juveniles show overlapping niches. A higher degree of trophic specialization is observed in juveniles related to larvae with narrower isotopic niche and higher TP's in summer juvenile. Differing feeding behavior between hake larval cohorts may influence growth differences, as made evident by the greater RNA:DNA ratios and the SL versus DW relationships of the summer larvae. The study provides the first estimates of nutritional condition by RNA:DNA ratios in European hake larvae enabling to verify interseasonal differences between seasonal hake larval cohorts. Its implementation may represent a means to analyze larval viability towards recruitment success.

ACKNOWLEDGMENTS

The authors express their gratitude to all the participants that work in both CRAMER-0312 and CRAMER-0712 surveys, including the crew of the RV "Cornide de Saavedra." The manuscript was significantly improved by feedback from A. Bode, E. Malca, and two anonymous reviewers that contributed to improve and clarify the manuscript. We are also grateful to M. Lema, from the Servicio de Análisis Instrumental (Universidad da Coruña, Spain), for the stable isotope determinations.

CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

RLC, AG, and CG designed the research. RLC, JM, AC, JG, AH, AG, and CG performed the research. RLC, AC, AH, AG, AU, and JM analyzed the data. RLC, JM, AU, AC, AH, AG, CG, and FS-R collected the data. All authors wrote, revised, and edited the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing applies to this article as new data were created in this study. The original data on sample location, micro- and meso-zooplankton biomasses as well as individual fish characteristics and stable isotope composition, can be accessed through the PANGAEA repository ([https://urldefense.com/v3/https://www.pangaea.de/!!N11eV2iwtfslpCOszh6fNQWDb-RxdLqeUI5UZz9BbuJMwpqj5tUo5qK44kDrKKx-a4wE71C5dZZpl1lwHJOp2QpgCWTbLkzkgA\\$](https://urldefense.com/v3/https://www.pangaea.de/!!N11eV2iwtfslpCOszh6fNQWDb-RxdLqeUI5UZz9BbuJMwpqj5tUo5qK44kDrKKx-a4wE71C5dZZpl1lwHJOp2QpgCWTbLkzkgA$)). Correspondence and requests for materials should be addressed to RLC.

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How to cite this article: Laiz-Carrión, R., Cabrero, Á., Quintanilla, J. M., Hernández, A., Uriarte, A., Gago, J., Rodríguez, J. M., Piñeiro, C., García, A., & Saborido-Rey, F. (2022). Shifts in the seasonal trophic ecology of larvae and juveniles of European hake (*Merluccius merluccius*): From the Galician upwelling system (NW Spain). *Fisheries Oceanography*, 1–15. <https://doi.org/10.1111/fog.12603>