Trophic niche changes during settlement in the Argentine hake *Merluccius hubbsi* reveal the importance of pelagic food post metamorphosis

Florencia Botto[1],*, Esteban Gaitán[2], Oscar O. Iribarne[1], E. Marcelo Acha[1,2]

[1]**Instituto de Investigaciones Marinas y Costeras (IIMyC, UNMDP-CONICET), CC 1260 Correo Central, B7600WAG Mar del Plata, Argentina**
[2]**Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo No. 1, Escollera Norte, B7602HSA Mar del Plata, Argentina**

ABSTRACT: Among those factors that determine the success of a fish cohort, trophic interactions play a key role, especially during the larval and juvenile stages. The Argentine hake *Merluccius hubbsi* is the most abundant demersal fish in the southwestern Atlantic and also one of the main commercial resources for Argentina. By employing C and N stable isotope analysis, we evaluated *M. hubbsi* changes in trophic niche and trophic position throughout their early life from early larvae to juveniles 2+ (8–330 mm total length, TL) during their drift from the spawning to the nursery ground. We analyzed 121 individuals and 7 possible resources in different sectors as fish move from the spawning to the nursery ground in the coastal region of northern Argentine Patagonia. Our results show that Argentine hakes occupy different trophic niches during their ontogenetic development. While larval stages (8–34 mm TL) feed almost exclusively on copepods, larger juveniles showed shrimps as their main prey. Individuals between 35 and 89 mm TL showed the most generalist diet, with a mix of both pelagic and epibenthic prey. Therefore, our results indicate that the change from a planktonic to a demersal habitat (settlement), which is of paramount importance in the early life history of hakes, is a gradual process.

KEY WORDS: *Merluccius hubbsi* · Argentine hake · Stable isotopes · Ontogenetic development · Trophic interaction · Dietary changes

1. INTRODUCTION

Among those factors that determine the success of a fish cohort, trophic interactions play a key role, especially during larval and juvenile stages. Because of diet changes during early life, it is said that fish exhibit a life history omnivory (Pimm & Rice 1987), moving through different trophic niches during ontogeny. Indeed, differences in dietary preferences between the life stages of a species may be even greater than those between species (Livingston 1988). Moreover, these changes imply that one fish species may play varying ecosystem roles during its ontogeny. For example, different stages of one species can have distinct functional roles in nutrient and energy fluxes, oc-cupying more than 1 trophic position in the food web and changing interactions with other species from being a competitor to being a predator (Miller & Rudolf 2011). The use of different resources can even affect the connections between distant habitats or benthic–pelagic coupling. Moreover, it has been hypothesized that in the presence of species with ontogenetic niche shifts, complexity can increase community persistence (Mougi 2017). Therefore, it is important for the understanding and subsequent management of marine ecosystems to consider the variability within main populations that constitute communities (e.g. Miller & Rudolf 2011, Nakazawa 2015).

Settlement is one of the major changes during the early life history of fishes that are benthic or demer-

sal when adults (e.g. Kaufman et al. 1992, Able et al. 2006). To satisfy the ever-changing trophic demands, larvae, juveniles and adults migrate to new habitats and, in many cases, to an entirely different food web. During settlement, larvae and young juveniles move from pelagic to demersal food webs with subsequent changes in their foraging success and predation risks (Secor 2015). This process is also associated with the morphological transformation of larva into juvenile and, in some cases, with a displacement towards the place of settlement (Sánchez & Gil 2000). Therefore, those mechanisms that promote adequate availability of food as well as the processes involved in the transport or retention of larvae into favorable habitats are coupled during early ontogeny and become responsible for the success of a cohort (Cushing 1975, Bakun 1996).

The Argentine hake *Merluccius hubbsi* is the most abundant demersal fish in the southwestern Atlantic and the main Argentine marine commercial resource in terms of biomass. For example, the Argentine fleet landed 279 690 t in 2016, 281 108 t in 2017 and 265 897 t in 2018, always representing around 60% of the total national marine fish catch (www.minagri. gob.ar). Two hake stocks are recognized in the Argentine Sea, a northern and smaller one, restricted to 34–41° S, and a southern or Patagonian stock, distributed between 41° and 55° S, which sustains ca. 85% of the total hake catch. Hake is a batch spawner with a protracted spawning season during the southern summer, lasting from December to March and peaking in January to February (Macchi et al. 2004, Pájaro et al. 2005). High concentrations of eggs and larvae are often observed between 43° and 45° S in coastal and mid-shelf waters (Irusta et al. 2016). This location of the spawning area matches the bottom expression of a tidal front located parallel to the coastline at around 80 m depth (Pájaro et al. 2005, Macchi et al. 2010). Larvae attain retention by making daily vertical migrations in a region where flux is vertically structured, so early larval stages remain near the spawning ground. Then, during the following months, they are slowly advected southwestward by bottom currents to their nursery and settlement locations, particularly to the San Jorge Gulf (45° S, 65° W; Fig. 1), which is the main nursery ground (Álvarez-Colombo et al. 2011, 2014). The San Jorge Gulf is a semi-enclosed basin with a rather deep connection to the open shelf. Vertical mixing by wind and tides, and the entrance of southern diluted waters from the Magellan Strait, characterizes some regions of the gulf, generating frontal systems (Acha et al. 2004). Those fronts produce nutrient enrich-
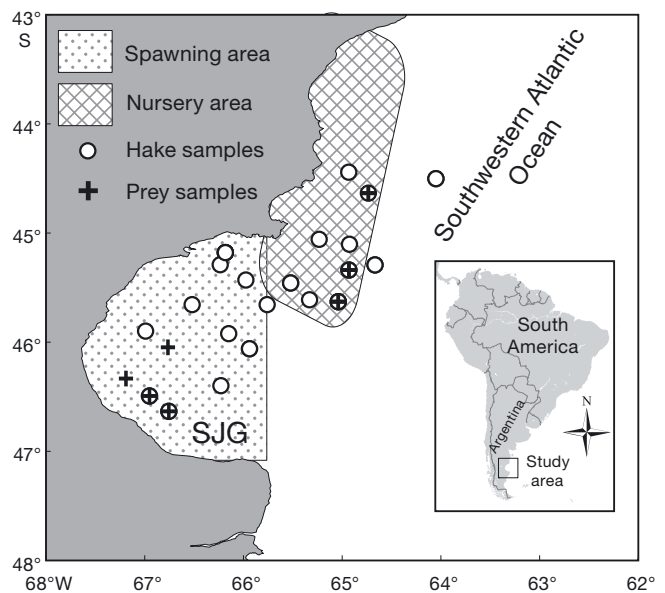

Fig.1. Argentine hake and potential prey, and the location of spawning and nursery areas. SJG: San Jorge Gulf

ment and retention mechanisms of ecological importance (Acha et al. 2004, Carreto et al. 2007).

Stomach content analyses performed on specific size groups of Argentine hakes in both spawning and nursery areas reveal different results. Early larvae (size <30 mm) have shown a diet based mainly on calanoid copepodites and small adult copepods (<1 mm) such as *Calanoides carinatus* and *Drepanopus forcipatus* (Viñas & Santos 2000, Temperoni & Viñas 2013), which are the dominant groups in the spawning ground (Sabatini & Martos 2002). Juveniles between 60 and 150 mm showed mainly amphipods in their diet (Temperoni & Viñas 2013, Temperoni et al. 2014), while larger hakes (between 150 and 300 mm) incorporate larger epibenthic fauna such as squat lobster (Belleggia et al. 2014). The type of available prey for hakes varies accordingly with a progressive increase in the abundance or availability of euphausiids, amphipods (Temperoni et al. 2018), the small sergestid shrimp *Peisos petrunkevitchi* (Bezzi et al. 1995) and the squat lobster *Munida gregaria* (Vinuesa & Varisco 2007) toward the nursery ground. On the other hand, Argentine hake individuals larger than 350 mm feed mainly on cephalopods and fishes (Belleggia et al. 2014). Although stomach content studies provided an accurate description of the hake's diet, this methodology is limited by the time that the contents remain in the stomach, and there are problems associated with differential digestion rates of prey (Cortés 1997). Therefore, stomach contents provide snapshot data that can be

biased to patchy distributed prey and can be of low value to evaluate broad changes in diet.

Although it was suggested that hakes settle soon after their metamorphosis (e.g. Buratti & Santos 2010), acoustic methods and sampling with midwater and bottom trawls show that a large proportion of juvenile hakes remain well above the seafloor during their first year (Álvarez-Colombo et al. 2014). Daily vertical movements of young-of-the-year (YOY) are hypothesized to be a response to both biological interactions and circulation patterns (Álvarez-Colombo et al. 2014). In other hake species, these vertical migrations follow the movements of their prey, mainly planktonic crustaceans (e.g. Bozzano et al. 2005). The shift from the pelagic to the demersal habitat of juvenile hakes appears to be a gradual process (Álvarez-Colombo et al. 2011), and therefore the shift in diet is also expected to be gradual, with a period during which individuals may be exploiting resources from both habitats. To evaluate this hypothesis, we performed carbon and nitrogen stable isotope analysis (SIA), which is an effective tool to investigate niche shifts because it provides a temporally and spatially integrated representation of diet (Hammerschlag-Peyer et al. 2011). The main goal was to evaluate ontogenetic changes in the diet of Argentine hakes throughout their early development: from early larvae (8 mm, when they start feeding) to juveniles 2+. We encompass Argentine hake movement from the spawning to the nursery ground while the settlement takes place. We evaluated the hypothesis that the shift in Argentine hake diet from pelagic to demersal prey is a gradual process, and the pelagic prey remain important in juveniles during a period after metamorphosis.

2. MATERIALS AND METHODS

Larval and juvenile Argentine hakes and their potential prey were collected in the region of northern Patagonian tidal fronts (spawning area) and the San Jorge Gulf (nursery area, Fig. 1) in April 2009, during a research survey carried out by the RV 'Capitán Oca Balda' of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP). Hake specimens (8–320 mm in total length, TL) and potential prey were collected with different samplers: bottom trawl, rectangular mid-water trawl, epibenthic sledge and mini bongo (with nets of 67 and 220 μm mesh to catch a broad size spectrum of items). All specimens were separated, measured to the lower millimeter with a caliper and frozen on board for sub-

sequent processing. Pooled samples (between 3 and 20 individuals of the same size per pool) were performed for small entire individuals (8–50 mm), while muscle tissue was used for the other size groups. All samples were oven dried to a stable weight (70°C). One milligram of dried material was weighed and packed into tin capsules for SIA. The most abundant zooplankton taxa and potential epibenthic prey were separated (including copepods, amphipods, euphausiids, mysids, squat lobsters and shrimps) and prepared for SIA as described in this paragraph. All samples were analyzed at the Stable Isotope Facility, University of California, Davis (USA), using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon). Laboratory standards (nylon, glutamic acid, bovine liver) were run every 5 samples, and measurement error was estimated to be less than 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Ratios relative to the standard were calculated (Pee Dee Belemnite for carbon and air [N_2] for nitrogen) using the standard equation $\delta X = \{(R_{sample} / R_{standard}) - 1\} \times 1000$, where X is ^{13}C or ^{15}N, and R is the ratio of the heavy to light isotope for the sample (R_{sample}) and standard ($R_{standard}$) in units of parts per thousand (‰).

We evaluated the relationship between both stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) and size classes (TL) of Argentine hake with simple linear regression (Zar 1999). Although the geographic range is small and within the same biogeographic domain, we evaluated the variation of stable isotope signatures of hake with respect to latitude and longitude (with Pearson's correlation analysis, Zar 1999). The relationship between size of sampled hakes and latitude and longitude was also analyzed, but given that it was not a random population sample, the analysis was performed only to account for the possible effects of size influencing the relationship between isotope signatures and location. Because of logistical constraints prey were not sampled along the entire gradient, so stable $\delta^{15}N$ and $\delta^{13}C$ of potential prey were compared between the 2 sampled areas (spawning and nursery areas, Fig. 1) using Welch's t-tests (Zar 1999).

Bayesian mixing models were performed to evaluate the contribution of the potential prey to the hake diet using the MixSIAR package version 3.1.10 (Stock & Semmens 2016). We fitted different models including fixed and continuous effects as covariates explaining variability in the stable isotopes of hake and to calculate relative support for multiple models via information criteria (Stock et al. 2018). We therefore ran 5 models: (1) null model: a model with no covariates, (2) continuous model: a model with size as a
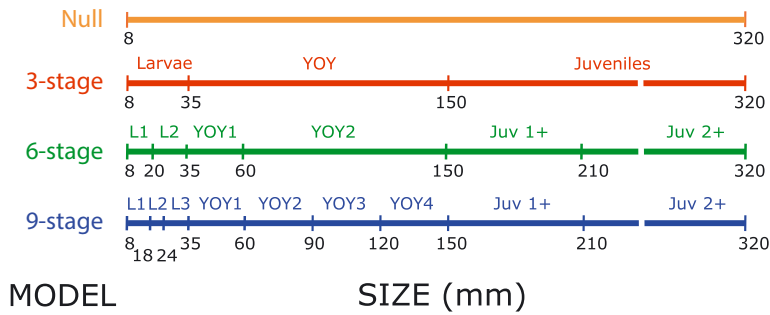
Fig. 2. Size range of the different models compared with MixSIAR. YOY: young-of-the-year; L: larvae; Juv: juveniles

continuous effect, and 3 models with size stages as fixed factors: (3) a 3-stage model, (4) a 6-stage model, and (5) a 9-stage model. The 3-stage model considered larvae, YOY and juveniles as stages (see Fig. 2 for size ranges). Given that during these stages hake are under multiple morphological and behavioral changes, we considered models with subdivisions of these stages (Fig. 2). The 6-stage model considered a subdivision of larval stage at 20 mm, the size at which all larvae metamorphose (Bezzi et al. 2004), and 2 YOY stages, one smaller than 60 mm, which is a size range not included in previous studies of stomach contents, and one 60 to 150 mm, following the size range studied for stomach contents (Temperoni et al. 2013). Juveniles were divided according to age (Vaz-dos-Santos & Rossi-Wongtschowski 2007). In the 9-stage model, larval size stages were divided following classification based on morphological changes (Betti et al. 2009): between 8 and 18 mm, when larvae complete development of the vertebral column and acquire the majority of fin rays; between 18 and 24 mm, the transformation stage, in which pectoral fin development is completed; and between 25 and 34 mm, when the fin-ray complements are complete and squamation begins. YOY were subdivided into size classes following Temperoni et al. (2013). We ran the 5 models including concentration dependence means and uninformative priors and using trophic fractionation factors (TFFs) of 3.4 ± 0.4‰ for $\delta^{15}N$ and 0.3 ± 1.3‰ for $\delta^{13}C$ (Post 2002). We selected the best model, after convergence, calculating the leave-one-out (LOO) cross-validation, which evaluates the relative support for the candidate models fitting the mixture data (Stock et al. 2018).

After selecting the 9-stage Bayesian mixing model and to evaluate the possible different contributions of sources as food for the 9 size groups of hake, we calculated the 95% confidence intervals of the *a posteriori* distributions for each group. Trophic level was also calculated for the different size groups of hake

as follows: trophic level = ($\delta^{15}N_{hake}$ − $\delta^{15}N_{baseline}$) / 3.4‰ + 2, where 3.4‰ is the TFF as used in models and $\delta^{15}N_{baseline}$ = 8.65‰, which is the mean value of the benthic filter-feeder scallop *Zygochlamys patagonica*, taken from the same area in the previous year (Gaitán 2012). This scallop is a good baseline (trophic level = 2), given that filter-feeder bivalves can integrate the possible variation of primary productivity of the area (Vander Zanden & Rasmussen 1999).

Using MixSIAR posterior distributions, we also calculated a specialization index for each size group following Newsome et al. (2012). This specialization index varies from 0 in complete generalist consumers (i.e. consumers feed on all available prey resources in equal proportions) to 1 in consumers that are ultra-specialists (i.e. consumers feeding on only 1 of the available prey resources, regardless of which is the preferred item). To evaluate the isotopic shift from pelagic to benthic environment of the different hake size stages, we combined sources *a posteriori* in 2 groups: pelagic and epibenthic prey. We evaluated the proportional contribution of both groups of prey to the diet of different size stages of hake.

3. RESULTS

The isotopic signature of hake showed $\delta^{13}C$ values between −19.5 and −11.5‰ and $\delta^{15}N$ values between 13.6 and 17.5‰ (Table 1, Fig. 3). Hake showed a positive logarithmic relationship between $\delta^{15}N$ and total length ($F_{1,117}$ = 92, p < 0.05; Fig. 4A) and a negative logarithmic relationship of $\delta^{13}C$ with increasing total length ($F_{1,117}$ = 498, p < 0.05; Fig. 4B). Several relationships were detected along the way, from the spawning to the nursery ground. The $\delta^{15}N$ values of hake increase with latitude (r^2 = 0.1, p < 0.05; Fig. 5A) and with longitude (r^2 = 0.26, p < 0.05; Fig. 5B), and there was a negative relationship between $\delta^{13}C$ and both latitude (r^2 = 0.11, p < 0.05; Fig. 5C) and longitude (r^2 = 0.47, p < 0.05; Fig. 5D). Also, there was a positive relationship between size and latitude (r^2 = 0.18, p < 0.05) and longitude (r^2 = 0.39, p < 0.05) with larger individuals in the southwestern stations (Fig. 6).

Species of zooplankton captured were unidentified cyclopoid copepods, calanoid copepods *Calanoides carinatus*, euphasiids *Euphausia lucens*, amphipods *Themisto gaudichaudii* (Hyperiidae), chaetognaths *Parasagitta friderici* and juveniles of the pelagic morphotype of squat lobster *Munida gregaria*. This spe-

Table 1. Carbon and nitrogen stable isotope ratios of the different hake stages and potential prey, size ranges and estimated trophic level. YOY: young-of-the-year; pel: pelagic morphotype; ben: benthic morphotype; –: not measured

| Species or stage	Size range (mm)	Trophic level [mean (±SD)]	δ^{13}C [mean (±SD)]	δ^{15}N [mean (±SD)]	N
Merluccius hubbsi					
Larvae 1	8–19	3.5 (0.1)	−12.1 (0.44)	14.6 (0.42)	18
Larvae 2	20–24	3.8 (0.3)	−12.8 (0.89)	15.7 (1.04)	8
Larvae 3	25–34	3.8 (0.1)	−12.7 (0.92)	15.7 (0.25)	6
YOY 1	35–59	3.9 (0.1)	−14.8 (0.99)	16.1 (0.43)	25
YOY 2	60–89	3.8 (0.1)	−15.0 (0.39)	15.9 (0.3)	13
YOY 3	90–119	3.9 (0.1)	−17.8 (0.72)	16.9 (0.52)	6
YOY 4	120–149	4.1 (0.1)	−17.7 (1.04)	16.9 (0.24)	10
Juveniles 1+	150–209	4.1 (0.1)	−17.2 (0.86)	16.2 (0.55)	19
Juveniles 2+	210–320	4.0 (0.2)	−17.8 (0.62)	16.3 (0.65)	10
Cyclopoid copepods	–	–	−14.2	8.6	1
Calanoides carinatus	>2	2.7 (0.1)	−13.1 (0.11)	11.6 (0.17)	6
Euphausia lucens	4–10	3 (0.2)	13.7 10.30	13.1 (0.65)	6
Themisto gaudichaudi	5–13	2.8 (0.1)	−16.1 (0.15)	12.5 (0.41)	3
Parasagitta friderice	15–25	3.8 (0.0)	−15.5 (0.12)	15.5 (0.28)	3
Munida gregaria pel	10–13	2.7 (0.0)	−18.9 (0.29)	12.1 (0.18)	3
Munida gregaria ben	8–12	3.3 (0.2)	−15.6 (0.52)	14.2 (0.69)	3
Peisos petrunkevitchi	45–55	3.9 (0.0)	−18.4 (0.21)	14.3 (0.04)	3
Mysidacea	15–20	3 (0.5)	−18.5 (0.45)	13.0 (1.74)	3
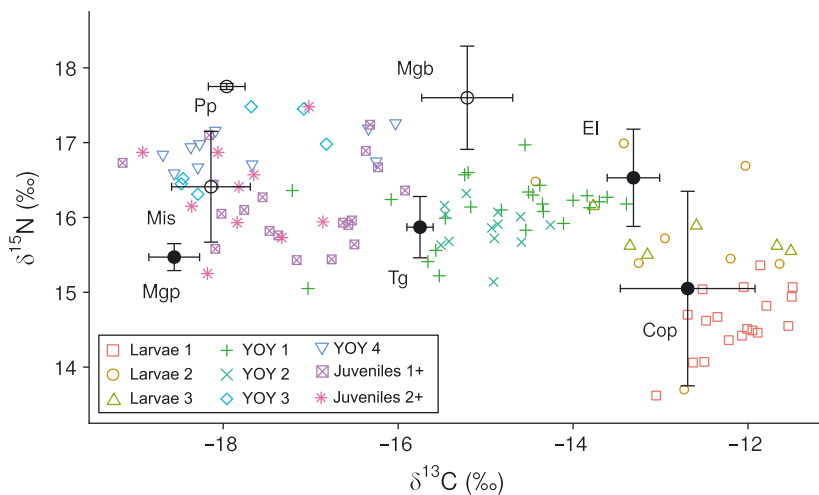
Fig. 3. Mean (±SD) δ^{13}C and δ^{15}N of hake size classes and evaluated prey (adjusted by fractionation). Cop: copepods (including cyclopoids and calanoids); El: *Euphausia lucens*; Mgb: *Munida gregaria* benthic morphotype; Tg: *Themisto gaudichaudii*; Pp: *Peisos petrunkevitchi*; Mis: mysids; Mgp: *Munida gregaria* pelagic morphotype; YOY: young-of-the-year. Black circles are pelagic prey; white circles are benthic prey

cies presents 2 morphotypes (Baba et al. 2008, Pérez-Barros et al. 2008) previously considered as distinct species, and given their differences in isotope signals, we considered both morphotypes as different sources. Epibenthic species caught were the shrimp *Peisos petrunkevitchi* (Sergestidae), unidentified mysids (Mysidacea) and juveniles of the benthic morphotype of the squat lobster. These potential prey showed a wide range of δ^{13}C values (between −11 and −19‰,

Table 1), with lower mean values in the spawning area (−16.7‰, SD = 2.18) than in the nursery zone (−14.9‰, SD = 1.09; t = 2.32, df = 22.7, p < 0.05). δ^{15}N showed no difference between sites (13.1‰, SD = 1.51; t = 1.1, df = 29, p > 0.05).

Mixing models were performed with all potential prey except *Parasagitta friderici*, since it was never found as a major prey of hakes and the mean isotopic values were out of the isotopic polygon (δ^{13}C = −15.5‰, SD = 0.11; δ^{15}N = 15.5‰, SD = 0.07; Table 1). Copepods were included as a single group, given that we obtained only 1 sample of cyclopoid copepods. Most models converged after running for 3×10^5 iterations with a burn in of 2×10^5. The continuous model did not converge after 3×10^6 iterations and therefore was excluded from further analysis. The model with the lowest LOO value and 100% of weight was the 9-stage model (see Table 2), followed by the 6-stage model, the 3-stage model and, finally, the null model.

The posterior distributions of the selected 9-stage model showed differences in the proportional contribution of each prey to the different groups of hake. The small individuals corresponding to larval stages showed a very high contribution of copepods to their diet (Fig. 7, Table 3). The importance of copepods as
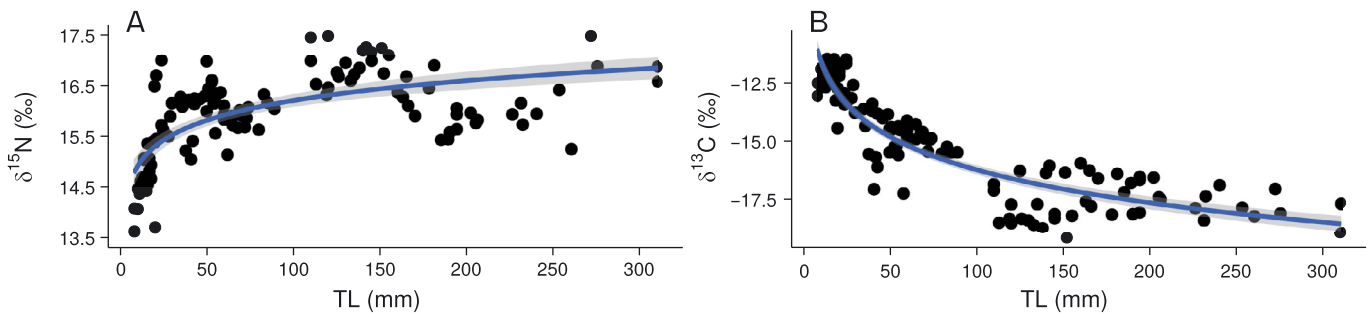
Fig. 4. Relationship between hake stable isotope signatures and fish size as total length (TL). (A) $\delta^{15}N$ and (B) $\delta^{13}C$. Line and shades are the adjusted regression line and its 95% CI
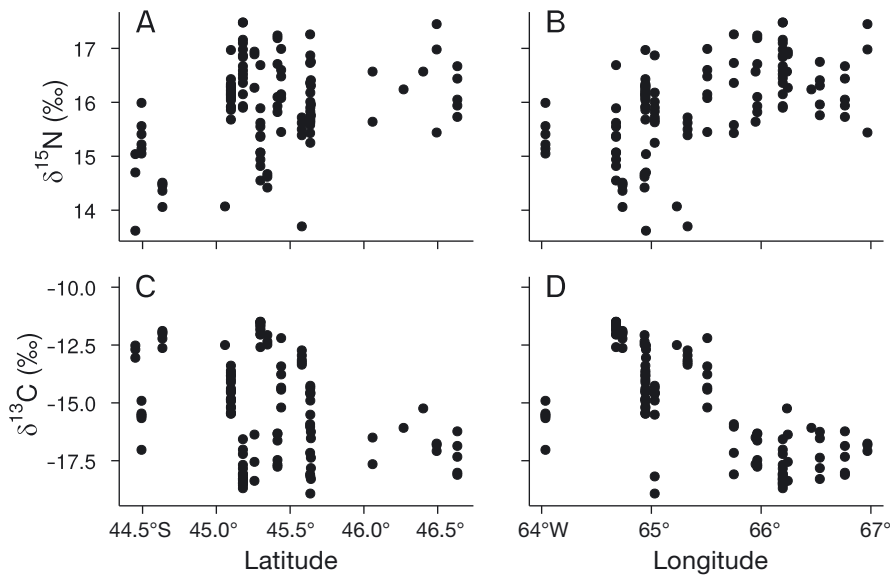

Fig. 5. Relationship between stable isotope signatures of hake with latitude and longitude. $\delta^{15}N$ with (A) latitude and (B) longitude; $\delta^{13}C$ with (C) latitude and (D) longitude

Fig. 6. Relationship between size of hake and (A) latitude and (B) longitude. TL: total length

prey diminished as hake increased in size with very small contributions in the larger size classes. Size classes YOY 2 and YOY 3 showed the most uncertainty in diet contribution, with the amphipod *T. gaudichaudii* and euphasiids *E. lucens* gaining importance in the diet. YOY 4 and juveniles 1+ and juveniles 2+ showed high contributions from squat lobster, shrimps and mysids in their diet (Fig. 7, Table 3). When sources were combined in epibenthic and pelagic prey, there was a higher pelagic contribution in larval stages of hake; YOY 1 and YOY 2 had a mixed contribution of epibenthic and pelagic prey; and larger hake fed mainly on epibenthic prey, but a minor contribution of pelagic prey was still evident (Fig. 8A). The trophic level of ha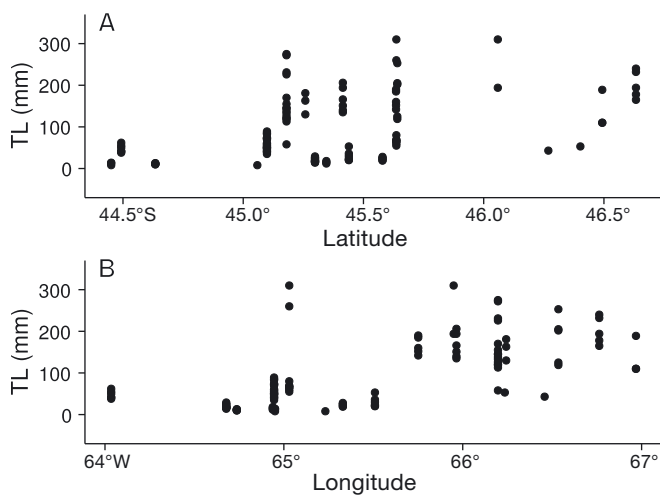kes was 3.5 in small larvae and between 3.8 and 4 in the other stages (Fig. 1, Table 1). The specialization index showed the highest values in the larval stages, indicating that these were the most specialist feeding stages. The index decreased in the YOY 1 stage and

Table 2. Comparison of mixing model fit with MixSIAR. LOOic: leave-one-out cross-validation information criterion. dLOOic is the difference between each model and the model with the lowest LOOic. Continuous model did not reach convergence and was discarded. NA: not applicable

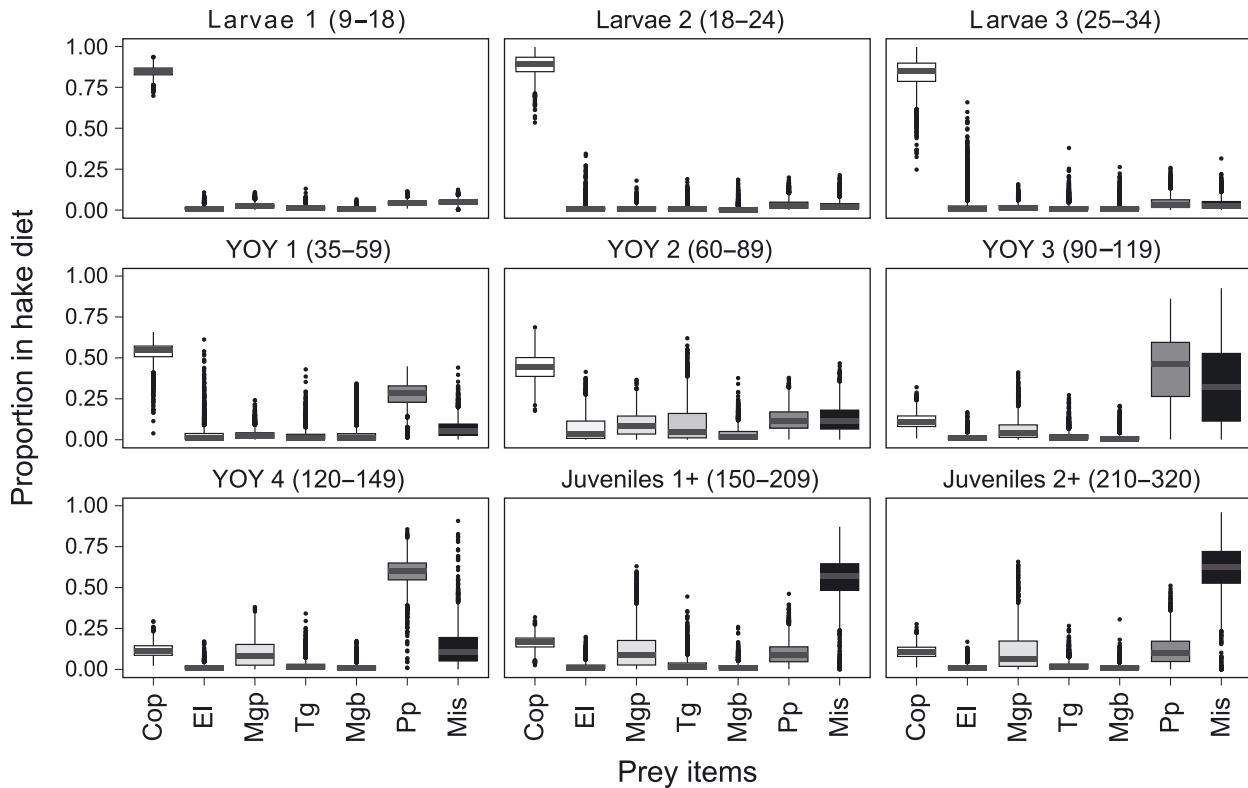| Model	LOOic	SE (LOOic)	dLOOic	SE (dLOOic)	Weight
Null	554.9	20.6	277.8	18.5	0
3-stage	384.9	19.2	107.8	18.3	0
6-stage	358.4	19.4	81.3	14.4	0
9-stage	277.1	22.0	0.0	NA	1

Fig. 7. Results of the MixSIAR model showing proportional contribution of main prey to the diet of different stages of hakes (total length range in millimeters in parenthesis). Cop: Copepods; El: *Euphausia lucens*; Mgb: *Munida gregaria* benthic morphotype; Tg: *Themisto gaudichaudii*; Pp: *Peisos petrunkevitchi*; Mis: mysids; Mgp: *Munida gregaria* pelagic morphotype; YOY: young-of-the-year. Box boundaries are the 75th and 25th percentiles, vertical lines are the minimum and maximum, points are outliers and line within box is the median

Table 3. Confidence intervals (95%) of the proportional contribution of the different potential prey on the different size classes of hakes *Merluccius hubbsi* as estimated by Bayesian mixing model. YOY: young-of-the-year; Juv: juveniles; ben: benthic morphotype; pel: pelagic morphotype

| Potential prey | Stages | | | | | | | | |
	Larvae 1	Larvae 2	Larvae 3	YOY 1	YOY 2	YOY 3	YOY 4	Juv 1+	Juv 2+
Copepods	0.77–0.90	0.75–0.98	0.60–0.97	0.38–0.62	0.28–0.59	0.04–0.22	0.04–0.21	0.07–0.20	0.04–0.2
Euphausia lucens	0.00–0.05	0.00–0.10	0.00–0.26	0.00–0.21	0.00–0.27	0.00–0.07	0.00–0.06	0.00–0.10	0.00–0.6
Munida gregaria ben	0.0–0.03	0.00–0.06	0.00–0.09	0.00–0.23	0.00–0.17	0.00–0.09	0.00–0.07	0.00–0.09	0.00–0.07
Themisto gaudichaudii	0.00–0.05	0.00–0.06	0.00–0.10	0.00–0.14	0.00–0.40	0.00–0.11	0.00–0.12	0.00–0.15	0.00–0.11
Peisos petrunkevitchi	0.02–0.07	0.00–0.12	0.00–0.15	0.05–0.38	0.02–0.28	0.01–0.75	0.41–0.75	0.01–0.25	0.00–0.32
Mysids	0.02–0.08	0.00–0.11	0.00–0.13	0.00–0.22	0.01–0.31	0.01–0.80	0.01–0.41	0.05–0.76	0.17–0.85
Munida gregaria pel	0.00–0.06	0.00–0.06	0.01–0.08	0.00–0.11	0.00–0.25	0.00–0.26	0.00–0.27	0.00–0.43	0.00–0.42

showed the lowest values (most generalist) in the YOY 2 stage. In turn, larger stages showed intermediate values (Fig. 8B).

4. DISCUSSION

Our results emphasize the existence of different trophic niches occupied by Argentine hake during their ontogenetic development and show that the change from a planktonic to a demersal habitat (settlement), which is of paramount importance in the early life history of hakes, is a gradual process. Argentine hake within this study go through several processes simultaneously: drift from the spawning to the nursery ground, change from a planktonic to a demersal habitat (settlement), development from larvae to juveniles and growth (from 9 to 320 mm TL).
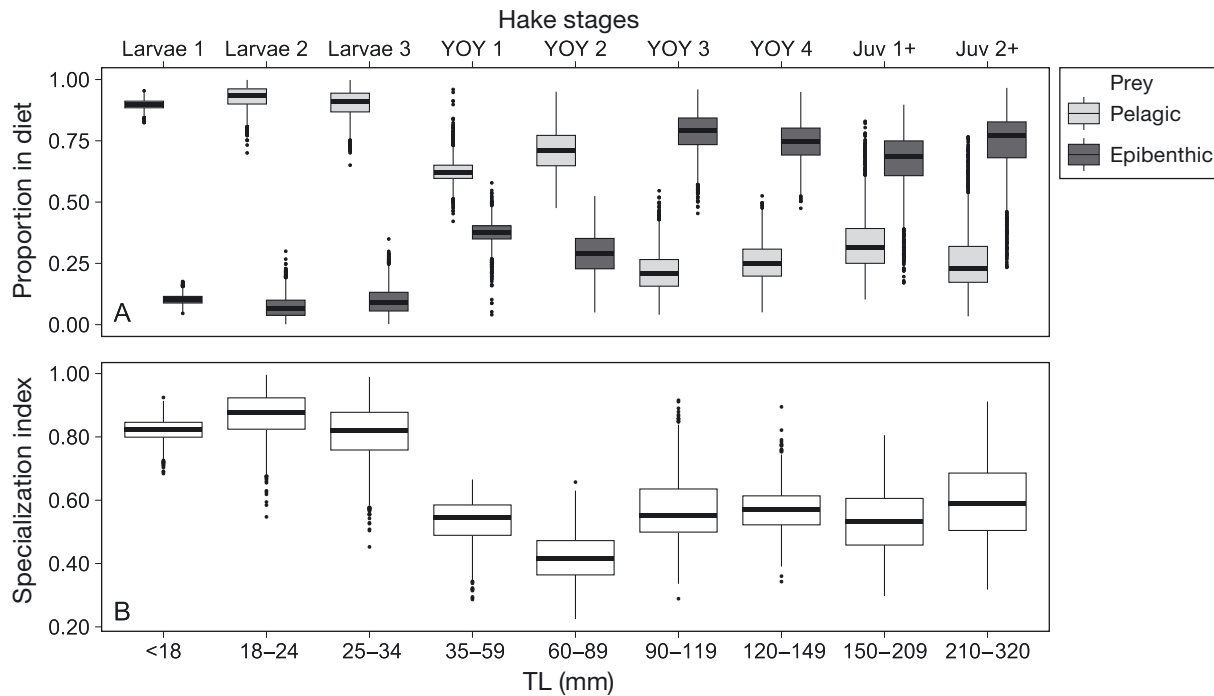
Fig. 8. (A) Result of combined *a posteriori* distributions of the MixSIAR model considering the contribution of pelagic and epibenthic prey to the diets of different size stages of hake. (B) Specialization index following Newsome et al. (2012) of the different size stages of hake. This index varies from 0 to 1, with 0 being a complete generalist consumer to 1 being an ultra-specialist consumer. Box boundaries are the 75th and 25th percentiles, vertical lines are the minimum and maximum, points are outliers and line within box is the median. YOY: young-of-the-year; Juv: juveniles; TL: total length

Isotopic signals showed changes related to these processes, mainly attributable to changes in diet.

As expected, trophic level increased with size and development. Similar results were found for this species in Cabo Frio, Brazil (Muto & Soares 2011), and for other *Merluccius* species (Le Loc'h & Hily 2005, Iitembu et al. 2012, Van Der Lingen & Miller 2014), in which $\delta^{15}N$ changed linearly with size. However, our study is the first to include larvae, which showed high variability in $\delta^{15}N$ values and an accelerated increase in trophic level with size in the early larval stages. Juveniles larger than 90 mm have $\delta^{15}N$ values slightly lower than values reported for adults by other studies in the Patagonian shelf ($\delta^{15}N$ of adults found in other studies: 17.5 ± 0.04‰, Forero et al. 2004; 17.0 ± 0.1‰, Ciancio et al. 2008; 17.1 ± 0.4‰, Drago et al. 2009; 18.2 ± 0.66‰, Gaitán 2012; 16.3 ± 0.4‰, Mariano-Jelicich et al. 2014). Stomach contents, however showed that adults of Argentine hakes incorporate fish and squid in their diet (Belleggia et al. 2014). Also, cannibalistic behavior of juveniles 1+ and 2+ that has been previously shown (Davenport & Bax 2002, Sherwood & Rose 2005) could explain increased $\delta^{15}N$ values of YOY stages.

The $\delta^{13}C$ values allowed us to determine the hake diet but did not reflect a clear pattern associated with the pelagic or benthic environment. Smaller pelagic organisms such as hake larvae and copepods had higher $\delta^{13}C$ values than the benthic organisms. This contrasts with other studies that showed lower $\delta^{13}C$ (more negative) values in pelagic environments relative to those in benthic environments (Davenport & Bax 2002, Sherwood & Rose 2005, Le Loc'h et al. 2008). Previous studies along the southwestern Atlantic also showed high variability of $\delta^{13}C$ (Botto et al. 2006, 2011), particularly in the northern Patagonian shelf, where $\delta^{13}C$ of particulate organic matter was highly variable (Lara et al. 2010). The primary source of organic matter was not evaluated in this study, but the role of microbial loops would be important (Temperoni et al. 2019), and dinoflagellates (such as *Polykrikos* sp. and *Dinophysis* sp., Carreto et al. 2007) and bacteria (Krock et al. 2015) are the main components, usually associated with high $\delta^{13}C$ values (Wada et al. 2012), which could explain the carbon isotope ratios found in this study.

The mixing model (Fig. 6) reflected that the relative importance of the potential prey changes along ontogeny. According to this model, larval stages feed

almost exclusively on copepods, the results of which coincide with previous studies based on stomach content analysis (Viñas & Santos 2000, Temperoni & Viñas 2013). Copepods were also important in the diet of YOY 1 and YOY 2 (35–89 mm TL); hakes in this size range showed the most diverse diet, incorporating planktonic (copepods, amphipods and euphausiids) as well as epibenthic prey (mainly mysids and the small shrimp), showing the transitional nature of these stages. Larger and already settled juveniles (>90 mm) showed the prevalence of epibenthic prey (small shrimps, mysids and the squat lobster). The mixing models therefore showed a clear pattern of diet shifts in hakes through growth and ontogeny.

The different stages of hake showed different degrees of specialization (Fig. 7B). The group of YOY between 60 and 89 mm were the most generalist, expressed isotopically as feeding on planktonic and epibenthic prey. This agrees with the behavior demonstrated for the YOY juveniles, which perform daily vertical migrations from the near-bottom depth towards the surface during darkness (Álvarez-Colombo et al. 2014). Ontogenetic change in fish can imply 2 possible outcomes: (1) an increase in niche width due to the possibility to ingest more prey while growing or (2) a complete shift in niche in those species with a change in feeding habits (Hammerschlag-Peyer et al. 2011). In the case of the Argentine hake, it is hard to determine which is the main process because both occur simultaneously.

Settlement is a decisive event in the early life history of fishes, throughout which important morphological and behavioral changes as well as niche shifts take place. Based on different arguments, there are several estimations about the size range for settlement in the Argentine hake. Metamorphosis and settlement seem to be associated in several species (e.g. Werner 2002); by using osteological analysis, the size at the end of the metamorphosis in Argentine hake was estimated in the range of 27 to 32 mm TL (Betti et al. 2009). In close agreement, the formation of accessory growth centers in otoliths (that would be related to the transition to demersal habits) started at about 15 mm TL (50 d old) and finished at about 30 mm TL (80 d old, Buratti & Santos 2010). Both approximations may indicate that settlement occurs at significantly smaller sizes than our estimation. However, our results suggest that settlement in the Argentine hake is a gradual process, occurring in the size range of 35 to 89 mm TL. On the other hand, fish sampled employing bottom and mid-water trawls showed that the smaller benthic individuals were 40 mm TL and the larger pelagic individuals 110 mm TL (Álvarez-Colombo et al. 2014). This range (40–110 mm TL) is much closer to our results (35–89 mm TL). Perhaps the reasons for discrepancies rely on settlement being a 2-component process: a morpho-anatomical one (fishes need an adequate size to achieve competence for benthic life and to develop anatomical structures and physiological capacities) and an ecological one (individuals move from pelagic to demersal food webs with attendant changes to their foraging success and predation risk). That is, once individuals become competent for a bottom-oriented lifestyle, ecological conditions determine the size and moment of settlement.

Growth is the dominant process during the juvenile period and is a fitness imperative. Analysis of otolith rings shows that juvenile hake grow fast as they acquire demersal habits (Buratti & Santos 2010). On the other hand, predation risks increase near the bottom, and cannibalism over the smaller sizes is intense; so delaying settlement to attain a larger size would diminish predations risks. Cannibalism could not be included in the isotopic models, but it may explain the high $\delta^{15}N$ values. Cannibalism in this species has been proved from stomach content analysis, with the smallest juvenile hake detected in the stomachs of cannibals being around 30 to 80 mm TL (e.g. Ocampo et al. 2011), close to our estimation of the settlement size range. Fine sediments and smooth bathymetric gradients characterize the settlement grounds of the Argentine hake. In most of their extension, there is a lack of shelters (e.g. rocky reefs, macroalgae forests, sponge gardens, shell banks). The pelagic domain, with its vastness and lack of hiding places, could nevertheless act as a refuge for the smaller hakes due to the preference of the bottom surroundings by larger specimens (cannibals).

Settlement implies movement into a new habitat, where young fish face a new array of prey and predators (e.g. Secor 2015). Settlement of Argentine hake seems to be ruled by the balance between 2 antagonist forces: improving growth performance and diminishing predation risk. Settlement is of paramount importance in the early life history of hake: a decision to settle or to remain pelagic would have great implications for survivorship and the eventual level of recruitment (e.g. Cushing 1996, Houde 2009). Because of the low habitat complexity of hake settlement grounds, it could be expected that density dependent mortality of settling individuals could be increased. In other Gadidae, such as the Atlantic cod, there is evidence that density-dependent mortality in the juvenile stage can dampen variability

generated by the earlier stages and act to finely adjust recruitment levels (Folkvord 1997). Typically, strong density dependence is assumed to operate during the juvenile stages and is modeled through stock–recruitment relationships. That is, as the parental stock size increases, there is an increase in recruitment, but it generates an increased density-dependent mortality (e.g. the Ricker model). Consequently, recruitment begins to decline above a certain spawner population size (e.g. Hilborn & Walters 1992), resulting in dome-shaped stock–recruitment curves. So identifying the processes that allow juveniles to escape or diminish predation pressure is important to adequately understand marine species population dynamics. Moreover, the ontogenetic shift in the diets of organisms overcomes species implications and should be considered in studies of community dynamics and ecosystem functioning (Miller & Rudolf 2011, Nakazawa 2015). Thus, the different roles that hakes occupy during early life on the food web could also have implications in species interactions, pelagic–benthic coupling, and energy and nutrient fluxes through the ecosystem.

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