Elucidating trophic pathways of the most abundant fish larvae in northern Patagonia using δ^{13}C and δ^{15}N isotopes

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ABSTRACT: The alternation of the classic and microbial food-webs in spring and winter, respectively, and the trespass towards higher trophic levels represented by fish early stages, are not well understood in Patagonia. These trophic routes were investigated in the inner Sea of Chiloe, an estuary of high ecological relevance in northern Patagonia. The isotopic values of δ^{13}C and δ^{15}N of ichthyoplankton and particulate organic matter were analyzed in late winter and spring 2017 to evaluate whether seasonal changes (e.g. in the composition of the freshwater discharge) were reflected in the isotopic signals of fish larvae. For this purpose, larvae of dominant fish species with contrasting feeding strategies were collected up to 100 m depth. The inshore zone of northern Patagonia was characterized by a dominance of marine carbon production, with increasing input of terrestrial organic matter during winter. δ^{13}C values < −25 ‰ at the outermost estuary stations indicated the influence of allochthonous carbon exported from the inshore area in spring. The δ^{13}C–larval signature of the species of the lightfish *Maurolicus parvipinnis*, the pipefish *Leptonotus blainvilleanus*, and the rockfish *Sebastes oculatus* followed the isotopic signature of the particulate organic matter in both seasons, at inshore and the exchange (outer) zone. Food partitioning was detected between species, with *Merluccius* spp. at the highest trophic position and *L. blainvilleanus* at the lowest. The fish larval community reached more diverse and higher δ^{15}N values in winter, when larvae likely fed on prey items of higher trophic level, or instead when the food-web was partly sustained by microbial sources. Our results showed seasonal variations in δ^{13}C values, suggesting differences in the source of organic carbon incorporated by the studied fish larvae. Moreover, trophic plasticity at larval stages may be an important characteristic of this type of estuarine environment.

KEY WORDS: Carbon sources · Ichthyoplankton · Stable isotopes · Sea of Chiloé · Estuarine systems · Southern Chile · Fjords

1. INTRODUCTION

Estuaries have diverse functional uses for fish such as breeding, feeding, and reproduction, bringing together residents, marine, and migratory species (Franco et al. 2008). In a scenario of climate change, estuaries are prone to be strongly affected (e.g. Tello & Rodríguez-Benito 2009). The trophodynamics studies of fish larvae are fundamental to understand how populations of diverse origin occurring within estuaries might be affected. In the south-east Pacific Ocean, many fish species with high ecological or commercial value, such as the lightfish *Maurolicus parvipinnis*, the hake *Merluccius australis*, the rockfish *Sebastes oculatus*, and the pipefish *Leptonotus blainvilleanus* reproduce in nursery areas off northern Patagonia. The larval stages of these fish species are exposed to seasonal environmental stresses produced by the multiple freshwater inputs such as water and nival precipitation, river flux variations, and runoffs. Particularly,

fjord estuaries like the West Patagonian coastal systems receive important inputs of nutrients from glacier melting (Arimitsu et al. 2016, Meire et al. 2017, Wadham et al. 2019). The Sea of Chiloé in northern Patagonia (see Fig. 1a) is surrounded by rivers and receives also the terrigenous discharge of a fjord (Reloncaví Fjord) in its north that modulate the composition of the particulate organic matter (POM) of the adjacent coasts (Iriarte et al. 2007). During the less productive season (austral winter), the marine food-web relies on a major carbon contribution from the microbial loop (González et al. 2010, 2011, 2016). Instead, during the spring and summer, production is mostly based on diatoms and the classical food-web is reestablished. The concentration of particulate organic carbon (POC) doubles from winter (4–8 g C m^{-2}) to spring (10–30 g C m^{-2}) in Reloncaví Fjord (González et al. 2010). The POC locally produced by phytoplankton or microbes, along with the terrigenous carbon (allochthonous) from the glaciers and rain runoffs, create a complex pool of organic matter at disposal of planktonic consumers in the coastal areas (Eisma 1993). Also, the permanent and vertical intrusion throughout the channels of oceanic sub-Antarctic waters from the adjacent continental shelf at the latitudes where the northward moving Humboldt Current and the southward moving Cabo de Hornos Current originate (38–46° S; Strub et al. 2019), and with higher salinity and temperature than that from the inner coastal area, enriches the food-web baseline with phosphates and nitrates. Thus, fjords and channels contain up to 50 % of terrestrial organic carbon in their surface sediments, which is the result of this mixture of allochthonous and marine organic sources at the epipelagic waters (Sepúlveda et al. 2011, Silva et al. 2011).

In this Patagonian zone, the zooplankton biomass and the abundance and diversity of ichthyoplankton are typically greater inshore than offshore (Palma & Silva 2004), although large abundances of eggs and larvae of *M. parvipinnis*, as well as other marine fishes such as Gadiformes, have been also described in the offshore shelf-break (Osorio-Zúñiga et al. 2018). Several fish species take advantage of the favorable conditions in spring-summer to spawn in regions with high productivity and food available such as the Sea of Chiloé (González et al. 2010, Vargas et al. 2011, Flores et al. 2019). Ice thawing increases the drift of developing larval stages into the channels. Thereafter, higher exportation of larvae that incorporate terrigenous carbon to outer locations could be expected (González et al. 2011). There are hardly any reports on the winter fish activity, and larval feeding habits have been uniquely described for

some species in other fjords southwards (Landaeta et al. 2019). The strong variations in the physicochemical characteristics and productivity of the area set restrictions on the development of fish during winter due to the oligotrophic waters that typify the inner sea during this season (Landaeta et al. 2019).

The fish species that occur in the Sea of Chiloé enter the inland area to spawn, but dwell in diverse environments. The majority of marine fishes reported in this inner sea spawn during the spring season, which is characterized by vertical water stratification caused by the seasonal thermal regime and cold freshwater inputs (modifications in salinity). These conditions, in turn, ensure higher availability of food for fish larvae (Lasker & Zweifel 1978, Kondo 1980, Lasker 1981). Conversely, *Merluccius* spp. spawn over the shelf-break in winter and have a second spawning in spring in the channels and fjords (Landaeta & Castro 2006a, Flores et al. 2019, Landaeta et al. 2015, Osorio-Zúñiga et al. 2018, 2019).

Ichthyoplankton of this zone is composed of a variety of species, from demersal and mesopelagic fish species such *Merluccius* spp., or *Maurolicus parvipinnis*, which constitutes a key link between plankton and top predators. Finally, coastal-demersal species such as *L. blainvilleanus*, and *Sebastes oculatus*, or small pelagic fishes such as *Sprattus fuegensis*, *Engraulis ringens*, and *Strangomera bentincki* also occur in this estuary (Landaeta & Castro 2006b). A few fish species are often found in lower abundances, e.g.: *Odontestes regia*, common in estuaries or marine coastal environments (Fierro et al. 2014); *Hippoglosina macrops*, which extends from the coastal zone to 600 m depth (Yañez & Barbieri 1974); *Scyasis sanguineus,* distributed mainly in coastal waters of Chile between the rocky subtidal and intertidal seabed and *Stromateus stellatus*, a coastal pelagic species and the only representative of the Stromateidae family in the Chilean coasts (Chirichigno & Cornejo 2001).

The larval feeding habits of some of the Patagonian fish have been determined by the analysis of gut contents, providing information on the prey captured in recent time (e.g. Anderson 1994, Valenzuela et al. 1995, Landaeta et al. 2011a,b, 2014, 2015, Contreras et al. 2014). Sometimes, these prey items cannot be identified because they are in an advanced degradation stage in the guts. The use of isotope analysis provides additional information on trophic levels and diet composition integrated through space and longer periods (Post 2002, Bearhop et al. 2004, Olivar et al. 2019).

During the last decade, the use of stable isotopes has been incorporated into trophodynamics studies.

The isotopic fraction of organic carbon (δ^{13}C) is used as a tracer to indicate the influence of terrigenous carbon on the pelagic system (West et al. 2006). In particular, low δ^{13}C signatures (−28 to −25‰) are indicators of allochthonous (terrigenous) inputs to the marine systems, whereas higher δ^{13}C values (−19 to −22‰) show the greater contribution of oceanic carbon produced by phytoplankton (Kline 2009, Boecklen et al. 2011). Thus, larval diets are expected to reflect the seasonal fluctuations of the ecosystem through an integrated isotopic signal (Grey et al. 2001). δ^{15}N provides information on the trophic position of fish larvae. Studies on δ^{15}N variations are also used to identify ontogenetic changes in the diet of larvae and juvenile fish and their relationship with growth (Uriarte et al. 2014).

In this study, we described δ^{13}C and δ^{15}N values of the most frequent fish larvae collected in the inner Sea of Chiloé, northern Chilean Patagonia (41° to 44° S; 73° W). Our interest was to ascertain whether the seasonal changes at the base of the food-web were reflected in the larval isotopic compositions. We intended to identify changes in organic carbon sources and potential variations in the larval trophic positions. We hypothesized that the estuarine food-web would be more complex in winter than in spring, with a higher partitioning of food resources, due to low primary production, while it would be shorter and have a higher contribution of allochthonous organic carbon in spring.

Disentangling the trophic role of larval fish stages in this Patagonian system is important to understand the dynamics, recruitment, and growth of key species of fish. Further, this study can serve as a precedent to our understanding of trophic relationships at the base of the food-web, since there is little knowledge at this level, and what factors condition the type of food-web that is established in estuaries, where seasonal variations involve different concentrations of nutrients.

2. MATERIALS & METHODS

2.1. Sample collection

The study was conducted in northern Patagonia, Chile (41° S−44° S, 73° W; Fig. 1a). Samples of the zoo- and ichthyoplankton were collected at 4 inshore stations, of which 2 were located at Reloncaví Fjord and 2 at Ancud Gulf in late winter 2017 (6−8 September). Ichthyoplankton was also investigated in spring 2017 (24−27 November), when the primary production and zooplankton abundances were maximum at the 4 inshore stations and when 2 new stations off Guafo Mouth were added to the study (Fig. 1a). Guafo Mouth is the main connection to the ocean, although, of less importance, there is also water inflow through the Chacao Channel, north of Chiloé Island. Hydrographic data were obtained by a Seabird SBE-19 profiler equipped with fluorescence and oxygen sensors. For plankton hauls, a Tucker Trawl with a 300 µm mesh-size and 1 m² mouth was employed. The plankton was collected employing oblique tows with the Tucker Trawl in 3 depth strata, 0−25, 25−50, and 50−100 m, to detect potential variations of the isotope carbon signatures throughout the first meters of the water column. Seawater was collected with Niskin bottles at depths of 5, 40, and 75 m, and then filtered through precombusted GFF filters to obtain POM at each station.

The samples of plankton were split into 2 volumes: one subsample was preserved in 5% buffered formalin for the later identification of broad potential prey groups (i.e. small copepods, large copepods, euphausiids, and amphipods) plus the identification of fish larvae to the lowest possible taxonomic level, using the descriptions by Orellana & Balbontín (1983), Moser (1996), and Balbontín et al. (2004). The second subsample was immediately frozen at −80°C for bulk isotope analysis.

2.2. Isotope analyses

C and N bulk isotope analyses in POM, planktonic crustaceans, fish eggs, and fish larvae collected in the study area (see Table 1 for an entire list of fish species and other larger taxonomic groupings) during 2 cruises performed in late winter and spring 2017 are presented to examine (1) organic matter sources (inshore vs. zone with direct oceanic influence—outer estuary, corresponding to the stations located in the Guafo Mouth), (2) larval isotopic niche width, (3) potential trophic partitioning among the most common fish larvae, and (4) seasonal differences in carbon and nitrogen signatures at inshore locations.

The preparation of samples for isotope analyses (species identification, weighing, drying, grinding, homogenization, and encapsulation) was carried out in the Oceanography Fisheries and Larval Ecology laboratory (LOPEL) of the University of Concepción (Chile) following the protocols established by the US Davis Stable Isotope Facility (http://stableisotope facility.ucdavis.edu/). One liter of seawater from each Niskin bottle was filtered through 0.7 µm GFF filters and then half of each filter was acidified with
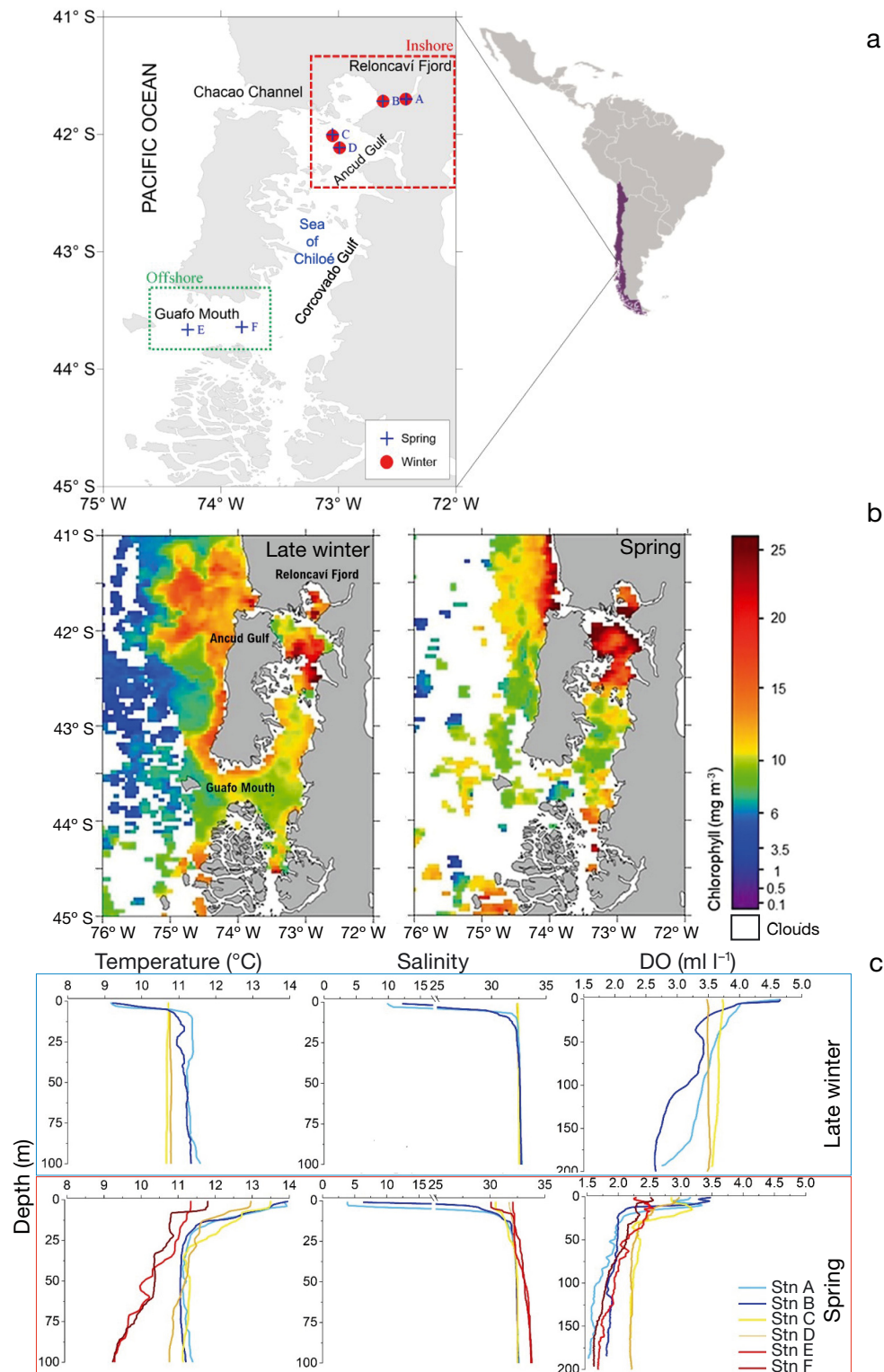
Fig. 1. (a) Area of study at the Sea of Chiloé, depicting the inshore (A, B, C, D) and offshore-influenced (E, F) stations sampled during 2 seasons. (b) Mean chlorophyll *a* (mg m^{-3}) satellite images exhibited higher primary production at Reloncaví and Ancud areas than offshore. http://oceancolor.gsfc.nasa.gov/ (c) Temperature (°C), salinity, and dissolved oxygen (DO; ml l^{-1}) profiles in winter and spring. Sampling areas with freshwater influence are depicted in blue, considered as inshore sites (Stn A and Stn B: Reloncaví Fjord; Stn C and Stn D: Ancud Gulf), and those under the oceanic influence (Stn E and Stn F: Guafo Mouth) are depicted in warm colors

1% v/v of HCl to erase the inorganic carbon from the POM samples at each depth. The whole larvae were used for isotope analyses, except for the larger larvae and post-larval stages where the guts could be dissected and removed. Samples were dried overnight in an oven at 60°C, then ground to a fine powder and encapsulated, taking 3 replicates of each if possible. Bulk isotope analyses were performed in the UC Davis analytical facility.

Fish larvae collected at both seasonal surveys were scarce. Consequently, stratified samples from the surface to 100 m depth were pooled when the number of larvae per species was insufficient to provide the optimal sample weight required for minimum detection by the mass spectrometer. A statistical test was run before pooling isotopic values across the water column. Non-significant differences between depth strata were detected for POM ($F = 0.616$, $p = 0.439$). Each output deviation was expressed in parts per thousand (‰) of ^{13}C and ^{15}N and represented with the usual δ notation. A mathematical approach to correct for lipid $\delta^{13}C$-alteration was applied when the C:N ratio was higher than 3.5 (Post et al. 2007) using the following formula for aquatic animals:

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C:N \quad (1)$$

Lipid normalization allows comparisons among organisms with variable amounts of lipids in their bodies. POM values were also corrected by this method.

We used the following equation to estimate the trophic level (TL):

$$TL = \frac{\delta^{15}N_{consumer} - \delta^{15}N_{baseline}}{3.4} + 1 \quad (2)$$

where $\delta^{15}N_{baseline}$ is the mean of the $\delta^{15}N$ values of POM, and 3.4 ‰ is assumed to be the $\delta^{15}N$ trophic fractionation factor (Post 2002).

2.3. Statistical analyses

Mean isotopic values of $\delta^{13}C$ and $\delta^{15}N$ of the main fish species, i.e. *Maurolicus parvipinnis*, *Sebastes oculatus*, a group of other mesopelagic fish larvae (likely composed of the dominant myctophid in the zone, *Lampanyctodes hectoris*; hereafter, mesopelagics), and *Leptonotus blainvilleanus*, were simultaneously tested through a MANOVA routine in R v3.4.4 software (R Core Team 2018). The relationship between $\delta^{13}C$ and $\delta^{15}N$ values was analysed using least-squares linear regressions. Spatial and temporal variations in $\delta^{13}C$ and $\delta^{15}N$ between inshore and oceanic-influenced locations, and between seasons, were tested using 1-way ANOVA ($\alpha = 0.05$), followed by post-hoc Tukey multiple comparisons of the C and N means for each fish group. Because rough weather conditions precluded the collection of plankton samples during winter at Guafo Mouth, a 1-way ANOVA test was run separately for the season (winter–spring) and location (inner waters–exchange zone) (see Table 1). Stable isotope ratios were tested for normal distribution using the Shapiro-Wilk test and Q-Q plots, while standardized residual plots were used to check for homogeneity. In our study, the ANOVA test is robust enough to tolerate moderate deviations from the assumption of homogeneity of variances (Sahai et al. 2011, Blanca et al. 2017) and thus data were not tested for this assumption. Location was defined as 'inshore' from Reloncaví Fjord to Ancud Gulf, and as 'outer estuary' for the Guafo Mouth, the area with more oceanic influence. Basins were determined as follows: 'northern basin', comprising Reloncaví Fjord and Sound, and 'central basin', comprising Ancud Gulf. Standard ellipses (SEA) were calculated using the R-package 'SIBER' (Jackson et al. 2011) to determine the trophic niche breadth and diet overlap of the main fish species. Average Euclidean distance of each species to the $\delta^{13}C/\delta^{15}N$ centroid to define the isotopic niche is subjected to sampling biases and sample-size sensitive. On the other hand, SEA takes into account those biases by calculating the ellipse parameters based on Markov-Chain Monte Carlo bootstrapping. Thus, Bayesian inference was applied to calculate the standard area of ellipses that represent the isotopic niche of the consumer. Niche areas were corrected for small sample sizes by using 2 standard deviations.

3. RESULTS

3.1. Oceanographic conditions

The hydrographic characteristics of the water column changed markedly between winter and spring, particularly at the inshore stations of Reloncaví Fjord. There, while winter temperature showed an inverse thermocline and salinity a clear halocline (deeper than in spring) at the inshore stations, in spring, temperature showed a normal thermocline and there were lower salinity values than in winter, indicating an influence of freshwater input probably resulting from recent rainfall events. The salinity did not vary much between winter and spring at the Ancud Gulf stations (no halocline present), but the temperature increased at the surface as solar radiation augmented.

Dissolved oxygen concentrations (DO) were higher in winter than in spring, reaching values lower than $2 \text{ ml O}_2 \text{ l}^{-1}$ at a depth as shallow as 12 m. These values were likely a combined result of the high primary production and organic matter degradation below the strong halocline and the ingress of cold, salty, and low-oxygen equatorial subsurface waters from the Guafo Mouth, mixed with sub-Antarctic water from the shelf, which occasionally enter sub-superficially the inshore zone in spring and summer (Silva & Vargas, 2014) (Fig. 1b). The highly abundant phytoplankton in the inshore area was revealed by the high surface chlorophyll *a* (chl *a*) concentrations observed in the satellite images that show an inshore zone of extremely high values (>20 mg chl *a* m^{-3}) that coincides with the site of larval fish collections inshore.

Chl *a* concentration indicated high inshore primary production in both seasons. In winter, high chl *a* concentrations occurred inshore and over the shelf off Chiloé Island. A spring phytoplankton bloom, with higher chl *a* concentrations, occurred inshore at Reloncaví Fjord and Ancud Gulf but not at the estuary mouth. Mean weekly satellite images showed higher values of chl *a* (10 to >25 mg m^{-3}) inshore at lower latitudes (41° to 42.5° S), corresponding to Reloncaví Fjord and Ancud Gulf (Fig. 1b). At higher latitudes, with more oceanic water influence (42.5° S to 45° S), chl *a* decreased towards the Guafo Mouth (3 to 13 mg m^{-3}). Both surface chlorophyll and POC

concentrations were higher at Reloncaví Fjord and Sound than at the central basin (González et al. 2010).

3.2. Isotope analysis

3.2.1. Baseline values

There were significant differences between treated and untreated POM subsamples (depth: $F_{(1,30)} = 0.616$; p = 0.439; season × depth: df: 1; $F_{(1,30)} = 0.309$; p = 0.582). Thus, only the δ^{13}C acidified POM and the δ^{15}N untreated POM subsamples were used for subsequent analyses.

An ANOVA analysis (W, Shapiro-Wilk test on ANOVA = 0.758, p = 2.644e^{-16}) detected significant differences in δ^{13}C-POM between seasons ($F_{(1,30)} = 3582.9$; p = 2e^{-16}) and between the inshore and exchange zones ($F_{(1,30)} = 105.4$, p = 2.47e^{-11}). In winter, most inshore stations reached the most negative δ^{13}C values (±SD) (−25.4 ± 0.9‰). In spring, when values were lower than in winter at the whole sampling area, POM was instead more depleted in δ^{13}C at the mouth (−23.29 ± 1.7‰) than inshore (−19.3 ± 1.5‰) (Figs. 2 & 3).

The δ^{15}N isotopic signatures of POM showed significant differences between seasons ($F_{(1,30)} = 47.8$, p = 1.13e^{-07}), with lower values in winter, and
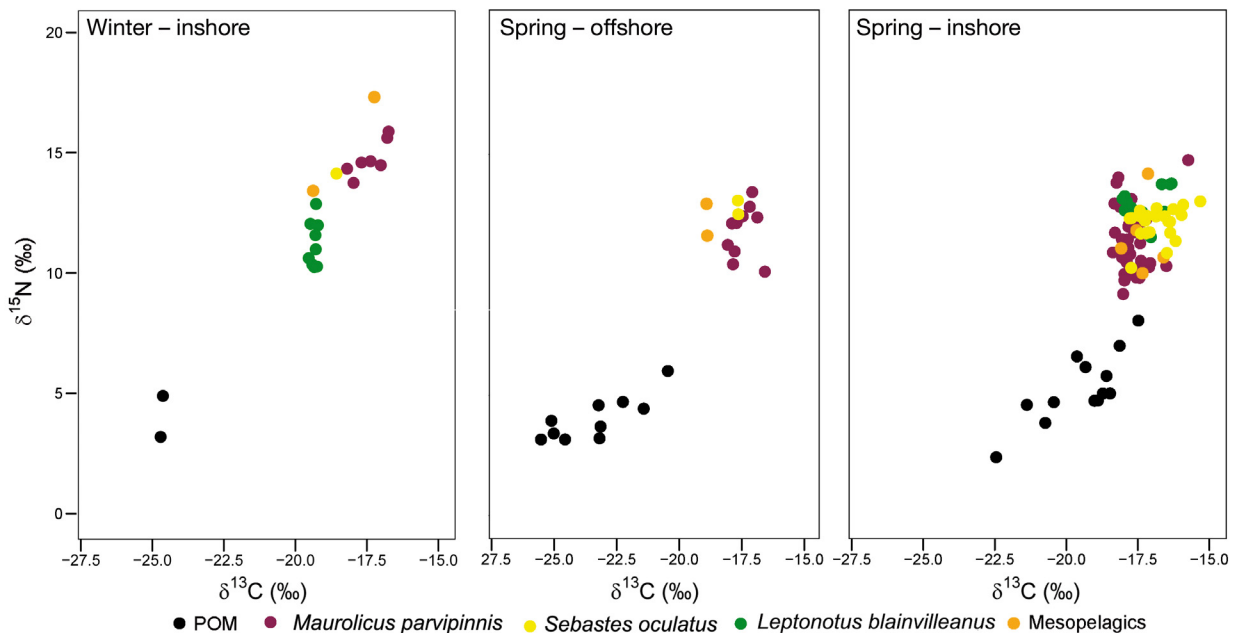

Fig. 2. Scatterplot of the bulk δ^{13}C and δ^{15}N values for particulate organic matter (POM) and the 4 main fish larval categories of this study
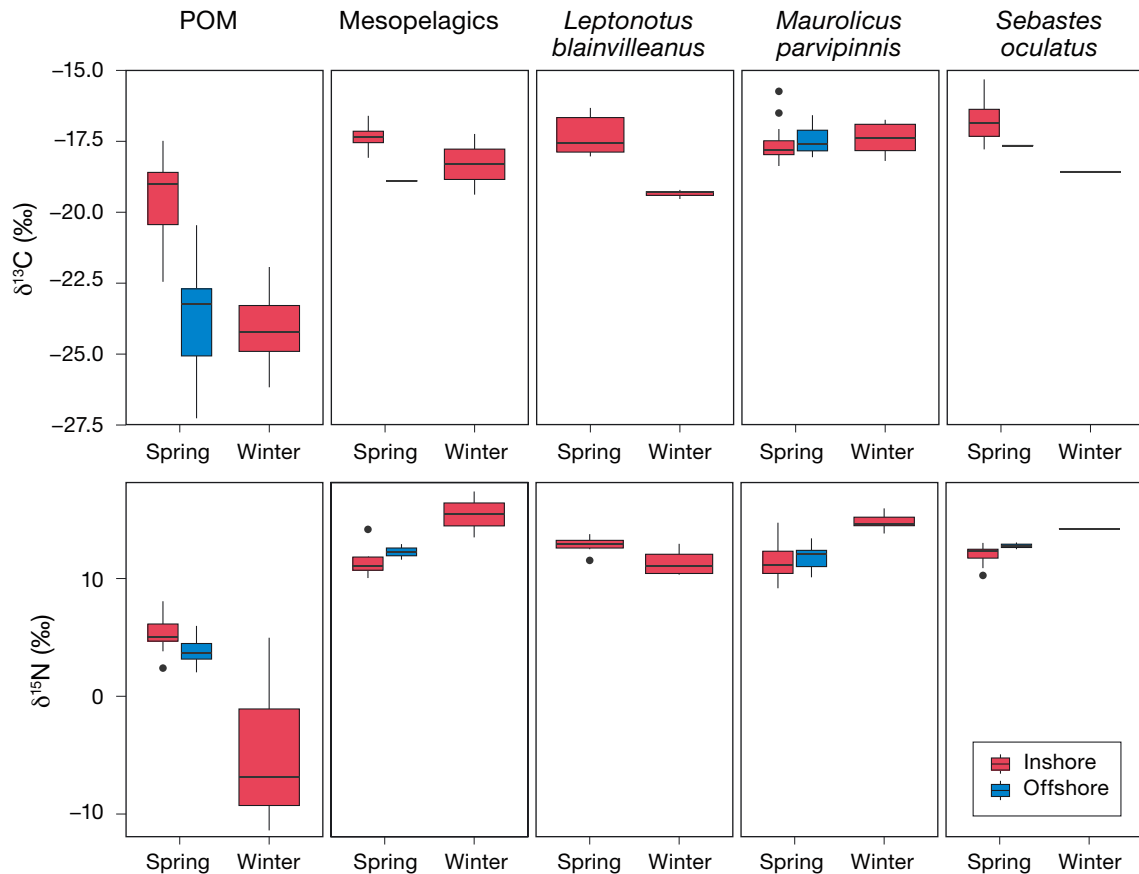
Fig. 3. Boxplots of the $\delta^{13}C$ and $\delta^{15}N$ values for POM and the 4 main fish larval categories. The horizontal line that divides the box into 2 parts represents the median, the ends of the box show the upper (Q3) and lower (Q1) quartiles, the whiskers indicate Q3 + 1.5 × interquartile range to Q1 − 1.5 × interquartile range, and black dots are outliers of isotopic values (inshore: red boxes; offshore: blue boxes)

between the inshore and outer estuary locations ($F_{(1,30)}$ = 5.4, p = 0.027), with lower $\delta^{15}N$ values at the outer estuary (Fig. 3).

3.2.2. Stable isotope composition of fish larvae between areas and seasons

Fish larval $\delta^{13}C$ values ranged from −19.5 ‰ in a post-larva of *Leptonotus blainvilleanus* to −16‰ in a preflexion individual of *Sebastes oculatus*. The $\delta^{13}C$ values were significantly more negative for *L. blainvilleanus* ($F_{(1,20)}$ = 4.7; p = 0.042) in winter (−19.5‰) than in spring (−17.5‰), while non-significant differences were noted for *M. parvipinnis* ($F_{(1,58)}$ = 2.071; p = 0.155). Moreover, when comparing between POM and other taxonomic groups (larval fish and copepods), significant differences were found for mean seasonal $\delta^{13}C$ values between POM and the larvae of *Maurolicus parvipinnis*, *Merluccius* spp., and *S. oculatus* (Tukey multiple comparisons of

means, p < 0.0001) (Figs. 2 & 3), as well as between POM and small copepods (p = 0.039).

Fish larval $\delta^{15}N$ values ranged from 9.2‰ in a clupeid larva, through *L. blainvilleanus* with the lowest $\delta^{15}N$ values (winter: 11.3 ± 0.9‰; spring: 12.9 ± 0.6‰), to the highest values of over 18‰ for *Merluccius* spp. The range of $\delta^{15}N$ values was very similar for fish between inshore and outer estuary locations in spring, while it varied largely in winter, generally reaching higher values (Table 1 shows mean values of all the fish species collected). Post-hoc pairwise mean comparisons exhibited significant differences between (1) winter and spring individuals of *M. parvipinnis* (p < 0.0001), (2) *M. parvipinnis* and *Merluccius* (p < 0.0001), and (3) *Merluccius* with Clupeidae (p = 0.001). However, the different fish species/categories showed no significant trends in the isotope values of $\delta^{13}C$ and $\delta^{15}N$ between basins (northern and central basins, and the exchange zone). The TL values for the different fish species collected at inshore stations in spring ranged from 1.1 for the clupeid larva to 2.3 for

Table 1. Mean ± SD $\delta^{13}C$ values (‰), $\delta^{15}N$ values (‰), and C:N ratios of plankton sampled in winter and spring 2017. N: number of samples analyzed and obtained by grouping several individuals of the same species (and other larger taxonomic groups), similar size, and zone in most cases. SL: Standard length range in mm. Empty cells mean that no data were available

| Taxa/category | — Late winter (6–8 September)— | | | | Spring (24–27 November) | | | | | | | | |
| | SL | —Inshore— | | | SL | —Inshore— | | | | —Offshore— | | |
		N	$\delta^{13}C$	$\delta^{15}N$	C:N		N	$\delta^{13}C$	$\delta^{15}N$	C:N	N	$\delta^{13}C$	$\delta^{15}N$	C:N
Maurolicus parvipinnis (larva)	6–11	1	−18.2	14.4	4.9	6–11	41	−17.7 ± 0.5	11.3 ± 1.2	5.8 ± 1.3	7	−17.5 ± 0.5	12.1 ± 0.85	5 ± 0.7
M. parvipinnis	20–25	6	−17.3 ± 0.5	14.9 ± 0.8	5.8 ± 0.2	20–25	3	−18.3 ± 0.1	13.6 ± 0.6	5.2 ± 0.3	3	−17.3 ± 0.7	11 ± 1.25	4.2 ± 0.7
Sebastes oculatus (larva)	21	1	−18.6	14.2	10.9	6–13	23	−16.8 ± 0.7	12.1 ± 0.7	6.5 ± 1.1	2	−17.6 ± 0.0	12.8 ± 0.40	4.1 ± 0.3
Mesopelagic fish (Myctophidae)	6–11	2	−18.3 ± 1.51	15.4 ± 2.3	7.4 ± 0.5	6–11	5	−17.3 ± 0.5	11.6 ± 1.6	6.6 ± 0.9	2	−18.9 ± 0.0	12.3 ± 0.9	3.7 ± 0.4
Leptonotus blainvillianus	16.5–36	9	−19.3 ± 0.11	11.3 ± 0.9	4.7 ± 0.7	50–75	13	−17.3 ± 0.7	12.9 ± 0.6	5.4 ± 0.8				
Merluccius spp. (larva)	6–9	2	−16.6 ± 0.07	18.4 ± 0.2	6 ± 1.2									
Clupeidae (larva)						20	1	−19.3	9.2	4.8				
Hippoglossina macrops (larva)						25	1	−17.1	13.1	7.1				
Odontesthes regia (larva)						9–10	7	−17.5 ± 0.6	12.6 ± 0.5	5.2 ± 0.7	1	−16.9	12.7	4.1
Sicyases sanguineus (larva)		1	−17.1	15.5	5.1									
Stromateus stellatus (larva)						13	3	−18.2 ± 0.2	12.7 ± 0.5	6.2 ± 0.2				
Prionotus sp. (larva)											3	−16.7 ± 0.1	14.8 ± 0.6	3.6 ± 0.2
Fish eggs		12	−17.3 ±1.27	19.5 ± 2.3	4.7 ± 0.9		62	−17.2 ± 0.4	13.2 ± 0.8	5.5 ± 1.1	4	−17.4 ± 0.3	12.4 ± 1.93	4.9 ± 0.5
Small copepods		3	−20.7 ± 0.1	6.7 ± 0.1	6.1 ± 0.1									
Large copepods		90	−20.9 ± 0.7	12.6 ± 0.5										
Amphipods		34	−18.9 ± 0.1	10.9 ± 0.9										
Euphausiids		6	−18.6 ± 0.7	10.3 ± 0.2										

Hippoglossina macrops; *M. parvipinnis*, mesopelagics, and *S. oculatus* experienced lower TLs than in winter (1.8, 1.9, and 2.0, respectively). In winter, the TL value calculated for *M. parvipinnis* was 3.3, for mesopelagics, 3.4, for *S. oculatus*, 3.1, for *Sicyases sanguineus*, 3.5, and for *Merluccius* spp., 4.3. *L. blainvilleanus* showed the same TL (2.2) in both seasons.

3.3. Isotopic niche

The SEA of the fish larvae of the different species overlapped, except for *L. blainvilleanus* in winter, with an ontogenetic niche markedly segregated. Differences in $\delta^{15}N$ were more patent among species than in $\delta^{13}C$ (excluding the pipefish). In spring, the value for the median SEA in mesopelagics was the highest (3.79), followed by *M. parvipinnis* (1.88), *S. oculatus* (1.34), and *L. blainvilleanus* (1.21). In winter, SEA values were lower than in spring (*M. parvipinnis*: 0.76; *L. blainvilleanus*: 0.31). In general, the isotopic niches of these species overlapped to a large extent in spring (Fig. 4). Winter and spring isotopic niches of *M. parvipinnis* differed, with wider niche ranges in winter. *L. blainvilleanus* occupied a completely different trophic niche in winter than spring: the range of $\delta^{13}C$ values was narrower in winter, whilst it was wider for nitrogen. *S. oculatus* showed the widest core of the foraging niche in spring.

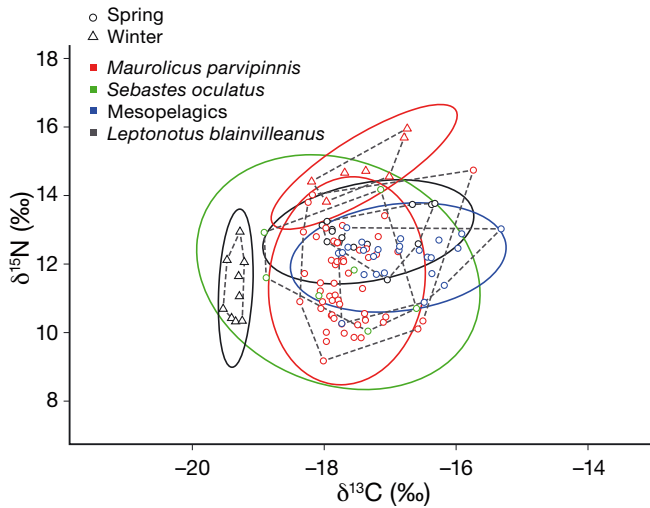Fig. 4. δ^{13}C and δ^{15}N values for *Maurolicus parvipinnis* (n = 61), *Sebastes oculatus* (n = 25), mesopelagics (n = 9), and *Leptonotus blainvilleanus* (n = 22). 95 % confidence interval bivariate ellipses denote a significant isotopic niche for each group. *S. oculatus* has only 1 individual in winter and is thus not plotted. The lines that enclose the area of an ellipse are assumed to be a measure of the isotopic niche of consumers. Polygons indicate convex hull areas (broken lines); ellipses indicate standard ellipse areas (solid lines)

3.4. Intraspecific body size influence on isotope values

C:N mean values decreased in older developmental stages of *M. parvipinnis* and *S. oculatus* (Fig. 5). δ^{13}C mean values tended to decrease from younger to later developmental stages in the 3 species. Mean δ^{15}N of *M. parvipinnis* was higher in post-larvae than in larval stages (apparently also in *L. blainvilleanus*). Juveniles of *M. parvipinnis* showed higher δ^{13}C and δ^{15}N mean values in winter than in spring.

4. DISCUSSION

For the first time, we document carbon and nitrogen isotopic values of some of the dominant species in the ichthyoplankton in northern Patagonia during 2 seasons of contrasting freshwater input. Our results showed that seasonal variations were observed in δ^{13}C values, suggesting differences in the source of organic carbon entering the coastal zone reached by
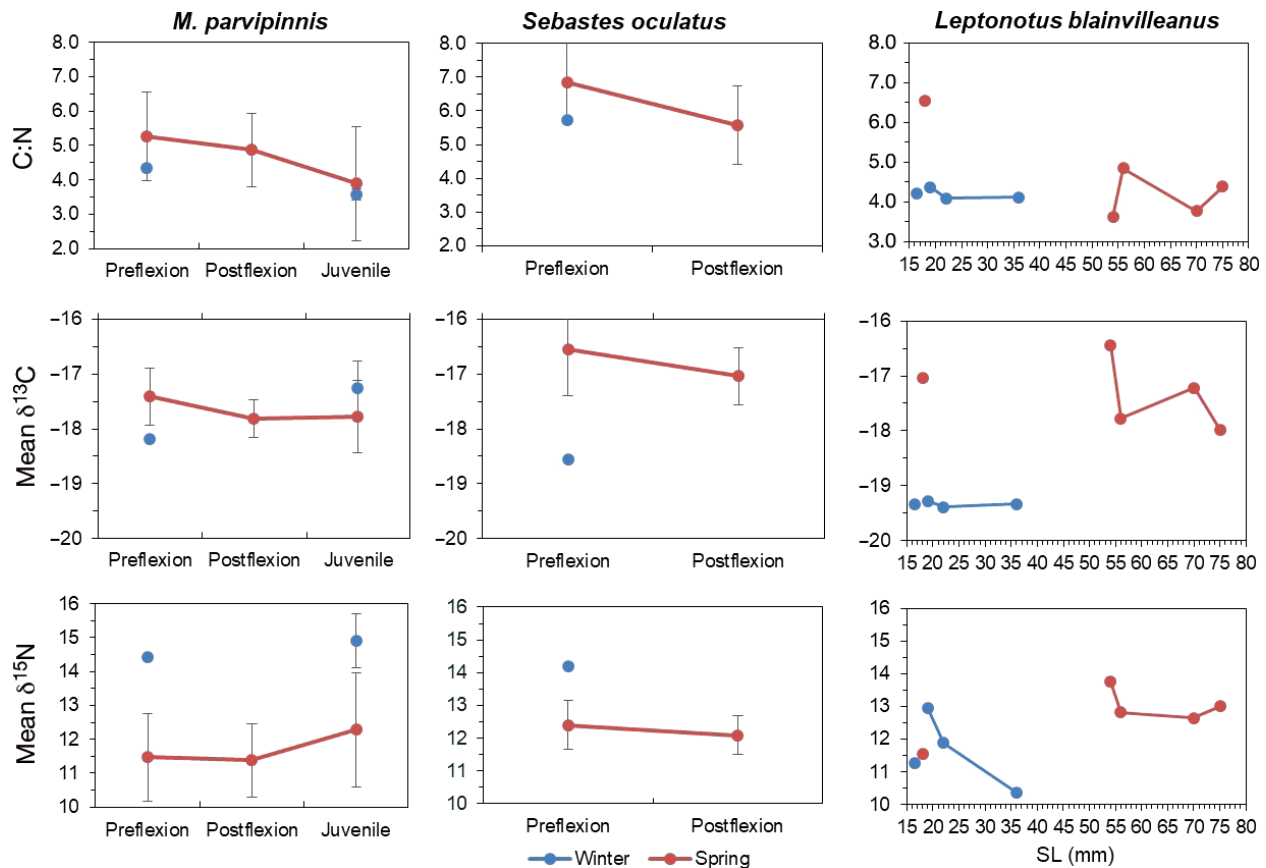

Fig. 5. Seasonal differences in the mean δ^{13}C and δ^{15}N values and C:N with the developmental stages of *Maurolicus parvipinnis* and *Sebastes oculatus*; δ^{13}C and δ^{15}N values were depicted against standard length (SL) in *Leptonotus blainvilleanus* since the range of body sizes was greater. Error bars are SD

the main fish species studied. We also identified changes in their isotopic niche breadth between seasons, suggesting that trophic plasticity at larval stages may be an important characteristic in this type of environment.

The trophic dynamics of fish larvae are not well understood due to the scarcity of studies that focus on their links with the pelagic food-webs worldwide. This is due to the effort needed to work with early stages, since sampling and identifying them phenologically might be challenging (Kelso et al. 2012). However, fish larvae are a main component of the plankton and marine trophic-webs, and particularly sensitive to climate-driven fluctuations. In areas where climate change has potentially strong effects on ecosystem functioning, such as Patagonia, due to the rapid melting of glacial ice (Iriarte et al. 2014, Iriarte 2018), trophodynamic studies on ichthyoplankton are scarce, but needed in order to increase our understanding on how environmental variations affect the trophic-web base and propagate to higher levels. In estuarine environments, changes of the freshwater influx may have a large influence on the trophic-web by modifying, for instance, the relative importance of the different carbon sources (allochthonous and autochthonous) or the TL at the base of the food-web during different seasons and longer-term periods.

4.1. Seasonal and spatial $\delta^{13}C$ signature variations in POM and larval fish

Seasonality demarcates strong physico-chemical changes in freshwater outflows in the inner Sea of Chiloé. These seasonal variations in hydrographic conditions affected the $\delta^{13}C$ isotopic signal of POM. Although the salinity between both seasonal periods inshore did not show large variations, it was lower in spring throughout the first 10 m of the water column due to the higher input of freshwater. The interpretation of the isotopic signatures in relation to freshwater input variations does not seem straightforward when considering that the most $\delta^{13}C$–depleted POM (terrestrially derived $\delta^{13}C$ mean value: −25.4 ± 0.9‰) was observed in winter (as expected considering that the austral winter is the rainy season in the area), whereas lower salinity values were registered in surface waters in spring. This apparent discrepancy was the result of a few rainy days before our sampling dates in spring that, although surface salinity decreased, were not likely so intense as to induce major changes in the $\delta^{13}C$-POM values in the inshore area. The wider salinity and temperature gradients at the inshore stations

suggest that any increased amount of terrestrial POM at the surface did not sink inshore but drifted offshore. This explanation is supported by both the low salinity values (<30) observed in surface waters at the Guafo Mouth and the low $\delta^{13}C$ POM-values (mean: −23.3 ± 1.7‰) at the oceanic-influenced stations in spring. The inshore area of the Ancud Gulf was characterized by $\delta^{13}C$ POM-values that ranged from −17.5 to −23‰, inferring a dominance of the autochthonous oceanic production of organic carbon. The C:N ratio was low (4 to 7), which corresponds to an oceanic influence (Silva et al. 2011). Towards open waters, the $\delta^{13}C$-POM signatures were lower (mean: −23.3 ± 1.7‰), being interpreted as a combination of both allochthonous and autochthonous organic matter in the complex. However, the C:N ratio was very low in the oceanic-influenced stations, ranging between 3 and 6. Hence, there is a prevalence of marine organic carbon along the inner sea, except for Reloncaví Fjord (−24.9 ± 0.96‰), where the allochthonous terrestrial matter seemed to be more retained. Carbon values were close to those reported by Silva et al. (2011) for allochthonous organic matter in northern Patagonian waters off Chiloé. $\delta^{13}C$ also coincided with those reported by Montecinos et al. (2016) for small copepods (−20.7‰). Other studies have found low $\delta^{13}C$-POM values near the Chilean coasts and fjord food-webs associated with river discharges, where bacteria incorporate the organic matter from a benthopelagic source, or by the fractionation of carbon via heterotrophic bacteria (Vargas et al. 2011, Zapata-Hernández et al. 2014, Quiroga et al. 2016).

Alternatively, atypically more depleted ^{13}C-POM values in the oceanic-influence area might be associated with microbial activity. When using the seston as the base of the marine food-web, it is usually assumed that this material comprises microscopic algae, when in fact it might be composed of a mixture of cyanobacteria, microplankton, and detritus which are difficult to separate (Major et al. 2017). As isotopic signatures for zooplankton in this oceanic-influenced area are not available, we cannot disregard this possibility of zooplankton grazing on particulate matter composed of this mixture of organisms and detritus.

The $\delta^{13}C$ mean values in most fish species follow the same seasonal trend observed in POM: winter $\delta^{13}C$ values were lower in larval fish of *Sebastes oculatus* and *Leptonotus blainvilleanus* compared with those collected in spring. *Maurolicus parvipinnis*, instead, was the only species that did not show variations in $\delta^{13}C$ between seasons, probably as a result of its deeper residence in the water column (in agreement with observations for species of the same genera else-

where: e.g. Olivar et al. 1992, Landaeta & Castro 2002, Rodríguez et al. 2015). In all the species, the $\delta^{13}C$ values and range of variation were much smaller than those observed in POM, which corresponds with organic carbon that mostly originates in the marine environment with the scarce influence of allochthonous organic carbon of terrestrial origin.

The $\delta^{13}C$ comparisons between larvae collected in spring suggest that, while $\delta^{13}C$ values in fish larvae collected inshore are indicative of production by marine phytoplankton (fish larvae feed on nauplii that, in turn, feed on diatoms), which was highly abundant inshore, in larvae collected at Guafo Mouth, $\delta^{13}C$ values also were influenced by terrestrially originated carbon. This result is in agreement with the isotopic signature previously reported for POM in superficial waters of the Guafo Mouth.

4.2. Trophic web structure and $\delta^{15}N$ isotopic signature in early fish life stages

$\delta^{15}N$-POM values observed in both seasons were higher (>5 ‰) than those obtained in oligotrophic seas (e.g. Altabet 2001, Kerhervé et al. 2001) and higher in winter than in spring. Thus, the trophic-web structure in the area corresponded to the classical food-web during both seasons, but it was probably more influenced by the microbial loop and benthic waters that ascend and mix with surface waters in late winter. In Patagonia, the microbial loop dominates in winter, and the classical diatom-based food-web dominates in spring (González et al. 2010). However, $\delta^{15}N$-POM values were relatively high in both seasonal periods, similar to Olivar et al. (2019), supporting the idea of a classic food-web with a low influence of diazotrophs. Also, Aberle et al. (2010) detected seasonal $\delta^{15}N$ shifts in consumers in the transition from the water mixing (winter) to vertical stratification (spring), whereas $\delta^{15}N$ values in seston were high in agreement with low diazotrophic reliance.

Early life stages of *M. parvipinnis*, *S oculatus*, and most larvae of the dominant fish species showed more variable and higher late-winter $\delta^{15}N$ values compared with those in spring. The higher values of SEA for fish larvae in spring support the idea of greater use of food resources during this season. SEA values were lower and dissimilar among fish species in late winter, which points out that their trophic niches were segregated by $\delta^{15}N$. The TLs of the larval fish species were generally higher in winter (between 2.2 and 4.3) than in spring (1.1 to 2.3), when

the species showed a more homogeneous feeding strategy (see Table 2 for values at each TL). Thus, we could expect that larvae in winter fed on prey with higher $\delta^{15}N$ content, i.e. potential zooplankton prey with a higher trophic level (considering that it was ca. 1 or 2) such as a wider assortment of omnivorous and carnivorous crustaceans; basically, calanoid copepods. Otherwise, we could suggest that the food-web relied more on microbial resources following the lower biomass of prey organisms during the less productive winter season. High nitrogen values for potential zooplankton prey would indicate a low level of herbivory (i.e. Landaeta & Castro 2002, Landaeta et al. 2011a) in line with Aberle et al. (2010). In spring, the food-web was mainly maintained by crustaceans where fish larvae reflected the lower isotopic value of resources. They might have also been fueled with herbivorous crustaceans, which in turn preyed on a dominant primary trophic level consisting of diatoms (classic food-web).

Most larvae of pelagic fish feed on copepod stages. The preflexion and postflexion larvae of *M. parvipinnis* (Landaeta et al. 2011a, 2012, 2015, Bernal et al. 2020) and *S. oculatus* (i.e. Anderson 1994, Bernal et al. 2020) prey mainly on calanoids, eggs, and nauplii, and incorporate larger prey as their mouth gape increases with growth (Contreras et al. 2014). In this study, the highest trophic values of *Merluccius* spp. were coincident with the dietary preferences of hake larval species and consistent with the idea of preferably feeding on large and more nutritious copepods, and occasionally on fish larval stages (Livington & Baley 1985, Valenzuela et al. 1995, Morote et al. 2011, Bernal et al. 2020). Non-significant nitrogen isotope differences were found between larvae and post-larvae of *M. parvipinnis*. This species appeared to be more euriphagic than hake. The juvenile stages of the pipefish, *L. blainvilleanus*, occurred in shallow coastal waters, no deeper than 25 m depth. This species is known as an algal-bed inhabitant that preys selectively on small amphipods distributed on the

Table 2. $\delta^{15}N$ (‰) values at each trophic level (TL) as estimated using the general equation from Post (2002) for each season and location sampled

| TL | Inshore | | Outer estuary zone |
	Late winter	Spring	Spring
1	7.1	8.7	7.3
2	10.5	12.1	10.7
3	13.9	15.5	14.1
4	17.3	18.9	17.5

same algal beds (Bastidas 2000), similar to other syngnathids (Ryer & Orth 1987, Woods 2002, García et al. 2005), but which in this region also feeds on copepods (Landaeta et al. 2019). In our study, both copepods and amphipods showed low $\delta^{13}C$ values coinciding with the low $\delta^{13}C$ in the pipefishes that were collected in shallow waters (<25m). Nevertheless, larger individuals of pipefish might be feeding at benthic algal beds in spring, where $\delta^{13}C$ could be more enriched compared with phytoplankton (France 1995), explaining more depleted carbon content in winter individuals, since the species exploited a different habitat each season. The only clupeid larvae (i.e. *Strangomera bentincki* or *Sprattus fuegensis*) showed the lowest $\delta^{15}N$ value coinciding with the lowest trophic position of larvae of this fish family that feed on phytoplankton and small copepods (e.g. Kurtz & Matsuura 2001, Morote et al. 2008, Ara et al. 2011), which is in agreement with a lower energetic demand of their tubular and slender body.

The $\delta^{15}N$ signature was lower in fish larvae than in eggs, as expected, due to mother transference, and reflecting the higher trophic position of adult female fishes and their nutritional stage. Usually, egg-nitrogen values are quite depleted with respect to those reported in mature female fishes (ca. 3–5 ‰). Mei et al. (2019) also found large variability in $\delta^{15}N$ of early larval stages of mesopelagic fishes in the East China Sea due to the maternal effect. The $\delta^{15}N$ values of fish eggs were higher in spring than in summer in Mei et al. (2019), and in winter in the present study, showing part of the isotopic signal of the spawning adults in this nursery area, which possibly fed more selectively on larger prey with higher $\delta^{15}N$ values during that period.

$\delta^{15}N$ values overlapped between co-occurring larvae in spring and were more segregated during the less productive winter. The high degree of trophic overlap within the fish community suggests high connectivity of predators with common prey. Spring individuals of *M. parvipinnis* and *S. oculatus* had segregated niches from that of the winter larvae of *M. parvipinnis*. Moreover, the larvae of both species are partly distributed at different depths over the shelf, according to previous studies (e.g. Landaeta et al. 2009) and observations by the authors herein. The number of trophic groups was more diverse at inner waters in spring than at the outer estuary zone, which is in line with the highest ichthyoplankton richness and abundances reported near the coastline in the northern basin of the Sea of Chiloé. We believe that dissimilar spring and winter niches reflect the seasonal variations in the zooplankton community. Despite the abundance of larvae and phytoplankton

being much higher in spring, our results suggest the potential existence of greater trophic diversity in winter, with higher $\delta^{15}N$ values and a wider $\delta^{15}N$-range. The isotope niche amplitude for some species and the $\delta^{15}N$ seasonal differences of some larvae showed high plasticity in the diet of consumers in the inland area.

4.3. Isotopic variations with body-size

Other biological features, such as development, may also affect isotopic composition (Olivar et al. 2019). We found slightly higher mean values of $\delta^{13}C$ in the older stages analyzed of the 3 species: *M. parvipinnis* (7–25 mm standard length, SL), *S. oculatus* (6–13 mm SL), and *L. blainvilleanus* (50–75 mm SL). For $\delta^{15}N$, *M. parvipinnis* and *L. blainvilleanus* also showed a trend of increasing values with larval size, while in *S. oculatus* (6–13 mm SL) this trend was not clear, probably because any shift in diet can be detected as a result of the small range of sizes analyzed. For *L. blainvilleanus*, the isotopic differences had an ontogenetic pattern and can be explained regarding the habitat occupied by larvae and juveniles, the latter reaching deeper benthic waters while larvae occurred in the epipelagic zone. Such isotopic differences are also dependent on the habitat occurrence in other species (e.g. Bode et al. 2015, Demopoulos et al. 2017).

5. CONCLUSIONS

This study showed that there are variations in the $\delta^{13}C$ and $\delta^{15}N$ isotopic values of early fish stages and POM in coastal and outer oceanic-influenced locations of northern Patagonia and is the first approach towards analysis of fish larval trophodynamics. We have documented some of the seasonal changes in the isotopic signature and also potential processes conducive to variations between the inshore areas and zones connecting with the continental shelf and adjacent ocean. However, because of the complex dynamics and seasonality in the region, more extensive sampling over time is required in order to clarify the occurrence of seasonal variations in baseline values and the complexity of the trophic-web. It is also important to understand how the climatic changes predicted in the forthcoming decades will act as possible vectors of alteration for the dynamics in the inland sea and the trophic interactions in this large estuarine-like ecosystem.

LITERATURE CITED

Aberle N, Hansen T, Boettger-Schnack R, Burmeister A, Post AF, Sommer U (2010) Differential routing of 'new' nitrogen toward higher trophic levels within the marine food web of the Gulf of Aqaba, Northern Red Sea. Mar Biol 157:157–169

Altabet MA (2001) Nitrogen isotopic evidence for micronutrient control of fractional NO3 — utilization in the equatorial Pacific. Limnol Oceanogr 46:368–380

Anderson JT (1994) Feeding ecology and condition of larval and pelagic juvenile redfish *Sebastes* spp. Mar Ecol Prog Ser 104:211–226

Ara R, Arshad A, Musa L, Amin SMN, Kuppan P (2011) Feeding habits of larval fishes of the Family Clupeidae (Actinopterygii: Clupeiformes) in the Estuary of River Pendas, Johor, Malaysia. J Fish Aquat Sci 6:816–821

Arimitsu ML, Piatt JF, Mueter F (2016) Influence of glacier runoff on ecosystem structure in Gulf of Alaska fjords. Mar Ecol Prog Ser 560:19–40

Balbontín F, Uribe F, Bernal R, Braun M (2004) Descriptions of larvae of *Merluccius australis, Macruronus magellanicus*, and observations on a larva of *Micromesistius australis* from southern Chile. N Z J Mar Freshw Res 38:609–619

Bastidas K (2000) Dieta y selectividad alimentaria de la aguja de mar *Leptonotus blainvilleanus* (Osteichthyes, Syngnathidae) en praderas de *Gracilaria chilensis* del río Maullín, X Región, Chile. MS dissertation, Universidad Austral de Chile, Valdivia

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73:1007–1012

Bernal A, Castro L, Soto S, Cubillos L (2020) Ichthyoplankton distribution and feeding habits of fish larvae at the inshore zone of northern Patagonia, Chile. Mar Biodivers 50:56

Blanca MJ, Alarcon R, Arnau J, Bono R, Bendayan R (2017) Non-normal data: Is ANOVA still a valid option? Psicothema 29:552–557

Bode M, Hagen W, Schukat A, Teuber L, Fonseca-Batista D, Dehairs F, Auel H (2015) Feeding strategies of tropical and subtropical calanoid copepods throughout the Eastern Atlantic Ocean: Latitudinal and bathymetric aspects. Prog Oceanogr 138:268–282

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 42:411–440

Chirichigno N, Cornejo RM (2001) Catálogo comentado de los peces marinos del Perú. Publicación Especial, Instituto del Mar del Perú, Callao

Contreras T, Castro LR, Montecinos S, González HE, Soto S, Muñoz MI, Palma S (2014) Environmental conditions, early life stages distributions and larval feeding of Patagonian sprat *Sprattus fuegensis* and common sardine *Strangomera bentincki* in fjords and channels of the northern Chilean Patagonia. Prog Oceanogr 129: 136–148

Demopoulos AWJ, McClain-Counts J, Ross SW, Brooke S, Mienis F (2017) Food-web dynamics and isotopic niches in deep-sea communities residing in a submarine canyon and on the adjacent open slopes. Mar Ecol Prog Ser 578: 19–33

Eisma D (1993) Suspended matter in the aquatic environment. Springer, Berlin

Fierro P, Bertran C, Martinez D, Valdovinos C, Vargas-Chacoff L (2014) Ontogenetic and temporal changes in the diet of the Chilean silverside *Odontesthes regia* (Atherinidae) in Southern Chile. Cah Biol Mar 55: 323–332

Flores EA, Castro LR, Narváez DA, Lillo S, Balbontín F, Osorio-Zúñiga F (2019) Inter-annual and seasonal variations in the outer and inner sea spawning zones of southern hake, *Merluccius australis*, inferred from early life stages distributions in Chilean Patagonia. Prog Oceanogr 171: 93–107

France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124:307–312

Franco A, Elliott M, Franzoi P, Torricelli P (2008) Life strategies of fishes in European estuaries: the functional guild approach. Mar Ecol Prog Ser 354:219–228

García AM, Geraldi RM, Vieira JP (2005) Diet composition and feeding strategy of the southern pipefish *Syngnathus folletti* in a widgeon grass bed of the Patos Lagoon Estuary, RS, Brazil. Neotrop Ichthyol 3:427–432

González HE, Calderón MJ, Castro L, Clement A and others (2010) Primary production and plankton dynamics in the Reloncaví Fjord and the Interior Sea of Chiloé, Northern Patagonia, Chile. Mar Ecol Prog Ser 402:13–30

González HE, Castro LR, Daneri G, Iriarte JL and others (2011) Seasonal plankton variability in Chilean Patagonia fjords: carbon flow through the pelagic food web of the Aysén Fjord and plankton dynamics in the Moraleda Channel basin. Cont Shelf Res 31:225–243

González HE, Graeve M, Kattner G, Silva N and others (2016) Carbon flow through the pelagic food web in southern Chilean Patagonia: relevance of *Euphausia vallentini* as a key species. Mar Ecol Prog Ser 557:91–110

Grey J, Jones RI, Sleep D (2001) Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. Limnol Oceanogr 46:505–513

Iriarte JL (2018) Natural and human influences on marine processes in Patagonian Subantarctic coastal waters. Front Mar Sci 5, doi:10.3389/fmars.2018.00360

Iriarte JL, González HE, Liu KK, Rivas C, Valenzuela C (2007) Spatial and temporal variability of chlorophyll and primary productivity in surface waters of southern Chile (41.5–43° S). Estuar Coast Shelf Sci 74:471–480

Iriarte JL, Pantoja S, Daneri G (2014) Oceanographic processes in Chilean fjords of Patagonia: from small to large-scale studies. Prog Oceanogr 129:1–7

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80:595–602

Kelso WE, Kaller MD, Rutherford DA (2012) Collecting, processing, and identification of fish eggs and larvae and zooplankton. In: Zale AV, Parrish DL, Sutton TM (eds) Fisheries techniques, 3rd edn. American Fisheries Society, Bethesda, MD p 363–452

Kerhervé P, Minagawa M, Heussner S, Monaco A (2001) Stable isotopes (^{13}C/^{12}C and ^{15}N/^{14}N) in settling organic matter of the northwestern Mediterranean Sea: biogeochemical implications. Oceanol Acta 24:77–85

Kline TC (2009) Characterization of carbon and nitrogen sta-

ble isotope gradients in the northern Gulf of Alaska using terminal feed stage copepodite-V *Neocalanus cristatus.* Deep Sea Res II 56:2537–2552

Kondo K (1980) The recovery of the Japanese sardine — The biological basis of stock-size fluctuations. Rapp P-V Cons Int Explor Mer 177:332–354

Kurtz FW, Matsuura Y (2001) Food and feeding ecology of Brazilian sardine (*Sardinella brasiliensis*) larvae from the southeastern Brazilian Bight. Rev Bras Oceanogr 49:60–74

Landaeta MF, Castro LR (2002) Spring spawning and early nursery zone of the mesopelagic fish *Maurolicus parvipinnis* at the coastal upwelling zone off Talcahuano, central Chile. Mar Ecol Prog Ser 226:179–191

Landaeta MF, Castro LR (2006a) Variabilidad estacional en los patrones espaciales de las asociaciones ictioplanctónicas de la zona de fiordos de Chile Austral. Cienc Tecnol Mar 29:107–127

Landaeta MF, Castro LR (2006b) Larval distribution and growth of the rockfish, *Sebastes capensis* (Sebastidae, Pises), in the fjords of southern Chile. ICES J Mar Sci 63:714–724

Landaeta MF, Muñoz MI, Castro LR (2009) Seasonal and short-term variability in the vertical distribution of ichthyoplankton in a stratified fjord of southern Chile. Revista. Cienc Tecnol Mar 32:27–42

Landaeta MF, Suárez-Donoso N, Bustos CA, Balbontín F (2011a) Feeding habits of larval *Maurolicus parvipinnis* (Pisces: Sternoptychidae) in Patagonian fjords. J Plankton Res 33:1813–1824

Landaeta MF, Bustos CA, Palacios-Fuentes P, Rojas P, Balbontín F (2011b) Distribución del ictioplancton en la Patagonia austral de Chile: potenciales efectos del deshielo de Campos de Hielo Sur. Lat Am J Aquat Res 39:236–249

Landaeta MF, López G, Suárez-Donoso N, Bustos CA, Balbontín F (2012) Larval fish distribution, growth and feeding in Patagonian fjords: potential effects of freshwater discharge. Environ Biol Fish 93:73–87

Landaeta MF, Muñoz MJO, Bustos CA (2014) Feeding success and selectivity of larval anchoveta *Engraulis ringens* in a fjord-type inlet from northern Patagonia (Southeast Pacific). Rev Biol Mar 49:461–475

Landaeta MF, Bustos CA, Contreras JE, Salas-Berríos F (2015) Larval fish feeding ecology, growth and mortality from two basins with contrasting environmental conditions of an inner sea of northern Patagonia, Chile. Mar Environ Res 106:19–29

Landaeta MF, Vera-Duarte J, Ochoa-Muñoz MJ, Bustos CA, Balbontín F (2019) Feeding ecology of fish larvae from Chilean Patagonia during austral winter. Rev Biol Mar Oceanogr 54:221–226

Lasker R (1981) The role of a stable ocean in larval fish survival and subsequent recruitment. In: Lasker R (ed) Marine fish larvae, morphology, ecology and relation to fisheries. University Washington Press, WA, p 80–87

Lasker R, Zweifel JR (1978) Growth and survival of first-feeding northern anchovy (*Engraulis mordax*) in patches containing different proportions of large and small prey. In: Steele JH (dd) Spatial pattern inpPlankton communities. Plenum, New York, NY, p 329–354

Livington PA, Baley KM (1985) Trophic role of the Pacific whiting, *Merluccius productus.* Mar Fish Rev 47:16–22

Major Y, Kifle D, Niedrist GH, Sommaruga R (2017) An isotopic analysis of the phytoplankton–zooplankton link in a highly eutrophic tropical reservoir dominated by cyanobacteria. J Plankton Res 39:220–231

Mei W, Umezawa Y, Wan X, Yuan J, Sassa C (2019) Feeding habits estimated from weight-related isotope variations of mesopelagic fish larvae in the Kuroshio waters of the northeastern East China Sea. ICES J Mar Sci 76:639–648

Meire L, Mortensen J, Meire P, Juul-Pedersen T and others (2017) Marine-terminating glaciers sustain high productivity in Greenland fjords. Glob Change Biol 23:5344–5357

Montecinos S, Castro L, Neira S (2016) Stable isotope (δ^{13}C and δ^{15}N) and trophic position of Patagonian sprat (*Sprattus fuegensis*) from the Northern Chilean Patagonia. Fish Res 179:139–147

Morote E, Olivar MP, Villate F, Uriarte I (2008) Diet of round sardinella, *Sardinella aurita*, larvae in relation to plankton availability in the NW Mediterranean. J Plankton Res 30:807–816

Morote E, Olivar PO, Bozzano A, Villate F, Uriarte I (2011) Feeding selectivity in larvae of the European hake (*Merluccius merluccius*) in relation to ontogeny and visual capabilities. Mar Biol 158:1349–1361

Moser HG (1996) The early stages of fishes in the California Current region. CalCOFI Atlas 33. Allen Press, Lawrence, KS

Olivar MP, Rubiés P, Salat J (1992) Horizontal and vertical distribution patterns of ichthyoplankton under intense upwelling regimes off Namibia. Afr J Mar Sci 12:71–82

Olivar MP, Bode A, López-Pérez C, Hulley PA, Hernández-León S (2019) Trophic position of lanternfishes (Pisces: Myctophidae) of the tropical and equatorial Atlantic estimated using stable isotopes. ICES J Mar Sci76:649–661

Orellana MC, Balbontín F (1983) Estudio comparativo de las larvas de clupeiformes de la costa de Chile. Rev Biol Mar 19:1–46

Osorio-Zúñiga F, Landaeta MF, Angulo-Aros J, Balbontín F (2018) Spatio-temporal variability of ichthyoplankton and its relationship with oceanographic conditions at the shelf break off Chilean Patagonia (43°S–51°S). Mar Biol Res 14:191–202

Palma S, Silva N (2004) Distribution of siphonophores, chaetognaths, euphausiids and oceanographic conditions in the fjords and channels of southern Chile. Deep Sea Res II 51:513–535

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189

Quiroga E, Ortiz P, González-Saldías R, Reid B and others(2016) Seasonal patterns in the benthic realm of a glacial fjord (Martinez Channel, Chilean Patagonia): the role of suspended sediment and terrestrial organic matter. Mar Ecol Prog Ser 561:31–50

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rodríguez J, Cabrero A, Gago J, Guevara-Fletcher C and others (2015) Vertical distribution and migration of fish larvae in the NW Iberian upwelling system during the winter mixing period: implications for cross-shelf distribution. Fish Oceanogr 24:274–290

Ryer CH, Orth RJ (1987) Feeding Ecology of the Northern Pipefish, *Syngnathus Fuscus*, in a Seagrass Community of the Lower Chesapeake Bay. Estuaries 10:330–336

Sahai H, Ageel MI, Khurshid A (2011) Analysis of variance model, effects of departures from assumptions underly-

ing. In: Lovric M (ed) International encyclopedia of statistical science. Springer, Berlin p 46–51

Sepúlveda J, Pantoja S, Hughen KA (2011) Sources and distribution of organic matter in northern Patagonia fjords, Chile (~44–47° S): a multi-tracer approach for carbon cycling assessment. Cont Shelf Res 31:315–329

Silva N, Vargas CA (2014) Hypoxia in Chilean Patagonian fjords. Prog Oceanogr 129:62–74

Silva N, Vargas CA, Prego R (2011) Land–ocean distribution of allochthonous organic matter in surface sediments of the Chiloé and Aysén interior seas (Chilean Northern Patagonia). Cont Shelf Res 31:330–339

Strub PT, James C, Montecino V, Rutllant JA, Blanco JL (2019) Ocean circulation along the southern Chile transition region (38°–46° S): mean, seasonal and interannual variability, with a focus on 2014–2016. Prog Oceanogr 172:159–198

Tello A, Rodríguez-Benito C (2009) Characterization of mesoscale spatio-temporal patterns and variability of remotely sensed Chl a and SST in the Interior Sea of Chiloe 41.4–43.5° S. Int J Remote Sens 306:1521–1536

Uriarte A, García A, Ortega A, de la Gándara F, Laíz-Carrión R (2014) Maternal transference of isotopic signature (δ^{15}N, δ^{13}C) and its evolution during ontogenetic development of reared bluefin larvae, *Thunnus thynnus* (Linnaeus, 1758). In: Ríos P, Suárez LA, Cristobo J (eds) Proc XVIII Iberian Symp Mar Biol Stud, Gijón, Spain, Sep 2–5 2014, p 103

Valenzuela V, Balbontín F, Llanos A (1995) Diet composition and prey size of the larvae of eight species of fishes from the coast of central Chile. Rev Biol Mar 30:275–291

Vargas CA, Martínez RA, San Martín V, Aguayo M, Silva N, Torres R (2011) Allochthonous subsidies of organic matter across a lake–river–fjord landscape in the Chilean Patagonia: implications for marine zooplankton in inner fjord areas. Cont Shelf Res 31:187–201

Wadham JL, Hawkings JR, Tarasov L, Gregoire LJ and others (2019) Ice sheets matter for the global carbon cycle. Nat Commun 10:3567

West JB, Bowen GJ, Cerling TE, Ehleringera JR (2006) Stable isotopes as one of nature's ecological recorders. Trends Ecol Evol 21:408–414

Woods CMC (2002) Natural diet of the seahorse *Hippocampus abdominalis*. N Z J Mar Freshw Res 36:655–660

Yañez E, Barbieri MA (1974) Distribución y abundancia relativa de los recursos disponibles a un arte de arrastre camaronero frente a la costa de Valparaíso (invierno 1973). Invest Mar Valparaíso 5:137–56

Zapata-Hernández G, Sellanes J, Mayr C, Muñoz P (2014) Benthic food web structure in the Comau fjord, Chile (~42° S): Preliminary assessment including a site with chemosynthetic activity. Prog Oceanogr 129:149–158