

RESEARCH NOTE

Feeding ecology of fish larvae from Chilean Patagonia during austral winter

Ecología trófica de larvas de peces de la Patagonia chilena durante el invierno

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Abstract.- Feeding habits of the ichthyoplankton from Chilean Patagonia (44-46°30'S) were studied during June-July 2012 (austral winter). Ichthyoplankton assemblage was composed by 10 species, with low abundance (3.8 to 16.73 ind. 1000 m⁻³). Most abundant larvae were *Maurollicus parvipinnis*, *Leptonotus blainvillleanus* and *Sprattus fuegensis*. These three species fed mainly on calanoid copepodites, *Paracalanus indicus* and *Calanus* sp. copepodites, and cyphonautes. Trophic overlap among dominant fish larvae was high (Schoener's D > 0.69) and no significant differences were detected in prey composition and size. Therefore, no resource partitioning occurred in planktonic fish larvae during winter 2012 in Chilean Patagonia.

Key words: Feeding, overlap, ichthyoplankton, prey size

INTRODUCTION

The Chilean Patagonia is located in the southeastern border of the Pacific Ocean and covers more than 1,000 km between 41° and 55°S. Fjords therein usually range between 100 and 500 m depth, from 10 to 70 km long, and have a two-layer structure that is vertically limited by a strong halocline between 4 and 20 m depth (González *et al.* 2011). In the area, the productivity cycle can be divided into productive (late winter/early spring to late autumn) and non-productive (winter) seasons (Montero *et al.* 2011). In winter, integrated Chlorophyll-*a* concentration (Chl-*a*) in the mixed layer varies between 5 and 56 mg m⁻², whereas the spring range is 8.5-165 mg m⁻² (González *et al.* 2011). Picophytoplankton (< 2 µm) make the greatest contribution of all size-fractions to the total Chl-*a* in winter; on the other hand, large phytoplankton cells (> 20 µm) account for 51% of the total Chl-*a* during spring (González *et al.* 2011).

The strong temporal variations in oceanographic conditions and primary and secondary productivity pose restrictions to reproductive tactics of marine fish inhabiting this ecosystem. Tactics of most marine fish species from Chilean Patagonia encompass reproduction during austral spring in order to ensure food availability to their offspring during the first-feeding period, after the yolk-sac absorption (Landaeta & Castro 2006, Bustos *et al.* 2008a, Landaeta *et al.* 2015). Other species, mainly Gadiform fishes such as southern hake *Merluccius australis* and Patagonian grenadier *Macruronus magellanicus*, spawn during austral winter on the continental shelf-break, out fjords

and channels (Niklitschek *et al.* 2014, Osorio-Zúñiga *et al.* 2018), although a secondary spawning location in the inner seas is used during austral spring (Bustos *et al.* 2007, Landaeta *et al.* 2018). In this sense, the oligotrophic nature of the environmental conditions during austral winter may constraint feeding strategy of fish larvae. However, until now, no studies have been done regarding feeding ecology of fish larvae during winter. Therefore, using fish larvae collected during winter 2012, we evaluated the diet, prey size and trophic overlap of the early life stages of marine fishes of south Patagonia. Studying feeding ecology of fish larvae is important to establish potential constraints that the winter season may pose to the trophic interactions, particularly in those species with commercial interest.

MATERIALS AND METHODS

A bio-oceanographic survey was carried out between 16 June 2012 and 08 July 2012 (austral winter) in the inner fjords and channels of Chilean Patagonia between 44° and 46°30'S, on board RV Abate Molina (Fig. 1A). Ichthyoplankton samples were collected at each station (*n*= 31) with 1 m² Tucker trawl (300 µm mesh size), by oblique trawls from surface to 150 m depth performed for 15-20 min. A flowmeter (General Oceanics™, Florida USA) was installed in the frame of the net to estimate the filtered volume. All samples were fixed with 10% formaldehyde buffered with sodium borate, and after 12 h, they were preserved in 96% ethanol. Filtered seawater volume varied from 93.6 to 353.7 m³ (mean ± SD, 236.5 ± 67.6 m³ trawl⁻¹).

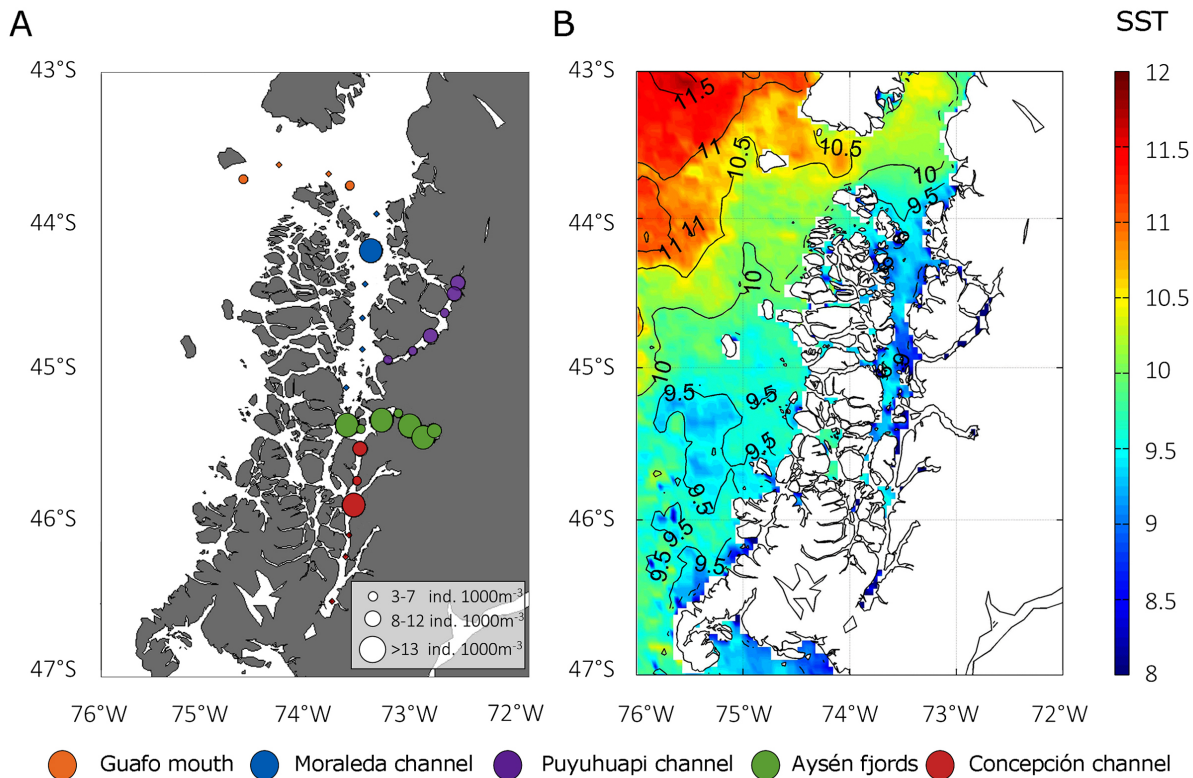


Figure 1. A) Location of the bio-oceanographic sampling stations and horizontal distribution of larval abundance (ind. 1000 m⁻³). B) Sea surface temperature (SST) field (°C), during austral winter in the Chilean Patagonia / A) Localización de las estaciones de muestreo bio-oceano-gráfico y distribución horizontal de la abundancia de larvas (ind. 1000 m⁻³). B) Temperatura superficial del mar (TSM) (°C) durante el invierno austral de la Patagonia chilena

Sea Surface Temperature (SST) was obtained through MODIS satellite systems (NASA), which were distributed by Ocean Color Web software¹. For this purpose, the average SST was calculated to show the surface thermal distribution of the Chilean Patagonian Sea during the cruise.

In the laboratory, all fish larvae from plankton samples were separated, counted and identified to the lowest possible taxon, under stereomicroscope following to Fisher (1959), de Ciechomski (1971), Sabatés & Olivar (1990) and Bustos & Landaeta (2005). The fish abundance was standardized to individuals 1000 m⁻³.

The standard length (SL, from the tip of the snout to the tip of the notochord or to the base of the hypural plates) and upper jaw length (UJL, from the tip of the premaxilla to the distal end of the maxilla) of undamaged larvae ($n=71$) were measured to the nearest 0.01 mm under a stereomicroscope Olympus® SZ-61 with a video camera Moticam® 2500 (5.0MPixel) connected to a PC with Moticam® Image Plus 2.0 software. Larvae measurements were not corrected for shrinkage. The relationships between

SL and UJL were determined separately for each species by linear regression analysis, and comparisons of adjusted means were performed with one-way ANCOVA (Zar 1999), setting significance at $P < \alpha = 0.05$.

The gut of each larva was dissected from the body and opened lengthwise with fine needles. Prey items were counted and identified to the lowest possible taxon under a Motic® BA310 microscope. The maximum length and width of each prey item were measured with an accuracy of 1 µm using the same equipment mentioned above. The maximum prey width (MPW) was compared among species with the non-parametric Kruskal-Wallis test. The volume of each prey item was estimated using the three-dimensional shape that most closely resembled the item, according to Cass-Calay (2003) and Sun & Liu (2003). The prosome length of copepodite prey was measured since the urosome was often missing.

Feeding incidence (FI) was calculated as the percentage of the total number of individuals examined with at least one prey item in the gut contents (Vera-Duarte & Landaeta 2017). All larvae with identifiable prey in their guts were used for the analysis. The diets were described with the

¹<https://oceancolor.gsfc.nasa.gov/>

index of relative importance (IRI) (Pinkas *et al.* 1971), calculated as $IRI = (\%N + \%V) \times \%F$, where %N is the percentage of the total numbers of prey items in their guts, %V is the percentage of volume of each item and %F is the frequency of occurrence of a prey item in larvae with food in their guts. To readily allow comparison among prey items, the IRI was standardized to %IRI for each prey item (Cortés 1997). Additionally, trophic overlap was measured using proportional similarity or Schoener's D index, $D = 1 - 0.5 \sum_i (|p_i - q_i|)$, where p_i is the proportion of prey item i used by species p and q_i is the proportion of prey item i used by species q . This index varies between 0 (no trophic overlap) and 1, where values above 0.6 indicate overlap (Schoener 1974, Balbontín *et al.* 1997).

RESULTS AND DISCUSSION

During the study period, 10 species of fish larvae were collected, belonging to 9 families (Table 1). Larval abundance was low, varying from 3.8 to 16.73 ind. 1000 m⁻³ (Fig. 1A). The most abundant larvae species found in the Chilean Patagonia during winter were *Maurolicus parvipinnis* and *Leptonotus blainvillanus* (Table 1). This result is in agreement with Bustos *et al.* (2011) and Landaeta *et al.* (2012), which have previously described the dominance of both species during austral spring in adjacent areas of the Patagonian fjords (41-45°S and 47-50°S, respectively).

Table 1. Larval abundance (ind. 1000 m⁻³), standard length (mm), upper jaw length (mm), feeding incidence (FI; %) and diet composition, in terms of the percentage of index of relative importance (%IRI) of fish larvae collected during austral winter in Chilean Patagonia / Abundancia de larvas (ind. 1000 m⁻³), rangos de tamaño corporal (mm), rangos de tamaño de longitud de mandíbula superior (mm), incidencia alimentaria (IA) y composición de la dieta en porcentajes del índice de importancia relativa de las presas (%IIR) de larvas de peces recolectadas en la Patagonia chilena durante un invierno austral

	<i>Maurolicus parvipinnis</i>	<i>Leptonotus blainvillanus</i>	<i>Sprattus fuegensis</i>	<i>Normanichthys crockeri</i>	<i>Patagonotothen tessellata</i>	<i>Meluccius australis</i>	<i>Merluccius gayi</i>	Macrouridae	<i>Lampanyctodes hectoris</i>	<i>Sebastes oculatus</i>
Larval abundance (ind. 1000 m ⁻³)	6.6 ± 3.9	7.8 ± 4.6	7.4 ± 4.6	7.9 ± 7.4	6.8 ± 2.8	3.8	5.8 ± 4.1	4.5 ± 1.6	16.73	4.8
Standard length (mm)	4.04-28.91	21.23-90.95	41.83-45.06	2.96-4.37	3.97-6.72	3.71	2.91-3.77	3.56-4.33	2.88-3.15	4.42
Upper jaw length (mm)	0.30-4.63	0.23-0.92	3.30-5.43	0.14-0.23	0.27-0.42	0.36	0.22-0.43	0.28-0.45	0.10-0.14	0.40
Feeding incidence (%)	80.76	80.90	100	33.3	25	100	50	100	0	0
N	26	21	3	6	4	1	2	2	5	1
Prey Item										
Dinoflagellate cysts	-	-	1.661	-	-	-	-	-	-	-
<i>Thalassiosira</i> sp.	0.287	-	-	-	-	-	-	-	-	-
Invertebrate egg	0.039	1.489	6.744	-	-	-	-	-	-	-
Bivalve larvae	1.802	-	0.825	26.356	-	-	-	-	-	-
Copepod egg	0.469	0.461	-	-	69.917	-	-	-	-	-
Copepod nauplii	0.194	0.249	-	-	-	-	-	-	-	-
Copepodite	1.292	3.650	-	-	-	-	-	-	-	-
Calanoid copepodite	14.522	57.545	37.911	-	-	11.610	42.227	-	-	-
Copepodite (<i>Acartia tonsa</i>)	0.502	-	1.484	-	-	-	-	-	-	-
Copepodite (<i>Calanus</i> sp.)	5.289	0.733	10.083	-	-	-	-	-	-	-
Copepodite (<i>Paracalanus indicus</i>)	11.259	4.238	9.514	-	-	-	-	-	-	-
Copepod	0.044	-	-	-	-	-	-	-	-	-
Calanoid copepod	14.933	2.565	-	-	-	-	-	-	-	-
Poecilostomatoid copepodite	0.149	-	-	-	-	-	-	-	-	-
<i>Oncaea</i> sp.	0.038	0.013	-	-	-	-	-	-	-	-
Cyclopoid copepodite	-	0.015	-	-	-	-	-	-	-	-
Copepod remains	17.290	13.097	12.603	-	-	88.390	-	88.504	-	-
Cyphonaute	3.678	1.198	1.128	-	-	-	-	-	-	-
Euphausiid nauplii	-	0.113	-	-	-	-	-	-	-	-
Calyptopis	-	0.116	-	-	-	-	-	-	-	-
Crustacean remains	-	-	2.740	-	-	-	-	-	-	-
<i>Evadne</i> sp.	-	0.048	-	-	-	-	-	-	-	-
<i>Podon</i> sp.	-	0.016	-	-	-	-	-	-	-	-
Ostracod	2.375	0.138	2.593	-	-	-	-	-	-	-
Chitinous remains	25.693	14.301	8.583	73.644	30.083	-	57.773	11.496	-	-
Unidentified prey	0.145	0.014	4.132	-	-	-	-	-	-	-

Size of larvae ranged from 2.91 to 90.95 mm SL (mean \pm SD, 27.6 ± 29.29 mm SL), while upper jaw length varied between 0.10 and 5.45 mm (UJL: 0.93 ± 1.12 mm). Linear regressions between SL and UJL were significant in almost all larval species ($P < 0.05$), except for *Sprattus fuegensis* larvae ($P = 0.425$). In addition, *M. parvipinnis* larvae showed a larger UJL compared with *L. blainvillaeus* (one-way ANCOVA, $F_{1,44} = 16.13$, $P < 0.001$). Larval size range in *M. parvipinnis* was narrower than in *L. blainvillaeus*, but the proportion of UJL/SL was smaller for the latter. Differences in mouth gape directly affect feeding incidence, and the ability to capture and handle prey of different size ranges (Sabatés & Saiz 2000, Salas-Berrios *et al.* 2013).

The spatial distribution of the larvae during winter was not homogeneous throughout the area (Fig. 1A). Fish larvae were detected at Guafo mouth, at the northern entrance of the Moraleda channel, along the Puyuhuapi and Aysén fjord, and at the mouth of the Concepción channel. Nonetheless, those abundances were lower compared to those found during austral spring in Patagonian fjords, which usually range between 3 and 370 ind. 1000 m⁻³ (Bustos *et al.* 2008b, Landaeta *et al.* 2009, 2011a). The average conditions of SST indicated the presence of relatively warmer waters in the Guafo mouth (> 10.5 °C), and slightly cooler waters along the Moraleda channel (< 9.5 °C). The lowest temperatures were registered inside the Puyuhuapi and Aysén fjords and in the Concepción channel (~ 8 °C) (Fig. 1B). SST in the area was lower compared to SST in the inner sea of Chiló during austral spring (> 11 °C; Landaeta *et al.* 2015).

Highest feeding incidences (100%) were observed in *Sprattus fuegensis*, *M. australis* and Macrouridae larvae (Table 1). The diet of *S. fuegensis* included 10 prey items, which was dominated by calanoid copepodites (%IRI, 37.91%), copepod remains (12.60%) and *Calanus* sp. copepodites (10.08%). Predation of this species upon copepodites has been previously described during austral spring in fjords and channels of southern Chile (Landaeta *et al.* 2012). *M. australis* diet was characterized by high IRI values on copepod remains (88.39%). However, low larval abundances were recorded, probably since spawning occurs in austral winter from July to September (Balbontín & Bravo 1993). Unidentified Macrouridae larvae showed presence of copepod remains (88.50%) and chitinous remains (11.49%). Nonetheless, FI results need to be taken with caution due to the small number of individuals collected. Slightly lower feeding incidences were observed in *M. parvipinnis* and *L. blainvillaeus* larvae ($\sim 81\%$). Diet composition of these species was similar (17 prey items); while *M. parvipinnis* showed presence of copepod remains (17.29%), calanoid copepodites (14.52%) and *Paracalanus indicus* copepodites (11.29%), diet of *L. blainvillaeus* was based on calanoid copepodites (57.54%), copepod remains (13.09%) and *P. indicus* copepodites (4.28%) (Table 1). Accordingly, feeding habits of larval *M. parvipinnis* during austral spring

in Patagonian fjords has been characterized by preying on calanoid and cyclopoid copepodites (Landaeta *et al.* 2011b, 2012, 2015).

On the other hand, lower feeding incidences ($< 50\%$) were registered in *Normanichthys crockeri*, *Patagonotothen tessellata* and *Merluccius gayi* larvae. The diet of *N. crockeri* (FI= 33.3%) was composed by bivalve larvae (26.35%) while *P. tessellata* (FI= 25.0%) mainly ate copepod eggs (69.91%). In both cases, chitinous remains were observed (73.64 and 30.08%, respectively) (Table 1). Larval *P. tessellata* were exclusively carnivorous, feeding on copepod nauplii and larger prey such as calanoid copepodites (Salas-Berrios *et al.* 2013). *M. gayi* diet was characterized by high values of IRI on chitinous remains (57.77%).

Prey size (MPW, μm) of larval *M. parvipinnis* ranged between 14.94 and 575.44 μm (median \pm median absolute deviation, 249.93 ± 86.73 μm), while in larval of *L. blainvillaeus* it varied between 38.47 and 500.64 μm (233.75 ± 53.79 μm). Finally, in larval *S. fuegensis*, prey size ranged between 43.37 and 493.70 μm (255.78 ± 43.37 μm). Larval fishes predated on similar prey size ranges (Kruskal-Wallis H test, $H = 3.901$, $P = 0.144$; Fig. 2). Particularly, *blainvillaeus* has larger sizes and *M. parvipinnis* has relatively larger mouth opening, both species fed upon similar prey size. This similarity may be caused by the low abundance of microzooplankton during winter (1.5 ± 2 mg C m⁻²) compared to spring (163 ± 148 mg C m⁻²) (González *et al.* 2011). Therefore, low abundance of potential prey suggests that several components of the ichthyoplankton assemblage are predated on small crustacean copepods.

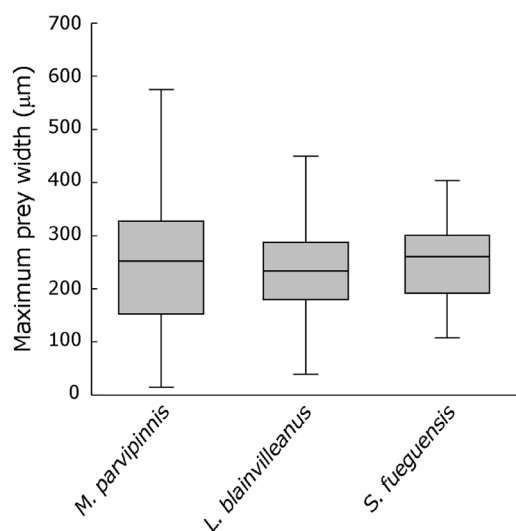


Figure 2. Maximum prey width (μm) of *Maurollicus parvipinnis*, *Leptonotus blainvillaeus* and *Sprattus fuegensis* larvae collected during austral winter in the Chilean Patagonia. Median (middle line), 25-75% percentiles (box) and minimum and maximum values (whiskers) are presented / Ancho máximo de las presas (μm) de larvas de *Maurollicus parvipinnis*, *Leptonotus blainvillaeus* y *Sprattus fuegensis* recolectadas durante el invierno austral en la Patagonia chilena. La línea media representa la mediana, el cuadrado indica los percentiles 25-75%, y los bigotes el valor mínimo y máximo

Trophic overlap among *M. parvipinnis*, *L. blainvillanus* and *S. fuegensis* larvae was high. Low sampling size precluded the comparison amongst other species. In particular, considerable overlaps were found between *M. parvipinnis* and *S. fuegensis* ($D=0.803$), *L. blainvillanus* and *S. fuegensis* larvae ($D=0.790$), and *M. parvipinnis* and *L. blainvillanus* ($D=0.696$). These results suggest that resource partitioning was not occurring between larvae collected in Chilean Patagonia (44-46°30'S) during austral winter conditions. Therefore, the main components of the winter larval fish assemblages are feeding on similar prey items and size ranges. It is plausible to consider that the low abundance of larval fishes and the habitat complexity (channels, fjords, inner seas) may reduce the probabilities of competition among species. It is expected that the trophic overlap maintains during spring, because the dominant larval fish fed on copepods (Landaeta *et al.* 2012, 2014, 2015), but the large abundance of prey items potentially preclude food competition. Additionally, some oceanographic structures, such as salinity and tidal fronts, may help to increase the aggregation of prey items in oligotrophic conditions. However, to test this hypothesis, it is necessary to collect plankton samples and environmental conditions at smaller spatial and temporal scales.

ACKNOWLEDGEMENT

We want to thank the field work and cooperation of María Inés Muñoz and Dr. Leonardo Castro, as well as all the crew of the RV Abate Molina. We appreciate the satellite image analysis by Dr. Jaime Letelier (IFOP). This research was funded by Comité Oceanográfico Nacional de Chile (CONA-CHILE), grant CIMAR 18-F 12-07 to MFL, CAB and FB. Comments and suggestions of two anonymous reviewers and Editor Gabriela Muñoz improved an early version of the manuscript.

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Received 1 February 2019 and accepted 8 April 2019

Associated Editor: Gabriela Muñoz C.