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Short Communication

Effects of salinity gradients on larval growth and otolith asymmetry of austral hake *Merluccius australis*

Mauricio F. Landaeta[1,2], Gissella Castillo-Hidalgo[1] & Claudia A. Bustos[1]
[1]Laboratorio de Ictioplancton (LABITI), Escuela de Biología Marina
Facultad de Ciencias del Mar y de Recursos Naturales, Universidad de Valparaíso
Viña del Mar, Chile
[2]Centro de Observación Marino para Estudios de Riesgos del Ambiente Costero (COSTA-R)
Corresponding author: Mauricio F. Landaeta (mauricio.landaeta@uv.cl)

ABSTRACT. Austral hake *Merluccius australis* sustain an important demersal fishery in South Pacific and Atlantic oceans. The main spawning and nursery areas are in the Chilean Patagonia, which is characterized by large inputs of freshwater from rivers and ice melting. The spatial distribution of larval austral hake *M. australis* was studied during spring, and the potential effects of oceanographic conditions (temperature, salinity, vertical stability and turbidity) on early life traits, derived from sagitta otolith microstructure analysis, size-at-age (SAR), individual larval growth (IGR) and bilateral asymmetry (FA6). Larval abundance ranged between 0.86 and 41.55 ind 1000 m^{-3} (median: 4.08 ind 1000 m^{-3}) and population larval growth estimated by micro-increment counts of sagitta otoliths was 0.129 ± 0.010 mm d^{-1}, with age varying from 4 to 24 days old. All three indicators derived from otolith analysis (SAR, IGR, and FA6) showed positive and significant correlations with vertical salinity gradients; IGR and FA6 were also positively correlated to Brunt-Väisälä frequency (vertical stability) and FA6 was correlated to turbidity. Therefore, larval *M. australis* were larger-at-age, grew faster and showed more asymmetry in areas with more influence of freshwater runoff in south Pacific Patagonia.

Keywords: ichthyoplankton, otolith, fluctuating asymmetry, freshwater runoff, Baker River larvae, Chilean Patagonia.

The austral hake *Merluccius australis* (Hutton, 1872) sustain an important fishery in New Zealand, Chile and around Falkland Islands (Horn, 2015). In South America, it inhabits from 40°S (Chiloé Island) in the Pacific Ocean, southward around the southern tip, to the continental shelf north of 49°S and the slope north to 38°S in the Atlantic Ocean (Brickle *et al.*, 2016). The main spawning area is located along the shelf-break and canyons in the northern area of Chilean Patagonia, close to Guafo and Guamblin islands (43-45°S), and spawning occurs in austral winter from July to September (Balbontín & Bravo, 1993). However, the inner seas of southern Chile are also used as secondary areas of spawning (Medina *et al.*, 2014) and larval nursery grounds (Bustos *et al.*, 2007, 2015). This has been recently confirmed by Brickle *et al.* (2016) who found a large range in Sr/Ca ratios in the otolith cores, suggesting complex salinity structuring in the austral hake spawning and/or larval grounds.

The southern Patagonia is characterized by large inputs of freshwater through river discharge and runoff in coastal marine zones, besides marine precipitation, causing salinity and density anomalies that affect the dynamics of the coastal circulation (Dávila *et al.*, 2002). The Baker and Pascua rivers are the main rivers of Chilean Patagonia, with mean annual outflows of 1133 and 753 $m^3 s^{-1}$, respectively (Meerhoff *et al.*, 2015). Additionally, particulate organic matter inputs from the rivers to the fjord ecosystem may partially sustain pelagic food webs during periods of food limitation (Vargas *et al.*, 2011). The salinity field distribution along the Patagonian coast may affect the dispersion (Landaeta & Castro, 2006), feeding habits (Landaeta *et al.*, 2011) and condition (Landaeta *et al.*, 2012; Zenteno *et al.*, 2014) of early life stages of marine fishes.

The analysis of microstructure pattern of sagittal otoliths has proven to be a useful tool in investigating

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different aspects of the early life history of hakes (Palomera *et al.*, 2005; Grote *et al.*, 2012; Betti *et al.*, 2014; Bustos *et al.*, 2015). It can be used to estimate back-calculated hatch dates, growth rates at population and individual levels and developmental instability (Campana, 1990; Díaz-Gil *et al.*, 2015). Therefore, the main goal of this research was to test the effect of freshwater discharge from Baker and Pascua rivers on larval traits of marine fish using austral hake *M. australis*.

Between 10 and 22 October 2014, a bio-oceanographic sampling was carried out along Chilean Patagonia, Southeast Pacific Ocean (Fig. 1). Samples were collected in 40 stations, where environmental data of the water column (temperature, salinity, density) were collected from 1000 m depth or 10 m above the bottom with to the surface a Seabird SBE-19 CTD profiler. Turbidity was measured with a Hach® 2100q portable turbidimeter from water samples taken by Niskin bottles at 0, 10, 30, 50 and 100 m depth. Ichthyoplankton samples were collected from 100 m depth or 10 m above the bottom to the surface through oblique tows with a Bongo net (60 cm diameter, 300 µm mesh size) with a TSK flowmeter mounted in the frame of the net to estimate the volume of the seawater filtered. Towing speed was ~2 knots. The volume filtered in each tow (range = 272.1-1158.2 m^3 tow^{-1}, mean ± SD = 612.8 ± 181.6 m^3 tow^{-1}) was calculated from flowmeter counts. Once over the vessel, the nets were washed and all samples were fixed in 5% formalin buffered with sodium borate. Once in the laboratory, all plankton samples were washed and preserved in 96% ethanol.

In the laboratory, larval hake *Merluccius australis* were identified based on descriptions of Balbontín *et al.* (2004) and Bustos & Landaeta (2005). Larval abundance was standardized to individuals (ind) 1000 m^{-3} using the flowmeter counts for further analysis. All larvae were measured, from the tip of the premaxilla to the tip of the notochord (standard length, SL), to the nearest 0.01 mm under a stereomicroscope Olympus SZ-61 with a video camera Moticam 2500 (5.0 MPixel) connected to a PC with Moticam Image Plus 2.0 software. Later, extraction and measurement of left and right sagittal otoliths were carried out; a detailed description of the methods can be found elsewhere (Bustos *et al.*, 2015). From the 138 larvae, undamaged otoliths of 100 individuals were available, and 88 were readable. The daily periodicity of the micro-increment deposition has not been validated for *M. australis*; nonetheless, it has been validated in postlarval and juvenile *M. productus* and *M. merluccius* (Bailey, 1982; Morales-Nin *et al.*, 2005).

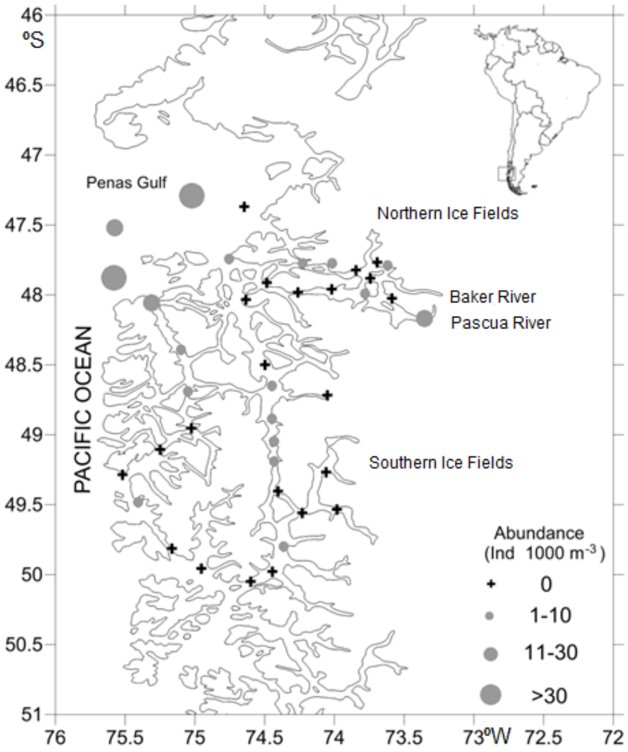

Figure 1. Location of the study site, Chilean Patagonia, abundance and distribution of larval austral hake *Merluccius australis* during October 2014.

Estimations of the age of larval austral hake were compared among those collected over the shelf and those from channels and fjords, using a t-test, because data showed a normal distribution (Shapiro-Wilk test, W = 0.973, $P = 0.063$). A least-squares linear regression model was fitted to the relationship micro-increment counts (MC, days) and standard length (SL, mm), where the intercept corresponds to the larval size-at-hatch and the slope corresponds to the population larval growth rate. Residuals of the regression correspond to individual variability of the size-at-age (size-at-age residuals, SAR). Individual Growth Rates (IGR, mm d^{-1}) were estimated as follows: individual size-at-hatch (SAH) was estimated using the relationship $SL = 1.1183(OR)^{0.3813}$ (Fig. 2a), where OR is the otolith radius at hatch (*i.e.*, hatch mark). Then, IGR was calculated as IGR = [SAC-SAH]/MC, where SAC is size-at-capture (mm, SL) and MC is micro-increment counts (days).

The sagittal otolith area was used as a trait to test fluctuating asymmetry (Díaz-Gil *et al.*, 2015). Because the asymmetry of the trait (*i.e.*, right, R, minus left, L, sagitta area) was positively correlated with the trait size ([R+L]/2). At the individual level (Spearman $rs = 0.426$; $P = 5.72\times10^{-5}$), FA2 (R-L/([R+L]/2)) was used

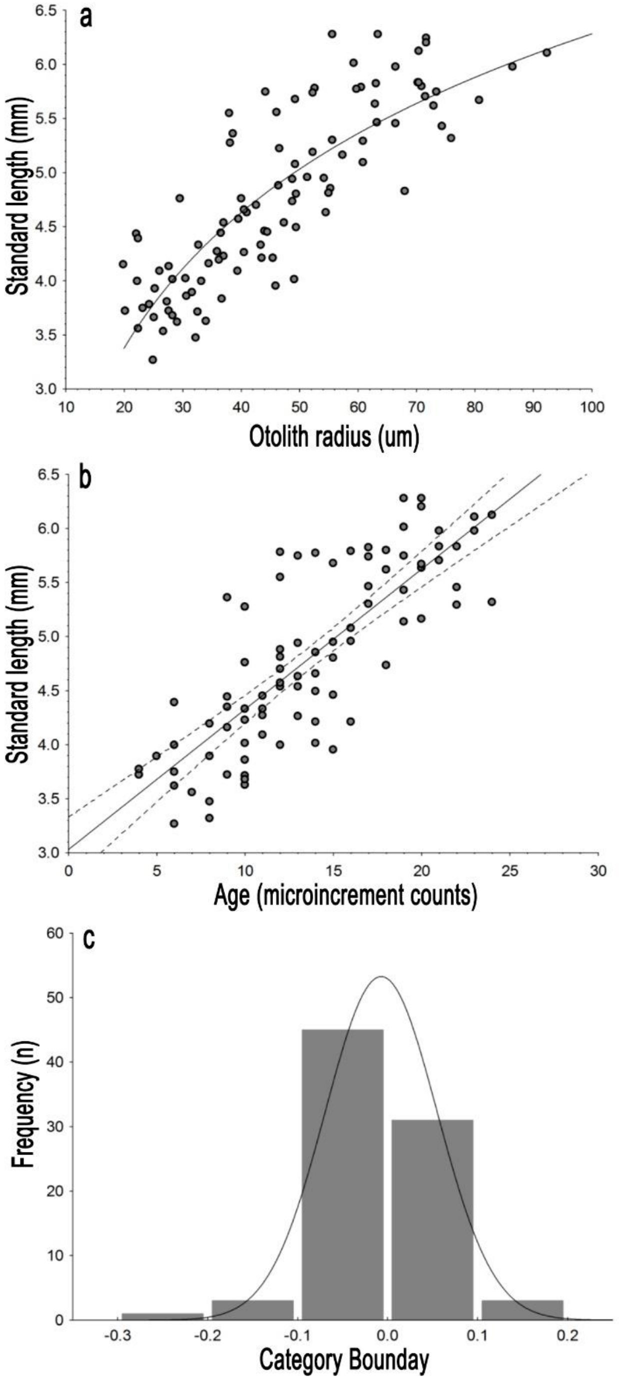

Figure 2. a) Potential relationship between sagitta otolith radius (OR, µm) and larval size (standard length, SL, mm), $SL = 1.1183(OR)^{0.3813}$, n = 100, b) Linear relationship between age (microincrement counts, A, days) and larval size (SL, mm), $SL = 3.031 + 0.1297(A)$, n = 88. Dotted lines correspond to 95% confidence interval; c) frequency of signed asymmetry of left and right sagitta area from larval *Merluccius australis* from south Patagonia.

to test fluctuating asymmetry (normality, skewness and kurtosis), and FA6 ($|R-L|/([R+L]/2)$) was used for further comparisons with the environmental conditions of the water column (Δtemperature, Δsalinity, maximum stability, maximum turbidity) derived from the CTD and turbidity data. Vertical stability was estimated using the Brunt-Väisälä frequency ($N^2 = [g/\rho]*[d\rho/dz]$, where g = gravity, ρ = water density, and z = depth). Spearman correlation rank tests (*rs*) were run to test the independence of variables.

A total of 138 larval *Merluccius australis*, measuring between 2.77 and 6.07 mm SL (mean ± SD, 4.69 ± 0.90) were collected in larger abundance in open waters of Penas Gulf, but also along channels, fjords and zones influenced by the discharge of Baker and Pascua rivers (Chilean Patagonia), during austral spring 2014 (Fig. 1). Larval abundance ranged between 0.86 and 41.55 ind 1000 m^{-3} (mean ± SD, median; 11.97 ± 14.33, 4.08 ind 1000 m^{-3}).

Mean age of larvae collected in the shelf were slightly older (mean = 14.83 days) than those collected in channels and fjords (mean = 12.40 days) (*t*-test, $t = 2.28$, $P = 0.024$). This age difference suggests that: 1) spawning occurred in the shelf and later eggs/larvae were transported to channels and fjords, or 2) spawning occurred in both areas, but sequentially first in the shelf and then in channels. Population larval growth estimated by micro-increment counts of sagitta otoliths was 0.129 ± 0.010 mm d^{-1}, with age varying from 4 to 24 days old (Fig. 2b).

Estimated individual growth rates (IGR) varied from 0.111 to 0.345 mm d^{-1} (0.181 ± 0.049 mm d^{-1}). IGR was slightly significantly faster for those individuals collected in fjords and channels (median = 0.128 mm d^{-1}) compared to those collected in shelf waters (median = 0.126 mm d^{-1}, Mann-Whitney test, U = 1737, $P = 0.012$). The fluctuating asymmetry (FA2) showed normal distribution (Kolmogorov-Smirnov test, KS = 0.091, $P = 0.20$, Fig. 2c), scarce positive skewness (0.337 ± 0.264) and platykurtic (kurtosis = 1.697 ± 0.522). FA2 of otolith area from larval hake collected in fjords and channels showed larger variance (*i.e.*, more asymmetry) than those otoliths from larvae collected in shelf waters (*F* test, $F = 2.03$, $P = 0.024$). IGR and FA6 were not correlated (Spearman *rs* = 0.222; $P = 0.119$).

When all three indicators derived from otolith analysis (SAR, IGR, and FA6) were compared with environmental conditions of the water column (Table 1), all showed positive and significant correlations with vertical salinity gradients (Fig. 3, Table 2). IGR and FA6 were also positively correlated with the vertical stability (B-V frequency, Fig. 3, Table 2). Finally, FA6 was also positively correlated with turbidity (Table 2).

Therefore, in areas with large freshwater input from the Baker and Pascua rivers (Fig. 1), larval hakes were

Table 1. Summary of water column (first 100 m depth) environmental conditions, during austral spring 2014 in the study area (south Chilean Patagonia). SD: one standard deviation. *P* values correspond to results of Mann-Whitney U tests.

	Shelf		Channels and fjords		
	Mean	SD	Mean	SD	*P*
Temperature (°C)	9.405	0.508	9.068	0.673	5.54×10-35
Salinity	32.953	0.825	31.538	2.927	5.31×10-59
Stability (cycles s^{-1})	1.44×10^{-4}	2.45×10^{-4}	6.62×10^{-4}	3.49×10^{-3}	7.86×10-31
Turbidity (NTU)	0.777	0.754	0.967	2.117	0.238

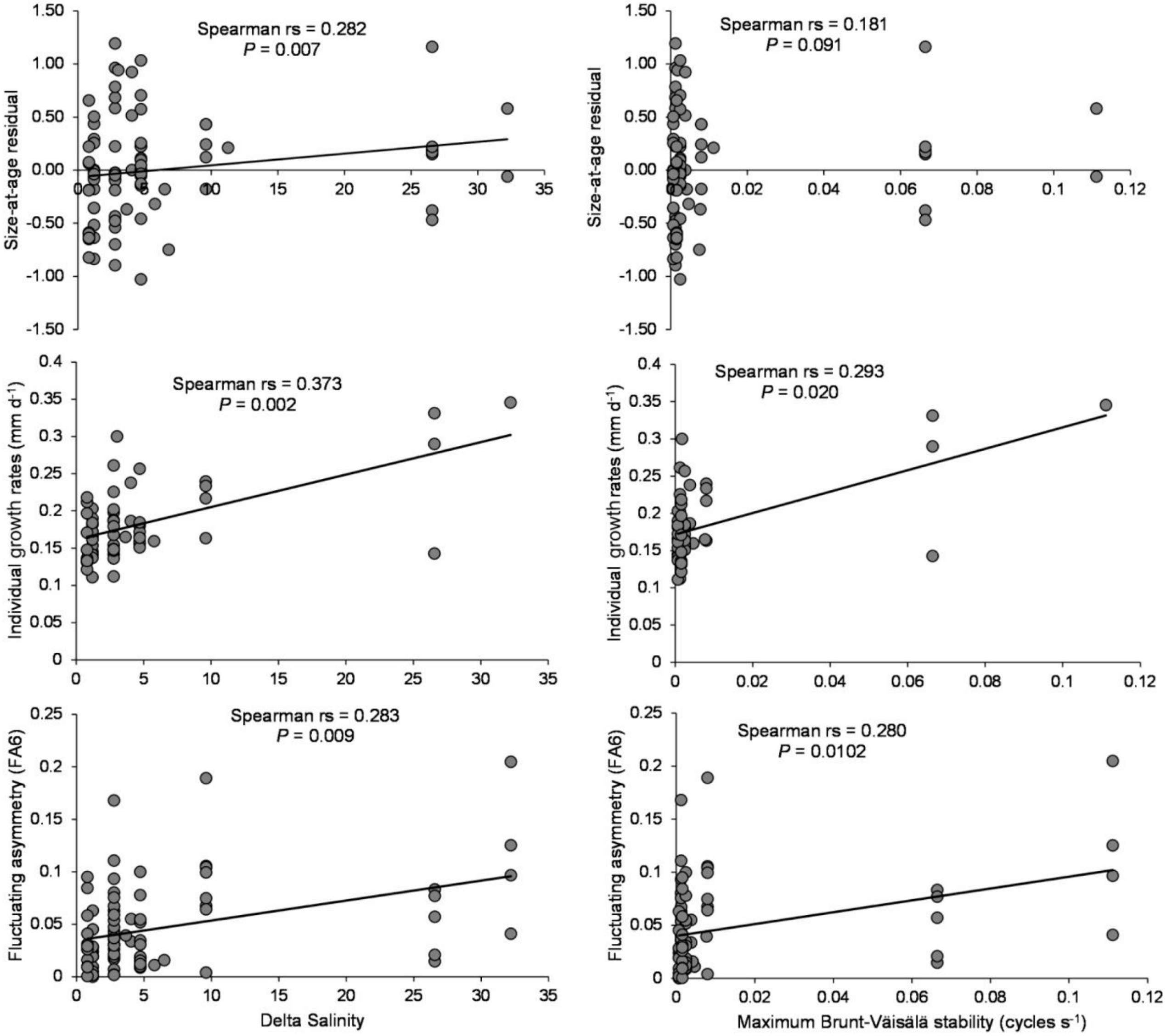

Figure 3. Relationships between size-at-age residuals (from the linear growth model), individual growth rates and otolith fluctuating asymmetry with vertical gradients of salinity (Δsalinity) and stability (Brunt-Väisälä frequency).

less abundant, but larger-at-age showed faster growth rates and were more asymmetrical than those individuals collected in the open sea and along channels of Chilean Patagonia. This bio-physical interaction seems to be species-specific; freshwater runoff in fjords may reduce recent growth rates in larval stages of rockfish *Sebastes oculatus* and lightfish *Maurolicus parvipinnis*, but it has no effect in clupeid larvae *Sprattus fuegensis* (Landaeta & Castro, 2006; Landaeta *et al.*, 2012).

Why does freshwater input appear to benefit larval growth rate in austral hake from this region? Large

Table 2. Spearman correlation rank tests (rs) between size-at-age residuals (SAR), individual growth rates (IGR), and fluctuating asymmetry (FA6) and environmental conditions of the water column. Bold numbers indicate significant correlations ($P < 0.05$)

	SAR	IGR	FA6
Delta temperature	0.139	0.187	0.103
Delta salinity	**0.287**	**0.373**	**0.283**
Maximum stability	0.181	**0.294**	**0.280**
Maximum turbidity	0.032	0.072	**0.222**

water stability in fjords ecosystems caused by freshwater discharges could have a potential benefit for larval hake in terms of predator-prey encounter rates and a subsequent increase of feeding success (large ingested prey volume, Landaeta *et al.*, 2011). Additionally, hake larvae have a wide visual acuity from its early stages (Morote *et al.*, 2011), aiming them to successfully capture prey at low light intensities in fjords and channels. Near the Baker River mouth, net flux estimates showed that small meroplankton, such as barnacle nauplii are advected landward (Meerhoff *et al.*, 2015), indicating that suitable prey for small hake larvae is more abundant inside fjords and channels than over the shelf.

Channels and fjords had sea water colder than shelf waters (Table 1). The lack of significance of the relationship between IGR of austral hake and sea temperature has been previously described for larval hake elsewhere (Cass-Calay, 1997; Brown *et al.*, 2004; Alvarez & Cotano, 2005). Timescale changes for growth rate in fish larvae and temperature in the water column are different. For larval hake during spring 2014, growth seemed to respond more to food availability than temperature variations.

Recently, the use of otolith fluctuating asymmetry (OFA) to assess fitness has been questioned (Díaz-Gil *et al.*, 2015). Similarly, OFA was unsuccessful to find any significant difference between fast-growing and slow-growing larval Atlantic menhaden *Brevoortia tyranus* (Fey & Hare, 2008), or in larval *M. parvipinnis* inhabiting fjords and channels of southern Chile (Zenteno *et al.*, 2014). Nonetheless, the level of the stress factors, in the present case, low salinity (as fresh as 1.15) and high turbidity (from 0 to 15 NTU) must be significant to affect OFA, and these stressors may affect differentially to early life stages of epipelagic or benthic species (Grønkjær & Sand, 2003; Fey & Hare, 2008). Austral hake larvae can be found in the first 100 m depth of the water column, with largest abundances from the surface to 25 m depth (Castro *et al.*, 2011), and therefore it is plausible that larvae experienced fresher and turbid waters.

Present results confirm recently published evidence based on trace element signatures of otolith cores of austral hake collected in Chilean and Falkland Islands' waters (Brickle *et al.*, 2016). These authors suggest that there is only one spawning stock of austral hake with spawning situated in and around the Chilean fjords, and the variation in Sr/Ca ratios likely suggest that survivors were moving across salinity gradients during early ontogeny, *i.e.*, they used nursery areas with different salinities (open areas, channels, fjords) prior to the migration to their feeding grounds in southern South Atlantic (Arkhipkin *et al.*, 2003).

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