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Spatial patterns of morphological variability of the eelpout Austrolycus depressiceps (Teleostei: Zoarcidae) across western Patagonia

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Corresponding Author:	Mauricio F. Landaeta, Dr. Universidad de Valparaiso Viña del Mar, Valpararaiso CHILE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universidad de Valparaiso
Corresponding Author's Secondary Institution:	
First Author:	Fernanda S. Orrego, BSc
First Author Secondary Information:	
Order of Authors:	Fernanda S. Orrego, BSc
	Mathias Hüne, MSc.
	Hugo A. Benítez, PhD
	Mauricio F. Landaeta, Dr.
Order of Authors Secondary Information:	
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Abstract:	Phenotypic variation in organisms depends on the genotype and the environmental constraints of the habitat that they exploit. Therefore, for marine species inhabiting contrasting aquatic conditions, it is expected to find covariation between the morphospace and hydrography. We studied the phenotypic variability of the head and cephalic sensorial canals of the eelpout Austrolycus depressiceps (4.5-22.5 cm TL) across its latitudinal distribution in western Patagonia (45°S-55°S). Geometric morphometric analyses show that morphospace varied from individuals with larger snout and an extended suborbital canal to individuals with shorter snouts and frontally compressed suborbital canal. The ontogenetic allometry was low, with a decrease of the relative size of the eye. Individuals from the northernmost locations showed robust heads while those from the south had slender head, with a pointed snout. Seawater salinity covaried with shape of the head; in brackish waters, eelpouts had slender head and snout, smaller eyes, and horizontally oriented mouth, while in saltier waters individuals had robust head, shorter snout, bigger eyes and oblique mouth. The morphospace of marine fish with shallow distribution across Patagonia are influenced by salinity (i.e., phenotypic modulation), and may be sensitive to the ice melting of Northern and Southern Icefields caused by global warming.
Suggested Reviewers:	Alberto T. Correia, PhD Researcher, Universidade Fernando Pessoa acorreia@ufp.edu.pt Dr. Correia has vast experience in the utilization of geometric morphometrics in describing fish phenotypic variability
	Brian Sidlauskas, PhD Researcher, Oregon Agricultural College: Oregon State University

	brian.sidlauskas@oregonstate.edu Dr. Sidlauskas has vast experience in fish morphology and ecological and evolutionary variation
	Dominique Ponton, PhD Professor, Universite de Toliara dominique.ponton@ird.fr Dr. Ponton has wide experience in the use of geometric morphometrics in marine fish morphological variation

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Spatial patterns of morphological variability of the eelpout *Austrolycus depressiceps* (Teleostei: Zoarcidae) across western Patagonia

Fernanda S. Orrego[1], Mathias Hüne[2], Hugo A. Benítez[3], Mauricio F. Landaeta[1,4,5*]

[1]Laboratorio de Ictioplancton (LABITI), Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile

[2]Centro de Investigación para la Conservación de los Ecosistemas Australes (ICEA), Punta Arenas, Chile

[3]Laboratorio de Ecología y Morfometría Evolutiva, Centro de Investigación de Estudios Avanzados del Maule, Universidad Católica del Maule, Talca, Chile

[4]Centro de Observación Marino para Estudios de Riesgos del Ambiente Costero (COSTA-R), Universidad de Valparaíso, Viña del Mar, Chile

[5]Millennium Nucleus for Ecology and Conservation of Temperate Mesophotic Reef Ecosystems (NUTME)

*Corresponding author. Email: mauricio.landaeta@uv.cl

Mauricio F. Landaeta ORCID-ID: 0000-0002-5199-5103; Mathias Hüne ORCID-ID: 0000-0003-0089-7623; Hugo Benítez ORCID-ID: 0000-0001-7553-9893

Short title: Phenotypic variation in zoarcid *Austrolycus depressiceps*

Abstract

Phenotypic variation in organisms depends on the genotype and the environmental constraints of the habitat that they exploit. Therefore, for marine species inhabiting contrasting aquatic conditions, it is expected to find covariation between the morphospace and hydrography. We studied the phenotypic variability of the head and cephalic sensorial canals of the eelpout *Austrolycus depressiceps* (4.5-22.5 cm TL) across its latitudinal distribution in western Patagonia (45°S-55°S). Geometric morphometric analyses show that morphospace varied from individuals with larger snout and an extended suborbital canal to individuals with shorter snouts and frontally compressed suborbital canal. The ontogenetic allometry was low, represented by a decrease of the relative size of the eye, and a depression of the posterior margin of the head. Individuals from the northernmost locations showed robust heads while those from the southern tip of South America had a slender head, with a pointed snout. Seawater salinity covaried with shape of the head; in brackish waters, eelpouts had a slender head and snout, smaller eyes, and horizontally-oriented mouth, while in saltier, oceanic-influenced waters, individuals had robust head, shorter snout, bigger eyes and oblique mouth opening. Two of the canals of the cephalic lateralis pores and the head shape showed modularity in its development. This study shows that the morphospace of marine fish with shallow distribution across Patagonia are influenced by salinity (i.e., phenotypic modulation), and may be sensitive to the ice melting of Northern and Southern Icefields caused by global warming.

Keywords: latitudinal gradient; geometric morphometrics; morph; freshwater input; phenotype

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Conflict of interest

The authors have no financial or proprietary interests in any material discussed in this article

Availability of data and material

Data will be available upon reasonable request.

Code availability

Not applicable.

Author's contribution

MH collected the fish, measured it and took the photographs, **FSO** digitized the landmarks and performed data analysis, **HAB** performed modularity data analysis, **MFL** wrote the first version of the manuscript; the whole team wrote the final version of the manuscript.

Introduction

Phenotypic plasticity is one of the mechanisms for explaining why populations or species may differ phenotypically, in which a single genotype expresses different phenotypes in response to different environmental signals (Sultan 2011). Two types of phenotypic plasticity can be defined, developmental conversion, and phenotypic modulation. The first one results in discrete morphs in response to an environmental cue. In contrast, phenotypic modulations result in continuous variation of a particular trait in response to an environmental stimulus (Stearns 1989). Thus, at large distributional scales, trait variation patterns can provide insights into micro- and macroevolutionary patterns, indicating selective agents responsible for major trends for phenotypic evolution and local adaptation (Riesch et al. 2018). Latitudinal variation in body size (the Bergmann's rule) has been linked to fitness, with larger body size in colder environments, i.e., towards higher latitudes (Ouyang et al. 2018).

The extensive Chilean Patagonia (41ºS-56ºS) is characterized by high degree of geomorphological and hydrographic complexity, with innumerable channels and fjords, which have been molded by ice expansion and contraction during the Quaternary glacial period (Hulton et al. 2002; Sudgen et al. 2002). These generate highly heterogeneous marine habitats that are heavily influenced by freshwater discharges from precipitation and glacier melting (Dávila et al. 2002), which give this region high environmental variability. Two large temperate icefields, the Northern Patagonian icefield and Southern Patagonian icefield, represent 83% of the total ice loss in the southern Andes, and are responsible for a total of 3.3 mm of sea-level rise between 1961 and 2006 (Bravo et al. 2021). Additionally, and because of the latitudinal extension of Patagonia, important sea surface temperature gradients also occur, varying from 4 to 18 °C (Bruno et al. 2018, Molina-Valdivia et al. 2021). This environmental variability as well as coastal geometry may impact on the body shape variability in terrestrial (i.e., rodents, Valladares-Gómez et al. 2020) and marine species with shallow vertical distribution, such as some endemic eelpouts.

Eelpouts of the family Zoarcidae are a group of 47 genera and about 240 valid species of marine perciform fishes. Twenty-one of these fish species have been recorded in the Chilean

Patagonia (Hüne 2019). They are the most diverse family of the suborder Zoarcoidei and exhibit a greater degree of character plasticity than the other families (Anderson and Fedorov 2004). It is considered as monophyletic and has four subfamilies, Lycozoarcinae, Zoarcinae, Gymnelinae and Lycodinae. These fish can be found over a wide range of depths: most zoarcids are deep-water benthic species, but some southern hemisphere species inhabit intertidal areas (Gosztonyi 1977). Zoarcids have elongated, eel-like bodies and as a group show modest diversification in form (Lannoo and Eastman 2006).

Vision in zoarcids is reduced, and specimens rely on olfaction and taste to detect prey (Fanta et al. 2001, Lannoo and Eastman 2006), based on the peripheral lateral-line system. This system is composed of canal neuromasts (CNs) occurring in canals on fishes' heads and trunks. CNs function as pressure gradient detectors, i.e., they respond to pressure differences between neighbouring canal pores (Mogdans 2019), and are distributed into supra and infraorbital, the otic and postotic and the mandibular and preopercular canals (Anderson 1994, Mogdans 2019). It is unknown whether morphological integration occurs between sensory canals and head shape during ontogeny of Zoarcidae. In this sense, morphological integration describes the correlation among traits and occurs when changes in one trait are accompanied by changes in other traits that are affected by common mechanisms such as developmental pathways, genetic linkages and functional activities (Adams 2016).

The genus *Austrolycus* belongs to Lycodinae, and it is composed of two species, *A. depressiceps* Regan, 1913 and *A. laticinctus* (Berg, 1895). The genus is characterized by the absence of pyloric caeca, 6-7 suborbital pores, and a single postorbital pore above the gill slit (Anderson 1994). The latter species have more caudal vertebrae (80-89) than the first (72-79) (Anderson and Gostonyi 1991). *Austrolycus depressiceps* is a medium-size zoarcid (4-41 cm length), living among macroalgae, below rocks and stones, in the subantarctic waters of South America, from the intertidal down to 12 m depth (Moreno and Jara 1984, Hüne et al. 2021). They are sedentary and sluggish (Vanella and Calvo 2005). Females deposit egg masses below stones in the intertidal zones. Eggs are nearly spherical and large (9.2-9-8 mm), as well as the size of the recently hatched larvae (22 to 25 mm, Matallanas et al. 1990). Juveniles feed on small crustaceans (isopods, amphipods, polychaetes), while adults are ichthyophagous, predating on *Harpagifer bispinis* and *Patagonotothen cornucola* (Matallanas 1988; Lloris and Rucabado 1991). This species has a wide geographic range throughout the west Patagonia, from Aysen (47°S) to Diego Ramírez Island (56°30'S), and off Argentina and Malvinas/Falkland Islands (Pequeño 1986, Reyes and Hüne 2012).

Based on the large spatial distribution of *A. depressiceps* along the western Patagonia, we expect to find latitudinal gradients of the shape of the head and the relative location of the sensory canals, expressed as well as in changes in body mass condition. Our goal is to describe the variability of the morphospace of the head and some cephalic lateralis canals located in the head, at ontogenetic and large spatial scales (hundreds of kilometres), and the integration/modulation of the head and its canals throughout the life history of the eelpout.

Materials and Methods

Field collection and measurement

Adult fish (n = 126) were collected between 2007 and 2020 at 11 intertidal locations across the western coast of Patagonia, covering most of the spatial distribution of the species (Fig. 1). Sea temperature and salinity were obtained at each sampling site with multiparameter sondes (YSI 556 and YSI 6920 v2) (Table 1). All fish were captured by hand and with hand nets during diurnal low tide periods (Fig. 1 A-B). Specimens were preserved in 96% ethanol for subsequent examination. All specimens were measured for total length (TL, cm) and total weight (g) and photographed from a lateral view using a digital SLR camera (Nikon D850) fitted with a microlens (AF-S Nikkor 60 mm). The species is sexually monomorphic, so we did not distinguish between males and females in the following analyses.

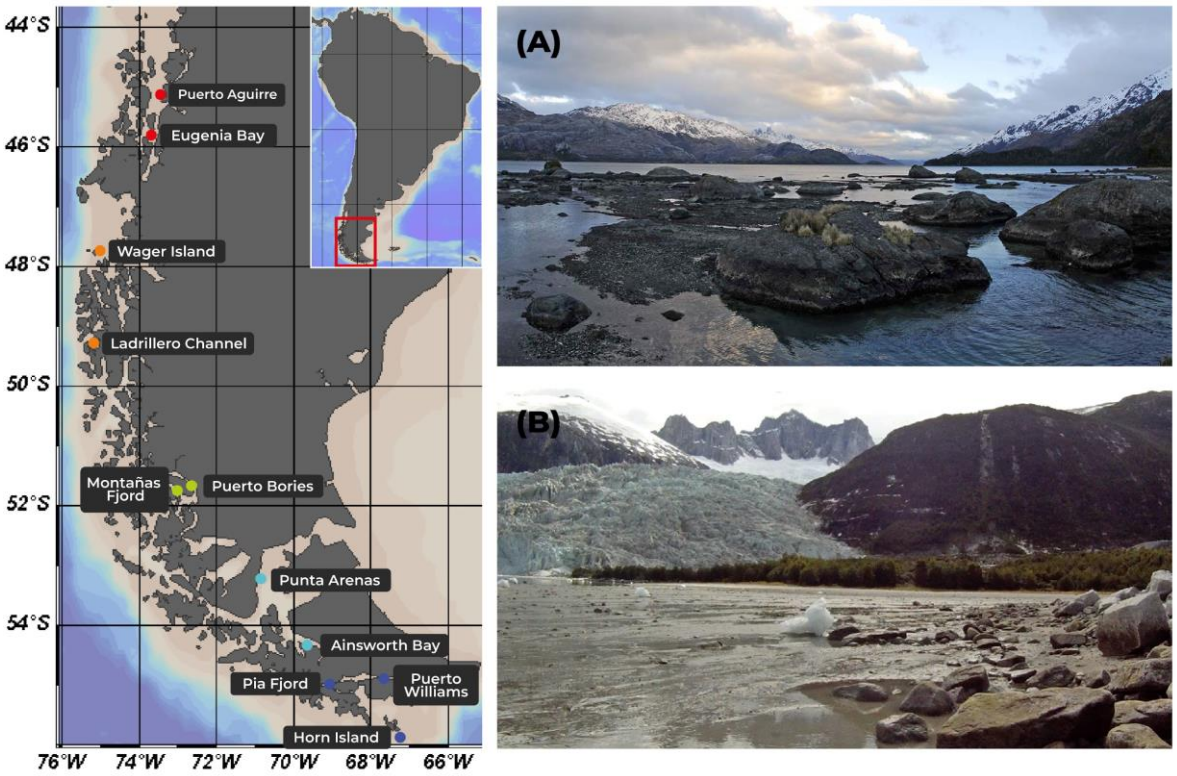


Figure 1. Sampling locations of *Austrolycus depressiceps* along the western coast of Patagonia. Photos of some intertidal locations: (A) Montañas Fjord, and (B) Pia Fjord.

Data analysis

Length-weight relationship was fitted by a power model: $Y = aX^b$, where Y = weight, and X = total length. The Fulton index of condition, $K = 1000 \times (M / L^3)$, where M is the mass and L is the length, was estimated separately for each specimen. Additionally, the Relative condition (K_n) was computed as the observed individual mass (M_i) divided by the predicted mass (M_i*, where $M_i^* = aL_i^b$, and a and b are the parameters of the length-weight relationship).

Locations were grouped into zones in order to represent a latitudinal gradient (Fig. 1, Table 1): North of Taitao Peninsula (NTP, Puerto Aguirre, Eugenia Bay), Central Channels (CCh, Wager Island, Ladrillero Channel), Central Fjords (CF, Montañas Fjord, Puerto Bories), Magellan Strait (MS, Punta Arenas, Ainsworth Bay), and Beagle Channel (BC, Pia Fjord, Puerto Williams, Horn Island). Comparison of K and K_n were made among zones using one-way ANOVA and Tukey's post hoc tests, because data showed normal distribution (Shapiro-Wilk´s test, W = 0.98, $P > 0.085$).

155 Table 1. Locations and sampling dates where the eelpout *Austrolycus depressiceps* were collected
156 across the Chilean Patagonia, as well as the *in situ* hydrographic conditions.

Zones	Location	Sampling date	Latitude	Longitude	Temperature (°C)	Salinity (PSU)
NTP	Puerto Aguirre	14-01-2017	-45.159	-73.531	12.61-12.63	27.33-27.32
NTP	Eugenia Bay	30-10-2007	-45.948	-73.569	11.51	26.1
CCh	Wager island	02-02-2016	-47.753	-74.999	11.83-11.94	30.15-30.22
CCh	Ladrillero Channel	20-03-2010	-49.178	-75.397	11.03-11.64	31.20-31.60
CF	Montañas Fjord	04-04-2009	-51.778	-73.330	7.2	16.45
CF	Puerto Bories	10-03-2008	-51.690	-72.542	8.23-9.93	14.11-14.51
MS	Punta Arenas	03-03-2009	-53.307	-70.934	7.80-7.87	30.19-30.62
MS	Ainsworth Bay	10-01-2020	-54.406	-69.621	7.13	28.42
BC	Pia Fjord	18-12-2011	-54.793	-69.600	6.55	14.1
BC	Puerto Williams	10-12-2008	-54.931	-67.577	7.75-7.86	28.62-28.70
BC	Horn Island	15-02-2017	-55.959	-67.230	9.16	33.51

157

158 For the geometric morphometric analyses, a database with images of 126 specimens was
159 created using the program TPSUtil 1.60. A 20-landmark configuration (Fig. 2) was then digitized
160 onto each photograph and a replica of each image using the software TPSDig 2.17. This landmark
161 configuration aimed to reflect the shape of the mandible apparatus (premaxilla, maxilla, dentary, LM
162 1-4), eye (LM 16-19), head (LM 1 and 20), as well as the location of the nostril (LM 9) and the
163 arrangement of some cephalic lateralis canals, such as the suborbital canal (LM 10-15), the
164 preopercular portion of the preoperculo-mandibular canal (LM 5-7) and the postorbital pore 4 (LM
165 8). Shape information was extracted from the landmark coordinates with a Generalized Procrustes
166 analysis (GPA) using MorphoJ 1.06d (Klingenberg 2011), in which coordinates were scaled,
167 translated and rotated onto a common coordinate system to extract the shape variation from each
168 individual and remove size and rotation influences (Rohlf and Slice 1990). Residuals from the
169 consensus configuration were modeled with the thin-plate spline (Bookstein 1992). To assess the
170 measurement error, a Procrustes analysis of variance (Procrustes ANOVA) was carried out using
171 MorphoJ software.

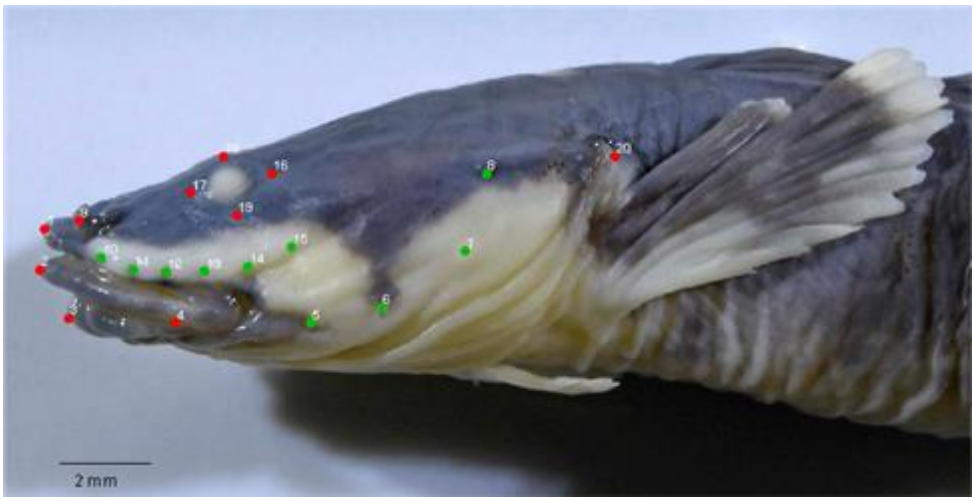


Figure 2. Eelpout *Austrolycus depressiceps* from west Patagonia, showing the distribution of landmarks on the head (red dots) and on selected sensory canals (green dots). 1. Mesethmoid; 2. Anterior margin of the premaxilla; 3. Anterior margin of the dentary; 4. Posterior margin of the dentary; 5-7. preoperculo-mandibular pores; 8. postorbital pore; 9. nostril; 10-15. Suborbital pores; 16. Anterior margin of the eye; 17. Posterior margin of the eye; 18. Lower margin of the eye; 19. Upper margin of the eye; 20. Posteriormost margin of the operculum.

A Principal component analysis (PCA) was performed using the shape coordinates to identify the main axes of shape variation, as well as specific changes in the head and pores. For the visualization, a warping transformation grid was used following Klingenberg (2013). To quantify the ontogenetic effect in the shape change (ontogenetic allometry), a multiple regression between Procrustes coordinates and log centroid size (a proxy of size) was done (Loy et al. 1998). Residuals of the ontogenetic allometry relationship were used for comparisons of the morphospace among zones, by a Canonical Variate Analysis (CVA) and Bonferroni-corrected multiple comparisons using the Procrustes distances and permutation tests.

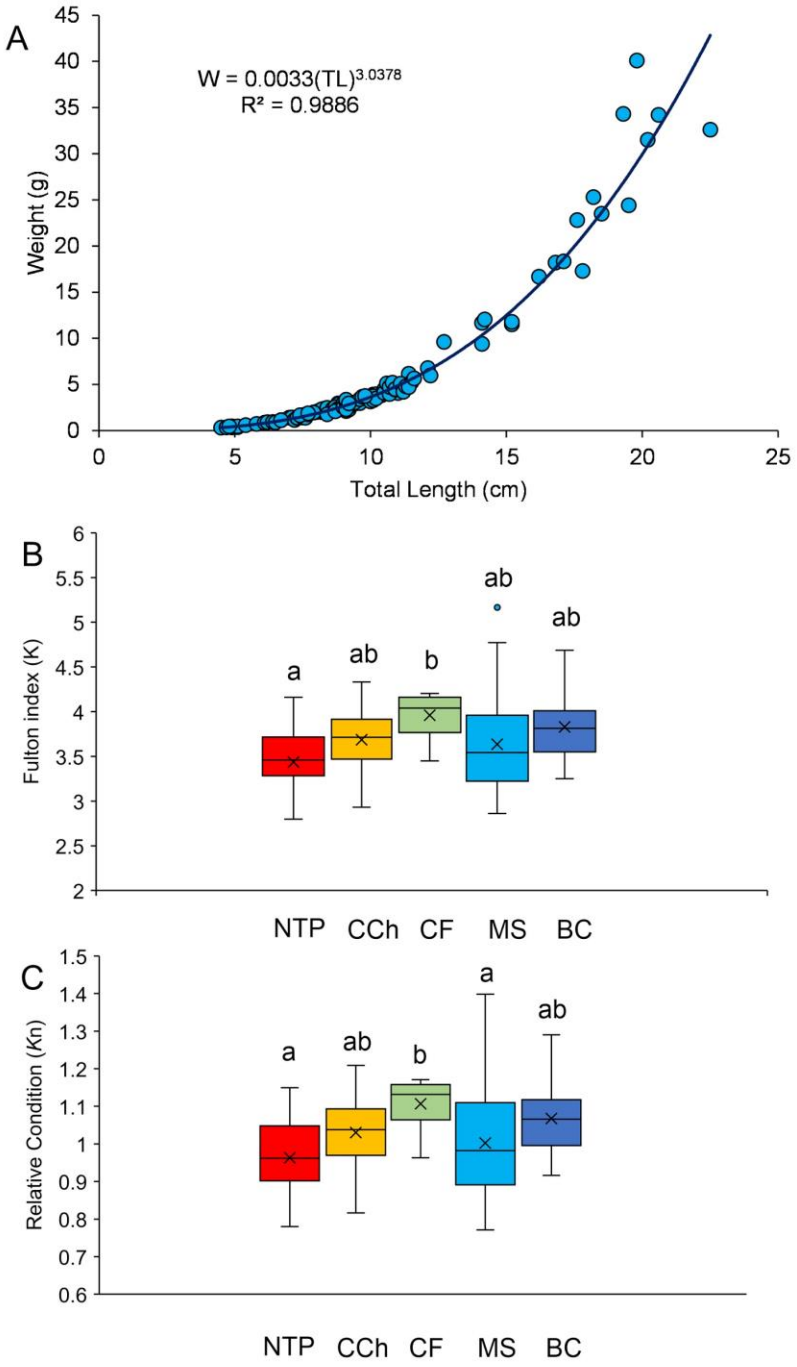
Additionally, a two-block, partial least squares (PLS) between condition indices (*K* and *Kn*), sea temperature and salinity (Block 1) and Procrustes coordinates (Block 2), were run, for testing for covariation between shape, body mass condition of the eelpout and sea water conditions across western Patagonia. PLS is a method for extracting the eigenvectors and eigenvalues from two blocks of covarying data. Also, for the estimation of the strength association between blocks, the Escoufier's RV coefficient and a permutation test were estimated (Goswami and Polly 2010, Klingenberg 2009).

Finally, two modularity hypotheses were tested between the head (red landmarks in Fig. 2) and sensory canals (green landmarks in Fig. 2), a *functional hypothesis* (H1) and a *structural hypothesis* (H2). H1 assumes that the sensory canals and the anterior morphology (neurocranium, oral jaw apparatus) have different, but complementary functions (i.e., prey detection and capture, respectively), especially under low-light conditions (Mogdens 2019), such as the turbid, coastal waters of west Patagonia (>4 NTU, Castillo-Hidalgo et al. 2018). By contrast, H2 is a hypothesis of developmental modularity derived from the observation that zoarcid have different rates of ossification during larval development (Voskoboinikova and Laius 2003), and the opercular region (where the preoperculo-mandibular canal is located) increases its relative size during early development (Marcinkevicius and Gosztonyi 2013). Integration between modules will be measured using the covariance ratio test (CR), which can be defined as the ratio of the overall covariation between modules relative to the overall covariation within modules (Adams 2016). All statistical analyses were performed using MorphoJ 1.06d and *geomorph 4.0* R package (Adams & Otárola-Castillo 2013), and the new package *gmShiny* using the new modules of geomorph (Baken et al. 2021).

Results

Size distribution, length-weight relationships and condition

Specimens collected varied between 4.5 and 22.5 cm TL (mean ± SD, 9.94 ± 3.69 cm) and from 0.316 to 40.100 g (5.43 ± 7.75 g). The length-weight relationship showed a positive allometry (b = 3.03), and a larger dispersion in bigger specimens (between 13-23 cm TL, Fig. 3A). The analysis of K indicated that those specimens collected north of Taitao Peninsula (NTP) were significantly in poor condition than those from Central Fjords (CF; one-way ANOVA, F = 7.24, $P < 0.0001$; Tukey test, $P = 0.002$) (Fig. 3B). Similarly, Kn varied significantly (one-way ANOVA, F = 7.55, $P = 0.001$) among zones, with specimens from CF being in better condition than those from NTP and MS (Fig. 3C).



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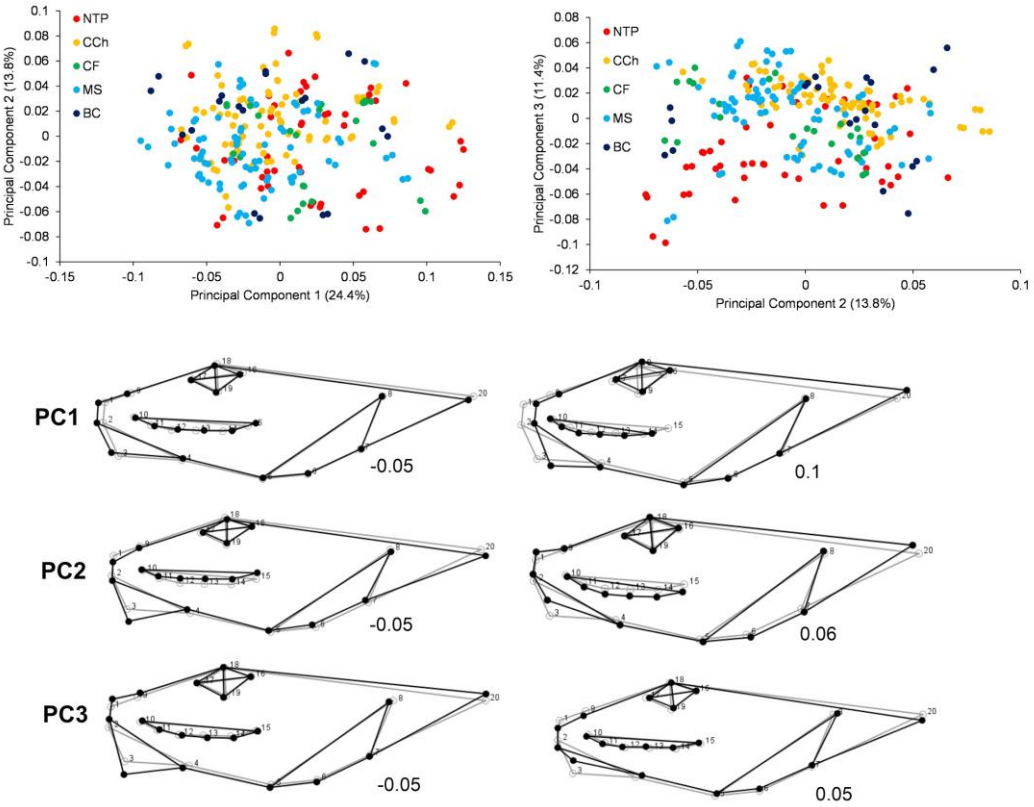
221 Figure 3. A. Length-weight relationship of the eelpout *Austrolycus depressiceps* from western
222 Patagonia; B. Comparison of the Fulton's index of condition (*K*); C. Comparison of the Relative
223 Condition (*K*n) among sites, North of Taitao Peninsula (NTP), Central Channels (CCh), Central
224 Fjords (CF), Magellan Strait (MS) and Beagle Channel (BC). Different letters indicate significant
225 differences ($P < 0.05$).

226

227 Shape changes and ontogenetic allometry

228 Procrustes ANOVA estimated that the measurement error (i.e., landmark digitalization)
229 explained 3.09% of the total variance ($F = 31.29$, $P < 0.0001$). The variability of the morphospace of
230 adult *A. depressiceps* was explored using PCA. The PCA was explained by 36 principal components,
231 from which the first 14 explained 90% of the total variance.



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233 Figure 4. Shape changes of the head and sensory canals of the eelpout *Austrolucys depressiceps* from
234 west Patagonia. NTP: North of Taitao Peninsula, CCh: Central Channels, CF: Central Fjords, MS:
235 Magellan Strait, BC: Beagle Channel. Grey wireframes represent the consensus morph (0,0), while
236 the black wireframes represent the final morph. The number under each wireframe represents the
237 scale along each principal component.

238 PC1 (24.49%) was explained mainly by the horizontal displacement of the suborbital pores
239 4, 5 and 6 (LM 14-16), and the horizontal displacement of the anterior margin of the dentary (LM 3),
240 mesethmoid (LM 1) and premaxilla (LM 2) (Table 2). These relative displacements indicate the
241 presence of specimens with larger snout and an extended suborbital canal (i.e., suborbital pores 5 and
242 6 located behind the posteriormost margin of the eye) *vs.* specimens with shorter snouts and frontally
243 compressed suborbital canal (i.e., suborbital pores 5 and 6 posteriorly extended to the level of the

middle of the eye), respectively (PC1 Fig. 4). The PC2 explained 13.8% of the shape variance and represented the vertical displacement of the anterior margin of the dentary (LM 3), and the mesethmoid (LM 1) and oblique displacement of the operculum (LM 20). This means a shape variability from individuals with pointed snout, closed mouth and smaller head to specimens with rounded snout, open mouth and larger head (PC2 Fig. 4, Table 2). Finally, the PC3 explained 11.4% of the variance, and represented the opposite of the PC2, specimens with pointed snout, open mouth and shorter head *vs.* specimens with rounded snout, closed mouth and larger head (Fig. 4, Table 2). Because the position of the dentary (closed *vs.* opened mouth) depends on the *post-mortem* conditions rather than the morphological features of the individual, both PCs represent specimens with pointed snout and shorter head and those with rounded snout and larger head.

Table 2. Landmark configuration in the head of the eelpoul *Austrolycus depressiceps*. Bold numbers indicate those landmarks showing the most noticeable changes at each Principal Component (PC).

Morphological trait	LM	PC1	PC2	PC3
Mesethmoid	x1	0.282	0.026	-0.076
	y1	0.016	**0.281**	**-0.342**
Anterior margin of the premaxilla	x2	0.276	-0.026	-0.011
	y2	0.029	0.228	**-0.343**
Anterior margin of the dentary	x3	**0.302**	-0.077	0.020
	y3	-0.149	**0.554**	**0.572**
Posterior margin of the dentary	x4	-0.033	0.148	**0.282**
	y4	-0.085	-0.173	0.093
Preoperculo-mandibular pore 5	x5	0.002	0.114	0.063
	y5	-0.057	-0.074	0.115
Preoperculo-mandibular pore 6	x6	-0.019	0.154	0.006
	y6	-0.027	-0.099	0.100
Preoperculo-mandibular pore 7	x7	-0.053	0.118	0.035
	y7	-0.025	-0.108	0.072
Postorbital pore 4	x8	0.026	-0.024	0.180
	y8	-0.005	0.092	0.046
Nostril	x9	0.189	0.042	-0.099
	y9	0.025	0.065	-0.201
Suborbital pore 1	x10	-0.058	0.044	-0.085
	y10	0.020	-0.042	-0.055
Suborbital pore 2	x11	-0.173	0.033	-0.098
	y11	0.025	-0.110	-0.031
Suborbital pore 3	x12	-0.270	0.001	-0.143

	y12	-0.014	-0.203	0.021
Suborbital pore 4	x13	**-0.351**	-0.029	-0.168
	y13	-0.023	-0.229	0.087
Suborbital pore 5	x14	**-0.405**	-0.050	-0.132
	y14	-0.032	-0.243	0.064
Suborbital pore 6	x15	**-0.355**	-0.056	-0.075
	y15	-0.115	-0.274	0.025
Anterior margin of the eye	x16	0.141	-0.027	0.026
	y16	0.086	0.032	0.010
Posterior margin of the eye	x17	0.078	-0.086	0.143
	y17	0.037	-0.005	-0.014
Inferior margin of the eye	x18	0.108	-0.081	0.057
	y18	0.080	0.096	-0.012
Superior margin of the eye	x19	0.094	-0.014	0.103
	y19	0.041	-0.076	0.073
Posteriormost margin of the operculum	x20	0.220	-0.211	-0.027
	y20	0.173	**0.289**	**-0.280**

The multiple regression between shape (Procrustes) coordinates and log centroid size was significant ($P < 0.001$) and 4.71% of the total variance was explained by the ontogenetic allometry (Fig. 5A). The main changes through ontogeny were the decrease of the relative size of the eye, and a deepening of the upper margin of the operculum, which may be representing a depression of the posterior margin of the head (Fig. 5B). A slight elevation of the suborbital pores 5 and 6 (LM 14 and 15, respectively) and a frontal displacement of the nostril (LM 9) were also observed.

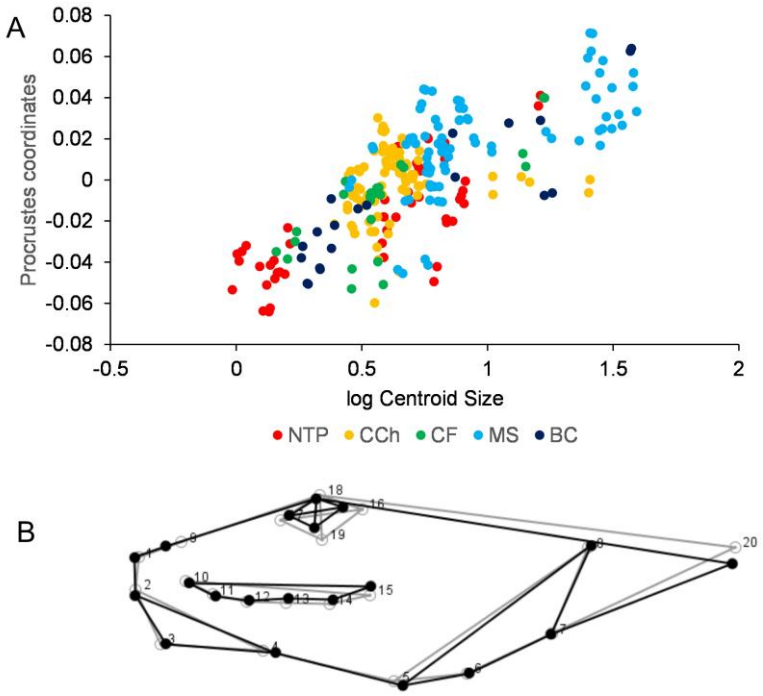

Figure 5A. Ontogenetic allometry of the eelpout *Austrolycus depressiceps* (Zoarcidae) from west Patagonia. NTP: North of Taitao Peninsula, CCh: Central Channels, CF: Central Fjords, MS: Magellan Strait, BC: Beagle Channel. B. Shape changes during ontogeny. Grey wireframes represent the starting morph, while the black wireframes represent the final morph.

Spatial variability of *A. depressiceps* shape

The comparison of the shape among zones was explained by four Canonical Variates, in which the first two CVA showed noticeable differences in the shape of the eelpout among zones (Fig. 6). A permutation test based on Procrustes distances detected significant differences in the morph among zones (Bonferroni corrected P values, P <0.001). The differences were more pronounced between the northern (NTP) and southern (BC) extremes of the spatial distribution: while in NTP (red in Fig. 6) the shape of the head was robust, expressed by a dorsal location of the eye and open mouth (vertical displacement of the point of the dentary), specimens from BC showed a slender head, with a more pointed snout (blue in Fig. 6). Additionally, specimens from channels and fjords (CCh and CF) showed a concave snout, caused by a deeper position of the nostril (LM9) (Fig. 6).

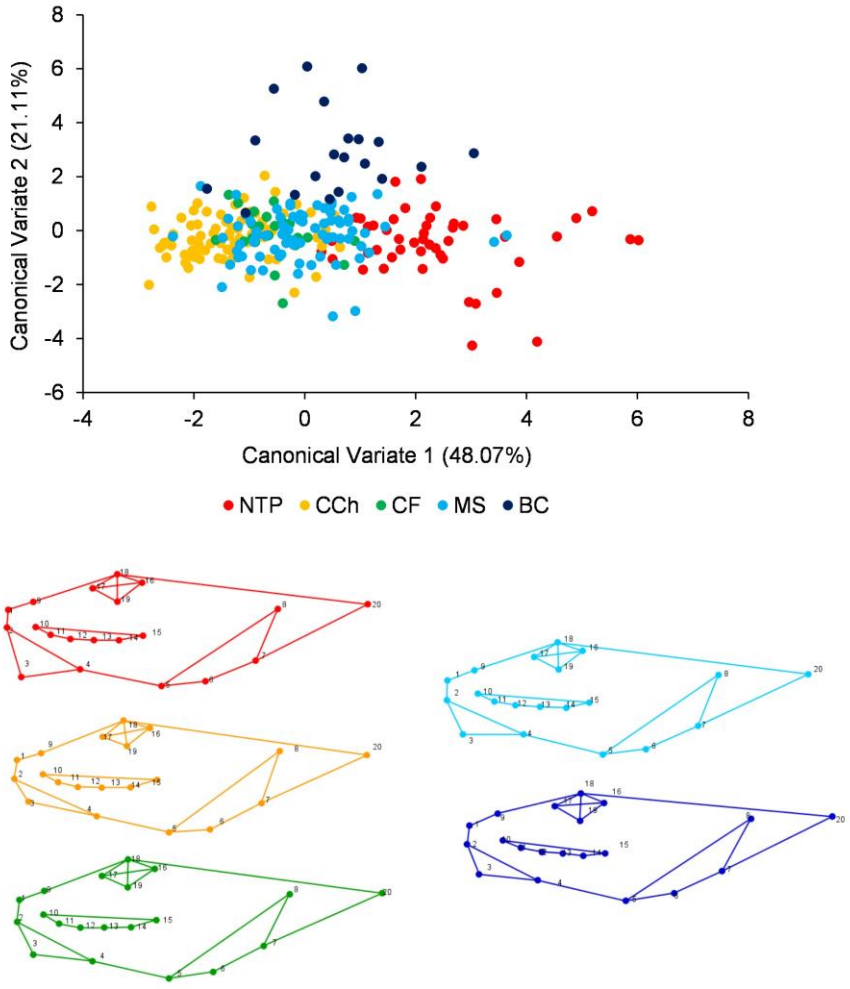

Figure 6. Canonical Variate Analysis (CVA) comparing the morph of the eelpout *Austrolycus depressiceps* among zones of west Patagonia. NTP: North of Taitao Peninsula, CCh: Central Channels, CF: Central Fjords, MS: Magellan Strait, BC: Beagle Channel. The consensus morph of each zone is shown by different colours.

Shape, body mass and sea water conditions

The PLS showed covariance between Block 1 and Block 2, explained by four PLS. The PLS1 explained 76.4% of the covariance, with significant correlation between PLS1 of block 1 (hydrography+condition) and PLS1 of block 2 (Procrustes coordinates; correlation = 0.421, $P < 0.001$). The maximum covariance related changes in salinity with displacements of the maxilla-dentary (LM3 and 4), mesethmoid (LM1) and opercle (LM20). This means that in brackish waters, eelpouts showed a slender head and snout, smaller eyes, and horizontally oriented mouth (Fig. 7, left

panel), while in saltier waters (more oceanic influenced areas), individuals had more robust head, shorter snout, bigger eyes and more oblique mouth opening (Fig. 7, right panel). The overall strength of association between blocks was low (Escoufier's RV = 0.058), but significant (permutation test, *P* < 0.001).

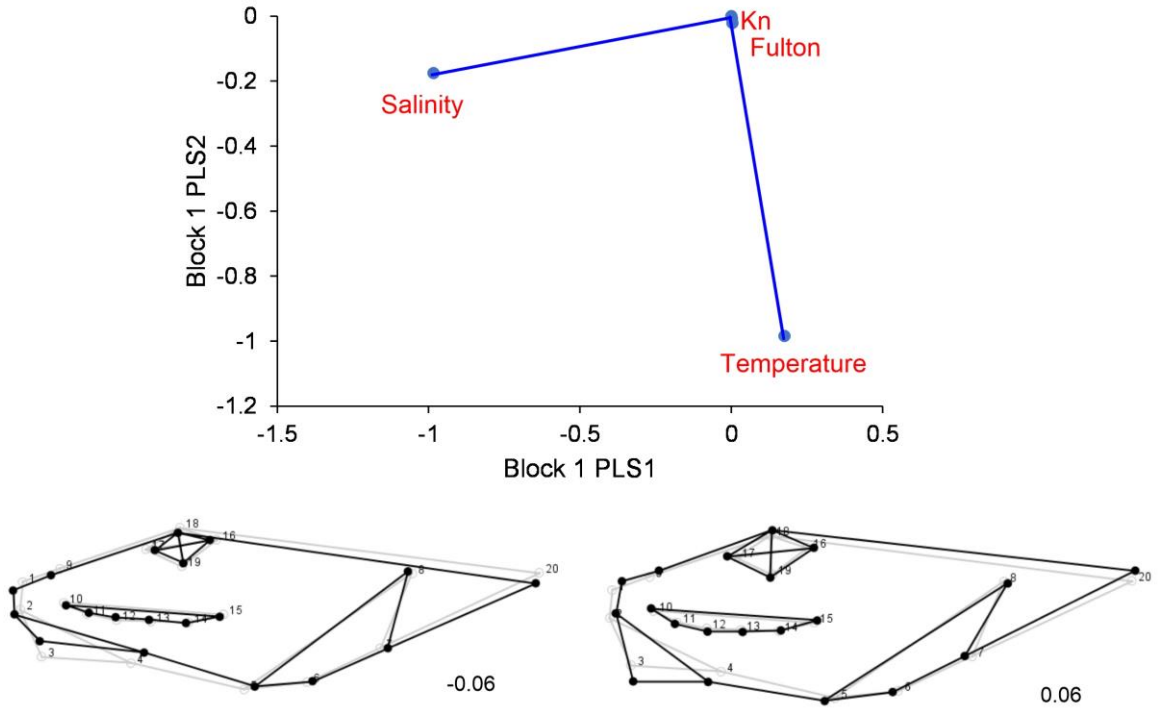

Figure 7. PLS showing covariance between condition indices (Fulton, Kn) and hydrographic conditions (sea temperature and salinity) (upper panel), and shape variability (lower panel). Grey wireframes represent the consensus shape, while the black wireframes represent the target shape related to fresher (left panel) and saltier waters (right panel).

Integration and modularity

Two hypotheses of modularity were tested. H1 (the *functional hypothesis*) compared the modularity between the shape of the head and the location of two peripheral lateral-line canals. Under this scenario, the observed CR was 0.954, showing that both head and canals are integrated in the development (P = 0.061, Fig. 8A, C). Nonetheless, As CR gets smaller (CR < 1), that means that within-module covariation is greater than between module covariation and that the structure is more

modular, H2 (the *structural hypothesis*) indicated that there is a significant modularity among three modules: head, suborbital pores, and preoperculo-mandibular pores (CR = 0.698, *P* = 0.001, Fig. 8B, D).

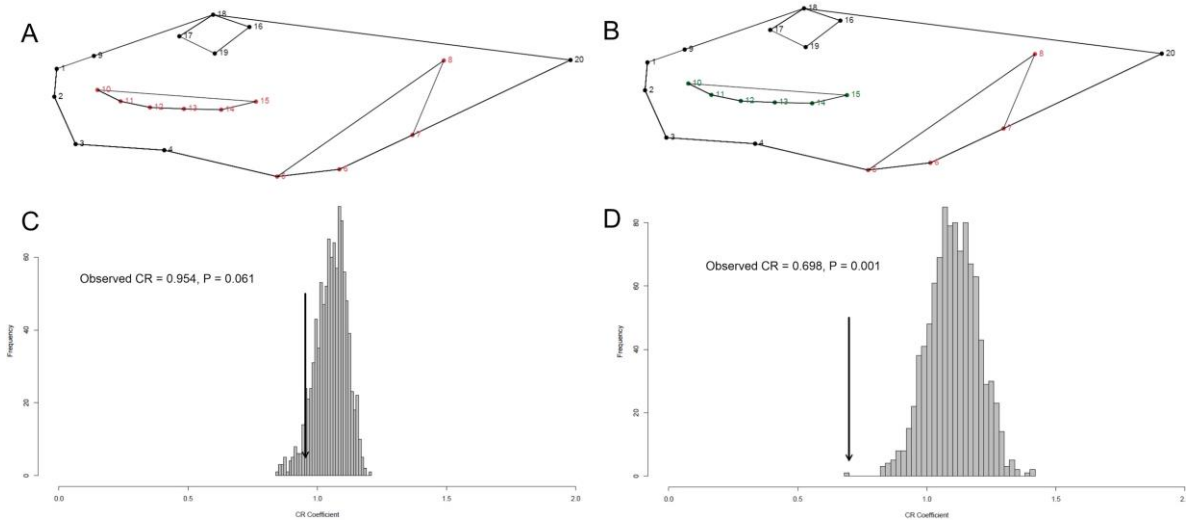

Figure 8. Hypothesis of integration and modularity between A, C) head (black dots) and sensory canals (red dots), and B, D) head (black dots), suborbital pores (green dots) and preoperculo-mandibular pores (red dots).

Discussion

In the eelpout *Austrolycus depressiceps* (4.5-22.5 cm TL), intraspecific phenotypic variability of morphospace was detected in terms of individuals with larger, pointed snout and an extended suborbital canal to individuals with shorter, rounded snout and frontally compressed suborbital canal. These morphs represent ~40% of the shape variation, while the ontogenetic allometry was low (~5%), represented by a decrease of the relative size of the eye, and a depression of the posterior margin of the head. At scales of hundreds of kilometres, individuals inhabiting the northernmost locations (45ºS) showed robust heads while those from the southern tip of South America (55ºS) showed a slender head, with a pointed snout. Seawater salinity covaried with shape variability of the head; in brackish waters, eelpouts showed a slender head and snout, smaller eyes, and horizontally oriented mouth, while in saltier waters (more oceanic-influenced areas), individuals had more robust head, shorter snout, bigger eyes and more oblique mouth opening. Additionally, two of the canals of the cephalic lateralis pores and the head shape showed modularity in its development. This evidence

suggest ecomorphological interactions between the changing coastal environment and the eelpout phenotype.

There are no previous studies using geometric morphometrics describing phenotypic variability in the morphology of Zoarcidae. Nonetheless, recent studies using classic morphometrics, suggests that Zoarcidae show high rates of shape evolution across several traits (Price et al. 2019). In the family, phenotypic variation has been described in terms of vertebral counts of *Lycodapus mandibularis* (Peden 1979), the positive allometry for body width and the complex of head characters of *Lycodes yamatoi* (Savelyev et al. 2011), and the fluctuating asymmetry in the morphological variation of *Zoarces viviparus* (Lajus et al. 2003). Our results indicate that the snout showed the largest source of shape variation (large *vs*. short; pointed *vs*. rounded snout), as well as the relative location of the two last pores of the suborbital canal. These morphological differences may cause intraspecific variations in the feeding habits, like in some gobies (Vera-Duarte et al. 2017), and may be linked to variations in salinity and turbidity along fjords and channels of west Patagonia (Castillo-Hidalgo et al. 2018).

The ontogenetic allometry was low and mainly related to the change of the relative size of the eye diameter. The eye size variability was previously described by Lloris and Rucabado (1992), who detected that it might be contained between 9 and 16 times in the size of the head in specimens between 9.8 and 41.1 cm TL. Generally, juveniles have larger eyes and heads relative to body size, whereas, adults have smaller eyes and heads relative to body size (Ahlnelt et al. 2020). The change in size of the head in relation to the eyes suggests the relevance of the sensory canals of the head as the main source of information for predators and prey detection in adulthood.

Intraspecific variations in lateral-line anatomy and morphology, and their origin are not well studied. Differences can be attributed to epigenetic effects (changes that affect gene activity and expression without altering the DNA sequence) or to phenotypic plasticity (Mogdans 2019). In terms of modularity, analyses showed a modular development between the suborbital and the preoperculo-mandibular canals of the cephalic lateralis system. Pores of the cephalic system can be observed from the hatching (Matallanas et al. 1990; Marcinkevicius & Gosztonyi 2013), but its relative location in the head vary throughout early development, with larger displacements of the preoperculo-mandibular canal than the suborbital canal in larval stages (Marcinkevicius & Gosztonyi 2013). Additionally, intraspecific shape variability in juvenile and adults of *A. depressiceps* was focused on the suborbital canal, while the preoperculo-mandibular canal showed slight variation only in PC2 (14% of the shape variation). Evidence, therefore, suggests an ontogenetic effect in the modularity of head shape, suborbital and preoperculo-mandibular canals.

The West Patagonia shows important latitudinal gradients in the hydrographic features of the coastal waters, such as temperature and salinity (Dávila et al. 2002; Saldías et al. 2019). The major content of freshwater is located off central Patagonia, where the low-salinity surface layer dominates the stratification of the upper ocean (Saldías et al. 2019). This latitudinal variability has significant effects on several characteristics of marine fishes throughout the ontogeny, such as growth, survival and bilateral symmetry (Landaeta et al. 2012, 2018; Molina-Valdivia et al. 2021). Additionally, species with large latitudinal distribution show shallower bodies with a smaller head at higher latitudes (Riesch et al. 2018), similar to our results. Other species that inhabit southern Patagonia, such as the pike icefish *Champsocephalus esox*, express similar phenotypic plasticity (Landaeta et al. 2021). Therefore, the hydrographic gradients may have imposed some constraints for the shape variability of the head and sensory canals of *A. depressiceps*, i.e., phenotype modulation. In phenotypic modulation, non-specific phenotypic variation results from the relative sensitivity of various parts of the developing organism to primarily physical factors in the environment. Therefore, this mechanism typically produces continuous phenotypic variation (Smith-Gill 1983), such as the eelpout's morphs observed across Patagonia for a large salinity gradient (from 14 to 33).

A morphospace pattern related to water temperature has been observed in larval stages of some blennioid species, showing taller bodies (head and trunk) when water temperature is warmer (labrisomids genus *Auchenionchus* and *Calliclinus*, Landaeta et al. 2019, Galeano-Chavarría et al. 2020). Similarly, specimens collected in the southernmost locations (Beagle channel and Horn Island), which show low seawater temperature (<4-9°C, Bruno et al. 2018), had hydrodynamic heads. In the covariance analysis, temperature only had a secondary influence on the shape variability (PLS2). However, temperature might be covarying also with salinity, particularly in central Patagonia, where Icefields' retreat causes the intrusion of fresher, and colder waters (Landaeta et al. 2011, Bown et al. 2019).

Finally, future scenarios of climate change in the area will cause the freshening and cooling of the coastal waters, because of the rapid retreat of Northern and Southern Patagonian icefields (Muto & Furuya 2013, Bravo et al. 2021), and may cause important variations in the morphology of the marine fish inhabiting Patagonia, particularly those utilizing shallow, coastal waters.

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