NOTE

Using head, pectoral girdle bones and otoliths to estimate length and weight of Argentine anchovy (*Engraulis anchoita*), a key species in Patagonian marine ecosystem

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ABSTRACT. The Argentine anchovy, *Engraulis anchoita*, plays a vital role as a key prey species for several marine predators in the north Patagonian marine ecosystem of the Atlantic Ocean. Reconstructing the length and weight of each consumed specimen is essential to provide a detailed description of the trophic ecology of top marine predators. Predictive linear regression equations were calculated for the Patagonian stock of Argentine anchovy to estimate parameters of length-weight relationships using measurements of whole individuals and diagnostic elements such as otoliths, head bones and pectoral fin bones. Among the diagnostic elements analyzed, the cleithrum and dentary exhibited the best fit. This study validates the use of head and pectoral girdle bones as reliable indicators for predicting the weight and length of Argentine anchovy across a wide size range, which corresponds to the target range of various predators. These relationships can contribute to the determination of body condition, estimation of consumed biomass, and calculation of energy density, providing valuable insights into the trophic ecology of predators in the southern Atlantic Ocean.

Key words: Head bones, otoliths, regression, Patagonia, measurements, length-weight.

Utilización de los huesos de la cabeza, cintura pectoral y otolitos para estimar la talla y el peso de la anchoíta (*Engraulis anchoita*), especie clave en el ecosistema marino patagónico

RESUMEN. La anchoíta, *Engraulis anchoita*, juega un papel vital como especie presa clave para varios depredadores marinos en el ecosistema marino patagónico norte del Océano Atlántico. Reconstruir la longitud y el peso de cada espécimen consumido es esencial para proporcionar una descripción detallada de la ecología trófica de los principales depredadores marinos. Se calcularon las ecuaciones de regresión lineal predictiva para el *stock* patagónico de anchoíta, a fin de estimar los parámetros de las relaciones talla-peso utilizando medidas de individuos completos y elementos de diagnóstico como otolitos, huesos de la cabeza y huesos de la aleta pectoral. Entre los elementos diagnósticos analizados, el cleitro y el dentario exhibieron el mejor ajuste. Este estudio valida el uso de los huesos de la cabeza y la cintura pectoral como indicadores confiables para predecir el peso y la talla de la anchoíta en un amplio rango de tamaños, que corresponden al rango objetivo de varios depredadores. Estas relaciones pueden contribuir a la determinación de la condición corporal, la

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estimación de la biomasa consumida y el cálculo de la densidad de energía, proporcionando información valiosa sobre la ecología trófica de los depredadores en el Océano Atlántico sur.

Palabras clave: Huesos de la cabeza, otolitos, regresión, Patagonia, mediciones, talla-peso.

Diet studies are crucial for understanding trophic dynamics, foraging behaviors and life history patterns of most species within ecosystems (Hódar 1997). Descriptions in terms of prey size are essential for determining predator consumption rates, total prey biomass consumed, selectivity of a predator towards a specific prey size, and modeling consumer energy use (Hansel et al. 1988). Additionally, the diet of marine mammals and seabirds can provide early indications of fluctuations in fish populations, making them sentinel species for marine ecosystem variability (Velarde et al. 2013; Ciancio et al. 2021; Pirotta et al. 2022; Ramos and Furness 2022).

Direct measurement and weighing of prey items is challenging due to fragmentation or digestion (Hódar 1997). Therefore, reconstructing the length or weight fragmented preys in diet samples is often necessary. Despite advanced digestion in predators, certain prey parts exhibit slower digestion and maintain a constant relationship with prey body size, enabling reliable identification and reconstruction of most prey items (Hansel et al. 1988). This is the case of bones, for which several studies use marine fish diagnostic bones to estimate original lengths and weights of prey consumed by predators through regressions (Sinovčić et al. 2004; Tapella and Lovrich 2006; González-Zevallos et al. 2010; Pérez Comesaña et al. 2014; Riestra et al. 2020).

In the marine ecosystem of northern Patagonia, the dominant schooling fish is the Patagonian stock of Argentine anchovy, *Engraulis anchoita* (Hubbs and Marini, 1935). This stock is distributed from 41° S to 48° S, and a large fraction is located in front of Península Valdés during the last quarter of the year in waters ranging from 50 to 80 m deep (Hansen 2004). They play a key role as an important prey item for several fishes, seabirds, and marine mammals (Crespo et al. 1997; Koen Alonso et al. 2000; Gatto and Yorio 2009; Belleggia et al. 2012; Loizaga de Castro et al. 2016; Ibarra et al. 2018; Fernández et al. 2019). To provide a detailed description of the trophic ecology of top predators, it is essential to reconstruct the length and biomass of each consumed specimen. Therefore, our goals were to estimate parameters of length-weight relationships using measurements of whole individuals, as well as diagnostics elements such as otoliths, head bones and pectoral fin bones from the Patagonian stock of Argentine anchovy.

A total of 125 specimens from the Patagonian stock of Argentine anchovy were studied. Sixteen specimens were captured in April 2013 using a net in Golfo Nuevo, Península Valdés, Chubut Province. Additionally, specimens from commercial fishery vessels in Rawson, Chubut Province during October 2014 (n = 23), December 2019 (n = 28), and June-July 2021 (n = 41) were included. All specimens were frozen for further analysis. Finally, 17 samples preserved in formalin from the same stock were obtained from the Ichthyological Collection of the Centro Nacional Patagónico (CENPAT) (n = 17, CNPICT 1992/75, CNPICT 1992/76 and CNPICT 2000/23).

Furca length (FL) from individuals was measured to the closest millimeter with an ichthyometer and weighted (W) in grams using an analytical balance. For samples obtained since 2019, total length (TL) was also measured (n = 69). Furca length refers to the measurement from the tip of the longest jaw of a fish to the center of the fork in the caudal fin, while total length is measured to the longest caudal lobe.

Dissection techniques were employed to disarticulate anchovies after they have been soaked in boiling water (100 °C) for at least ten minutes. Selected diagnostic elements (Table 1) were those that commonly appear in stomach contents based on Gosztonyi and Kuba (1996) and our personal experience. Photographs of elements were taken using a digital camera with a scale. Images were subsequently digitally measured with ImageJ 1.48v software (Schindelin et al. 2015) according to the description in Table 1 and the scheme delineated in Figure 1.

Statistical analyses were conducted using the R software (R Core Team 2021). Relationships were modeled using 'lm' function from car package (Fox et al. 2007). Normality and homoscedasticity assumptions were tested using graphical methods. Prior to model fitting, log-transformed data plots were used to detect outliers following the suggestion of Froese (2006). The performance of the regression models was assessed using the root mean squared error (RMSE) during repeated K-fold cross-validation (10 folds with 100 repeats) (Linhart and Zucchini 1986). The RMSE provides a measure of the average difference between the observed and predicted values of the model, allowing for the evaluation of how well the model fits the data. In our study, RMSE values that were a small fraction of the data (less than 10%) were considered as low, indicating a good fit of the model and accurate predictions.

Length-weight relationship was examined using the equation $W = aL^b$ (Froese 2006), where W represents the wet weight, L is the furca length or total length (TL, when data was available), a is the intercept, and b is the scaling exponent. To establish the relationship, the exponential model was linearized by applying logarithmic transformation ($\log[W] = \log[a] + b \log[L]$). The scaling exponent (b) of the length-weight relationship determined the growth pattern, indicating isometric growth ($b = 3$), positive allometric growth ($b > 3$), or negative allometric growth ($b < 3$). The growth status was determined using a t-test according to the $b = 3$ isometric growth hypothesis (Sokal and Rohlf 1987), providing insights into the species' growth patterns and indicating its status in a given environment (Froese 2006). Total length is more widely used than furca length in fisheries research (Carlander and Smith 1945). Therefore, establishing a more precise relationship between these two measurements was aimed for lengths conversion. The equation $TL = a + b\,L$, with TL and L in mm, was used.

Simple linear regression equations were calculated to estimate the furca or total length versus the length of diagnostic elements using the equa-

Table 1. Measurement description for the diagnostic elements of the Argentine anchovy.

Measurement	Abbreviation	Description
Parasphenoid	LPar	Length from the tip of the anterior process to the posterior incisure
Dentary	LDen(1)	Length from the rostral tip to the external incisure
	LDen(2)	Maximal length measured from the rostral tip to the caudal tip of the ventral process
Maxilla	LMax	Maximal length, from the rostral tip of the external process to the tip of the caudal process
Anterior ceratohyal	LCer	Maximal length of the anterior ceratohyal
Cleithrum	LCle	Length from the dorsal to the ventral tip
Otolith	LOto(1)	Maximal length (rostro-cauda axis)
	LOto(2)	Maximal width (dorso-ventral axis)

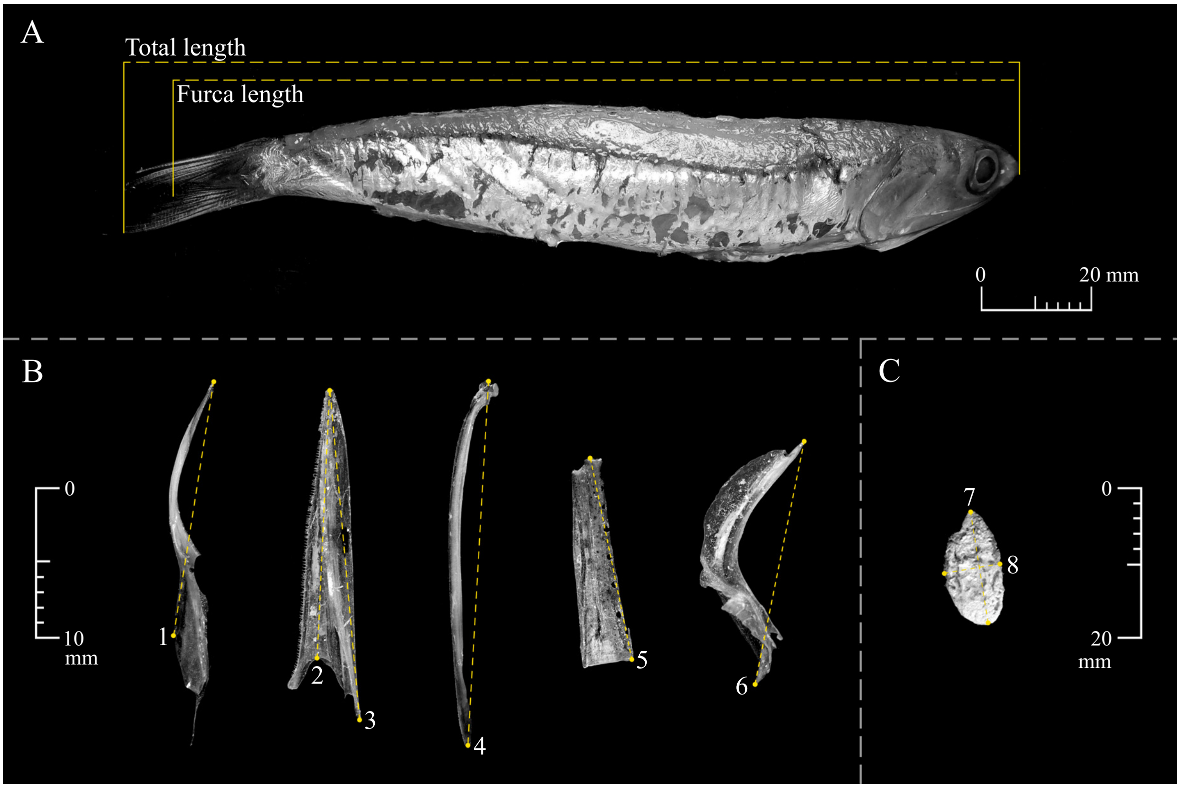

Figure 1. Scheme of measurements performed on cranial bones, pectoral girdle bones, and otoliths of the Argentine anchovy. A) Fish body measurements. B) Parasphenoid and the left paired bones (from left to right): dentary, maxilla, anterior ceratohyal, and cleithrum. C) Left otolith indicating the two measurements used. 1) LPar: length of the parasphenoid. 2) LDen(1): length of dentary. 3) LDen(2): maximal length of dentary. 4) LMax: maximal length of the maxilla. 5) LCer: maximal length of the anterior ceratohyal. 6) LCle: length of cleithrum. 7) LOto(1): maximal length of otolith. 8) LOto(2): maximal width of otolith. Measurements of right bones and otoliths were taken in the same manner as shown in the diagram.

tion $L = a + b\ DL$, where DL is the length (mm) of the diagnostic element. Additionally, linear regression on log-transformed data was used to determine relationships between wet weight and the length of diagnostic elements. An ANCOVA was performed to test for significant differences between left and right measurements of pair bones and otoliths, as they may not always provide the same estimation of fish length (e.g. Martínez-Polanco et al. 2022).

Population studies comprised Argentine anchovies with furca lengths ranging from 40 to 170 mm (mean ± SD: 120.50 ± 30.89 mm, n = 125), total lengths between 87 and 195 mm (mean ± SD: 159.92 ± 24.75 mm, n = 69), and wet weight that ranged from 0.23 to 47.54 g (mean ± SD: 20.57 ± 13.62 g, n = 125) (Table 2).

Length-weight relationships for furca and total length were statistically significant (R^2 = 0.989 and 0.922, respectively). Estimated values of b (b ± SE: b_L = 3.69 ± 0.03 and b_{TL} = 3.41 ± 0.12) differed significantly from 3 (t-test, p-values < 0.05), indicating positive allometric growth in our samples. The linear relationship between total length and furca length was also significant (R^2 > 0.914, p-value < 0.05) (Table 2).

Fifteen linear regressions were conducted to estimate furca and total length using the length of

Table 2. Parameters of morphometric equations calculated for the Argentine anchovy. Mean, standard deviation, minimum and maximum size of the fish (lengths in mm, weight in g), R^2, and the root mean squared error (RMSE) value are presented. The level of significance is not shown since all equations had a p-value < 0.05. For length equations, use the formula $L = a + b * DL$, and for weight equation, use $W = aDL^b$, where L can be total length (TL) or furca length (FL), and DL is the length of the diagnostic element. To estimate the relationship between total length and fork length, use the equation $TL = a + b * FL$.

Diagnostic element	Mean	SD	Min.	Max.	N	Relationship	Parameters [CI 95%inf, CI 95%sup]		R^2	RMSE
							a	*b*		
Furca length (FL)	120.50	30.89	40	170	125					
Total length (TL)	159.93	24.76	87	195	69	TL versus FL	13.787 [2.726, 24.858]	1.074 [0.994, 1.154]	0.914	6.966
Wet weight (W)	20.57	13.62	0.23	47.57	125	W versus FL	3.31×10^{-7} [2.41×10^{-7}, 4.55×10^{-7}]	3.692 [3.625, 3.759]	0.989	0.113
					69	W versus TL	7.54×10^{-7} [2.22×10^{-7}, 2.55×10^{-6}]	3.415 [3.173, 3.656]	0.922	0.168
Parasphenoid	13.06	3.69	5.65	20.87	68	LPar versus TL	-13.206 [-24.639, -1.772]	10.087 [9.425, 10.748]	0.934	6.461
					122	LPar versus FL	-12.841 [-18.590, -7.093]	8.649 [8.287, 9.012]	0.949	6.647
						LPar versus W	2.51×10^{-4} [1.50×10^{-4}, 4.00×10^{-4}]	4.038 [3.865, 4.211]	0.947	0.241
Dentary left (1)	15.41	3.5	6.46	20.44	69	LDenL(1) versus TL	-13.137 [-28.317, 2.044]	10.106 [9.228, 10.985]	0.887	8.192
					123	LDenL(1) versusFL	-7.287 [-11.355, -3.219]	8.373 [8.114, 8.633]	0.971	5.275
						LDenL(1) versus W	5.34×10^{-4} [4.00×10^{-4}, 7.00×10^{-4}]	3.778 [3.676, 3.879]	0.978	0.167
Dentary left (2)	19.26	4.51	7.71	26.65	69	LDenL(2) vs. TL	-3.998 [-18.342, 10.345]	7.635 [6.973, 8.296]	0.888	8.211
					123	LDenL(2) versus FL	-4.524 [-8.101, -0.947]	6.553 [6.372, 6.735]	0.977	4.852
						LDenL(2) versus W	2.77×10^{-4} [2.10×10^{-4}, 3.70×10^{-4}]	3.714 [3.615, 3.814]	0.978	0.167
Dentary right (1)	15.27	3.68	4.4	20.65	69	LDenR(1) versus TL	-11.649 [-26.551, 3.253]	9.978 [9.119, 10.837]	0.889	8.192
					125	LDenR(1) versus FL	-6.017 [-9.871, -2.164]	8.286 [8.041, 8.532]	0.973	5.094
						LDenR(1) versus W	5.98×10^{-4} [4.60×10^{-4}, 7.80×10^{-4}]	3.736 [3.637, 3.836]	0.978	0.166

Table 2. Continued.

Diagnostic element	Mean	SD	Min.	Max.	N	Relationship	Parameters [CI 95%inf, CI 95%sup] *a*	*b*	R^2	RMSE
Dentary right (2)	19.10	4.66	6.25	26.23	69	LDenR(2) versus TL	-6.313 [-20.570, 7.944]	7.727 [7.071, 8.384]	0.892	8.135
					124	LDenR(2) versus FL	-5.034 [-8.468, -1.601]	6.577 [6.402, 6.752]	0.979	4.610
						LDenR(2) versus W	2.43×10^{-4} [1.80×10^{-4}, 3.20×10^{-4}]	3.760 [3.669, 3.851]	0.982	0.149
Maxilla left	20.86	5.2	6.37	28.86	69	LMaxL versus TL	-4.137 [-18.195, 9.921]	7.010 [6.415, 7.605]	0.892	8.073
					121	LMaxL versus FL	-2.096 [-5.604, 1.411]	5.911 [5.747, 6.075]	0.977	4.722
						LMaxL versus W	2.62×10^{-4} [2.00×10^{-4}, 3.40×10^{-4}]	3.632 [3.546, 3.718]	0.983	0.147
Maxilla right	20.82	5.11	6.44	28.72	69	LMaxR versus TL	-2.965 [-18.712, 12.782]	6.957 [6.291, 7.623]	0.866	8.859
					124	LMaxR versus FL	-0.922 [-4.918, 3.074]	5.864 [5.677, 6.051]	0.969	5.410 5.410
						LMaxR versus W	2.91×10^{-4} [2.20×10^{-4}, 3.80×10^{-4}]	3.601 [3.509, 3.693]	0.980	0.161
Anterior ceratohyal left	11.62	2.86	3.39	16.03	66	LCerL versusTL	-0.172 [-15.169, 14.824]	12.350 [11.213, 13.488]	0.880	8.544
					122	LCerL versus FL	-4.349 [-8.526, -0.172]	10.779 [10.429, 11.129]	0.969	5.599
						LCerL versus W	1.79×10^{-3} [1.36×10^{-3}, 2.35×10^{-3}]	3.710 [3.597, 3.823]	0.972	0.188
Anterior ceratohyal right	11.66	2.84	3.45	16.02	68	LCerR versus TL	-5.87×10^{-3} [-14.521, 14.509]	12.285 [11.181, 13.388]	0.882	8.526
					124	LCerR versus FL	-2.398 [-6.398, 1.602]	10.613 [10.277, 10.948]	0.970	5.382
						LCerR versus W	2.17×10^{-3} [1.67×10^{-3}, 2.82×10^{-3}]	3.632 [3.525, 3.739]	0.974	0.182
Cleithrum left	14.07	3.76	5.38	20.47	68	LCleiL versus TL	22.841 [11.251, 34.431]	8.550 [7.837, 9.262]	0.897	7.971
					123	LCleiL versus FL	6.031 [3.218, 8.844]	8.163 [7.969, 8.357]	0.983	3.985
						LCleiL versus W	1.25×10^{-3} [1.00×10^{-3}, 1.57×10^{-3}]	3.581 [3.496, 3.666]	0.983	0.144

Table 2. Continued.

Diagnostic element	Mean	SD	Min.	Max.	N	Relationship	Parameters [CI 95%inf, CI 95%sup]		R^2	RMSE
							a	*b*		
Cleithrum right	14.13	3.67	5.95	20.36	67	LCleiR versus TL	20.772 [9.764, 31.781]	8.694 [8.016, 9.372]	0.910	7.423
					120	LCleiR versus FL	7.147 [4.278, 10.016]	8.076 [7.878, 8.273]	0.982	3.949
						LCleiR versus W	1.59×10^{-3} [1.30×10^{-3}, 1.93×10^{-3}]	3.491 [3.416, 3.567]	0.986	0.124
Otolith left (1)	3.9	0.55	2.35	4.96	68	LOtoL(1) versus TL	-9.957 [-27.033, 7.118]	41.914 [37.738, 46.089]	0.859	9.223
					104	LOtoL(1) versus FL	-22.267 [-31.911, -12.624]	38.916 [36.469, 41.364]	0.907	6.865
						LOtoL(1) versus W	0.070 [0.048, 0.101]	4.179 [3.910, 4.449]	0.903	0.206
Otolith left (2)	2.02	0.27	1.39	2.59	68	LOtoL(2) versus TL	-4.477 [-30.045, 21.092]	78.052 [66.009, 90.095]	0.717	13.361
					108	LOtoL(2) versus FL	-26.236 [-39.495, -12.977]	76.866 [70.359, 83.374]	0.838	9.209
						LOtoL(2) versus W	0.948 [0.717, 1.253]	4.361 [3.967, 4.755]	0.820	0.277
Otolith right (1)	3.9	0.57	2.38	5.08	68	LOtoR(1) versus TL	-4.449 [-22.143, 13.244]	40.468 [36.154, 44.782]	0.842	10.021
					104	LOtoR(1) versus FL	-19.839 [-29.523, -10.157]	38.183 [35.727, 40.638]	0.903	7.143
						LOtoR(1) versus W	0.077 [0.053, 0.111]	4.106 [3.836, 4.376]	0.899	0.211
Otolith right (2)	2.02	0.27	1.43	2.74	68	LOtoR(2) versus TL	-0.024 [-27.748, 27.700]	76.251 [63.143, 89.359]	0.671	14.159
					106	LOtoR(2) versus FL	-23.083 [-37.588, -8.578]	75.464 [68.337, 82.589]	0.809	10.101
						LOtoR(2) versus W	0.992 [0.733, 1.342]	4.307 [3.879, 4.736]	0.793	0.305

diagnostic elements, as well as to estimate the wet weight of anchovies using bones and otoliths (Table 2). All relationships were significant (p-value < 0.05). Generally, a strong correlation was observed between diagnostic elements and lengths, with R^2 values exceeding 0.81 for furca length and 0.67 for total length. The R^2 values greater than 0.793 for the regressions with the wet weight as the response variable were obtained. The slope *b* did not significantly differ between measurements of the right and left diagnostic elements (ANCOVA, p-value > 0.05). Overall, the prediction error (RMSE) was low, ranging from 0.11 to 14.159, indicating the reliability of the proposed regressions for predicting anchovy size and weight.

This study contributes to the use of different diagnostic elements such as otoliths, pectoral and head bones to estimate the length and weight of Argentine anchovy, a highly relevant species in the southwestern Atlantic Ocean and a significant prey item for numerous top predators (Velasco and Castello 2005). The analyzed length range encompasses the maximum size recorded for the Patagonian stock (198 mm, Hansen 2004) and the prey size range observed in the diet of various important marine predators in the region, including fish (Koen Alonso et al. 2001; Sánchez 2009), seabirds (Gatto and Yorio 2009; Castillo et al. 2019), and marine mammals (Koen Alonso et al. 1998; Koen Alonso et al. 2000).

Traditionally, otoliths have been commonly used in diet studies to estimate length and/or weight of consumed prey. However, regressions conducted in this study exhibited lower coefficients of determination (R^2) and higher RMSE for otoliths compared to other elements. This discrepancy may be attributed to differences in the otolith length and fish growth relationship (Laidig and Ralston 1995). Typically, otoliths exhibit a linear relationship until fish reaches its maximum size, after which it begins to increase in thickness (Blacker 1974). In contrast, cranial bone lengths exhibited stronger relationships, with R^2 values ranging from 0.866 to 0.989. This is because fish length and the length of some growth bones are constant (Prenda and Granado-Lorencio 1992; Carss and Elston 1996). Moreover, cranial and pectoral bones are easily recognizable and identifiable at the species level (Prenda and Granado-Lorencio 1992) and more resistant to erosion compared to otoliths (Carss and Elston 1996), which can introduce lower biases in size calculation (Scharf et al. 1998). For this reason, durable bones like cleithrum and dentary are preferred. In our study, the best regression estimates were obtained using these two bones.

Our study indicated positive allometric growth in Patagonian anchovy stock samples, suggesting that its weight increases more than its predicted total length. The *b* parameter estimated in previous studies for the Patagonian anchovy stock ranged from 3.21 to 3.35 (Hansen 2004), while it was estimated from 2.97 to 3.40 for the Bonaerensis stock (Hansen 1997; Haimovici and Velasco 2000; Garciarena et al. 2002; Hansen 2004). Differences in these values may be attributed to various sampling-related factors, such as sample size, length range, conservation method, temporal resolution of sampling, sex, and stage of specimens, as well as variations between stocks or those from different geographical regions (Weatherley and Gill 1987; Froese et al. 2011; Kuriakose 2017).

Although this study provides valuable insights, it should be noted that collection methods used did not allow for the acquisition of smallest sizes of Argentine anchovy (< 40 mm furca length), which are particularly challenging to obtain. However, fortuitous stranding events of this species in Golfo Nuevo, Chubut, in 1997 and 2000, did allow for the collection of some of the smallest sizes (2023 pers. comm. A Gosztonyi). Additionally, it should be noted that these samples (17 of the 125 individuals) were preserved in formalin, so caution should be exercised when estimating wet weight based on our relationship within this size range, as it may underestimate individual weight.

These relationships can be valuable in comparative studies of growth, determination of body condition and calculation of total biomass consumed in diet studies, such as those of Fernández et al. (2019) and Ibarra et al. (2022), and estimation of energy density in this Patagonian population (Ciancio et al. 2020), contributing to the understanding of the trophic ecology of predators in one of the world's most productive oceans.

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Author contributions

Fernández J. Santiago: conceptualization, data curation, formal analysis, investigation, methodology, roles/writing-original draft, writing-review and editing. Cynthia Ibarra: conceptualization, data curation, formal analysis, investigation, methodology, roles/writing-original draft, writing-review and editing. Ximena Navoa: formal analysis, methodology, supervision, roles/writing-original draft, writing-review and editing. Javier E. Ciancio: project administration, supervision, validation, visualization, writing-review and editing.

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