Age structure for Patagonian Toothfish *Dissostichus eleginoides* around the Falkland Islands:

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AGE- 2021-TOO

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Table of Contents

1. Introduction

The age structure in a fish population provides the basic information for mortality rates, recruitment and growth (Hussy *et al.*, 2016). These parameters are essential inputs in age-structured stock assessment models that provide the basis for management advice in many world fisheries (Payne *et al.*, 2005; Lorenzen, 2016). Due to the key role of age in stock assessment models, bias in age estimates can therefore fundamentally influence the perception of the stock and fishing mortality, resulting in erroneous predictions of stock size and related management advice.

Patagonian toothfish *Dissostichus eleginoides* is a large (Total Length could reach up to 200 cm) bentho-pelagic notothenioid fish, occurring across islands, seamounts and shelf areas of the Sub-Antarctic (Collins *et al*. 2010). Its distribution spans the Antarctic Polar Front and extends north over the Patagonian Shelf in the Atlantic Ocean, off Chile in the Pacific and to 40°S in the south-western Indian Ocean (Laptikhovsky *et al.*, 2006). Its depth range is the widest of any teleost fish, being found from 10 m to 2500 m (Péron *et al.*, 2016).

It is a species of primary importance in the Falkland Islands longline fishery due to its high commercial value and abundance with an annual total allowable catch of 1040 t since 2015 (Skeljo, 2023). Juvenile and sub-adult Patagonian toothfish are also captured as bycatch in the finfish and squid trawl fisheries.

This annual report presents a reliable ageing methodology for the construction of age length keys and estimation of growth and mortality parameters from Patagonian toothfish samples obtained in the Falkland Islands during 2021. It also aims to provide estimates of inter- and intra-reader bias and precision in the age estimation in order to establish the reliability of the age estimation protocol and their potential use in stock assessment and subsequent management advice.

2. Materials and methods

2.1. Data Collection

Patagonian toothfish *Dissostichus eleginoides* were sampled by scientific observers and other scientific staff of the Falkland Islands Government Fisheries Department. Data were collected on board the licensed longliner and commercial fishing vessels operating bottom trawls under various license types. In addition, data were collected on board the RV 'Castelo' operating bottom trawls during research surveys.

Randomly sampled Patagonian toothfish were measured to the nearest cm (Total Length: TL), sexed and the stage of reproductive maturity assigned according to an eight-stages scale (I and II – immature, III and IV – maturing, V – mature, VI – running, VII – post spawning and VIII – spent). Otoliths were stored in paper envelopes in four quarterly time periods (A: Jan – Mar, B: Apr – Jun, C: Jul – Sep and D: Oct – Dec). This macroscopic key was defined by Nikolsky (1963) and adjusted for Falkland Islands species according to Brickle *et al.* (2005).

A minimum of 60 male and female otoliths for each quarterly collection are selected to cover the length distribution of sampled fish for age estimation (n = 480). This ensures that sufficient otoliths are aged for all lengths on a temporal basis.

2.2. Preparation of otoliths

Otoliths were embedded in rows of five in blocks of clear epoxy resin (West system epoxy: 105 epoxy Resin and 206 Slow Hardener; https://www.westsystem.com/) and left to set for 24 hours. Fully dried blocks are ground in order to provide smooth linear surfaces and the nuclei were delineated using a pencil. This was undertaken to guide the cutting angle and ensure that sections were cut precisely at right angles. Resin blocks were subsequently sectioned using a BUEHLER Isomet Low Speed Saw. Between two and six sections of 0.35 mm were taken per resin block and mounted on microscope slides under coverslips with clear epoxy resin.

2.3. Reading methodology

Sections were viewed under reflected light at 20 to 40 times magnification. All sections of each row of otoliths were inspected and the section closest to the primordium was used for subsequent ageing.

Following previous work on age estimation of this species, the sector from the primordium to the proximal edge of the section, on the ventral side of the sulcus was chosen as the area in which to count increments. However, for some preparations, increments formed on the dorsal side were at least as clear as those on the ventral side. Each otolith was aged at least twice by the primary reader. For otoliths where the first two readings did not agree, a third reading was realised. When annual rings cannot clearly be delineated and/or counted, otoliths were considered as unreadable and were eliminated from the age estimation process.

All counts of annuli were made without prior knowledge of fish size or previous age estimates.

2.4. Precision of the age estimates

Repeated readings of the same otoliths provide a measure of intra-reader or inter-reader variability. They do not validate the assigned ages but provide an indication of size of the error to be expected with a set of age estimates, due to variation in interpretation of an otolith. To examine the precision among age estimations, we calculated the four following indices across all age estimations: Average Standard Deviation (ASD), Average Absolute Deviation (AAD), Average Coefficient of Variation (ACV), and Average Percent Error (APE) (Ogle, 2016).

The ASD is the average (across all fish) of standard deviation of ages within a fish (Ogle, 2016). The AAD is the average (across all fish) absolute deviation of ages within a fish (Ogle, 2016).

The APE is the average (across all fish) percent error of ages within a fish using the mean as the divisor (Beamish and Fournier, 1981). The APE was calculated as:

$$APE = 100 * \left[\frac{1}{n} \sum_{j=1}^{n} \left(\frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j} \right) \right]$$

where n is the number of fish aged, R is the number of times fish are aged, Xij is the ith determination for the jth fish, and Xj is the average estimated age of the jth fish. APE was calculated for all repeated readings undertaken by the primary reader.

To avoid the inherent assumption in the APE that the standard deviation of age is proportional to the mean age for individual fish, the ACV should be measured Chang (1982). The ACV was defined as:

$$ACV = 100 * \frac{1}{n} \left(\sum_{j=1}^{n} \frac{s_j}{\bar{x}_j} \right)$$

where *sj* is the standard deviation of the *R* age estimates for the *j*th fish.

2.5. Estimation of von Bertalanffy parameters

A von Bertalanffy growth function was fitted to the observed length-at-age data:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right)$$

where L_t is length (TL in cm) at time t (years), L_∞ the asymptotic length, K is the rate (year^{-1}) by which L_∞ is approached, and t_0 is the theoretical age at length zero. Growth curves were fitted for males and females by non-linear least-square regression in R (R Core Team, 2021). Likelihood ratio tests were used to estimate whether a combined sex growth model or sex-separated models better described the length-at-age data. A parametric bootstrapping procedure with 1,000 iterations was employed to estimate 95% confidence intervals for final parameter estimates (Baty *et al.*, 2015).

2.6. Mortality estimates

Catch-at-age frequency plots for each sex and fishery were obtained from representative length frequency data, by applying age-length keys derived from the aged otoliths (Ogle *et al.*, 2018). The annual mortality rate (A) and instantaneous rate of total mortality (Z) were estimated from catch-at- age data on the descending limb of the catch-curve using a weighted regression to reduce the relative impact of older ages with fewer fish on Z. Initial ascending points representing fish that were not fully recruited to the fishery were excluded from the analysis.

3. Results and discussion

3.1. Distribution of samples

Biological information was obtained from a total of 2391 Patagonian toothfish samples. Of these, 1397 and 994 were sampled from within the trawl and longline based fisheries, respectively. We randomly selected 481 Patagonian toothfish to estimate their ages. However, we used only 470 otoliths to perform age estimation analyses as 11 otoliths were considered unreadable and were eliminated of the age estimation process.

In the trawl-based fisheries, Patagonian toothfish were sampled from across the shelf to the south and west of the Falkland Islands at depths between 100 and 428 m depth (mean = 220 m; Figure 1). In the longline fishery, Patagonian toothfish were sampled at depths between

300 and 1804 m (mean = 1347 m) predominantly to the south over the north of Burdwood Bank, and to the east and north-east of the Falkland Islands (Figure 1).

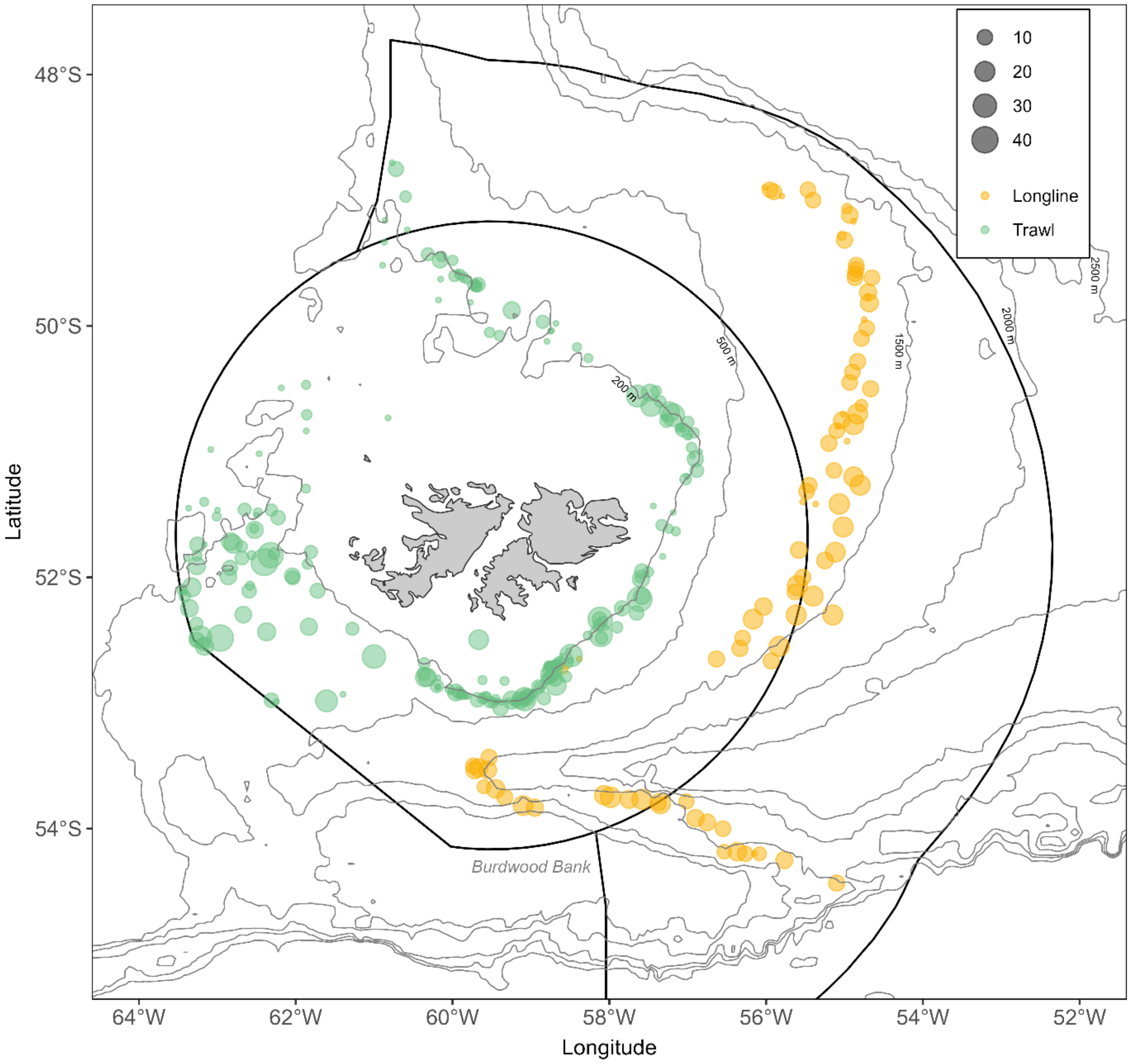

Figure 1: Positions from which Patagonian toothfish were sampled around the Falkland Islands during 2021 (n=2391).

3.2. *Length and Age composition*

In the trawl-based fisheries, lengths ranged between 9 and 97 cm TL (mean ± sd = 47.4 ± 16.6 cm). The distribution showed a clear modal peak occurring around 23 cm TL, reflecting newly recruited (age-1) fish (Figure 2). The length frequency distribution was skewed to the right, displaying a multimodal distribution reflecting older cohorts of juvenile and sub-adult fish inhabiting the shallow waters wherein trawl-based fisheries occur. The trawl-based fisheries predominantly captured fish aged less than 6 years old (yo; quantile 0.1 = 1 yo and quantile 0.9 = 5 yo; Figure 3).

The longline-based fishery appeared to target a different part of the Patagonian toothfish stock with lengths ranging from 49 to 214 cm TL (mean ± sd = 106 ± 24.6 cm), with a clear modal peaks occurring around 76 and 106 cm TL for both male and female fish and a third modal peak could be highlight around 212 cm for female (Figure 2). These modal peaks of longline caught Patagonian toothfish seemed to reflect fish around 7 yo and 14 yo (Figure 3). The majority of Patagonian toothfish being caught within the longline fishery were older than 8 yo (quantile 0.1 = 7.9 yo and quantile 0.9 = 23.1 yo; Figure 3).

Combining all the fisheries data, ages estimated ranged from 1 to 33 yo for females and 1 to 29 yo for males (Figure 3). Newly recruited age-0 fish were also present in the sampled catch, although these were not identified by sex.

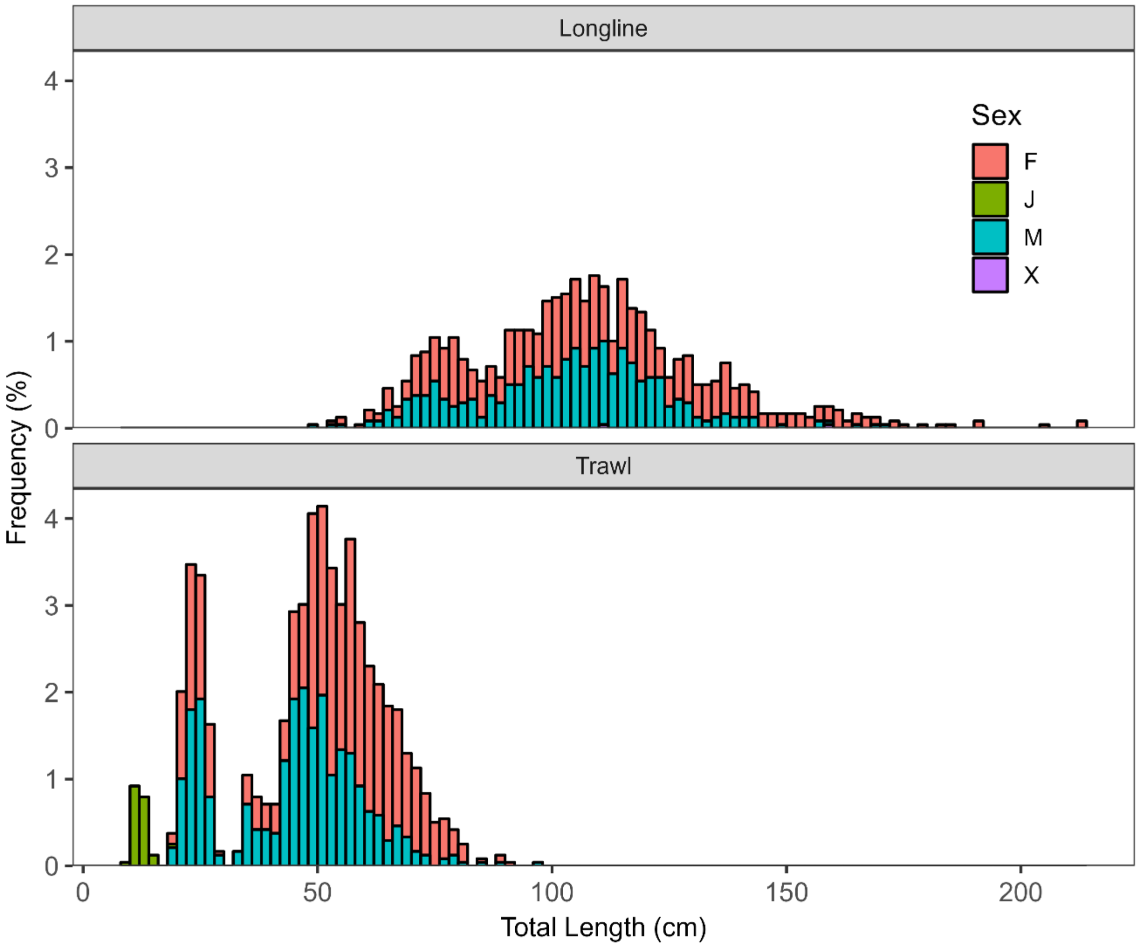

Figure 2: Length frequency distribution for Patagonian toothfish sampled in the longline (n=994) and trawl-based (n=1397) fisheries.

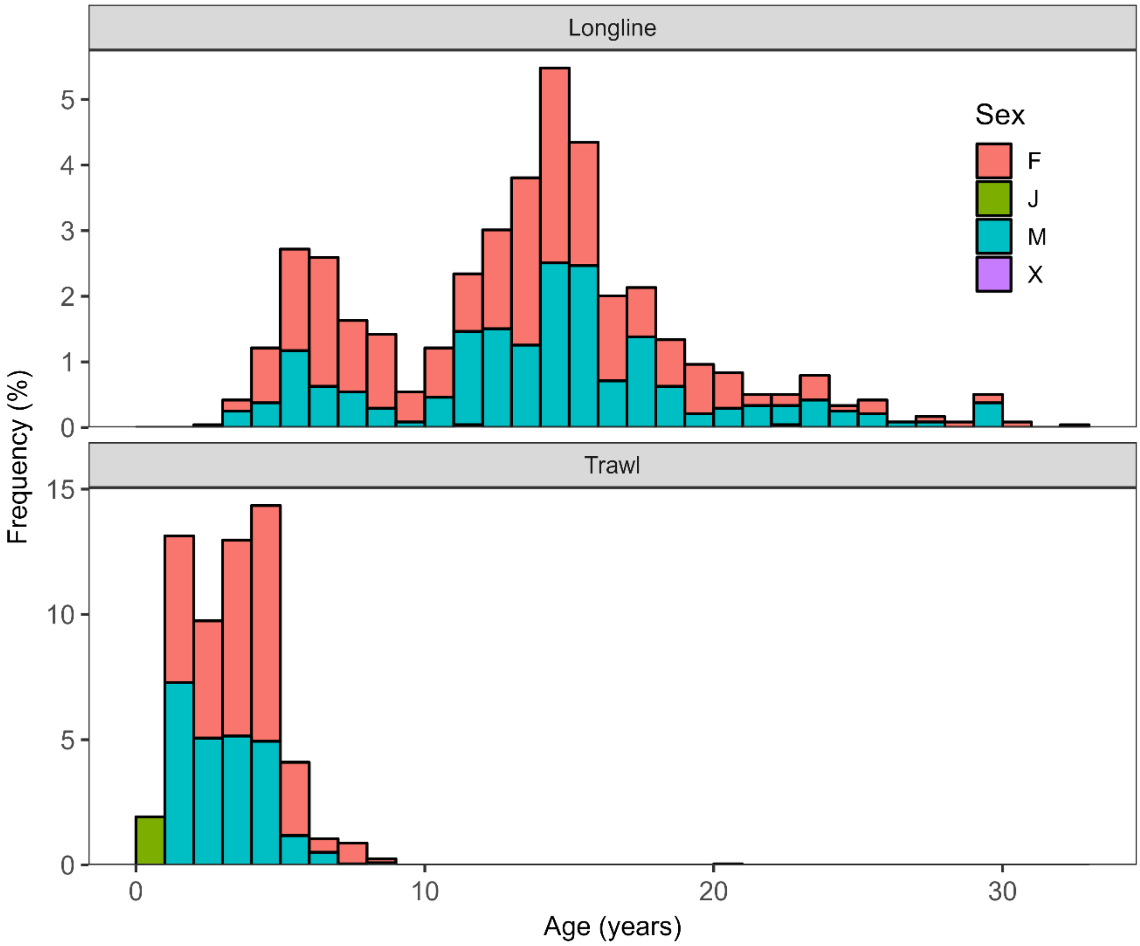

Figure 3: Age frequencies estimated from the total sampled catch of Patagonian toothfish in the longline (n=240) and trawl-based (n=230) fisheries.

3.3. Otolith interpretation

The clarity of zonation patterns in Patagonian toothfish otoliths varied greatly with complex patterns. Some otoliths displayed distinct banding with distinct wide annuli nearer to the core, becoming increasingly narrow with age (Figure 4A). Frequently, otoliths displayed a distinct zonal structure with a dark region closer to the core, a transition zone, followed by a more translucent region extending to the otolith margin (Figure 4B). The inner dark zone of the otoliths usually contained between 3 and 5 annuli, with this region often containing macrostructures considered to be false rings (also noted by Horn, 2002). The outer, translucent region of the otolith usually consisted of narrow yet regular annuli. In some otoliths annuli within this region could barely be discernible due to the extent of translucence. Interpretation of the banding structure across the transition zone was often complex with variable rates of change from the wider annuli present in the dark zone compared to the narrow banding structure observed in the outer translucent region of the otolith.

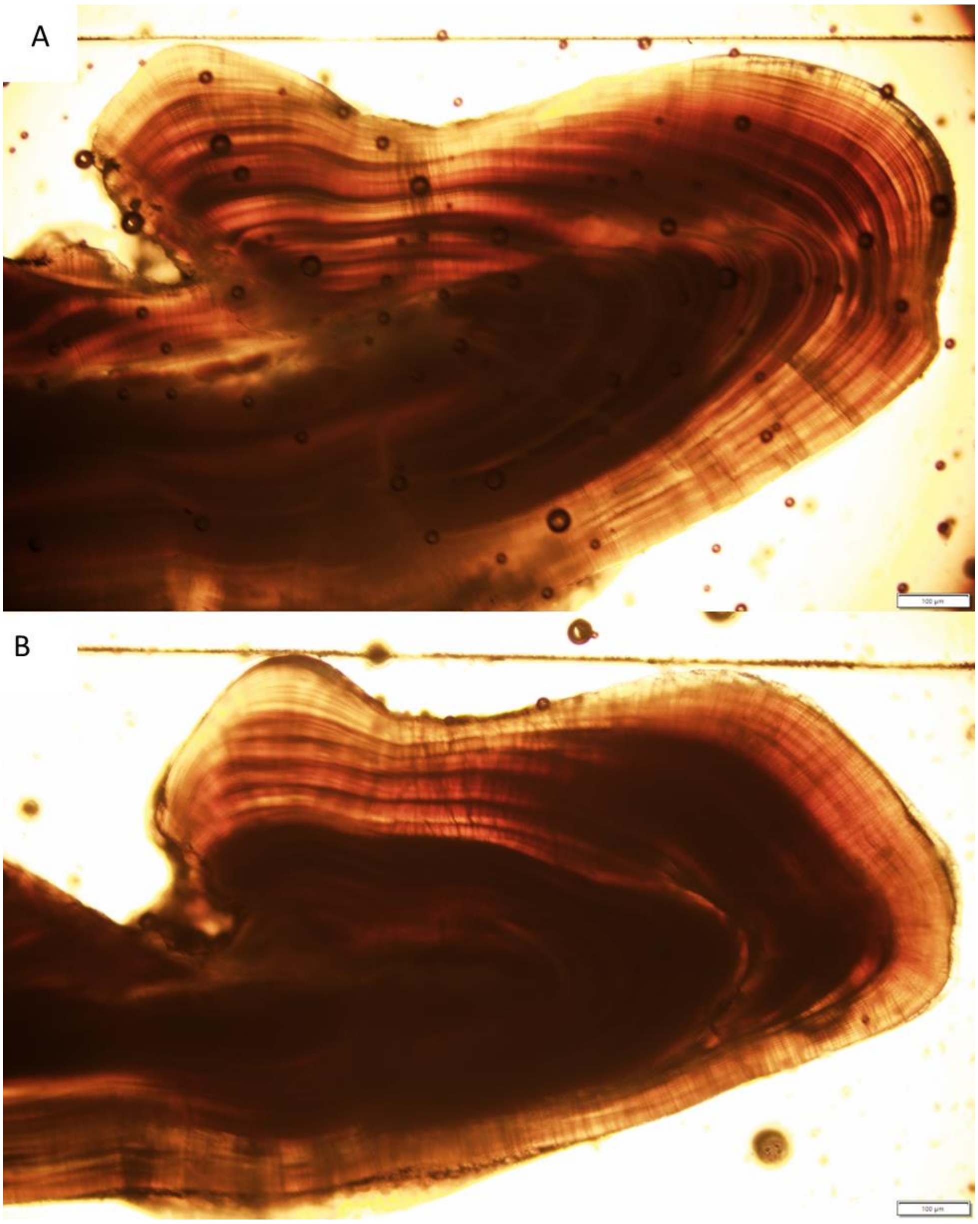

Figure 4: Sectioned otolith from (A) a 93 cm and (B) 88 cm male Patagonian toothfish with estimated ages of 10 and 11 years, respectively.

3.4. Age and growth

Likelihood ratio tests indicated significant differences in growth between male and female Patagonian toothfish ($\chi^2_{(df=3,\ n=467)}$ = 153.81; p < 0.001 ***). Likelihood ratio tests indicated significant differences in the $L\infty$ ($\chi^2_{(df=1,\ n=467)}$ = 25.98; p < 0.001 ***) parameter estimates for male and female fish. Likelihood ratio tests indicated significant differences in the *K* ($\chi^2_{(df=1, n=467)}$ = 13.40; $p < 0.001$ ***) parameter estimates for male and female fish.

Calculated von Bertalanffy growth parameters and their 95% confidence intervals for male and female fish were presented in Table 1 and Figure 5. Female fish generally grew to a larger size and age compared to males (Table 1 and Figure 5).

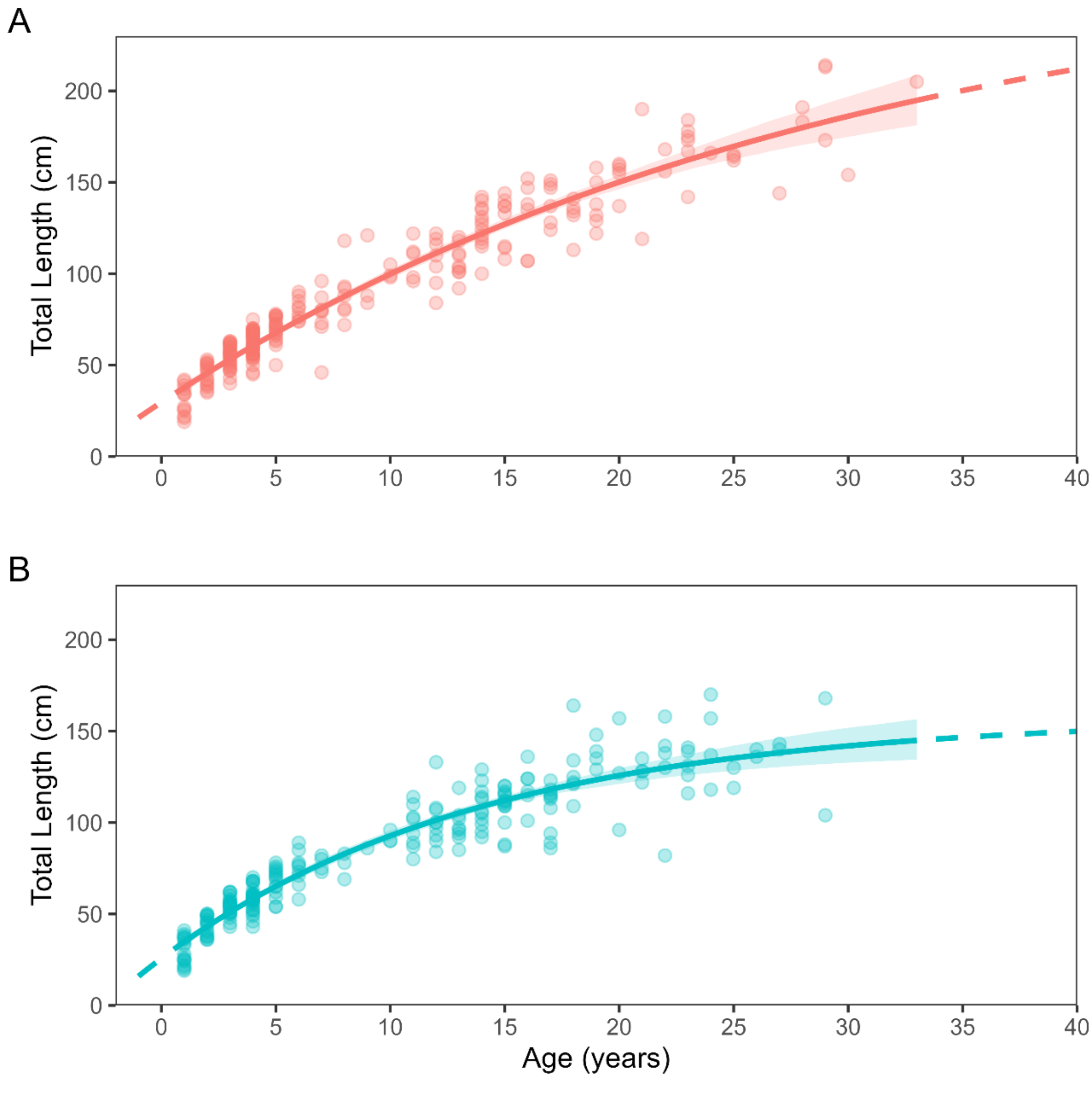

Figure 5: Length versus age with superimposed best-fit von Bertalanffy growth model and 95% confidence bands for (A) female (n=235) and (B) male (n=232) Patagonian toothfish sampled during 2021.

Table 1: von Bertalanffy parameters estimates for (A) female (n=235) and (B) male (n=232) Patagonian toothfish sampled during 2021 with their 95% confidence intervals, 95% LCI (Lower Confidence Interval) and 95% UCI (Upper Confidence Interval).

Sex	Parameter	Estimate	95% LCI	95% UCI
Female	L_∞	277.435	215.384	414.432
	K	0.033	0.019	0.052
	t_0	-3.394	-4.409	-2.38
Male	L_∞	157.58	138.23	188.58
	K	0.071	0.05	0.098
	t_0	-2.499	-3.555	-1.676

3.5. Mortality estimates

The threshold age for the mortality estimates for Patagonian toothfish was 1 yo for both male and female (Figure 6). The mortality estimates obtained from the catch curves was significantly different between sexes (p = 1.466e-09 ***; Table 2, Figure 6).

Table 2: Estimates of total annual mortality rate (A) and instantaneous total mortality rate (Z) for Patagonian toothfish using the catch-curve (regression) method and their 95% confidence intervals, 95% LCI (Lower Confidence Interval) and 95% UCI (Upper Confidence Interval).

Sex	Parameter	Estimate	Std Error	95% LCI	95% UCI
Female	A	13.92	NA	10.87	16.87
	Z	0.15	0.017	0.12	0.18
Male	A	10.73	NA	6.32	14.93
	Z	0.11	0.0234	0.07	0.16
Combined	A	12.85	NA	10.14	15.48
	Z	0.14	0.015	0.11	0.17

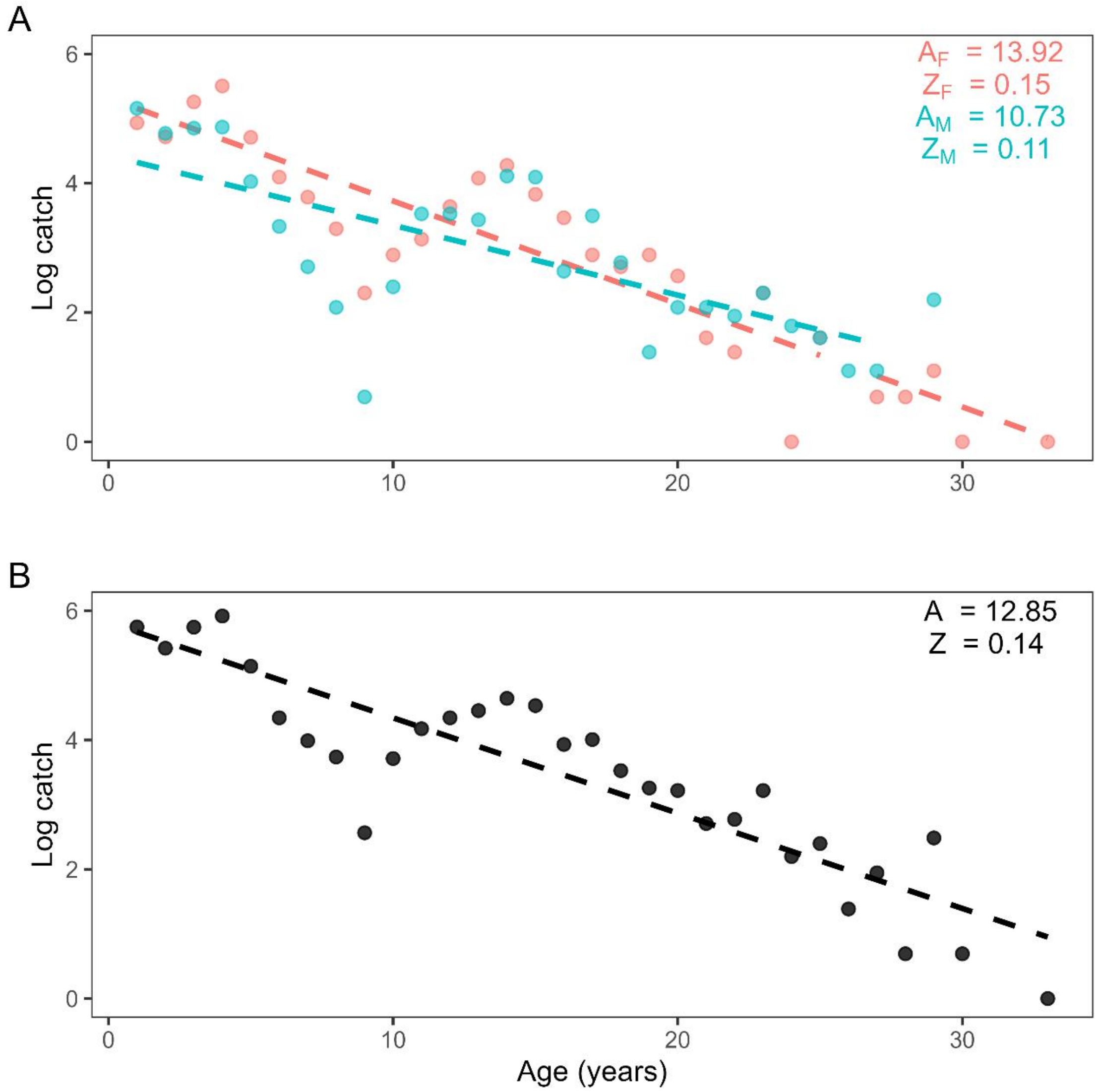

Figure 6: Catch at age of Patagonian toothfish for female and male (A) and for combined sex (B) captured around the Falkland Islands. The points were used to compute the regression-based (dashed line) estimates of *A* and *Z*.

3.6. Precision of the age estimates

The two readings (age estimation) by the primary reader were similar at 55.74% and a difference of one year between these two readings was found in 26.38% of the otolith readings (n = 470; Table 3). APE and ACV results, respectively 4.28 and 6.05 (Table 4), indicated that ageing precision was relatively high in Patagonian toothfish.

Table 3: Percentage table of raw differences between multiple readings of Patagonian toothfish otoliths (n=470).

	Age difference (%)						
	0	1	2	3	4	5	6+
Age1 v. Age 2	55.74	26.38	7.23	4.68	3.19	0.85	1.91

Table 4: Precision indices for age estimates of Patagonian toothfish. ASD = The average (across all fish) standard deviation of ages within a fish; ACV = The average (across all fish) coefficient of variation of ages within a fish using the mean as the divisor. AAD = The average (across all fish) absolute deviation of ages within a fish; APE = The average (across all fish) percent error of ages within a fish using the mean as the divisor.

n	R	Agreement (%)	ASD	ACV	AAD	APE
470	2	55.74	0.6	6.05	0.43	4.28

4. Conclusion

Results of the current study provide biological parameters for Patagonian toothfish in the Falkland Islands for 2021. Our findings indicate that the prescribed ageing protocol provides a reliable method for age estimation for the successful application of empirical age-length keys for the assessment of the Patagonian toothfish stock. Nonetheless, difficulties remain in interpreting patterns of annuli formation.

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Otolith age estimation: Brendon Lee

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