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

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AGE- 2022-TOO

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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). Given the critical role that age plays in these models, any bias in age estimates can significantly affect the perception of the stock and fishing mortality, leading to inaccurate predictions of stock size and related management advice.

The Patagonian toothfish (*Dissostichus eleginoides*) is a large, bentho-pelagic notothenioid fish that can reach a total length of up to 200 cm. It is found across various islands, seamounts, and shelf areas of the Sub-Antarctic (Collins et al., 2010). Its distribution covers the Antarctic Polar Front and extends northward over the Patagonian Shelf in the Atlantic Ocean, off the coast of Chile in the Pacific, and to 40°S in the southwestern Indian Ocean (Laptikhovsky et al., 2006). This species has the broadest depth range of any teleost fish, inhabiting waters from 10 m to 2500 m deep (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 1,040 tons since 2015 (Skeljo, 2023). Additionally, juvenile and sub-adult toothfish are often captured as bycatch in finfish and squid trawl fisheries.

This annual report presents a reliable methodology for Patagonian toothfish age estimation, providing age-length keys and growth parameters estimations from samples collected in the Falkland Islands during 2022. It also aims to provide estimates of intra-reader bias and precision in age estimations, establishing the reliability of the age estimation protocol and its potential application in stock assessment and subsequent management advice.

2. Materials and methods

2.1. Data collection

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

On the longliner, sampling consists in measuring the length frequency of 40 random individuals per day, and collecting the otoliths, length, weight and maturity of 10 random individuals (FIFD, 2023.a). On the trawler, there is no target number for the sampling of Patagonian toothfish. They are opportunistically sampled for otolith collection, such as the other bycatch species (FIFD, 2023.b).

Sampled Patagonian toothfish are 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). This macroscopic key was defined by Nikolsky (1963) and adjusted for Falkland Islands species according to Brickle *et al.* (2005). Otoliths are collected to meet the otolith's collection requirement of 5/sex/cm/quarter on trawlers and 3/sex/cm/quarter on longliner. Quarterly time periods consisted in A: January – March, B: April – June, C: July – Sep and D: October – December. Otoliths are stored in paper envelope, and otoliths belonging to individuals smaller than 80 cm are stored in eppendorf filled with 96% ethanol to avoid them to break during transportation.

A minimum of 60 otoliths per sex and quarterly collection are selected to cover the length distribution of sampled fish for age estimation (n = 480). The selection of otoliths for age estimation consists in going across the otolith collection manually and selecting 60 otoliths/sex/quarter to cover the length frequency of the analysed year, and ensuring that the selection overlap the trawl and longline fisheries for the length shared between the two fisheries. This ensures that sufficient otoliths are aged for all lengths on a temporal and spatial basis. Noted that we did not actively select otoliths in different spatial areas. We considered that the spatial distribution is covered by selecting otoliths in each quarterly collection because the fishing vessels are targeting different areas on a weekly or monthly basis.

2.2. *Preparation of otoliths*

Otoliths were cleaned and embedded in rows of five within blocks of clear epoxy resin (West system epoxy: 105 epoxy Resin and 206 Slow Hardener; https://www.westsystem.com/). The blocks were then ground to create smooth linear surfaces to guide the cutting angle and ensured that sections were cut precisely at right angles. Nuclei locations were delineated, and blocks were subsequently sectioned using a BUEHLER IsoMet® Low Speed Saw. Between two and six sections, each with a thickness of 0.35 mm, were taken from each resin block and mounted onto microscope slides beneath coverslips using clear epoxy resin.

2.3. *Otolith interpretation and age estimation*

Sections of otoliths were examined under reflected light at magnifications ranging from 20 to 40 times. For each row of otoliths, the section closest to the primordium was selected for age estimation. The primordium is the initial complex structure of an otolith, formed during the embryonic stage of fish development. It is the origin point for all growth in the otolith. Photographs of the best section were taken, and age estimation was performed using ImageJ software.
Following established methods for estimating the age of this species, we selected the sector from the primordium to the proximal edge of the section on the ventral side of the sulcus to count the increments (Figure 1). In some preparations, however, increments on the dorsal side were equally clear as those on the ventral side.

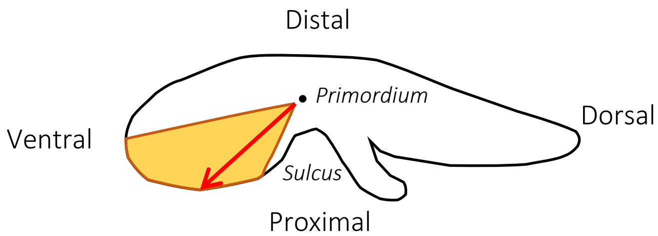

Figure 1. Localisation of the preferred zone (in orange) for the reading of Patagonian toothfish otolith for age estimation.

For each reading, we recorded the otolith's readability, the estimated number of rings, the reader's name, and the date of the reading. A five-point readability scale, as described by Sutton et al. (2012) (Table 1), was used.

Table 1. Five-point otolith readability scores used to characterise otolith's reading (Sutton *et al*., 2012).

Readability	Synthetic description	Description
1	Very easy	Otolith very easy to read; excellent contrast between successive opaque and translucent zones.
2	Easy/Good	Otolith easy to read; good contrast between successive opaque and translucent zones, but not as marked as in 1; potential error ± 1 opaque zone.
3	Readable/Fair	Otolith readable; less contrast between successive opaque and translucent zones than in 2, but alternating zones still apparent; potential error ± 2 opaque zones.
4	Readable with difficulty/Poor	Otolith readable with difficulty; poor contrast between successive opaque and translucent zones; potential error ± 3 opaque zones.
5	Unreadable	Otolith unreadable.

The first and most crucial step is to identify the first increment. In Patagonian toothfish, this can be challenging due to a large dark region at the center of the otolith (Figure 2.a). The first opaque zone is most easily located by identifying the first translucent zone outside of the dark core, followed by the adjacent dark (opaque) band. In sections that include the primordium, the sulcus acusticus usually extends to the edge of the first increment (Kalish and Timmiss, 2000). Starting from the nucleus, each annulus comprises one opaque and the next adjacent translucent zone. The opaque zone inhibits light passage, appearing dark under transmitted light and bright under reflected light. Conversely, the translucent zone, also referred to as the hyaline zone, allows light to pass through, appearing bright under transmitted light and dark under reflected light.

Most of the Patagonian toothfish otoliths exhibit a clear zonal structure, characterised by a dark region near the core, a transition zone, and a more translucent area extending to the edge of the otolith (Figure 2). The annuli near the core tend to be wider and gradually become narrower with age (Figure 2.b). Typically, the inner dark zone contains between three to five annuli and often includes macrostructures known as false checks (as noted by Horn, 2002). False checks are translucent zones that can be confused with annuli but lack consistency in their appearance. These false checks should not be counted as annulus. The outer translucent region generally comprises narrow but consistent annuli. However, in some otoliths, the annuli in this area may be difficult to distinguish due to the level of translucence.

When annual rings cannot be clearly delineated or counted, otoliths are considered unreadable and eliminated from the age estimation process. All counts of annuli were made without prior knowledge of fish size or previous age estimates.

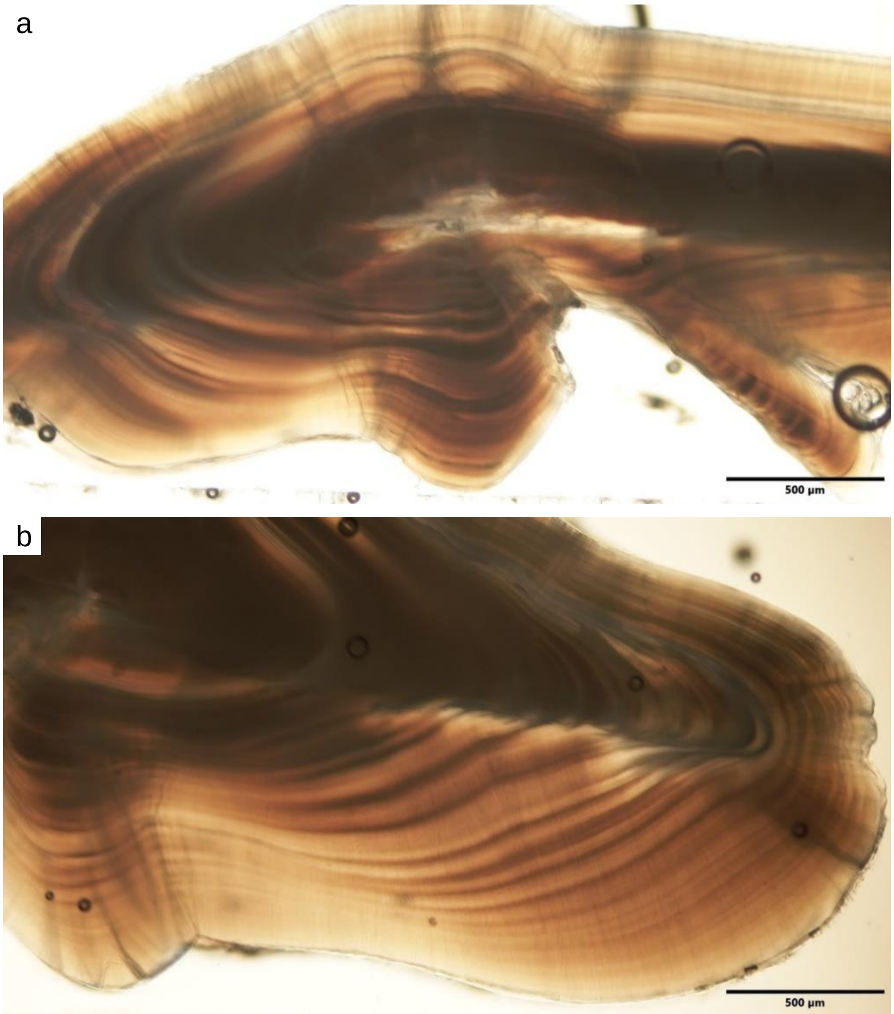

Figure 2. Patagonian toothfish sectioned otolith from (a) 60 cm female and (b) 136 cm male with estimated ages 5 and 20 years old, respectively.

Estimated ages were determined based on the number of ring estimates and the quarter of the year in which the individual was captured. For individuals caught between July and December, the age is calculated as the estimated number of rings plus one (La Mesa, 2007; Sutton et al., 2012). This adjustment accounts for the timing of ring deposition (La Mesa, 2007).

2.4. Precision of the age estimates

Repeated readings of the same otoliths provide a measure of intra-reader or inter-reader variability. While these readings do not validate the assigned ages, they provide an indication of the expected error associated with a set of age estimates due to variations in the interpretation of an otolith.

After the first reading of each otolith, we randomly sampled 30% of the aged otoliths for a second reading by the primary reader. This subsample is used to calculate the reading precision (Morison *et* *al*., 1998; Campana, 2001). Although the literature suggests that 25% of the otoliths could be used for a second reading (Morison et al., 1998; Campana, 2001), we opted to increase this sample size to 30% due to the recent training of the primary reader.

To examine the precision among age estimations, we calculated the four following indices across all age estimations using ageBias function of the FSA package (Ogle, 2016): 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, X_{ij} is the i^{th} determination for the j^{th} fish, and X_j is the average estimated age of the j^{th} 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 jth fish.

When the calculated Average Percent Error (APE) and Average Coefficient of Variation (ACV) are close to the 5% threshold, it indicates that the intra-reader precision in age estimation is relatively good (Morison et al., 1998; Campana, 2001). In such cases, the first reading is retained for age estimation.
For otoliths where the two readings differ by more than 4 years, a third reading is conducted, and this reading is used as the estimated age.
If the calculated APE and ACV exceed 7% (Morison et al., 1998; Campana, 2001), a third reading is performed on 30% of the selected otoliths. Additionally, 10% of the otoliths that were already aged are

aged a second time to calculate APE and ACV with a higher percentage of the second reading. Subsequently, APE and ACV are recalculated for 40% of the re-aged otoliths.

If the new APE (APE2) and ACV (ACV2) are still close to the 5% threshold, we follow the same procedure mentioned earlier. However, if APE2 and ACV2 indicate that the age estimation is inadequate, all otoliths are reread.

A collection of otolith images, representing a range of lengths, ages, and readability from otoliths collected in the Falkland Islands fisheries, is set to be completed by June 2025. This reference collection will enhance the accuracy of otolith readings, support reader training, and ensure consistency in the reading process for Patagonian toothfish. The collection will be developed in collaboration with scientists from the British Antarctic Survey, who will provide a second expert review of the otoliths.

2.5. Estimation of von Bertalanffy parameters

A von Bertalanffy growth function (VBGF) 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.

VBGFs were compared among sexes following the 'likelihood ratio test' approach outlined in Ogle (2016). The process requires fitting up to eight models to examine differences in VBGFs among sexes (Table 2). The most complex model $\{L_\infty, K, t_0\}$ (Table 2) represents the case where all three parameters differ among sexes. The simplest model {Ω} (Table 2) represents the case where no parameters differ among sexes. Between these two extremes are three models where two parameters differ among sexes ($\{L_\infty, K\}$, $\{L_\infty, t_0\}$, and $\{K, t_0\}$; Table 2) and three models where a single parameter differs among sexes ($\{L_\infty\}$, $\{K\}$, and $\{t_0\}$; Table 2). More complex models (i.e. with more parameters different among sexes) are sequentially tested against the simpler nested models using likelihood ratio tests. Any nested model that is not statistically different from the more complex model is considered better because it fits equally (statistically) well, but is more parsimonious.

A parametric bootstrapping procedure with 1,000 iterations was then used to estimate 95% confidence intervals for the final parameter estimates (Baty *et al.*, 2015).

Table 2. The family of models considered when examining differences in VBGFs among sexes. The abbreviations denote which parameters differ among groups for that model. No parameters differ among groups for the Ω model.

Abbreviation	Model
$\{L_\infty, K, t_0\}$	$L_t \sim L_\infty$ [sex]*(1- e (-K [sex]*(age- t_0 [sex])))
$\{L_\infty, K\}$	$L_t \sim L_\infty$ [sex]*(1- e (-K [sex]*(age- t_0)))
$\{L_\infty, t_0\}$	$L_t \sim L_\infty$ [sex]*(1- e (-K *(age- t_0 [sex])))
$\{K, t_0\}$	$L_t \sim L_\infty$ *(1- e (-K [sex]*(age- t_0 [sex])))
$\{L_\infty\}$	$L_t \sim L_\infty$ [sex]*(1- e (-K *(age- t_0)))
$\{K\}$	$L_t \sim L_\infty$ *(1- e (-K [sex]*(age- t_0)))
$\{t_0\}$	$L_t \sim L_\infty$ *(1- e (-K *(age- t_0 [sex])))
$\{\Omega\}$	$L_t \sim L_\infty$ *(1- e (-K *(age- t_0)))

3. Results and discussion

3.1. Spatial and length-frequency distribution of samples

Biological information was obtained from a total of 6295 Patagonian toothfish samples. Of these, 5301 and 994 were sampled from the longline and trawl-based fisheries, respectively. We randomly selected 478 Patagonian toothfish to estimate their ages. However, we used only 477 otoliths to perform age estimation analyses as 1 otolith was considered unreadable and was 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 140 and 382 m depth (mean = 231 m; Figure 3). In the longline fishery, Patagonian toothfish were sampled at depths between 830 and 1820 m (mean = 1368 m) across the FI waters where the Toothfish fishery usually occurs (Figure 3Figure).

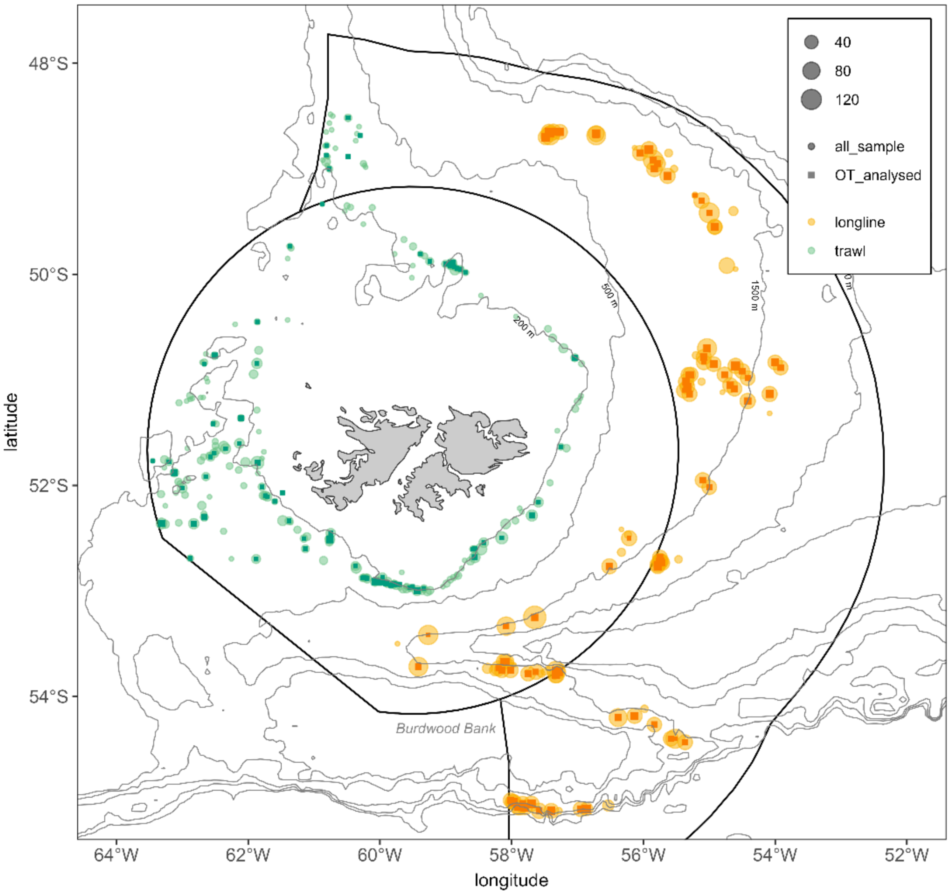

Figure 3. Positions of Patagonian toothfish sampling for otolith and length frequency around the Falkland Islands during 2022 (n=6295). Circle with a light colour indicates the position of the collected samples and square with a dark colour the position of the otoliths selected for age estimation.

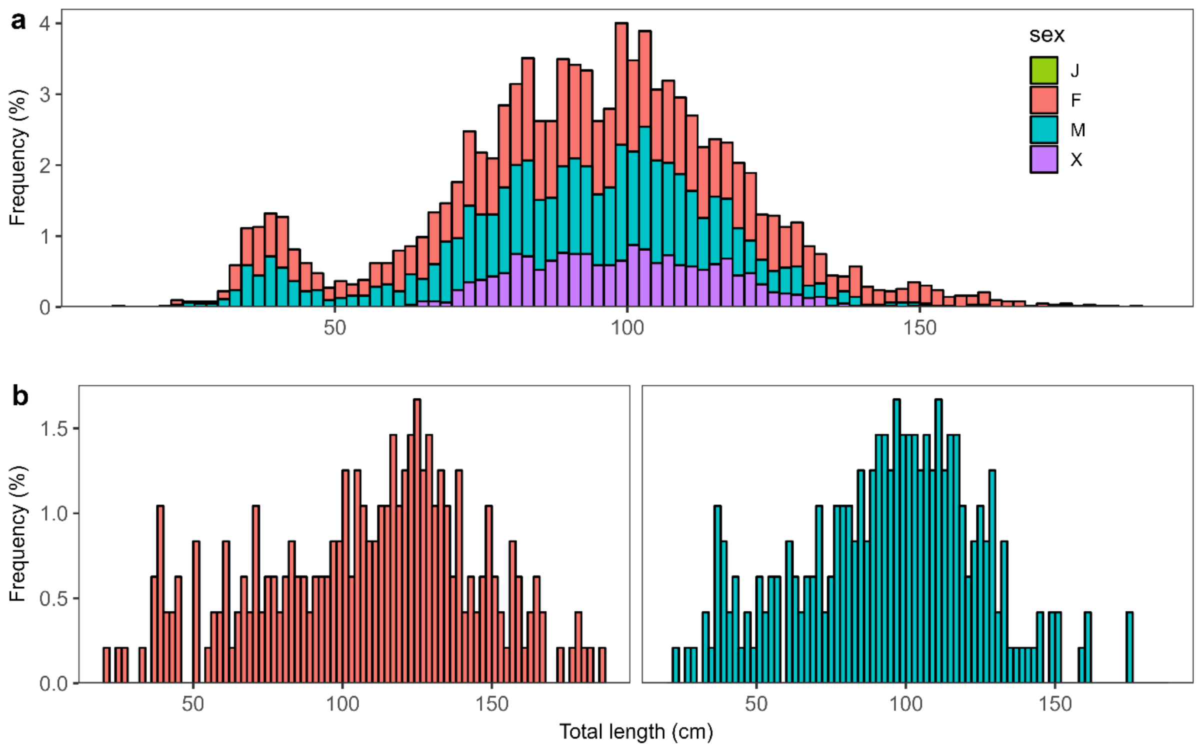

Figure 4. Length frequency distribution for Patagonian toothfish (a) sampled in the longline and trawl-based fisheries (n=6295), and (b) female (red) and male (blue) selected for age estimation (n= 478).

3.2. Length and age composition

In the trawl-based fisheries, lengths ranged between 12.5 and 120 cm TL (mean ± sd = 51.6 ± 16 cm). The distribution showed a clear modal peak occurring around 38 cm TL, reflecting age-1 and age-2 fish (Figure 5). 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 9 years old (yo; quantile 0.1 = 2 yo and quantile 0.9 = 9 yo; Figure 6).

The longline-based fishery targeted a different part of the Patagonian toothfish stock with lengths ranging from 54 to 187 cm TL (mean ± sd = 100 ± 19.7 cm), with clear modal peaks occurring around 90 and 101 cm TL for both male and female fish and a third modal peak could be highlight around 149 cm for female (Figure 5**Error! Reference source not found.**). These modal peaks of longline caught Patagonian toothfish seemed to reflect fish around 7 yo and 16 yo (Figure 6). The majority of Patagonian toothfish being caught within the longline fishery were older than 9 yo (quantile 0.1 = 9 yo and quantile 0.9 = 21 yo; Figure 6).

Combining all the fisheries data, ages estimated ranged from 1 to 42 yo for females and 1 to 27 yo for males (Figure 6).

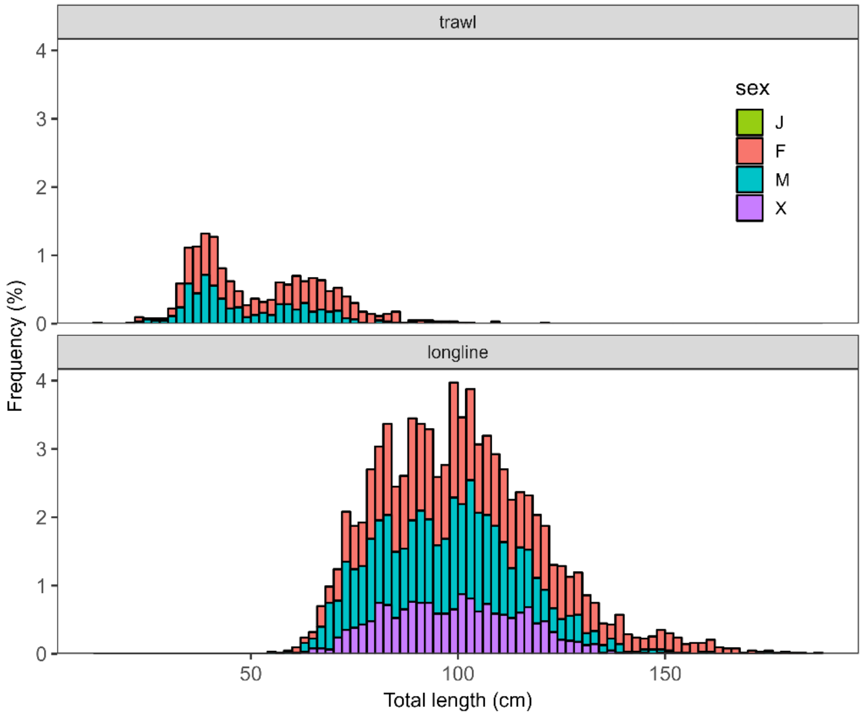

Figure 5. Length frequency distribution for Patagonian toothfish sampled in the trawl-based (n=994) and longline (n=5301) fisheries

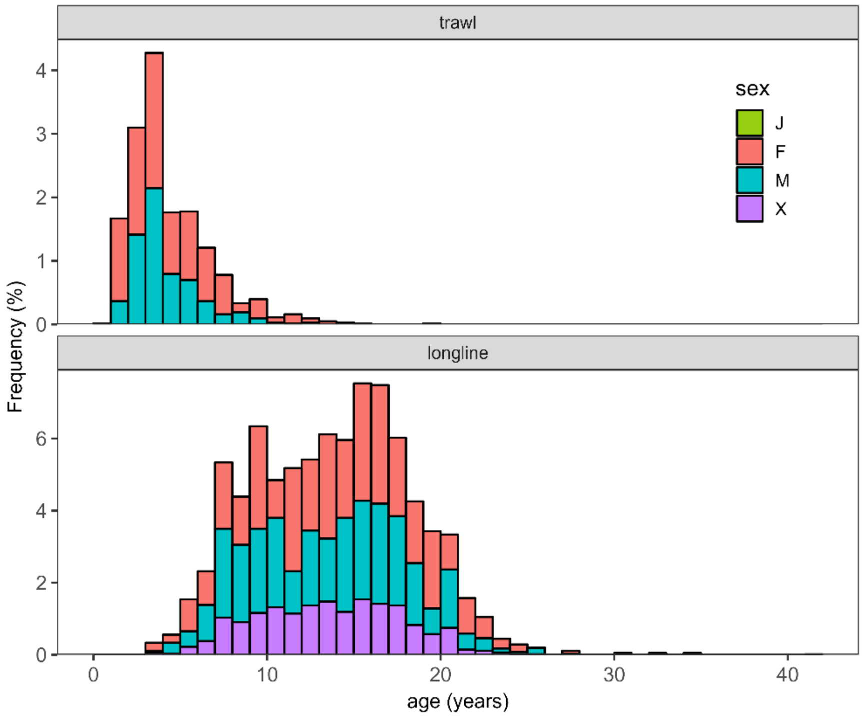

Figure 6. Age frequencies estimated from the total sampled catch of Patagonian toothfish in the trawl-based (n=994) and longline (n=5301) fisheries (based on a sub-sample of 367 otoliths from the longline and 110 otoliths from the trawl-based fisheries).

3.3. *Age at length - von Bertalanffy growth model*

The {L_∞} model (Table 2; only L_∞ differs between the sexes) fitted the data statistically equally well as more complex models (where two or all three parameters differ between the sexes), but better than the simpler nested model (where no parameters differ between the sexes). Therefore, it was considered the best model, and the growth was described by two growth curves with shared K and t_0, but different L_∞ for males and females.

Calculated von Bertalanffy growth parameters were presented in Figure 7 and Table 3, and their 95% confidence intervals in Table 3. Female fish generally grew to a larger size and age compared to males (Table 3 and Figure 7).

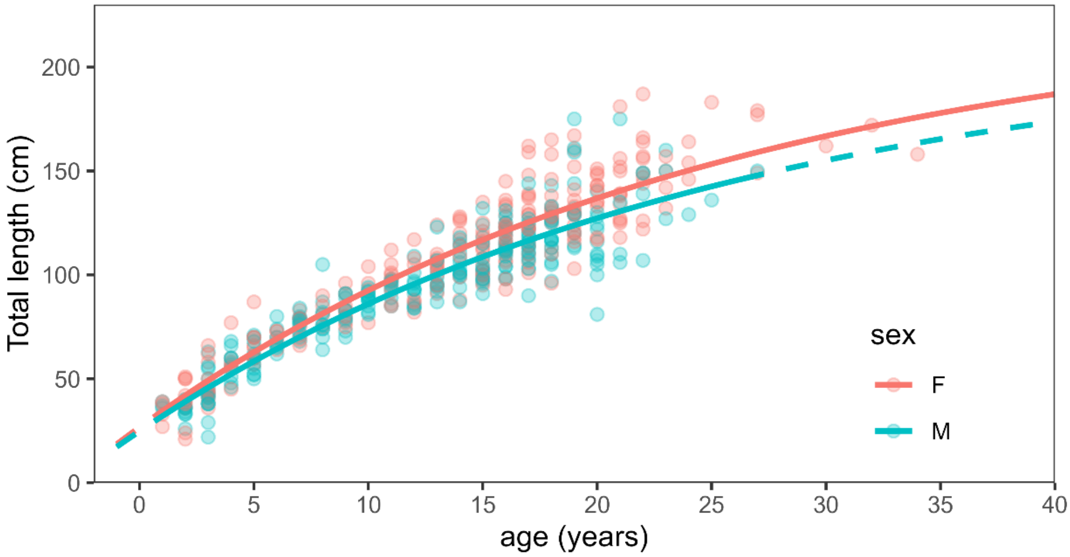

Figure 7. Length versus age with superimposed best-fit von Bertalanffy growth model for female (n=238) and male (n=239) Patagonian toothfish sampled in 2022.

Table 3. von Bertalanffy parameters estimates for female (n=238) and male (n=239) Patagonian toothfish sampled during 2022 with their 95% confidence intervals, 95% LCI (Lower Confidence Interval) and 95% UCI (Upper Confidence Interval).

Sex	Parameter	Estimate	95% LCI	95% UCI
Female	$L\infty$	228.565	204.400	269.991
Male	$L\infty$	212.517	189.159	251.664
Both	K	0.039	0.029	0.049
	t0	-3.147	-4.028	-2.379

3.4. Precision of the age estimates

A sub-sample of 30% of the otoliths used for age estimation was randomly selected to measure the precision of age estimate (n = 143; Table 4 and 5Table). The two readings (age estimation) by the primary reader were equal at 53.85% (n = 143; Table 4 and 5). A difference of 1 year and more than 2 years between these two readings was found in 30% and 16.15 % of the otolith readings, respectively (n = 143; Table 4). APE and ACV results, respectively 3.34 and 4.72 (Table 5), were both below the 5% which indicated a relatively good precision in Patagonian toothfish age estimation (Morison *et al.*, 1998; Campana, 2001).

Table 4. Percentage table of raw differences between multiple readings of Patagonian toothfish otoliths (n=143).

	0	1	2	3	4	5
Age1 v. Age 2	53.85	30.07	12.59	1.4	1.4	0.7

Table 5. 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
143	2	53.85	0.48	4.72	0.34	3.34

4. Conclusion

The results of the current study provide biological parameters for Patagonian toothfish in the Falkland Islands for 2022. Our findings indicate that the prescribed ageing protocol provides a reliable method for age estimation, and for the successful application of empirical age-length keys for the Patagonian toothfish stock assessment.

Emilie Le Luherne: Writing, Data analysis, Otolith age estimation, and Otoliths pictures.
Zhanna Shcherbich and observers: Selection of otoliths, and Otoliths processing.

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