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

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AGE- 2025-TOO

Emilie Le Luherne

Falkland Islands Government
Directorate of Natural Resources
Fisheries Department
Stanley, Falkland Islands

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Emilie Le Luherne: otoliths selection, processing, pictures, and age estimation, data analysis, and writing.

Louis Desmet, Riccardo Minichino, and Niccolo Orlandi: otoliths processing.

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 2025. 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

All the following steps are more detailed in the "Manual for routine age estimation of Patagonian toothfish (*Dissostichus eleginoides*) (Le Luherne, 2026).

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 F/V Argos Vigo (ZDLU1) operating bottom trawls during research surveys in 2025.

On the longliner, sampling consists in measuring the length of 40 random individuals per day, and collecting the otoliths, length, weight and maturity of 10 random individuals (FIFD, 2023.a). On trawlers, there is no target number for the sampling of Patagonian toothfish. They are opportunistically sampled for otolith collection, same 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 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 envelopes, and otoliths belonging to individuals smaller than 80 cm are stored in eppendorf tubes filled with 96% ethanol to avoid them to break during transportation.

A minimum of 60 otoliths per sex and quarterly collection were selected to cover the length distribution of sampled fish for age estimation (n = 480). Using a R script, we selected 20 otoliths/sex/quarterly collection for trawl and 40 otoliths/sex/quarterly collection for longliner to cover the length frequency of the analysed year, and ensuring that the selection overlapped the trawl and longline fisheries for the length shared between the two fisheries. We selected otoliths to have at least one individual per 5 cm length class by every sex/activity/quarterly collection. To reach the selection of 500 otoliths, we additionally randomly selected large individuals with a total length greater than 120 cm. This is to account for the variability of the age distribution of larger individuals.

This selection 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 ensure 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 four sections, each with a thickness of 0.4 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.

In accordance with the CCAMLR Age Determination Workshop guidelines (WS-ADM3-2025, CCAMLR, 2025), annual increments are estimated in the proximal area on both the ventral and dorsal side (reading path ventral = rpv and reading path dorsal = rpd, Figure 1). However, if one side is unclear, age estimation could be done only on one side.

For each reading, we recorded the otolith's readability, the estimated number of rings on both rpv and rpd, the reader's name, and the date of the reading. All counts of annuli were made without prior knowledge of fish size or previous age estimates. A five-point readability scale, as described by Sutton et al. (2012) (Table 1), was used.

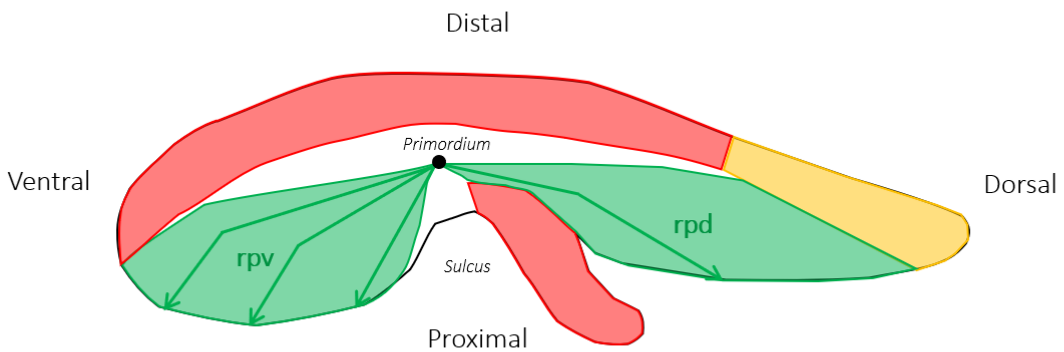

Figure 1. Localisation of the preferred zone (in green) for the reading of Patagonian toothfish otolith for age estimation, reading path ventral (rpv) and reading path dorsal (rpd). Red areas are unsuitable for age estimation and orange area may be helpful to confirm annuli in some specimens, but this area is prone to split and create double bands.

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. 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.

It is useful to make measurements of the distance from the primordium to the outer proximal edge, and from the primordium to the proximal edge of the first increment using the image measurement tool in ImageJ. Measurements taken on the juvenile Patagonian toothfish used in Lee et al. (2024), indicated that the distance from the primordium to the first opaque zone ranged from 0.28 to 0.32 mm when measured proximally (Le Luherne, 2026). These known distances can be a useful reference when identifying the first increment.

Most 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. The annuli near the core tend to be wider and gradually become narrower with age. 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.

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 25% 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).

To examine the precision among age estimations, we calculated the four following indices across all age estimations using the 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 *j*th 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 the limits for acceptable age discrepancies between reading (Nowara et al., 2024), 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.
Once the reference collection becomes available, the procedure described above will be updated, and the reader will need to retrain using the reference collection before rereading the routine collection.

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 between 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_∞, 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_∞, K}, {L_∞, t_0}, and {K, t_0}; Table 2) and three models where a single parameter differs among sexes ({L_∞}, {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 1000 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_∞, K, t_0}	L_t ~ L_∞ [sex]*(1- e (-K [sex]*(age- t_0 [sex])))
{L_∞, K}	L_t ~ L_∞ [sex]*(1- e (-K [sex]*(age- t_0)))
{L_∞, t_0}	L_t ~ L_∞ [sex]*(1- e (-K *(age- t_0 [sex])))
{K, t_0}	L_t ~ L_∞ *(1- e (-K [sex]*(age- t_0 [sex])))
{L_∞}	L_t ~ L_∞ [sex]*(1- e (-K *(age- t_0)))
{K}	L_t ~ L_∞ *(1- e (-K [sex]*(age- t_0)))
{t_0}	L_t ~ L_∞ *(1- e (-K *(age- t_0 [sex])))
{Ω}	L_t ~ L_∞ *(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 10842 Patagonian toothfish samples in 2025. Of these, 4802 and 6040 were sampled from the longline and trawl fisheries, respectively.

We randomly selected 484 Patagonian toothfish to estimate their ages. However, we used only 483 otoliths to perform age estimation analyses, because 1 otolith was considered unreadable and was eliminated from the age estimation process. The selected 483 fish included 3 juveniles, 255 females, and 225 males.

In the trawl fisheries, Patagonian toothfish were sampled from across the shelf to the south and west of the Falkland Islands at depths between 125 and 307 m depth (mean = 213 m; Figure 2). In the longline fishery, Patagonian toothfish were sampled at depths between 770 and 1950 m (mean = 1332 m) across the FI waters where the toothfish fishery usually occurs (Figure 2).

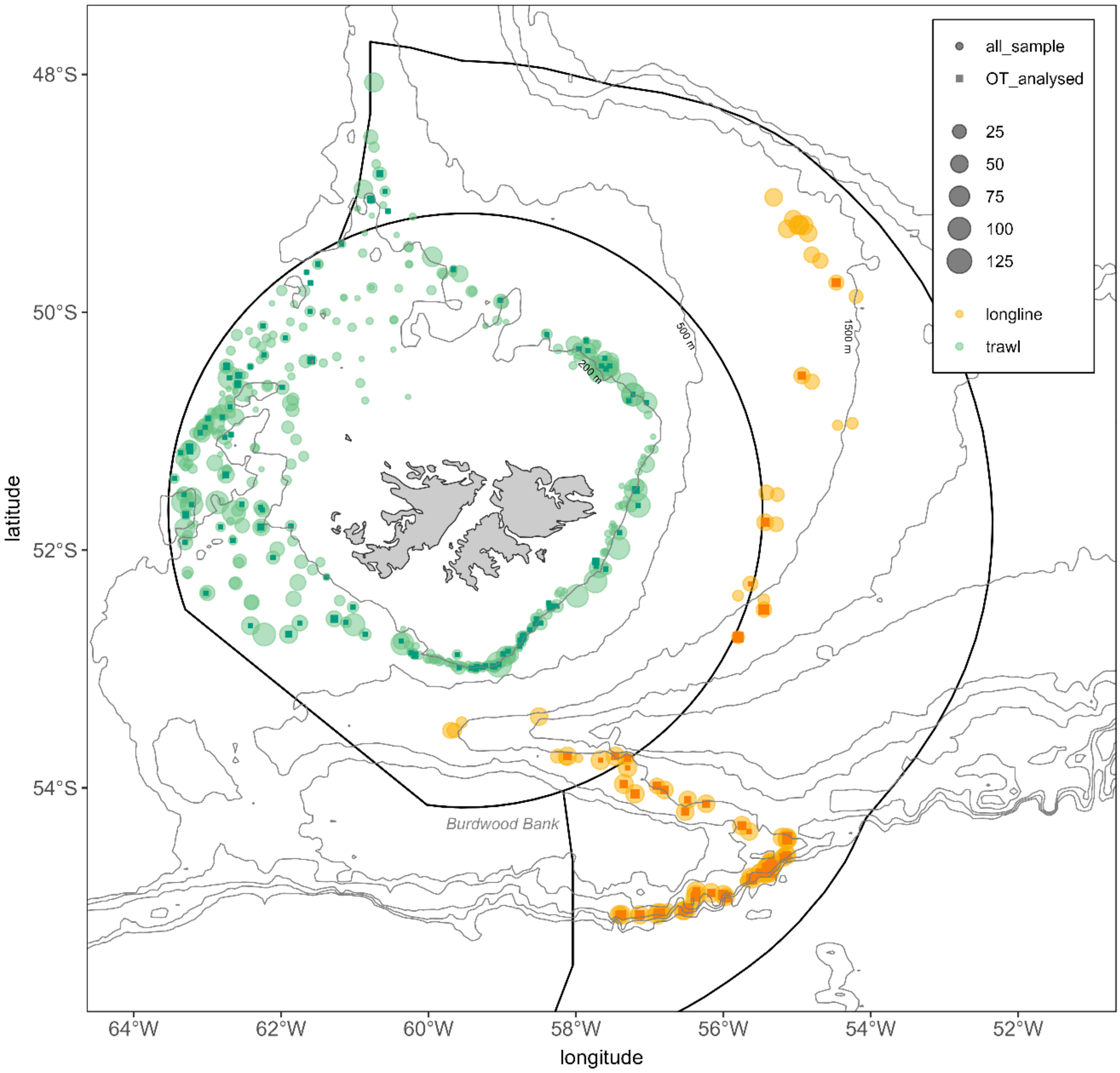

Figure 2. Positions of Patagonian toothfish sampled in the trawl (n=6040, in green) and longline (n=4802, in orange) fisheries for otolith and length frequency in the Falkland Islands waters during 2025 (n=10842). Circle with a light colour indicates the position of the collected samples and square with a dark colour the position of the otoliths selected for age estimation.

The length distribution of Patagonian toothfish sampled from combined longline and trawl fisheries (n=10842) showed two distinct modal peaks occurring around 45 and 100 cm (Figure 3.a). The peak at 100 cm indicates the targeted length for the longline fishery, while the peak at 45 cm represents juvenile Patagonian toothfish captured as bycatch in trawl fisheries on the continental shelf at depths shallower than -400 m. The larger individuals are predominantly female (Figure 3). This trend is also evident in the length distribution of females and males selected for age estimation (Figure 3.b).

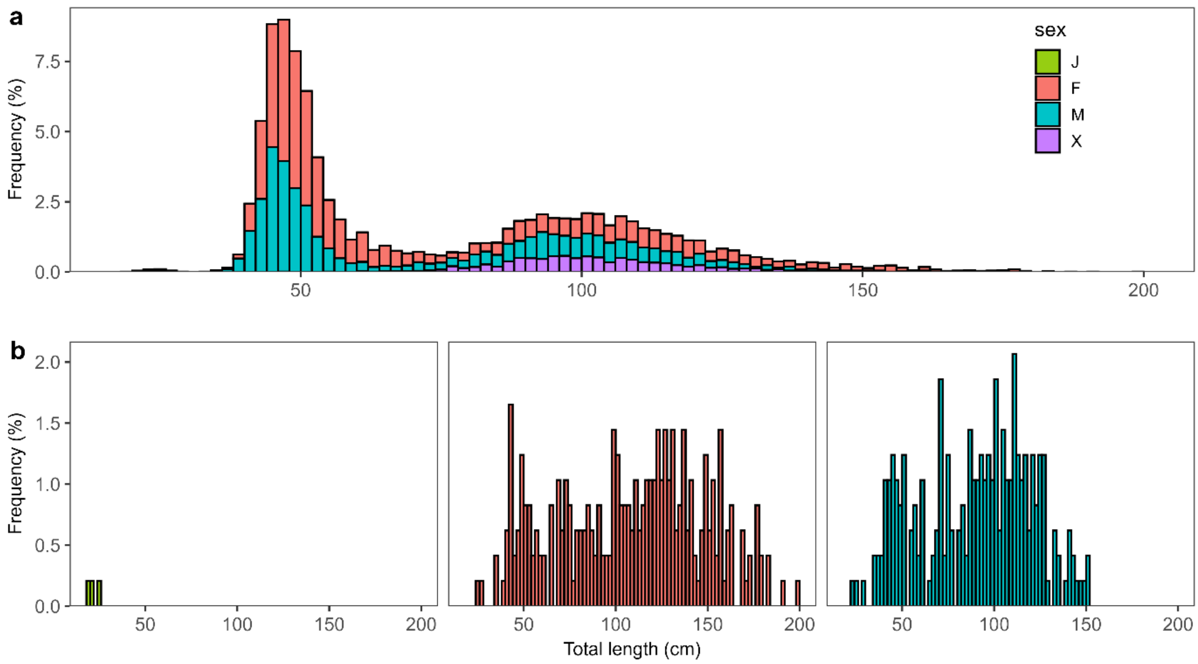

Figure 3. Length-frequency distribution for Patagonian toothfish (a) sampled in the longline and trawl fisheries (n=10842), and (b) juvenile (green), female (red), and male (blue) selected for age estimation (n= 484). The individuals not sex-identified (X) were Patagonian toothfish tagged and released alive.

3.2. Length and age composition

In the trawl fisheries, lengths ranged between 19 and 108 cm TL (mean ± sd = 49.2 ± 7.27 cm). The distribution showed a clear modal peak occurring around 46 cm TL, reflecting recruited age-2 and 3 fish (Figure 4). A small peak is also revealed around 23 cm TL, reflecting newly recruited and age-1 fish (Figure 4). 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 fisheries occur. The trawl fisheries predominantly captured fish aged less than 6 years old (min = 1 yo and max = 15 yo; quantile 0.1 = 2 yo and quantile 0.9 = 6 yo; Figure 5).

The longline fishery targeted a different part of the Patagonian toothfish stock with lengths ranging from 46 to 200 cm TL (mean ± sd = 105 ± 20.1 cm), with modal peaks occurring around 100 and 153 cm TL for both male and female fish (Figure 4). These modal peaks of longline caught Patagonian toothfish seemed to reflect fish around 12 and 23 yo respectively (Figure 5). The majority of Patagonian toothfish being caught within the longline fishery were older than 8 yo (min = 4 yo and max = 43 yo; quantile 0.1 = 8 yo and quantile 0.9 = 21 yo; Figure 5).

Combining all the fisheries data, ages estimated ranged from 1 to 43 yo for females and 2 to 36 yo for males (Figure 5).

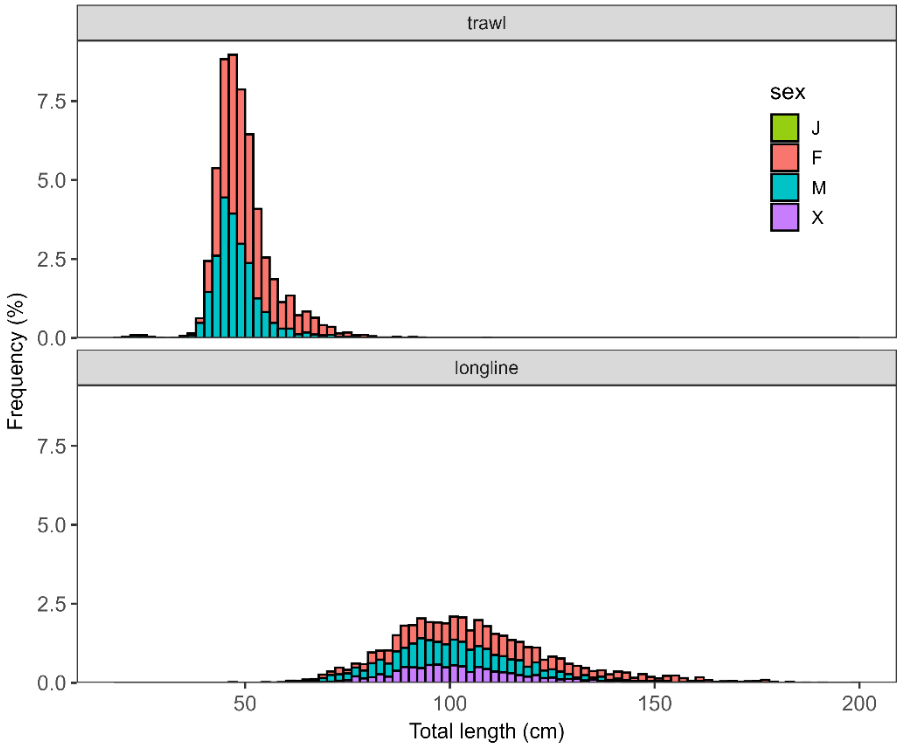

Figure 4. Length frequency distribution for Patagonian toothfish sampled in the trawl (n=6040) and longline (n=4802) fisheries.

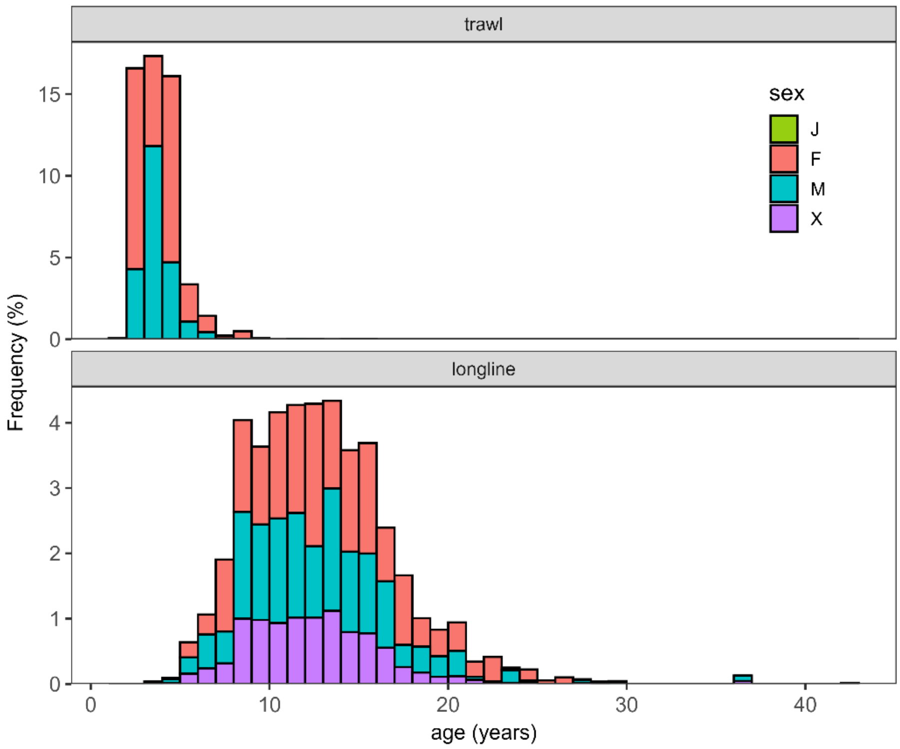

Figure 5. Age frequencies estimated from the total sampled catch of Patagonian toothfish in the trawl (n=6040) and longline (n=4802) fisheries (based on a sub-sample of 358 otoliths from the longline and 125 otoliths from the trawl fisheries).

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

The {L_∞, K} model (Table 2; L_∞ and K differs between the sexes) fitted the data statistically equally well as more complex models (where all three parameters differ between the sexes), but better than the models where a single parameter differs among sexes and 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 t_0, but different L_∞ and K for males and females. Calculated von Bertalanffy growth parameters were presented in Figure 6 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 6). Growth models were fitted on 255 females and 225 males.

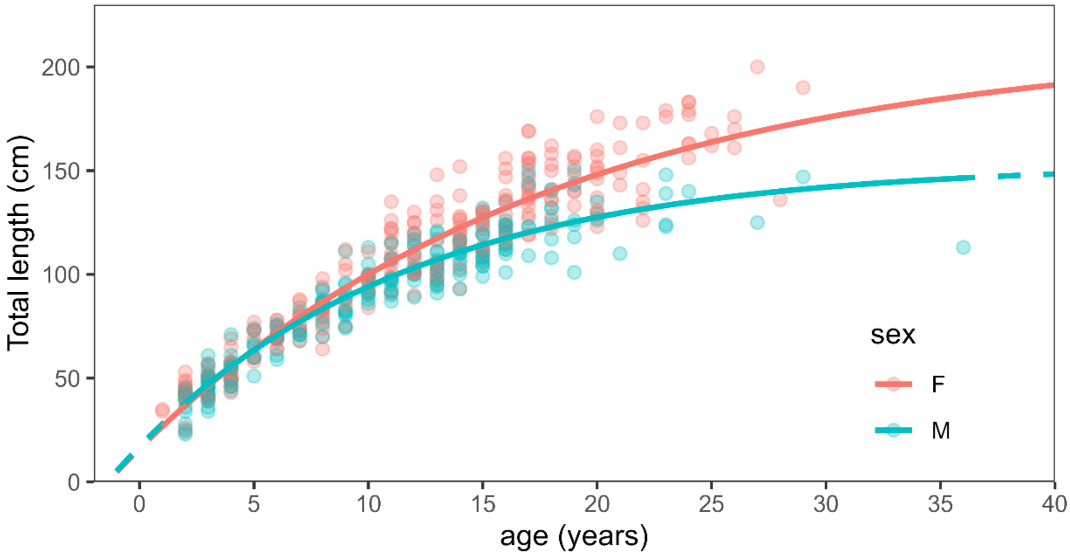

Figure 6. Length versus age with superimposed best-fit von Bertalanffy growth model for female (n=255) and male (n=225) Patagonian toothfish sampled in 2025.

Table 3. von Bertalanffy parameters estimates for female (n=255) and male (n=225) Patagonian toothfish sampled during 2025 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_∞	212.359	196.963	231.623
	K	0.056	0.048	0.065
Male	L_∞	153.183	143.768	165.863
	K	0.083	0.07	0.097
Both	t_0	-1.412	-1.916	-0.955

3.4. *Precision of the age estimates*

A sub-sample of 25% of the otoliths used for age estimation was randomly selected to measure the precision of age estimate (n = 120; Table 4 and 5). The two readings (age estimation) by the primary reader were similar at 60% and a difference of one year between these two readings was found in 27.5% of the otolith readings (number of otoliths read twice = 120; Table 4 and 5). APE and ACV results, respectively 2.82 and 3.99 (Table 5), were both below or close to 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=120).

	0	1	2	3	4
count 1 v. count 2	60	27.5	9.17	2.5	0.83

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
120	2	60	0.4	3.99	0.28	2.82

4. Conclusion

The results of the current study provide biological parameters for Patagonian toothfish in the Falkland Islands for 2025. 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.

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