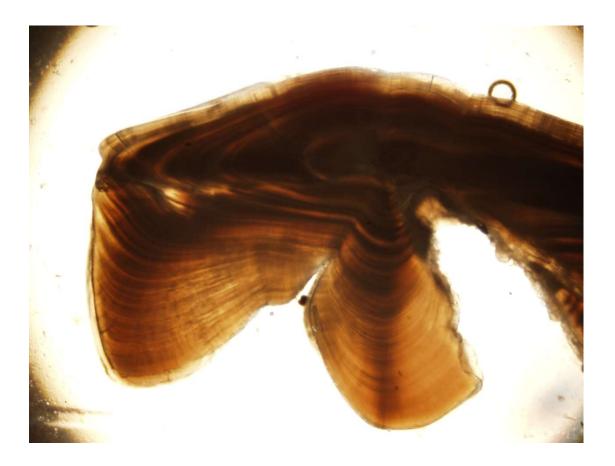
Age structure for Patagonian toothfish *Dissostichus eleginoides* from Falkland Island waters:

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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 (Lorenzen, 2016; Payne et al., 2005). 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 (>200 cm TL) 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 (APF) 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. Significant amounts are also captured as bycatch in the finfish and squid fisheries, although the true extent of this remains unknown.

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 2017. 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. Methods

2.1. Data Collection

Patagonian toothfish were sampled by scientific observers and other scientific staff of the Falkland Islands Government Fisheries Department. Data were collected on

board licensed longliners and commercial fishing vessels operating bottom trawls under various license types. In addition data were collected on board RV 'Monteferro' operating bottom trawls during research cruises.

Randomly sampled Patagonian toothfish were measured to the nearest cm (TL), sexed and the stage of reproductive maturity assigned according to an eight-stage scale (I and II – immature, III and IV – maturing, V – mature, VI – running, VII – post spawning and VIII – spent). Each annual collection of otoliths are stored in paper envelopes in four quarterly time periods (A: Jan – Mar, B: Apr – Jun, C: Jul – Sep and D: Oct – Dec).

Otoliths for ageing are selected to cover the length distribution of sampled fish from each quarterly otolith collection. 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 amber coloured polyester resin and left to set for 24 hours. Fully dried blocks are ground in order to provide smooth linear surfaces and the nucleus marked using a pencil. This is undertaken in order to guide the cutting angle and ensure that sections are cut precisely at right angles. Resin blocks were subsequently sectioned using a Buehler Isomet Low Speed Saw. Between two and six sections of 0.35mm were taken per resin block and mounted on microscope slides under coverslips with clear polyester 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. Images were taken for the best section for each otolith and enhanced to provide assistance in 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 undertaken. All counts of annuli were made without prior knowledge of fish size, date of capture or previous age estimates.

2.4. Precision of the age estimates

Repeated readings of the same otoliths provide a measure of intra-reader or interreader 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. Beamish and Fournier (1981) have developed an index of average percent error (IAPE), which has become a common method for quantifying this variation. The IAPE is 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.

An IAPE was calculated for all repeated readings undertaken by the primary reader.

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, 2018). 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 survival rate (S) and instantaneous rate of total mortality (Z) were estimated from catch-at- age data on the descending limb of the catch-curve using the Chapman- Robson approach (Chapman and Robson, 1960) with the variance estimate corrected for overdispersion as recommended by Smith et al. (2012). 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 24644 Patagonian toothfish samples. Of these, 11873 and 12771 were sampled from within the trawl and longline based fisheries, respectively.

In the trawl-based fisheries, Patagonian toothfish were sampled from across the shelf at depths between 58 and 829 m depth (Mean = 245.95 m; Figure 1). In the longline fishery, Patagonian toothfish were sampled at depths between 920 and 2210 m (mean = 1307.99 m) across the slope of the Burdwood Bank, through the Falklands Trough and extending along the slope to the east and north-east of the Falkland Islands (Figure 1).

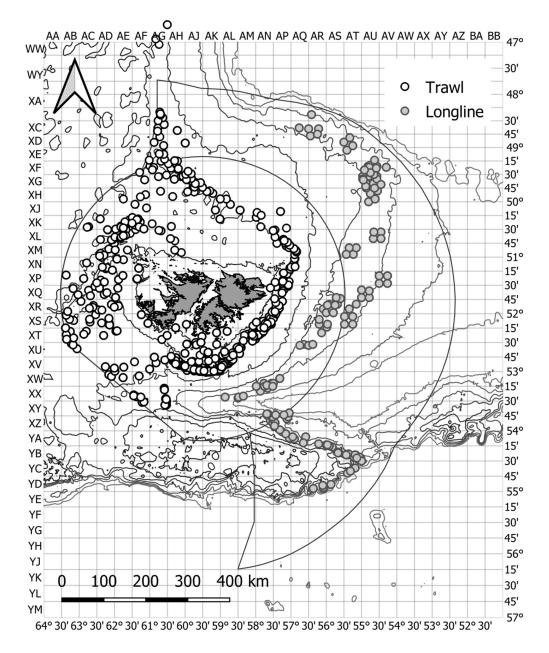


Figure 1: Positions from which Patagonian toothfish were sampled around the Falkland Islands during 2017 (n=24644).

3.2. Length and Age composition

In the trawl based fisheries, lengths ranged between 6 and 123 cm TL with three clear modes, occurring at 10, 22 and 46 cm TL (Figure 2). These modal groupings appear to reflect 0+, 1+ and 2+ year old fish (Figure 3). The longline-based fishery targeted a different part of the Patagonian toothfish stock with lengths ranging from 22 to 207 cm, with a single clear mode occurring between 98 and 102 cm TL for both male and female fish (Figure 2).

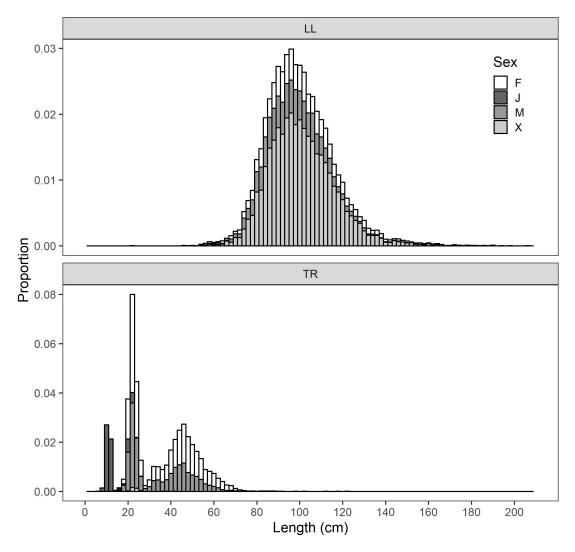


Figure 2: Length frequency distribution for Patagonian toothfish sampled in the longline (LL; n=12771) and trawl-based (TR; n=11873) fisheries.

The age frequency distribution was derived from the age length keys. The modal age of longline caught Patagonian toothfish appeared to reflect older fish of between 8 and 10 years old (Figure 3). Ages ranged from 0 to 29 years for females and 1 to 28 years for males. The majority of Patagonian toothfish being caught within the longline fishery were older than 6 years old.

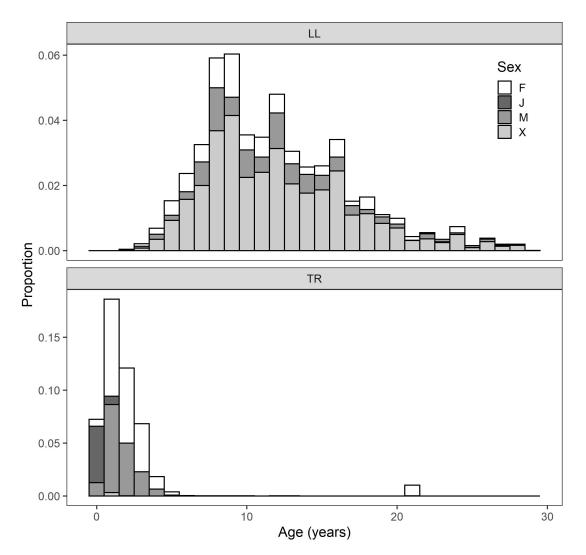


Figure 3: Age frequencies estimated from the total sampled catch of Patagonian toothfish in the longline (LL; n=12771) and trawl-based (TR; n=11873) fisheries.

3.3. Otolith interpretation

The clarity of zonation patterns in Patagonian toothfish otoliths varied greatly with complex patterns. Otoliths often 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 4A). 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. The internal structure usually displayed distinct banding with wide annuli nearer to the core, becoming increasingly narrow with age (Figure 4B).

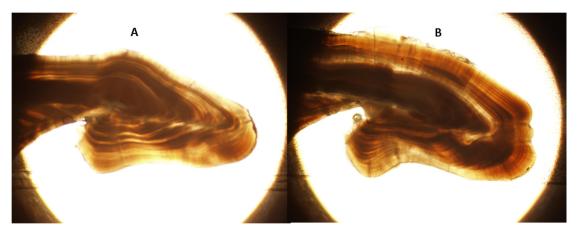


Figure 4: Sectioned otolith from (A) a 118 cm and (B) 134 cm female Patagonian toothfish with estimated ages of 12 and 16 years, viewed at 20 x magnification.

3.4. Age and growth

Likelihood ratio tests indicated significant differences in growth between male and female Patagonian toothfish (χ^2 =118.02; *P*<0.001; AIC=5159.22). Likelihood ratio tests indicated significant differences in the L^{∞} (χ^2 =33.99; *P*<0.001; AIC=5191.21) and *K* (χ^2 =10.75; *P*=0.001; AIC=5167.97), but not in the t_0 (χ^2 =1.41; *P*=0.24; AIC=5158.63) parameter estimates for male and female fish.

Calculated von Bertalanffy growth parameters and their 95% confidence intervals for male and female fish are presented in Table 1 and Figure 5. Female fish generally grew to a larger size and age compared to males. Male fish, however reached their asymptotic size at a faster rate compared to females.

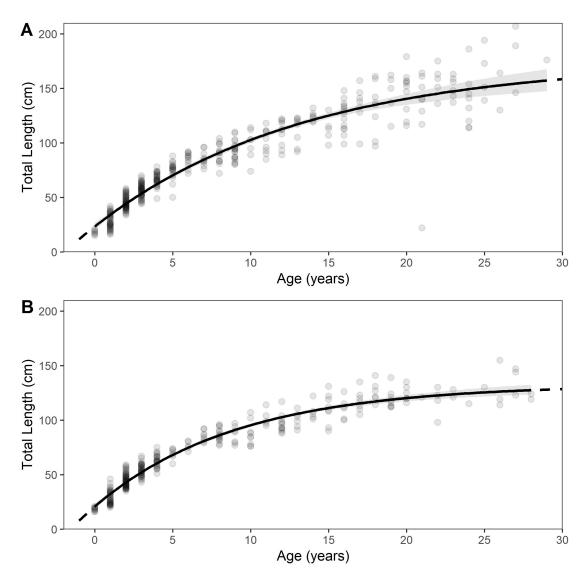


Figure 5: Length versus age with superimposed best-fit von Bertalanffy growth model and 95% confidence bands for (A) female (n=374) and (B) male (n=295) Patagonian toothfish sampled during 2017.

Parameters	Estimate	LCI	UCI
Females			
L∞	174.89	155.74	201.01
К	0.074	0.056	0.094
t_O	-1.93	-2.58	-1.48
n	374		
Males			
L∞	132.68	236.62	141.04
К	0.11	0.094	0.12
t_o	-1.55	-1.94	-1.28
n	295		

Table 1: Von Bertalanffy parameters (with 95% confidence intervals) for Patagonian toothfish sampled during 2017.

3.5. Mortality estimates

The threshold ages for the mortality estimates for Patagonian toothfish were 1 year in the trawl-based fisheries, 9 years in the lonline fishery for females and 8 years for male fish in the longline fishery, respectively (Figure 6). The mortality estimates obtained from the catch curves did not appear to differ greatly between sexes. In the trawl-based fisheries, the total annual survival and mortality rate for females was 47.52% (Z=0.74 year-1) compared to 42.16% (Z=0.86 year-1) for males (Table 2, Figure 6). In the longline fishery, the total annual survival and mortality rate for females (Z=0.20 year-1) compared to 81.53% (Z=0.20 year-1) for males (Table 2, Figure 6).

Fishery	Parameter	Estimate	Std. Error	95% LCI	95% UCI	
Trawl	Females					
	S	47.52	0.48	46.59	48.46	
	Z	0.74	0.08	0.59	0.89	
	Males					
	S	42.16	0.59	41.00	43.32	
	Z	0.86	0.08	0.70	1.03	
Longline	Females					
	S	81.68	0.44	80.82	82.55	
	Z	0.20	0.02	0.16	0.24	
	Males					
	S	81.53	0.39	80.76	82.30	
	Z	0.20	0.02	0.17	0.24	

Table 2: Estimates of survival rate and total mortality for Patagonian toothfish using the Chapman-Robson catch-curve estimator.

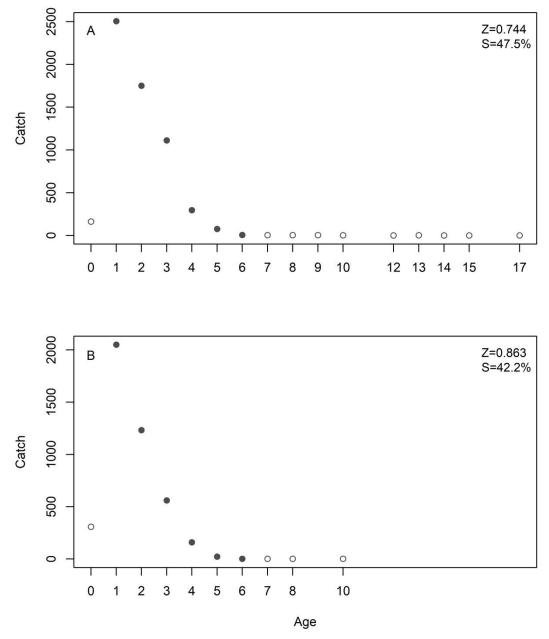


Figure 6: Catch at age for (A) female (n=5935) and (B) male (n=4341) Patagonian toothfish sampled from within the trawl fishery. The solid points were used to compute the Chapman-Robson estimates of S and Z.

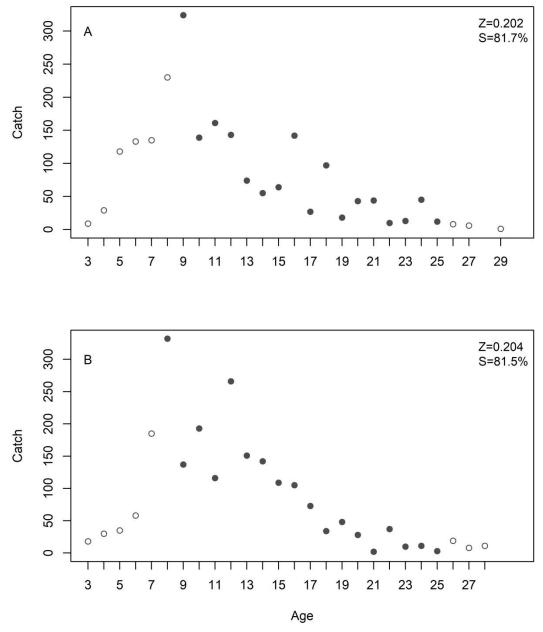


Figure 7: Catch at age for (A) female (n=2080) and (B) male (n=2161) Patagonian toothfish sampled from within the longline fishery. The solid points were used to compute the Chapman-Robson estimates of S and Z.

3.6. Precision of the age estimates

The percentage agreement table indicates that multiple estimates of ages by the primary reader agreed for 64.82 to 94.46% of the fish otoliths, while 1.80 and 22.46% differed by one year (Table 3). The APE was 3.23% and the ACV was 4.31% (Table 4). These indicate that ageing precision was reasonable in Patagonian toothfish.

	Age differ	Age difference (%)									
	0	1	2		3		4	5		6+	
Age v. Age1	74.70	14.37		4.94		2.54	1.65	5	1.05		0.75
Age v. Age2	83.68	8.68		3.29		1.05	1.35	5	0.90		1.05
Age v. Age3	94.46	1.80		0.90		0.60	1.20)	0.60		0.45
Age1 v. Age2	64.82	22.46		4.64		3.29	2.25	5	0.60		1.95
Age1 v. Age3	73.20	15.42		5.69		2.40	1.65	5	0.60		1.05
Age2 v. Age3	82.34	9.43		3.74		2.10	1.20)	0.30		0.90

Table 3: Percentage table of raw differences between multiple readings of Patagonian toothfish otoliths (n=668).

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
668	4	64.82	0.38	4.31	0.29	3.23

4. Conclusion

Results of the current study provide biological parameters for Patagonian toothfish in the Falkland Islands for 2017. 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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