REPORT

Age structure of rock cod Patagonotothen ramsayi in January – December 2014

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1. Introduction

Growth parameters, estimates of age at maturity and recruitment, and population age structures are essential inputs in fisheries yield models that provide the basis for management advice in many world fisheries (Ashford, 2002; Horn, 2002).

Methodology for age determination should be applied across the catch and over a sequence of years while minimizing sources of error (Morison et al., 1998). In addition, quantitative estimates of variability in age determination should be provided for incorporation into decisions on analyses and modelling and interpretation.

This annual report, therefore presents a reliable ageing methodology for rock cod, *Patagonotothen ramsayi*, to calculate growth parameters from samples obtained in the Falkland Islands during 2014. It also presented an assessment of the bias and precision in order to ensure error does not exceed a quality threshold.

2. Methods

2.1. Data Collection

P. ramsayi were sampled by scientific observers and other scientific staff of the Falkland Islands Government Fisheries Department. Data were collected on board licensed commercial trawlers operating bottom trawls under various license types. In addition data were collected on board RV 'Castello' operating bottom and semi-pelagic trawls during two research cruises.

Randomly sampled rock cod were measured to the nearest cm (Lt), 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 10 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 saved for image enhancement and 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 preparation, increments formed on the dorsal side were at least as clear as those on the ventral side. A readability index of 1 - Easy, 2 - Medium and 3 - Difficult was assigned to each otolith. Each otolith was aged twice by the primary reader. All counts of annuli were made without prior knowledge of fish size, date of capture or previous age estimates.

2.4. Estimation of von Bertalanffy parameters

Von Bertalanffy growth parameters were estimated for male and female fish from the length-at-age data using non-linear least squares regression procedure using R software (R core Team, 2015). 95% confidence was calculated for the von Bertalanffy parameters along with upper and lower 95% prediction limits of the derived curve using bootstrap methods with 1000 iterations. Differences in growth between male and female fish were estimated using likelihood ratio tests. The age structure of the total sample of *P. ramsayi* captured in 2014 was estimated by constructing an age-length key (ALK) using the FSA package in R (Ogle, 2016). The

relationship between length and weight was estimated using linear regression analysis.

2.5. 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 (1987) have developed an index of average percent error (IAPE), which has become a common method for quantifying this variation. The IAPE is calculated as:

$$IAPE = \frac{100}{N} \sum_{j=1}^{N} \left[\frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_{j}|}{X_{j}} \right]$$

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.

Chang (1982) suggested that precision should be measured by the mean coefficient of variation (ACV) which is defined as:

$$ACV = 100 * \frac{\sum_{j=1}^{n} \frac{S_j}{X_j}}{n}$$

where sj is the standard deviation of the R age estimates for the jth fish.

An IAPE and ACV were calculated for all repeated readings undertaken by the primary reader. The distributions of the differences between repeat readings were also inspected as another indicator of ageing errors, and of any bias between readings. Precision of repeated age estimates was also examined using an age bias plot (Campana *et al.*, 1995).

3. Results and discussion

3.1. Distribution of Samples

During 2014, biological information was obtained from a total of 38 598 *P. ramsayi* samples. Otoliths were extracted from a total of 1208 fish of which 446 were processed for age determination.

Aged *P. ramsayi* were captured in trawls over the Falkland Islands shelf between 110 and 612 m depth (Mean = 220.41 m; Figure 1). Comparatively, the total samples of *P. ramsayi* were captured at an average depth of 193.42 m, ranging between 95 and 612 m.



Figure 1: Positions from which *P. ramsayi* samples were obtained over the Falkland Islands shelf.

3.2. Length and Age composition

The length frequency distribution of *P. ramsayi* sampled during 2014 was unimodal (28 cm) with an average total length of 22.19 cm, ranging between 6 and 44 cm (Figure 2A). The average length of male fish captured within the trawl fishery was 22.93 cm (28cm mode) while the average length for females were 23.22 cm (26 cm mode).

The age distribution of *P. ramsayi* captured during 2014 as estimated from the age length key had a mode of 4+ years (Figure 2B). The average age of the total sample of *P. ramsayi* was 3.41 years. The average age of males (3.58 years) was slightly lower compared to that of females (3.65 years).



Figure 2: Length (A) and age frequencies (B) estimated from the total (aged and unaged) sampled catch of *P. ramsayi* as estimated from the age-length key.

Results of the length-weight regression indicate a significant relationship between length and weight (P<0.001) with low variability around the line (R^2 =0.981, Table 1, Figure 3).

 Table 1: Parameter estimates for the length-weight regression for *P. ramsayi* sampled during 2014.

Coefficient	Estimate	Std error	t-value	Р
Intercept	-2.51	0.0075	-334.5	<0.001
LogL	3.38	0.0056	599.5	<0.001
R2	0.981			



Figure 3: The log10 – log10 transformed length-weight data with the best fit line superimposed (left) and the length-weight data with the back-transformed best-fit line superimposed for *P. ramsayi* sampled during 2014.

3.3. Size and Age

P. ramsayi ages ranged from 0 to 14 years (Figure 4). Likelihood ratio tests indicated no significant differences in growth between male and female *P. ramsayi* (χ^2 =4.96; *P*=0.17) and as such, growth for sexes were pooled together. A representation of the age-length key for *P. ramsayi*, smoothed using a multinomial logistic regression model is presented in Appendix A. Calculated von Bertalanffy growth parameters and their 95% confidence intervals are presented in Table 2 and Figure 5.



Figure 4: Length versus age with superimposed best-fit von Bertalanffy growth model and 95% confidence bands (dashed lines) for *P. ramsayi* sampled during 2014.

Table 2: Von Bertalanffy parameters (with 95% confidence intervals) for *P. ramsayi* sampled during 2014.

Parameters	Estimate	Std Error	LCI	UCI
Linf	42.71	1.05	40.69	45.16
К	0.20	0.012	0.18	0.23
tO	-0.54	0.089	-0.73	-0.39



Figure 5: Histograms of parameter estimates for the von Bertalanffy growth function from bootstrapped samples for *P. ramsayi* sampled during 2014 (A: Linf, B: K and C: t0).

3.4. Precision of the age estimates

The percentage agreement table indicates that multiple estimates of ages by the primary reader agreed for 88.34% of the fish, 9.42% differed by one year and 1.34% differed by two years (Table 3). Figure 6 shows a tendency for the second age estimate to be higher than the accepted age estimate, particularly for older fish. The APE was 1.18% and the ACV was 1.67% (Table 4). These represent a high level of precision.

Table 3: Percentage table of raw differences between multiple readings of *P. ramsayi* otoliths for 2014.

Years	-1	0	1	2	3	4
Frequency (%)	1.57	88.34	7.85	1.35	0.67	0.22



Figure 6: Age-bias plot for comparing the mean estimated ages from multiple readings of *P. ramsayi* for 2014.

 Table 4: Estimates of average coefficient of variation and average percent error for

 multiple ageings of *P. ramsayi* otoliths for 2014

					Percent
n	Valid n	R	ACV	APE	agreement
516	446	2	1.67	1.18	88.34

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5. Acknowledgements

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6. Appendix A

Bubble plot representation of the observed age-length key for *P. ramsayi* sampled during 2014 and smoothed using a multinomial logistic model. The area of each circle is proportional to the proportion of fish in a length interval that are a given age.