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COMPARISON OF AGE READINGS FROM SCALES AND OTOLITHS OF THE PATAGONIAN TOOTHFISH (*DISSOSTICHUS ELEGINOIDES*) FROM SOUTH GEORGIA

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Abstract

Scale and otolith age readings were compared for 105 specimens of the Patagonian toothfish (*Dissostichus eleginoides*) from the South Georgia region (Subarea 48.3). In 44% of specimens, age readings from both structures were identical. A Wilcoxon test for paired data ($Ts = 0.256$, $n = 59$) conducted on the remaining 56% showed that differences in age estimates between the two methods were not significant. Otoliths frequently appeared totally opaque, making it impossible to read them, while scale images were always clear. The scale method is considered most appropriate for determining the age of *D. eleginoides* because it does not underestimate age and is the most effective with regard to time and cost. Values of fish growth parameters using the generalised von Bertalanffy equation estimated from 1 000 data records are: $L_t = 207.01[1 - e - 0.0748\{t + 0.2898\}]$; where $L_\infty = 207.01$ cm, $K = +0.0748$ and $t_0 = -0.2898$. The maximum age of *D. eleginoides* was found to be 24 years. This is very close to the maximum age of 22.8 years calculated from Taylor's formulae which relates maximum observed age to natural mortality. In this case we used M = 0.13, which is the value recommended by CCAMLR.

Résumé

Comparaison des lectures d'âges d'écailles et d'otolithes de 105 spécimens de légines australes (*Dissostichus eleginoides*) de la région de la Géorgie du Sud (sous-zone 48.3). Pour 44% des spécimens, les lectures d'âges des deux structures sont identiques. Les 56% restants ont été soumis au test de Wilcoxon pour les données couplées ($Ts = 0{,}256$, $n = 59$) qui a indiqué que les différences entre ces estimations d'âges calculées par ces deux méthodes étaient minimes. Souvent, les otolithes étaient opaques, ce qui les rendait impossibles à lire, alors que les écailles présentaient des images toujours claires. La méthode fondée sur les écailles est considérée comme la plus appropriée pour déterminer l'âge de *D. eleginoides* car elle ne sous-estime pas l'âge et se révèle la plus efficace en matière de temps et de coût. Les valeurs des paramètres de croissance des poissons estimées par l'équation de von Bertalanffy à partir de 1 000 enregistrements de données sont les suivantes : $L_t = 207{,}01[1 - e - 0{,}0748\{t + 0{,}2898\}]$; lorsque $L_\infty = 207{,}01$ cm, $K = +0{,}0748$ et $t_0 = -0{,}2898$. L'âge maximum de *D. eleginoides* rencontré serait de 24 ans, ce qui se rapproche des 22,8 ans estimés par la formule de Taylor qui met en rapport l'age maximum observé et la mortalité naturelle. Dans ce cas nous avons utilisé M = 0,13, valeur recommandée par la CCAMLR.

Резюме

Были сравнены результаты определения возраста по чешуе и отолитам 105 экземпляров патагонского клыкача (*Dissostichus eleginoides*) района Южной Георгии (Подрайона 48.3). У 44% рыб результаты определения возраста по обеим структурам были идентичными. Критерий Вилкоксона с помощью парных данных ($Ts = 0{,}256$, $n = 59$) показал, что у остальных 56% особей оценки возраста, полученные с использованием двух структур, различались только в небольшой степени. Часто оказывалось невозможным считывать отолиты в связи с их абсолютной непрозрачностью, в то время как чешуя всегда была прозрачной. Метод определения возраста *D. eleginoides* с помощью чушуи считается самым надежным, так как он не приводит к недооценкам и является наиболее эффективным с точки зрения времени и стоимости. Величины параметров роста рыб, полученные в результате применения обобщенного уравнения фон Берталанффи к 1000 записям данных, следующие: $L_t = 207{,}01[1 - e - 0{,}0748\{t + 0{,}2898\}]$; где $L_\infty = 207{,}01$ см, $K = +0{,}0748$ и $t_0 = -0{,}2898$. Максимальный возраст *D. eleginoides* – 24 года. Этот результат очень близок к возрасту 22,8 года, рассчитанного по уравнению Тейлора, которое соотносит максимальный наблюденный возраст с естественной смертностью. В данном случае мы применили рекомендуемое АНТКОМом значение M = 0,13.

Resumen

Se compararon las estimaciones de la edad efectuadas a partir de otolitos y de escamas para 105 ejemplares de bacalao de profundidad (*Dissostichus eleginoides*) capturados en la región de Georgia del Sur (Subárea 48.3). La edad estimada a partir de ambas estructuras fue idéntica en 44% de los ejemplares. Se sometió el 56% restante a una prueba Wilcoxon para datos pareados (Ts = 0,256, n = 59) que demostró que las diferencias entre las estimaciones de la edad efectuadas por ambos métodos no eran significativas. En tanto que los otolitos presentaron con frecuencia una apariencia opaca que no permitía realizar las estimaciones de la edad, las escamas siempre presentaron imágenes claras. Se considera que el método que utiliza escamas es el más apropiado para la estimación de la edad de *D. eleginoides* porque no la subestima y es más efectivo en términos del tiempo empleado y los costes. Los parámetros de crecimiento del pez, estimados a partir de 1000 registros de datos según la ecuación general de von Bertalanffy son: $L_t = 207{,}01[1 - e - 0{,}0748\{t + 0{,}2898\}]$; donde L_∞ = 207,01 cm, K = +0,0748 y t_0 = -0,2898. La edad máxima observada de *D. eleginoides* fue 24 años. Esta estimación se asemeja mucho a la edad máxima de 22,8 años calculada a partir de la fórmula de Taylor, que relaciona la edad máxima observada con la mortalidad natural. En este caso se ha utilizado el valor recomendado por la CCRVMA de M = 0,13.

Keywords: *Dissostichus eleginoides*, Patagonian toothfish, otoliths, scales, age, growth, South Georgia, CCAMLR

INTRODUCTION

Patagonian toothfish (*Dissostichus eleginoides* Smitt, 1898) inhabit cold waters of the Pacific Ocean along the coasts of Peru and Chile (Fischer and Hureau, 1985), waters of the Atlantic Ocean off Argentina between 36°30'S and 55°S (Cassia and Perrotta, 1996) and waters around sub-Antarctic islands and seamounts from South Georgia and Shag Rocks in the west to Macquarie Island in the east (Gon and Heemstra, 1990). The bathymetric range of this species extends to deeper than 2 500 m (Moreno, 1991), with the smallest specimens being found above 500 m. An increase in mean length of fish with increasing depth has been observed (Zhivov and Krivoruchko, 1990; Duhamel, 1992; Cassia and Perrotta, 1996).

The maximum recorded size of *D. eleginoides* is 223 cm. Sexual maturity is reached at about 90 to 100 cm total length (TL) (9–10 years) in most fish, although a few mature males smaller than 80 cm TL (Gon and Heemstra, 1990) have been observed.

The diet of *D. eleginoides* varies considerably from area to area and includes mesopelagic and demersal species of fish and squid. This suggests that *D. eleginoides* is an opportunistic feeder that takes advantage of any locally abundant species.

Many methods of assessing fish populations require the use of age/length keys. Such keys for *D. eleginoides* from the South Georgia region have been derived using age data obtained from scale readings. Beamish and McFarlane (1987) cited several studies indicating that age readings from scales could underestimate actual age. The need for refining methods of age determination for *D. eleginoides* was highlighted by the CCAMLR Working Group on Fish Stock Assessment (SC-CAMLR, 1991, 1992, 1993, 1994). It was noted that this refinement could be achieved by comparing age readings from scales and otoliths of the same fish, as well as readings conducted by different readers.

According to Moreno and Rubilar (1992), variations in parameters of the von Bertalanffy growth equation, mainly K and L_∞, are potential causes of error in estimates of mortality rates. They concluded that the highest priority for research in *D. eleginoides* should be the validation of age and growth data from scale readings using alternative methods.

The aim of this paper is to establish whether scales or otoliths are more reliable for age determination of *D. eleginoides* and to estimate growth parameters for specimens collected in Subarea 48.3.

Table 1: Distances from otolith nucleus (Mean O.R.) and scale nucleus (Mean S.R.) to each annual ring in μ from ages 1 to 24. Standard deviation for otoliths (SD O.R.) and scales (SD S.R.).

Age	Mean O.R.	SD O.R.	Mean S.R.	SD S.R.	Number
1	294.35	91.54	2 013.60	767.50	105
2	460.41	130.62	2 981.05	1 072.02	105
3	572.69	153.93	3 579.58	1 245.36	105
4	656.44	167.58	4 104.30	1 387.63	104
5	727.50	170.52	4 565.27	1 544.60	102
6	802.09	170.25	5 120.28	1 621.12	92
7	877.29	172.13	5 655.08	1 544.22	87
8	934.41	182.38	6 002.62	1 598.85	83
9	1 009.79	189.32	6 325.08	1 688.65	80
10	1 078.44	184.64	6 707.18	1 769.47	70
11	1 138.89	188.02	7 069.12	1 744.33	65
12	1 197.19	190.18	7 482.33	1 805.49	55
13	1 228.15	187.39	7 770.29	1 764.36	49
14	1 270.38	190.02	8 044.16	1 894.60	43
15	1 334.08	191.39	8 156.02	2 122.08	35
16	1 356.08	214.23	8 246.66	2 526.24	25
17	1 398.05	217.11	8 799.16	2 072.69	22
18	1 438.60	223.97	9 023.39	2 470.50	15
19	1 402.83	190.14	10 071.54	1 529.85	8
20	1 322.46	108.79	10 352.16	1 593.63	7
21	1 316.02	117.65	10 640.58	1 838.90	5
22	1 345.54	120.51	10 317.30	2 173.46	3
23	1 362.38	142.87	10 461.83	2 149.28	3
24	1 300.40		9 326.20		1

MATERIAL AND METHODS

Scales and otoliths of *D. eleginoides* were collected by Argentinian scientific observers on board Chilean bottom longliners operating within Subarea 48.3 during January, March, April and May 1995, and by the INIDEP research vessel *Dr Eduardo L. Holmberg* during November 1995. For this study, scales and otoliths from 105 individuals between 38 and 223 cm total length were collected for age reading (see Table 1).

Sagittal otoliths were attached to a pasteboard with double-sided tape and quick-drying cement (Proxilina 10'), and covered with another layer of cement. Usually no more than four otoliths were attached to one piece of board, which were then sectioned at very low speed using a Isomet-Buehler saw with a blade of diameter 76 mm and thickness of 0.15 mm. A thin transverse section was taken through the nucleus, which was then examined under incident light. Rings were counted in the dorsal area (Hecht, 1987) as hyaline rings were clearly visible here. The distance of each ring from the nucleus was measured along a standard radius in this area. It appears that otoliths grow longitudinally for about seven years, followed by a period when an increase in thickness is the main result of growth.

Scales were sampled from beneath the pectoral fin. Scales were soaked for one week in a solution of 20% alcohol (96°), 5% glycerin, a few drops of domestic detergent and 70% tap water. After rinsing, they were mounted on a slide with gelatine and analysed in transmitted light. Only 105 scales with observable rings were analysed because the majority were impossible to read owing to the presence of opaque rings. Only complete, distinct annual rings were counted. The paper by Hureau and Ozouf-Costaz (1980) was referred to when conducting annual ring readings of *D. eleginoides* scales.

Inter-ring measurements, the total number of rings on scales, as well as otolith images and radii counts, were obtained using the Otolith Daily Ring Measurement System from RATOC System Engineering. Otolith and scale readings were made separately. Statistical comparison of age readings determined using both structures was made using Wilcoxon's test for paired data in large samples (>50) (Sokal and Rolf, 1969). The von Bertalanffy model was used to describe growth; parameters were calculated using the radius-age method (Aubone and Hernandez, 1996).

RESULTS

Scales and otoliths from the same individual of *D. eleginoides* were used to determine age.

It was observed in transverse cross sections of otoliths that the nucleus was not well defined and was surrounded by incomplete rings. Sometimes

Table 2: The relationships between scale radius (μ) and total fish length (cm), and otolith radius (μ) and total fish length (cm) of *D. eleginoides* from South Georgia.

Structure	Regression	*a*	*b*	R^2	*N*	*P*
Otolith	Linear	217.3783	8.7105	0.4221	98	0.0000
	Exponential	0.2166	0.8769	0.6567	98	0.0000
Scale	Linear	1054.3711	55.3934	0.8758	104	0.0000
	Exponential	104.147	0.9008	0.8816	104	0.0000

opaque rings, corresponding to summer growth, are interrupted by weak hyaline rings, especially at ages 1, 2 and 3 (Figure 1a).

Figure 1 shows selected transverse cross sections of otoliths corresponding to different age groups of *D. eleginoides.* Reflected light was used in (b) and (c), and transmitted light in (a) and (d).

Scales are the traditionally used structure for determining the age of this species and do not present major difficulties for preparing and interpretation. Figure 2 shows scales corresponding to different age groups of *D. eleginoides.* Hureau and Ozouf-Costaz (1980) recommend the use of polarised light as the best method of detecting false rings, and it was this method that was used to the select the first ring, due to the fact that false rings around the nucleus mix together with the first ring.

Annual periodicity in the formation of hyaline rings could not be validated because the samples had not been continuously collected over the year and also because of the characteristics of the species. Other validation methods would require very complicated procedures. However, as no more information is available in this study, it is assumed that the rings are formed annually as concluded by North (1988), i.e. that an adjacent opaque and hyaline zone in otoliths, and an adjacent wide and closely-spaced sclerite zone in scales, comprise an annulus which represents one year of life. Counting such annuli is a valid technique for determining the age of Antarctic fish. On the other hand, Young et al. (1992) demonstrated that for *D. eleginoides* the greatest density of hyaline rings corresponded to winter months.

The mean distance and standard deviation along a standard radius between the otolith or scale nucleus to each of the growth annuli are shown in Table 1. The distances at each age are relatively constant, which helps to verify age readings. Rings have a definite and similar formation pattern in both structures. These results are shown in Figures 3 and 4.

Comparison between Both Structures

Results obtained using scales and otoliths are compared in Figure 5. Throughout the age range, results deviate only slightly from complete agreement between the two methods. Scale and otolith readings agreed in 43.81% of the samples. In the 56% of cases where readings did not agree, there was no evidence of significant differences between results from both methods using the Wilcoxon test (Ts = -0.256, n = 59).

The otolith radius:fish length relationship and the scale radius:fish length relationship were calculated from 105 individuals between 38 and 223 cm total length. The highest correlation coefficients (0.8758 for linear regression and 0.8816 for exponential regression) were obtained between total length of fish and scale radius. Conversely, the otolith radius/fish length relationship showed low linear and exponential correlation. The output was fitted to a linear regression (Figure 6 and Table 2), which allowed us to use the radius-age method to estimate growth parameters.

Estimation of Growth Parameters

The radius-age method applied to the estimation of growth parameters was developed by Aubone and Hernandez (1996). It is based on least squares and requires that the relationship between the radius of the utilised structure and the fish length be a linear one.

On the other hand, this method does not make any back-calculation of fish length. In this study, however, the size:age curve is estimated using data from the entire sample, i.e. using sample data rather than individual estimations. This method is not sensitive to variations in the smaller size classes. This is very important because small individuals generally are not well represented in the samples.

The following equation relates the length of an individual fish to its age:

$$l_t = g(t,\phi) + n_t \qquad (1)$$

where l_t is the length of an individual fish at age t; n_t is the random variable which represents the length-at-age variations among individuals whose mean and variance are given by $E\ (n_t) = 0$ and $V(n_t) = \sigma_t^2$ for each age t ; $g(t,\phi)$ is the growth model and represents the mean length at age t of the individuals, ϕ represents the vector of the parameter.

Furthermore, the relationship was considered

$$rl = A + BL + EL \qquad (2)$$

where rl is the radius of the HS of an individual of length, L (in this case HS is scale), EL is the random variable.

From equations 1 and 2 we obtain

$$Rt = A + B\, g(t,\phi) + h\ (t,\theta) \qquad (3)$$

where Rt is the theoretical mean radius of the HS at age t, and $h\ (t,\theta)$ is the residuals trend corresponding to equation (3) at age t, i.e. $h\ (t,\theta) = E\ (EL/t)$ (average of all individuals at age t), θ is the vector of parameters of h.

If it is considered that $g(t,\theta) = L_\infty\ .\ (1 - e^{-K(t-T0)})$ and $h\ (t,\theta) = 0$, then equation 3 takes the form

$$Rt = A + B\ L_\infty\ (1 - e^{-K(t-T0)}) \qquad (4)$$

In equation 4 A is replaced with its estimate. Having obtained 'a' from the regression of the HS radius on length, and $C = B.L_\infty$, the corresponding model for the observed HS mean radius at age can be written as

$$Rt = a + C(1 - e^{-K(t-T0)}) + \eta_t \qquad (5)$$

Considering the covariance structure of η_t, estimations can be obtained from C, K and T_0 starting from generalised least squares. The corresponding estimation of L_∞ is obtained from $L_\infty = c/b$ when c and b are the estimates of C and B.

The growth equation calculated for *D. eleginoides* from Subarea 48.3 (South Georgia) was $Lt = 207.01[1 - e^{-0.0748(t + 0.2898)}]$. The maximum age among the fish examined was 24 years.

DISCUSSION AND CONCLUSIONS

Young et al. (1995), who compared age readings of scales and otoliths of *D. eleginoides* from the south of Chile, reported difficulties in cutting otoliths due to their fragility, although no reference was made to their cut width. The technique of cutting otolith sections of 0.3 mm gave good results as there were no losses due to breakage. Most difficulties with otolith readings arose because the otolith was totally opaque, making it impossible to see the rings. For this reason, only specimens with clearly visible time markers were chosen.

When comparing ages determined using both structures, no significant differences were found, therefore the scale reading method did not underestimate the age of *D. eleginoides* in the South Georgia area. Although Young et al. (1995) found differences between both types of readings of *D. eleginoides* from the south of Chile, they were not significant because the age range using both methods was very similar, the only differences being in the relative frequencies at the age class level.

Growth parameters of the von Bertalanffy equation were estimated using a non-linear method, as was recommended by CCAMLR's Working Group on Fish Stock Assessment (WG-FSA). According to Young (1992), male and female growth can be represented by a common equation; consequently data from both sexes were pooled.

Although growth parameters were estimated using an innovative method (Aubone and Hernandez, 1996) of utilising the relationship between annulus and age, it can be seen that the results are very close to the other three existing growth equations for *D. eleginoides:*

Zakharov and Frolkina (1976)	$Lt = 204.3[1-e^{-0.0563(t + 0.545)}]$
Shust et al. (1990)	$Lt = 174.8[1-e^{-0.07117(t - 0.0049)}]$
Aguayo (1992)	$Lt = 210.8[1-e^{-0.0644(t + 0.7832)}]$
This paper	$Lt = 207.01[1-e^{-0.0748(t + 0.2898)}]$

According to CCAMLR, the most appropriate value for M is 0.13. Applying this value of M to the Taylor formulae gave an age limit of 22.8. This figure is very close to the maximum age found in this paper.

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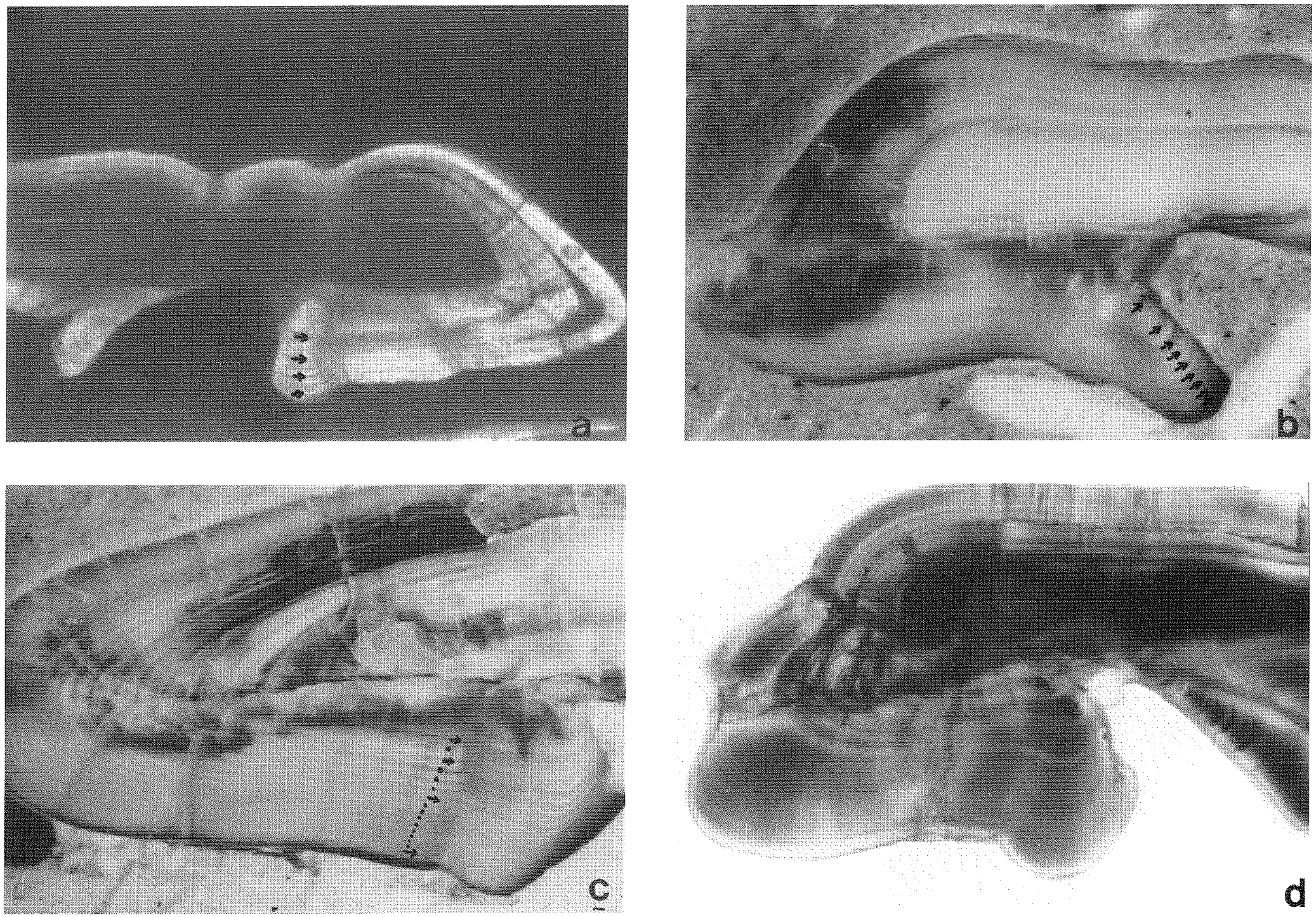

Figure 1: Interpretation of age from otoliths of *D. eleginoides* from South Georgia (with annuli indicated): (a) age estimated as 4 years, total length 48 cm; (b) age estimated as 9 years, total length 92 cm; (c) age estimated as 17 years, total length 142 cm (indicated ages: 1, 3, 7 and 16); (d) age estimated as 21 years, total length 173 cm.

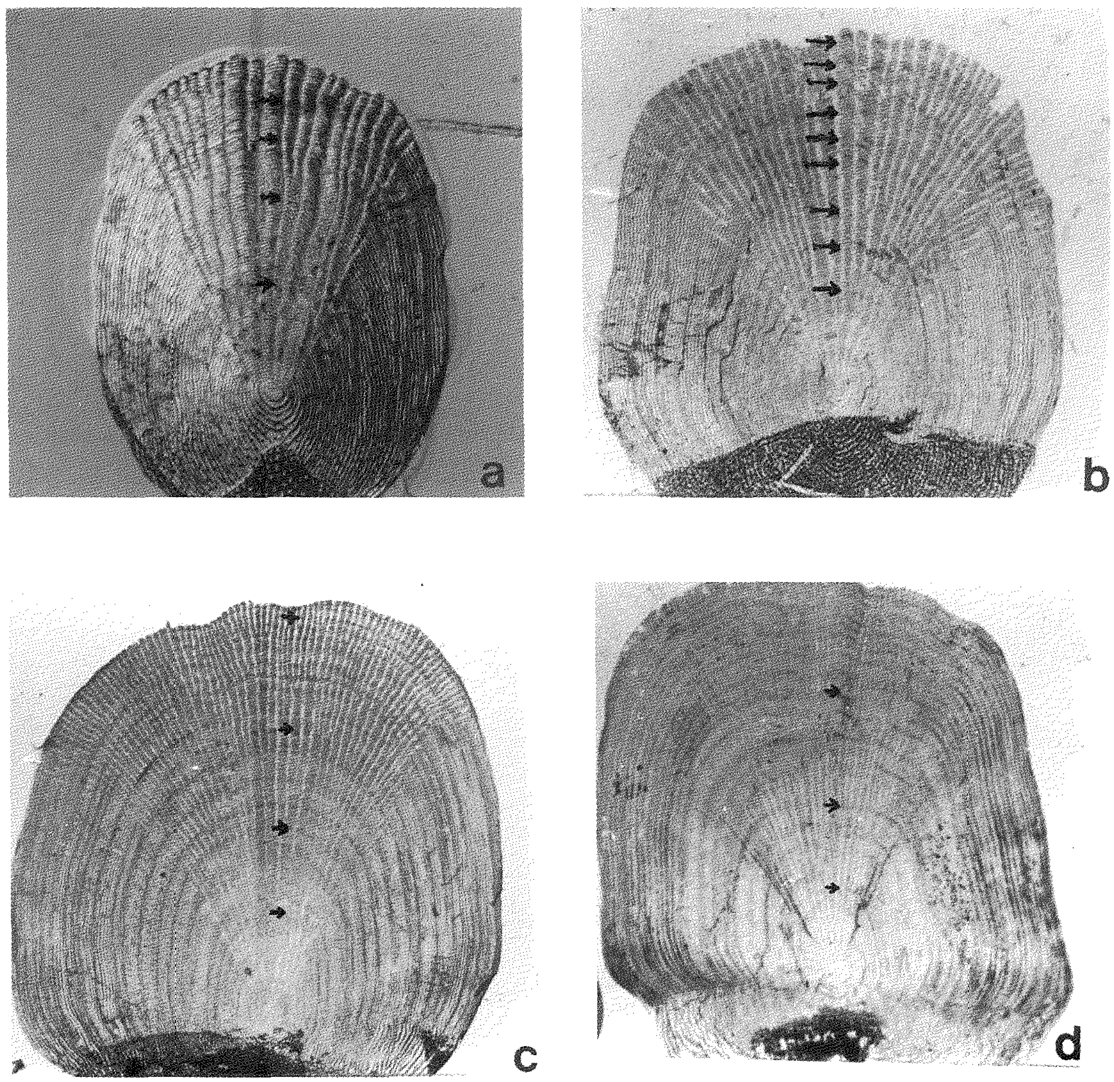

Figure 2: Interpretation of age from scales of *D. eleginoides* from South Georgia (with annuli indicated): (a) age estimated as 4 years, total length 48 cm; (b) age estimated as 9 years, total length 92 cm; (c) age estimated as 17 years, total length 142 cm (indicated ages: 1, 3, 7 and 16); (d) age estimated as 21 years, total length 173 cm (indicated ages: 1, 3, 8 and 21).

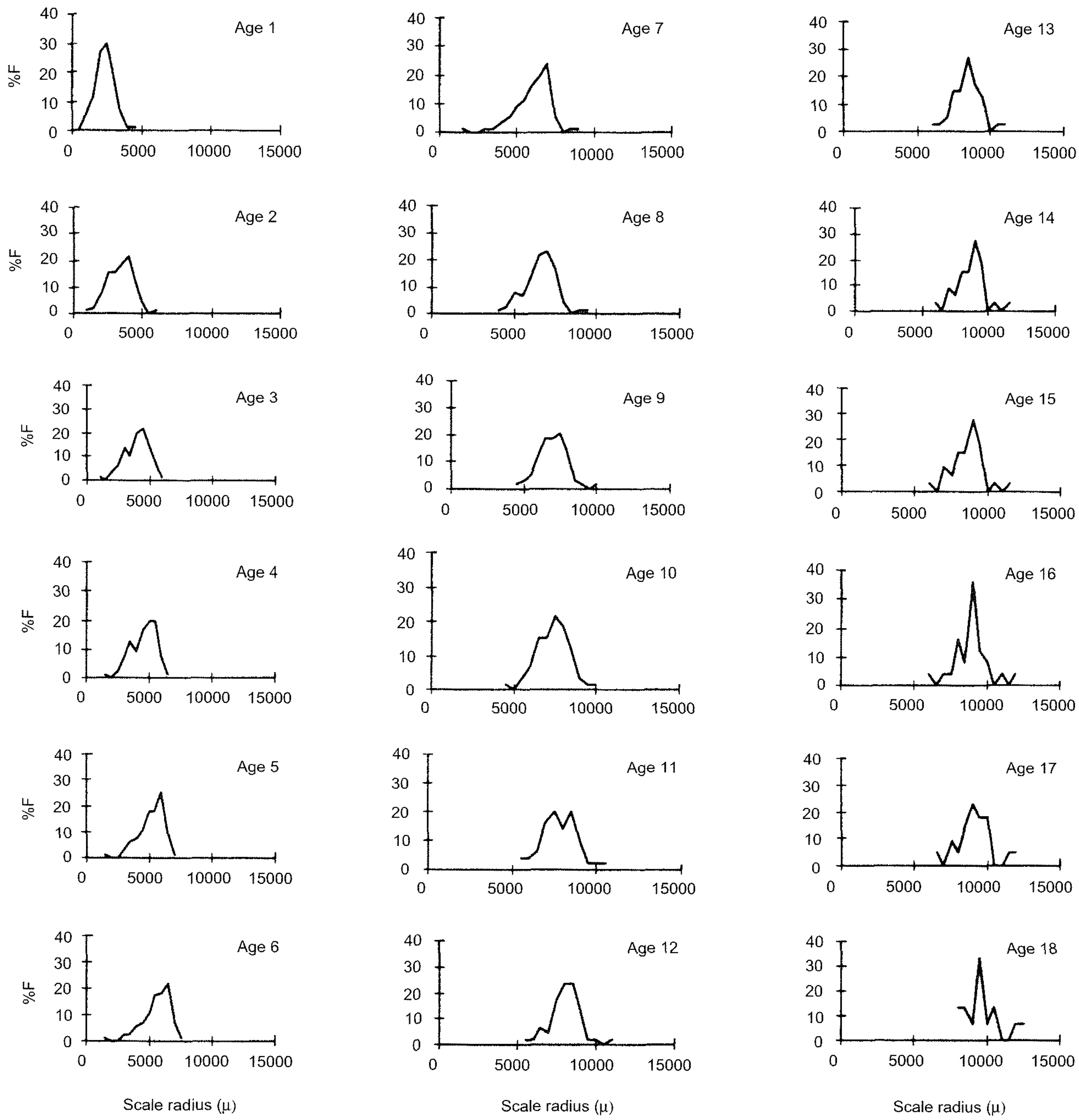

Figure 3: Frequency (%) of scale radii (µ) in *D. eleginoides* from South Georgia, ages 1 to 18.

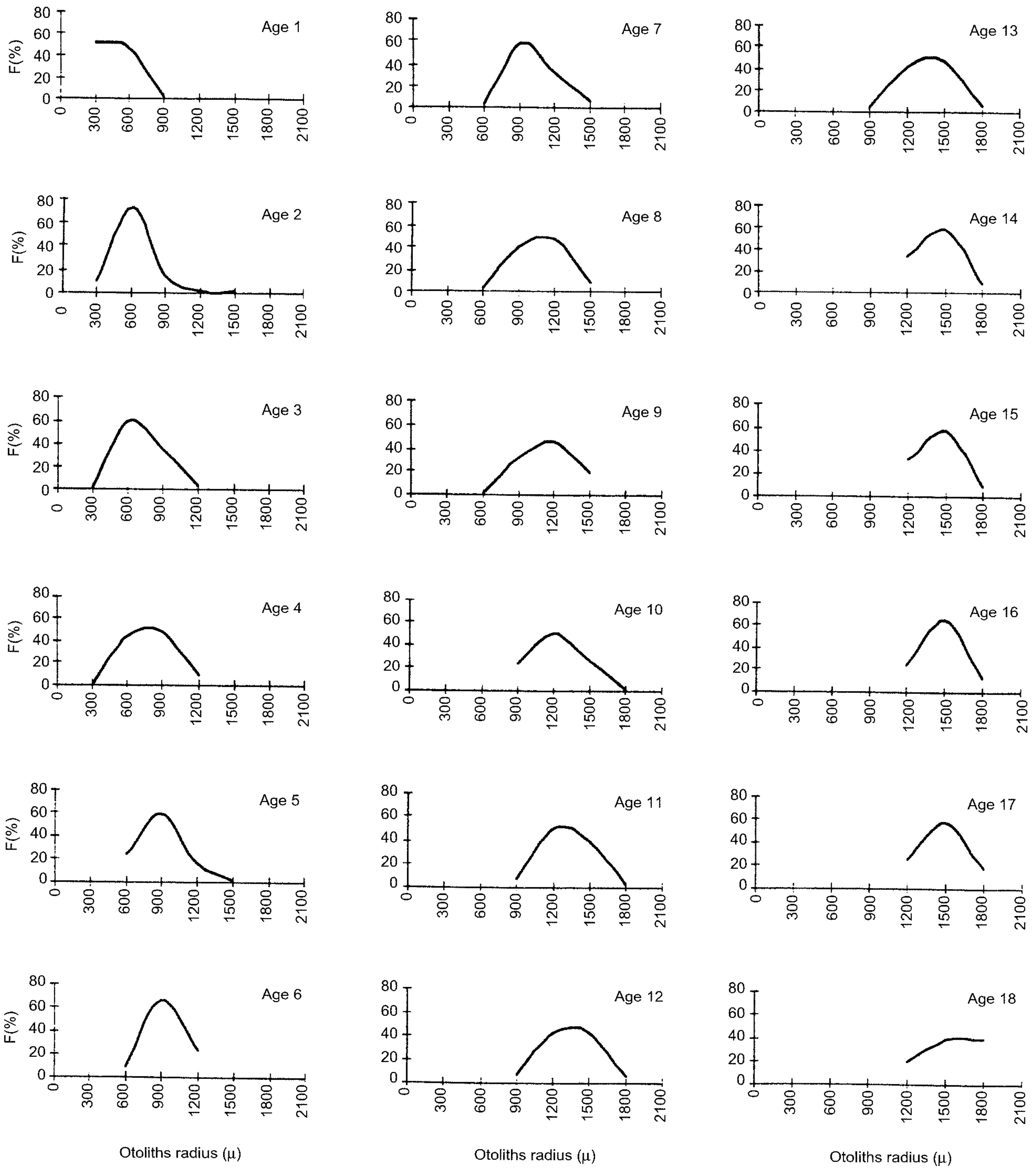

Figure 4: Frequency (%) of otolith radii (μ) in *D. eleginoides* from South Georgia, ages 1 to 18.

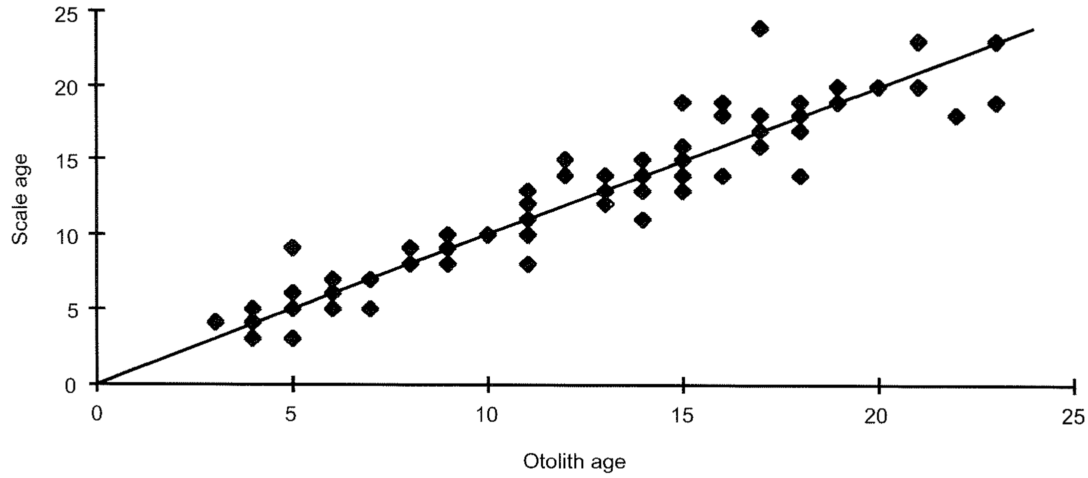

Figure 5: Comparison of age readings from scales and otoliths of *D. eleginoides* from South Georgia.

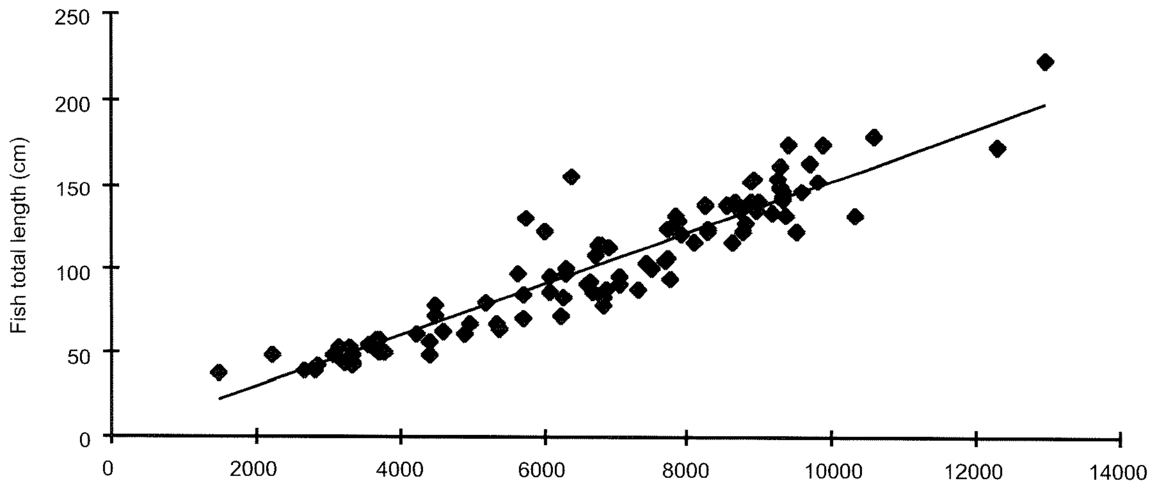

Figure 6: Linear relationship between scale radii (μ) and total fish length (cm) of *D. eleginoides* from South Georgia.

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