LENGTH-AT-AGE IN JUVENILE PATAGONIAN TOOTHFISH (*DISSOSTICHUS ELEGINOIDES*)

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Abstract

We used the otoliths from a sample of *Dissostichus eleginoides* pre-recruits, whose length density was distinctly polymodal, to see whether ages estimated by reading otoliths were congruent with the length modes observed. Length densities by age were compared graphically with the overall length density observed. Additionally, ages were predicted for each fish based on length, and compared with ages estimated from reading otoliths in a goodness-of-fit test. The majority of the otoliths (83.6%) were estimated to be from fish 1+ or 2+ years old, with mean total lengths of 32.5 cm and 41.3 cm respectively. No difference was found between predicted and estimated ages. We conclude that the two dominant modes observed in the length density represent different age cohorts separated by one year.

Résumé

À partir des otolithes d'un échantillon de pré-recrues de *Dissostichus eleginoides* dont la densité était nettement polymodale, nous avons cherché à déterminer si les âges estimés par la lecture des otolithes étaient congruents avec les modes de longueurs observés. Les densités de longueurs en fonction de l'âge ont été comparées de manière graphique à la densité générale des longueurs observée. De plus, pour chaque poisson, un âge a été présumé en fonction de sa longueur, puis cet âge à été comparé à celui qui avait été estimé par une lecture des otolithes dans un test d'ajustement statistique. Il est estimé que la plupart des otolithes (83,6%) appartiennent à des poissons d'âge 1+ et 2+, avec des moyennes respectives de 32,5 cm et 41,3 cm de longueur totale. Aucune différence n'est apparue entre les âges présumés et les âges estimés. Nous en concluons que les deux modes dominants observés dans la densité des longueurs représentent des cohortes de deux années consécutives.

Резюме

Чтобы установить, соответствуют ли оценки возраста по результатам считывания отолитов наблюдавшимся модам длин, мы изучили отолиты из выборки еще не вступивших в промысел особей *Dissostichus eleginoides* с явно полимодальной плотностью длин. Было проведено графическое сравнение плотности длин по возрастам с общей наблюдавшейся плотностью длин. Кроме этого, для каждой рыбы был сделан прогноз возраста по длине, и полученный результат сопоставлен с оценкой возраста, полученной при считывании отолитов, путем проверки степени соответствия. По оценкам, большинство отолитов (83.6%) принадлежало рыбе возрастом 1+ и 2+ со средними значениями общей длины соответственно 32.5 см и 41.3 см. Разницы между прогнозным и оценочным возрастами обнаружено не было. Нами был сделан вывод, что 2 доминирующих моды, наблюдаемых в плотности длин, соответствуют различным возрастным когортам с разницей в 1 год.

Resumen

Se utilizaron los otolitos de una muestra de prerreclutas de *Dissostichus eleginoides* cuya curva de distribución de la densidad de tallas era claramente polimodal, para determinar si las edades estimadas de las lecturas de otolitos eran congruentes con las tallas modales observadas. Se comparó gráficamente las densidades por talla según la edad con la densidad por talla total observada. Además, se utilizaron las tallas para predecir la edad de cada pez, y se comparó con la edad estimada de las lecturas de otolitos en una prueba estadística de la bondad del ajuste. Se calculó que la mayoría de los otolitos (83,6%) correspondían a peces de 1+ ó 2+ años de edad, con un promedio de longitud total igual a 32,5 cm y 41,3 cm respectivamente. No se encontraron diferencias entre las edades predichas y las edades estimadas. Hemos concluido que las dos modas dominantes observadas en la densidad de tallas representan distintas cohortes con un año de diferencia en edad.

Keywords: Southern Ocean, South Georgia, age validation, length-frequency modal analysis, population age structure, CCAMLR

INTRODUCTION

In recent years a fishery for Patagonian toothfish (*Dissostichus eleginoides*) has developed in the Southern Ocean. When this fishery began, information on the species was extremely limited (e.g. Everson, 1977; Kock et al., 1985; Kock, 1992). Due to the value of the resource and the rapid increase in the total catch of the fishery, better information was urgently required. Reliable information on size-at-age is critical to many stock assessment models, and the topic came into sharp focus during the 2000 meeting of the CCAMLR Working Group on Fish Stock Assessment (WG-FSA). The discussion at WG-FSA indicated that there was a clear need for validation of the age of individual fish and an assessment of the precision of the ages estimated.

Although otoliths and scales have been used in the past to estimate the age of *D. eleginoides,* it has been demonstrated (Ashford et al., 2001) that, over the lifespan of the fish, age estimates derived from scale readings are lower than those from otoliths. Several techniques for preparing otolith sections have been tested at the Center for Quantitative Fisheries Ecology, including the original method of Bedford (1983), the adaptation of Bedford's (1983) method for scanning electron microscopy (SEM) use developed by Ashford et al. (1993), and variations of Christensen's (1964) 'crack and char' method developed by Ashford and Wischniowski (1998). Of these, grinding thin sections using a Hillquest thin sectioning machine was considered to provide the best and most consistent pattern for readers estimating age (Ashford, 2001). However, although the nucleus and annuli are easily recognisable to an experienced reader, until now there has been insufficient information to confirm that the annuli are formed over a yearly period.

We obtained a series of samples of *D. eleginoides* pre-recruits whose length density was distinctly polymodal, taken during a demersal trawl survey off South Georgia in January 2000 (Everson et al., 2000). The modal values were similar to those which had been assumed to represent year classes in previous assessments. In this study we have used the otoliths from these fish to estimate their age to see whether these estimated ages are congruent with the modes.

METHODS AND MATERIALS

Otolith sections from 281 *D. eleginoides* were prepared according to the method of Ashford (2001). The left or right otolith from sampled fish was randomly selected, baked and ground using a Hillquest thin section grinding machine to reveal a transverse plane through the otolith nucleus and between crenellations. Sample otoliths were all small; manipulating the smallest during grinding was difficult, so these were mounted in epoxy using square moulds, before being ground individually. Sections were mounted on slides for reading and storage. Before being presented to readers, all otoliths were sorted into random order, using SAS PROC PLAN to generate random numbers. The sections were viewed under reflected light using a Leica MZ8 microscope at 25x magnification, and the age recorded. Age readings were undertaken by one experienced reader. Slides from this study were interspersed with 104 control slides from a previous study to test for consistency in the reader's age estimation.

This study was carried out prior to the CCAMLR Workshop on Estimating Age in Patagonian Toothfish (SC-CAMLR, 2001) at which standard criteria for age estimation were established. As a result, criteria for interpreting age from otolith structure were as described in Ashford (2001), with the edge of the nucleus and the first annulus as identified by Ashford and Wischniowski (1998). Fish showing the region of the otolith from the centre of the nucleus to the outer edge of the first opaque zone were designated as 0+ years old. Fish showing the outer edge of the first opaque zone were designated as one year old. Fish showing the outer edge of the first opaque zone to the outer edge of the second opaque zone were defined as 1+ year old. As in previous years, apart from the dates of the groundfish survey, no auxiliary information was made available to the readers until after completion of all readings of the otoliths. The experienced reader read each otolith once. The trainee made four readings but, during subsequent quality control procedures, evidence of severe bias was found in his readings and these were not used in this analysis.

To test whether the modes observed in the length-frequency data corresponded to age classes, we predicted ages for each fish based on its length. Predicted ages were then compared with ages estimated from reading otoliths to test the null hypothesis that there was no difference between predicted and estimated ages in a goodness-of-fit test using:

$$\chi^2 = \sum_{1}^{n} \frac{(y_{ri} - y_{pi})^2}{y_{pi}}$$

where n = the number of fish, y_{ri} = the age of fish i estimated by reading its otolith, and y_{pi} = the age of fish i predicted from the length-frequency distribution.

An age–length key was constructed using length data collected along with the otoliths during the groundfish survey. From the resulting age-at-length matrix the proportions of the population at each age (Fridriksson, 1934; Kimura, 1977) were calculated using the following equation:

$$\hat{p}_i = \Sigma_{j=1} \hat{l}_j \hat{q}_{ij},$$

where p_i is the proportion of a population of age $i = 1,2,3,...,n$; l_j is the proportion of length j; and q_{ij} is the proportion of fish of age i within an age subsample for a length j.

RESULTS

Of the sample of 281 otolith pairs, one was unusable because both otoliths were overground in preparing the section. In the remaining pairs, the nucleus was sufficiently distinct to allow age estimation (Figure 1). The outer edge of the otolith in most sample fish showed a well-developed opaque zone, interpreted from our criteria as corresponding to fast growth during the latter part of the 1998/99 season, with evidence of a translucent zone on the very edge corresponding to slow growth during winter 1999. The otoliths of some fish had a further fine opaque zone on the edge of the otolith that was interpreted as corresponding to the initiation of faster growth during late 1999/2000.

No bias was detected in the readings of the 104 control slides. For the majority of the otoliths (83.6%), ages estimated by reading the otolith were 1+ or 2+ years old. The length-frequency plot for all fish in the sample is shown in Figure 2. The length-frequency plots for cohorts of estimated age 0+ to 4+ years are shown in Figure 3. The cohorts 0+ to 2+ years corresponded closely to the peaks evident in Figure 2, although the 0+ cohort comprised only six fish. The mean total length (TL) for each cohort is shown in Table 1. Length-at-age is plotted in Figure 4.

Based on the length-frequency modes observed, fish TL <23 cm were predicted to be 0+ years old, fish TL 23–37 cm were predicted to be 1+ years old, and fish TL 38–46 cm were predicted to be 2+ years old. Fish >46 cm were considered to lie outside the modes found, and were not included in the analysis. Fish <23 cm were also not included in the analysis because of their paucity; however, estimated and predicted ages were the same for all six fish. The goodness-of-fit test included n = 232 fish of TL 23–46 cm; no difference was found between predicted and estimated ages ($\chi^2 = 15.5$, $\alpha = 0.05$).

To generate the age–length key, ages were pooled into 5 cm TL groupings as the study covered only 280 otoliths. Proportions of lengths within each age class are shown in Table 2.

DISCUSSION

Ages estimated by reading the otoliths conformed well to the length-frequency peaks shown in Figure 2, indicating that the two large peaks represent year classes containing fish 1+ and 2+ years old respectively. A much smaller peak at TL ca. 20 cm contained fish 0+ years old. Similar peaks

Table 1: Mean total length (TL) for each age cohort.

Age	Mean TL (cm)	SE	*n*
0+	19.8	**0.83**	**6**
1+	32.5	**0.17**	**121**
2+	41.3	**0.38**	**113**
3+	55.0	**1.39**	**26**
4+	61.3	**1.21**	**10**

Table 2: Age–length key for *Dissostichus eleginoides*, based on 280 fish sampled off South Georgia. TL = maximum total length in each 5 cm group (i. e. TL = 40 is for grouping 36–40 cm). Prop. = proportion.

TL	Age									
	0+	1+	2+	3+	4+	5+	6+	7+		14+
20	4									
25	2									
30		12	1							
35		102	7							
40		6	33							
45		1	65	3						
50			5	3						
55				7	1					
60			2	9	3					
65				2	5	1				
70				2	1	1				
70+								1		1
Total	6	121	113	26	10	2		1		1
Prop.	0.021	0.432	0.404	0.093	0.036	0.007	- - -	0.0035	- - -	0.0035

Table 3: Length-at-age (cm) estimated for *Dissostichus eleginoides*. Estimates from Zakharov and Frolkina (1976) and Shust et al. (1990) are based on their published von Bertalanffy parameters (VBGF). The mean TL in the polymodal distributions from 1990 and 1991 survey data are based on these von Bertalanffy parameters (Everson, 1991). The mean TL by cohorts for the 2000 survey is based on ages estimated directly in this study.

VBGF Age	Zakharov and Frolkina (1976)	Shust et al. (1990)	1990 Survey	1991 Survey	2000 Survey	Direct Estimates of Age
1	17.02	12.15				
2	27.27	23.40	20.1	17.3	19.8	0+
3	36.96	33.87	32.0	32.0	32.5	1+
4	46.12	43.62	41.1	40.0	41.3	2+
5	54.78	52.69	48.3	50.5	55.0	3+
6	62.97	61.14			61.3	4+

were found also in the South Georgia surveys of 1990 and 1991 (Everson, 1991). These are shown in Table 3. When comparing the 1990 and 1991 data with lengths-at-age predicted from von Bertalanffy growth estimates by Zakharov and Frolkina (1976) and Shust et al. (1990), Everson (1991) concluded that if the estimates were correct, these peaks represented 2-year-old and 3-year-old fish. These same peaks correspond to those estimated as containing fish 0+ and 1+ years old in the present study.

Ages were estimated in this study using criteria given by Ashford (2001) which were different from those agreed at the CCAMLR Workshop on Estimating Age in Patagonian Toothfish (SC-CAMLR, 2001). As part of the initial work of the CCAMLR Otolith Network arising from the workshop, readers from the New Zealand National Institute of Water and Atmospheric Research and the Australian Central Ageing Facility were consulted and agreed that the translucent zone on the otolith edge in January 2000 corresponded to the winter of 1999, indicating a delay of several months in the formation of the opaque zone at South Georgia.

If this is correct, and fish are in fact spawned in July–August at South Georgia, the fish 0+ years old in this study may have been spawned in July 1999, in which case the ages given in this study are correct under the agreed criteria. This implies substantial growth of the nucleus in only a few months before the appearance of the first translucent zone. An alternative is that fish designated 0+ years old may have been spawned in July 1998, in which case the nucleus corresponds to a period of over a year, and ages given in this study are underestimated by a year. Evseenko et al. (1995) reported four larvae caught at South Georgia in November–December 1975 whose lengths were 19.8–22.1 mm; if this is indicative of the mean length of fish spawned the previous July, fish estimated as 0+ in January in this study are in fact most likely to be 1+. However, no fish were caught in the sample taken by the 2000 survey which could correspond to the missing cohort predicted under this scenario, and the toothfish larvae and post-larvae that have been sampled at South Georgia are too few to establish if they are representative. Furthermore, there is little evidence from the otolith structure supporting the idea that the nucleus corresponds to a period of more than one year.

Under either alternative the ages estimated from otoliths indicate that the frequency modes represent different age cohorts separated by one year, and that the previous ages estimated using published von Bertalanffy parameters are too high. At present, fish showing the first translucent zone on the edge of the nucleus in January are considered to be 0+. However, the alternative, that they are 1+, cannot be discarded on the basis of our results, and the timing of events in the early life history of toothfish needs to be clarified. Specifically, more work is needed to determine: (i) when spawning and hatching occur; (ii) the timing of the formation of opaque and translucent zones; and (iii) the period to which the nucleus corresponds in toothfish at South Georgia.

Length-at-age for the cohorts containing fish 3+ and 4+ years old from the present study corresponded reasonably well to fish estimated as 5 and 6 years old by Everson (1991), but peaks for these cohorts are not clearly defined in Figure 2. This may be due to a variety of causes such as: movement away from the area covered by the survey, possibly into deeper water; high early mortality during 1998 and 1999; or low recruitment in 1997 and 1996 compared to 1998 and 1999. Alternatively, it may be due to sampling effects through changes in population distribution, for instance due to oceanographic concentrating processes or aggregating behaviour. Clearly, further sampling of toothfish together with information on migration patterns and water circulation in the region will be needed to clarify this matter.

A product of this study is an age–length key for juvenile toothfish. Year classes containing fish 1+ and 2+ years old were well represented, but older year classes were found in few or no samples. Monitoring the recruiting cohorts will allow predictive assessment of the age structure of the population, including the influence of good recruitment years on the subsequent biomass available to the fishery. In future, samples covering older age classes will also be needed, as accurate estimation of the overall age proportions for a full age–length key will depend on representative sampling of these older age classes, and validation of the age estimation methodology for all ages. Kimura (1977) warned that age–length keys will give biased results if applied to a population whose age composition differs from that of the population from which the age–length key was drawn: care should be taken in applying an age–length key outside the inferential limits of its sampling design (Ricker, 1975). To apply the age–length key in this study, further work is therefore needed to assess interannual variation in the age-frequency distribution of juvenile toothfish caught by trawl at South Georgia.

CONCLUSIONS

(i) The two dominant frequency modes represent different age cohorts separated by one year.

(ii) Previous ages estimated using published von Bertalanffy parameters are too high.

(iii) Age estimation criteria for South Georgia currently adopted by the CCAMLR Otolith Network imply substantial growth of the nucleus in only a few months before the appearance of the first translucent zone. Alternatively, the nucleus corresponds to a period greater than one year, and the current criteria lead to underestimation of age by one year.

(iv) Further research is needed to test these alternatives to establish whether the present CCAMLR Otolith Network criteria are correct. Further research is needed to determine: when spawning and hatching occur; when opaque and translucent zones are formed, and variation in the timing with age and between years; and the period to which the nucleus corresponds in *D. eleginoides* at South Georgia.

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Figure 1: Otolith section showing micro-structure used to estimate the age of juvenile *Dissostichus eleginoides*. Black dashes mark the edge of each yearly annulus (end of opaque zone and beginning of the following year's translucent zone).

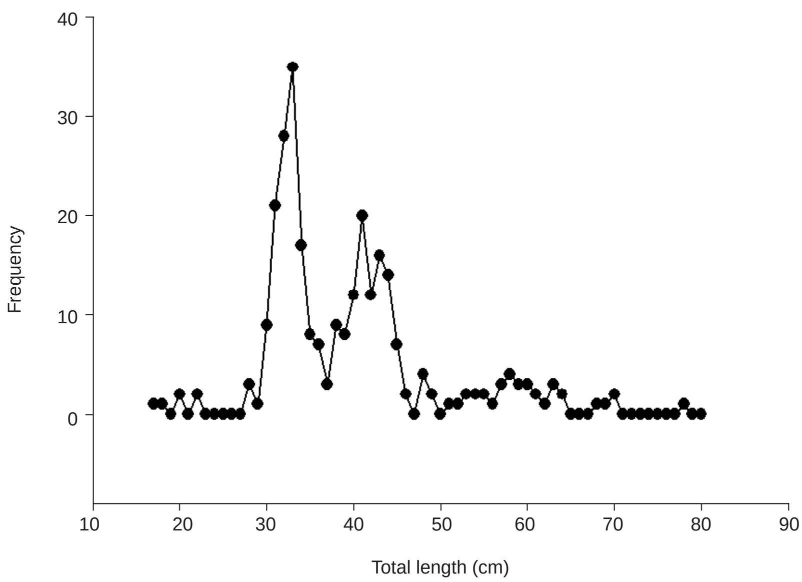

Figure 2: Length frequencies for the sample of *Dissostichus eleginoides* taken

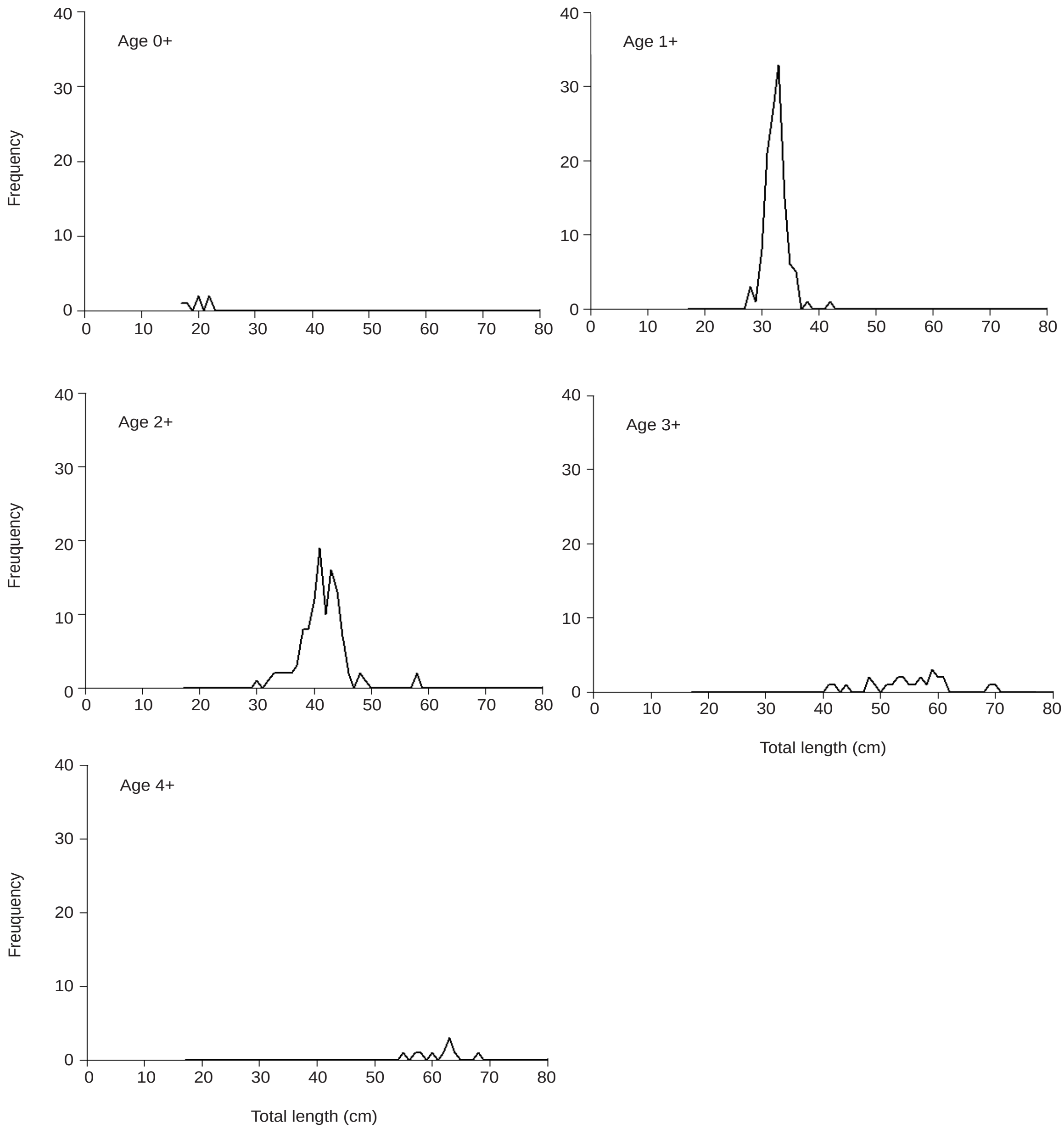

Figure 3: Length frequencies by age estimated from otoliths for the sample of *Dissostichus eleginoides* taken off South Georgia in January 2000.

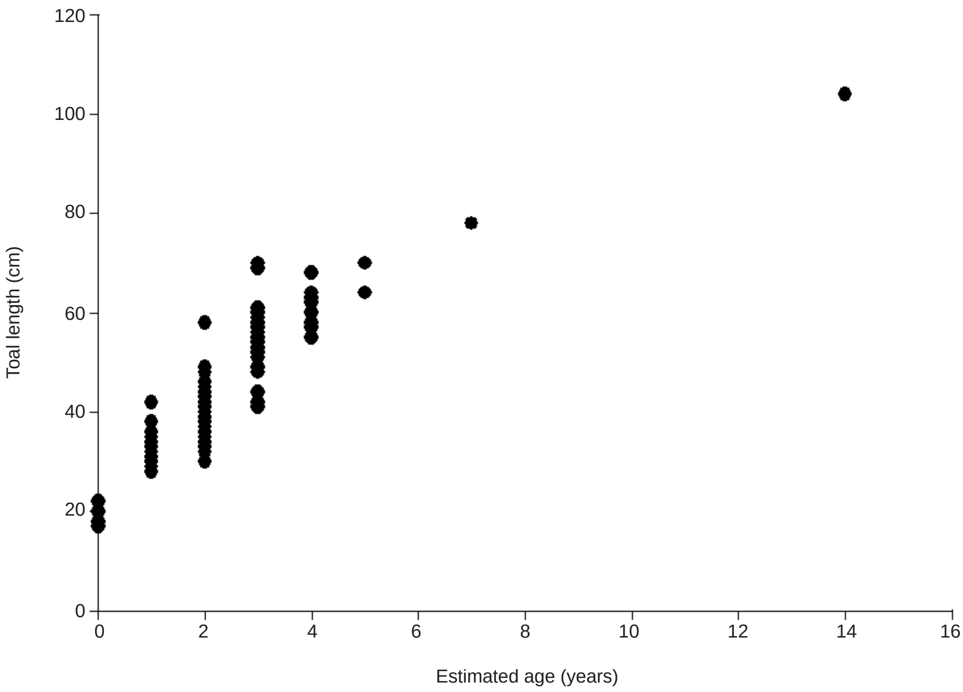

Figure 4: Size-at-age for the sample of *Dissostichus eleginoides* taken off South Georgia in January 2000.

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