

## SHORT NOTE

### EVIDENCE TO SUPPORT THE ANNUAL FORMATION OF GROWTH ZONES IN OTOLITHS OF ANTARCTIC TOOTHFISH (*DISSOSTICHUS MAWSONI*)

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#### Abstract

Antarctic toothfish (*Dissostichus mawsoni*) from the Ross Sea (CCAMLR Subarea 88.1) have been aged assuming that one translucent zone is formed annually in the otoliths of this species. However, no evidence to validate this assumption has previously been presented. In the current work, otoliths from four distinct juvenile length-frequency modes were examined, and the translucent zones counted. Zone counts were consistent within modes, and increased by one in each consecutive mode. This indicates that the modes represented year classes and that one translucent zone is formed annually in otoliths of juvenile *D. mawsoni*. Mean fish lengths at ages 0.5, 1.5, 2.5 and 3.5 years were estimated to be 14, 28, 37 and 46 cm total length (TL) respectively. These estimates agree with growth curves calculated previously from a sample of data that was large but lacked any fish younger than 3.5 years. Counting translucent zones in the otoliths of *D. mawsoni* appears to be a valid method for determining the age of this species. In addition, sagittal otoliths were examined from *D. mawsoni* that had been injected with oxytetracycline some years before recapture. The six recaptured fish were all large (129–145 cm TL at tagging) and had been at liberty from 1 to 7 years. The number of zones between the fluorescent oxytetracycline line and the otolith margin indicates that one translucent zone is formed annually in otoliths of post-mature *D. mawsoni*.

#### Résumé

L'âge de la légine antarctique (*Dissostichus mawsoni*) de la mer de Ross (sous-zone 88.1 de la CCAMLR) a été déterminé en présumant la formation d'une zone translucide chaque année sur les otolithes de cette espèce. Or, aucune évidence n'a encore été présentée pour valider cette hypothèse. Dans les travaux exposés ici, les otolithes de quatre modes distincts des fréquences de longueur de juvéniles ont été examinés et les zones translucides ont été comptées. Le dénombrement des zones concordait pour chaque mode, et l'on comptait un accroissement pour chacun des modes suivants. Il en découle que les modes représentent des classes d'âge et qu'une nouvelle zone translucide apparaît chaque année sur les otolithes des juvéniles de *D. mawsoni*. Les longueurs moyennes respectives des poissons à l'âge de 0,5, 1,5, 2,5 et 3,5 ans étaient estimées à 14, 28, 37 et 46 cm de longueur totale (TL). Ces estimations concordent avec les courbes de croissance calculées antérieurement

à partir d'un échantillon de données qui, malgré sa taille importante, ne couvrirait pas les poissons de moins de 3,5 ans. Il semblerait que le dénombrement des zones translucides sur les otolithes de *D. mawsoni* représente une méthode valable de détermination de l'âge de cette espèce. De plus, des coupes sagittales d'otolithes de *D. mawsoni* ayant subi une injection d'oxytétracycline plusieurs années avant d'être recapturé ont été examinées. Les six poissons recapturés étaient tous de grande taille (129–145 cm de TL au marquage) et avaient été relâchés entre 1 et 7 ans plus tôt. Le nombre de zones séparant la ligne fluorescente d'oxytétracycline de la marge de l'otolithe met en évidence la formation d'une zone translucide annuelle sur les otolithes de *D. mawsoni* post-matures.

#### Резюме

При определении возраста антарктического клыкча (*Dissostichus mawsoni*) моря Росса (Подрайон АНТКОМа 88.1) предполагается, что в отолитах этого вида образуется одна полупрозрачная зона в год. Тем не менее данных, подтверждающих это предположение, ранее представлено не было. В рамках данной работы было проведено исследование отолитов для четырех различных мод частотного распределения длин молоди и пересчитаны полупрозрачные зоны. Число зон было постоянным внутри моды и возрастало на 1 в каждой следующей моде. Это говорит о том, что зоны представляют годовые классы и что в отолитах молоди *D. mawsoni* формируется одна полупрозрачная зона в год. По оценкам, средняя длина особей возрастом 0,5, 1,5, 2,5 и 3,5 года составляла соответственно 14, 28, 37 и 46 см (общая длина – TL). Эти оценки согласуются с кривыми роста, рассчитанными ранее по большому набору данных, в котором, однако, отсутствовали данные по рыбе, моложе 3,5 лет. Подсчет полупрозрачных зон в отолитах *D. mawsoni* представляется адекватным методом для определения возраста этого вида. Помимо этого, были исследованы сагиттальные отолиты особей *D. mawsoni*, которым за несколько лет до повторной поимки были сделаны инъекции окситетрациклина. Все шесть повторно пойманных особей были крупными (129–145 см TL при мечении) и находились на воле от 1 до 7 лет. Количество зон между люминесцентной окситетрациклиновой линией и границей отолита свидетельствует о том, что в отолитах половозрелых *D. mawsoni* образуется одна полупрозрачная зона в год.

#### Resumen

La determinación de la edad de la austromerluza antártica (*Dissostichus mawsoni*) del mar de Ross (Subárea 88.1 de la CCRVMA) se ha basado en la formación anual de una zona translúcida en los otolitos de esta especie. Sin embargo, esta hipótesis hasta ahora no había sido demostrada. En este trabajo se examinaron los otolitos de peces juveniles de cuatro modas bien definidas de la frecuencia de tallas; y se contaron las zonas translúcidas. Los recuentos de zonas en los otolitos de cada moda fueron congruentes, aumentando en una zona con la moda siguiente. Esto denota que las modas representan clases anuales y que una zona translúcida se forma anualmente en los otolitos de los juveniles de *D. mawsoni*. Las tallas promedio de los peces a los 0,5, 1,5, 2,5 y 3,5 años se estimaron en 14, 28, 37 y 46 cm de longitud total (TL) respectivamente. Estas estimaciones concuerdan con las curvas de crecimiento calculadas a partir de numerosos datos de una muestra que no incluyó peces menores de 3,5 años. El recuento de las zonas translúcidas en los otolitos de *D. mawsoni* parece ser un método efectivo para determinar la edad de esta especie. Se examinaron además los otolitos sagita de ejemplares de *D. mawsoni* que habían sido inyectados con oxitetraciclina años antes de su captura. Los seis peces capturados después de haber estado en libertad de 1 a 7 años eran de talla grande (marcados a los 129–145 cm de longitud total). El número de zonas entre la línea fluorescente producida por la oxitetraciclina y el margen del otolito demuestra que se forma una zona translúcida anual en los otolitos de *D. mawsoni* después de alcanzada la madurez.

Keywords: age validation, length-frequency modal analysis, oxytetracycline, CCAMLR

## INTRODUCTION

The Antarctic toothfish (*Dissostichus mawsoni*) occurs around mainland Antarctica, generally south of 60°S. Growth parameters for this species were recently reported by Horn (2002) based on readings from the otoliths of approximately 1 500 fish sampled in the Ross Sea region (CCAMLR Subarea 88.1). Differences in growth rates between sexes were apparent, with females having a significantly faster growth rate and reaching a larger size than males. A growth curve for *D. mawsoni*, based on otolith zone counts from 46 fish caught in McMurdo Sound, had previously been reported by Burchett et al. (1984). The calculated growth curves from the two studies were similar (see Horn, 2002). However, neither study used a validated methodology, and the importance of validating ageing methodologies is well understood (Campana, 2001). Also, while Horn's (2002) growth curves were based on large sample sizes, few small fish were available. This was because the samples were obtained from the Ross Sea commercial longline fishery in which fish less than 60 cm total length (TL) are seldom caught (Hanchet et al., 2001).

Horn (2002) concluded that multiple banding was often present within the presumed annual growth zones in *D. mawsoni* otoliths, particularly in the early years of growth. However, because of the lack of small *D. mawsoni*, it was not possible to link the interpreted pattern of juvenile annual growth zones to fish of specific lengths. Multiple banding in juvenile zones has also been noted in the otoliths of the closely related Patagonian toothfish (*Dissostichus eleginoides*) (Cassia, 1998; Kalish and Timmiss, 2001; Horn, 2002). Because the interpretation of the zones in *D. mawsoni* otoliths is not straightforward, the validation of any ageing methodology is crucial.

Since Horn's (2002) study, two sources of data with potential to validate the periodicity of growth zones in *D. mawsoni* otoliths have become available. They are a set of length-frequency data (with associated otoliths) exhibiting distinct juvenile modes (Jones et al., 2001), and a set of otoliths from recaptured *D. mawsoni* that had previously been injected with oxytetracycline. This paper presents the analysis of these two datasets.

## METHODS

### Trawl Survey Samples

Juveniles of *D. mawsoni* were captured during a research trawl survey targeting a suite of demersal fish species, conducted in the region of the South Shetland Islands in March 2001 (Jones et al., 2001).

Otoliths from 31 of these fish were weighed to the nearest 0.1 mg. They were prepared for reading by being baked whole in an oven at 280°C for about 5 minutes until they were a light amber colour. The otoliths were embedded in clear epoxy resin, and sectioned transversely through the nucleus. The sectioned surfaces were coated with paraffin oil and examined using reflected light under a binocular microscope at  $\times 25$  and  $\times 40$  magnification. The number of complete translucent (dark) zones was counted and the otolith margin was recorded as either opaque or translucent. Decimal fractions of age were assigned to each year class based on the time elapsed between the estimated end of the spawning season (September) and capture (March). Hence, true age was assumed to be  $x + 0.5$  years, where  $x$  is the number of translucent zones counted. Otolith radii were measured, using a micrometer eyepiece, from the primordium to the outer edge of each of the translucent zones, on the longest axes of both the dorsal and ventral sides of the section.

The reader was not aware of fish length or otolith weight at the time the zone counts were made.

### Tagged Fish Samples

Specimens of *D. mawsoni* captured on hooks attached to a vertical 2.3 mm stainless steel set line in McMurdo Sound (78°S 167°E) have been tagged and released since 1972 (DeVries and Eastman, 1998). Some of these tagged fish were also injected with oxytetracycline hydrochloride (OTC). The injected OTC was undiluted vet grade Liquamycin, 50 mg OTC cc<sup>-1</sup>. Dosages were approximately 1 mg OTC per kg of fish weight. Some injected fish were later recaptured a year or more after tagging.

Otoliths from the recaptured fish were embedded whole and unbaked in clear epoxy resin. A thin transverse section (approximately 0.6 mm thick) was cut through the primordium of each otolith using a Struers saw fitted with two diamond-edged circular blades separated by a spacer. The section was mounted on a glass slide using quick-setting epoxy resin and ground down until it was about 0.35 mm thick. The exposed section surface was polished using alumina paste. The otoliths had been stored in darkness following extraction from the fish, and exposure to light was also minimised during the preparation process. The prepared sections were examined under a compound microscope at  $\times 40$  magnification with illumination by transmitted ultraviolet light. Oxytetracycline, when incorporated into the otolith in sufficient quantity, shows up as a fluorescent line on the

otolith section. Pairs of colour photographs were taken of each section. Each pair was of the same portion of the section, at the same magnification, but one of the pair was illuminated by ultraviolet light (to highlight the fluorescent zone) while the other was illuminated by transmitted white light (to highlight the presumed annual zones). The otolith reader measured the position, on the photograph, of the fluorescent zone from the otolith edge in the ultraviolet image and transferred it to the white light photograph. The number of complete annual zones between the estimated position of the fluorescent zone and the otolith edge was then counted, giving an estimate of the number of years between the oxytetracycline injection and recapture. Estimates were made initially without reference to the time at liberty between tagging and recapture.

Estimates of age at recapture were made by counting zones in the prepared slides examined under a binocular microscope with illumination by transmitted light at  $\times 40$  magnification.

## RESULTS

### Trawl Survey Samples

Juveniles of *D. mawsoni* (i.e. fish less than 55 cm TL) were captured at depths of 113 to 363 m, in an area bordered by latitudes  $61^{\circ}36'S$  and  $62^{\circ}56'S$ , and longitudes  $58^{\circ}35'W$  and  $61^{\circ}49'W$ . The length-frequency distribution of these fish is presented in Figure 1 (from Jones et al., 2001). It comprises at least four distinct and separate modal groups, i.e. 13–14, 26–30, 35–40 and 42–48 cm TL. The distribution of the modes was assumed to represent consecutive year classes.

Based on zone counts in the otoliths, the 31 juvenile *D. mawsoni* were estimated to be aged between 0+ and 3+ years. A length-frequency distribution of the aged fish by estimated year class (Figure 2), when compared with the trawl survey length frequency (Figure 1), supports the hypothesis that the length modes represent distinct year classes. The smallest fish (13–14 cm) were 0+, and the 3+ group comprised fish of 42 to 51 cm TL. Details of fish length, estimated age, mean radial measurements to the translucent zones, and otolith weight are presented in Table 1. Clarity of zones was generally better on the ventral side of the otolith section. The first annual zone on the dorsal side was sometimes too faint or indeterminate to enable a radial measurement to be made. Otolith sections from 1+, 2+ and 3+ fish are shown in Figure 3, with the positions of the translucent zones indicated on the ventral sides. The mean

radial measurements could be used to help interpret otoliths with unclear or confused juvenile banding structure.

The relationship between otolith weight and estimated age is linear and intercepts the axes close to the origin (Figure 4). There is no overlap between age classes of the ranges of otolith weights. Mean fish length by assumed year class, calculated from the raw data used to create Figure 1, was plotted against age on the growth curve (Figure 5) estimated previously by Horn (2002).

### Tagged Fish Samples

Details of the six tagged recaptured fish that had been injected with oxytetracycline are given in Table 2. Five had been tagged and recaptured in McMurdo Sound ( $78^{\circ}S$   $167^{\circ}E$ ). One was recaptured by a longline vessel in the northern Ross Sea ( $71^{\circ}S$   $176^{\circ}30'E$ ), approximately 450 n miles north of the tagging site. Two of the six fish had no immediately apparent fluorescent zones in the ultraviolet light photographs. For three of the remaining four fish, the estimated number of years between tagging and recapture was the same as the integer value of the actual number of years between these two episodes (Table 2). Images from the otolith of fish A00950 are shown in Figure 6. However, for the fish with tag A00993, the time at liberty estimated from the photographs was one year less than the actual time. On re-examination it was apparent that the fluorescent zone coincided with a translucent zone that was broad relative to the zones immediately before and after it, and that in some places this 'single' zone was split by a very narrow band of opaque material. It appears likely that the opaque zone expected to be laid down in the otolith in the summer immediately after tagging was poorly formed and not apparent in the photographs, leading to an underestimate of time at liberty.

Following receipt of data on the dates of tagging and recapture of all six fish, the photographs for fish with tags 790242 and 840061 were re-examined for fluorescent marks, given that the likely positions of the marks were now known. Fluorescence was still not apparent for fish 840061. However, the fluorescent zone could be identified in the photographs for fish 790242. The mark was faint and, being only one zone in from the margin, was largely obscured by the flaring effect of light refracted by the otolith edge (see Figure 6b for an example of this effect).

Age at recapture for the six fish was estimated to be between 19 and 28 years (Table 2). Growth

Table 1: Data derived from the 31 juvenile *Dissostichus mawsoni* and their otoliths. Radii are measured from the otolith primordium to the outer edge of the translucent zones, along the dorso-ventral axis of the section. Means (with standard deviations and sample sizes) of the radial measurements are also presented. ‘-’ denotes undeterminable radii or ages.

Otolith Number	Fish Length (TL, cm)	Estimated Age (years)	Ventral Radii (mm)			Dorsal Radii (mm)			Otolith Weight (g)
			R1	R2	R3	R1	R2	R3	
379	26	1	0.80			1.24			0.0123
404	46	3	0.76	1.20	1.52	1.12	2.00	2.52	0.0422
405	29	-							0.0126
406	29	1	0.96			1.36			0.0132
407	30	1	0.88			-			0.0151
408	28	1	0.92			-			0.0119
409	27	1	0.88			-			0.0124
410	27	1	0.92			1.40			0.0122
411	28	1	0.84			-			0.0128
412	27	1	0.84			1.28			0.0130
414	35	2	0.72	0.96		1.16	1.88		0.0222
415	29	1	0.92			-			0.0148
416	28	1	0.76			1.60			0.0149
500	-	1	0.64			-			0.0112
1945	14	0							0.0019
2501	27	1	0.96			1.28			0.0125
2701	26	1	0.80			1.12			0.0122
2702	28	1	0.92			1.28			0.0142
4317	48	3	1.00	1.44	1.76	1.28	2.28	2.80	0.0414
4321	51	3	1.00	1.40	1.72	1.20	2.08	2.60	0.0429
4322	46	3	0.84	1.28	1.44	1.40	2.16	2.76	0.0411
4323	35	2	0.76	1.20		1.48	2.12		0.0225
4324	48	3	0.92	1.32	1.52	1.04	1.92	2.48	0.0359
4325	29	1	0.80			1.60			0.0132
4326	44	3	0.88	1.20	1.40	1.36	2.12	2.68	0.0374
4363	42	3	0.88	1.28	1.44	1.28	2.16	2.64	0.0346
4364	38	2	0.84	1.36		1.28	2.08		0.0230
4365	27	1	1.04			1.60			0.0131
4366	27	1	0.92			1.60			0.0118
4371	26	1	0.88			1.56			0.0127
4372	28	1	0.88			1.48			0.0149
mean			0.87	1.26	1.54	1.35	2.08	2.64	
SD			0.09	0.14	0.14	0.17	0.12	0.12	
n			29	10	7	23	10	7	

Table 2: Details of the six tagged and recaptured fish used in this analysis. ‘-’ denotes unavailable data.

Tag Number	Date of		Time at Liberty (years)		Length (TL, cm) at		Sex	Age at Recapture (years)
	Tagging	Recapture	Actual	Estimated	Tagging	Recapture		
790242	24 Nov 78	5 Dec 79	1.0	-	129	127	-	19
840061	2 Dec 83	21 Oct 84	0.9	-	139	140	-	21
840066	28 Nov 82	22 Oct 84	2.0	2	133	133	-	19
A00950	27 Nov 87	24 Nov 94	7.0	7	133	155	-	28
A00993	8 Dec 87	17 Nov 91	4.0	3	145	152	-	28
A001229	30 Oct 97	28 Feb 01	3.3	3	131	137	F	28

increments (based on the total lengths measured on the two occasions each fish was captured) are plotted on the growth curves (Figure 5) estimated previously by Horn (2002).

## DISCUSSION

Two sources of data have been analysed to examine the likely validity of the method currently being used to age *D. mawsoni*. That method (counting translucent zones in sagittal otoliths) was described by Horn (2002).

The analysis of length data and otoliths of juvenile *D. mawsoni* from the South Shetland Islands did not confirm the juvenile growth rate, because the age at formation of the first translucent zone is still unknown. An examination for daily growth zones was unable to provide any information on the likely time of formation of the first translucent zone. However, several sources of information support the hypothesis that the four length-frequency modes represent consecutive year classes, and that the first mode (at 13–14 cm TL) represents 0+ fish. The relationship between otolith weight and estimated age is linear and intersects the axes relatively close to the origin (see Figure 4). Similar characteristics have been shown for otolith weight-to-age relationships for various fish species with validated ages (e.g. Anderson et al., 1992; Francis, 1992). This is logical, as a hatchling (i.e. a fish close to age 0) would be expected to have an otolith of negligible weight. If fish classified as 1+ were actually a year younger or a year older, the subsequent otolith weight-to-age relationships would imply either a positive otolith weight at age 0, or zero otolith weight more than a year after hatching. It therefore appears likely that the modes have been aged correctly. Otolith weights are grouped into four relatively distinct groups, and each weight group corresponds to one of the four length modes in Figure 1. Counts of translucent zones increase by an increment of one in otoliths of fish from each consecutive length mode. It therefore appears likely that one translucent zone is formed annually in the otoliths of *D. mawsoni* aged from 1 to 3 years.

The estimation of the time elapsed between hatching and capture of the juvenile fish is somewhat arbitrary but, in the light of all available data, is likely to be about half a year. No published information confirming the spawning season of *D. mawsoni* at the South Shetland Islands is available. Available information on gonad maturity stages in Ross Sea fish indicates that spawning does not occur between October and December (Eastman and DeVries, 2000) or between

January and April (Hanchet et al., 2001). Patchell (2002) recorded some spawning fish in May at the end of the 2001/02 fishing season. Yukhov (1982, cited by Eastman and DeVries, 2000) captured *D. mawsoni* 4–6 cm long in January and suggested that spawning by this species occurs in late winter to early spring (i.e. August–September). Spawning by *D. mawsoni* in the Ross Sea may, therefore, be protracted over the months May to September, but the times of peak spawning or hatching of the larvae are unknown.

The juvenile *D. mawsoni* otoliths from the South Shetland Islands all had a well-defined, and often quite broad, opaque margin (see Figure 3). It was also apparent that otoliths from fish taken in the Ross Sea longline fishery (i.e. from January to April) predominantly exhibited opaque margins (authors' unpublished data). It appears likely that *D. mawsoni* deposit translucent otolith material in winter and opaque material in summer, as shown for *D. eleginoides* by Horn (2002).

For three of the four fish with clear oxytetracycline marks in their otoliths, estimated and actual times between tagging and recapture, based on counts of translucent zones between the fluorescent mark and the otolith margin, were identical. In the one example where estimated and actual time at liberty differed, it appeared likely that otolith growth had been interrupted (possibly as a consequence of the capture and tagging process), resulting in the virtual non-deposition of one band of opaque material. A fluorescent zone in the 'correct' position was subsequently identified in photographs for one of the two fish that were initially classified as having no fluorescence, once the actual time at liberty was known. These results strongly support the hypothesis that, for the size range examined, one translucent zone is formed annually in otoliths. It is estimated that these fish were 17 to 25 years old when tagged and injected. A fluorescent zone was not visible in the otolith from one fish that had been injected with oxytetracycline. There are various reasons why this might occur, but the most likely causes are that the oxytetracycline dosage was insufficient or that exposure to white light had faded the fluorescence.

The estimates of mean length at ages from 0.5 to 3.5 years match relatively well the von Bertalanffy curves calculated previously by Horn (2002) (see Figure 5). Those curves were derived from fish aged from 3.5 to more than 50 years. The actual growth increments of the six tagged fish also fit well to the growth curves (although the times between the two measurements for each

fish are relatively short in most cases). Growth by the tagged fish ranged from  $-2.0$  to  $3.1$   $\text{cm yr}^{-1}$  with a mean of  $1.0$   $\text{cm yr}^{-1}$ . DeVries and Eastman (1998) estimated a mean annual growth rate of  $2.3$   $\text{cm yr}^{-1}$  from 13 recaptured tagged *D. mawsoni* from McMurdo Sound. The expected growth rate indicated by the von Bertalanffy curves is from  $0.6$  to  $4.0$   $\text{cm yr}^{-1}$  for fish aged from 15 to 30 years. It is interesting that all the points from the tagged and recaptured fish fall below the lower (male) growth curve calculated by Horn (2002), yet fit reasonably well around the growth curve calculated by Burchett et al. (1984) for McMurdo fish (see Figure 5). This raises the question of whether *D. mawsoni* might be relatively sedentary and growth might be slightly faster in the northern Ross Sea (where Horn's data are derived from) relative to that for fish in McMurdo Sound. It is noted, however, that there is at least some movement of fish from south to north, indicated by the toothfish tagged at McMurdo Sound but captured about 450 n miles to the north in one of the main commercial longlining areas.

It should be noted that although data from *D. mawsoni* from both the Ross Sea and the South Shetland Islands are incorporated in Figure 5, there is currently no information showing that growth is similar in the two areas. Growth of juveniles in the South Shetland Islands fits the growth curves calculated for the Ross Sea population, but no data are available on size at age of mature fish from the South Atlantic. However, it is likely that the inclusion of data on juvenile growth (from the South Shetland Islands fish) with the available length-at-age data from the Ross Sea would produce a more accurate growth curve than that reported by Horn (2002).

Marking otoliths of tagged fish with oxytetracycline successfully showed that one translucent zone forms annually in otoliths of older *D. mawsoni*. Marking small, young fish with the same method could help interpret the presumed multiple banding pattern apparent in some of the growth zones formed in young otoliths (Horn, 2002). This characteristic appears to be most common in zones formed from ages 3 to 10; after this age the zones are often clear and regularly spaced. Oxytetracycline marking (and later recapture) of fish younger than 10 years would essentially complete the validation of the ageing method for this species.

## CONCLUSIONS

1. Otoliths from distinct length modes of juvenile *D. mawsoni* from the South Shetland Islands provided a good indication of the juvenile

growth rate and indicated that one zone was also formed annually in the otoliths of these fish.

2. *D. mawsoni* that had been tagged and injected with oxytetracycline in McMurdo Sound, and later recaptured, had fluorescent zones in their otoliths indicating that post-mature fish laid down one zone annually.
3. Counting translucent zones in *D. mawsoni* otoliths is a valid method of ageing this species.

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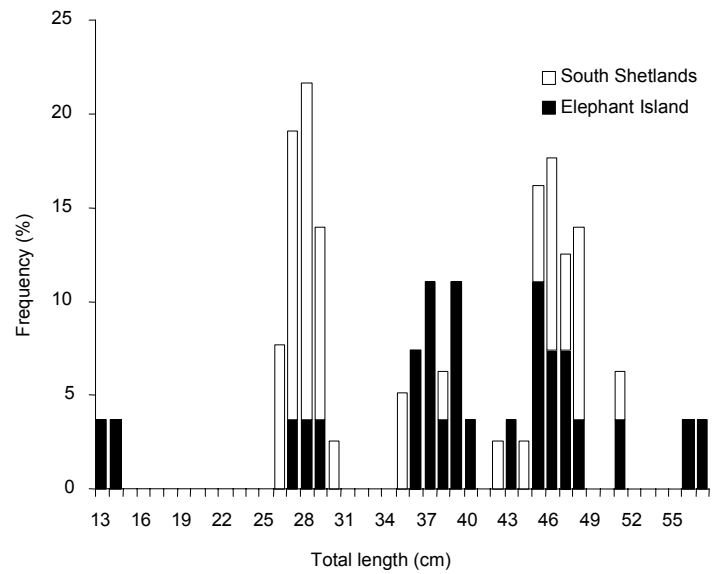


Figure 1: Length-frequency distribution for *Dissostichus mawsoni* from a trawl survey adjacent to the South Shetland Islands in March 2001. (Figure 4D from Jones et al., 2001.)

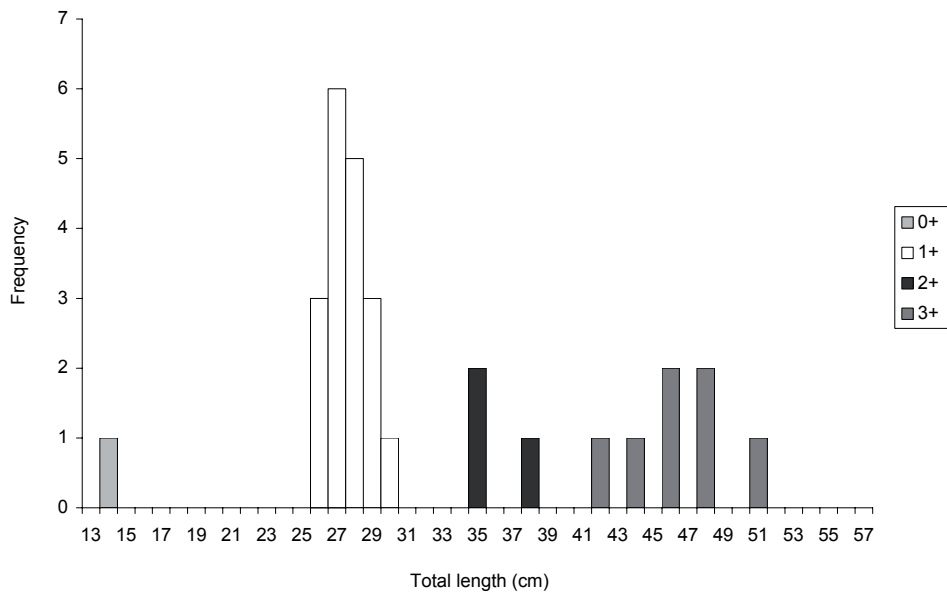


Figure 2: Length-frequency distribution of aged fish from the South Shetland Islands survey sample by year class.

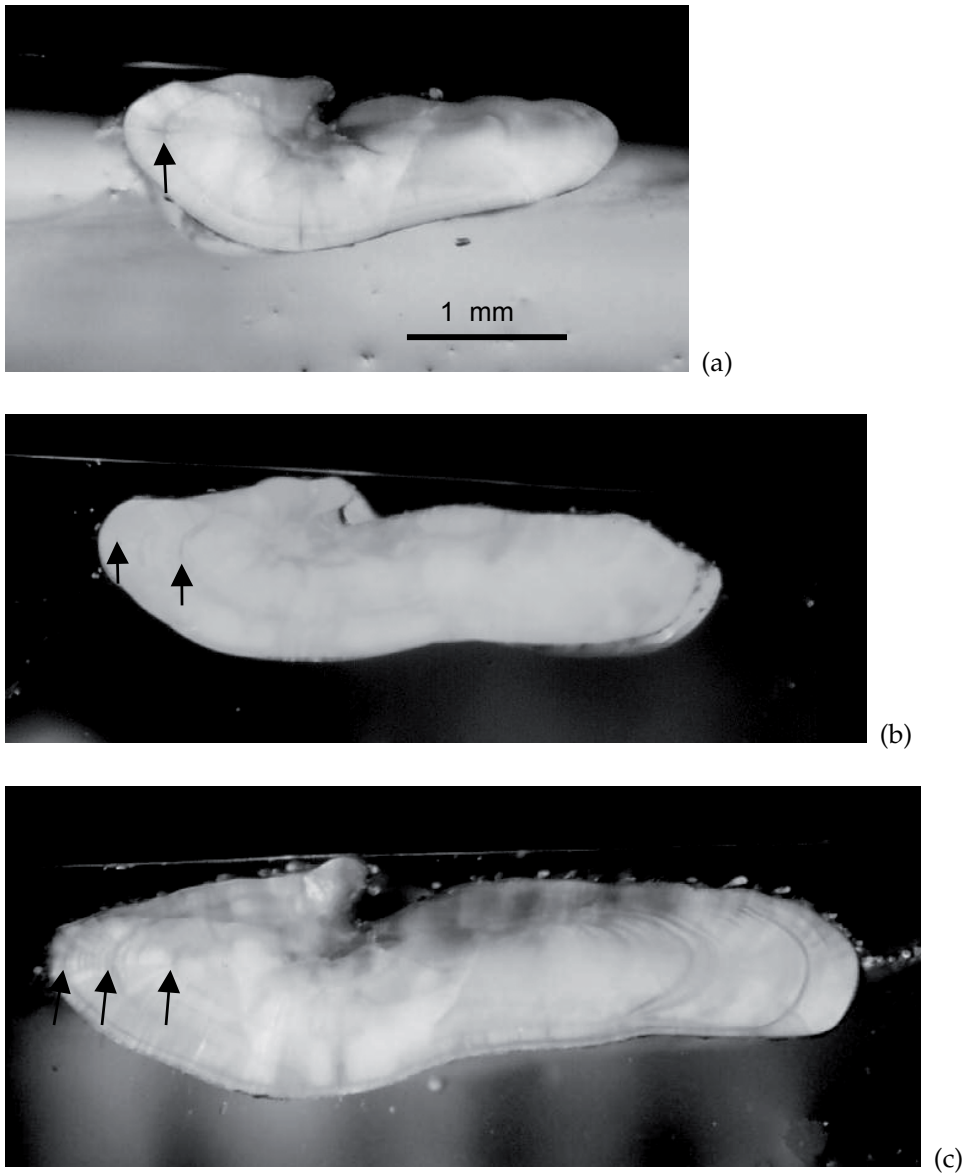


Figure 3: Transverse sections through baked otoliths from *Dissostichus mawsoni* estimated to be ages 1+ (a), 2+ (b) and 3+ (c). Arrows mark the positions of the translucent zones on the ventral sides of the sections. The 1 mm scale bar is applicable to all the micrographs.

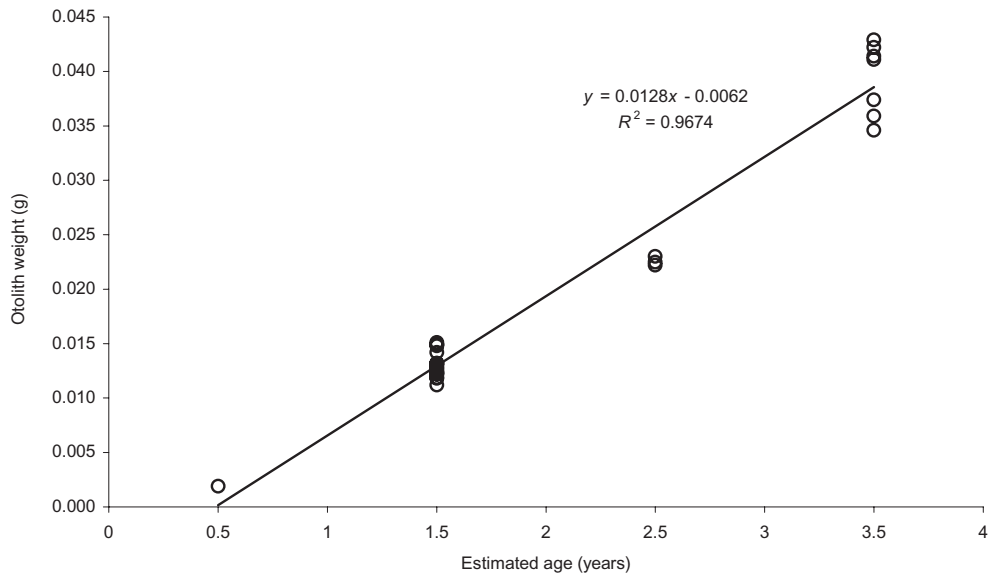


Figure 4: The linear relationship between estimated age and otolith weight for the 31 juvenile *Dissostichus mawsoni* from the South Shetland Islands.

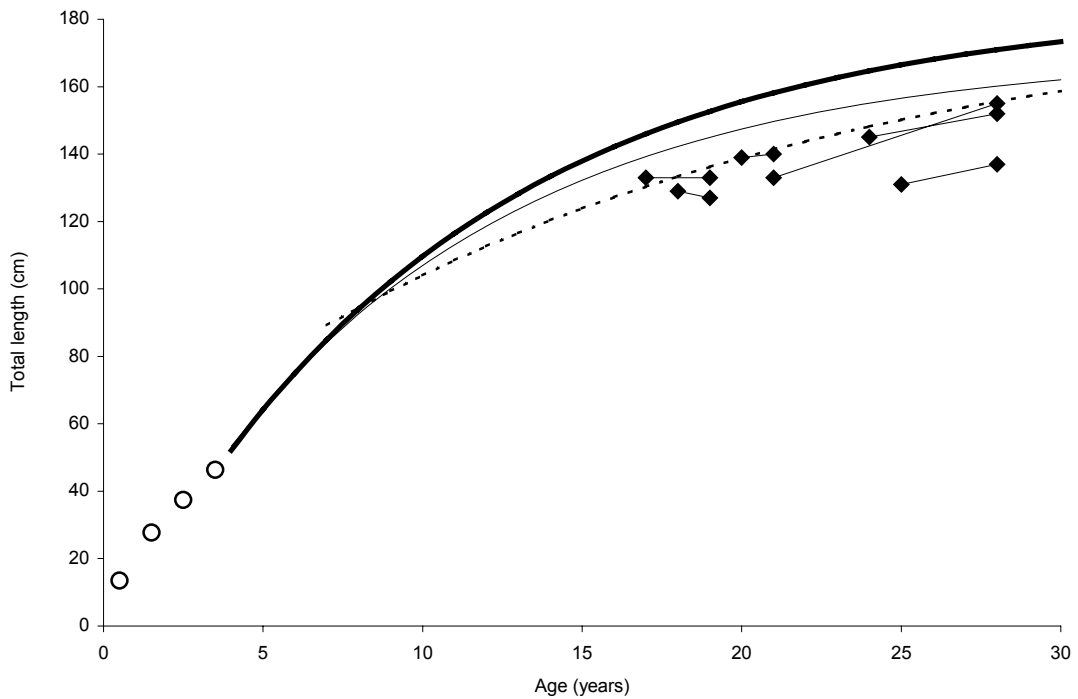


Figure 5: Estimates of mean length at age (open circles) for juvenile *Dissostichus mawsoni* from the South Shetland Islands, presented with growth curves for Ross Sea fish calculated by Horn (2002). Growth curves for both sexes are shown; the thicker line is the curve for females. The growth curve from Burchett et al. (1984) is presented as a broken line. Growth increments for the six recaptured tagged fish from McMurdo Sound are shown as diamonds connected by lines.

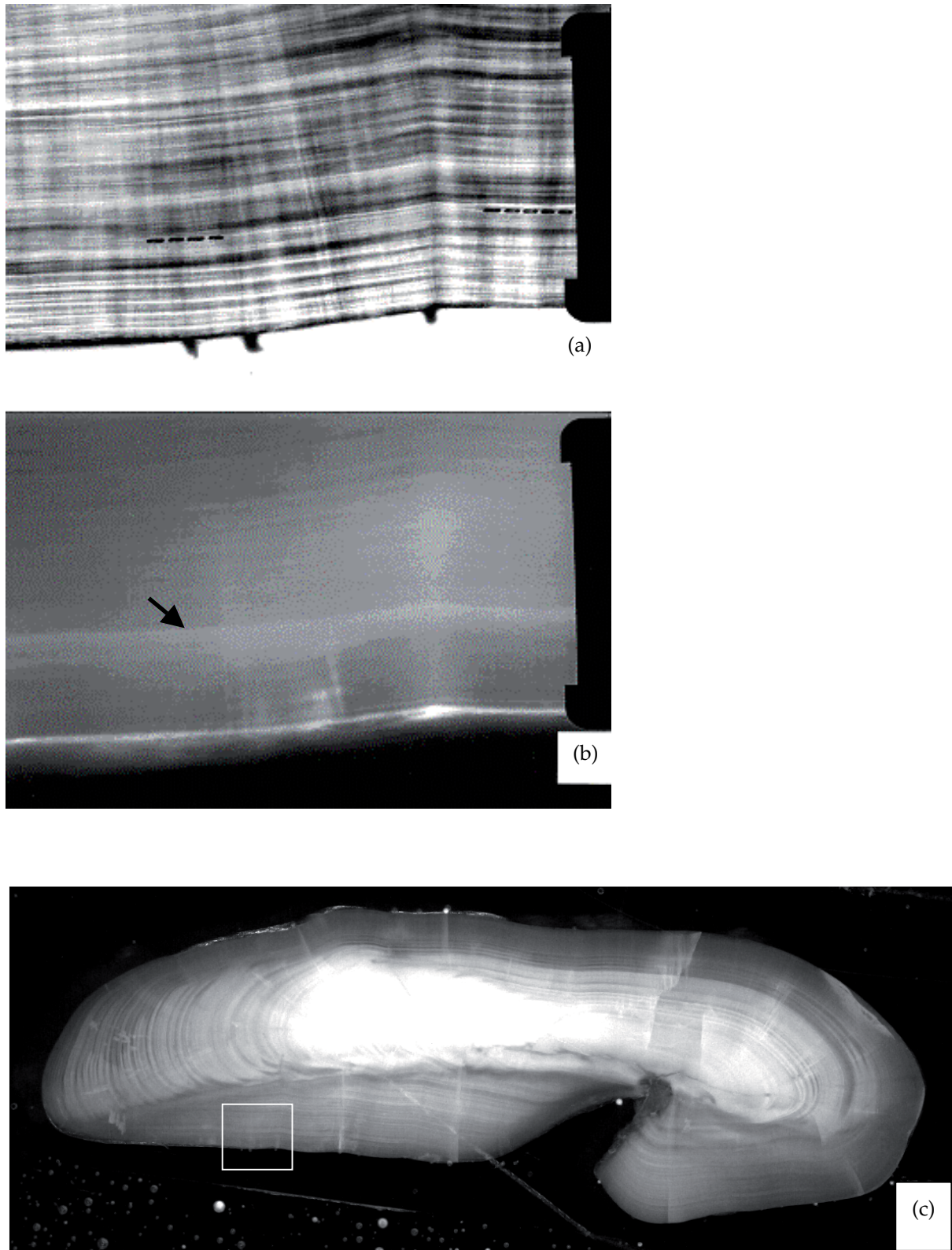


Figure 6: A portion of a thin section through the otolith of a *Dissostichus mawsoni* (tagged fish A00950) injected with oxytetracycline, with illumination by white light (a) and ultraviolet light (b), and the whole otolith section (c) showing the position of the micrographs as a white rectangle. The broken lines in (a) mark the position of the line of fluorescence (indicated by the arrow in (b)). The scale caliper on both micrographs is 320  $\mu\text{m}$ . Note the seven pairs of opaque and translucent zones between the broken line and otolith margin on (a), indicating a period of 7 years between injection and recapture.

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