SHORT NOTE

AGE ESTIMATION AND MATURITY OF THE RIDGE-SCALED MACROURID (*MACROURUS WHITSONI*) FROM THE ROSS SEA

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Abstract

Juveniles of the macrourid *Macrourus whitsoni* were collected by the NIWA research vessel *Tangaroa* during the BioRoss survey of the western Ross Sea and Balleny Islands in 2004. Intensive analysis of otoliths from these specimens greatly increased confidence in the interpretation of otolith growth zone structure, supporting the otolith interpretation protocol used in previous work on this species. Von Bertalanffy growth models assuming different growth by sex and by year-within-sex were fitted separately to a revised length-at-age dataset and compared using the likelihood-ratio test. Von Bertalanffy parameters for the pooled dataset with unsexed juveniles are L_∞ 76.12, K 0.065 and t_0 –0.159 for males and L_∞ 92.03, K 0.055 and t_0 0.159 for females. Revised estimates of the mean total length-at-maturity (38.8 and 46.4 cm) and mean age-at-maturity (10.6 and 13.6 years) are presented for males and females respectively, using a reduced probit model.

Résumé

Lors de la campagne d'évaluation BioRoss de l'ouest de la mer de Ross et des îles Balleny, en 2004, le navire de recherche *Tangaroa* de NIWA a collecté des juvéniles du grenadier *Macrourus whitsoni*. L'analyse intensive des otolithes de ces spécimens a grandement amélioré la confiance dans l'interprétation de la structure des zones de croissance des otolithes, confortant ainsi le protocole d'interprétation des otolithes utilisé lors des travaux antérieurs sur cette espèce. Des modèles de croissance de von Bertalanffy supposant une croissance différente selon le sexe et selon l'année pour le même sexe sont ajustés séparément à un jeu de données révisé de longueurs selon l'âge puis comparés au moyen du test du rapport de vraisemblance. Pour les jeux de données cumulés, les paramètres de von Bertalanffy, lorsque les juvéniles sont de sexe indéterminé, sont L_∞ 76,12, K 0,065 et t_0 –0.159 pour les mâles et L_∞ 92,03, K 0,055 et t_0 0,159 pour les femelles. Les estimations révisées de la longueur totale moyenne à la maturité (38,8 et 46,4 cm) et de l'âge moyen à la maturité (10,6 et 13,6 ans) sont présentées respectivement pour les mâles et les femelles, au moyen d'un modèle Probit réduit.

Резюме

Образцы молоди макруруса *Macrourus whitsoni* были получены научно-исследовательским судном *Tangaroa* института NIWA во время съемки «BioRoss» на западе моря Росса и у о-вов Баллени в 2004 г. Интенсивный анализ отолитов этих особей существенно повысил достоверность интерпретации зональной структуры

роста отолитов, подтвердив протокол интерпретации отолитов, использовавшийся в предыдущих работах по этому виду. Модели роста по Берталанфи, допускающие различный рост в зависимости от пола и от возраста особей одного пола, были отдельно подобраны к пересмотренному набору данных о длине по возрастам и сопоставлены с использованием критерия отношения правдоподобия. Параметры Берталанфи для объединенного набора данных с не разделенной по полу молодью были L_∞ 76.12, K 0.065 и t_0 −0.159 для самцов и L_∞ 92.03, K 0.055 и t_0 0.159 для самок. Приводятся пересмотренные оценки средней общей длины по достижении половозрелости (38.8 и 46.4 см) и среднего возраста по достижении половозрелости (10.6 и 13.6 лет) соответственно для самцов и самок, полученные по упрощенной пробит-модели.

Resumen

El barco de investigación *Tangaroa*, del Instituto Nacional de Investigación Hidrográfica y Atmosférica (NIWA), tomó muestras de ejemplares juveniles del granadero *Macrourus whitsoni* durante la prospección BioRoss del sector occidental del Mar de Ross y las Islas Balleny realizada en 2004. El análisis exhaustivo de los otolitos de estos especímenes contribuyó significativamente a la confianza con que se interpreta la estructura de la zona de crecimiento de los otolitos, y concuerda con el protocolo para la interpretación de la lectura de otolitos utilizado en estudios anteriores de muestras de esta especie. Se realizaron ajustes separados de dos modelos de crecimiento de von Bertalanffy a un conjunto revisado de datos de talla por edad, uno suponiendo que hay diferencias entre el crecimiento de ambos sexos, y el otro suponiendo que hay diferencias entre el crecimiento de peces de un mismo sexo de un año a otro. La comparación se hizo con la prueba de la razón de la verosimilitud. Los parámetros de von Bertalanffy para el conjunto de datos agrupados de peces juveniles sin diferenciación de los sexos fueron L_∞ 76.12, K 0.065 y t_0 −0.159 para los machos y L_∞ 92.03, K 0.055 y t_0 0.159 para las hembras. Se presentan estimaciones revisadas del promedio de la talla total de madurez (38.8 y 46.4 cm) y del promedio de la edad de madurez (10.6 y 13.6 años), para machos y hembras respectivamente, obtenidas mediante un modelo probit reducido.

Introduction

A longline fishery targeting toothfish (*Dissostichus* spp.) in the waters in and near the Ross Sea, Antarctica, has operated each year since 1998 (Hanchet et al., 2002). Toothfish landings increased steadily from 41 tonnes in 1998 to 2 426 tonnes in 2004 (Hanchet et al., 2004). Antarctic toothfish (*D. mawsoni*) accounted for about 85% of total landings, while Patagonian toothfish (*D. eleginoides*) made up less than 1% of the catch. Macrourids, almost exclusively *Macrourus whitsoni*, comprised a substantial by-catch of the fishery, accounting for about 10% of the total landed catch. In the 2004 season, about 297 tonnes of *M. whitsoni* were reported as having been caught (Hanchet et al., 2004). Although a by-catch, this species is clearly of commercial importance, and is in need of monitoring and management. However, little is known about its biology.

Some of the critical parameters of fisheries population biology are the estimation of age and growth, fishing selectivity and the age structure of the commercial catch. From 2000 to 2002, species identification and preliminary age and growth research were carried out on *M. whitsoni* by Marriott and McMillan (2002) and Marriott et al. (2003). While the authors were reasonably confident in their age estimates, there was still some degree of doubt as to the accuracy of their results, as the method they used to interpret *M. whitsoni* otoliths was unvalidated and few otoliths from small fish (<40 cm) were read. To increase confidence in the accuracy of their age estimates, it was proposed to prepare and read otoliths from a sample of small fish. Juvenile *M. whitsoni* were collected by scientific staff on board NIWA's research vessel *Tangaroa* during the 2004 BioRoss expedition to the Ross Sea (Mitchell and Clark, 2004).

This paper presents the results of the analysis of these specimens, and compares these results with those from the earlier studies. Revised estimates of age-at-maturity are also presented. Otolith terminology in this paper follows Kalish et al. (1995).

This work was carried out for the New Zealand Ministry of Fisheries under project ANT2004/04 Objective 2, as part of its commitment to Antarctic research.

Methods

Sample collection and preparation

Macrourus whitsoni were collected from 16 stations in the western Ross Sea and Balleny Islands, using an orange roughy bottom trawl. Trawl gear parameters are described in Mitchell and Clark (2004). *Macrourus whitsoni* specimens collected were immediately frozen and returned to NIWA Wellington for processing. A total of 149 fish were kept for ageing work, ranging from 6.8 to 83.5 cm in length. Of these, 45 fish were less than 40 cm in total length, the majority of which had been collected from six stations predominantly around the Balleny Islands.

Specimens were thawed, weighed to the nearest gram, and total length and snout–vent length were measured to the nearest millimetre. Otoliths were removed, cleaned, air-dried and stored in paper envelopes prior to processing. Otoliths from small fish less than 40 cm in total length were examined and aged from whole preparations, thick sections and thin sections, enabling comparisons to be made between the three preparation techniques for each otolith.

Whole otoliths were examined and read intact under a dissecting microscope with transmitted light. The otoliths were immersed in water to enhance visualisation of the internal zone structure.

Thick-section preparations were produced using a standardised resin embedding technique. The otoliths were baked at 285°C for 8 minutes, then embedded in epoxy resin (Araldite K142) and cured at 50°C for 24 hours. The cured resin blocks were then sectioned transversely through the otolith nucleus using a diamond-edged wafering blade, and polished on the cut surfaces. The prepared otolith surfaces were coated with paraffin oil and observed through a stereomicroscope at approximately x50 magnification, with illumination by reflected light.

Thin-section preparations were produced using a standardised resin embedding technique. Dried otoliths were embedded in small blocks of epoxy resin (Araldite K142) and cured at 50°C for 24 hours. Twin diamond-edged wafering blades were mounted on a Struers Accutom-2 saw to produce wafer sections 230 µ thick. The resin blocks were sectioned transversely through the otolith nucleus and the sections mounted on a glass slide under a coverslip using Araldite K142 as a mounting medium. These otoliths were observed through a stereomicroscope at approximately x50 magnification, with illumination by reflected light.

Zone count estimates

Whole-otolith preparations, thick and thin otolith sections generally exhibited a regular pattern of opaque and translucent zones. Translucent zones in the whole preparations appear as light bands, while in thick- and thin-section preparations the same zones appear as dark bands. This is due to the differing preparation and viewing techniques employed in these three methods (Figure 1). Counts were made of complete translucent zones in all cases.

Otoliths consist principally of aragonite, a form of calcium carbonate. The accretion of aragonite to the otolith surface is controlled by protein macromolecules (Campaña, 1999). The opaque zones are the summer growth zones and are characterised by a higher proteinaceous component in the aragonite matrix than occurs in adjacent winter zones.

Zone counts were generated on the assumption that each opaque and translucent zone couplet represents a single year's growth in the otolith. For each preparation method, the number of fully-formed translucent zones present, an estimate of the relative width of the terminal translucent zone (narrow, medium or wide) and a readability score on a five-point scale were recorded for each otolith read.

All readings were made with no prior knowledge of the fish length, sex, weight, or any previous readings made on the fish otoliths by other reading methods.

Converting zone counts to age estimates

Otolith age estimates were generated from the translucent zone counts using the following method. An arbitrary 'birth date' of 1 January was assumed for all fish. Although the temporal extent of the spawning season is poorly known, *M. whitsoni* larvae have been caught in the western Ross Sea during the austral summer, December to January (La Mesa et al., 1999), and *M. whitsoni* gonad condition data (Marriott and McMillan, 2002) suggested that spawning extends into the late austral summer.

As all aged fish in the combined dataset were collected between 15 January and 6 March, or close to the assumed birth date, age estimations were only generated as whole integers, as the actual spawning date of this species in this region was unknown.
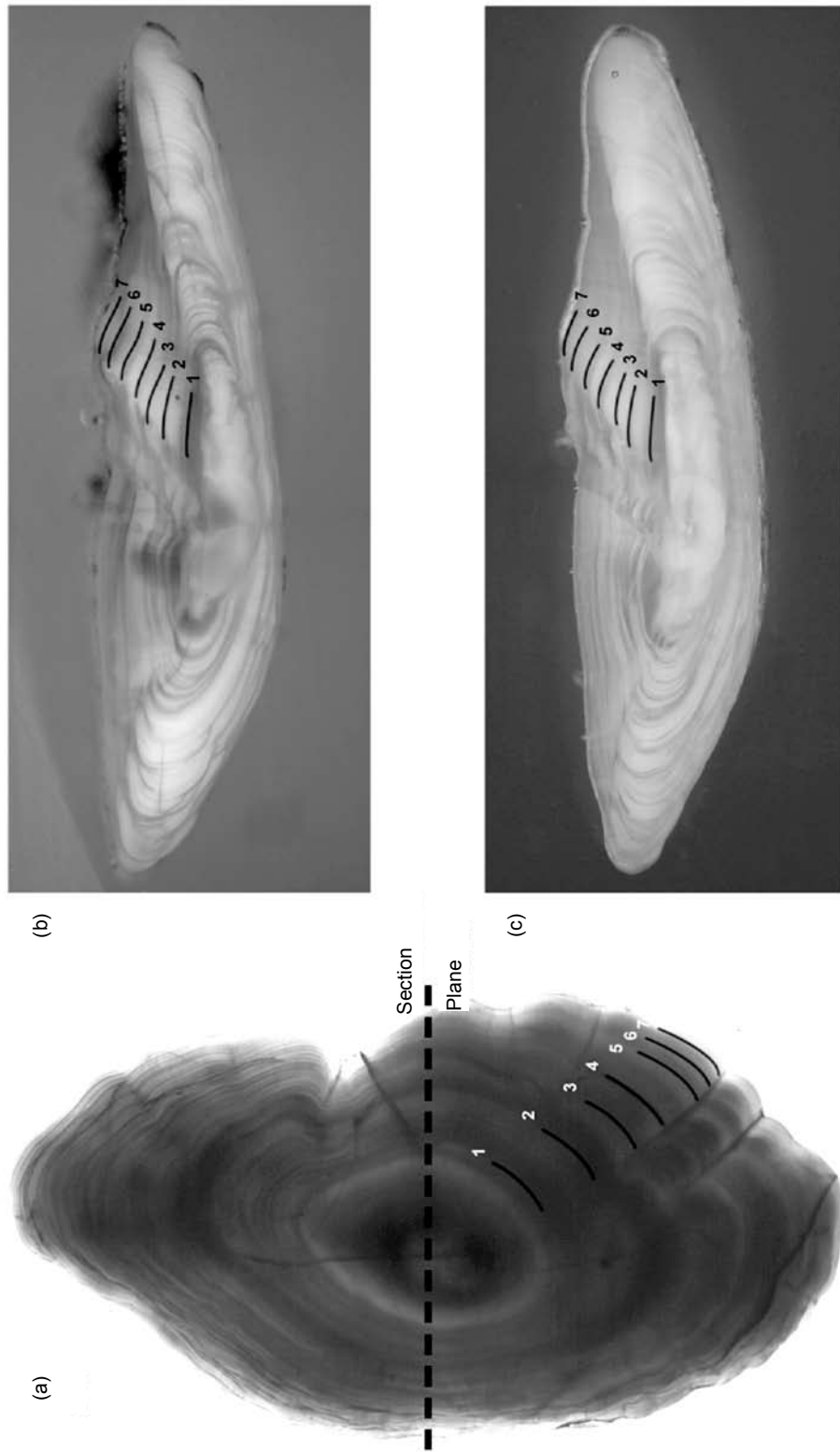
Figure 1:   The three otolith preparation methods used. All examples are from the same unsexed juvenile fish, total length 304 mm, snout–vent length 99 mm. (a) Whole preparation, also marked is the sectioning used for the thick and thin sections, (b) thick-section preparation, (c) thin-section preparation.

It can be difficult to define, in a summer-caught fish, whether the terminal opaque zone is visually discernable, especially when zones are narrow, as the whole margin can become transparent from light refracting in from the sides of the preparation. This is compounded by the fact that tetracycline studies in blue mackerel have shown that there is a time lag of a few months from when material is laid down until it becomes visually apparent (Stewart et al., 1999). To determine the state of the marginal annulus, a proxy was used by assessing the relative width of the adjacent terminal translucent zone. If the terminal translucent zone is very wide compared to previous translucent zones, then the otolith is said to be not yet visually exhibiting the new summer's growth zone, so the attributed age is equal to the zone count plus one.

Fitting von Bertalanffy growth models

The dataset produced during this study was merged with a dataset from 2002 (Marriott et al., 2003) to test for differences in growth between years within the sexes. A von Bertalanffy growth model that assumed separate parameters by year and sex and a model that assumed separate growth by sex only was fitted to the combined dataset. Maximum likelihood methods were used to fit the models to the data. The relative fits of the models to the data were then compared using the likelihood-ratio test (Kimura, 1980).

All models fitted assumed a single common variance parameter. Two likelihood-ratio tests were carried out. The full model for each test assumed separate parameters by year and sex. The reduced model for each test assumed separate parameters by sex only. The null hypothesis for each test was that the full and reduced model obeyed a set of constraints such that their parameters were equivalent; the alternative hypothesis was that the full and reduced model obeyed no such constraints. If fish were unsexed, or the age or length data was missing, they were dropped from the dataset. No other grooming of the data was performed. The combined dataset was also used in the length- and age-at-maturity analyses carried out below.

Reader comparisons for the 2004 data

Otolith-reading precision was quantified by carrying out within- and between-reader comparison tests following Campaña et al. (1995). A sample of 50 otoliths was randomly selected from the set of prepared otoliths and then re-read by the primary reader (P.M. Marriott). All otoliths were then read by a second reader (P.L. Horn) and both

sets of results compared with the primary reader's first set of results. The primary and second readers re-read a protocol set prior to carrying out their readings. The index of average percentage error (IAPE) (Beamish and Fournier, 1981), and mean coefficient of variation (CV) (Chang, 1982), were calculated and bias plots drawn for each test.

Length- and age-at-maturity

A large dataset of observer-collected maturity observations from 1999 was used to compute length- and age-at-maturity. These data were matched to the groomed combined length-at-age dataset described above. Fish reproductive maturity was determined by macroscopic examination of the gonads. A five-stage scale was used to classify reproductive maturity for both males and females: (1) immature; (2) maturing; (3) ripe; (4) running ripe; or (5) spent. Fish were designated as being mature in this analysis if they were classified as being at maturity stage 2 or greater.

Length-at-maturity ogives were fitted to the maturity data using probit analysis (Pearson and Hartley, 1962). A probit model was fitted to the data using a generalised linear model (GLM) (McCullagh and Nelder, 1989). The model assumed that maturity was modelled as a function of length and assumed separate intercepts for each sex. The model's slope was regressed through the origin.

$L_{50\%x}$, the length at which 50% of sex x are mature, was derived from the fit of the model. Male and female maturity ogives are overlaid on the raw data in Figure 5. Lengths used in the estimation of $L_{50\%x}$ were converted from snout–vent length to total length using the regressions in Marriott et al. (2003). The converted length values were substituted into the reduced von Bertalanffy model and solved for age to yield $A_{50\%x}$, the estimated age at which 50% of sex x are mature.

Results and Discussion

Sample selection

Most specimens analysed (75%) came from stations within the Ross Sea. This means that they are directly comparable to specimens collected by observers in the 2002 season. Although 80% of the fish less than 40 cm in total length were sourced from seamounts in the Balleny Islands group, it is assumed here that there is minimal difference in growth between fish collected from the two areas.

Otolith interpretation

It is not well known what defines the translucent and opaque zone structure apparent in deep-water fish, and the timing of the opaque zone deposition varies with species and environment (Gordon et al., 1995; Morales-Nin, 2001), but a number of studies on a large range of species have validated its annual nature (Bergstad, 1995; Gordon et al., 1995; Swan and Gordon, 2001).

The strongest environmental fluxes occurring on an annual basis at depth in Antarctic waters would be the seasonal peaks in food availability, which are driven by short, highly productive summer-season blooms in the surface waters. Similar peaks in food availability are thought to be a major controlling factor in zone formation in other deep-water rattails (Swan and Gordon, 2001). Spawning, and/or fasting associated with spawning, is another strong seasonal physiological stressor and could also have a major influence on the timing and appearance of annual zones, or sub-annual checks in the otolith (Høie and Folkvord, 2006).

The difficulty lies in differentiating between the annual and sub-annual checks apparent in the observed otolith when it is used for ageing purposes.

Of the three preparation methods investigated (Figure 1), the whole-otolith preparations were found to be the most difficult to interpret. This was due to the thick nature of the central region of the otolith, which made it hard to get a clear view of the central zones and the edge zones simultaneously. Differentiating between true, fully-formed translucent zones and false growth checks was more difficult in whole otoliths than in either the thick- or thin-section preparations. This method was judged to be inferior to both the thick- and thin-section preparation methods, and should only be applied to juvenile fish, which limits its application.

Both readers found the thick sections to be the easiest to interpret. Defining discrete zones was achieved with a high degree of confidence in most thick sections. Juvenile fish (<40 cm TL) were ascribed counts ranging from 0 to 16 zones. Otoliths from the very young fish, where 0–5 zones were recorded, were particularly important to the development of the reading protocol. Defining the zone structure of these fish greatly increased the confidence with which inner zones were defined in all fish aged. Good clear zone formation could be observed in most sections from the nucleus through to the otolith margins.

Thin sections showed the fine banding structure and false checks much more clearly than the corresponding thick section for each otolith. However, this extra detail often made it more difficult to obtain a confident zone count in the thin sections, and required more time to define the zone boundaries.

The remaining specimens from the BioRoss survey were aged using thick sections only. The observed zone structure in these juvenile fish strongly supported the zone structure that was inferred in previous ageing work on mature *M. whitsoni* (Marriott et al., 2003), so age estimates generated from that study are still deemed to be valid.

Fitting von Bertalanffy growth models

Translucent-zone counts in the combined 2002 and 2004 dataset were converted to age estimates using the methods described above. Results of likelihood-ratio tests comparing the fits of the full and reduced models to data suggested that growth does not appear to be statistically significant ($p > 0.05$) between the years within the sexes (Table 1).

This result supported the authors' belief that their interpretation of the otolith structure had not changed from the 2002 work, and suggested that the increased sample size of the reduced model dataset could be utilised to obtain a more robust estimate of the von Bertalanffy growth characteristics. Residual plots suggest a lack of fit to the very low and very high ages (Figure 2).

In the last run of the growth model, the groomed, combined 2002 and 2004 dataset was supplemented with observations from 26 unsexed juvenile fish after Stevens et al. (2004). Each observation in the set of 26 juvenile fish observations was added to both the male and female datasets. The von Bertalanffy models were then refitted to the supplemented dataset.

The juvenile end of the male and female growth curves exhibit very little difference when overlaid, so inclusion of the juvenile unsexed fish in both the male and female curves greatly improved the proximity of the t_0 values to zero (Table 2, Figure 3), suggesting that this von Bertalanffy model better estimates juvenile growth.

Reader comparisons for the 2004 data

The IAPE and mean CV for the between-reader test were 9.55 and 13.51% respectively, for the within-reader test they were 6.91 and 9.77%. Test results are shown in Figure 4.

Table 1: Von Bertalanffy model fits and likelihood-ratio test results for the full and reduced models.

Von Bertalanffy model fits:

Full model	2002 males	Std error	2004 males	Std error
L_∞	80.90	5.33	88.37	14.72
K	0.045	0.012	0.038	0.017
t_0	−6.18	3.70	−4.26	4.39
N	144		59	
	2002 females	Std error	2004 females	Std error
L_∞	90.88	4.12	101.63	11.54
K	0.056	0.012	0.041	0.011
t_0	−0.21	2.31	−1.18	1.64
σ^2	47.398	3.356		
N	156		64	
Reduced model	Pooled males	Std error	Pooled females	Std error
L_∞	81.03	4.47	91.89	3.01
K	0.047	0.010	0.055	0.006
t_0	−3.98	2.48	0.09	1.02
σ^2	48.793	3.455		

Likelihood-ratio test:

Model	Max. log-likelihood	Chi	DF	p (X > Chi)
Full	−1335.92	11.62	6	0.0711
Reduced	−1341.72			

(a)

(b)
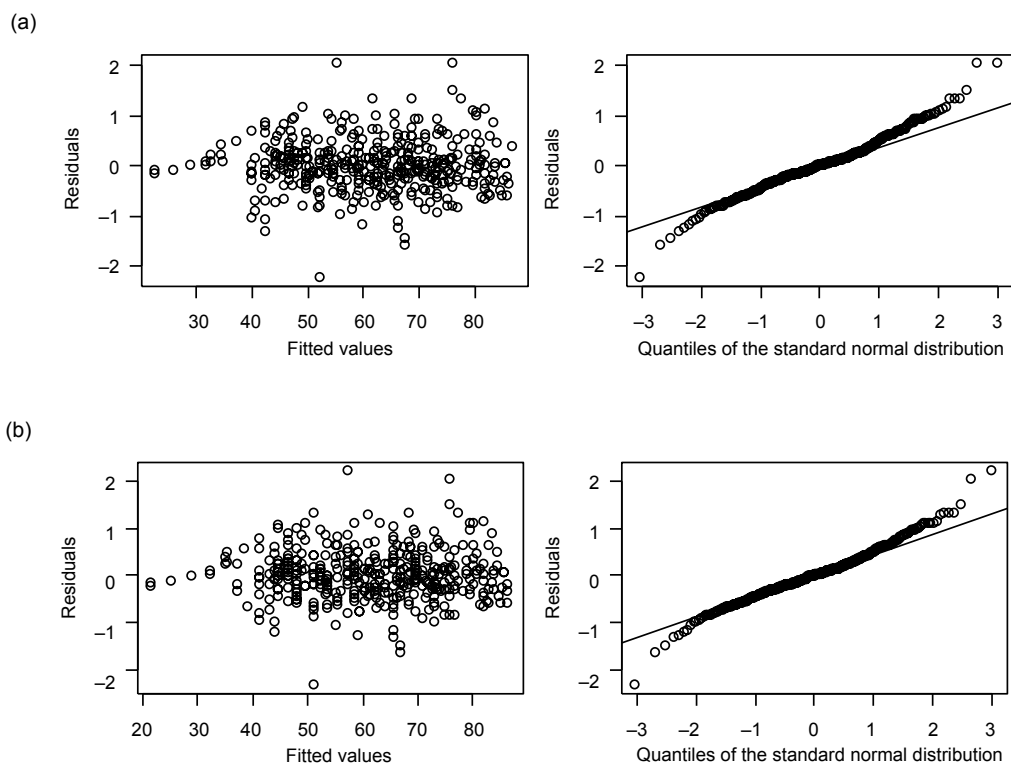
Figure 2: Diagnostic Pearson residual plots for the fits of (a) the full (separate growth by year and sex) and (b) the reduced (separate growth by sex only) von Bertalanffy growth models to the combined 2002 and 2004 dataset.

Table 2: Von Bertalanffy model fits for the reduced model, includes unsexed juveniles.

Reduced model including juveniles	Males + juveniles	Std error	Females + juveniles	Std error
L_∞	76.12	1.93	92.03	2.27
K	0.065	0.005	0.055	0.004
t_0	−0.159	0.489	0.159	0.411
σ^2	44.339	2.959		
N	229		246	
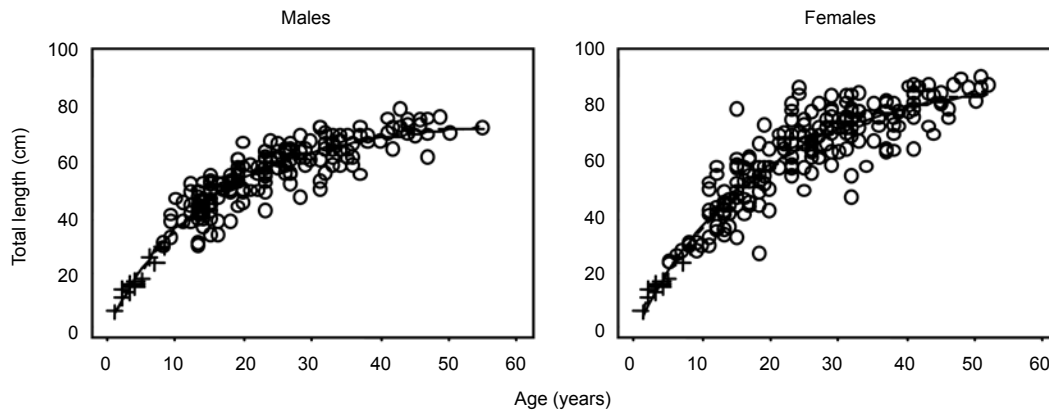
Figure 3: Length-at-age for all *Macrourus whitsoni* by sex, including unsexed juveniles (plotted as '+' signs).
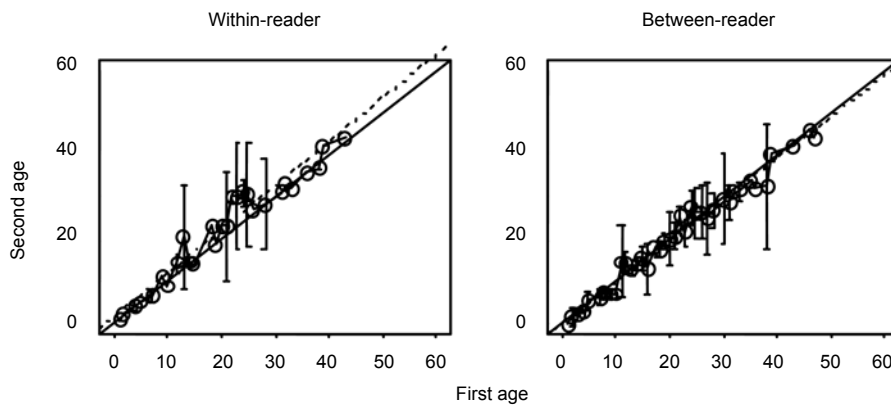
Figure 4: Bias plots of within- and between-reader comparison tests. The expected 1:1 (solid line) and actual relationships (dashed line) between ages produced during the first and second reading in each test are overlaid.

Within- and between-reader precision appears to be relatively low, but these results compare favourably with other long-lived deep-sea species with hard-to-read otoliths, e.g. cardinal fish (*Epigonus telescopus*), with a between-reader mean CV of 12.35% (Tracey et al., 2000), and black-gill rockfish (*Sebastes melanostomus*), which has a between-reader mean CV of 14.6% and within-reader mean CV of 7% (Stevens et al., 2004).

The bias plots show that most of the perceived error occurs in fish that were between 13 and 30 years old. There is some evidence of divergence between the primary reader's initial and subsequent readings, suggesting that interpretation changed to some degree over the course of the study. The within-reader bias plot (Figure 4) suggests that the reader generally assigned higher ages to fish that were between 10 and 30 years old in the second reading.
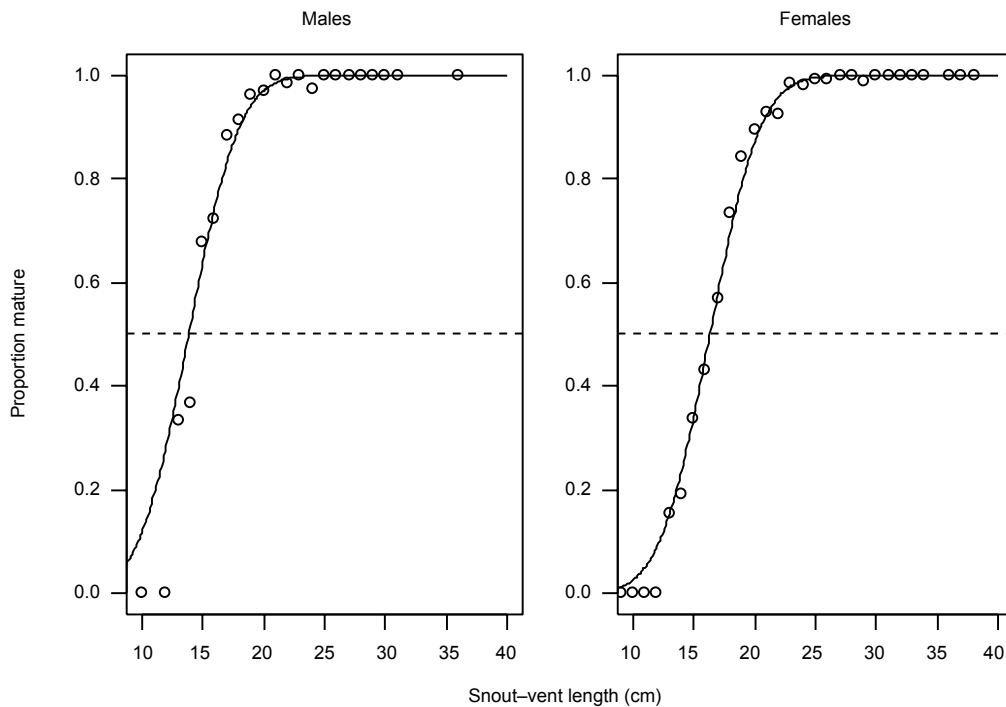
Figure 5: Fitted maturity ogives derived using probit analysis.

Table 3: Length (cm) and age (years) at maturity parameters. $L_{50\%x}$ – snout–vent length at which 50% of sex x are mature; $A_{50\%x}$ – estimated age at which 50% of sex x are mature.

Length-at-maturity		Males	Std error	Females	Std error
Snout–vent length	$L_{50\%x}$	13.84	0.208	16.32	0.138
Total length	$L_{50\%x}$	38.75	-	46.43	-
Age-at-maturity					
Years	$A_{50\%x}$	10.6	-	13.6	-

It was found that the most difficult region to interpret in *M. whitsoni* otoliths was around the transition zone. In this region the perceived growth in transverse sections changes from wide zones with many false checks, to narrower, more regular zones with few false checks. The primary axis of growth also changes from the dorso-ventral axis to the proximal-medial axis. This change in growth pattern generally occurred in the region of zones 10–13. This transition in growth pattern was not usually abrupt, but usually occurred as a gradual change in growth over a few zones.

The difficulties in interpreting individual otoliths are exhibited in the bias plots as the region where increased error occurs. It is assumed that the error observed in fish older than the transition zone-aged fish was largely accrued while attributing zones through this transitional growth area. This assumption is supported by the fact that the

errors observed do not get demonstrably larger as fish get older and accrue more zones. In fact, as the fish become older the associated errors actually become much smaller. It is assumed that a large number of outer, more narrow, zones actually improves the interpretation of the zones through the transitional growth region.

Length- and age-at-maturity

Length-at-maturity ogives were fitted to the maturity data (Figure 5). Length-at-maturity and age-at-maturity are presented in Table 3. Total length-at-maturity was derived from the snout–vent length values using the regression equations from Marriott et al. (2003). The length-at-maturity estimates were derived from length values substituted into the reduced von Bertalanffy model and solved for the age yield estimates.

Ogives derived from the fit of the reduced probit model were used to generate the length-at-maturity statistic $L_{50\%}$. This method gives the exact length, or age, at which 50% of the fish would be at maturity stage 2 or greater. The method used in Marriott et al. (2003) gave the length class, or age class, at which more than 50% of fish were at maturity stage 2 or greater. The two different methods produced slightly different $L_{50\%}$ estimates (15 and 17 cm snout–vent length or 46 and 50 cm total length respectively for the 2002 data; total length data being derived from the snout–vent length data size classes) even though the same observer-collected maturity dataset was used in both instances.

Age-at-maturity corresponds well with the observed transition zone (zones 10–13) in the otolith growth morphometrics, so it is likely that the onset of maturity is the cause of this transition.

Conclusions

Studying a sample of otoliths from small *M. whitsoni* has given much more confidence in the interpretation of the zone structure displayed in each year's growth, especially in the formative years. The otolith interpretation protocols developed during this work supported the interpretation used in previous ageing work on this species. Analysis of the von Bertalanffy growth curves showed that there was no significant difference between the 2002 and 2004 datasets within sexes. Various von Bertalanffy models were fitted to the data and compared. The reduced additive model with added unsexed juveniles should be used in future analyses, as this model is robust to pooling data across years, and adequately models the juvenile end of the growth curve. Revised estimates of length-at-maturity and age-at-maturity were estimated from ogives fitted to observer maturity data.

Acknowledgements

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