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Discrimination of *Macrourus whitsoni* and *M. caml* (Gadiformes, Macrouridae) using otolith morphometrics

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Abstract

That the predominant by-catch of grenadier by the toothfish fishery in the Ross Sea region is comprised of two species, rather than being solely *Macrourus whitsoni*, was recognised in 2010 with the identification of a new species, *M. caml*. Grenadier are the main by-catch in the toothfish fishery and modelling suggests that they may be subject to predation release as the abundance of toothfish reduces. In order to improve information on the spatial distribution, age-frequency and relative importance of grenadiers as prey to toothfish, a method of distinguishing between otoliths of the two species was sought. Based on 220 *M. whitsoni* and 307 *M. caml* otoliths, a linear function of fish total length, the depth of the otolith and the otolith vertically projected area gave excellent discrimination between the two species (92% success). A method was also developed to discriminate between otoliths of *M. whitsoni* and *M. caml* using otolith parameters alone. This method correctly identified 90% of otoliths to species and could be applied to otoliths removed from toothfish stomachs to estimate the relative consumption of the two species of grenadier. Models to estimate fish length, weight and age from otolith parameters are also presented.

Introduction

Grenadiers (Family Macrouridae) are taken as by-catch in many of the toothfish fisheries in the Southern Ocean (e.g. Duhamel et al., 1997; Morley et al., 2004; Stevenson et al., 2014). Grenadiers are the main by-catch in the fishery for Antarctic toothfish (*Dissostichus mawsoni*) in the Ross Sea region (CCAMLR Subareas 88.1 and 88.2) (Stevenson et al., 2014). A by-catch of 10 tonnes of grenadiers was reported in 1998, which increased to 480 tonnes in 2005 (Hanchet et al., 2008) and was 130 tonnes in 2014 (Stevenson et al., 2014). Grenadiers are also a major prey species of Antarctic toothfish in the region (Fenaughty et al., 2003; Stevens et al., 2014). Ecosystem modelling of the Ross Sea region suggests the potential for a 'predation release' of grenadiers as the number of toothfish reduces due to fishing, especially on the Ross Sea slope where fishing for toothfish is most intense (Pinkerton et al., 2010; Pinkerton and Bradford-Grieve, 2014; Mormede et al., 2014).

Grenadier caught as by-catch and found in stomachs of Antarctic toothfish in the Ross Sea region were considered to be almost exclusively *Macrourus whitsoni* (Regan, 1913) (Figure 1a) until samples collected on New Zealand's International Polar Year Census of Antarctic Marine Life (IPY-CAML) voyage in 2008 led to the identification of a new species: *Macrourus caml* (Smith et al., 2011; McMillan et al., 2012) (Figure 1b). Although two additional species of grenadiers, *M. carinatus* and *M. holotrachys*, occasionally occur in the region, these appear to be more abundant north of about 65°S and it is likely that *M. whitsoni* and *M. caml* are the main grenadiers occurring in the Ross Sea region at depths between approximately 500 and 2 100 m where fishing takes place (McMillan et al., 2012). The first differentiated information on the distribution, morphology, age, growth, staging, diet and trophic position of *M. whitsoni* and *M. caml* was given by Pinkerton et al. (2013) based on fish collected in 2008.

The recent recognition that there are two main species of grenadier in the Ross Sea region means that, at the time of writing, there are insufficient data to assess the potential effects of the toothfish fishery on these species. For example, there is inadequate information on the spatial distributions of the two species of grenadiers, no information on changes to the by-catch rates of the two species and no capacity to investigate changes to the age-frequency distributions of the species since the fishery started.

The capacity to differentiate between the two species of grenadiers using their otoliths would provide new information to help fill these knowledge gaps. Otoliths form *M. whitsoni* and *M. caml* cannot be distinguished from simple observation by the naked eye. However, differentiating on the basis of otolith morphology is increasingly used between species (e.g. Tuset et al., 2003a; De La Cruz-Agüero et al., 2012; Lord et al., 2012), for within-species stock discrimination (e.g. Tuset et al., 2003b; Cardinale et al., 2004; Morat et al., 2012; Leguá et al., 2013) and even to assigning age class (Petursdottir et al., 2006; Doering-Arjes et al., 2008).Where it works, this approach is relatively cheap (compared to genetics, for example), requires little specialised equipment and potentially (as in this case) allows archived otoliths to be used when a cryptic species is identified. Otoliths are commonly collected for ageing purposes by Members of CCAMLR (and other fisheries management agencies) and are often archived as they are small and cheap to store.

Therefore, the aim of this study was to determine whether it was possible to readily discriminate between *M. whitsoni* and *M. caml* on the basis of the shape of their otoliths. There is an archive of grenadier otoliths from the Ross Sea fishery presently stored at NIWA, Wellington, New Zealand. Marriott et al. (2003) aged 213 otoliths (117 males, 96 females) from fish collected in 2002 and subsequently Marriott et al. (2006) augmented this dataset with 149 aged otoliths (59 male, 64 female, 26 unsexed juveniles) collected in 2004. The ability to retrospectively identify these otoliths to species level will allow research such as: (i) examination of changes in the age and length distribution of grenadiers ('catch-curve analysis'); and (ii) mapping the spatial distributions of the two species in the Ross Sea in 2002 and 2004. Otolith morphometrics may also provide information on the species, sizes or ages of grenadiers consumed by toothfish, as otoliths removed from the stomachs of toothfish are often in good condition.

Materials and methods

Sampling

A total of 864 samples of grenadier were obtained for analysis from scientific observers on board four New Zealand autoline fishing vessels (between December 2011 and February 2012): *Antarctic Chieftain*, *Janas, San Aotea II* and *San Aspiring*. Scientific observers were asked to collect 10 grenadiers selected randomly from one haul every second day. Ignoring the location of the catch on the longline is assumed not to introduce a bias into the sampling. The observers were asked to make a preliminary identification of each specimen and then to place whole fish in individually labelled bags with identification, station number, small-scale research unit (SSRU), observer name and vessel name. The fish were frozen below –20°C and returned to NIWA (Wellington). These fish were the same ones used for the analysis presented in Pinkerton et al. (2013). All subsequent laboratory analysis was carried out between April and July 2012, less than six months after sampling. Samples were identified to species in the laboratory following McMillan et al. (2012) using the number of pelvic fins rays on the left (usually) or right (if the left was damaged) sides and the number of rows of teeth in the lower jaw. This morphological identification was found to be unambiguous in 98.8% of cases (n = 864, Pinkerton et al., 2013). The following biological information on each specimen was collected (where possible): total length (*TL*, cm), pre-anal length (*PAL*, cm), fish total wet weight (*fish_wgt*, g) and sex.

Observer logbooks were used to obtain the location of capture of each sample, which is taken to be midway between the start setting position and end setting position of the set on which the sample was caught. Samples of both species of macrourid were obtained from the northern seamounts of Subarea 88.1 (SSRUs 881B and C), from the Ross Sea continental slope (SSRUs 881H, J and K) and from Subarea 88.2 (SSRU 882H) (Figure 2; Table 1). Otolith samples from *M. whitsoni* and *M. caml* were taken from the same set 47% of the time (40 sets from a total of 86 sets). Within the sample of *M. whitsoni*, 37 fish were male (17%) and 183 were female (83%); for *M. caml*, 123 fish were male (40%) and 183 were female (60%). Lengths of *M. whitsoni* in the sample were 34.5–65.1 cm *TL* and 12.0–24.0 cm *PAL*. Lengths of *M. caml* in the sample were 34.5–81.5 cm *TL* and 11.0–30.0 cm *PAL* (Figure 3).

Table 1: Number of samples by SSRU and species used in this study.

SSRU	*M. whitsoni*	*M. caml*
881B	5	19
881C	61	48
881H	67	80
881J	4	19
881K	16	33
882H	67	108
Total	220	307

Otolith sampling and measurements

Otoliths were extracted from 220 *M. whitsoni* and 307 *M. caml* as described by Marriott et al. (2006). As the left otolith had usually been used for ageing studies, the right otolith was usually used for morphometric measurements in this study. Otolith studies show little difference between left and right otolith morphology in grenadiers or other species (e.g. Hunt, 1992; Cardinale et al., 2004; Morley and Belchier, 2002; Megalofonou, 2006). Whole undamaged otoliths were viewed lateral face uppermost under a stereomicroscope using standard illumination (3 000°K; 180 ms shutter; 1.006 gamma). Otolith images were captured with a c-mounted digital camera using calibrated magnifications and analysed with ImageJ software (Abramoff et al., 2004). The set of measurements made on each otolith (see below) followed recent published studies of otolith discrimination (Tuset et al., 2003a; De La Cruz-Agüero et al., 2012; Leguá et al., 2013) and included five commonly used shape indices (Russ, 1990; Tuset et al., 2003b; De La Cruz-Agüero et al., 2012). The names of the parameters as used in data analysis are given in italics:

(i) Otolith depth (*oto_depth*): depth of whole otolith measured using digital calipers (mm).

(ii) Otolith weight (*oto_weight*): weight of whole otolith to 0.1 mg on a Mettler AE163 analytical balance (mg).

(iii) Area (*Area*): Area of whole otolith projected onto a horizontal plane ('vertically projected' area) in mm^2. Note that *Area* is slightly different from 'cross-sectional area', with the difference depending on where the otolith was sectioned.

(iv) Maximum and minimum feret (or caliper) measurement (*MaxFeret*, *MinFeret*): the maximum or minimum distance between any two points on the otolith boundary (mm).

(v) Perimeter (*Perimeter*): The length of the outside boundary of the whole otolith (mm).

(vi) Bounding rectangle dimensions (*BRwidth*, *BRheight*): The smallest rectangle enclosing the outside boundary of the otolith (mm).

(vii) Fitted ellipse dimensions (*FEmajor*, *FEminor*): The major and minor axes of an ellipse fitted to the outside boundary of the otolith (mm).

(viii) *Circularity* = $4\pi Area/Perimeter^2$ (dimensionless).

(ix) *Aspect ratio* = *FEmajor*/*FEminor* (dimensionless).

(x) *Roundness* = $4Area/\pi/FEmajor^2$ (dimensionless).

(xi) *Ellipticity* = (*FEmajor* – *FEminor*)/(*FEmajor* + *FEminor*) (dimensionless).

(xii) *Rectangularity* = *Area*/*MaxFeret*/*MinFeret* (dimensionless).

Statistical analysis

Linear discriminant analysis was used to test for differences in otolith morphology between *M. whitsoni* and *M. caml*. In some cases, basic biological data of grenadiers (*TL*, *PAL*, fish weight) will be available in subsequent application of the results of this study; this information was assumed available to the discriminant model in addition to the otolith parameters. Sex was not used in developing the discriminant functions because of the few male *M. whitsoni* in the dataset. Location of capture, and associated information such as depth of capture, were also offered to the discrimination analysis.

The linear combination of otolith, fish biology and environmental parameters that provided greatest discrimination between species was calculated and tested using K-fold testing of the discriminatory power. In the interests of robustness, a relatively parsimonious model was sought, i.e. one that includes as few parameters as possible without sacrificing discriminatory performance. In testing the performance of each model, the data for *M. whitsoni* and the data for *M. caml* were each split randomly into 10 parts of approximately equal sizes. A linear model was developed on the dataset excluding the first 10% of the data for *M. whitsoni* and the first 10% of the data for *M. caml*. The model was applied to the withheld data. This was repeated 10 times, excluding each part of the data in turn, and the discrimination tested on the models applied to the withheld data. This was repeated 100 times with different random groupings of data to test the sensitivity to the random division of data. Three measures were used to test the discriminator power: (i) the multiple regression coefficient (R^2, higher values indicating better discrimination); (ii) the proportion of cases where species were correctly identified; and (iii) the area under the receiver operating characteristic (ROC) curve (Swets, 1988). ROC analysis is a widely used tool in the evaluation of the discriminatory power of a binomial model (Fawcett, 2006). The ROC value is the area under a plot of the fraction of true positives against the fraction of false positives. An ideal model has a ROC value of 1; a model with no discriminatory power has a ROC value of 0.5; a model with ROC value greater than 0.7 is considered 'useful' (Swets, 1988), although this depends on the particular application. The final models reported were then fitted to all data (none withheld).

Repeating the analysis without offering any biological information to the model was used to investigate if knowledge of otolith morphology alone could be used to discriminate between species or provide information on *TL*, *PAL* or weight. This analysis is applicable to otoliths retrieved from the stomachs of toothfish. Assuming species could be determined from these otoliths, models were developed to estimate fish length, weight and age based on otolith morphometrics alone using stepwise linear regression analysis.

Results

Otolith morphology

For both species, otolith size, depth, weight and vertically projected area increased with fish total length (Figure 4) and fish age (Figure 5) but there was little change in otolith shape (roundness) with fish size. In general, otoliths of *M. caml* were larger, deeper and heavier than otoliths of *M. whitsoni* for fish of a similar total length (Figure 4). To a lesser extent, otoliths of *M. caml* were also larger than otoliths of *M. whitsoni* for fish of the same age (Figure 5). Otoliths of *M. whitsoni* and *M. caml* were very similar in shape irrespective of fish length or age.

Discrimination function using all data

Using linear discriminant analysis and with biological data available to the model, the best discrimination was given using equation 1 (coefficients in Table 2 based on fitting to all data), where α greater than 0.5 indicates *M. whitsoni* and α less than 0.5 indicates *M. caml*. The summary statistics for a discriminant function based on these parameters (*TL*, *oto_depth* and *Area*) are given in Table 2 for the K-fold validation case. The ROC for this discriminant function is shown in Figure 6; the area under the ROC curve was 0.968. More details of the model using *TL*, *oto_depth* and *Area* are given in Table 3. While there was no significant difference between the discrimination function for either species by sex, or for *M. caml* between subareas, there was a significant difference between the discrimination function for *M. whitsoni* from Subareas 88.1 and 88.2.

$$\alpha = A_1 + A_2 TL + A_3 oto\_depth + A_4 Area \qquad (1).$$

The best discrimination function based on *PAL* was determined for those cases where *TL* was not available (Table 2). The discrimination using *PAL* was slightly poorer than using *TL* (e.g. 90% rather than 92% correctly identified).

Table 2: Discriminant analysis results for the model shown in equation 1 using fish total length (*TL*), otolith depth (*oto_depth*) and otolith vertically projected area (*Area*). The table also shows the model using *PAL* instead of *TL*. Performance statistics are based on K-fold validation with 10 folds. The coefficients are fitted to all data (n = 527).

	Model using *TL*		Model using *PAL*	
Coefficients	Constant	1.254	Constant	1.330
	TL	0.03512	*PAL*	0.09022
	oto_depth	–0.7668	*oto_depth*	–0.6915
	Area	–0.02463	*Area*	–0.02453
Multiple linear regression coefficient, R	0.776		0.770	
Proportion correctly identified	92% (483/527)		90% (475/527)	
Area under ROC	0.968		0.967	

Table 3: Contingency tables based on K-fold validation with 10 folds, for the model shown in equation 1. WGR = *Macrourus whitsoni*. WG2 = *M. caml*. One specimen of *M. caml* could not be sexed. A two-sided *t*-test with uneven variances was used to test whether we could reject the null hypothesis that α was the same in the groups. First set of rows: testing α between WGR and WG2. Second set of rows: testing α between sexes within WGR and WG2. Third set of rows: testing α between Subareas (88.1, 88.2) within WGR and WG2. * indicates a significant difference in the discriminant function at the 5% level.

Test	Actual	Model					Reject the null hypothesis that α is the same between groups?
		Number of cases			Proportion of cases		
		WGR	WG2	Total	WGR	WG2	
1. Testing between species	WGR	204	16	220	0.93	0.07	$t(525) = –30.29$, $p < 0.001$ *
	WG2	27	280	307	0.09	0.91	
	Total	231	296	527			
2. Testing between sexes within species	Male WGR	36	1	37	0.97	0.03	$t(218) = 1.26$, $p > 0.05$
	Female WGR	168	15	183	0.92	0.08	
	Male WG2	8	115	123	0.07	0.93	$t(304) = 1.56$, $p > 0.05$
	Female WG2	19	164	183	0.10	0.90	
	Total	231	295	526			
3. Testing between areas within species	88.1 WGR	146	7	153	0.95*	0.05	$t(218) = 4.20$, $p < 0.001$ *
	88.2 WGR	58	9	67	0.87*	0.13	
	88.1 WG2	17	182	199	0.09	0.91	$t(305) = 0.64$, $p > 0.05$
	88.2 WG2	10	98	108	0.09	0.91	
	Total	231	296	527			

Models based on otolith morphometrics alone

Discriminant models were derived which did not use biological data (such as fish length, fish weight or sex) for cases where these biological data were not available. Using otolith morphometrics alone, it was possible to distinguish otoliths of *M. whitsoni* from otoliths of *M. caml* nearly as effectively as in Table 2 (which used fish length), but more parameters were needed (Table 4). Models to estimate of fish length, weight and age based on otolith morphometrics are given in Table 5.

Discussion

The spatial scale of the sampling used in this study was set by the length of the longlines in the toothfish fishery, i.e. 5–10 km. At this scale of sampling, *M. whitsoni* and *M. caml* are almost completely sympatric by depth, both occurring widely over the continental slope in Subareas 88.1 and 88.2 and both appearing to be abundant between depths of approximately 900 and 1 900 m (Pinkerton et al., 2013). Samples of grenadier otoliths used in this study were drawn widely from across the Ross

Table 4: Discriminant analysis results using otolith morphometrics alone. Performance statistics are based on K-fold validation with 10 folds. The coefficients are fitted to all data (n = 527).

	Model using no biological data	
Coefficients	Constant	5.983
	oto_depth	–1.620
	oto_weight	0.01255
	Area	–0.04611
	Roundness	0.8840
	Rectangularity	–3.406
Multiple linear regression coefficient, R	0.776	
Proportion correctly identified	90% (476/527)	
Area under ROC	0.959	

Table 5: Predicting total length (*TL*, cm), pre-anal length (*PAL*, cm), fish weight (*fish_wgt*, g) and age of fish (*Age*, years) from otolith morphometrics. 'RMS' is the root-mean square error of the prediction.

Species	Equation	Performance	
		R^2	RMS
Macrourus whitsoni	$TL = 22.76 + 0.3425\ oto\_weight - 0.0007581\ oto\_weight^2$	0.694	3.37
	$PAL = 7.078 + 0.1283\ oto\_weight - 0.0002893\ oto\_weight^2$	0.647	1.38
	$fish\_wgt = -336.1 + 11.43\ oto\_weight - 0.02525\ oto\_weight^2$	0.575	146
	$Age = 15.76 + 0.1426\ oto\_weight - 0.3468\ Area$	0.460	2.90
Macrourus caml	$TL = 34.03 + 0.1082\ oto\_weight$	0.585	4.83
	$PAL = 10.32 + 0.05207 oto\_weight - 3.815e\text{-}5\ oto\_weight^2$	0.534	1.80
	$fish\_wgt = 238.4 + 1.030\ oto\_weight + 0.01285\ oto\_weight^2$	0.518	327
	$Age = 27.80 + 0.1980\ oto\_weight - 0.6714\ Area$	0.589	4.76

Sea region but no otoliths were available from areas where the highest historical catch of grenadiers has been taken by the fishery (SSRU 881I), nor the region of highest catch rates of grenadiers (northern part of SSRU 881K) (Stevenson et al., 2014). However, the small variation in otolith properties between Subareas 88.1 and 88.2 (Table 3) suggests that the limitations imposed by this sampling are likely to be small. The small but significant difference in otolith properties of *M. whitsoni* between Subareas 88.1 and 88.2 suggests that these may be different stocks.

Examination of the otoliths of the two species of grenadier showed that although they could not be distinguished by the naked eye, it was possible to discriminate the species using the size of the otolith (a combination of its depth and vertically projected area) relative to the size of the fish. The discrimination was tested by withholding 10% of the data, fitting a multiple linear regression and using this relationship to predict the species for the withheld data. The proportion of species correctly identified was 92% and the area under the ROC curve was 0.968, indicating excellent discrimination. Sometimes *PAL* rather than *TL* will be available for grenadiers. In these cases, an alternative discriminant function based on *PAL* rather than *TL* performs almost as well (90% samples correctly discriminated rather than 92%; area under ROC curve 0.967 rather than 0.968) (Table 2).

The successful discrimination of species using fish length and otolith size parameters is due to the fact that *M. caml* otoliths are substantially larger and heavier than *M. whitsoni* otoliths for fish of a similar length (Figure 4). Much of this difference is because *M. caml* caught by the toothfish fishery are older than *M. whitsoni*; otolith size and weight increase as material is added around the outside of the otolith each year (Figure 5). While *M. whitsoni* approaches full size at about 17 years and can live to at least 27 years, *M. caml* takes more than 30 years to reach 80% of the mean maximum length and one specimen was aged at 62 years (Pinkerton et al., 2013).

For cases where neither *TL* nor *PAL* of the grenadier was available (for example, otoliths retrieved from the stomachs of toothfish), an alternative model was developed using five otolith parameters (*oto_depth*, *oto_weight*, *Area*, *Roundness* and *Rectangularity*) and this allowed 90% successful discrimination between *M. whitsoni* and *M. caml*. Note that *Roundness* in this study was based on the length of the major axis of the fitted ellipse, and that *Rectangularity* was based on the maximum and minimum ferret (caliper) dimensions of the otolith. Measures of *Roundness* and *Rectangularity* based on other otolith size metrics are not interchangeable.

Eight models were developed which used otolith parameters alone to explain between 46% and 59% of the variance in fish length, weight and age, assuming that the species had first been determined correctly (Table 5). Applying these models to grenadier otoliths taken from toothfish stomachs will enable the size and age of both species of grenadier consumed by toothfish to be estimated. This information will be used to assess the potential effects of the toothfish fishery on these grenadier species, for example, through further minimum-realistic modelling of toothfish and its prey in the Ross Sea region (Mormede et al., 2014).

Although the limits of inference for the present study strictly only cover the period from which samples were collected (2011/12), the results are likely to be applicable retrospectively, unless there have been very substantial changes in growth rates (length-at-age) or condition of grenadiers to date.

Observers in the Ross Sea should continue to identify a proportion of *Macrourus* by-catch to species level using morphological keys (Pinkerton et al., 2013), but otoliths could be collected to check this at-sea identification. The positive result that *M. whitsoni* and *M. caml* can be discriminated using their otoliths suggests that similar methods could be used elsewhere in the Southern Ocean where grenadiers of the genus *Macrourus* are taken as by-catch in toothfish fisheries, for example on the Kerguelen Plateau (Duhamel et al., 1997) and around South Georgia (Morley et al., 2004). Fish of the *Macrourus* genus are also found outside the Southern Ocean. For example, *M. berglax* is targeted in the North Atlantic Ocean (Lorance et al., 2008) and is taken as a by-catch in the Barents Sea (Dolgov et al., 2008) and around east Greenland (Fossen et al., 2003). We recommend that otoliths of *Macrourus* spp. caught both inside the Southern Ocean and elsewhere should be examined for bimodality in case other cryptic species are present.

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Figure 1: (a) *Macrourus whitsoni*, 47 cm total length (*TL*) specimen caught on a longline from the Ross Sea, Antarctica, 77°20’S 169°25’E, 780–848 m, 22 January 1999. (Photograph by Andrew Stewart, Museum of New Zealand Te Papa Tongarewa.) (b) *Macrourus caml*, 26 cm TL specimen caught in a trawl from the Ross Sea, Antarctica, 73°15’S 178°44’E, 760–770 m, 19 February 2008. (Photograph by Peter Marriott, NIWA.)

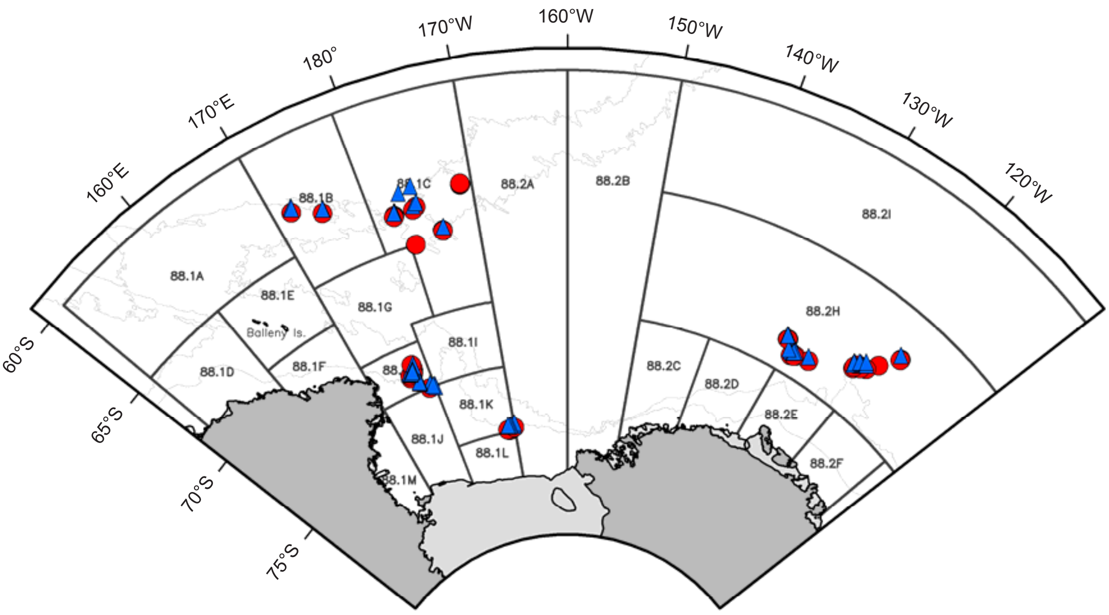

Figure 2: Locations of *Macrourus whitsoni* (red circle) and *M. caml* (blue triangle) obtained by random sampling from the toothfish longline fishery by-catch by observers in the 2011/12 fishing year. Also shown are CCAMLR small-scale research units (SSRUs). Depth contours are plotted at 1 000 and 3 000 m.

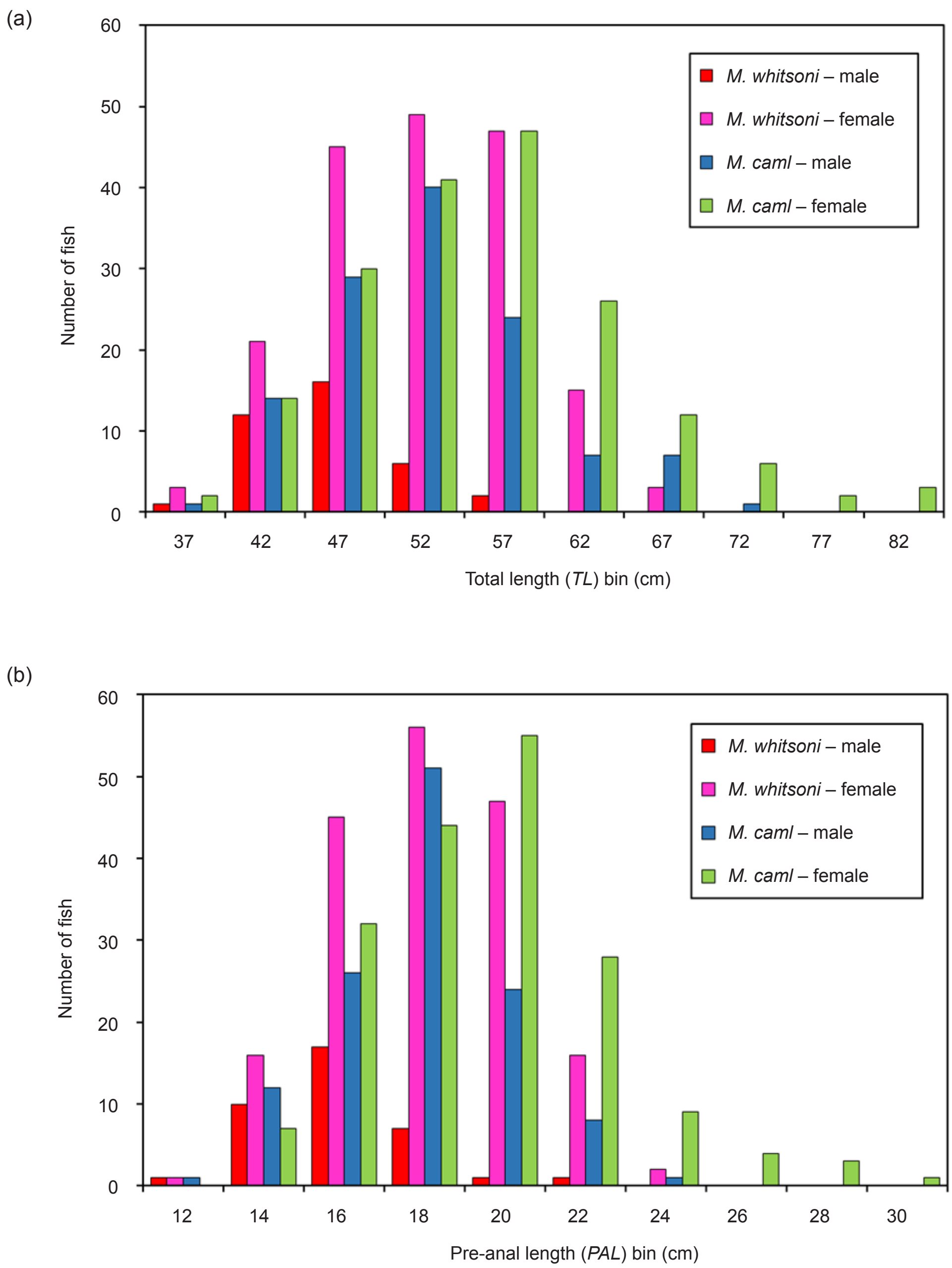

Figure 3: (a) Total lengths (*TL*, cm) and (b) pre-anal lengths (*PAL*, cm) of fish in this study by species and sex, grouped into ten equal-width bins (centre value of bin shown on x-axis).

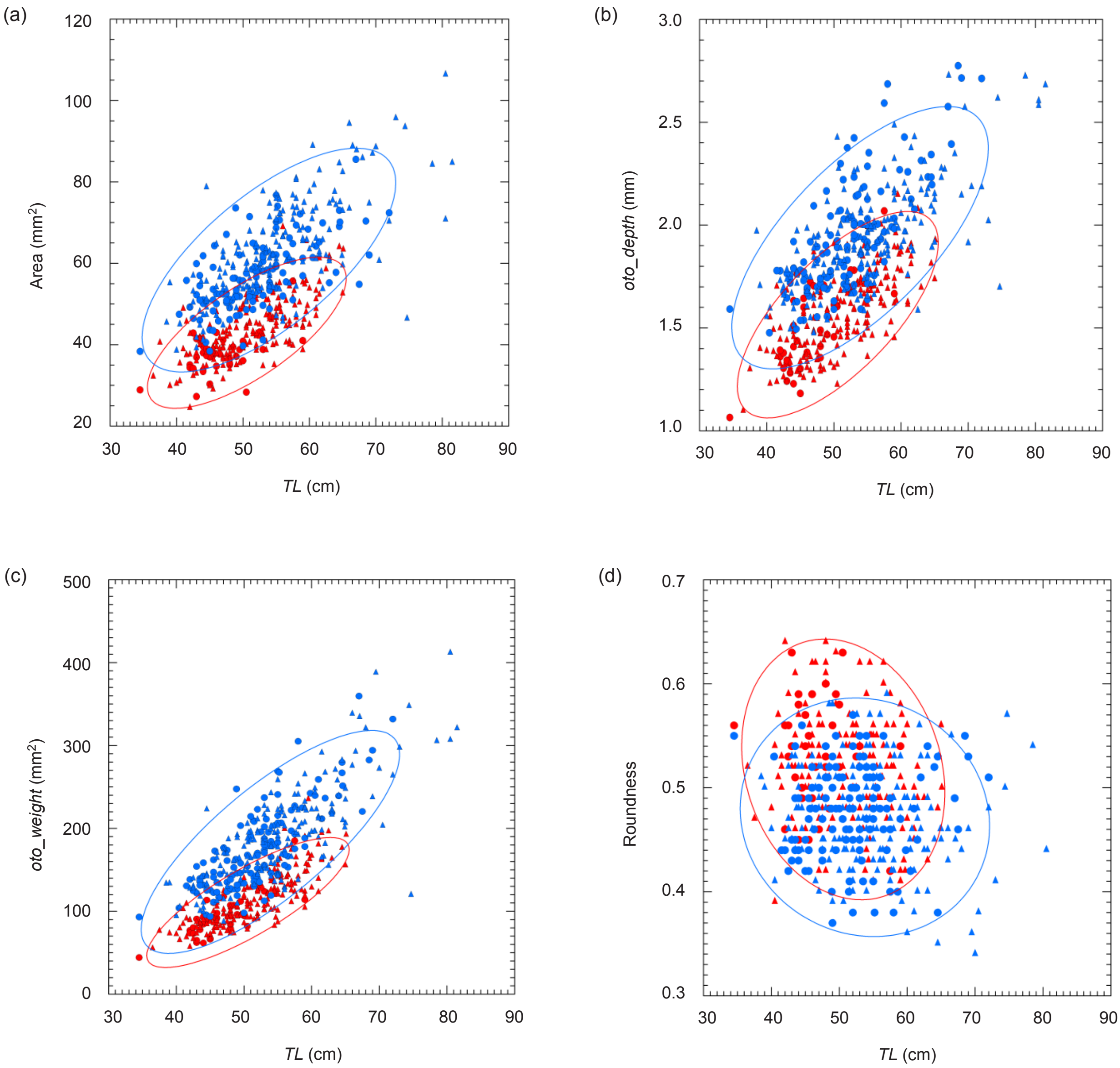

Figure 4: Variation in otolith parameters with fish total length (*TL*): (a) otolith cross-sectional area (*Area*, mm^2); (b) otolith depth (*oto_depth*, mm); (c) otolith weight (*oto_weight*, mg); (d) roundness. *Macrourus whitsoni*, red; *M. caml*, blue; males, circles; females, triangles. Confidence ellipses enclose 95% of the data for each species.

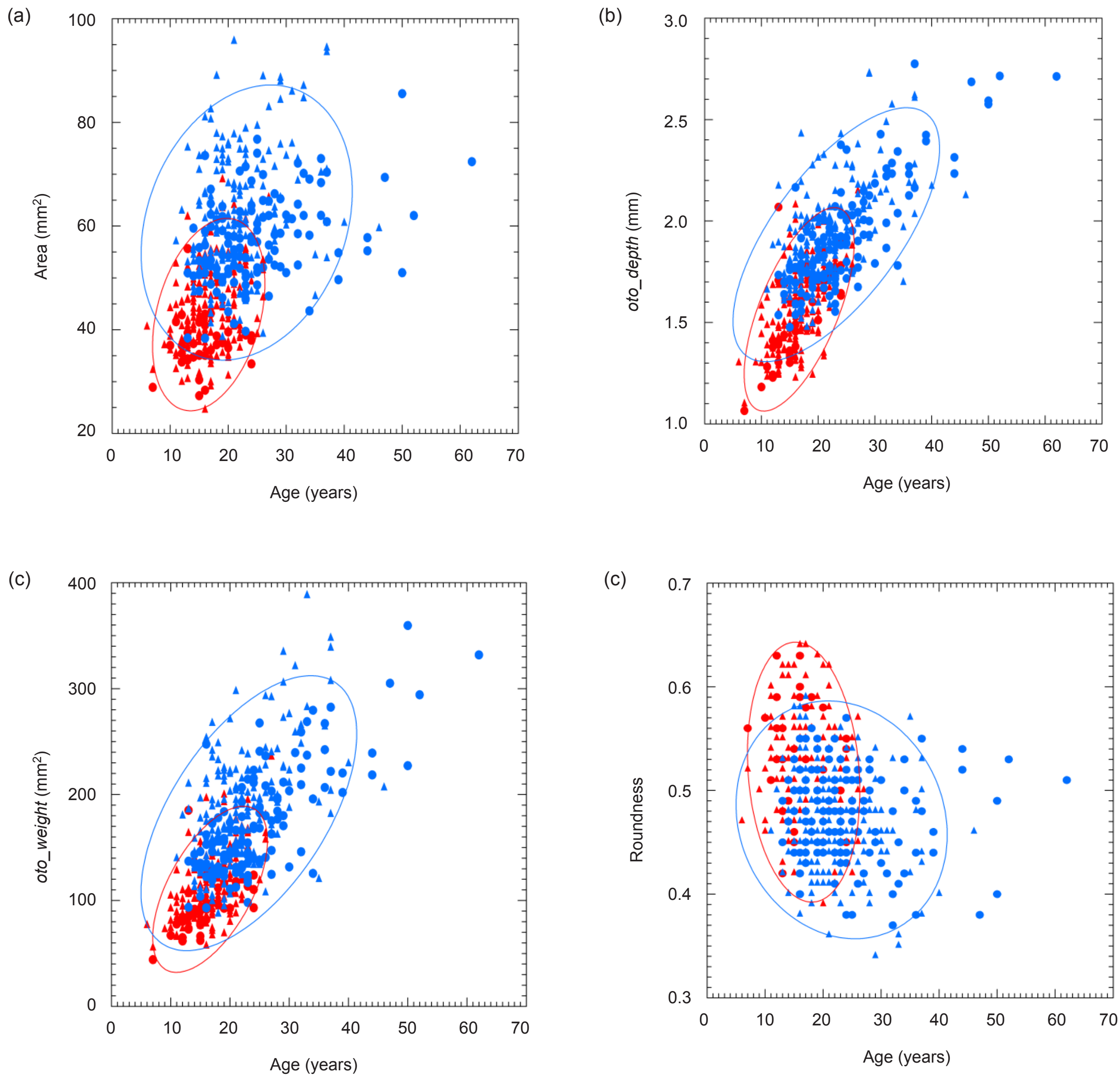

Figure 5: Interspecific variation in otolith parameters as for Figure 3, but shown as a function of fish age (years).

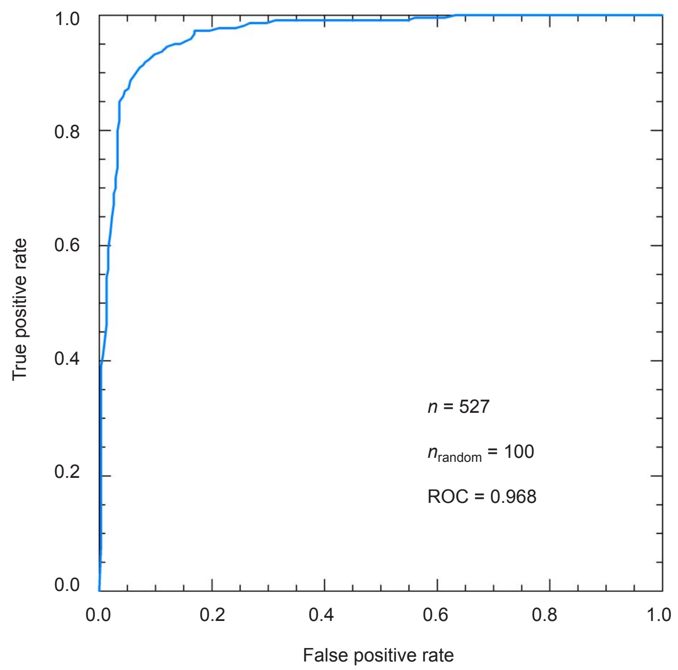

Figure 6: Receiver operating characteristic (ROC) curve based on K-fold validation with 10 folds, using fish total length (*TL*), otolith depth (*oto_depth*) and vertically projected area of the otolith (*Area*). The plot shows the fraction of true positives against the fraction of false positives. The blue line is the median ROC from the 100 randomisations of the selections of the withheld data; the range from these randomisations was very small. The ROC values of 0.968 refers to the area under the blue curve between x = 0 and x = 1.