J. Mar. Biol. Ass. U.K. (2003), **83**, 883–886
Printed in the United Kingdom

Otolith microchemistry of *Nezumia aequalis* (Pisces: Macrouridae) from widely different habitats in the Atlantic and Mediterranean

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Otoliths were obtained from *Nezumia aequalis*, a small macrourid that is widely distributed throughout the Atlantic and Mediterranean—two very different physical environments. Microchemical analysis of the otoliths was carried out using solution-based inductively coupled plasma mass spectrometry of whole otoliths. Significant differences between fish populations were found for concentrations of the elements Li and Sr. Only 54% of the samples were correctly classified by area using discriminant analysis. Otolith samples from the Reykjanes Ridge were most easily distinguished. The results are discussed in relation to trace element concentrations in the waters of the north-eastern Atlantic Ocean and the Mediterranean Sea.

INTRODUCTION

The otolith grows throughout the life of a fish and is mostly composed of $CaCO_3$ precipitated in a protein matrix (Campana, 1999). As the otolith grows, it incorporates many trace elements derived from the aquatic environment, either directly from the surrounding water, or indirectly through the diet. Because the otolith is regarded as being metabolically inert (Campana, 1999), the elemental signature embedded in the otolith can be an indicator of the water mass or masses occupied by the fish throughout its life. The microchemical analysis of otoliths is increasingly being used to discriminate between fish populations or stocks (Thresher, 1999) and this paper describes the elemental concentrations of *Nezumia aequalis* (Günther, 1878) otoliths measured using inductively coupled plasma mass spectrometry (ICP-MS).

Nezumia is a diverse and globally distributed genus of the family Macrouridae. *Nezumia aequalis* is distributed along the continental margins of the Atlantic to about 10°S and in large areas of the Mediterranean, at depths ranging from about 500 m to 1700 m. The distribution and abundance of *N. aequalis* have been summarized by Massutí et al. (1995), Moranta et al. (1998), Coggan et al. (1999) and Magnússon et al. (2000). *Nezumia aequalis* is probably not a long-lived species and unvalidated age estimates using otoliths give an approximate maximum age of nine or ten years (Massutí et al., 1995; Coggan et al., 1999).

The areas from which *N. aequalis* samples were obtained, the north-east Atlantic and the western Mediterranean, provide some interesting contrasts. Both have seasonal and permanent thermoclines, but below the thermocline the temperature regime is markedly different. In the Mediterranean the temperature remains at about 13°C irrespective of depth, whereas in the Atlantic, temperature generally decreases steadily with depth. These differing temperature profiles could mediate the uptake of trace elements into otoliths.

MATERIALS AND METHODS

Sagittal otoliths from 50 *Nezumia aequalis* were obtained from five different locations throughout the north-eastern Atlantic and western Mediterranean between 1998–1999 (Figure 1, Table 1). Fish samples from the Galician Bank were caught during an exploratory survey carried out by commercial trawlers. Pre-anal length (L_{PA}), and in some cases, pre-anal fin length (L_{PAF}) (Reykjanes Ridge samples), was measured for all fish (Table 1), but for this species they are effectively the same. Head length (L_H) was measured for samples from the Rockall Trough area and was converted to L_{PA} using the equation:

$$L_{PA} = (1.6825 \times L_H) - 0.7028, N = 177 \quad (1)$$

Although the size distribution of *N. aequalis* in the Mediterranean is generally smaller compared to that in the Atlantic, it was possible to obtain specimens with overlapping length ranges for all areas. Otoliths were extracted using plastic forceps and stored dry in acid-washed plastic vials, except for those from the Galician Bank, which were extracted using metal forceps and stored in paper envelopes. To remove any surface contamination, the otoliths were dipped in 2% HNO_3 (Romil Ultrapure) for 15 s, rinsed in 18 mega-ohm doubly deionized water (ELGA) and then air-dried and weighed to the nearest 0.01 mg.

Individual otoliths were placed in Teflon microwave tubes and a known volume of concentrated HNO_3 (Romil Ultrapure) was added. The tubes were placed in a microwave (CEM Microwave Accelerated Reaction System), heated and maintained at a temperature of approximately 90°C for 10 min, to ensure the contents of

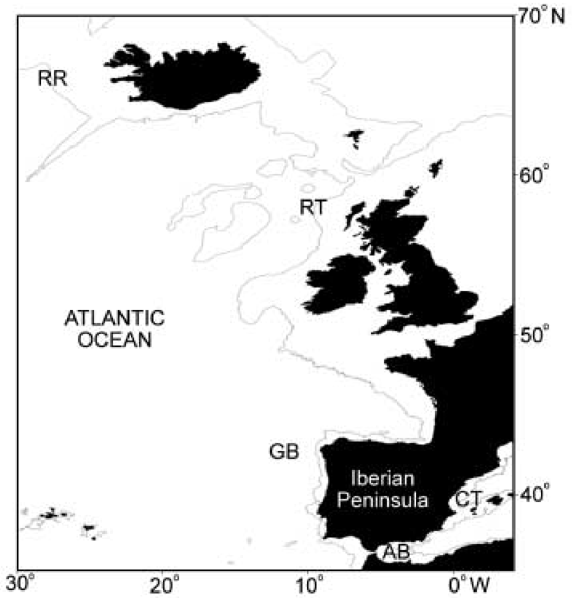

Figure 1. Location of *Nezumia aequalis* sampling sites in the north-east Atlantic and western Mediterranean. Reykjanes Ridge (RR), Rockall Trough (RT), Galician Bank (GB), Alboran Slope (AB) and Catalan Slope (CT). The 1000 m contour is shown.

the tubes were fully dissolved. After cooling, the dissolved otoliths were then rinsed into polypropylene volumetric flasks and diluted with ELGA water to obtain a final acid concentration of 2% HNO_3. The samples were diluted to give a total solids concentration of 0.77 mg ml^{-1}. A National Institute of Standards and Technology Certified Reference Material of $CaCO_3$ (SRM915a) was concurrently digested and analysed following the same procedures.

A VG PlasmaQuad 3 (S-Option) ICP-MS, operated in scanning mode, was used to determine elemental concentrations in the otoliths. Values were obtained from an average of three readings. When more than one isotope was available for the same element, those with least interference were used. An internal standard of ^{115}In and ^{209}Bi at a concentration of 10 μg l^{-1} was added to all solutions. Samples from each area were distributed throughout the whole analysis to avoid possible sequence effects. To minimize matrix effects caused by the high concentrations of some elements, standard addition was used to create a calibration curve.

To obtain accurate values for Ca and Sr, which were present at higher concentrations, the otolith solutions were further diluted to give a total solids concentrations of 0.005 mg ml^{-1} and the ICP-MS was calibrated with a set of solution standards. The limits of detection (LOD) were calculated from the standard deviation (multiplied by three) of the average value for a series of procedural acid blank solutions that were regularly interspersed throughout the samples. Data are expressed as fully-quantitative element concentrations, based on isotope measurements and are calculated using natural abundance ratios.

RESULTS

Measured Ca concentrations for SRM915a were an average of 75% ($\pm$4) of the certified value. Mean acid blank values and the limits of detection were separately calculated for each ICP-MS analysis session. Data were acid blank subtracted and solution elemental concentrations were converted to otolith elemental concentrations using the dilution factors obtained for each individual otolith. Values that fell below the LOD for a solid sample were set to zero prior to statistical analysis. Although the isotope ^{47}Ti was measured and the element Ti was apparently present in concentrations >1 μg g^{-1}, it was thought to be interference from ^{40}Ar and ^{7}Li and was therefore excluded from analysis. Measured concentrations of ^{60}Ni were in the order of >14 μg g^{-1}, although highly variable, possibly due to interference from ^{44}Ca^{16}O and the presence of Ni cones in the instrument, and this element was also excluded. Concentrations of the elements Be, Al, Sc, Co, Y, Zr, Mo, Ag, Cs, La, Ce, Nd, Sm, Th, and U were mostly below the LOD.

Concentrations of Zn, Rb and Pb were also mostly below the LOD, but some very high values were obtained for Reykjanes Ridge samples. Although ^{52}Cr has an interference from ^{35}Cl^{16}O^{1}H and the accuracy of the values is doubtful (analysis of a spiked sample of SRM915a carried out as part of a previous experiment gave a Cr recovery rate of 145%), it was decided to include Cr in the statistical analysis in order to examine the trends between areas and not absolute values.

Elemental concentrations for the otolith samples were grouped by area and $Log_{10}(y+1)$ transformed in order to achieve normal data distributions and homogeneous variances between groups. Analysis of covariance was used to determine the effect of area of collection on single element concentration, whilst controlling for effects due to fish length (L_{PA}) (Minitab v13.1). No significant interactions were identified between area of collection and fish

Table 1. *Details of the* Nezumia aequalis *samples and location of sampling sites. Hebridian Terrace temperature data from Ellett et al., 1986.*

Location		No. fish	Date	Latitude (°N)	Longitude	Depth range (m)	Pre-anal length range (mean) of samples (cm)	Temperature range (°C)
Reykjanes Ridge	**RR**	10	02–04 Oct. 1998	64.29–65.26	28.04–28.32°W	999–1252	5.4–7.1 (6.2)	4.2°–4.7°
Rockall Trough	**RT**	10	24 Sept. 1998	58.22	09.29°W	852	5.6–7.5 (6.1)	8–9°
Galician Bank	**GB**	10	09 Apr. 1999	N/A	N/A	1356–1574	7.0–8.0 (7.3)	6–7°
Alboran Slope	**AB**	10	05–08 May 1999	36.16–36.36	03.04–04.50°W	550–740	4.5–6.3 (5.6)	13.24°–13.29° (at approx. 280 m)
Catalan Slope	**CT**	10	03 Jun. 1999	38.59	00.30°E	735	5.5–6.6 (6.0)	approx. 13°

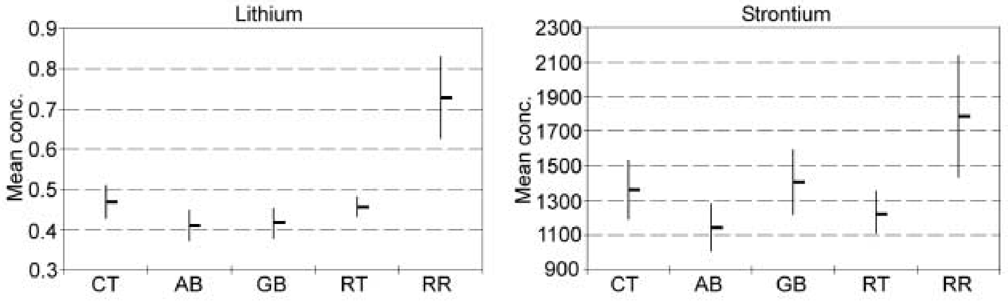

Figure 2. Variation in mean elemental concentration ($\mu g\, g^{-1}$) between otoliths of *Nezumia aequalis* from the Atlantic and Mediterranean (with 95% confidence intervals).

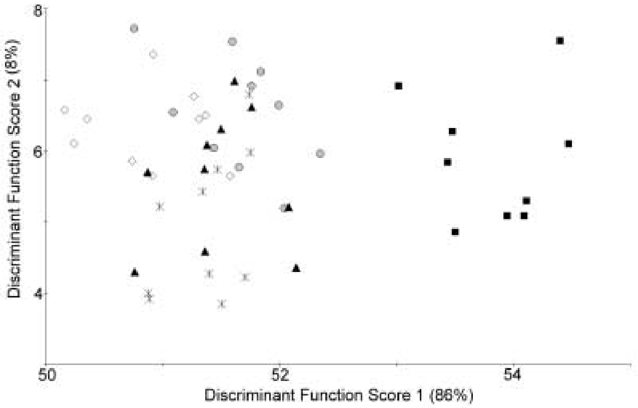

Figure 3. Discrimination between whole otoliths of *Nezumia aequalis* based on the concentrations of eight elements. ◇, Alcoran Slope; ●, Catalan Slope; ▲, Galician Bank; ■, Reykjanes Ridge; ⁎, Rockall Trough.

length. Elements found to be significantly different between some areas were Li and Sr ($P<0.001$) (Figure 2).

Discriminant analysis was used to test whether the assumed membership of an otolith sample to a categorical group (area) was justified using explanatory variables composed of element concentrations. The first two series of discriminant scores, based on the concentrations of the elements Li, Mg, Cr, Mn, Cu, Cd, Sr and Ba explained 86% and 8% of the variance, respectively (Figure 3) and 72% of the samples were correctly classified by area. A less biased technique known as cross-validation was also used, in which single observations were removed and the classification function built from the remaining data before the omitted observation was classified (SAS v8). Only 54% of the samples were correctly classified by area using this method. Li, Sr and Cr contributed most to group separation. The most distant group from all others was the Reykjanes Ridge and it was possible to correctly classify almost 90% of the samples from this group using the cross-validated method. When the remaining four areas (Alboran Slope, Catalan Slope, Galician Bank and Rockall Trough) were combined in a single data set, 45% of samples could be assigned to the correct group using cross-validation. Of the Galician Bank samples, only 30% were correctly classified, with 40% being assigned to the Alboran Slope and 20% to the Catalan Slope. Alboran Slope samples were misclassified as being from the Catalan Slope (30%) or the Galician Bank (20%), and 30% of the Catalan Slope samples were assigned to the Alboran Slope group. The most easily distinguished group was the Rockall Trough, with 60% of samples being correctly classified. Only one element, Li, was identified as being statistically significant in distinguishing between the fish from these four areas ($P=0.036$).

DISCUSSION

From the samples analysed, it was only possible to confidently discriminate fish from one sampling location. The Reykjanes Ridge otoliths were characterized by having higher concentrations of most elements compared to samples from other areas. Measured values of elements such as Zn, Rb and Pb were often above the LOD in otoliths from Reykjanes Ridge, but below the LOD in otoliths from other sample groups. Concentrations of Li and Sr were significantly higher in the Reykjanes Ridge samples. It may be that seawater interactions with the basaltic and ultramafic rocks of newly-formed oceanic crust at the Reykjanes Ridge are responsible for higher concentrations of these elements in the surrounding water. Hydrothermal activity is also associated with the input of relatively high levels of trace elements, including Li, Rb, Zn, Cu and Pb (Honnorez, 1983).

At the location and depth (approximately 1100 m) from which the Reykjanes Ridge samples were obtained, the bottom temperature is relatively cold (4–5°C) and the water masses in this area are a complex mixture of warm, saline Modified North Atlantic Water (MNAW) and Denmark Strait Overflow Water (DSOW). The MNAW originates west of the Mid-Atlantic Ridge and is carried northwards by the Irminger Current, and the deeper DSOW, which crosses the sill at a depth of 640 m, contributes to the North Atlantic Deep Water. The DSOW is cold and low in salinity and comprises water from more than one source. It incorporates Arctic Ocean Deep Water carried south by the East Greenland Current, with input from the intermediate waters of the Iceland and Greenland Seas. By contrast, the waters at the northern end of the Rockall Trough are warmer and more saline, originating from Eastern North Atlantic Water, with a small contribution from the colder Norwegian Sea Deep Water that overflows the Wyville Thomson Ridge. Although both salinity and temperature are known to influence the uptake of some elements into otoliths, there is little consistency in the reported literature (Thresher, 1999) and there are other factors such as growth rate and reproductive status that may have an effect.

Despite the contrasting environments of the north-east Atlantic and the western Mediterranean, there were no apparent trends in elemental concentrations between the Atlantic and Mediterranean groups of otoliths, apart from those of the Reykjanes Ridge. In general, surface trace element concentrations in the Mediterranean are high compared with those in the Atlantic, but at depths >300 m concentrations appear quite homogeneous, unlike the open ocean (Béthoux et al., 1990). The latter has been ascribed to limited recycling of nutrients and surface enrichment from sources outside the Mediterranean (Sherrell & Boyle, 1988). There is an interchange of water through the Strait of Gibraltar and high salinity Mediterranean outflow, with its characteristic density, is known to extend as a plume into the Atlantic at depths from about 1000 m and deeper, at least as far north as the Porcupine Seabight (52°N). Heavy metal input occurs during passage of surface water from the Atlantic to the Mediterranean through the coastal region (Gulf of Cadiz) (Sherrell & Boyle, 1988). There is also a considerable input into the Mediterranean from both atmospheric and anthropogenic sources, and mean surface concentrations of Zn, Cu, Cd and Pb can be higher or equal to deep-water concentrations (Béthoux et al., 1990). However, heavy metal concentrations in the Mediterranean otoliths were not significantly higher than in those from the Atlantic. High concentrations of Al have also been observed in the water column in the western Mediterranean, but there was no evidence of elevated values in the Mediterranean samples, and very few Al values were above the LOD for all areas. It might be expected that Sr levels would be higher in the otoliths from the warmer, more saline Mediterranean waters, but this was not the case. The only element found in higher concentrations in the Mediterranean otoliths was Ba, but this trend was not significant. Within the western Mediterranean, the oceanographic conditions at the two sampling sites are different. The Alboran Sea is the first basin encountered by inflowing Atlantic waters and low salinity Atlantic water usually occupies the upper 200–300 m. This interacts with the underlying Levantine Intermediate Waters (LIW) of characteristically high salinity, and with the surface Mediterranean Waters to the east. The Catalan Slope in the Balearic Basin is influenced by the Northern Current, which flows south-west along the Iberian Slope, and the LIW is found between 300 and 700 m. Otoliths obtained from the Catalan Slope had higher Li and Sr concentrations than those from the Alboran Slope, but concentrations were similar for most other elements.

Although there was no clear discrimination between sites in the Rockall Trough, Galician Bank, Alboran Slope and Catalan Slope, this does not necessarily indicate that the fish belong to a single population. A common origin appears to be unlikely due to the physical separation of the sampling areas. The Galician Bank otoliths collected using metal forceps did not show significantly different elemental concentrations compared to other areas, although there was more variability in some elements (Mg, Cr, Cd and Pb).

As part of this study, otoliths were also obtained from the Balearic Slope, north of Majorca. Although the size range of *Nezumia aequalis* in the Balearic Basin overlaps with those from other Mediterranean areas (E. Massutí, oral communication), only fish of a small size were caught. The ICP-MS analysis indicated a range of elements at higher concentrations compared to otoliths from other Mediterranean sites, although concentrations of Sr were lower. The fish sampled from the Balearic Slope were much smaller and younger than the other samples in the study and thus the results obtained from the analysis of the whole otolith will be more influenced by the otolith nucleus. The differences in element concentrations may be due to ontogenetic changes, or may be a consequence of the bigger-deeper phenomenon. The age and length of *N. aequalis* increases with depth (Massutí et al., 1995; Coggan et al., 1999) and it is probable that the otolith nucleus will be laid down while the fish is occupying shallower water. Thus, the Balearic Slope samples may reflect higher trace element concentrations in surface waters.

This study results from a European Commission research project to investigate the value of otolith microchemistry for stock discrimination in deep-water fish: Otolith Microchemistry as a Means of Identifying Stocks of Deep-water Demersal Fish (OTOMIC) FAIR CT98-4365. The authors would like to thank Enric Massutí and Luis Gil de Sola for providing some of the Mediterranean samples. Reykjanes Ridge samples were provided by Klara Jakobsdóttir and Galician Bank samples by Instituto Español de Oceanografía, Spain.

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Submitted 20 November 2002. Accepted 12 June 2003.