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www.publish.csiro.au/journals/mfr *Marine and Freshwater Research*, 2005, **56**, 1–9

Age-related trends in otolith chemistry of *Merluccius merluccius* from the north-eastern Atlantic Ocean and the western Mediterranean Sea

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Abstract. Sagittal otoliths of European hake obtained from five geographic locations in the north-eastern Atlantic and western Mediterranean were examined using laser ablation and inductively coupled plasma mass spectrometry. Otolith sections were analysed for the isotopes ^{24}Mg, ^{55}Mn, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{138}Ba and ^{208}Pb, measured relative to ^{43}Ca counts. These analyses considered only age 0 (core area) and ages 1 to 3. Age-related trends in otolith elemental composition were observed in hake from all areas, but were masked by variability between locations. Elemental concentrations generally decreased outside the core, with some increase at age 3. The composition of the otolith core was very distinct from that of the other growth increments. In the Mediterranean, part of this differentiation was a result of Mn, which was present in the core at high concentrations compared with the rest of the otolith. Mediterranean otoliths also had higher concentrations of Sr, Zn and Ba in the core. For most samples a similar trend was observed, although samples from one of the Mediterranean areas showed some differences, mainly in the concentrations of Mg and Sr. These results provide new empirical evidence of the variation in elemental concentrations across hake otoliths with age, at least throughout the first 3 years of life.

Extra keywords: inductively coupled plasma mass spectrometry, laser-ablation analysis, population.

Introduction

Otoliths grow throughout the life of a fish and are mostly composed of $CaCO_3$ precipitated in a protein matrix, which acts as a template for otolith growth (Campana 1999). As the otolith grows, it incorporates many trace elements, which are incorporated within the crystal, adsorbed onto its surface or directly bonded to the organic matrix polymers. Incorporation within the crystal may occur by one of two mechanisms; substitution, which occurs for cations of similar size and charge (e.g. Sr can substitute Ca), or co-precipitation (Mg, Li, Ba) (de Pontual and Geffen 2002). Elements such as Na, Cl, Zn and K can be absorbed onto the crystal surface in the interstitial space (Campana 1999). These trace elements, which represent less than 1% of the otolith weight, are derived from the environment in which the fish lives, either directly through the water, or indirectly from the diet (Campana 1999). Chemical analysis of otoliths can be useful as a means of determining several individual fish and population characteristics, as the elemental signature embedded within them can be used as an indicator of the water masses occupied by the fish throughout its life (Campana 1999). The elemental composition has been used to discriminate stocks (Thresher 1999; Swan *et al*. 2003) and for age estimation and validation using radiometric methods based in natural radioisotopes disequilibria and ^{14}C tracer (see citations in de Pontual and Geffen 2002).

Moreover, the otolith composition reflects the combined effects of endogenous processes such as development, and external conditions associated with changes in habitat, behaviour and diet. The metabolic rate of the fish affects the growth of its otoliths and thus may determine otolith composition, either directly by affecting element incorporation during growth, or indirectly by mediating the exposure to environmental elements through food consumption and the exchange rate across the gills and epithelia (de Pontual and Geffen 2002). Although some previous studies have examined variation in relative elemental composition along an otolith transect with respect to fish age (e.g. Papadopoulou *et al*. 1980; Kalish 1989), many have investigated only the core area (Campana *et al*. 1994; Milton *et al*. 1997), or have provided quantitative transect information concentrating on

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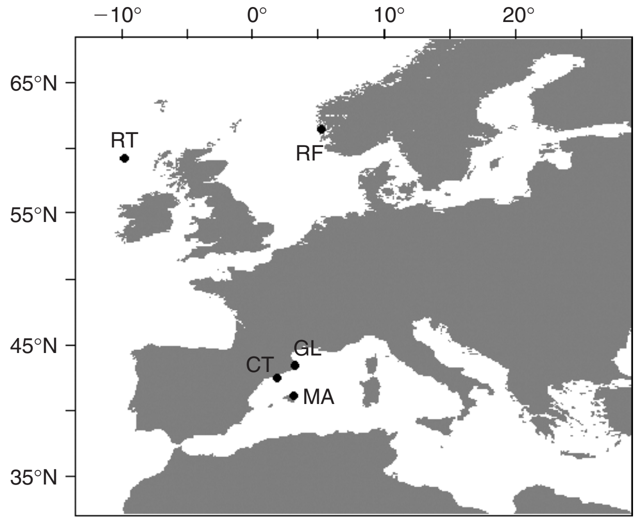

Fig. 1. Location of the sampling areas.

the identification of seasonal patterns (e.g. Kalish 1991*a*) rather than exploration of larger scale age-related trends within populations. However, techniques such as wavelength dispersive spectrometry (WDS) and increasingly, laser-ablation inductively coupled plasma mass spectrometry (ICP-MS), enable sophisticated investigations of element composition, otolith growth, and fish age. A study of the variation in otolith elemental concentrations at different points of the life cycle may provide a better understanding of the process of incorporation. The European hake *Merluccius merluccius* (Linnaeus, 1758) was selected as a case study, because the species has a wide geographic distribution (Casey and Pereiro 1995; Oliver and Massutí 1995; Recasens *et al*. 1998; Arneri and Morales-Nin 2000) and some spatial hetereogeneity has been demonstrated (Lo Brutto *et al*. 2004; Mattiucci *et al*. 2004). The aim of the current paper is to increase understanding of the influence of age on element incorporation into the otolith, and to determine if age-related trends in otolith composition are consistent across geographic locations.

Materials and methods

Sagittal otoliths were obtained from five different areas throughout the north-eastern Atlantic (Rockall Trough (RT), Romsdal Fjord (RF)) and western Mediterranean (Catalan slope (CT), Mallorca (MA), Gulf of Lions (GL)) (Fig. 1) from bottom trawl samples collected at depths ranging between 55 to 627 m (Table 1). The water temperatures and salinity at the collection areas are included in Table 1. Total length was measured for all fish and sex was determined (Table 1). To remove any surface contamination, the otoliths were dipped in 2% HNO_3 (Romil Ultrapure) for 15 s, rinsed in 18 mega-ohm doubly deionised water (ELGA, Bucks, UK) and then air-dried. The otoliths were set in polyester resin blocks and sectioned through the nucleus along the sagittal plane using a low-speed diamond saw. The sections were then rinsed in 2% HNO_3 (Romil Ultrapure) for 15 s, followed by distilled water and finally air-dried. The Atlantic and Mediterranean otoliths were analysed in separate sessions and in both sessions they were randomly mixed before analysis.

Table 1. Summary of the area and date of sampling and fish size of the European hake used in the current study
The data of mean annual temperature and salinity at depths similar to sampling are also included as well as their source

Area	Area code	Latitude	Longitude	Date	Depth (m)	Mean water temperature (°C)	Mean water salinity	Source	No. fish	Sex	Length range (TL cm)	Mean length (TL cm (s.d.))
Catalan Slope	CT	41°10′N	01°58′E	25 Aug. 98	55	14.1	38.12	MEDATLAS	5	All male	38–47	41.9 (3.5)
Gulf of Lions	GL	42°07′N	03°22′E	21 Jan. 00	85–128	13.9	38.35	Gaudy *et al.* 2003	5	All male	31–36	33.4 (1.8)
Mallorca	MA	39°50′N	03°18′E	30 Jan. 02	~200	13.2	38.45	Pinot *et al.* 2002	5	All male	32–43	36 (4.8)
Rockall Trough	RT	57°56′N	09°40′W	25 Sep. 98	627	9.2	35.34	Holiday *et al.* 2000	5	Not known	58–66	55.8 (3.5)
Romsdal Fjord	RF	62°33′N	07°30′W	Apr. 99	300–360	8.5	35.3	Orvik *et al.* 2001	5	All male	57–64	60 (2.5)

TL, Total length.

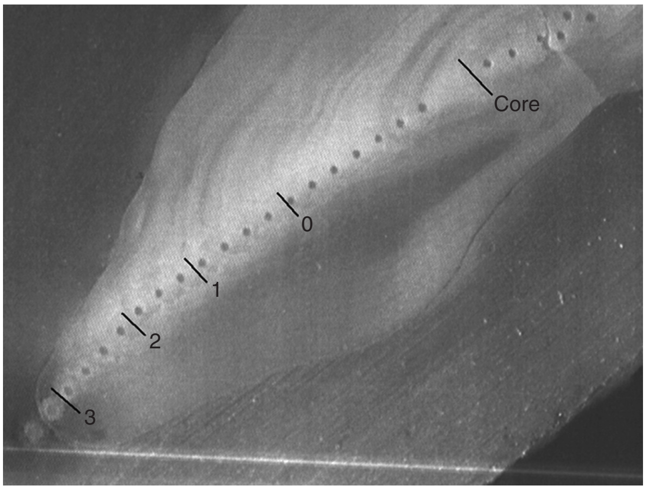

Fig. 2. Micrograph of a Mediterranean European hake otolith section showing the ablation spots (dots) and the growth increments considered as annuli (lines) (×20).

The laser-ablation analysis was carried out with a VG Plasma Quad 3 ICP-MS coupled to a VG Microprobe II pulsed Nd:YAG laser of wavelength 266 nm. The laser was operated in Q-switched, time resolved mode with an energy of 0.486 mJ, a scan speed of $10\,\mu m\,s^{-1}$ and a repetition rate of 10 Hz. The instrument was calibrated using standard reference National Institute of Standards and Technology glasses 610, 612 and 614, and a pressed limestone pellet (BCS CRM393) was analysed after each calibration set. Argon gas blanks were run before each assay and the mean blank counts per second (cps) was subsequently subtracted from the sample cps.

The otolith core was visually identified as the central part of the otolith surrounded by a clear check (Morales-Nin and Aldebert 1997) and a series of spots were ablated within the core area, usually in two parallel lines of four spots each. A radius from the core to the dorsal side of the section was identified and a further series of spots were ablated, separated by 150 μm. The individual spots were *c.* 50 μm in diameter and 20 μm deep. Before each set of spot ablation, the ICP-MS was pre-conditioned to carbonate material by ablating a line along the outer edge of the otolith for 40 s. Otolith material was also ablated for 120 s at the start of the experiment. Once a stable signal was reached, the time resolved analysis (TRA) signal was integrated over the same period for each sample. Results were obtained for the isotopes ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{138}Ba and ^{208}Pb. Data are presented in a semiquantitative approach as mean cps and normalised to ^{43}Ca for each isotope and not as absolute concentrations, as a result of the lack of matrix-matched standards.

Each analysed otolith was photographed and the location of each spot was identified (Fig. 2). The spots were grouped irrespective of the nature of the increment (translucent or opaque), but dependent on the age. These were grouped into the core (age 0) and ages 1 through to 3 years. The complex ring pattern in hake otoliths, with the formation of three major rings before what is considered to be the annulus and with the first annulus composed of a double ring, are common features for several hake species: *M. capensis* and *M. paradoxus* (ICSEAF 1983), *M. merluccius* in the Atlantic (Piñeiro and Sainza 2003) and in the Mediterranean (Morales-Nin *et al*. 1998). The core was therefore defined as the central otolith area enclosed within the translucent check formed at the end of the pelagic phase (Arneri and Morales-Nin 2000). Age 1 was considered to be from the outer edge of the core to the edge of the second translucent ring composing the double first growth increment. Ages 2 and 3 were considered to be to the edges of the subsequent translucent rings.

Session effects associated with analysing the Atlantic and Mediterranean samples separately prevented the analysis of the results as a single dataset. A preliminary Principal Components Analysis (PCA) on the normalised units per second of the seven elements was completed before the main statistical testing. This enabled obvious outliers to be removed from the data sets. A second PCA was completed to inspect the general patterns depicted by element composition. The PCA were performed on the correlation matrix (i.e. all elements were given equal weight on the results, irrespective of their relative concentrations).

The null hypothesis to be evaluated was that the age-related changes in elemental composition would show similar trends for all the sample areas analysed (i.e. the focus was on the interaction between age and sampling geographical area). The multivariate approach was chosen instead of a series of independent univariate tests for each of the elements analysed. Redundancy analysis (RDA) can be considered a multivariate extension of univariate linear models (such as ANOVA or regression). Hypothesis testing in RDA runs in an analogous way to univariate linear models: the value of a statistic (e.g. the *F*-ratio) that measures the departure of the observed data from those expected from the model is compared against a theoretical distribution. However, in this analysis a permutation-based test was used. Therefore, it was necessary to use completely balanced datasets, and to ignore some of the available data. The final data set covered five sample areas (three Mediterranean and two Atlantic), five fish from each area, and four (repeated) measurements from each otolith. Repeated-measurements correspond to the core, first, second and third ages, and they result from the average of a variable number of laser spots. The specific model assumed was a repeated-measures model with no interactions between fish and time (i.e. it is implicitly assumed that all fish from the same sample area change in a similar way with age; y = grand mean + area + fish (area) + age + area × age + error; Underwood 1997). Also, by analogy with partial univariate linear models, all variables other than the interaction between age and sample area (i.e. age and individual fishes) were considered as co-variables. Partial RDA and the permutation test were carried out using CANOCO 4.0 (ter Braak and Smilauer 1998). The relationships between response variables (matrix of elemental values) and explanatory variables (interactions between age and sample area) were visualised using an ordination diagram. The response variables (elements) were centred by samples (focusing on relative, i.e. within fishes, responses). Responses to age were not assumed to be linear and age was coded as four dummy variables. For completeness, some conventional univariate nested ANOVA were performed with Sr and Mg, because they showed between-area differences in age-related trends.

Results

Although the intention was to study otoliths of male fish with the same length and age ranges, there was some variation associated with the sampling (Table 1). In the western Mediterranean all the fish were 3-year-old males, although some differences were found in the mean length (GL = 33.4 cm (s.d. = 1.8), MA = 36 cm (s.d. = 4.8), CT = 41.9 cm (s.d. = 3.5)). In the Atlantic, mean age and length, as well as length range, were more variable (RT: 4 years, 55.8 cm (s.d. 3.5): RF: 6 years, 60 cm (s.d. = 2.5)).

The data sets contained the concentrations relative to ^{43}Ca for seven elements (^{24}Mg, ^{55}Mn, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{138}Ba and ^{208}Pb) measured at 551 discreet spots in 25 otoliths. The number of spots in each otolith growth increment was dependent on its relative size. The mean value for each area and otolith growth increment was calculated for the core and the first three growth increments or ages (Table 2).

Table 2. Mean values of isotopes normalised to ^{43}Ca

Data are multiplied by 100. Age −1 corresponds to the core area. For sampling zones code, see Table 1

Zone	Age	No. spots	Mg		Mn		Zn		Rb		Sr		Ba		Pb	
			Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
MA	−1	23	1.7570	0.3247	1.4480	1.5329	0.0520	0.0606	0.0093	0.0030	56.0168	3.9097	1.2356	0.7186	0.0003	0.0003
	1	41	1.9079	0.4378	0.3074	0.1795	0.0124	0.0320	0.0131	0.0091	41.9584	8.0001	0.3308	0.1421	0.0015	0.0031
	2	20	2.3594	0.3477	0.3423	0.1411	0.0055	0.0032	0.0139	0.0079	36.2192	5.7412	0.2315	0.0514	0.0017	0.0026
	3	11	1.9065	0.4410	0.2070	0.0831	0.0098	0.0084	0.0148	0.0121	41.7571	8.1649	0.2792	0.0995	0.0028	0.0030
CT	−1	24	2.4289	0.5326	1.2430	0.5607	0.0137	0.0152	0.0098	0.0020	51.1686	5.2891	0.5765	0.1382	0.0007	0.0004
	1	47	2.8861	0.6755	0.7007	0.3479	0.0078	0.0117	0.0128	0.0065	39.4411	8.0684	0.3674	0.2066	0.0008	0.0008
	2	21	2.7186	0.7375	0.3472	0.1403	0.0120	0.0378	0.0119	0.0050	36.8332	7.7554	0.2575	0.1057	0.0016	0.0018
	3	11	2.3229	0.6770	0.2460	0.1065	0.0072	0.0038	0.0140	0.0096	41.6652	4.5059	0.2337	0.0321	0.0025	0.0025
GL	−1	22	2.3753	0.5069	1.3228	0.6827	0.0970	0.1027	0.0073	0.0043	46.9856	6.6792	1.2509	0.4018	0.0015	0.0024
	1	35	2.8821	0.6755	0.7258	0.3020	0.0096	0.0070	0.0091	0.0056	39.8534	9.0977	0.5690	0.5354	0.0003	0.0003
	2	22	3.3346	0.6128	0.8308	0.4561	0.0075	0.0051	0.0110	0.0053	33.9901	4.9425	0.2946	0.1148	0.0004	0.0005
	3	13	3.4994	0.9192	0.6701	0.4047	0.0087	0.0081	0.0097	0.0038	31.0709	5.5005	0.1833	0.0673	0.0005	0.0004
RF	−1	61	1.7577	0.2637	0.5262	0.2524	0.0044	0.0053	0.0124	0.0050	49.6734	4.9319	0.7885	1.0475	0.0022	0.0018
	1	27	1.7405	0.3397	0.4975	0.1757	0.0042	0.0058	0.0117	0.0044	38.6775	8.1633	0.2623	0.1180	0.0023	0.0016
	2	18	1.7412	0.3219	0.4329	0.2259	0.0024	0.0026	0.0148	0.0037	38.8959	6.0376	0.2532	0.1364	0.0022	0.0024
	3	12	1.2950	0.3354	0.2902	0.1459	0.0033	0.0038	0.0144	0.0057	45.2754	8.2812	0.3605	0.3012	0.0040	0.0034
RT	−1	69	2.8339	0.7497	0.8786	0.6072	0.0221	0.0133	0.0107	0.0033	48.0443	8.2457	1.3383	1.6718	0.0064	0.0044
	1	31	2.6552	0.7735	0.9904	0.6765	0.0247	0.0225	0.0118	0.0056	37.4789	8.4014	0.3999	0.1712	0.0077	0.0056
	2	26	2.3417	0.4577	0.3469	0.3850	0.0182	0.0108	0.0120	0.0036	35.6695	4.1337	0.2386	0.0680	0.0079	0.0090
	3	17	2.2777	0.4153	0.1836	0.0974	0.0193	0.0108	0.0106	0.0055	39.0113	3.7715	0.2205	0.0517	0.0084	0.0071

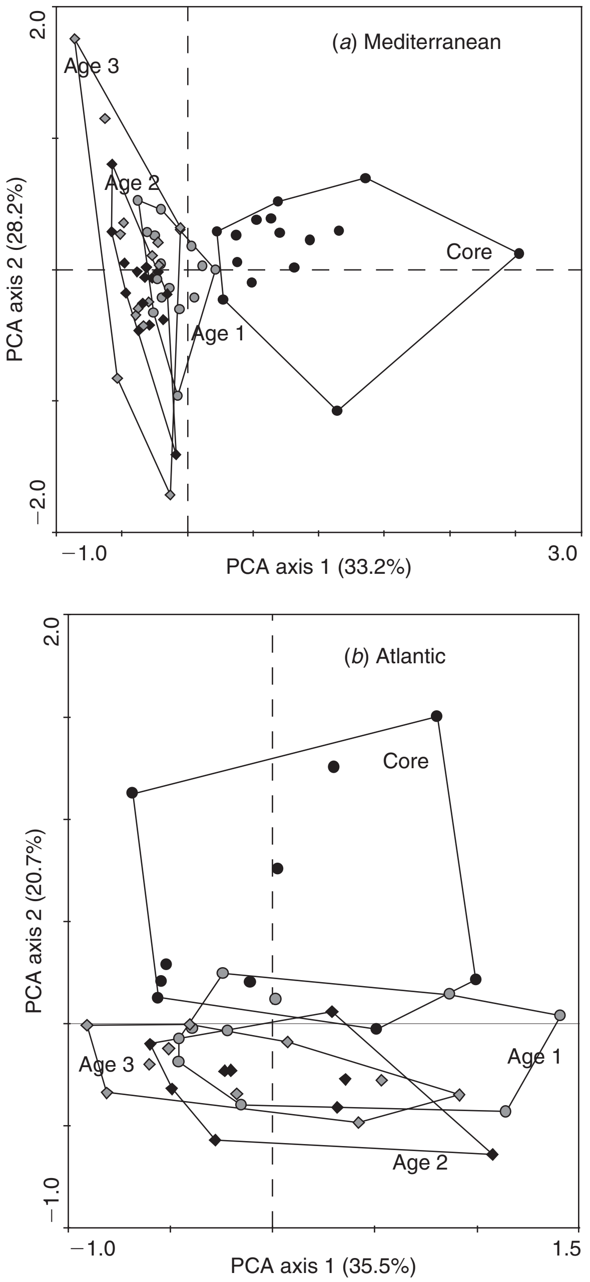

Fig. 3. Ordination diagrams corresponding to principal components analyses. Samples corresponding to the same age class have been enclosed by an envelope. The Mediterranean samples (*a*) show a clear age-related trend (horizontal axis), whereas in the case of the Atlantic fishes (*b*), the main trend (horizontal axis) seems to correspond to between-area differences, and the age-related differences are secondary (vertical axis).

The results of the PCA analysis on the combined data for the three Mediterranean areas are shown in Fig. 3*a*. Drawing an envelope around all samples corresponding to the same age (irrespective of the sampling area they came from), shows that an easily recognisable temporal sequence exists, from core to age 2 samples. Assuming that the first (horizontal) axis is related to time, age 3 samples have a greater vertical variation and their envelope covers age 2 and some of age 1. This pattern suggests that some elements could experience divergent age-related trends depending on the sampling area. The analysis for the pooled Atlantic data shows a similar trend for the core, but no trends were identified for later ages (Fig. 3*b*). This is probably because there are larger between-area differences, which are apparently related to the first PCA axis. In this case, the age-related pattern is secondary, and seems to be associated with the second (vertical) PCA axis.

The results of the statistical analysis were consistent with these descriptive patterns, and emphasised the differences between the Mediterranean and Atlantic samples. In the Mediterranean, the interactions between age and sample area explained 19.2% of the variance remaining after adjusting for co-variables (sample area and age). Thus the temporal trends in otolith elemental composition were not comparable between sampling areas ($F = 1.69$, $P < 0.01$, 1999 permutations).

These temporal trends and the main relationships between them and elemental composition are displayed in a biplot for the Mediterranean (Fig. 4*a*). There are two main gradients in elemental composition. The first one (corresponding to RDA axis 1) is defined by Sr, Ba, Zn and Mn. There is a tendency for a larger concentration of these four elements towards the otolith core (i.e. at otolith positions corresponding to younger ages). Interestingly, Sr and Mg show a different age-related pattern between two sampling areas (MA and CT) compared with GL. The univariate patterns for these two elements are shown in Fig. 5. The Sr concentration shows a similar between-area decrease from the core to age 2, but at age 3 it increases for the MA and CT samples and continues to decrease for GL. The plot for Mg shows the opposite trend at age 3, continuing to increase for GL and to decrease for the other areas. These differences are also apparent in Fig. 4, where the GL age-related trend is clearly different from the trend displayed by samples from the other two Mediterranean areas.

In the Atlantic the otoliths from the two sample areas did not differ significantly in their age-related trends ($F = 0.90$, $P = 0.29$, 1999 permutations). The variance explained by the interactions between age and area was only 7.8% of the variance remaining after adjusting for co-variables (sample area and age). The age-independent differences between otoliths from the two sampling areas were largely significant (F-ratio $= 15.8$; $P = 0.004$), with Zn, Mg, Pb and contributing most to differentiation (Fig. 4*b*). Age-related trends in Mg and Sr were comparable between Atlantic samples and fish from two of the Mediterranean sample areas (MA and CT, Fig. 5).

Discussion

Several studies on metal concentrations in fish otoliths have noted increased concentrations in the otoliths of fast growing

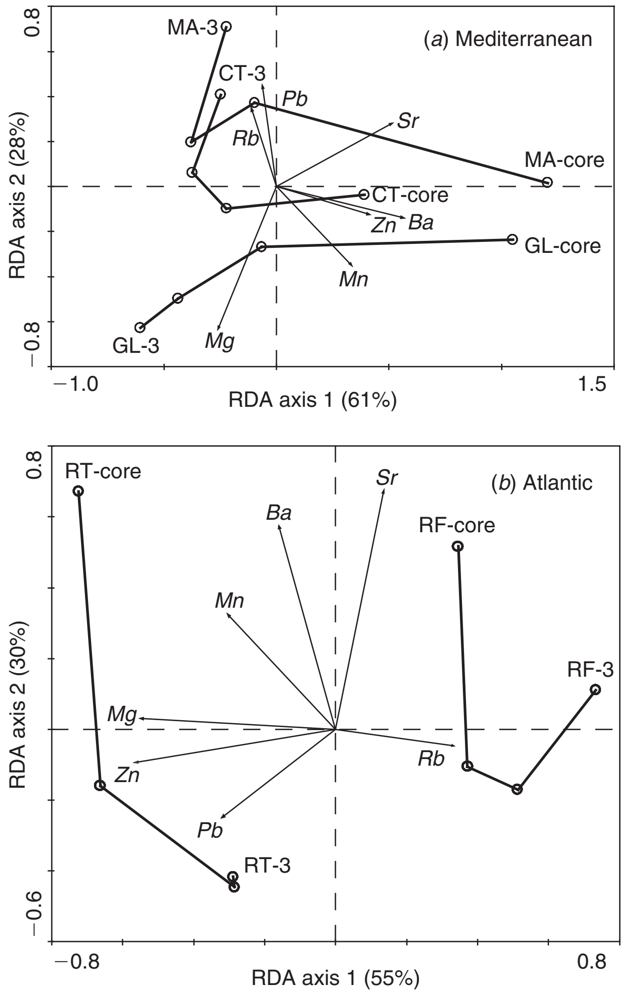

Fig. 4. (*a*) Age-related trends in the Mediterranean samples. Ordination diagram (biplot) corresponding to a partial redundancy analysis (RDA) based on seven elements (correlation matrix). The environmental variable in the model was the interaction between age (four categories from Core to age 3) and sample area (GL, Gulf of Lions, MA, Mallorca and CT, Catalan Slope). Age categories from the same sample area are connected. Scaling focuses on preserving the Euclidean distances between samples. The angle between arrows denotes the degree of correlation. Symbols of individual age classes can be projected perpendicularly onto the line overlaying the arrow of a particular element. Projection points are in the order of the predicted increase of the content of a particular element across the age classes. The first axis appears to correspond to an age-related gradient. The second axis seems to discriminate between sample areas. Note that the age-related pattern of GL differs from that displayed by the other two areas (especially at age 3). (*b*) Age-related trends in the Atlantic samples. Ordination diagram (biplot) with the same setting as Fig. 3*a*. The first axis discriminates between the two areas (RT, Rockall Trough and RF, Romsdal Fjord). The elements that contribute most to differentiation between the two areas are Mg, Zn, Pb and Rb.

fish (Papadopoulou *et al.* 1978; Protasowicki and Kosior 1988), which may indicate that higher metabolic rates are linked to higher rates of incorporation of some elements. There is direct experimental evidence that metabolism is a factor in determining the ∂^{13} C ratios in fish otoliths (Kalish 1991*b*; Thorrold *et al.* 1997). Variations in otolith Sr/Ca composition were initially related to seasonal temperature fluctuations (Radtke and Morales-Nin 1989) but recent evidence has indicated that variation in Sr concentrations are more likely linked to metabolic and ontogenetic changes (Sadovy and Severin 1994; Fowler *et al.* 1995; Friedland *et al.* 1998; de Pontual *et al.* 2003).

The elemental composition of the growth increments at the macrostructural level was determined with great precision by laser-ablation ICP-MS. The results show that age-related changes exist in the otolith elemental composition, although that this could be masked by inter-sample variability (e.g. Atlantic samples). A recent study that included more sample areas (S. Swan *et al.*, unpublished data) has shown that both core and whole otolith elemental composition enables European hake populations to be separated. However, data obtained for whole otolith composition was often found to be more accurate when predicting the geographical source of the samples and, as suggested by Fig. 4, this may be influenced by the wider variation in element concentrations in the otoliths of fish from different areas after the first few years of life.

Hake occupy a wide range of depths throughout their life and the otolith core represents the first weeks of age when the eggs and larvae are pelagic (Arneri and Morales-Nin 2000). The juveniles settle to the bottom at depths of 50–150 m. Our data showed that the otolith core composition was very distinct from the other growth increments (Fig. 3). In the Mediterranean, part of this differentiation is a result of Mn, which is present in the core at high concentrations compared with the rest of the otolith. In the Atlantic, Mn is also more abundant in the core but the differences are less pronounced. Sr, Zn and Ba also have higher concentrations in the core in the Mediterranean samples. It has been found that Cape hake (*M. capensis*) otoliths are richer in protein in the nuclear region of the otolith (Morales-Nin 1986), and it is possible that this could explain the observed differences between the elemental composition of the core and the outer growth zones in European hake. It could be that the protein matrix has an affinity for some elements such as Mn. In a study of aragonite and vaterite otoliths from herring, it was shown that vaterite otoliths were higher in Mn and lower in Sr content (Tomás and Geffen 2003). These authors also suggested that the Mn might act as a stabiliser of the vaterite, preventing its dissolution. Recent work with clupeid otoliths detected high concentrations of Mn in otolith cores and either embryonic effects or the presence of calcite were hypothesised as possible explanations (Brophy *et al.* 2004). No data are available at the small scale necessary to detect the possible presence of vaterite in the core area of the hake otolith. Thus, possible variations in the crystallisation of the otolith core should not be ignored.

Apart from the differences found in the otolith cores, an age-related trend exists in some elements for all sample areas (Fig. 4). This trend is similar in most cases, but the Gulf of

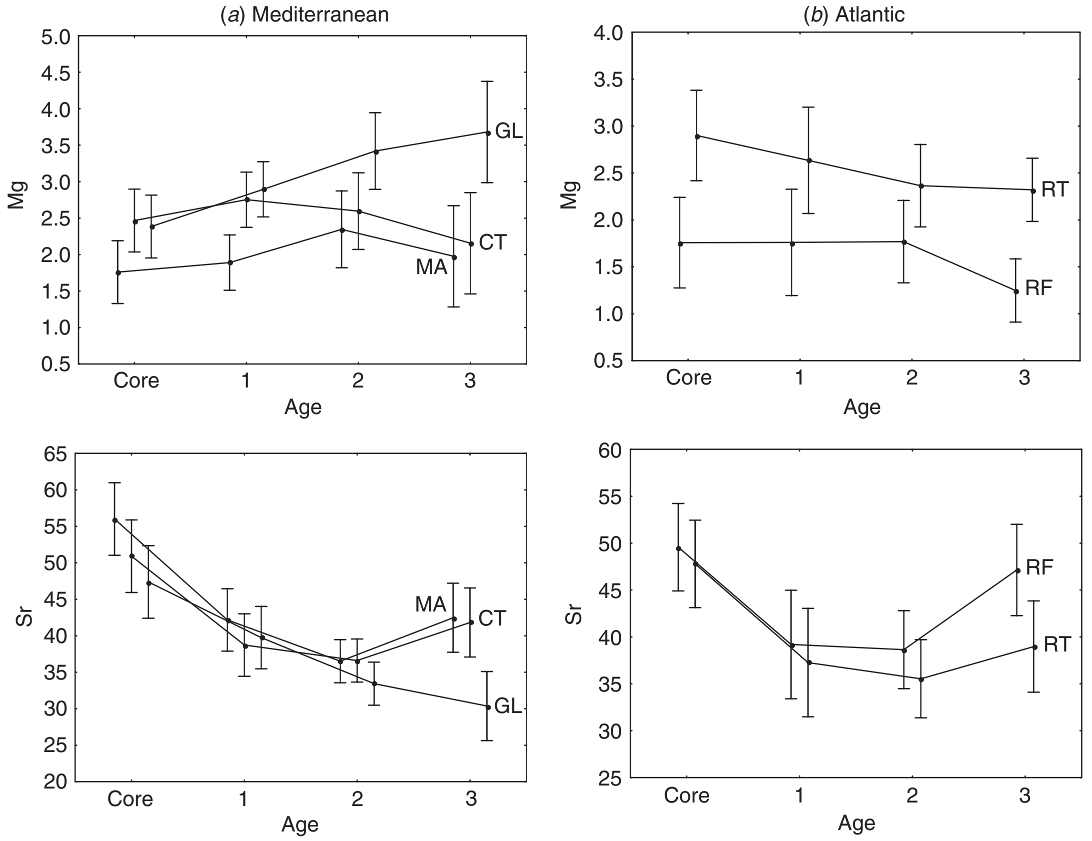

Fig. 5. Univariate age-related trends for Sr and Mg. (*a*) Results for the Mediterranean fishes. The Gulf of Lions (GL) samples show different age-related patterns in comparison with the other two areas (note the differences between age 3 classes). Vertical bars denote 95% confidence intervals. (*b*) Results for the Atlantic fishes. The two areas seem to display the same general age-related pattern.

Lions samples show some differences, mainly in the concentrations of Mg and Sr. In four of the sample areas (RF, RT, MA and CT) the relative concentration of Sr decreases from the core outwards, but increases again at age 3, although not reaching initial concentrations. In the Gulf of Lions the decreasing trend with age does not change (Fig. 5). Sr is mostly derived from the diet, diet being the origin of 70% of Sr in adult salmon calcified tissues with only 30% coming from the surrounding water (Kennedy *et al.* 2000). European hake experiences a shift in diet from euphausiids to fish at ~1-year-old (Bozzano *et al.* 1997). Thus, Sr changes at age 3 are probably not related to diet. An alternative explanation might be a result of changes in water temperature. This species migrates to deeper waters and mobility along the slope increases with age (Recasens *et al.* 1998). The climatic conditions prevailing in the Gulf of Lions during winter favour the formation of cold dense water in the area (Millot 1990), probably causing the presence of cold water at the depths occupied by European hake. Moreover, the freshwater influence of the river Rhône on the Gulf of Lions has been suggested as causing reduced Sr concentrations in whole otoliths of European hake, using solution-based ICP-MS analysis (S. Swan *et al.*, unpublished data). Another possible explanation might be related to sexual development. Both sexes mature at 3–3.5 years in the Gulf of Lions and 3–4 in the Balearic Sea (Oliver 1991). Adult distribution in the Gulf of Lions suggested that in peak spawning periods (autumn and winter) the adults, especially females, move towards the edge of the shelf, while in periods of lower reproductive activity they were scattered over the whole depth range (Recasens *et al.* 1998). Although it was not possible to draw any conclusions on the effect of sexual maturity on elemental concentrations, this aspect deserves further exploration.

Previous work has related the elemental composition of European hake otoliths with growth structures and with kinetic processes in otolith formation (Tomás *et al.* In press). An electron probe study based on Sr, Na and Ca in some European hake populations (Tomás *et al.* In press) has shown relative changes in composition associated with opaque and translucent increments. However, in the data presented here, there was no distinction made of seasonal growth structures because the number of spots in each kind of increment were irregular requiring a specific experiment design to allow the effect of age to be separated from structural effects.

This contribution shows that there are some age-related trends in the chemistry of hake otoliths. These differences may be related to structural changes associated with growth but the possibility of life history interactions cannot be ignored. This implies that if whole otolith composition is to be used to discriminate between stocks, the samples from the stocks must have the same age structure, or age-related trends could be mistaken for stock differences. Analysis across otolith transects such as in the current study may allow geographic variation to be assessed independently of age-related variation, thus yielding more information on stock structure than a whole otolith analysis.

Acknowledgments

The results presented in the present paper comprise parts of a project entitled *Otolith Microchemistry as a Means of Identifying Stocks of Deep-water Demersal Fish* (OTOMIC) (FAIR CT98–4365), which was part-funded by the European Commission. Part of Dr J. D. M. Gordon's contribution to the current paper was supported by a Spanish Ministry of Education Sabbatical Grant (SAB2002–0239).

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Manuscript received 13 July 2004; revised 22 December 2004; and accepted 2 March 2005.