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Otolith chemistry: an aid to stock separation of *Helicolenus dactylopterus* (bluemouth) and *Merluccius merluccius* (European hake) in the Northeast Atlantic and Mediterranean

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Helicolenus dactylopterus and *Merluccius merluccius* are widely distributed on the continental slopes of the Atlantic and Mediterranean and have quite different life histories. Both are commercially exploited, but little is known about their stock structure. Fish otolith composition is thought to reflect both endogenous processes and external factors, some of which relate to the surrounding environment, and therefore may be used as a tool for stock discrimination. The elemental composition of sagittal otoliths was examined using both solution-based inductively coupled plasma mass spectrometry of the whole otolith and laser-ablation analysis of the otolith nucleus. The relative concentrations of strontium, barium, and copper in dissolved whole otoliths contributed to the discrimination between *H. dactylopterus* samples from different geographic areas. Surface analysis of the otolith nucleus did not allow separation of geographic groups. For *M. merluccius*, separate analyses of the whole otolith data for the Atlantic and Mediterranean samples gave a clear distinction of the different groups within each ocean basin. Analysis of the *M. merluccius* nucleus composition indicated some differences in elemental concentration among both Atlantic and Mediterranean samples. Magnesium and lead were important elements in separating the groups in the Atlantic, and barium, strontium, and lead were important in the Mediterranean.

Keywords: deepwater fish, ICP-MS, otolith chemistry.

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Introduction

The deepwater fisheries of the North Atlantic and Mediterranean have developed rapidly in recent years, as the stocks of traditional shelf fisheries have declined, and the exploitation of some deepwater species is already considered to be outside safe biological limits. However, assessment and management of these fisheries is difficult because of the relatively short time-series of fishery information, inadequate biological knowledge, and a lack of information on stock structure (Large *et al.*, 2003). Stock discrimination is a complex concept that invokes numerous factors, ranging from large-scale geographic separation to small-scale differences in enzyme physiology, and the factors used pragmatically to separate and identify fish from different stocks integrate many endogenous and exogenous processes. Exogenous factors can be linked directly to physical features of the environment, such as bathymetry that affects lateral movement, or temperature and water chemistry that affect the elements available for incorporation into mineralized tissues, including otoliths.

Fish otoliths incorporate elements from the environment as they grow, either directly via the gills, indirectly through the diet (Campana, 1999), or from mobilized body stores (during vitellogenesis, for example; Kalish, 1989). Otoliths are assumed to be metabolically inert, so otolith composition may uniquely reflect populations that live and grow in a discrete area, or follow set migration routes

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(Thresher, 1999). The elemental signature of the otolith nucleus, the area lying within the first annual growth ring, is likely to be characteristic of the nursery areas of the species, and therefore could prove useful for discriminating between stocks (Milton *et al.*, 1997; Thorrold *et al.*, 1997, 2001; de Pontual *et al.*, 2000).

Two commercially exploited deepwater species that occur in both the Atlantic and Mediterranean, *Helicolenus dactylopterus dactylopterus* (Delaroche, 1809), the bluemouth or blackbelly rosefish, and *Merluccius merluccius* (Linnaeus, 1758), the European hake, were investigated during this study. They are representative of a range of habitats and life history characteristics making them suitable for assessing the effectiveness of otolith chemistry-based methodologies for determining the stock structure of deepwater fish.

Little is known about the stock structure of *H. dactylopterus*, although Eschmeyer (1969) showed that it has a complex pattern of distribution and identified two Atlantic subspecies, one of which is *Helicolenus dactylopterus dactylopterus*, composed of four separate populations (the Northeast Atlantic and the Mediterranean, the Gulf of Guinea, South Africa, and the Northwest Atlantic). Recent genetic evidence suggests that there are further subpopulations among the islands and seamounts of the Azorean archipelago (Aboim *et al.*, 2003).

The Mediterranean and Atlantic populations of *M. merluccius* are clearly differentiated both morphologically and genetically (Oliver and Massutí, 1995; Abaunza *et al.*, 2001; Lo Brutto *et al.*, 2004), and from parasitic tags (Mattiucci *et al.*, 2004). However, there is some evidence of gene flow between the Atlantic and Mediterranean in the vicinity of the Straits of Gibraltar (Roldán *et al.*, 1998). Although some authors have considered Mediterranean *M. merluccius* to be a subspecies (references cited in Lundy *et al.*, 1999), there is now a consensus that the small genetic differences and the potential for gene flow are insufficient to warrant subspecies status (Grant and Leslie, 2001). In the northeastern Atlantic within the ICES Area, *M. merluccius* is separated into two stocks for assessment purposes. A northern stock extends from the northern Bay of Biscay to the west of Norway, and a southern stock extends from the southern Bay of Biscay south along the Iberian Peninsula (Piñeiro and Saínza, 2003). The basis for the separation has been determined primarily on oceanographic and bathymetric features such as the Bay of Biscay and the Cape Breton Canyon. The behaviour and distribution of spawning adults and the dispersal of the juvenile stages also suggest two stocks (Casey and Pereiro, 1995). *Merluccius merluccius* along the North African coast spawn off northwest Morocco and are managed as a separate stock by CECAF (the Commission for the Eastern Central Atlantic Fisheries). The arbitrary boundary is the Straits of Gibraltar, although the extent of northward mixing with the southern ICES stock is unknown (Martos and Peralta, 1995).

Differences in the deepwater environments of the Atlantic and Mediterranean may be reflected in the elemental composition of the otoliths of the study species. Both areas have a complex layering of different water masses, and each water mass has a characteristic chemical signal (e.g. Bruland, 1983; Béthoux *et al.*, 1990). Both areas have seasonal and permanent thermoclines, but the temperature in the Mediterranean remains at about 13°C, whereas in the Atlantic temperature decreases steadily with depth. Temperatures at the sampling locations and depths in the Atlantic ranged from 7°C to 8°C in Romsdal Fjord to around 10°C in the Rockall Trough, and to 12°C on the Portuguese slope. There is an interchange of water through the Straits of Gibraltar, with high-salinity Mediterranean water extending into the Atlantic at depths of about 1000 m (Reid, 1994). One consequence of Atlantic inflow into the Mediterranean is that it creates a series of fronts in the Balearic region associated with local topography (López-Jurado *et al.*, 1996). The Algerian Basin acts as a reservoir for water of Atlantic origin, and its influence extends to the slope south of the Balearic Islands.

Otoliths were obtained from *H. dactylopterus* and *M. merluccius*, and two methods of otolith analysis were employed. Whole otolith analysis by solution-based inductively coupled plasma mass spectrometry (ICP-MS) permitted simultaneous measurement of the concentrations of many elements that are useful for stock identification (Thresher, 1999), and laser-ablation ICP-MS analysis of the otolith nucleus provided an indicator of the early life characteristics.

Material and methods

Fifty *H. dactylopterus* were obtained from surveys carried out by research vessels at locations throughout the Atlantic and Mediterranean (Table 1, Figures 1 and 2) and from market sampling (Azores). Both sagittal otoliths were extracted from fresh fish using plastic forceps, cleansed of adhering tissue, and stored dry in acid-washed polyethylene vials. Total length (L_T) was measured for all fish, except those from the Rockall Trough where standard length (L_S) was measured. This was converted to L_T using the equation

$$L_T = (1.209 L_S) + 0.5372, \quad n = 230 \text{ (SAMS, unpublished data)}.$$

Fish of similar size were chosen whenever possible to control for any size effects on otolith elemental concentrations (Edmonds *et al.*, 1989; Bronte *et al.*, 1996). It was not possible to obtain larger fish from the Rockall Trough, although the length range of this group does overlap with that of the samples from the Catalan Slope.

Table 1. Details of *H. dactylopterus* samples and location of sampling sites. Otolith pairs were used for both whole otolith solution-based analysis and laser-ablation analysis of transverse sections.

Sample area	Position	Number of fish	Total length range and mean L_T (cm)	Depth range(m)
Catalan Slope	39°53′N 00°53′E	10	15.4–27.3 (18.9)	152
Alboran Slope	36°15′–37°43′N 00°15′–04°58′W	10	20.5–30.0 (25.8)	402–635
Azores	39°N (approx.) 28°W (approx.)	10	25.0–42.0 (32.2)	180–396
Portugal	40°53′N 09°20′W	10	24.5–31.1 (27.3)	226
Rockall Trough	58°35′N 08°02′W	10	14.1–18.9 (15.7)	267

In all, 86 whole otoliths from *M. merluccius* were obtained from sampling sites in the Atlantic and Mediterranean (Table 2, Figures 1 and 2). Although care was taken to avoid contamination, otoliths from the Catalan Slope and Romsdal Fjord were extracted using metal forceps and stored in paper envelopes. Only those elements unaffected by storage and handling were used in the analysis (Swan *et al.*, in press). Total lengths of the fish from each area were measured, and there was little overlap in length between samples from the Mediterranean and the Atlantic, apart from those from off the Portuguese mainland, which were similar in size to Mediterranean samples. Although no sex data were recorded for the Rockall Trough or the Portuguese mainland samples, male fish were selected when available.

The whole otoliths for solution-based ICP-MS analysis were prepared and analysed following the method of Swan *et al.* (2003), using a National Institute of Standards and Technology (NIST) standard reference material of $CaCO_3$ (SRM915a; Table 3). For the analysis of all elements except calcium and strontium, the method of standard addition was used for calibration. Instrument drift was assessed using a 10-μg l^{-1} standard solution of ^{115}In and ^{209}Bi. Mean acid blank values and limits of detection (LOD) were produced from three times the standard deviation of a series of acid blanks interspersed throughout the ICP-MS analyses. Otoliths of *H. dactylopterus* and *M. merluccius* were analysed separately, although samples from each location were distributed throughout all the ICP-MS sessions, in order to avoid sequence effects. Solution data

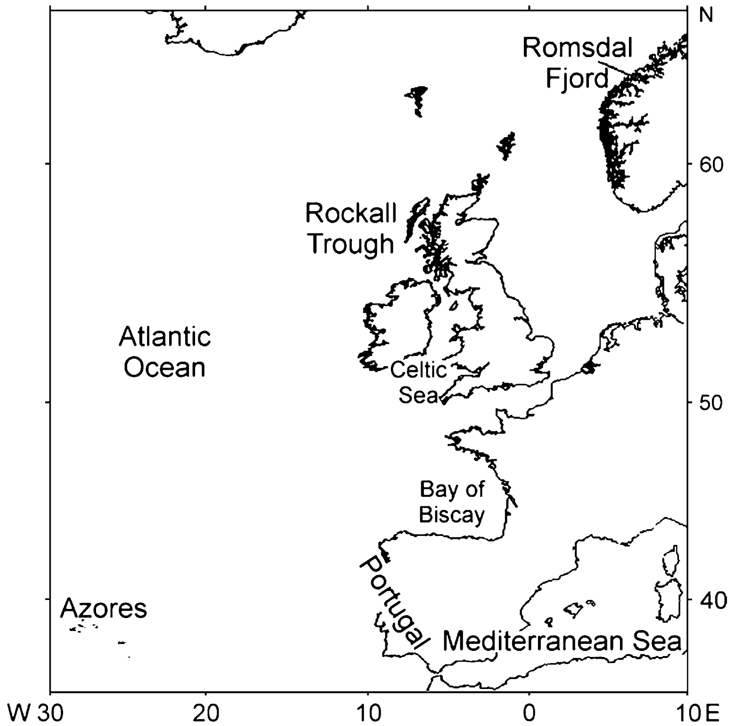

Figure 1. The Northeast Atlantic. *H. dactylopterus* were obtained from the Azores, the Rockall Trough, and off Portugal, and *M. merluccius* from Romsdal Fjord, the Rockall Trough, and off Portugal.

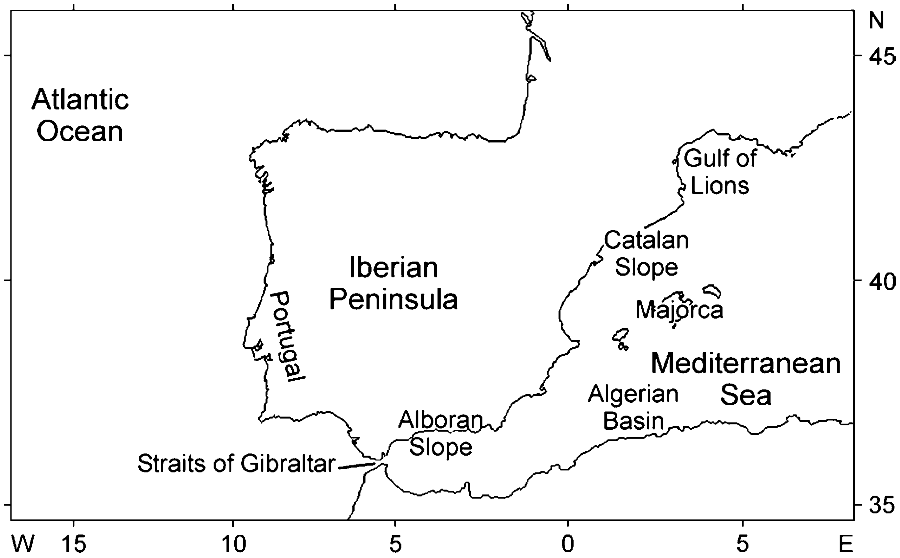

Figure 2. The Mediterranean. *H. dactylopterus* were obtained from the Alboran Slope and Catalan Slope, and *M. merluccius* from the Catalan Slope, Gulf of Lions, and south of Majorca.

were calculated using natural abundance ratios based on isotope measurements and expressed as fully quantitative element concentrations. These were standardized by adjusting for the weight of the otolith using the sample dilution factors obtained for individual otoliths.

The otoliths for laser-ablation analysis were set in polyester resin blocks and sectioned through the nucleus along the sagittal plane using an isomet double-blade diamond saw, then rinsed in 2% HNO_3 followed by deionized water, and allowed to dry. Analysis was carried out with a VG Microprobe II pulsed Nd:YAG laser (Table 3), using NIST glasses 610, 612, and 614, and a pressed limestone pellet (BCS CRM393) for calibration. Ablation parameters were optimized for a $CaCO_3$ matrix using otolith material instead of NIST glass. Argon gas blanks were run before each assay, and the mean blank counts per second (cps) was subsequently subtracted from the sample cps. A series of spots was ablated within the nuclear area of the otolith (three spots for *H. dactylopterus*, between three and six for *M. merluccius*), 50 µm in diameter and 20 µm deep. Atlantic and Mediterranean *M. merluccius* were analysed separately. Semi-quantitative data were calculated for the isotopes ^{7}Li, ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{138}Ba, and ^{208}Pb as mean cps normalized to the cps of ^{43}Ca. The relative standard deviation (RSD) for ^{43}Ca between the three spots for each otolith was used to evaluate the ablation efficiency for the different otoliths and the precision of measurement of the isotopes.

Elements that were consistently measurable above the LOD values from both the whole otolith solution and

Table 2. Details of *M. merluccius* samples used for whole otolith solution and otolith nucleus laser-ablation ICP-MS analyses. L_T and depth range are given for the samples used in the solution-based analysis.

Sample area	Position	Number of fish (solution)	Number of fish (laser)	Total length range and mean L_T (cm)	Depth range (m)
Gulf of Lions	42°07′N 03°22′E	11	6	30.0–38.0 (34.0)	85–128
Catalan Slope	41°04′–41°10′N 01°41′–01°58′E	11	10	29.5–41.0 (35.3)	255–300
Majorca	39°20′–39°50′N 02°20′–03°18′E	10	5	25.0–33.0 (29.0)	250
Portugal	36°50′–37°57′N 08°16′–09°25′W	10	10	30.6–33.5 (31.4)	127–371
Rockall Trough	57°56′–58°35′N 08°02′–09°40′W	15	10	36.0–68.0 (53.5)	267–627
Romsdal Fjord	62°30′N 07°30′W	8	8	46.0–64.0 (57.5)	300–360

Table 3. Instrument operating conditions for ICP-MS for solution-based analysis of whole otoliths and laser-ablation analysis of otolith nuclei.

Solution-based ICP-MS	VG PlasmaQuad 3 (Thermo Elemental, Winsford, UK)
Acquisition mode	Scan
Acquisition time	60 s run^{-1} (three repeats)
Mass range	7–240
Gas flow rate	0.90 l min^{-1}
Gas type	Argon
Laser-ablation ICP-MS	**VG PlasmaQuad 3**
Acquisition mode	Peak jumping
Acquisition time	50 s (blanks and standards), 30 s (spots)
RF power	1 350
Cool gas flow rate	12.8 l min^{-1}
Auxiliary gas	0.85 l min^{-1}
Nebulizer gas	1.05 l min^{-1}
Gas type	Argon
Laser	**VG Microprobe II**
Laser type	Nd:YAG wavelength 266 nm
Laser mode	Q-switched, time resolved mode (TRA)
Laser energy	0.486 mJ
Ablation type	Spot
Spot size	50 µm
Depth	20 µm
Scan speed	10 µm s^{-1}
Output	80%
Repetition rate	10 Hz

laser-ablation ICP-MS analyses were $\log_{10}(y+1)$ transformed to obtain normal distributions and homogenous variances. Analysis of covariance (ANCOVA) was used to determine the effect of area of collection on single element concentration in whole otoliths, while controlling for effects attributable to fish length (Minitab v13.1). All comparisons were significant at $p < 0.05$. A forward stepwise discriminant analysis (Manly, 1992) was used to determine a classification model built from the length-adjusted data matrix of elements. Classifications to area were made using cross-validation (SAS v8) and the predicted group membership compared directly with the actual source to give a rate of correct classification (expressed as a percentage of fish in each group).

Results

Some differences between sample groups of *H. dactylopterus* were attributable to fish length, and where significant effects were identified [Mg, Mn, Zn, Cu, and Ba (negative correlation), and Sr (positive correlation)], element concentrations were adjusted using the common slope (Edmonds *et al.*, 1989). Whole *H. dactylopterus* otoliths from different areas were significantly different in Sr, Ba, Sc, Y, and Cu concentrations. Both Sr and Ba concentrations were higher in Alboran Slope samples than in samples from all other areas (Figure 3).

Sr, Ba, Cu, Mn, and Li contributed most to group separation, and the overall cross-validated classification rate was 60% using only these elements (Table 4). Most of the classification errors were associated with the Azorean samples, because 30% of these were classified as being from the Catalan Slope group. The most distant groups were the Alboran Slope and the Rockall Trough. The most distinct group was the Catalan Slope, with 80% of the samples being correctly classified.

The relative standard deviation (RSD) of ^{43}Ca measured from the repeated laser-ablated samples from the nucleus area for each *H. dactylopterus* otolith ranged from 0.8% to 32.8%, suggesting that ablation efficiency varied among spots within the same otolith area. Analysis of variance (ANOVA) indicated no significant differences for single isotopes (ratioed to ^{43}Ca) between areas, but ^{55}Mn and ^{86}Sr values were higher in the Atlantic samples than in those from the Mediterranean. The cross-validated analysis produced a correct overall classification rate of only 18% (Table 5). The most distant groups were the Catalan Slope and the Rockall Trough. The Portuguese group was more similar to the Catalan Slope group than any of the Atlantic groups.

M. merluccius whole otoliths from separate areas differed not only in the concentrations of elements, but also in the distribution of elements between different individuals. For example, Pb was not detected in the whole otoliths from most areas, although measurable concentrations were found in 9% of the Gulf of Lions samples and 60% of the Rockall Trough samples. For the whole otolith analysis, the key elements contributing to separation between all areas were Mg, Mn, Sr, Ba, and Rb (Figure 4). Length-adjusted concentrations of Sr, Rb, and Ba were all significantly lower in the Rockall Trough samples than in those of the other Atlantic areas, whereas Romsdal Fjord samples had the highest concentrations of these elements. ANCOVA identified significant differences in otolith concentrations of Mg, Mn, and Sr between individual Mediterranean areas. Strontium concentrations were highest, and Mg and Mn lowest in Majorcan samples than in samples from the other Mediterranean areas, whereas Gulf of Lions samples were characterized by higher Mg and Mn, and lower Sr. No single element had a higher concentration in all samples from either of the ocean basins with the exception of Ba, which was lower in the Mediterranean groups.

A cross-validated discriminant analysis of the dissolved whole otolith data for all areas produced an overall correct classification rate of 64.7%, with Sr, Mg, Ba, and Mn

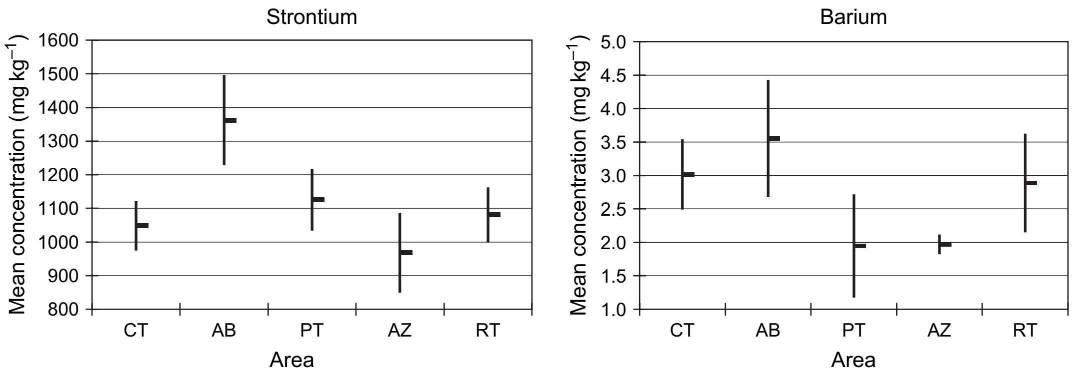

Figure 3. Variation in the concentration of Sr and Ba (mg kg^{-1}) with 95% CI in otoliths of *H. dactylopterus* from the five sampling areas (CT: Catalan Slope; AB: Alboran Slope; PT: Portugal; AZ: Azores; RT: Rockall Trough).

contributing most to group separation. Although 82% of Gulf of Lions and 80% of Rockall Trough samples were correctly assigned, most of the classification errors were associated with Romsdal Fjord samples (correct rate 25%), which were frequently grouped together with Mediterranean samples. However, separate analyses of the Atlantic and Mediterranean samples gave clear identification of the different groups within each ocean basin. The Atlantic samples were easily separated, with 79% assigned by cross-validated analysis to the correct area (Table 6). Sr, Sc, Cu, and Ba contributed most to group separation. For the Mediterranean samples, 65.5% of samples were correctly assigned, with Mg and Mn the most important elements in identifying the different groups (Table 7).

Some *M. merluccius* sampling areas were more readily distinguished, based on the composition of the otolith nuclei. The RSD of ^{43}Ca was 7.89% for the Atlantic group session and 10.58% for the Mediterranean group session, indicating efficient ablation of the nucleus material. Within the Atlantic samples, discriminant analysis produced a correct cross-validated classification rate of 67%, with ^{24}Mg and ^{208}Pb contributing most to group separation (Table 8). All the Romsdal Fjord samples were correctly classified, owing to there being significantly less ^{24}Mg and ^{66}Zn (ANOVA) than in samples from the other groups. ^{208}Pb was higher in Rockall Trough samples, and ^{86}Sr and ^{138}Ba were lower in Portuguese samples, although not significantly. For the Mediterranean *M. merluccius* otolith nuclei, stepwise discriminant analysis indicated that ^{138}Ba, ^{86}Sr, and ^{208}Pb contributed most to group separation. Cross-validated classification assigned 59% of samples to their correct groups (Table 9). Most of the classification errors were associated with a lack of clear distinction between the Catalan Slope and Majorcan groups for many isotopes. However, Catalan Slope samples were significantly lower in ^{138}Ba than in samples from other Mediterranean groups, and more ^{86}Sr was present in Majorcan samples than in those from the Catalan Slope, although the difference was not significant. Gulf of Lions samples were lower in ^{86}Sr, and also had higher levels of ^{208}Pb, ^{24}Mg, and ^{66}Zn than in samples from other Mediterranean groups. The two approaches, using the whole dissolved otoliths and laser ablation of the otolith nucleus, produced consistent area trends within the Mediterranean samples for Mg, Rb, and Sr. Only Mn gave consistent patterns among the Atlantic samples for the two sampling approaches.

Table 4. *H. dactylopterus*: separation between groups based on stepwise cross-validated discriminant analysis scores from whole otolith solution data.

Actual group	Number of samples	Predicted group membership (%)				
		AB	AZ	CT	RT	PT
Alboran Slope, AB	10	60.00	0.00	20.00	0.00	20.00
Azores, AZ	10	10.00	40.00	30.00	20.00	0.00
Catalan Slope, CT	10	0.00	10.00	80.00	0.00	10.00
Rockall Trough, RT	10	0.00	10.00	10.00	60.00	20.00
Portugal, PT	10	20.00	20.00	0.00	0.00	60.00

Table 5. *H. dactylopterus*: separation between groups based on stepwise cross-validated discriminant analysis scores from otolith nuclei data.

Actual group	Number of samples	Predicted group membership (%)				
		AB	AZ	CT	RT	PT
Alboran Slope, AB	10	10.00	10.00	30.00	20.00	30.00
Azores, AZ	10	10.00	30.00	20.00	30.00	10.00
Catalan Slope, CT	10	20.00	20.00	10.00	0.00	50.00
Rockall Trough, RT	10	40.00	20.00	0.00	30.00	10.00
Portugal, PT	10	10.00	10.00	50.00	20.00	10.00

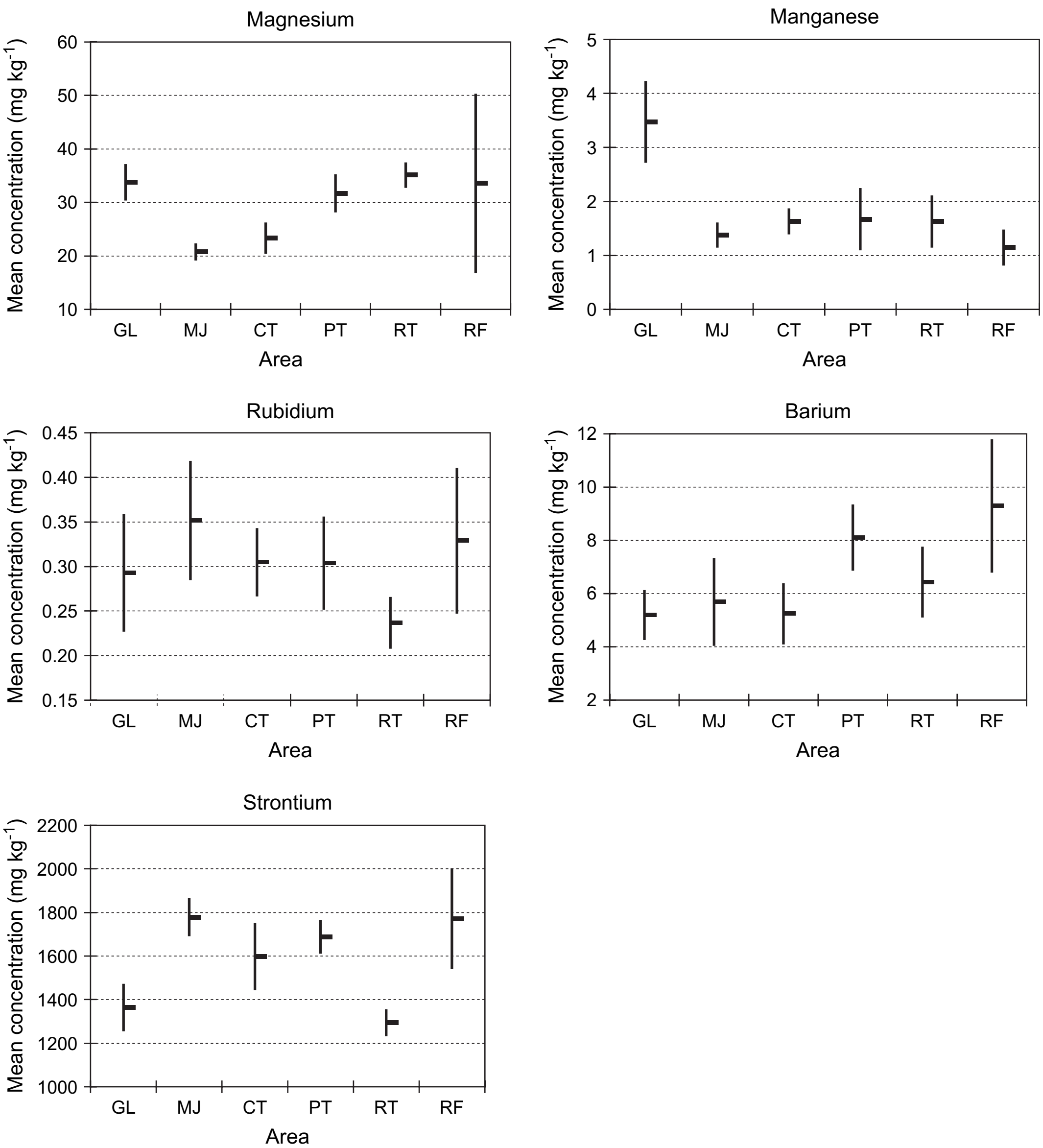

Figure 4. Variation in the concentration of key elements (mg kg^{-1}) with 95% CI in otoliths of *M. merluccius* from the six sampling areas (GL: Gulf of Lions; MJ: Majorca; CT: Catalan Slope; PT: Portugal; RT: Rockall Trough; RF: Romsdal Fjord).

Table 6. *M. merluccius*: separation between Atlantic groups based on stepwise cross-validated discriminant analysis scores from whole otolith solution data.

		Predicted group membership (%)		
Actual group	Number of samples	PT	RF	RT
Portugal, PT	10	80.00	20.00	0.00
Romsdal Fjord, RF	8	37.50	62.50	0.00
Rockall Trough, RT	15	0.00	6.67	93.33

Discussion

The basis of using otolith chemistry for stock separation applications is that the elemental composition of the water from separate geographical areas will influence otolith composition for fish living in those areas. The dissolved whole otoliths of adult *Helicolenus dactylopterus* from the Alboran Slope samples were distinguished from those of the Catalan Slope by having significantly higher concentrations of Sr and Y. Cr, Cu, and Ba concentrations tended to be higher in Alboran Slope samples, and Mg concentrations were lower. Portuguese mainland samples had lower

Table 7. *M. merluccius*: separation between Mediterranean groups based on stepwise cross-validated discriminant analysis scores from whole otolith solution data.

Actual group	Number of samples	Predicted group membership (%)		
		MJ	CT	GL
Majorca, MJ	10	60.00	40.00	0.00
Catalan Slope, CT	11	27.27	54.55	18.18
Gulf of Lions, GL	11	0.00	18.18	81.82

Table 9. *M. merluccius*: separation between Mediterranean groups based on stepwise cross-validated discriminant analysis scores from otolith nuclei data.

Actual group	Number of samples	Predicted group membership (%)		
		MJ	CT	GL
Majorca, MJ	5	40.00	40.00	20.00
Catalan Slope, CT	10	30.00	70.00	00.00
Gulf of Lions, GL	6	16.67	16.67	66.67

concentrations of Mg, Sc, Mn, and Cu than either the Azorean or Rockall Trough samples, and were generally more similar in composition to the Mediterranean groups. Rockall Trough samples were characterized by high concentrations of Ba compared with other Atlantic areas.

The Alboran Sea is the first basin encountered by inflowing low-salinity Atlantic water (Massutí *et al.*, 2001). At depths of 300−700 m, there is a layer of Levantine Intermediate Water (LIW), characterized by high salinity that may result in the increased Sr concentrations found in the *H. dactylopterus* otoliths sampled from those depths, whereas the Catalan Slope samples were obtained from a depth of about 150 m on the edge of the continental slope in water of primarily Atlantic origin (Ruiz-Pino *et al.*, 1991). Barium is often associated with high primary productivity and exhibits a nutrient-type profile, with surface depletion and enrichment at depth. Otolith uptake of Ba shows a positive response to environmental concentrations (Bath *et al.*, 2000), although concentrations may also be associated with diet (Buckel *et al.*, 2004). Both Mn and Cu concentrations can be influenced by terrestrial sources and are generally higher in shelf waters than in oceanic surface waters (Bruland, 1983). However, dissolved Cu and Mn in the eastern Mediterranean have shown complex patterns related to surface circulation, and Mn is occasionally enriched over the shelves (Zeri and Voutsinou-Taliadouri, 2003). It is possible that the higher concentrations of elements such as Cu present in the Alboran Slope samples are a result of heavy metal input during passage of surface water through the coastal region (Gulf of Cadiz) and into the Straits of Gibraltar (Sherrell and Boyle, 1988; van Geen *et al.*, 1991). Although surface trace element concentrations in the Mediterranean are generally high compared with those in the Atlantic (Béthoux *et al.*, 1990), there were few identifiable trends between the ocean basins, although whole otolith concentrations of Li and Ba were generally higher in both *H. dactylopterus* Mediterranean groups than in the Atlantic groups.

Table 8. *M. merluccius*: separation between Atlantic groups based on stepwise cross-validated discriminant analysis scores from otolith nuclei data.

Actual group	Number of samples	Predicted group membership (%)		
		PT	RF	RT
Portugal, PT	10	60.00	30.00	10.00
Romsdal Fjord, RF	8	0.00	100.00	0.00
Rockall Trough, RT	10	40.00	20.00	40.00

The composition of the *H. dactylopterus* otolith nuclei was not sufficiently different for consistent discrimination between fish from the different sampling sites. Initially, it was assumed that *H. dactylopterus*, in common with other scorpaenid fish, was viviparous and, as such, a degree of elemental uniformity, related to body fluid composition, might have been expected within the primordial nucleus. However, recent evidence of zygoparity (oviposition of early embryo instead of larvae; Sequeira *et al.*, 2003, and references therein) and the unusual discovery of juveniles in the shallow North Sea are indicative of an extensive pelagic dispersal stage (Heessen *et al.*, 1996), although evidence from photographs and submersibles suggests that the adults are mostly sedentary (Uiblein *et al.*, 2003). The lack of discrimination among the *H. dactylopterus* nuclei sampled from different areas does not necessarily indicate that the fish have a common origin; indeed, this is highly unlikely given the widespread distribution of mature fish. It may be that factors other than environmental considerations play a more important role in elemental uptake for this species. In summary, the analysis of the chemistry of whole *H. dactylopterus* otoliths showed some separation in adult fish, but the composition of the nuclei was not sufficiently different to permit discrimination between fish from widely separated locations.

Discriminant analysis produced a good separation of *M. merluccius* from the different sampling sites, based on the composition of both whole otoliths and otolith nuclei. The results obtained from the chemistry of *M. merluccius* otoliths are consistent with current views on the separation of western Mediterranean *M. merluccius* populations based on spawning and juvenile distributions (Recasens *et al.*, 1998; Maynou *et al.*, 2003). The separation of the northern northeastern Atlantic samples (represented by the Rockall Trough) from the southern northeastern Atlantic samples (represented by Portugal) is consistent with the current treatment of these areas by ICES as separate stocks (Piñeiro and Saínza, 2003). The otolith elemental signature of *M. merluccius* sampled from Portuguese waters was most consistently unique, supporting the separation of these fish from more northern populations. The potential existence of distinct fjordic populations, with little mixing with Atlantic populations was also indicated by the distinct chemical signature of the Romsdal Fjord samples. Lundy *et al.* (1999) compared samples from widespread locations in the Atlantic and found significant differences between fish from Norwegian waters and the Celtic Sea, populations that are currently treated as a single stock for assessment purposes. In the Mediterranean, the Gulf of Lions was the most distinctive group, and whole otoliths were characterized by significantly higher concentrations of Mg and Mn, and significantly lower concentrations of Sr, which may be due to the influence of freshwater input from the River Rhône. An examination of *M. merluccius* otoliths along a transect from the core to the outer edge using laser-ablation ICP-MS indicated that, although trends in Sr concentrations were similar for all three Mediterranean areas during the first two years of life, Gulf of Lions samples had reduced Sr levels by age 3, compared with the other sample areas (Morales-Nin *et al.*, 2005). Gulf of Lions samples also had higher levels of Zn, which has a nutrient-type distribution in seawater, and Pb, which has both anthropogenic and atmospheric sources and decreases with depth (Bruland, 1983).

There are many possible explanations for the differences in whole otolith composition between Atlantic and Mediterranean *M. merluccius*. The Atlantic *M. merluccius* samples tended to consist of fish of a larger size and from a greater depth range, so the associated temperature differences might have had an effect on otolith composition. Factors such as sex, age, and growth rate also influence elemental uptake. Ontogenetic differences in diet and a greater importance of crustaceans in the Mediterranean may also be a contributory factor. For both *H. dactylopterus* and *M. merluccius*, the patterns of Mg and Sr concentration in whole otoliths and nuclei provide some evidence for a physiological influence on the incorporation of these elements into calcium carbonate.

Conclusion

The unbiased correct classification rates were somewhat dependent on species and on whether whole or otolith nuclei were used in the analysis, but in some cases (e.g. Atlantic *M. merluccius*) good separation was achieved between all sample groups. Data obtained from the elemental analysis of otoliths may provide as much information on stock identity as other methods currently in use, e.g. parasitic tags, morphometrics (see Begg and Waldman, 1999, for a description), and the ability to discriminate between samples groups using otolith chemistry is certainly comparable with these methods and may provide a basis for further work using other techniques. The term "stock" does not automatically imply genetic differentiation, and genetic studies may not necessarily be suitable, because only a small amount of mixing between populations can produce genetic homogeneity (Edmonds *et al.*, 1989; Swan *et al.*, 2004). There are likely to be complex interactions between endogenous and exogenous factors that can lead to stock-specific characteristics exhibited as differences in morphology, behaviour, life history strategies, and the elemental composition of mineralized tissues, especially otoliths. Even without a clear understanding of the regulatory mechanisms that govern otolith composition, it is possible to utilize observed differences for stock separation applications. As long as consistent differences are observed, they can be exploited irrespective of whether the elemental signature is created directly by physico-chemical characteristics of the water, or by a complex interaction of stock-specific growth, physiology, and behaviour.

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