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Preliminary Investigations on the Uses of Otolith Microchemistry for Stock Discrimination of the Deep-water Black Scabbardfish (*Aphanopus carbo*) in the North East Atlantic

by

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Abstract

The black scabbardfish (*Aphanopus carbo*: family Trichiuridae) is widely distributed in the North Atlantic at depths between about 700 and 1000 m. There is a long-established longline fishery off the island of Madeira, and another that began off mainland Portugal in 1983. It is an important species in the landings from the mixed bottom trawl fishery that developed in the Rockall Trough in the 1990s. The stock composition of this species is unknown, as are the eggs, larvae and smallest juveniles. Length composition and reproductive maturity vary between areas and these were among the criteria that led the ICES Deep-water Study Group to carry our preliminary assessments on tentative s outhern and northern stocks.

An objective of the BASBLACK Project (EC DG Fisheries 97/0084) was to investigate stock discrimination in this species. The hypothesis to be tested was that there is one stock of black scabbardfish, which has a main spawning area in the south and a sub-adult feeding area in the north. One method used was otolith microchemistry and this paper describes the methodology and some of the results. The elemental composition of the otoliths was determined using solution based ICP-MS. The method used to minimize suppression or enhancement effects of the otolith matrix is described. Multivariate analysis was used to compare the results between areas, after taking into account possible differences in element concentration associated with fish length. Although the results are inconclusive, they suggest that this could be a useful technique for this species.

Introduction

Black scabbardfish (*Aphanopus carbo*) is a deep-water trichiurid fish first described from Madeiran waters, where a commercial longline fishery can be traced back to the 17th Century. Merrett and Haedrich (1997) give an interesting account of the development and present status of this fishery. Although the species was known to be widely distributed throughout the North East Atlantic at depths of between about 200 and 1600 m (Nakamura and Parin, 1993), the fishery was slow to develop in other areas. A directed longline fishery developed off mainland Portugal in 1983 and has continued to the present day (Anon., 2001a). Exploratory deep-water trawling surveys to the west of the British Isles in the 1970s and early 1980s showed that the black scabbardfish was one of the dominant species at depths of around 600-1000 m (Bridger, 1978; Ehrich, 1983). Russia also carried out exploratory deep-water trawl surveys in international waters during the 1960s, starting from Hatton Bank and following the continental margin around to the Reykjanes and Mid-Atlantic Ridges (Pechenik and Troyanovsky, 1970), and black scabbardfish was a bycatch of the subsequent fishery. The commercial fishery for deep-water species in waters under coastal state jurisdiction (mainly the European Union and Faroese sectors of the Rockall Trough) developed in 1989. Black scabbardfish is an important bycatch and sometimes a target species of this fishery (Gordon, 2001). Information on the landings from these fisheries is summarized by ICES (Anon., 2000).

The eggs and larval stages of the black scabbardfish are unknown and juvenile fish are seldom caught. Mature fish are caught at Madeira, where spawning takes place from November to December (Morales-Nin and Sena-Carvalho, 1996). Only spent fish have been found off mainland Portugal. These fish are typically larger than those from further north in the Rockall Trough (Figure 1). In the trawl fishery of the Rockall Trough the fish are smaller, about 80 to 110 cm total length, and are immature, although one mature fish was reported in a German survey (Ehrich, 1983). The situation on the Hatton Bank and on the Reykjanes Ridge is less clear. Recent Spanish landings from the Hatton Bank had a length range from about 80 to 120 cm (Anon., 2000). Earlier Russian data from the same general area reported spawning fish from November to April (Zilanov and Shepel, 1975). On the Icelandic slope and the Reykjanes Ridge, the majority of females were 90 to 110 cm in length and the males were 85 to 105 cm (Magnússon *et al*., 2000). Most fish were immature, although some spawning and newly spent fish were found between January and September. Unvalidated age estimates suggest that, unlike many other deep-water fishes, the black scabbardfish has a relatively rapid growth rate and longevity of about 8 years (Morales-Nin and Sena-Carvalho, 1996).

To assess the black scabbardfish in the northeastern Atlantic, ICES has arbitrarily divided the stock into a northern and southern component (Anon., 2001a). The stock in the northern areas is considered to be outside safe biological limits, with a steady decline in catch per unit of effort. In the southern area off mainland Portugal, total catches appear to be declining. However, given the differences in length composition and reproduction and also recognizing the different methods of capture between the two areas, it is questionable whether a north/south separation is valid. An investigation of stock discrimination was one of the components of a European Commission supported project on black scabbardfish (Environment and biology of deep-water species *Aphanopus carbo* in the NE Atlantic: basis for its management (BASBLACK) Study Contract 97/0084 (Santos, 2000). Current knowledge suggests that the black scabbardfish has its main spawning area at the southern range of its distribution (e.g. Madeira, mainland Portugal). The early life history stages are unknown and sub-adult stages dominate the landings from the commercial deep-water fisheries in the northern areas (e.g. Rockall Trough). The black scabbardfish is a piscivore and in the Rockall Trough it appears to feed mainly on semipelagic species such as blue whiting (*Micromesistius poutassou*) and argentine (*Argentina silus*), which can be highly aggregated on the slope at certain times of the year (Mauchline and Gordon, 1984). These species also occur at Hatton Bank and on the Reykjanes Ridge. The hypothesis to be tested is that black scabbardfish comprise a single stock with a southern spawning area, an unknown nursery ground and sub-adult feeding grounds in the northern areas.

In recent years, advances in analytical techniques have led to the use of otolith microchemistry as an aid to stock discrimination in fishes (Campana *et al*., 2000 and references therein). This application relies on the assumption that otoliths incorporate elements from the environment throughout the life of the fish. It is also assumed that otoliths are metabolically inert, such that any element deposited during growth of the otolith is fixed (Campana and Neilson, 1985). Otoliths are composed of about 96% calcium carbonate by weight. The remainder consists of about 3-4% organic matrix and <1% non-organic trace impurities. With the exception of Sr, the minor elements (>100 ppm) are likely to be under physiological regulation via the endolymph, which bathes the otolith (Campana, 1999). This does not appear to be the case for the trace elements. It is these trace elements which may provide a useful record of the environment to which the fish was exposed. If black scabbardfish carries out extensive migrations, it would pass through and reside in several different water masses. The chemical signature of the otolith may reflect these differing phases of the life cycle. There is also a suggestion that appears frequently in the literature that black scabbardfish is associated with high salinity Mediterranean water, which forms a layer at about 1000 m depth and extends in a plume out into the Atlantic (Pissarra *et al*., 1983; Leite, 1988; Reid, 1994). A component of this water mass extends northward into the deep water off Portugal and can be detected in the Porcupine Seabight (Rice *et al*., 1991) and the Rockall Trough (Ellett *et al*., 1986). In the Rockall Trough it was identified by its low oxygen content and slightly enhanced salinity. However, the presence of Mediterranean water in the Rockall Trough has been questioned recently (New and Smythe-Wright, 2001).

Materials and Methods

The sagittal otoliths from 98 black scabbardfish were obtained from six different locations throughout the North East Atlantic, namely Reykjanes Ridge **RR** (sampling depth range 795-2079 m), Hatton Bank **HB** (1285-1395 m), Rockall Trough **RT** (890-1134 m), Mid-Atlantic Ridge **MA** (823 m), Portuguese mainland (Sesimbra) **PT** and Madeira **MD** (Figure 2). Samples from Sesimbra and Madeira were obtained from the markets and these specimens would have been caught at a depth of approximately 1000 m. The total lengths (cm) of all fish were measured (Figure 3) and both

sagittal otoliths were extracted using plastic forceps and stored in acid-washed plastic vials. With the exception of the samples from the Mid-Atlantic Ridge, all otoliths were extracted from fish that had previously been frozen. To avoid possible contamination, all equipment was acid-washed prior to use, and at no stage were the otoliths exposed to metal. Prior to dissolution, the otoliths were dipped in 2% HNO_3 (Romil Ultrapure) for 15 s to remove any surface contamination, rinsed in 18 mega-ohm doubly deionized water (ELGA) to neutralize the acid and then air-dried and weighed to the nearest 0.01 mg.

Preliminary trials indicated that a cold acid digest of the otolith left a residue in the container, presumably consisting of the organic protein matrix. To ensure that this organic matrix and any associated elements were completely dissolved, a technique used by geochemists to dissolve sediment samples was adopted. The otoliths were placed in individual 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) set at 300 W 90% power, with a ramp time of 5 mins. The maximum temperature achieved (approximately 90º C) was maintained for 10 mins. The contents of the microwave tubes were then rinsed into polypropylene volumetric flasks and diluted with ELGA water to obtain a final acid concentration of 2% HNO_3. The quantities of acid used and final volumes of the digests were proportional to the sample weights, to insure that all resulting solutions were of a similar composition. A final dilution factor of approximately 0.83 mg of otolith material per 1 ml of 2% HNO_3 was obtained (i.e. dilution = H 1200). Procedural acid blanks, a multi-element spiked $CaCO_3$ sample (SRM915a) and an in-house homogeneously mixed sample of ground cod otoliths were concurrently digested and analyzed following the same procedures.

Elemental concentrations in the otolith were determined using a VG Elemental Plasma Quad 3 (S-Option) Inductively Coupled Plasma Mass Spectrometer (ICP-MS), operated in scanning mode with an acquisition time of 60 seconds. Values were obtained from an average of three readings. When more than one isotope was available for the same element, those with least interferences were used. Concentrations of 30 elements were determined, based on measurements of the following isotopes: ^{7}Li, ^{9}Be, ^{26}Mg, ^{27}Al, ^{44}Ca, ^{45}Sc, ^{47}Ti, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{64}Zn, ^{75}As, ^{85}Rb, ^{86}Sr, ^{90}Zr, ^{95}Mo, ^{107}Ag, ^{111}Cd, ^{133}Cs, ^{138}Ba, ^{139}La, ^{140}Ce, ^{146}Nd, ^{147}Sm, ^{208}Pb, ^{232}Th, and ^{238}U.

It is known that the more abundant elements present in the otolith can distort measurements, causing the enhancement or suppression of elements present at low concentrations (Dove *et al*., 1996). In order to minimize these matrix effects, otolith sample solutions were spiked with known concentrations of elemental standards at 0, 2, 16 and 60 µg/L and a calibration curve was produced. The calibration curve was applied as appropriate to the concentration of each element found in the sample. This method of standard addition was used for the analysis of trace elements at the H 1200 dilution. The otolith solutions were further diluted to concentrations of 0.005 mg per 1 ml of 2% HNO_3 (i.e. dilution = H 200 000) for the analysis of Ca and Sr and standard addition was not used to measure these elements.

The high concentrations of Ca in the solutions may result in the formation of deposits on the instrument itself, causing drift and a decrease in sensitivity over time. An internal standard of ^{115}In and ^{209}Bi at a concentration of 10 µg /L was added to all solutions and for all elements under investigation at the H 1200 dilution, the ICP-MS was re-calibrated after every 12 samples using an external set of standards to compensate for possible instrument drift. Changes in the sensitivity of the instrument were monitored every 6 samples with a non-matrix matched multi-element solution at concentrations of 1 and 20 µg/L. For samples analyzed for Ca and Sr at the H 200 000 dilution, the ICP-MS was re-calibrated every 24 samples and the instrument performance monitored every 12 samples with a non-matrix matched multi-element solution at concentrations of (i) 25 µg/L Sr, 50 µg/L Ca; (ii) 150 µg/L Sr, 400 µg/L Ca; (iii) 500 µg/L Sr, 1000 µg/L Ca. Instrumental limits of detection (LOD) were calculated from 3 H the standard deviation on the average value for a series of procedural acid blank solutions that were regularly interspersed throughout the samples. These were multiplied by the average sample dilution of H 1200 to produce values for minimum detectable concentrations for a solid sample. The ICP-MS analysis was carried out over 14 separate sessions and samples from each area were evenly distributed throughout the whole analysis to avoid possible sequence effects.

Results

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. Some elements were excluded from further analysis if

concentrations were mostly below the LOD. Otolith samples were also excluded from the data set if there were outliers present for many of the element concentrations. The in-house ground cod otolith material gave consistent results throughout the entire analysis and the results of the spiked $CaCO_3$ sample were similar to previously obtained values for this material. For the otolith samples, useable data were obtained for Li, Be, Mg, Ca, Sc, V, Cr, Mn, Co, Ni, Cu, Sr, Mo, Cs, Ba and Pb.

Elemental concentrations for the otolith samples were grouped by area and examined for normality of distribution and homogeneity of variances and $\log_{10}(y+1)$ transformations performed as appropriate to enable statistical analysis. Analysis of covariance (ANCOVA) was used to determine the effect of area of collection on single element concentration, whilst controlling for effects due to fish length (Minitab v13.1). No significant interactions were identified between area of collection and fish length for any of the elements examined. A significant negative length effect was identified for Li ($p=0.020$) and corrected for, by using the common linear slope (Edmonds *et al*., 1989). Elements found to be significantly different between areas were Mg and Sr ($p<0.001$), Mn ($p=0.019$) and Cu ($p=0.045$) (Figure 4).

Discriminant analysis (described by Manly, 1992) was used to test whether the assumed membership of an otolith sample to a categorical group (area) was justified using some explanatory variables (element concentration). Data with non-normal distributions or unequal variances were excluded from this analysis. The complete log-transformed data matrix was examined using the elements Li, Mg, Sc, Cr, Mn, Co, Ni, Cu, Sr and Ba (XLSTAT v4.4). The results of the discriminant analysis are shown in Figures 5 and 6. Axis 1 and axis 2 explained 52% and 26% of the variance, respectively. The classification matrix (SAS v8) is shown in Table 1. The number of samples correctly classified by area was 62%. A less-biased technique known as cross-validation was used, in which single observations were removed and the classification function built from the remaining data before the omitted observation was classified. The cross-validated classification was able to classify only 41% of the samples correctly. The number of samples correctly classified using cross-validation was increased to 45% by performing a stepwise discriminant analysis (Table 2). This assesses the relative importance of individual elements among groups using F-statistics (Wilks' lambda) estimated by the discriminant analysis procedure. Sr, Mg, Cu, Li, Co and Sc contributed most to group separation. Madeira was the most distant group from both Rockall Trough and Reykjanes Ridge. Mainland Portugal, Madeira and Mid-Atlantic Ridge were the closest groups.

Discussion

This preliminary study was based on the microchemical analysis of whole otoliths. The assumption is that the chemical composition of the otolith is related to the chemical composition of the water mass occupied by the fish. The exponential growth of the fish body weight tends to be reflected in the growth of the otolith and hence, for a long-lived species, a greater part of the otolith mass will be associated with the first years of life. If the fish in a stock are all derived from the same spawning area and have shared a common nursery ground, differences in chemical composition of the whole otolith associated with migration to other areas might be relatively small. If, as has been hypothesized, the black scabbardfish is associated with Mediterranean water throughout the greater part of its life, the variation between otoliths from different areas ought to minimal. The results of the discriminant analysis showed that there were only small differences between the overall chemical signatures of the otoliths from the study areas, and this is consistent with the single stock hypothesis.

There have been a number of studies that have successfully used whole otoliths for stock identification (Edmonds *et al*., 1989, 1991,1992, 1994; Campana and Gagné, 1994; Campana *et al*., 1995, 2000; Begg *et al*., 1998). These stock separations should not be considered as proxy for genetic identity nor should the chemical signal be considered to be stable over long periods (Campana *et al*., 2000). Only one of these studies (Edmonds *et al*., 1991) concerns a deep-water species, *Hoplostethus atlanticus*, in Australian waters. Some preliminary data suggests that the technique is useful for separating possible stocks of *Coryphaenoides rupestris* in the North East Atlantic (Anon., 2001b). In a study of whole otolith microchemistry on hake (*Merluccius merluccius*) from several sampling locations throughout both the Atlantic and the Mediterranean, it was found that discriminant analysis separated the samples from different locations when the Atlantic and Mediterranean areas were treated separately (Swan *et al*., unpublished). However, when both areas were combined in the analysis, samples from some Atlantic locations could not be discriminated from some Mediterranean locations, even though Atlantic and Mediterranean hake are highly unlikely to belong to the same genetic stock. The lack of discrimination between the two areas does not necessarily imply that the fish have a common origin.

For discriminating between the different sampling locations for black scabbardfish, the key elements appear to be Sr and Mg. These two elements were significantly negatively correlated ($p=0.009$). Mg concentrations were higher in more northern samples (except Hatton Bank) and also in the Mid-Atlantic Ridge samples. Sr concentrations were highest in Madeira and Portuguese samples. Cu concentrations were higher in samples from Madeira and the Reykjanes Ridge. In general, the Hatton Bank area had the lowest concentrations for all the key elements except Sr and also the least variance in element concentration. It is not clear to what extent the concentrations of trace elements in the water masses might influence the chemical composition of the otolith, or whether diet could be an important factor. It is known that fish accumulate trace elements in muscle, liver, gills, gonads and eye lenses (Willis and Sunda, 1984; Mormede and Davies, 2001) from both the environment and through diet. There is often a correlation between element concentrations in these other organs and those of the otoliths, although this is not always the case (Limburg, 1995; Dove and Kingsford, 1998; Geffen *et al*., 1998; Hanson and Zdanowicz, 1999; Milton *et al*., 2000). Little is known about the trace metal composition of the water masses of northeastern Atlantic oceanic waters and indeed many of the water masses themselves are composed by the mixing of other water masses and may exhibit strong intra- or inter-annual differences in their characteristics. For example, the water masses around the northern Hatton Bank area (in the region of the sample site) are complex and broadly influenced by a northeasterly flow of warmer Atlantic water and an overflow of colder Norwegian Sea water over the Faroe-Iceland Ridge (Hansen and Østerhus, 2000; van Aken and de Boer, 1995). Inter-annual variations in the properties of both these flows have been documented (van Aken and de Boer, 1995). Given the complexity of the hydrography in the Hatton Bank area, it is difficult to ascribe the lower elemental concentrations in the otoliths of fish caught at this location to any hydrographic phenomenon. On a broader North Atlantic scale, long term episodic events such as the North Atlantic Circulation are well-documented (Ellett, 1993). These oscillations can produce phenomena such as the 'Great Salinity Anomaly', which resulted in reduced salinity in the Rockall Trough in the mid 1970s (Dickson *et al*., 1998). It is important to consider long-term changes in the properties of water masses when utilizing the microchemistry of whole otoliths from adult fish of long-lived, deep-water species for stock discrimination, particularly if the otoliths are from historical collections.

The higher concentrations of Sr in the otoliths of the more southerly samples may be a result of the higher salinity Mediterranean Outflow Water. The higher Sr levels in the Hatton Bank samples may be related to the warmer, more saline water that exists in this area (Hansen and Østerhus, 2000; van Aken and de Boer, 1995). The incorporation of Sr into otoliths is known to be salinity and temperature dependent (Secor *et al*., 1995). In a series of experiments carried out to assess the influence of temperature and salinity treatments on the incorporation of Sr in the otoliths of Atlantic croaker, Fowler *et al*. (1995) found that the temperature effect was particularly evident, with higher concentrations being found for high-temperature treatments. High-salinity treatment also gave higher values of Sr, but in contrast, Mg gave higher values in the low-salinity treatment.

Microchemical analysis of otoliths is a useful tool, but needs to be developed in conjunction with other methods used for stock identification, such as genetics, morphometrics and otolith shape analysis. It is likely that a combination of techniques will be required in order to provide enough information on stock structures for fisheries management purposes. This preliminary study was based on the analysis of whole otoliths. If, in accordance with the hypothesis, black scabbardfish have a common origin in the North East Atlantic, it will be necessary to determine the elemental chemical signature in the otolith core. The preparation of otolith cores for solution-based ICP-MS has many potential difficulties and the use of laser-ablation ICP-MS as a means of measuring otolith elemental concentrations could be more effective in this regard.

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TABLE 1. Classification summary for the discriminant analysis and percentage of observations classified by area. The overall correct classification rate was 62%.

Actual group	No. of Samples	Predicted group membership (%)					
		HB	MA	MD	PT	RR	RT
Hatton Bank **HB**	16	**62.50**	12.50	0.00	12.50	6.25	6.25
Mid-Atlantic Ridge **MA**	10	10.00	**70.00**	0.00	10.00	0.00	10.00
Madeira **MD**	18	11.11	0.00	**61.11**	16.66	5.56	5.56
Portugal **PT**	20	5.00	5.00	15.00	**60.00**	10.00	5.00
Reykjanes Ridge **RR**	12	16.67	8.33	0.00	8.33	**58.33**	8.33
Rockall Trough **RT**	20	5.00	10.00	0.00	10.00	15.00	**60.00**

TABLE 2. Cross-validated (un-biased) classification summary for the stepwise discriminant analysis and percentage of observations classified by area. The overall correct classification rate was 45%.

Actual group	No. of Samples	Predicted group membership (%)					
		HB	MA	MD	PT	RR	RT
Hatton Bank **HB**	16	**50.00**	12.50	6.25	18.75	12.50	0.00
Mid-Atlantic Ridge **MA**	10	10.00	**40.00**	10.00	30.00	0.00	10.00
Madeira **MD**	18	11.11	11.11	**55.56**	16.66	0.00	5.56
Portugal **PT**	20	10.00	10.00	20.00	**45.00**	10.00	5.00
Reykjanes Ridge **RR**	12	25.00	0.00	8.33	16.67	**41.67**	8.33
Rockall Trough **RT**	20	5.00	15.00	0.00	10.00	30.00	**40.00**

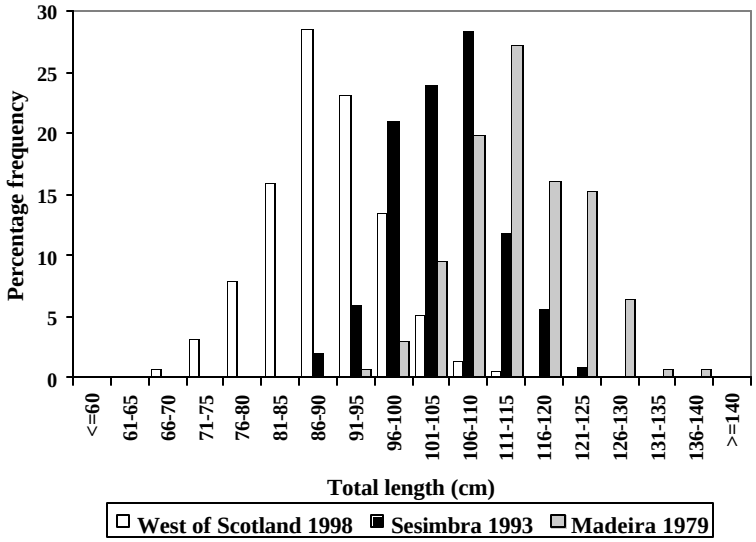

Fig. 1. Percentage length frequencies of black scabbardfish from three areas in the North East Atlantic. Madeira data from Anon. (1982), Sesimbra data from ICES Study Group Report (Anon., 1998), West of Scotland data courtesy of Fisheries Research Services, Aberdeen.

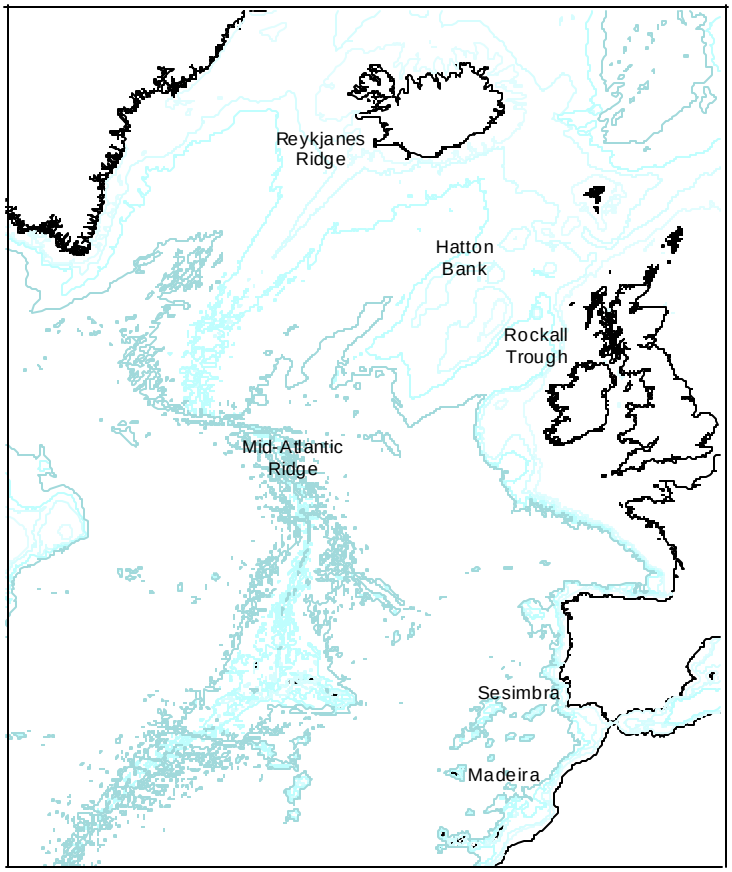

Fig. 2. Location of sites from which black scabbardfish samples were obtained.

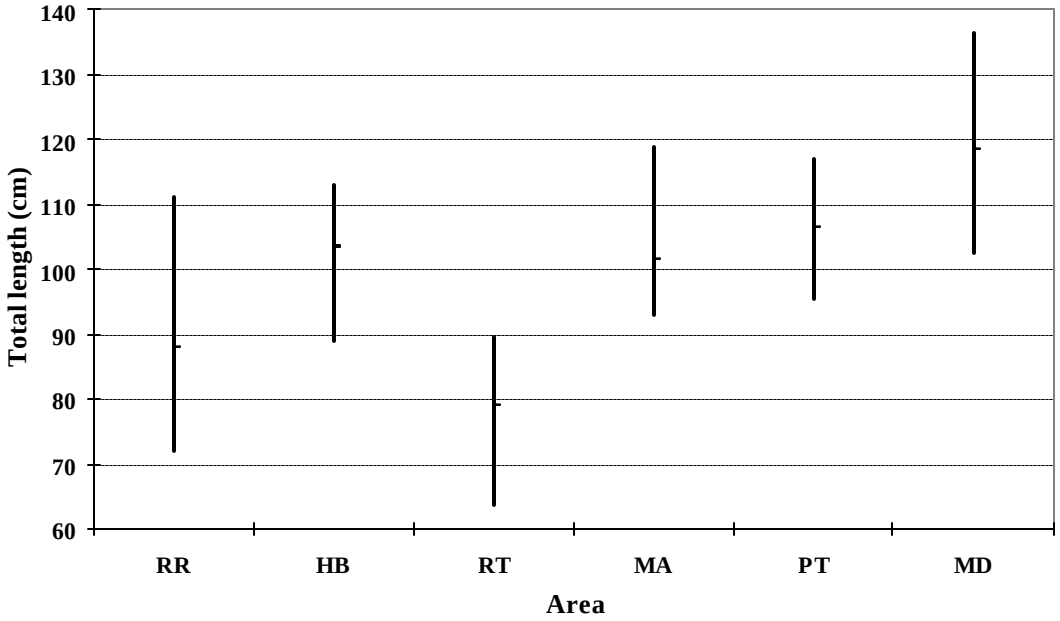

Fig. 3. Length ranges of samples obtained for this study.

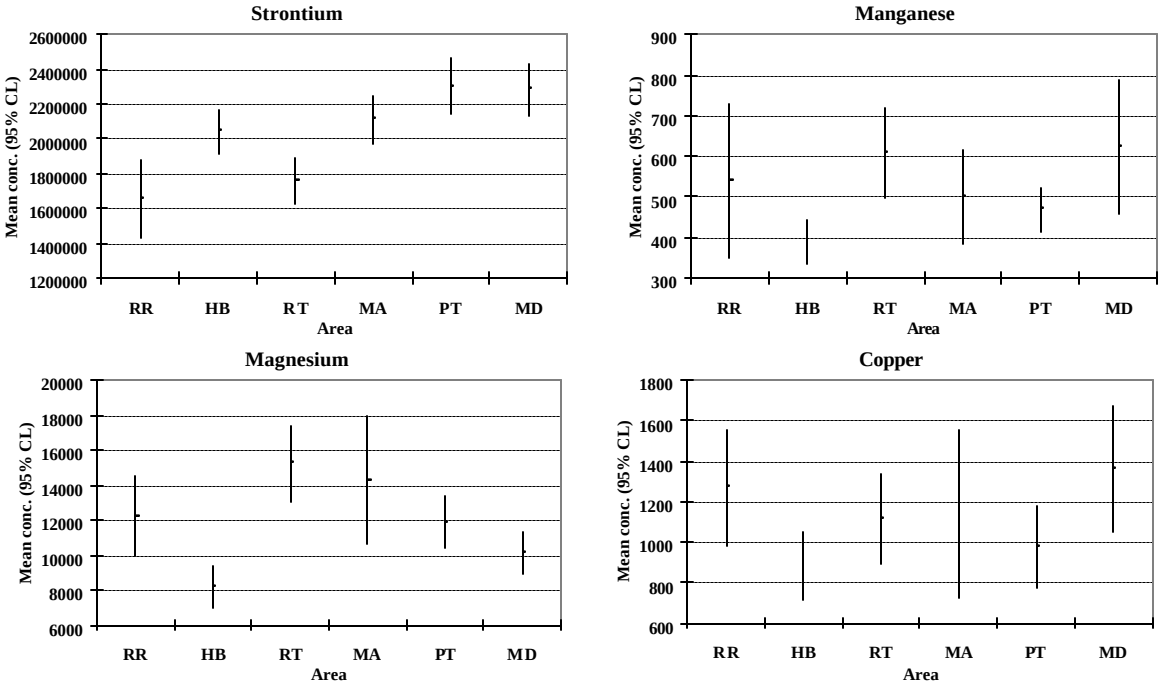

Fig. 4. Variation in mean (+-95% CL) elemental concentration (µg/L) between otolith samples for some key elements.

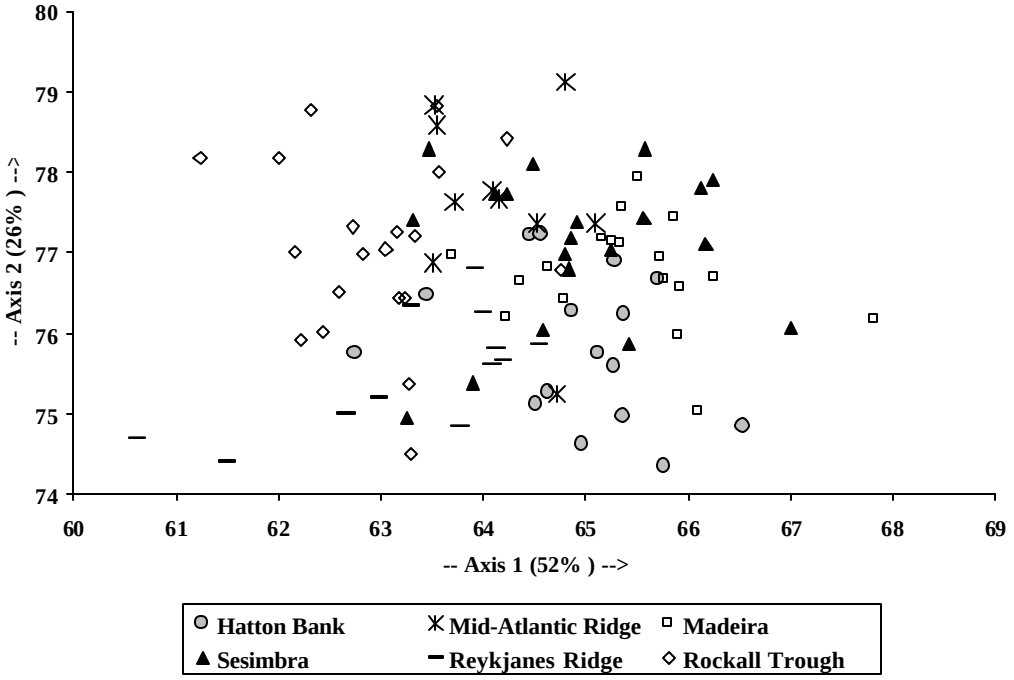

Fig. 5. Discrimination between black scabbardfish otolith samples based on the concentrations of 10 elements.

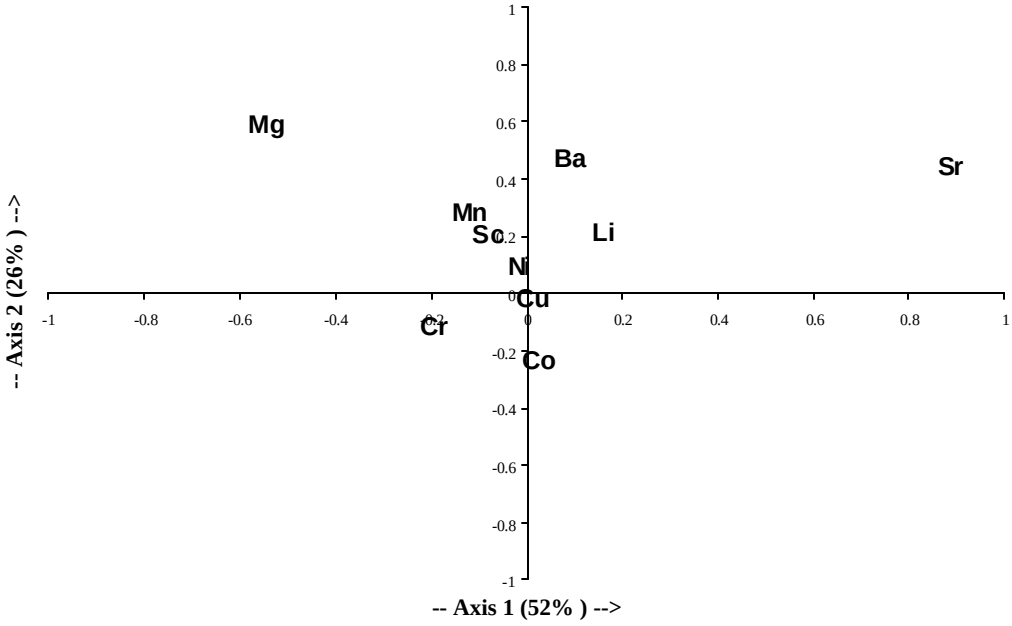

Fig. 6. Elements contributing to distribution pattern for black scabbardfish otoliths.