

Shape analysis and microchemistry of redfish otoliths: investigation of geographical differences in the North Atlantic

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Abstract

As part of an ongoing EU project on redfish, otolith shape analysis and otolith trace element assays were conducted to test for differences between distribution areas. Otolith morphometry and shape (Fourier) descriptors were compared between sampling areas of golden redfish (*Sebastes marinus*) and deep-sea redfish (*Sebastes mentella*) in the North Atlantic. A first series of trace element assays was performed using laser-ablation inductively coupled plasma mass spectrometry (LA-ICPMS) on cross-sections of *S. mentella* otoliths. Geographical separation by these methods appeared to be weak, although some distinction of western, central and eastern areas was apparent for otolith shapes of *S. marinus*. Trace element concentrations in *S. mentella* otoliths differed between three otolith zones (core, 3-year annulus and edge), giving first hints to physiological effects and/or migration. Differences in elemental concentrations between areas showed repeated patterns for some elements, indicating area-specific signatures. Multivariate analysis of these signatures, however, revealed no clear discrimination of distribution areas. Since the otolith shape analysis was limited to material from medium-sized fish (30-40 cm total length) and microchemical assays were only performed on a sub-set of *S. mentella* otoliths, further investigations will be carried out during the next months to reveal more information on possible natural markers for redfish.

Keywords: otolith shape analysis, Fourier analysis; trace element analysis, elemental assays, otolith microchemistry, LA-ICPMS; deep-sea redfish, golden redfish, *Sebastes mentella*, *Sebastes marinus*; North Atlantic

Introduction

Otolith shape analysis

Morphometric measurements of fish are commonly used to investigate phenotypic differences between species (e.g. Power and Ni 1985, Creech 1992) and stocks (Ihssen *et al.* 1981, ICES 1996 and 1999, Murta 2000). In addition to body morphometrics and meristic features, otolith shape analysis has become a popular tool for species and stock identification purposes. In numerous studies, otolith shapes were shown to be species-specific (Hecht & Appelbaum 1982, Gaemers 1984, L'Abée-Lund 1988) and also population-specific (Messieh 1972, McKern *et al.* 1974, Neilson *et al.* 1985). In many cases, geographic variations in otolith shapes could be related to stock differences (Bird *et al.* 1986, Castonguay *et al.* 1991, Campana and Casselman 1993, Friedland and Reddin 1994, Begg and Brown 2000, Turan 2000, Tuset *et al.* 2003).

Since the stock identification for North Atlantic redfish, particularly for *Sebastes mentella*, is still uncertain (e.g. ICES 1998), a multidisciplinary approach to investigate the stock structure of *Sebastes* species was implemented within an EU-funded research project. Apart from genetic studies and the investigation morphometric and meristic characters of the fish body, the otolith shapes of redfish, focusing on *S. mentella*, are analysed with regard to stock-specific differences. A sub-set of 878 otoliths was analysed for univariate and multivariate (Fourier) descriptors to investigate differences in otolith morphometry and shape between and within *S. marinus* and *S. mentella*.

Otolith microchemistry

Several recent studies have reported considerable success in fish stock separation by otolith microchemistry, e.g. Campana & Thorrold 2001, Gillanders 2001, Gillanders *et al.* 2001, Milton & Chenery 2001, Rooker *et al.* 2001, Forrester & Swearer 2002, Gillanders 2002, Sanchez-Jerez *et al.* 2002, Secor *et al.* 2002, Rooker *et al.* 2003. Provided that the elemental composition of the otoliths reflects that of the ambient seawater during a certain period of the fish's life (e.g. Hoff & Fuiman 1995, Campana 1999, Arai 2002), nursery habitats and migrations of the investigated fish can be tracked by otolith multi-element patterns. No elemental assays have been reported for *Sebastes* otoliths to date. This working document presents a preliminary study of otolith microchemistry for *S. mentella* from different sampling locations in the North Atlantic.

Materials and methods

Otolith shape analysis

The otolith outlines were digitised using an image analysis system consisting of a high resolution monochrome CCD video camera, mounted on a microscope and connected to a PC framegrabber card via BNC video cable. The microscope magnification was adjusted to the size of the otoliths to ensure as high resolution as possible, varying between 20x and 40x. The image analysis system was calibrated in horizontal and vertical direction separately to avoid possible distortion effects of the lens system. The otoliths were positioned onto a microscope slide with the sulcus down and the rostrum to the left in horizontal line to minimise distortion errors within the normalisation process. High-contrast video images were produced using transmitted light, delivering dark two-dimensional objects with bright background. The video signal was analysed using Optimas 6.51 (Media Cybernetics 1999) image analysis software. Images of right otoliths were mirrored vertically to allow pooling of right and left otoliths in the shape analysis. Shape digitalisation was performed by sampling 1000 equidistant points on each outline, representing the resolution of the video camera.

For the export of outline coordinates and univariate shape descriptors (otolith length, breadth etc.), Optimas macros were applied. Elliptical Fourier Analysis (EFA) (Kuhl and Giardina 1982, Rohlf and Archie 1984) was performed using C++ modules based on algorithms proposed by Ferson *et al.* 1985. The EFA represents a fitting of harmonic functions to the original otolith outlines with an ellipse as the first approximation step. The algorithm for normalising the rotation and starting angle of the outline was modified to account for deviations from the horizontal axis resulting from the positioning of the otolith on the microscope slide. During the EFA, the size, location and starting point of the object outlines within the two-dimensional space were normalised. Based on graphical representations of the fit of the reproduced outlines with the original shapes, the number of harmonic functions to be applied within EFA was set to 30. The resulting Fourier matrix consists of 120 descriptors (30 harmonics x 4 coefficients), of which 117 were used for multivariate analysis, since the first three descriptors become constants after the normalisation process.

The analysed material was limited to left-side otoliths (to minimise asymmetric variances within the same individual) from fish measuring 30-40cm in total length (to minimise length effects on the descriptors). The geographical coverage of these samples encompasses the entire distribution area of the two species (Table 1).

Otolith microchemistry

In a first attempt to investigate geographical variation in otolith microchemistry of redfish, 55 *S. mentella* otolith cross-sections were analysed by laser-ablation inductively coupled plasma mass spectrometry (LA-ICPMS) in collaboration with the University of Kiel/Germany. These samples, collected in seven wide-spread areas in the North Atlantic (Baffin Bay/Davis Strait to Barents Sea) were analysed in the nucleus region, along the third-year growth zone and along the marginal increments.

Results

Otolith shape analysis

From the 117 Fourier descriptors (4 descriptors * 30 harmonics, minus 3 constant descriptors), 37 (4* the first 10 harmonics, -3) were chosen for multivariate analysis, since they captured 95-99% of the observed variation. Multidimensional Scaling (MDS) of these data, based on Euclidean Distance matrices, revealed no clear separation between species and areas. To test the separation success, a Discriminant Analysis was performed on three different sub-sets of Fourier descriptors: [a] *S. marinus* and *S. mentella* combined, [b] *S. marinus* in different areas, [c] *S. mentella* in different areas. For all three comparisons, the differences between species and areas, respectively, are highly significant ([a] Pillai's trace = 0.510; $F = 23.619$; $df = 37, 840$; $p < 0.0001$. [b] Pillai's trace = 1.481; $F = 2.605$; $df = 148, 656$; $p < 0.0001$. [c] Pillai's trace = 1.479; $F = 3.391$; $df = 333, 5742$; $p < 0.0001$). The jackknifed classification matrix for the species comparison indicated that 90% of the *S. marinus* samples and 87% of the *S. mentella* samples were correctly allocated. The classification success within the *S. marinus* area comparison varied between 33% (West Greenland) and 87% (Barents Sea). The scores plot for the first two discriminant functions shows a distinct separation of the Barents Sea and Flemish Cap samples from the West/East Greenland and Iceland samples (Fig. 1). For *S. mentella*, the geographical discrimination was less pronounced, the classification success ranging from 14% (Davis Strait) to 68% (Barents Sea) and the areas showing more overlap (Fig. 2). Again, the Barents Sea samples are separating best from all other areas.

A set of univariate measurements and ratios was compared between species and areas, some of them providing considerable discriminatory power. The Barents Sea samples, for example, separated from all other areas by exhibiting the lowest a -values of the otolith length to otolith weight relationship for both *S. marinus* and *S. mentella* (Table 2), indicating growth differences between areas. Similar differences were found for the otolith length to otolith width ratio.

Otolith microchemistry

The mean concentration of nine elements, detected via LA-ICPMS with sufficient precision, differed considerably between areas and otolith zones (Fig. 3). In most cases, significant differences between the nucleus and edge zones were found, frequently showing a clear trend of either increasing (Sr, Ba) or decreasing concentrations (Mn, Cu) from the nucleus over the third-year growth zone to the edge. Most pronounced area-effects were observed for Li, Mn, Cu, Zn, Rb and Ba. Considering only the nucleus region, similar patterns can be detected for Mg, Mn and Cu: elemental concentrations elevating from the Baffin Bay (westernmost samples) to Iceland (central) and decreasing to the Barents Sea (easternmost). For a preliminary multivariate analysis, the concentrations of the nine detected elements were scaled to 1 by the maximum concentration of each element and an Euclidean Distances matrix was calculated. Based on this matrix, the dissimilarities between samples were ordinated in a 2D-multidimensional scaling (MDS) procedure. The MDS plot (Fig. 4) indicates weak separation of areas with large overlaps.

Discussion

Otolith shape analysis

While for *S. marinus*, geographical separation based on otolith shapes seems to be possible at least on a large spatial scale, the case for *S. mentella* is much less clear. Different distribution patterns of these species could be a reason for this observation. *S. mentella* is more wide-spread than *S. marinus*, showing a more continuous distribution across the North Atlantic due to pelagic occurrences. Since *S. marinus* only inhabits the shelf areas which are in most cases geographically separated, environmental and genetic differences between areas are likely to be more distinct than for *S. mentella*. A differentiation of the easternmost (Barents Sea) and westernmost area (Flemish Cap) from the central areas was observed for *S. marinus*. Recent genetic studies (Roques *et al.* 2002) found a similar pattern for *S. mentella*, describing population units in an eastern, western and so-called panoeceanic zone (Faroe Islands to the Grand Banks).

Additional univariate and multivariate analyses of the geographical variation in *S. marinus* and *S. mentella* otolith morphometry and shape will be carried out on sub-sets of other fish length groups and on a wider range of material with appropriate normalisation and size correction measures.

Otolith microchemistry

Only few studies on otolith microchemistry of North Atlantic fish have been carried out so far. The overall mean concentrations for the detected elements, however, are similar to values reported for cod (Campana & Gagné 1995) and bluefin tuna (Secor *et al.* 2002) in the Northwest Atlantic and deep-water black scabbardfish in the Northeast Atlantic (Swan *et al.* 2001). Whether the marked differences in some element concentrations of nucleus and edge zones are due to age-effects (accumulation or reduction of elements) or migratory patterns, has to be studied in a refined consideration of the physiological role of certain elements, pathways and assimilation from food sources (*e.g.* Kalish 1991, Olsson *et al.* 1998, Campana 1999). Further investigations into the separation power of sub-sets of elements and additional multivariate approaches, including elemental compositions of the third-year growth zone and edge, will be carried out in connection with subsequent LA-ICPMS analyses of *S. marinus* otoliths and samples from juvenile redfish.

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References

- Arai, T., Ikemoto, T., Kunito, T., Tanabe, S., Miyazaki, N. (2002) Otolith microchemistry of the conger eel, *Conger myriaster*. *J.Mar.Biol.Assoc.U.K.*, 82, 303-305.
- Begg, G.A. and Brown, R.W. (2000): Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. *Trans.Am.Fish.Soc.*, 129:935-945.
- Bird, J.L., Eppler, D.T. and Checkley, D.M. (1986): Comparisons of herring otoliths using Fourier-series shape-analysis. *Can.J.Fish.Aquat.Sci.*, 43:1228-1234.
- Campana, S.E. and Casselman, J.M. (1993): Stock discrimination using otolith shape-analysis. *Can.J.Fish.Aquat.Sci.*, 50:1062-1083.
- Campana, S.E. (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar.Ecol.Prog.Ser.*, 188, 263-297.
- Campana, S.E., Gagné, J.A. (1995) Cod stock discrimination using ICPMS elemental assays of otoliths. In: *Recent developments in fish otolith research.*, 671-691, University of South Carolina Press, Columbia.
- Campana, S.E., Thorrold, S.R. (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can.J.Fish.Aquat.Sci.*, 58, 30-38.
- Castonguay, M., Simard, P. and Gagnon, P. (1991): Usefulness of Fourier analysis of otolith shape for atlantic mackerel (*Scomber scombrus*) stock discrimination. *Can.J.Fish.Aquat.Sci.*, 48:296-302.
- Creech, S. (1992): A multivariate morphometric investigation of *Atherina boyeri* Risso, 1810 and *A. presbyter* Cuvier, 1829 (Teleostei, Atherinidae): Morphometric evidence in support of the two species. *J.Fish Biol.* 41:341-353.
- Ferson, S., Rohlf, F.J. and Koehn, R.K. (1985): Measuring shape variation of two-dimensional outlines. *Syst.Zool.*, 34:59-68.
- Forrester, G.E., Swearer, S.E. (2002) Trace elements in otoliths indicate the use of open-coast versus bay nursery habitats by juvenile California halibut. *Mar.Ecol.Prog.Ser.*, 241, 201-213.
- Friedland, K.D. and Reddin, D.G. (1994): Use of otolith morphology in stock discriminations of Atlantic salmon (*Salmo salar*) . *Can.J.Fish.Aquat.Sci.*, 51:91-98.
- Gaemers, P.A.M. (1984): Taxonomic position of the Cichlidae (Pisces, Perciformes) as demonstrated by the morphology of their otoliths. *Neth.J.Zool.*, 34:566-595.
- Gillanders, B.M. (2001) Trace metals in four structures of fish and their use for estimates of stock structure. *Fish.Bull.*, 99, 410-419.
- Gillanders, B.M. (2002) Temporal and spatial variability in elemental composition of otoliths: implications for determining stock identity and connectivity of populations. *Can.J.Fish.Aquat.Sci.*, 59, 669-679.
- Gillanders, B.M., Sanchez-Jerez, P., Bayle-Sempere, J., Ramos-Espla, A. (2001) Trace elements in otoliths of the two-banded bream from a coastal region in the south-west Mediterranean: are there differences among locations? *J.Fish Biol.*, 59, 350-363.
- Hecht, T. and Appelbaum, S. (1982): Morphology and taxonomic significance of the otoliths of some bathypelagic Anguilloidei and Saccopharyngoidei from the Sargasso Sea. *Helgoländer Meeresunters.*, 35:301-308.
- Hoff, G.R., Fuiman, L.A. (1995) Environmentally induced variation in elemental composition of red drum (*Sciaenops ocellatus*) otoliths. *Bull.Mar.Sci.*, 56, 578-591.
- ICES (1996): Report of the Study Group on Stock Identification Protocols for Finfish and Shellfish Stocks. *ICES C.M.*, 1996/M:1, Ref.: G,H,J,K, Assess: 140 pp.

- ICES (1998): Report of the Study Group on Redfish Stocks. *ICES C.M.*, 1998/G:3, Ref. H:30 pp.
- ICES (1999): Report of the Stock Identification Methods Working Group. *ICES C.M.*, G:1:14 pp.
- Ihssen, P.E., Booke, H.E., Casselman, J.M., McGlade, J.M., Payne, N.R. and Utter, F.M. (1981): Stock identification: materials and methods. *Can.J.Fish.Aquat.Sci.*, 38:1838-1855.
- Kalish, J.M. (1991) Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. *Mar.Ecol.Prog.Ser.*, 74, 137-159.
- Kuhl, F.P. and Giardina, C.R. (1983): Elliptic Fourier features of a closed contour. *Comp.Graph.Image Process.*, 18:236-258.
- L'Abée-Lund, J.H.L. (1988): Otolith shape discriminates between juvenile Atlantic salmon, *Salmo salar* L., and brown trout, *Salmo trutta* L. *J.Fish Biol.*, 33:899-903.
- McKern, J.L., Horton, H.F. and Koski, K.V. (1974): Development of steelhead trout (*Salmo gairdneri*) otoliths and their use for age analysis and for separating summer from winter races and wild from hatchery stocks. *J.Fish.Res.Board Can.*, 31:1420-1426.
- Media Cybernetics (1999): *Optimas 6.5 User guide and technical reference*. 9th ed., Media Cybernetics, Silver Spring, MD, 548 pp. + 717 pp.
- Messieh, S.N. (1972): Use of otoliths in identifying herring stocks in the Southern Gulf of St. Lawrence and adjacent waters. *J.Fish.Res.Board Can.*, 29:1113-1118.
- Milton, D.A., Chenery, S.R. (2001) Can otolith chemistry detect the population structure of the shad hilsa *Tenualosa ilisha* ? Comparison with the results of genetic and morphological studies. *Mar.Ecol.Prog.Ser.*, 222, 239-251.
- Murta, A.G. (2000): Morphological variation of horse mackerel (*Trachurus trachurus*) in the Iberian and North African Atlantic: implications for stock identification. *ICES J.Mar.Sci.*, 57:1240-1248.
- Neilson, J.D., Geen, G.H. and Chan, B. (1985): Variability in dimensions of salmonid otolith nuclei: implications for stock identification and microstructure interpretation. *Fish.Bull.*, 83:81-89.
- Olsson, P.E., Kling, P., Hogstrand, C. (1998) Mechanisms of heavy metal accumulation and toxicity in fish. In: Metal metabolism in aquatic environments. Langston, W.J. and Bebianno, M.J., eds., Chapman & Hall, London, pp. 321-350.
- Power, D.J. and Ni, I.-H. (1985): Morphometric differences between golden redfish (*Sebastes marinus*) and beaked redfishes (*S. mentella* and *S. fasciatus*). *J.Northw.Atl.Fish.Sci.*, 6:1-7.
- Rohlf, F.J. and Archie, J.W. (1984): A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Syst.Zool.*, 33:302-317.
- Rooker, J.R., Secor, D.H., Zdanowicz, V.S., de Metrio, G., Relini, L.O. (2003) Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. *Fish.Oceanogr.* 12(2):75-84.
- Rooker, J.R., Secor, D.H., Zdanowicz, V.S., Itoh, T. (2001) Discrimination of northern bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry. *Mar.Ecol.Prog.Ser.*, 218, 275-282.
- Roques, S., Sévigny, J.-M., Bernatchez, L. (2002) Genetic structure of deep-water redfish, *Sebastes mentella*, populations across the North Atlantic. *Mar.Biol.*, 140, 297-307.
- Sanchez-Jerez, P., Gillanders, B.M., Kingsford, M.J. (2002) Spatial variability of trace elements in fish otoliths: comparison with dietary items and habitat constituents in seagrass meadows. *J.Fish Biol.*, 61, 801-821.
- Secor, D.H., Campana, S.E., Zdanowicz, V.S., Lam, J.W.H., Yang, L., Rooker, J.R. (2002) Inter-laboratory comparison of Atlantic and Mediterranean bluefin tuna otolith microconstituents. *ICES J.Mar.Sci.*, 59, 1294-1304.

Swan, S.C., Gordon, J.D.M., Shimmield, T. (2001) Preliminary investigations on the uses of otolith microchemistry for stock discrimination of the deep-water black scabbardfish (*Aphanopus carbo*) in the North East Atlantic. *NAFO Sci. Counc. Res. Doc.*, 01/94, 12 pp.

Turan, C. (2000): Otolith shape and meristic analysis of herring (*Clupea harengus*) in the North-East Atlantic. *Arch. Fish. Mar. Res.*, 48:213-225.

Tuset, V.M., Lozano, I.J., González, J.A., Pertusa, J.F., García-Díaz, M.M. (2003) Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). *J. Appl. Ichthyol.*, 19, 88-93.

Table 1. Redfish otolith samples (from fish measuring 30-40 cm in total length) used in preliminary study on geographical variation of otolith shapes

Area	Area code	Number of samples	
		<i>S. marinus</i>	<i>S. mentella</i>
Davis Strait	1		14
Labrador Sea	8		2
Flemish Cap	5	18	13
West Greenland	10	33	13
East Greenland	3	48	73
Irminger Sea	6		318
Iceland	7	88	106
Faroe Islands	4		51
Spitzbergen	9		61
Barents Sea	2	15	25
Total		202	676

Table 2. Otolith length (mm) to otolith weight (mm) relationship for *S. marinus* (n=407) and *S. mentella* (n=1648) from different areas. a and b values of the exponential regressions ($y = a * e^{bx}$).

Area	<i>S. marinus</i>		<i>S. mentella</i>	
	a	b	a	b
Davis Strait			8.764	0.251
Labrador Sea			13.433	0.215
Flemish Cap	10.021	0.223	9.926	0.234
West Greenland	16.885	0.180	17.593	0.186
East Greenland	11.934	0.204	14.233	0.200
Irminger Sea			19.726*	0.184*
Iceland	15.605	0.185	20.197	0.178
Faroe Islands			31.944*	0.151*
Spitzbergen			21.178	0.180
Barents Sea	7.378	0.259	7.168	0.267
All	11.625	0.208	13.686	0.206

* juvenile fish (TL <20cm) missing from regression

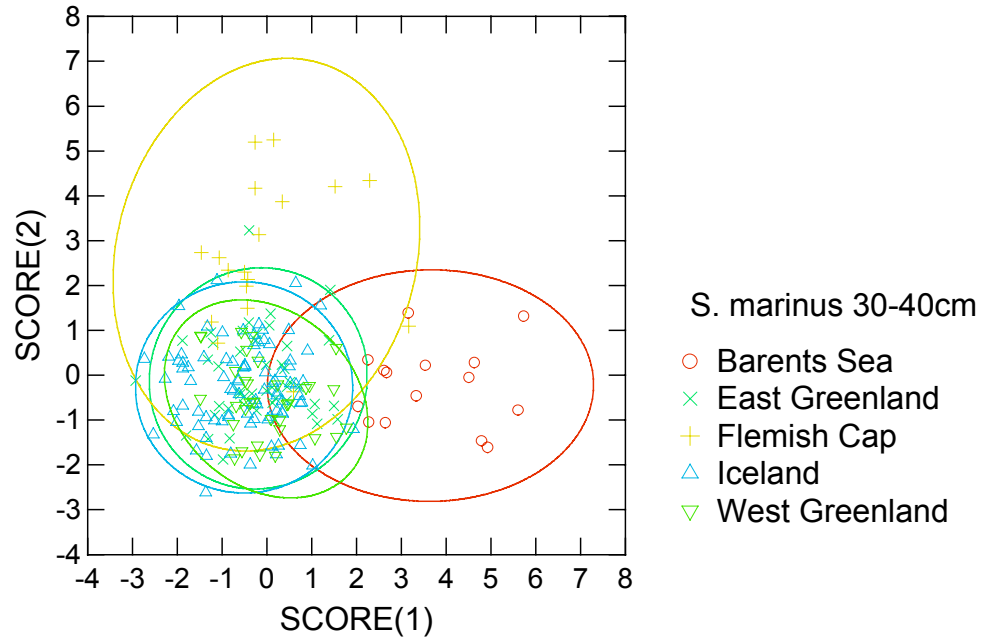


Fig. 1. Discriminant analysis function scores for *S. marinus* otolith samples from different areas in the North Atlantic, based on Fourier descriptors of the first 10 harmonics. The first two discriminant axes capture 72.2% of the observed variation. The 95% confidence ellipses are centred around the sample centroids.

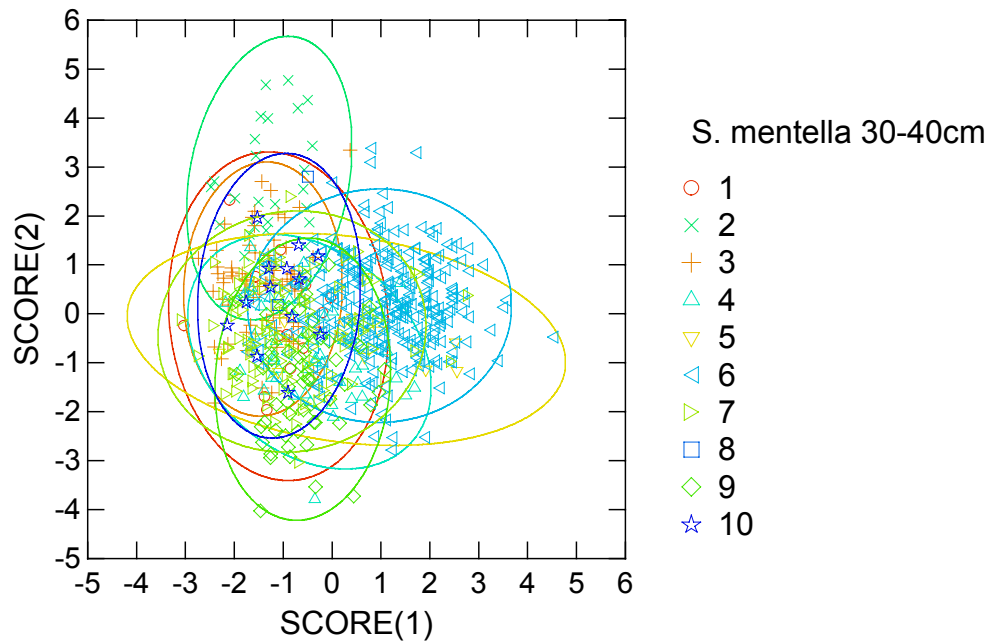


Fig. 2. Discriminant analysis function scores for *S. mentella* otolith samples from different areas in the North Atlantic, based on Fourier descriptors of the first 10 harmonics. The first two discriminant axes capture 67.7% of the observed variation. The 95% confidence ellipses are centred around the sample centroids. For area codes, see Table 2.

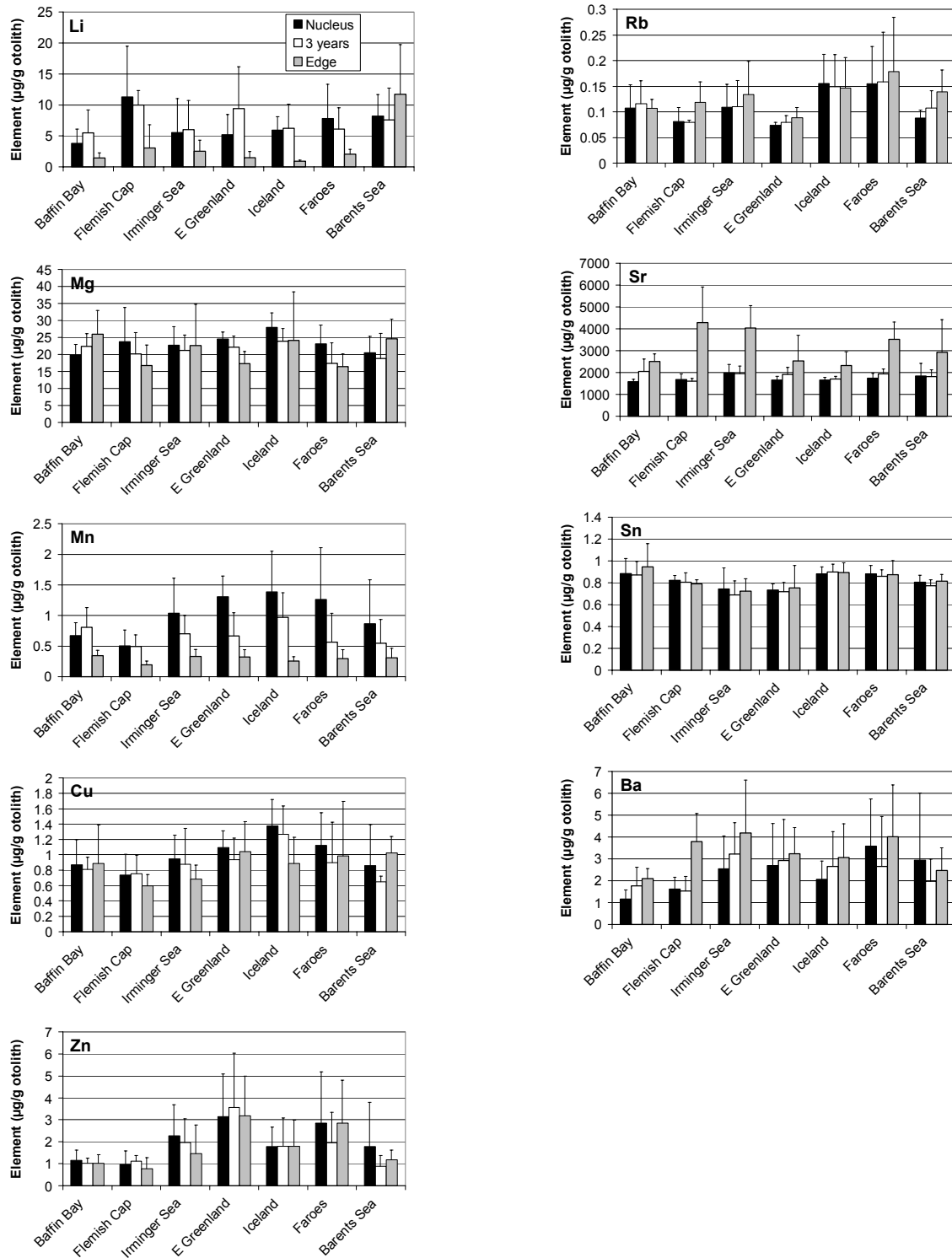


Fig. 3. Mean concentrations (+ standard deviation) of 9 elements determined in *S. mentella* otoliths from 7 different regions in the North Atlantic.

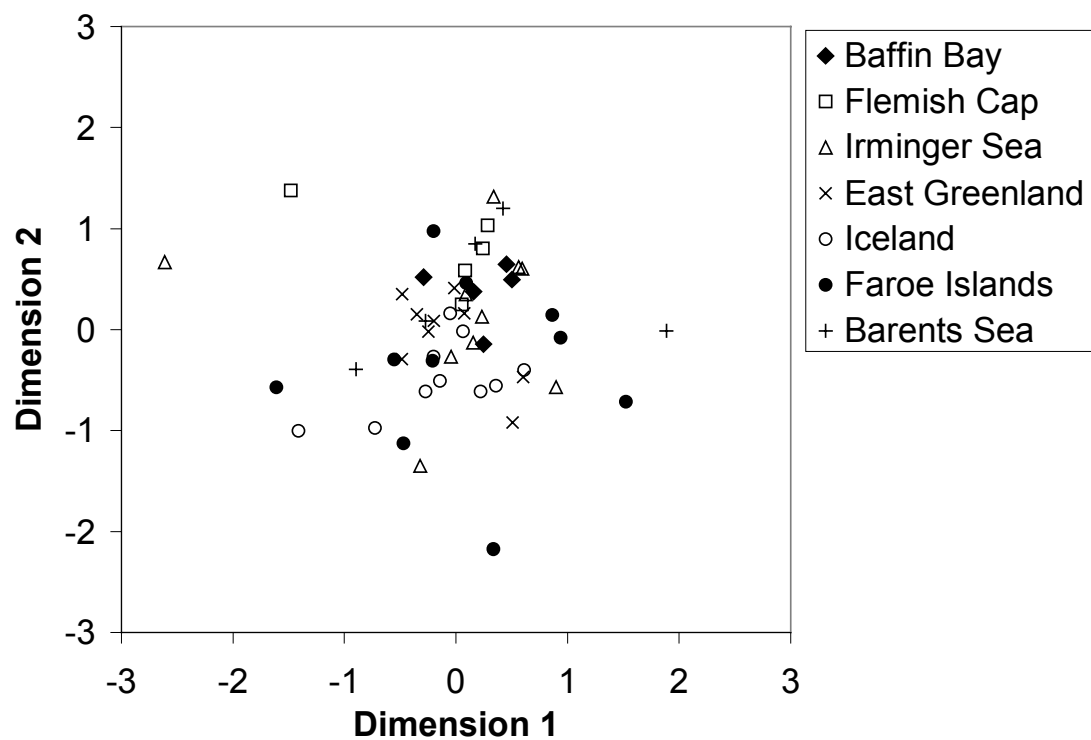


Fig. 4. MDS plot of 55 *S. mentella* otoliths, based on an Euclidean distances matrix of standardised concentrations of 9 elements (see Fig. 3) determined in the nucleus region.