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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 and 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 and Thorrold, 2001; Gillanders, 2001; Gillanders *et al.*, 2001; Milton and Chenery, 2001; Rooker *et al.*, 2001; Forrester and 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 and 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 30-40 cm 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 and 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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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 <20 cm) missing from regression

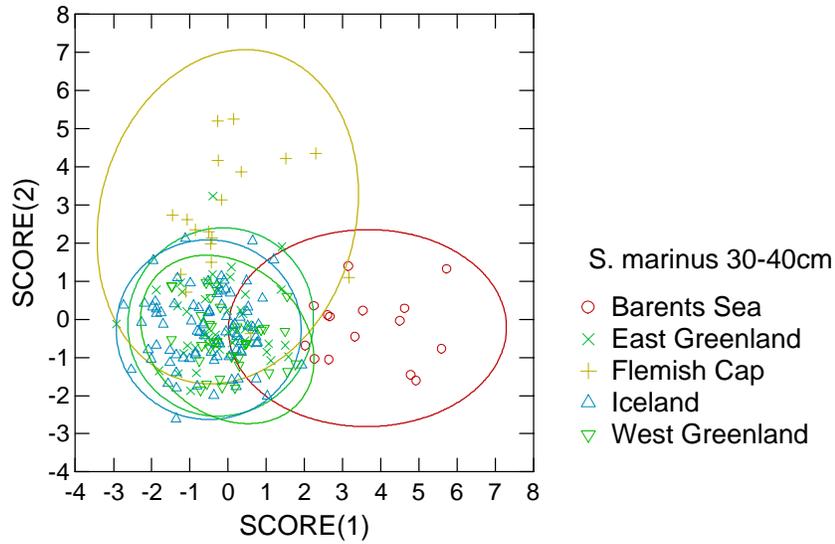


Fig. 1. Discriminant analysis function scores for *S. marinus* otolith samples from different areas in the North Atlantic, based on Fourier descriptors of the first 10 harmonics. The first two discriminant axes capture 72.2% of the observed variation. The 95% confidence ellipses are centred around the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

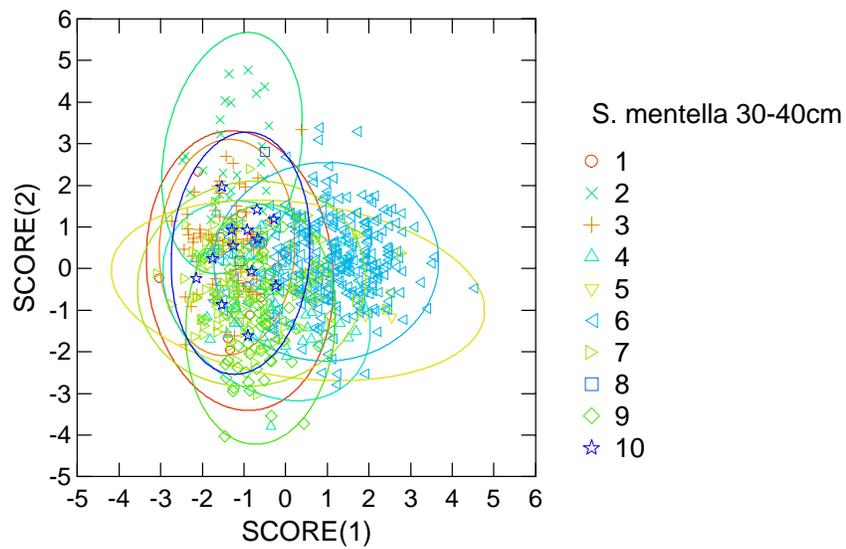


Fig. 2. Discriminant analysis function scores for *S. mentella* otolith samples from different areas in the North Atlantic, based on Fourier descriptors of the first 10 harmonics. The first two discriminant axes capture 67.7% of the observed variation. The 95% confidence ellipses are centred around the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

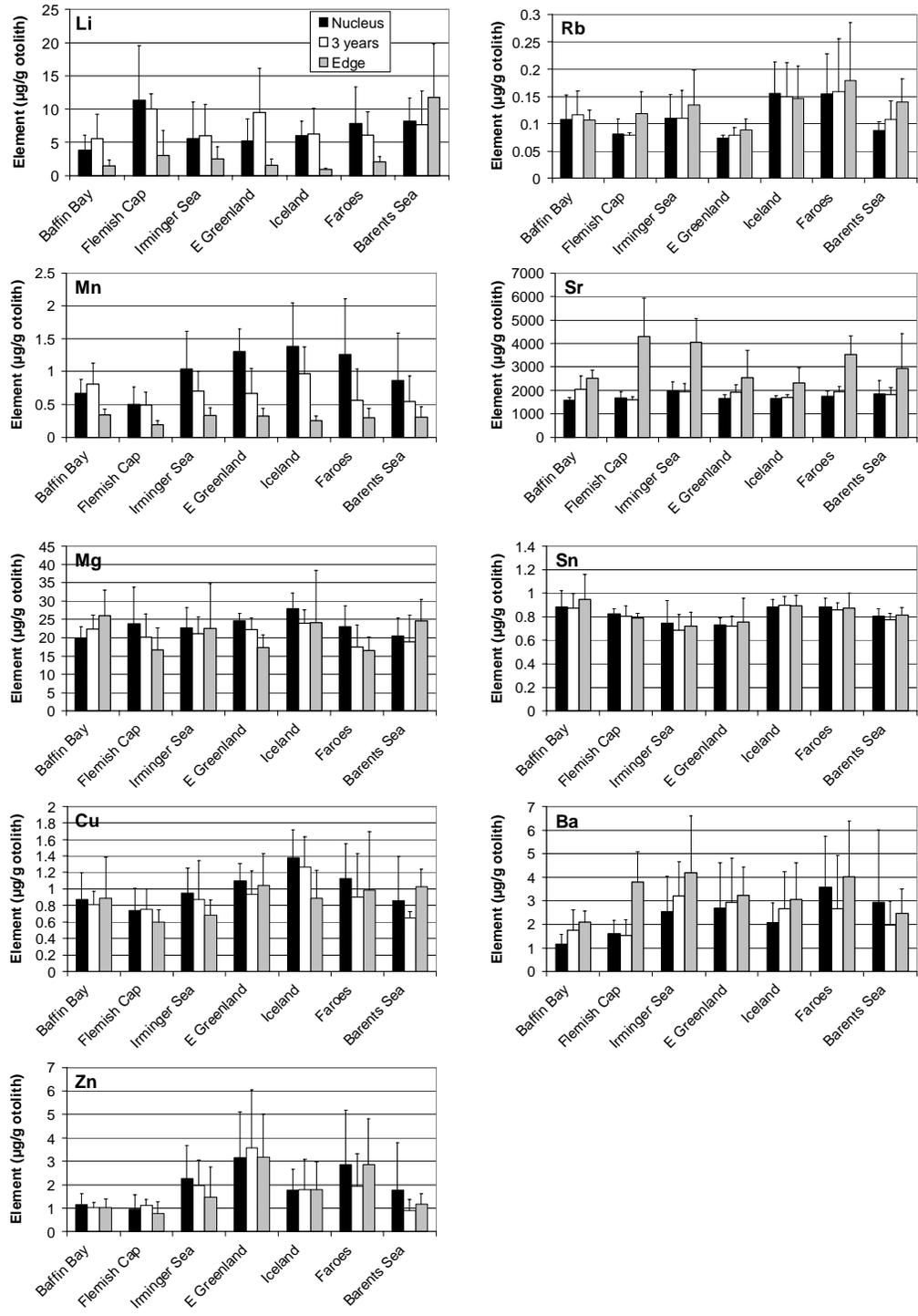


Fig. 3. Mean concentrations (+ standard deviation) of 9 elements determined in *S. mentella* otoliths from 7 different regions in the North Atlantic.

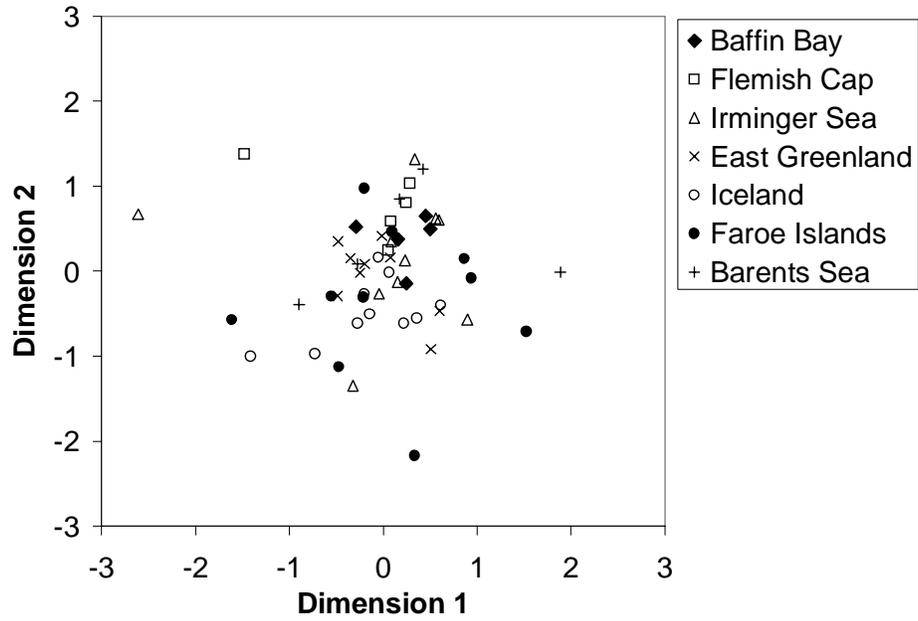


Fig. 4. MDS plot of 55 *S. mentella* otoliths, based on an Euclidean distances matrix of standardised concentrations of 9 elements (see Fig. 3) determined in the nucleus region.