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# Preliminary Results of a Shape Analysis of Redfish Otoliths: Comparison of Areas and Species

by

Christoph Stransky

Federal Research Centre for Fisheries, Institute for Sea Fisheries
Palmaille 9, D-22767 Hamburg, Germany
stransky.ish@bfa-fisch.de

## Abstract

This paper represents a preliminary otolith shape analysis of North Atlantic redfish, comparing sampling areas and species. Differences in univariate morphometric descriptors such as otolith length, breadth and weight could not be observed. Elliptical Fourier Analysis (EFA) was applied to describe digitised otolith outlines. The resulting Fourier descriptors were used as the basis for multivariate analysis to investigate dissimilarities in otolith shapes between samples. While the comparison of *Sebastes mentella* otolith shapes did not reveal any differences between sampling areas, the comparison of *S. mentella, S. marinus* and *S. viviparus* resulted in a considerable grouping of samples by species. The clearest separation was observed between *S. mentella* and *S. viviparus*. An inverse EFA of the mean Fourier shape descriptors within species illustrated this interspecific variation in otolith shapes, identifying the rostrum area as contributing most to the observed shape differences. Reproduced outlines of *S. mentella* otoliths, averaged over sampling areas, did not show any differences between supposed stock units. Effects of different length and age groups on otolith shapes have not been considered and might be confounding the observed shape variation. Ongoing shape analyses of *Sebastes* otoliths will first focus on the comparison of all available species, possibly including Pacific rockfish, before studying between-stock variation in more detail.

**Keywords**: Otolith shape analysis, Fourier analysis; redfish, *Sebastes mentella*, *Sebastes marinus*, *Sebastes viviparus*; North Atlantic

## Introduction

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).

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 in the research project "Population structure, reproductive strategies and demography of redfish (Genus *Sebastes*) in the Irminger Sea and adjacent waters (ICES V, XII and XIV; NAFO 1)", funded by the European Union (QLK5-

CT1999-01222). 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.

This paper represents a preliminary shape analysis of *S. mentella* otoliths, collected in three different sampling areas in the North Atlantic, and otoliths from three redfish species, namely *S. mentella*, *S. marinus* and *S. viviparus*, collected in the Barents Sea. The comparison of sampling areas, presented in the first part of this study, was focusing on a certain length group of *S. mentella* (26-29 cm), since there are indications that a part of these fish were migrating from the East Greenland shelf area into the Irminger Sea in 1998/1999 (Stransky 2000). The species comparison in the second part of this study was implemented for an estimation of interspecific variation in otolith shapes of the genus *Sebastes*, as observed in genetic studies (*e.g*. Nedreaas *et al*. 1994, Rocha-Olivares 1999).

## Materials and Methods

### *Data sets*

*S. mentella* otoliths, sampled on the East Greenland shelf, in the Irminger Sea and in the Barents Sea, were chosen for a comparison of redfish otolith shapes between sampling areas (Table 1). Otoliths of *S. mentella*, *S. marinus* and *S. viviparus* were used for a species comparison within the Barents Sea/Northern Norwegian Sea sampling area (Table 1).

### *Image and 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 graphical representation of average otolith shapes, characterising groups of samples (areas, species), was based on the reproduced outlines for the mean Fourier descriptors of each group.

### *Multivariate analysis of Fourier descriptors*

Dissimilarities between otolith samples were analysed by calculating Euclidean distances for the Fourier descriptor matrix and ordination to a two-dimensional Multidimensional Scaling (MDS) plot (Kruskal and Wish 1978).

*Otolith weights*

After removal of adhering tissue and blood remains from the otolith surface and a minimum of one day air drying at room temperature, otolith weights were recorded with a precision of 0.1 mg.

## Results

*Univariate measurements*

For a first investigation of morphometric differences between *S. mentella* otoliths from three different sampling areas in the North Atlantic, the relationship between otolith length and otolith breadth (Figure 1a) was plotted. No considerable differences between areas could be detected, apart from a shift in otolith lengths to the larger sizes for the Irminger Sea samples due to a close relation with fish length. Otolith weight can be regarded as a descriptor of three-dimensional growth (differences). The relationship of otolith length to otolith weight (Figure 1b), however, also provides no clear differences between areas.

The length to breadth and length to weight relationship for the species comparison (Figure 2) indicates no major differences between *S. mentella*, *S. marinus* and *S. viviparus* otoliths, sampled in the Barents Sea. The largest otolith length and weight range was found in *S. viviparus* otoliths, collected from fish covering almost all occurring body length classes.

*Shape analysis*

Classification results of dissimilarites (Euclidean distances) between individual otoliths, based on the Fourier descriptor matrix, were ordinated through Multidimensional Scaling (MDS). The comparison of *S. mentella* otolith shapes between sampling areas (Figure 3) showed no grouping of samples, whereas the species comparison (Figure 4) revealed some separation of species groups with large overlaps between these 'clusters'. The largest differences in otolith shapes were observed between samples of *S. mentella* and *S. viviparus*.

The (dis)similarities between areas and species, observed in the MDS ordination plots, are supported by the average shapes calculated by inverse EFA of the mean Fourier descriptors within groups. Figure 5 shows no major differences in the overall shape between *S. mentella* otoliths from the three sampling areas, although the Irminger Sea samples appeared to have a slightly smaller breadth than the East Greenland and Barents Sea samples. The average shapes of the otolith outlines drawn in Figure 6, however, show considerable differences between species. The reproduced outlines identify the rostrum area as being subject to highest variability and thus contributing most to the observed differences between *S. mentella*, *S. marinus* and *S. viviparus*.

## Discussion

The observed similarity in shapes of *S. mentella* otoliths from different sampling areas suggests that stock-specific differences, found in some genetic markers (Johansen 2000), do not exist or are not expressed in otolith shapes. While we could expect a linkage between the occurrences of *S. mentella* on the East Greenland shelf and the 'oceanic' *S. mentella* in the Irminger Sea (Stransky 2000), the analysed Barents Sea samples do not form a separate group, as one would anticipate considering geographic separation. Clear phenotypic differences in body morphometry of *S. mentella* in the Northeast Arctic, however, were described by Saborido-Rey and Nedreaas (2000).

A part of the observed otolith shape variation and inferring separation between *S. mentella*, *S. marinus* and *S. viviparus* is due to different fish length ranges chosen for this preliminary study. The otoliths of *S. mentella* were taken from 26-29 cm fish, while the *S. marinus* and *S. viviparus* otoliths were collected from fish with a wider length (and possibly age) range (Table 1). Growth-related differences in otolith shapes were described for several species (*e.g.* eel: Doering and Ludwig 1990, Baltic sprat: Aps *et al.* 1988 and 1989, herring: Messieh 1975), generally induced by an increasing complexity of the shape with increasing age.

Since this preliminary study revealed no obvious stock-specific differences in shapes of *S. mentella* otoliths, ongoing investigations on redfish otolith shapes will first focus on a species comparison of all four North Atlantic *Sebastes* species, adding otolith samples of *S. fasciatus*, before investigating stock differences in further detail (*e.g.* for a wider range of length/age groups). If available, material from the South Atlantic species *S. capensis* as well as Pacific rockfish such as *S. alutus*, *S. entomelas* and *S. flavidus* will be included into the species comparison to evaluate overall variability in otolith shapes within the genus *Sebastes*. To account for confounding effects of different age groups on the interpretation of otolith shape differences, age readings of the analysed otolith samples will be performed and included in the statistical analysis of the shape data.

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Table 1: Otolith samples used for shape analysis, comparing redfish sampling areas and species.

| Species | Area (ICES Sub-area, NAFO Div.) | Vessel (Nation) | Gear (Type) | Fishing depth | Date | No. of samples | Length range | Comparison (A=areas, S=species) |
|---|---|---|---|---|---|---|---|---|
| *S. mentella* | East Greenland (XIVb) | Walther Herwig III (GER) | demersal (140 BT) | 360-380 m | October 1998 | 58 | 26-28 cm | A |
| *S. mentella* | Irminger Sea (XII, XIVb, NAFO 1F) | Walther Herwig III (GER) | pelagic (Gloria) | 200-650 m | June/July 1999 | 108 | 27-29 cm | A |
| *S. mentella* | Barents Sea (I, IIa) | G.O. Sars (NOR) | demersal (Campelen) | 250-370 m | February 2000 | 48 | 26-29 cm | A, S |
| *S. marinus* | Barents Sea (IIa) | M. Sars (NOR) | demersal (Campelen) | 130-400 m | July/August 2000 | 57 | 18-36 cm | S |
| *S. viviparus* | Barents Sea (IIa) | M. Sars (NOR) | demersal (Campelen) | 150-360 m | July/August 2000 | 73 | 10-25 cm | S |

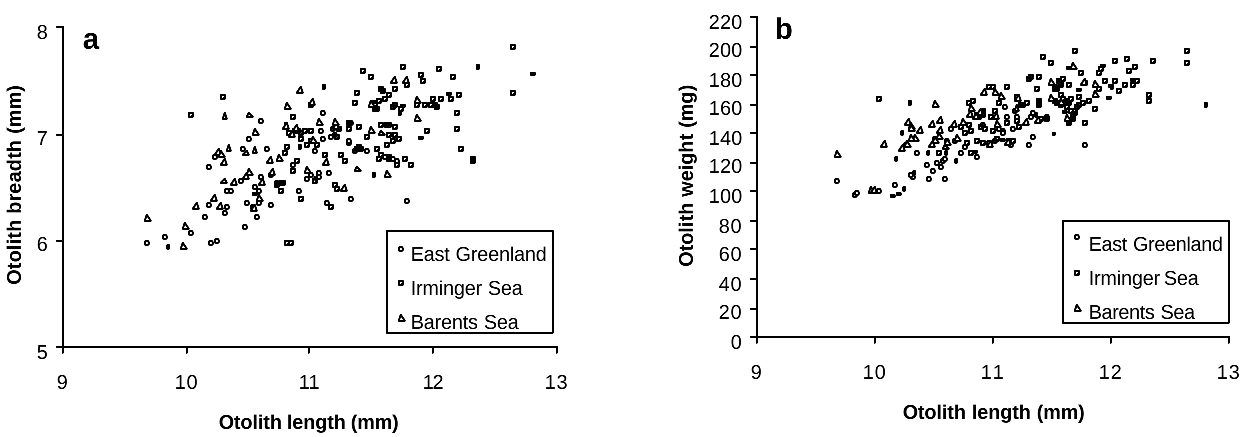


Fig. 1: Otolith length against otolith breadth (a) and otolith length against otolith weight (b) for *S. mentella* otoliths, collected in three areas in the North Atlantic.

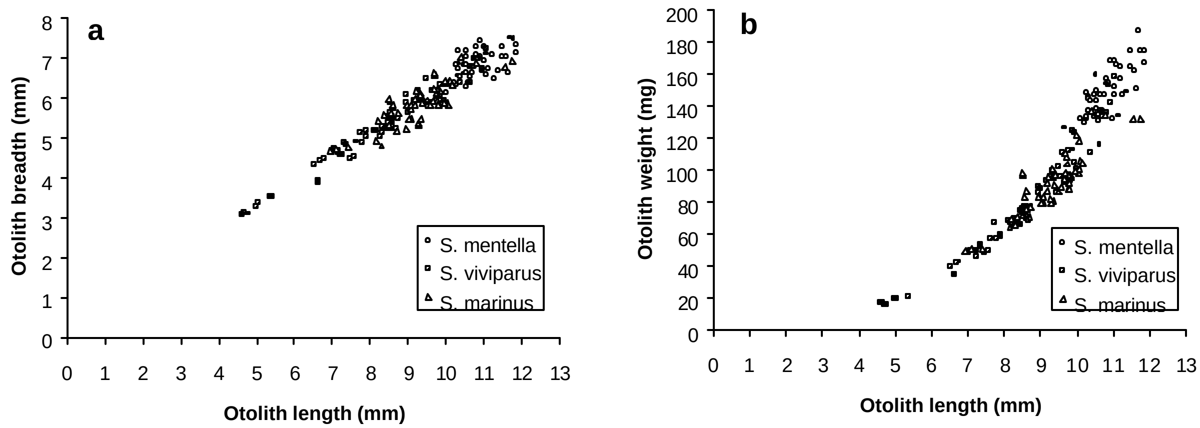


Fig. 2: Otolith length against otolith breadth (a) and otolith length against otolith weight (b) for *S. mentella*, *S. viviparus* and *S. marinus* otoliths, collected in the Barents Sea.

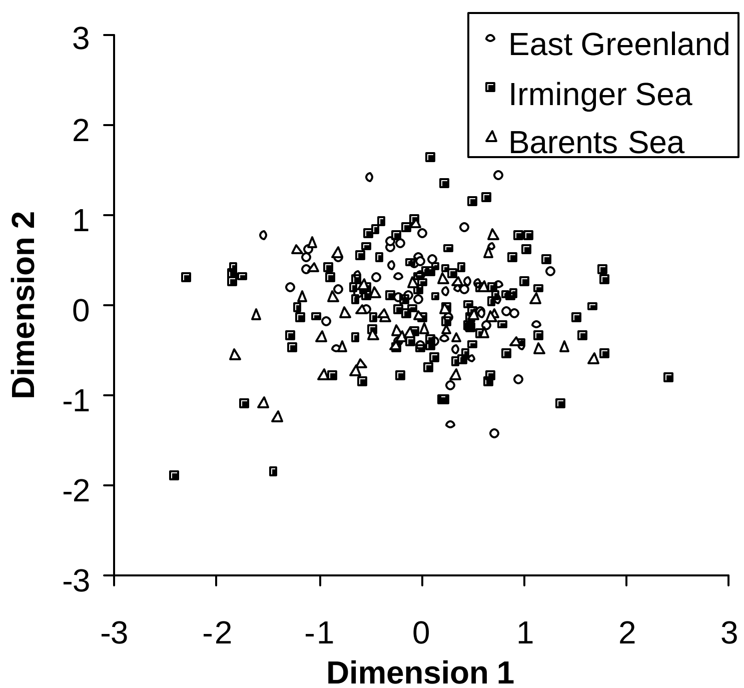


Fig. 3: Multidimensional Scaling (MDS) plot of the Euclidean distances between otolith shape (Fourier) descriptors for *S. mentella* otoliths, collected in three areas in the North Atlantic.

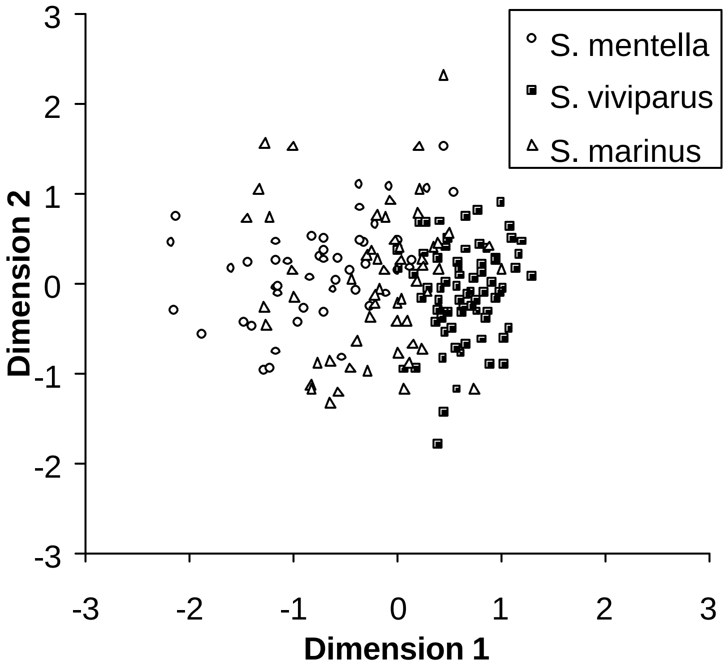


Fig. 4: Multidimensional Scaling (MDS) plot of the Euclidean distances between otolith shape (Fourier) descriptors for *S. mentella, S. viviparus* and *S. marinus* otoliths, collected in the Barents Sea.

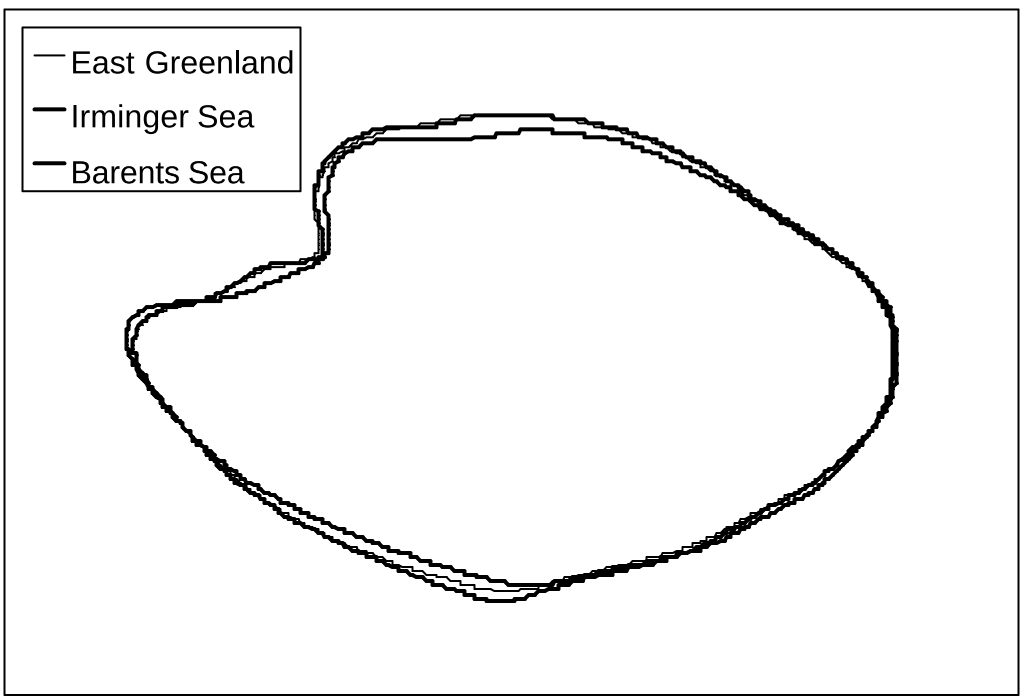


Fig. 5: Average shapes of *S. mentella* otoliths, collected in three areas in the North Atlantic. Otolith outlines were normalised for size, rotation, location and starting position.

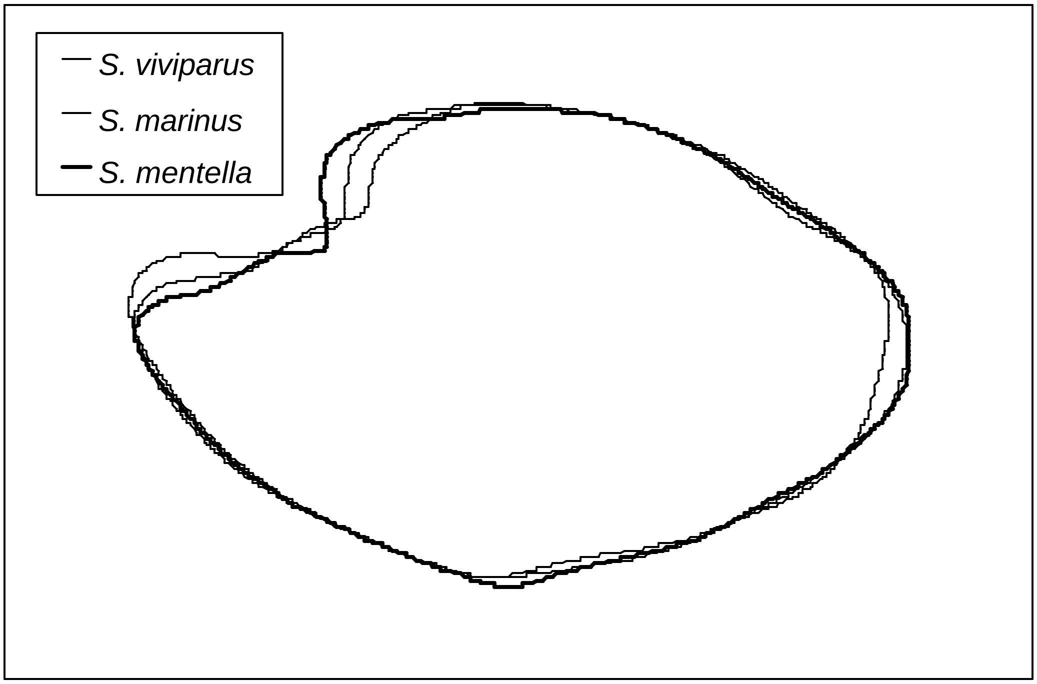


Fig. 6: Average shapes of *S. mentella*, *S. viviparus* and *S. marinus* otoliths, collected in the Barents Sea. Otolith outlines were normalised for size, rotation, location and starting position.