

**Stock separation and growth of redfish (genus *Sebastes*)
in the North Atlantic by means of shape and
elemental analysis of otoliths**

Dissertation

zur Erlangung des Doktorgrades
des Fachbereiches Biologie
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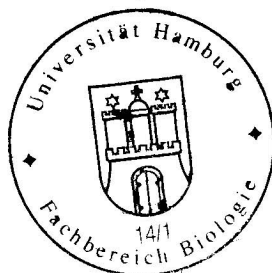
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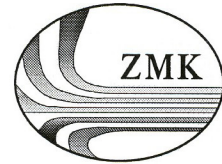


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Title: English Stock separation and growth of redfish (genus *Sebastes*) in the North Atlantic by means of shape and elemental analysis of otoliths.

The quality of English grammar and the vocabulary employed by the candidate fulfills the requirements for acceptance as a Ph. D. at the University of Hamburg.

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Summary

Within the species-rich genus *Sebastes*, four species are found in the North Atlantic, Acadian redfish (*S. fasciatus*), small redfish (or Norway haddock, *S. viviparus*), golden redfish (*S. marinus*) and deep-sea redfish (*S. mentella*). The latter two are of highest interest to commercial fisheries, especially the pelagic occurrences of *S. mentella* in the Irminger Sea that were explored in their full dimensions only recently. Despite the high fishing pressure on redfish resources, only patchy knowledge on their distribution, stock structure, reproductive cycles and growth exists, preventing optimum harvesting strategies. The vague nature of the scientific basis for redfish assessment, particularly the controversial concepts on the amount and delimitation of stocks in the Irminger Sea and adjacent waters, has motivated an EU-funded multidisciplinary research project on redfish. As part of this project, the work presented in this thesis was focusing on stock separation and growth of the two predominantly exploited species, *S. marinus* and *S. mentella*, utilising a suite of advanced techniques applied to their ear bones (otoliths) collected across the distributional range. Otolith shape analysis was used to examine species-specific differences and geographic variation, together with the analysis of the elemental composition of the otoliths. The bias and precision of age determinations and inferred growth of redfish was tested by comparisons between age reading experts and reading methods. Utilising the ratio of two naturally incorporated radioisotopes in otolith cores, radiometric age validation of redfish was achieved.

The first paper employing otolith shape analysis was studying interspecific variation within the genus *Sebastes* by univariate and multivariate techniques. Otolith samples from all four North Atlantic redfish species, six rockfish species from the North Pacific and *S. capensis* from the South Atlantic were compared for differences in linear otolith measurements and elliptical Fourier shape descriptors derived from digitised otolith outlines. A distinction between the North Atlantic and North Pacific/South Atlantic species was achieved by univariate and multivariate analyses of the shape variables. Discriminant analysis revealed correct classification of 88% between the four redfish species. High similarity of the North Pacific rockfish to the South Atlantic *S. capensis* and clear discrimination from North Atlantic species coincides with current zoogeographic theories and recently reported genetic results.

The complex stock structure of North Atlantic redfish species has raised several problems preventing a stock-adaptive fisheries assessment and management. Geographic variation of otolith shapes of *S. marinus* and *S. mentella* across the North Atlantic was analysed to evaluate this technique for stock separation. Multivariate analysis of Fourier descriptors revealed relatively small differences between sampling sites and high within-area variation. The overall classification success of the discriminant analysis was poor for both species (< 50%) but increased to 72-74% by combining sampling areas to

regions (west, central, east). The observed similarities within the central North Atlantic areas (Greenland, Iceland, Faroe Islands) and weak separation of western and eastern areas are in accordance with current fisheries management units. Employing the same methodology, considerably clearer small-scale geographic patterns were found for otolith shapes of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean, providing new information on stock boundaries that will have immediate impact for fisheries management.

Complementary to otolith morphometrics, otolith microchemistry was tested as a stock separation tool for redfish by means of determining minor and trace elements in different otolith zones of *S. marinus* and *S. mentella*. Relatively high temporal stability in otolith elemental composition was found for juvenile redfish from a major nursery area off East Greenland, collected during five consecutive years. Elemental concentrations, measured in the nucleus, juvenile and marginal otolith zones, were found to differ significantly between sampling areas and showed consistent longitudinal trends for several elements. Multivariate analysis of element constituents by area, however, revealed poor geographic separation (< 50% cross-validated classification success) for both species, comparable to recent studies on deep-sea fish in the Northeast Atlantic. Elevated Sr and Ba levels were observed in the otolith edge regions, as compared to the inner growth zones, whereas Li and Mn exhibited opposite patterns. Ontogenetic effects or changes in growth rate are most likely responsible for these phenomena. The effect of water chemistry or dietary uptake could not be tested directly due to insufficient resolution of available trace element and stomach content data. The recently found evidence for migration of juvenile *S. mentella* from the East Greenland shelf into the pelagic habitat of the Irminger Sea could be confirmed by similarity in nucleus chemistry, indicating a common natal origin. The connectivity within the central North Atlantic, inferred from otolith elemental signatures, and the observed weak separation from the Northwest and Northeast Atlantic are in accordance with the results of concurrently undertaken body and otolith morphometrics, as well as recent genetic studies, and support current fisheries management units.

Age determination of Atlantic redfish has proven to be difficult and led to inconsistent age and growth estimates in the past. Even with consensus on the use of otoliths as preferred structure for ageing, the error observed in redfish age readings has prevented reliable age-based stock assessment. Using otoliths of *S. marinus* and *S. mentella*, a series of exchange schemes was carried out to assess bias and precision of age readings between four readers and between two preparation methods, the break-and-burn and the thin-sectioning technique. Considerable bias between readers and moderate precision was observed in the *S. marinus* readings, especially for ages above 20 years. The percent agreement between readers increased from 17-28% to 45-61% when allowing deviations of ± 1 year and to 80-92% with ± 3 years tolerance. *S. marinus* aged from broken and burnt otoliths were estimated slightly younger than the same individuals scored from thin-sectioned otoliths. The bias and precision

estimates obtained from the *S. mentella* material were generally poorer than for *S. marinus* but similar to reported values for other long-lived fish species. Above 50% agreement were only achieved with ± 3 years tolerance. Growth functions for both species revealed only minor differences between readers and confirmed slower growth for *S. mentella*. Since some of the presented error in age determinations could be attributed to interpretational differences between readers, further intercalibration of redfish ageing is urgently needed in order to provide consistent input data for stock assessment.

Considering the observed error in age determinations of redfish, age validation is essential for a reliable age-based stock assessment. Validation studies for *Sebastes* species were predominantly focused on Pacific rockfish, whereas only few verification attempts have been undertaken for North Atlantic redfish. Using a radiometric ageing technique based on $^{210}\text{Pb}/^{226}\text{Ra}$ isotope ratios in otolith core samples (pooled by length groups), ages of *S. marinus* around Iceland as well as *S. mentella* off East Greenland and in the Irminger Sea were determined. In general, the isotope ratios corresponded well with expected radioactive ingrowth curves and with traditional age estimates for the same length group. A slight tendency of relative underestimation of ages by traditional annulus counts was indicated, with considerable discrepancies found for *S. marinus* over 40 cm length and *S. mentella* from deeper layers of the Irminger Sea. Irminger Sea redfish of the biggest investigated length group (41-45 cm) exhibited the maximum radiometric age recorded (41.3 years), in contrast to 34.8 years found by reading the annuli. This study confirms slow growth and high longevity of North Atlantic redfish.

Table of contents

	Page
English Language Evaluation	
Summary	
Table of contents	
1. Introduction	1
2. Species and stock separation	4
2.1 Species separation of the genus <i>Sebastes</i> by otolith shape analysis	4
Abstract	4
Introduction	4
Material and methods	6
Results	9
Discussion	11
Acknowledgements	15
References	16
Tables	21
Figures	23
2.2 Geographic variation of golden redfish (<i>Sebastes marinus</i>) and deep-sea redfish (<i>S. mentella</i>) in the North Atlantic based on otolith shape analysis	29
Abstract	29
Introduction	29
Material and methods	31
Results	33
Discussion	34
Acknowledgements	36
References	36
Tables	39
Figures	41
2.3 Methodological comparison: Otolith shape analysis as a tool for stock separation of horse mackerel (<i>Trachurus trachurus</i>) in the Northeast Atlantic and Mediterranean	43
Abstract	43
Introduction	44
Material and methods	45
Results	47
Discussion	48
Acknowledgements	50
References	50
Tables	52
Figures	54
2.4 Microchemistry of Atlantic redfish otoliths: temporal stability, geographic variation and migration	59
Abstract	59
Introduction	60
Material and methods	62
Results	65
Discussion	67
Acknowledgements	71
References	72
Tables	77
Figures	79

3. Age determination, validation and growth	87
3.1 Age and growth of Atlantic redfish (<i>Sebastes marinus</i> , <i>S. mentella</i>): bias and precision of age readers and otolith preparation methods	87
Abstract	87
Introduction	88
Material and methods	89
Results	91
Discussion	95
Acknowledgements	98
References	98
Tables	101
Figures.....	104
3.2 Radiometric age validation of golden redfish (<i>Sebastes marinus</i>) and deep-sea redfish (<i>S. mentella</i>).....	111
Abstract	111
Introduction	111
Material and methods	112
Results	117
Discussion	118
Acknowledgements	121
References	121
Tables	124
Figures.....	126
4. Conclusions and perspectives	127
5. Literature	130
6. Acknowledgements.....	132

1. Introduction

The species-rich scorpaenid genus *Sebastes* is found in the Atlantic and Pacific, with a marked antitropical distribution and preference to the North Pacific and North Atlantic coasts. Over 100 species of this genus have been described, most of them occurring in the Northeast Pacific. *Sebastes* in the Southern Hemisphere is limited to (at least) two closely related species. Only four species inhabit the North Atlantic, Acadian redfish (*S. fasciatus*; being limited to the Northwest Atlantic), small redfish (or Norway haddock, *S. viviparus*; being limited to the Northeast Atlantic), golden redfish (*S. marinus*) and deep-sea redfish (*S. mentella*). The latter two are of highest interest to commercial fisheries, especially the pelagic occurrences of *S. mentella* in the Irminger Sea that were explored in their full dimensions only recently (*e.g.* Sigurdsson *et al.* 1999).

Despite the high fishing pressure on redfish resources, only patchy knowledge on their distribution, stock structure, reproductive cycles and growth exists, preventing optimum harvesting strategies. The vague nature of the scientific basis for redfish assessment, particularly the controversial concepts on the amount and delimitation of stocks in the Irminger Sea and adjacent waters (ICES 1998), has motivated an EU-funded research project on redfish. Species and stock identification was investigated by body morphometry and meristics, as well as shape analysis and microchemistry of the otoliths, and a variety of genetic markers. Analyses of maturation and fecundity were carried out to gain knowledge on reproductive strategies. To obtain a clearer picture on the distribution and abundance of redfish, data from research surveys and commercial sampling were collated. Age determination and validation was employed to assess demography and growth. As part of this multidisciplinary approach, the work presented in this thesis was focusing on stock separation and growth of the two predominantly exploited species, *S. marinus* and *S. mentella*, utilising a suite of advanced techniques applied to otolith structures.

Since the two-dimensional outlines of otoliths were not only found to be species-specific, but also characteristic for stocks of *e.g.* mackerel, cod and haddock, otolith shape analysis was tested as a tool for species and stock separation of redfish. Species separation of redfish is not straightforward in some areas. *S. fasciatus* and *S. mentella* in the Northwest Atlantic are only differentiated reliably by inspection of their extrinsic gasbladder musculature (Ni 1981), whereas *S. marinus* and *S. mentella* around Greenland are often confused due to intermediate forms that exhibit a mixture of external features usually observed for one or the other species. The differentiation of all four occurring redfish species on the basis of their otolith shapes was attempted in the first study of this thesis (**chapter 2.1**), including *Sebastes* species from the North Pacific and *S. capensis* from the South Atlantic to evaluate overall variation within the genus. The main objective of this section, however, was to examine the use of otolith shape analysis for stock separation of redfish, under special consideration of material

from the Irminger Sea and adjacent waters (**chapter 2.2**). As a methodological comparison, the stock identification of horse mackerel (*Trachurus trachurus*) from the Northeast Atlantic and Mediterranean was approached by the same technique (**chapter 2.3**).

As *S. marinus* is limited to the shallower shelf areas, being more or less separated from each other, differentiation between stocks of this species has not been the primary interest of fishery assessment and management. The highly migratory and straddling nature of *S. mentella*, however, implies interbreeding between population units and thus increased difficulties in delineating stocks. Several perceptions of the stock structure of *S. mentella* in the Irminger Sea and adjacent areas have been put forward (ICES 1998):

- The single-stock hypothesis suggests that all *S. mentella* from the Faroe Islands to Greenland are belonging to one stock, although they may be segregated according to age/size.
- The two-stock hypothesis suggests that the *S. mentella* living on the shelves (shelf deep-sea *S. mentella*) and that living in deeper pelagic waters of Irminger Sea (usually > 500 m depth; pelagic deep-sea *S. mentella*) constitute one stock unit, which is separated from the oceanic *S. mentella* living in upper layers of the Irminger Sea (usually < 500 m depth).
- The three-stock hypothesis supports the idea that each of the described types constitutes a distinct stock.

Most of the studies on stock identification of *S. mentella* (early genetics work, morphometry, parasites) reported relatively weak separation between large geographic areas and a high degree of within-area variation, *i.e.* supporting the single-stock hypothesis. The two-stock hypothesis is mainly based on haemoglobin studies by Johansen *et al.* (2000). A further separation of the demersal stock from the two suggested pelagic stocks (three-stock hypothesis) is, in contrast, unlikely due to the complimentary length spectrum found between shelf and pelagic *S. mentella*: Around Iceland and off East Greenland, regarded as the main nursery areas, only few *S. mentella* larger than 35 cm in length are observed, whereas the usual length spectrum in the Irminger Sea is usually around 25-45 cm. By analysing variation in otolith shapes (**chapter 2.2**) and trace element composition (**chapter 2.4**) in otolith core (nucleus) regions of redfish from across the distributional range in the North Atlantic, patterns in stock structure and their possible use for stock identification were examined.

Traditionally, otoliths have been used for age determination, utilising regular growth increments laid down throughout the life of a fish. The inconsistencies in age determination of redfish, however, led to unreliable estimates of growth and productivity and thus prevented age-based stock assessment. The second section of this thesis aimed at the quantification of error in age readings based on *S. marinus* and *S. mentella* otoliths, as well as age validation using radioisotope measurements. Four readers and

two otolith preparation/reading methods were compared for bias and precision in age reading exchange programs (**chapter 3.1**). Complementary to the traditionally derived age and growth, age validation was achieved for both species by means of an improved technique for the determination of ^{210}Pb and ^{226}Ra in otolith cores, serving as radiometric chronometer (**chapter 3.2**). Maximum ages of up to 75 years were reported for *S. mentella* in the Northwest Atlantic (Campana *et al.* 1990), whereas *S. mentella* in the Barents Sea did not exceed ages of 48 years (Nedreaas 1990). By calculating the age of the analysed *S. marinus* and *S. mentella* samples from $^{210}\text{Pb}/^{226}\text{Ra}$ ratios, longevity of redfish was investigated and compared to traditional ageing results.

2.1 Species separation of the genus *Sebastes* by otolith shape analysis

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Abstract

Interspecific otolith shape variation was investigated within the species-rich genus *Sebastes* (redfish, rockfish) by univariate and multivariate techniques. Otolith samples from all four North Atlantic redfish species, six rockfish species from the North Pacific and *S. capensis* from the South Atlantic were compared for differences in linear otolith measurements and indices, such as relationships between otolith length and otolith breadth and otolith weight, circularity and rectangularity, as well as elliptical Fourier shape descriptors. A distinction between the North Atlantic and North Pacific/South Atlantic species was achieved by univariate analyses. Multivariate analysis of size-corrected Fourier descriptors confirmed the separation of these two groups and enabled quantification of interspecific shape differences. Discriminant analysis revealed correct jackknifed classification of 91% for species and 97% for the three regions (North Atlantic, North Pacific, South Atlantic). Overall classification success for the North Pacific species was 94%, while the North Atlantic samples were correctly classified by 88%, owing to relatively close relationships of *S. mentella* otolith shapes with *S. fasciatus* and *S. marinus*. From the Pacific rockfish samples, *S. alutus* otoliths showed the strongest affinity to the North Atlantic *Sebastes*. High similarity of the North Pacific *Sebastes* to *S. capensis* otolith shapes and clear discrimination from North Atlantic species coincides with current zoogeographical theories and recently reported genetic results. Since this study represents the first approach to quantitative otolith shape analysis within the genus *Sebastes*, more extensive studies based on material covering all known *Sebastes* species would enable complete evaluation of interspecific differences.

Keywords: *Sebastes*, rockfish, redfish; otolith shape, Fourier analysis; species identification

Introduction

The genus *Sebastes* Cuvier includes the highest number of species within the family Scorpaenidae, with over 100 species worldwide, most of them occurring in the North Pacific (Love *et al.* 2002). Along the North Pacific coasts, the commonly termed rockfish species range from Kyushu/Japan and Taiwan in the west (Matsubara 1943, Chen 1981) over the Bering Sea and the Alaskan coast (Barsukov and Lisovenko 1965, Kramer and O'Connell 1995) to Baja California and the Gulf of

California in the east (Chen 1975). Following an antitropical distribution, *Sebastes* in the Southern Hemisphere is limited to two closely related species with extraordinary zoogeographical anomalies (Eschmeyer and Hureau 1971). *S. oculatus* is found from the Pacific coast of Peru and Chile (Kong Urbina 1985) east to the Falkland Islands, marking the western distribution boundary of *S. capensis* which expands eastwards to the Tristan da Cunha-Gough Islands group and South Africa (Eschmeyer 1969, Andrew *et al.* 1995). While southern California represents the ‘hot spot’ of *Sebastes* species diversity in the North Pacific with up to 60 species (Love *et al.* 2002), the North Atlantic is inhabited by only four species, *S. fasciatus* (being limited to the Northwest Atlantic), *S. viviparus* (limited to the Northeast Atlantic), *S. marinus* and *S. mentella*, commonly known as redfish (*e.g.* ICES 1998).

Attempts of taxonomic division of the genus *Sebastes* have been numerous and subject to large revisions (reviewed by Kendall 2000). After the first use of the generic name by Georges Cuvier in 1829, up to 13 subgenera including 71 species had been described in the late 19th century (Jordan and Everman 1898). After major revisions by Matsubara (1943), Chen (1971), Hallacher (1974) and Barsukov (1981), mainly based on morphological characters, only few new species have been described recently (Eitner *et al.* 1999, Kai and Nakabo 2004). Various biochemical and genetic studies were employed since the 1960’s to resolve inter- and intra-specific relationships. Investigations on haemoglobin patterns (Barrett *et al.* 1966, Tsuyuki *et al.* 1968, Nefyodov 1971, Nædal 1978, Nedreaas *et al.* 1994) and protein electrophoresis (Johnson *et al.* 1970 and 1972, Wishard *et al.* 1980, Seeb 1986) were followed by more advanced techniques using mitochondrial DNA (mtDNA; *e.g.* Seeb 1998, Bentzen *et al.* 1998, Sundt and Johansen 1998) and more specifically the cytochrome *b* gene of the mtDNA control region (Johns and Avise 1998, Rocha-Olivares *et al.* 1999a, 1999b, 1999c and 2000, Kai *et al.* 2003), as well as microsatellite variation (Roques *et al.* 1999 and 2001, Asahida *et al.* 2004). Also fatty acid analyses were evaluated for use as species-specific markers of Atlantic *Sebastes* (Joensen and Grahl-Nielsen 2000 and 2001).

The traditional approach to species identification involves morphometric and meristic characters of the fish body and was predominantly applied to the morphologically very similar Atlantic redfish (Ni 1982, Power and Ni 1985, Saborido-Rey 1994, Valentin *et al.* 2002). Morphometric analyses on body hard parts such as otoliths, however, have the advantage of being relatively unaffected by short-term changes in fish condition, stress, starvation etc. (Campana and Casselman 1993). Descriptions of otolith morphometry have been used to investigate evolutionary aspects and phylogeny of various fish species (Casteel 1974, Hecht and Hecht 1978, Gaemers 1984, Nolf and Steurbaut 1989a), to identify fossil discoveries (Gaemers 1971, Nolf and Steurbaut 1989b, Nolf 1995) and recent species (Rybock *et al.* 1975, Wilson 1985, L’Abée-Lund and Jensen 1993, Gago 1993, Assis 2003, Volpedo and Echeverría 2003), as well as fish remains in stomachs of marine mammals or seabirds (Fitch and Brownell 1968, Ainley *et al.* 1981). More recently, linear otolith morphometrics (*e.g.* Lombarte and

Lleonardt 1993, Aguirre 2003) and otolith shape analysis (Lombarte and Castellón 1991, Torres *et al.* 2000, Gauldie and Crampton 2002) were adopted for species discrimination, allowing quantification of interspecific differences.

Rasmussen (1958) and Kotthaus (1961) were the first to describe differences in otolith morphology of Atlantic redfish. Species-characteristic otolith shapes are documented for most Northeast Atlantic fish (Harkonen 1986), but no attempt has been made yet to quantify the observed differences. Since the redfish species in the North Atlantic show high similarity between species and large intraspecific variation regarding morphological as well as genetic features (*e.g.* ICES 1998), the otolith shapes of Atlantic redfish were investigated as a tool for species identification. By comparing Atlantic and Pacific *Sebastes* species, the overall variation within the genus *Sebastes* was evaluated as a companion study of intraspecific variation in otolith morphology and inferred stock discrimination of the widely distributed and migrating Atlantic redfish species *S. marinus* and *S. mentella* (**chapter 2.2**). To achieve these goals, univariate shape indices and elliptical Fourier descriptors were analysed for differences between all four North Atlantic redfish species, a selection of six North Pacific *Sebastes* species and *S. capensis* from the South Atlantic. Current knowledge on zoogeographical relations within this genus and speciation theories are discussed in relation with the quantified inter-species variation in otolith shapes.

Material and methods

Sampling

Sagittal otoliths were taken from fish collected on research vessels or from commercial catches, using bottom trawl gears, with the exception of *Sebastes mentella* taken in the Irminger Sea with a pelagic, Gloria-type gear. Otolith samples from the four North Atlantic *Sebastes* species (redfish) were obtained from research cruises in typical fishing areas of the respective species between April 1997 and October 2001, aiming at a complete coverage of the distribution area (Table 1, Figure 1). Sampling periods of several weeks or months were necessary in some cases to achieve this coverage. Although *S. viviparus* is known to occur off East Greenland, Iceland and around the Faroe Islands, the number of otoliths available from these areas was too small to be added to the Norway/Barents Sea sample. The otolith samples were taken as pairs and stored dry in paper envelopes including individual fish data (*e.g.* length, weight, sex). The North Pacific samples were taken from six commercially important *Sebastes* species (rockfish) landed by commercial trawlers operating off British Columbia between June 1977 and December 1978 (Table 1, Figure 1). Since the exact positions of the trawls

were not available, the mid-points of the areas where the vessels operated (Groundfish Areas 3C, 5A, 5B, 5D, 5E; see Rutherford 1999) were drawn into the sampling map (Figure 1). These single otolith samples were stored in glass tubes filled with glycerine for conservation, with otoliths of a certain fork length cm class being separated from the neighbouring cm class by paper separators. In the South Atlantic, otoliths of *S. capensis* were collected on fishing trips off Gough Island (Tristan da Cunha) and near Cape Town/South Africa in February and July 2002, respectively, and stored in paper envelopes.

Only otoliths from sexually mature fish were included in the data analysis to minimise variability caused by size effects and to standardise the selected fish length range to a common life-history landmark. Length-at-maturity (L_{50}) data for the respective species and areas available from the literature were used to determine the minimum fish length at which the corresponding otoliths could be included. Average L_{50} values reported by Ni and Sandemann (1984), Ni and Templeman (1985), Saborido-Rey (1994) and St. Pierre and de Lafontaine (1995) were used for *S. fasciatus*, *S. marinus* and *S. mentella* in the Northwest Atlantic, while the corresponding values for *S. marinus*, *S. mentella* and *S. viviparus* in the Northeast Atlantic and Arctic were taken from Trout (1961), Sorokin *et al.* (1986), Rikhter (1987) and Magnússon and Magnússon (1995). When more than one estimate for the same species in the same area was available, the maximum value was selected. Since female redfish generally become mature with fish lengths 1-2 cm larger than the males, the data sets were divided by sex to account for differences in maturation. For the North Pacific *Sebastes* species off British Columbia, L_{50} data were provided by sex by Westrheim (1975), Archibald *et al.* (1981) and Leaman (1991). No L_{50} data were reported for *S. capensis*, but regular investigations off South Africa indicated length-at-maturity around 26 cm (Tim Andrew, Enviro-Fish Africa Ltd., Grahamstown, South Africa; pers. comm.). For most of the species, only fork length data were recorded. Thus, total length data provided for *S. marinus*, *S. mentella* and *S. viviparus* were converted to fork length based on available regressions (F. Saborido-Rey, Institute of Marine Research, Vigo, Spain; unpubl. data).

The selected otoliths were further restricted to the left body side to ensure that one sample represents one fish and to avoid possible asymmetric effects. Trial comparisons of shape descriptors derived from left otoliths and mathematically mirrored right otoliths, however, revealed non-significant discriminant analysis results (Wilks' $\lambda > 0.8$, $p > 0.05$). The reduced data set included a total of 1265 otoliths (North Atlantic: $n = 767$, North Pacific: $n = 459$, South Atlantic: $n = 39$; Table 1).

Image and shape analysis

Otolith outlines were digitised using an image analysis system consisting of a high resolution monochrome CCD video camera, mounted on an OlympusTM 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 30x and 50x. 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 OptimasTM 6.51 (Media Cybernetics 1999) image analysis software. 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, OptimasTM macros were applied.

A set of univariate descriptors was calculated based on the digitised x-y coordinates. In addition to otolith length (major axis length), otolith breadth (minor axis length) and area, two shape descriptors, circularity and rectangularity, were recorded. Circularity is a dimensionless value, defined as the area perimeter squared, divided by the area, resulting in a minimum of 4π (approx. 12.57) that is obtained from boundaries describing a circle (Media Cybernetics 1999). Rectangularity is a dimensionless number between 0 and 1, defined as the object's area divided by the area of the enclosing box orientated along the major axis. The rectangularity of a square is 0.5, whereas a circle reaches $\pi/4$ (approx. 0.79), and a long and narrow rectangle has a value of 1. After removal of adhering tissue and blood remains from the otolith surface and a minimum of 24 hours air drying at room temperature, all otoliths were weighed with a precision of 0.1 mg. Differences in univariate descriptors between species and regions were tested by analysis of variance (ANOVA).

The digitised outline coordinates were forwarded to Elliptical Fourier Analysis (EFA; Kuhl and Giardina 1982, Rohlf and Archie 1984), using C++ modules based on the algorithms of Ferson *et al.* (1985). The principle methodology of Fourier analysis has been described in detail by several authors (*e.g.* Full and Ehrlich 1982, Bird *et al.* 1986, Lestrel 1997) and will therefore not be presented here. 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. Only the first 17 harmonics were included in the statistical analysis since these were responsible for over 99% of the shape variation

(Lestrel 1997). This selection results in 65 Fourier descriptors (FDs) for each sample, consisting of 3*16 FDs (harmonics 2-17 each) of the sine and cosine parts of the x-direction and sine part of the y-direction (where FDs of harmonic 1 become constants after the normalisation process, see above) plus 17 FDs (harmonics 1-17) of the cosine part of the y-direction. Before analysing the FDs for differences between species, the distribution of these data was tested. All FD amplitudes were normally distributed (Kolmogorov-Smirnov test of normality; $p > 0.05$). Thus, no transformation of the FD data was necessary.

Size correction and multivariate analysis

Most important for morphometric analysis is the correction of the data by size, *i.e.* uncoupling of otolith shape and size in this case (Bookstein *et al.* 1985). This was accounted for by using the residuals of the common-within group slopes (Reist 1985, Claytor and MacCrimmon 1987) of the linear regressions of each FD on otolith length. Rather the effect of otolith length than fish length was removed from the variables, since fish length for the North Pacific species was found to be very variable within similar otolith length classes due to possible misinterpretation or mixing of fish length interval layers in the otolith storage tubes. The FD residuals were compared between groups (species or regions) using linear discriminant function analysis (*e.g.* Klecka 1980). The classification success into groups was tested by jackknifed cross-validation (SPSS Inc. 1999). Differences between sexes and temporal effects (for areas where more than one sampling interval was carried out) were also tested by discriminant analysis. Average otolith shapes of were drawn for each species from reproduced outlines of the average FDs within a species.

Results

Univariate shape descriptors

Clear differences in the otolith length-otolith breadth relationships between regions (North Atlantic, North Pacific, South Atlantic) and species were observed (Figure 2) and confirmed by ANOVA of the otolith breadth to otolith length ratios (main effect regions: $F_{2, 1262} = 1516.84$, $p < 0.0001$; species: $F_{10, 1254} = 571.63$, $p < 0.0001$). Otoliths of the North Atlantic species were considerably broader than otoliths of the North Pacific and South Atlantic species, with most of the species exhibiting distinctive within-region differences and *S. alutus* samples showing the greatest overlap with the North Atlantic

species. The *S. capensis* samples had an otolith length-otolith breadth relationship close to the North Pacific samples.

A similar separation of North Atlantic and North Pacific/South Atlantic species was obvious from the log-transformed otolith length-otolith weight regressions (Figure 3), with a clearer differentiation within the North Atlantic species. Especially the *S. marinus* samples appeared separated from *S. mentella*, showing strongest overlaps with *S. alutus*. Log otolith weight to log otolith length ratios indicated highly significant differences between regions (ANOVA; $F_{2, 1261} = 1022.37, p < 0.0001$) and species (ANOVA; $F_{10, 1253} = 507.78, p < 0.0001$).

The variation of the shape descriptors circularity and rectangularity generally increased with increasing otolith length (Figures 4 and 5). While a considerable overall trend of decreasing circularity (as given by higher values) with increasing otolith length was apparent (Figure 4), no clear trend could be derived from the rectangularity plot (Figure 5). Otoliths of the North Atlantic species, however, were characterised by higher circularity (lower values) and lower rectangularity than otoliths of the North Pacific and South Atlantic species (ANOVA; circularity: $F_{2, 1262} = 303.59, p < 0.0001$; rectangularity: $F_{2, 1262} = 230.39, p < 0.0001$).

Fourier shape analysis

The size-correction of the FDs by residuals reduced the correlation between FDs and otolith lengths very effectively. Pearson correlation before size-adjustment was up to $r = 0.49$ with more than two thirds of the FDs being significantly correlated ($p < 0.05$) with otolith length, while the residuals showed no correlation with otolith length ($r < 6 \cdot 10^{-14}, p > 0.05$). No significant differences were found between sexes (Wilks' $\lambda = 0.92, p > 0.05$).

Discriminant analysis based on the size-corrected FD data of all analysed species revealed a North Atlantic and a North Pacific/South Atlantic cluster with relatively small overlap (Figure 6). *S. viviparus* and *S. capensis* appear as considerably well separated groups in the lower part of each cluster. Overall jackknifed species classification success was 91%, ranging from 76% correct classification for *S. fasciatus* to 100% for *S. proriger* and *S. viviparus* (Table 2). Differences between species were highly significant (Wilks' $\lambda = 0.0016, p < 0.001$). Individual fish could be correctly classified into regions by 97% (Table 3), with the South Atlantic (*S. capensis*) being best discriminated from the other regions.

Species differentiation within regions was further investigated by separate analyses for the North Atlantic and North Pacific. The discriminant scores plot for the North Atlantic species (Figure 7) illustrates a considerable overlap of *S. marinus* and *S. mentella* otolith shapes, with the *S. fasciatus* samples showing high variability and *S. viviparus* being well discriminated from the other species. The North Atlantic species were classified with 88% accuracy (Table 4), ranging from 81% (*S. fasciatus*) to 100% (*S. viviparus*). The highest misclassification rates were found for *S. mentella*, 16% of the *S. fasciatus* samples and 9% of the *S. marinus* samples being allocated to this species. Within the North Pacific, overall classification success into species (94%; Table 5) was markedly higher as for the North Atlantic. *S. entomelas* otoliths were misclassified by 11% into the *S. proriger* group, representing the largest observed overlap with this region (Figure 8). 100% correct classification was achieved for *S. flavidus*.

The average otolith shapes by species, scaled to the same size, showed distinct differences between North Atlantic and North Pacific *Sebastes*, with considerable variation within these regions (Figure 9). Otoliths of North Atlantic redfish are generally broader than Pacific *Sebastes* otoliths and have a marked antirostrum indentation (upper left part of the outlines in Figure 9). *S. capensis* otoliths were very similar to the North Pacific otoliths.

Discussion

This study demonstrates clear differences in otolith shapes of North Atlantic and North Pacific *Sebastes* species and interspecific variation in univariate measurements and indices as well as multivariate shape descriptors.

Univariate shape descriptors

Two major species groups were determined by univariate measurements. Higher otolith breadth and otolith weight in relation to otolith length discriminate North Atlantic redfish from the North Pacific *Sebastes* and *S. capensis*. Differences in allometric otolith growth in length and breadth (Simoneau *et al.* 2000) as well as thickness, with otolith weight as a proxy for three-dimensional growth, are responsible for the observed differentiation. Representing the traditional and predominantly followed approach to otolith shape variation due to relatively straightforward data acquisition, differences in univariate (linear) otolith morphometrics have been used to discriminate fish species and stocks. A variety of measurements, such as otolith length and breadth (*e.g.* Einarsson 1951, Giedz 1982, Aps *et*

al. 1990), the size of the nucleus or first growth ring (Postuma 1974, Dawson 1991, Torres *et al.* 1996) were mainly employed to differentiate between stocks but also between species (Lombarte and Lleonardt 1993, Aguirre 2003).

Univariate shape indices calculated as a combination of otolith perimeter and area, such as circularity and rectangularity, have been used to evaluate otolith shape variation in other fish species (Bolles and Begg 2000, Tuset *et al.* 2003). In this study, North Pacific *Sebastes* generally showed higher circularity and rectangularity than North Atlantic *Sebastes*, with considerable more overlap between regions. The common trend of increasing shape complexity and variation with larger fish or otolith sizes was also supported in this study, indicated by higher circularity (high otolith perimeter in relation to otolith area) and increased variation in rectangularity for larger otoliths. The reason for an increased otolith perimeter with otolith size is a higher number of indentations and variations on the otolith outline as the fish grow older. While there are approaches to utilise this feature for fish ageing (Doering and Ludwig 1990, Hamrin and Doering-Arjes 2002), otolith complexity could be related to life-history effects, such as ear-dependent predation (Aguirre and Lombarte 1999) that gains relevance in greater depths (Gauldie and Crampton 2002).

Fourier and multivariate analysis

A complete description of otolith shape can only be accomplished by an outline technique that captures the entire shape variation and small-scale individual differences on the otolith outline. Fourier series analysis has proven to be the most powerful technique (*e.g.* Bird *et al.* 1986, Castonguay *et al.* 1991, Campana and Casselman 1993, DeVries *et al.* 2002), although the biological interpretation of these data is more complex than for linear morphometrics (Bookstein *et al.* 1982). The elliptical Fourier analysis used in this study appeared to be the most appropriate method for otolith outlines, since otolith shape can be best described by an ellipse (Chauvelon and Bach 1993) and thus approximation to original shapes is achieved by fewer steps than in conventional Fourier transformation. Similar applications of elliptical Fourier analysis, however, have been limited to very few studies on scales (de Pontual and Prouzet 1987 and 1988) and otoliths (Murta 1996, Petry 2001). Based on the interspecific differences in elliptic Fourier amplitudes, individual relationships between and within *Sebastes* species could be investigated and quantified in this study. The separation of the North Atlantic and North Pacific/South Atlantic species groups observed in the univariate data was supported by 97% classification success into regions using the multivariate FD data. The *Sebastes* otoliths investigated in this study could be allocated correctly to species by over 90%, the North Atlantic species exhibiting lower classification success than the North Pacific species due to relatively low discrimination of *S. fasciatus* from *S. mentella* and *S. marinus*. These species are known to be

closely related, as expressed by similar body morphometrics (*e.g.* Power and Ni 1985, Saborido-Rey 1994, Valentin *et al.* 2002), low interspecific genetic variation (*e.g.* Nedreaas *et al.* 1994, Bentzen *et al.* 1998, Sundt and Johansen 1998) and hybridisation (Roques *et al.* 2001). Classification success for the North Atlantic species did not improve significantly by leaving out sampling areas where intermediate types of *S. marinus* and *S. mentella* are found regularly, such as East Greenland (Barsukov 1973, Nedreaas *et al.* 1994).

As the final step of the multivariate shape analysis of *Sebastes* otoliths, the reconstruction of the average shapes by species, based on the FDs that explained over 99% of shape variation, highlighted major differences between North Atlantic and North Pacific samples and a strong similarity of *S. capensis* to the North Pacific *Sebastes*. This overlay technique also identified the breadth dimension and antistrostrum outline parts as being responsible for the observed differences between regions and interspecific shape variation. Albeit the advantage of a direct comparison of shapes, similar studies presenting average shapes (Lombarte and Castellón 1991, Campana and Casselman 1993) have not utilised this technique.

Confounding effects

Since the otolith samples from the selected North Pacific rockfish were taken in the late 1970's, over 20 years before the North Atlantic and *S. capensis* samples were collected, temporal effects could confound the presented results. Comparisons of FD data for *S. mentella* from East Greenland (where two sampling intervals were available), however, revealed no significant differences between sampling years. On an evolutionary scale, the time lag in collection dates between North Pacific and Atlantic samples, is negligible. The related effect of different age-groups or year-classes present in similar fish or otolith length groups could not be directly tested owing to missing ageing data for the investigated otoliths. Trial multivariate analyses of age-corrected FDs derived from available age-length keys (R. Stanley, DFO Namaimo, Canada, pers. comm.; F. Saborido-Rey, unpubl. data) showed species differences that were not significantly different from the presented patterns. On the other hand, uncertainty and high variation in ageing data for North Atlantic redfish (*e.g.* Nedreaas 1990, Saborido-Rey *et al.* 2004; **chapter 3.1**) and North Pacific *Sebastes* species (*e.g.* Kimura *et al.* 1979, Watters 1993, Andrews *et al.* 2002, Laidig *et al.* 2003) could have introduced additional error. The comparison of otolith length-otolith weight relationships allows limited evaluation of age-effects, since otolith weight can be employed as a proxy for age (Pawson 1990, Fletcher 1995, Cardinale *et al.* 2000, Araya *et al.* 2001, Pilling *et al.* 2003, Pino *et al.* 2004). Otolith weight related to otolith length of *S. mentella* was higher than for *S. marinus*, caused by a higher number of additional growth layers in proximal direction (thickness) with higher longevity. This can also be observed for several rockfish species (Boehlert 1985, MacLellan 1997).

Zoogeography and speciation

Unambiguous differentiation of the investigated *Sebastes* otolith shapes into a North Atlantic and North Pacific group, as well as the affinity of *S. capensis* otoliths to North Pacific species, support current theories on the speciation of the genus *Sebastes*. The Northwest Pacific appears to be the origin and centre of distribution for the genus *Sebastes* (Eschmeyer 1969), having spread to the north and east with the expansion of cold water about 10-13 million years ago (Kendall 1991, Kai *et al.* 2003). Recent genetic studies point to significant radiation occurring about 5 million years ago (Johns and Avise 1998), with the subgenus *Sebastomus* (including *S. capensis* from the Southern Hemisphere) having evolved latest (about 320000 to 1.1 million years ago; Rocha-Olivares *et al.* 1999b). The four North Atlantic *Sebastes* species have most probably originated from one common ancestor species, entering the Arctic basin at the end of the Miocene (10 million years ago) or at the beginning of the Pliocene after the formation of the Bering Strait (Barsukov 1981). Moser *et al.* (1977) suggested *S. jordani* having invaded the North Atlantic on the basis of similarity of larval characters, and Seeb (1986) found biochemical indications linking *S. wilsoni* to the North Atlantic species. The observed similarity of *S. alutus* otolith shapes with the North Atlantic *Sebastes*, especially with *S. marinus* in the univariate comparisons, is in accordance with Kendall (1991) who found that larvae of *S. alutus* have similar appearance to larvae of the North Atlantic species. There are also similarities between *S. alutus* and *S. marinus* in life-history parameters such as fecundity (Haldorson and Love 1991).

The transarctic movement of the ancestor of the North Atlantic *Sebastes* was complete by the end of the Pliocene (about 1 million years ago), when further speciation into the four extant species took place (Barsukov 1981). With the cooling of the Arctic waters, the North Atlantic species moved further south and spread into the Northeast Atlantic. Deep climatic changes in the Tertiary had greater influence in the Atlantic than in the Pacific (Briggs 1970), enabling higher radiation rates in the Pacific and thus differentiation of the numerous Pacific *Sebastes* species. Pacific rockfish have then occupied a large variety of different habitats, ranging from shallow kelp beds, rocky areas and reefs to depths exceeding 500 m (Matthews 1990, Krieger and Ito 1999, Yoklavich *et al.* 2000), whereas Atlantic redfish are mainly restricted to deeper demersal or pelagic habitats. Some bathymetric separation is apparent in the North Atlantic, *S. mentella* being distributed deeper than *S. fasciatus*, *S. marinus* and *S. viviparus*. The variation in otolith shapes within the North Atlantic was not much different to North Pacific *Sebastes*, as would have been expected from evolutionary aspects and genetic variation (Rocha-Olivares 1999a).

Otolith shapes of *S. capensis* from the South Atlantic showed strong similarities with the North Pacific samples, coinciding with zoogeographical considerations of Eschmeyer and Hureau (1971) and

genetic analyses of Rocha-Olivares *et al.* (1999c). During cooling and then rewarming of equatorial waters, ancestors of the present Southern Hemisphere *Sebastes* likely have crossed the tropics of the eastern Pacific and speciated into at least two sibling species. *S. oculatus* inhabited the coasts off Chile, Peru and the Falkland Islands, from where *S. capensis* speciated and moved eastwards via West Wind drift to Tristan da Cunha and South Africa (Rocha-Olivares *et al.* 1999c). In the Atlantic, however, a transequatorial movement of *Sebastes* from north to south is unlikely, since invasion of *S. capensis* into the South Atlantic probably took place before or during the North Atlantic species evolved. This theory was supported by Rocha-Olivares *et al.* (1999a) who compared the cytochrome *b* sequence of 54 *Sebastes* species and found distinct differences between *S. marinus* and *S. capensis*. Confirmation of these results on a phenotypic level was achieved for the first time in this study, showing clear separation of *S. capensis* otolith shapes from the North Atlantic *Sebastes* samples.

Although otoliths from all four North Atlantic species, from six typical North Pacific species and *S. capensis* from the South Atlantic were investigated in this study, full evaluation of the variability in otolith morphometry within the genus *Sebastes* will only be possible by a complete coverage of all known species. This would require an extensive sampling programme that will probably take several years but would enable comprehensive morphometric analysis of this incredibly diverse genus.

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Table 1. Sampling information. Number of otolith samples by species, selected fish length range (fork length; only mature fish).

Species	code	Region	Area	Sampling period	Mean fish length (range) [cm]	No. of otoliths
<i>Sebastes fasciatus</i>	fas	North Atlantic	S Newfoundland	April 1997	32.9 (26-43)	37
<i>Sebastes marinus</i>	mar	North Atlantic	Flemish Cap, Greenland, Iceland, Barents Sea	July 2000-July 2001	40.0 (36-57)	117
<i>Sebastes mentella</i>	men	North Atlantic	Flemish Cap, Davis Strait, Greenland, Iceland, Irminger Sea, Faroe Islands, Barents Sea	October 1998-October 2001	38.9 (34-49)	593
<i>Sebastes viviparus</i>	viv	North Atlantic	Barents Sea	July-August 2000	21.0 (19-25)	20
<i>Sebastes alutus</i>	alu	North Pacific	British Columbia	October 1977-October 1978	40.9 (36-49)	94
<i>Sebastes brevispinis</i>	bre	North Pacific	British Columbia	March-December 1978	53.0 (44-65)	106
<i>Sebastes entomelas</i>	ent	North Pacific	British Columbia	July-September 1978	51.7 (40-59)	111
<i>Sebastes flavidus</i>	fla	North Pacific	British Columbia	June 1977-August 1978	48.0 (41-55)	64
<i>Sebastes proriger</i>	pro	North Pacific	British Columbia	May-October 1978	36.3 (31-42)	46
<i>Sebastes reedi</i>	ree	North Pacific	British Columbia	August-November 1978	42.3 (38-47)	38
<i>Sebastes capensis</i>	cap	South Atlantic	Tristan da Cunha, South Africa	February-July 2002	29.3 (26-36)	39
Total						1265

Table 2. Jackknifed classification matrix of the discriminant analysis between species (see Table 1 for species codes). The percentages in rows represent the classification into the areas given in columns, sample sizes are given in parentheses. Overall classification success is 91.0%, Wilks' $\lambda = 0.0016$.

	fas	mar	men	viv	alu	bre	ent	fla	pro	ree	cap
fas	75.7 (28)	2.7 (1)	18.9 (7)	0 (0)	2.7 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
mar	7.7 (9)	82.9 (97)	5.1 (6)	0 (0)	2.6 (3)	0.9 (1)	0 (0)	0.9 (1)	0 (0)	0 (0)	0 (0)
men	2.4 (14)	4.4 (26)	91.4 (542)	0.2 (1)	0.7 (4)	0.3 (2)	0 (0)	0.3 (2)	0 (0)	0.2 (1)	0.2 (1)
viv	0 (0)	0 (0)	0 (0)	100.0 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
alu	2.1 (2)	2.1 (2)	1.1 (1)	0 (0)	83.0 (78)	1.1 (1)	2.1 (2)	4.3 (4)	0 (0)	4.3 (4)	0 (0)
bre	0 (0)	0.9 (1)	0 (0)	0 (0)	0 (0)	89.6 (95)	3.8 (4)	1.9 (2)	3.8 (4)	0 (0)	0 (0)
ent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	90.1 (100)	2.7 (3)	7.2 (8)	0 (0)	0 (0)
fla	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.6 (1)	0 (0)	98.4 (63)	0 (0)	0 (0)	0 (0)
pro	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	100.0 (46)	0 (0)	0 (0)
ree	0 (0)	0 (0)	0 (0)	0 (0)	5.3 (2)	0 (0)	0 (0)	0 (0)	0 (0)	94.7 (36)	0 (0)
cap	0 (0)	2.6 (1)	0 (0)	0 (0)	2.6 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	94.9 (37)

Table 3. Jackknifed classification matrix of the discriminant analysis between regions (see Table 1 for region allocations). The percentages in rows represent the classification into the areas given in columns, sample sizes are given in parentheses. Overall classification success is 97.0%, Wilks' $\lambda = 0.1156$.

	North Atlantic	North Pacific	South Atlantic
North Atlantic	97.0 (744)	2.6 (20)	0.4 (3)
North Pacific	2.2 (10)	96.5 (443)	1.3 (6)
South Atlantic	0 (0)	2.6 (1)	97.4 (38)

Table 4. Jackknifed classification matrix of the discriminant analysis between North Atlantic species. The percentages in rows represent the classification into the areas given in columns, sample sizes are given in parentheses. Overall classification success is 88.3%, Wilks' $\lambda = 0.1302$.

	<i>S. fasciatus</i>	<i>S. marinus</i>	<i>S. mentella</i>	<i>S. viviparus</i>
<i>S. fasciatus</i>	81.1 (30)	2.7 (1)	16.2 (6)	0 (0)
<i>S. marinus</i>	7.7 (9)	82.9 (97)	8.5 (10)	0.9 (1)
<i>S. mentella</i>	3.0 (18)	7.4 (44)	89.2 (529)	0.3 (2)
<i>S. viviparus</i>	0 (0)	0 (0)	0 (0)	100.0 (20)

Table 5. Jackknifed classification matrix of the discriminant analysis between North Pacific species. The percentages in rows represent the classification into the areas given in columns, sample sizes are given in parentheses. Overall classification success is 94.0%, Wilks' $\lambda = 0.0023$.

	<i>S. alutus</i>	<i>S. brevispinis</i>	<i>S. entomelas</i>	<i>S. flavidus</i>	<i>S. proriger</i>	<i>S. reedi</i>
<i>S. alutus</i>	91.5 (86)	1.1 (1)	0 (0)	4.3 (4)	0 (0)	3.2 (3)
<i>S. brevispinis</i>	0.9 (1)	94.3 (100)	0.9 (1)	0 (0)	3.8 (4)	0 (0)
<i>S. entomelas</i>	0 (0)	0 (0)	87.4 (97)	1.8 (2)	10.8 (12)	0 (0)
<i>S. flavidus</i>	0 (0)	0 (0)	0 (0)	100.0 (64)	0 (0)	0 (0)
<i>S. proriger</i>	0 (0)	2.2 (1)	4.3 (2)	0 (0)	93.5 (43)	0 (0)
<i>S. reedi</i>	2.6 (1)	0 (0)	0 (0)	0 (0)	0 (0)	97.4 (37)

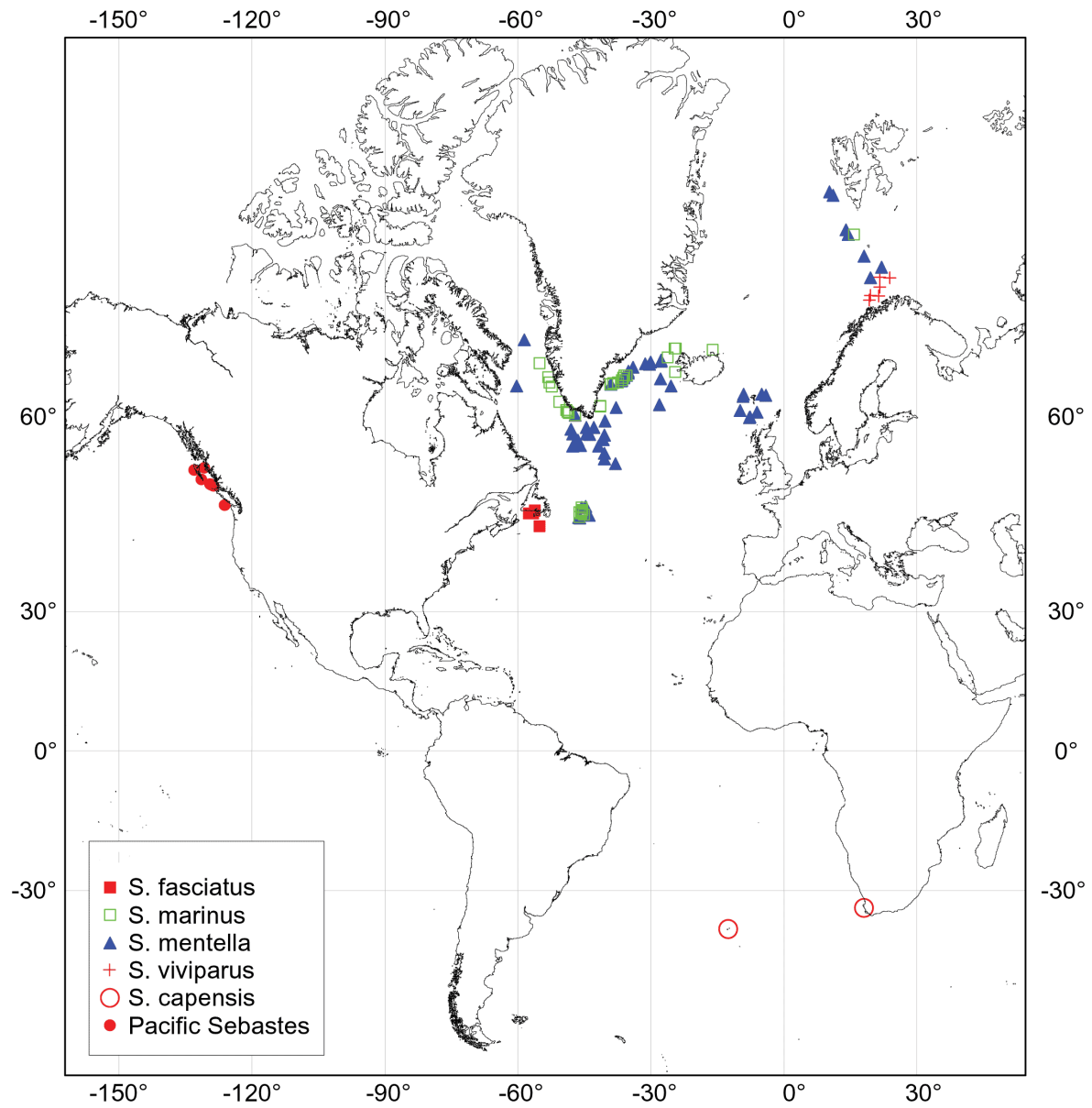


Figure 1. Sampling positions of *Sebastes* otoliths from the North Atlantic (redfish; *S. fasciatus*, *S. marinus*, *S. mentella* and *S. viviparus*; separately marked), North Pacific (rockfish; *S. alutus*, *S. brevispinis*, *S. entomelas*, *S. flavidus*, *S. proriger* and *S. reedi*) and South Atlantic (*S. capensis*).

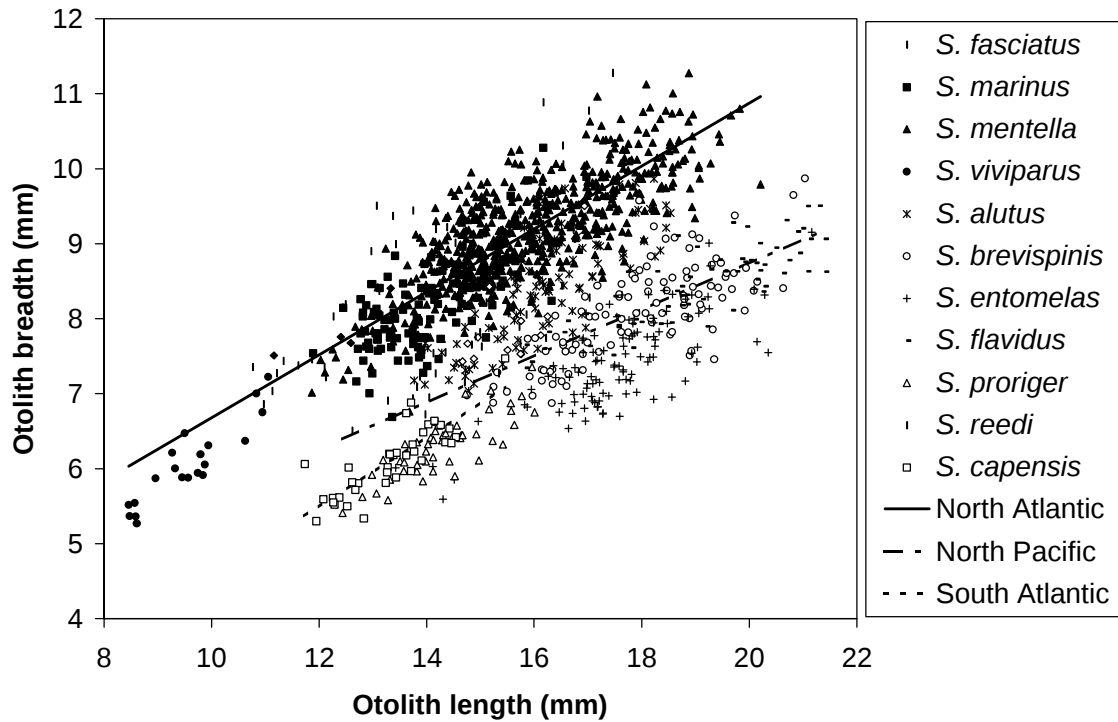


Figure 2. Relationship between otolith length and otolith breadth for all *Sebastes* species. Linear regressions were fitted through all individuals belonging to the same region (North Atlantic: $OB = 0.420 \cdot OL + 2.484$, $r^2 = 0.734$; North Pacific: $OB = 0.311 \cdot OL + 2.522$, $r^2 = 0.446$; South Pacific: $OB = 0.463 \cdot OL - 0.061$, $r^2 = 0.714$).

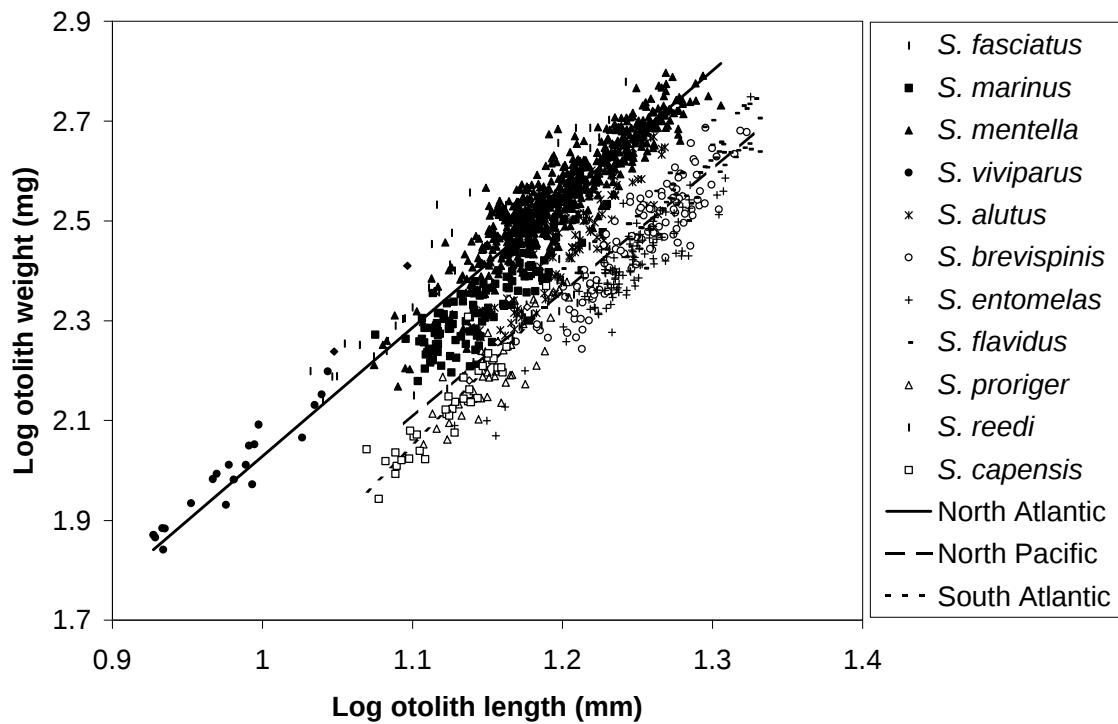


Figure 3. Relationship between otolith length and otolith weight for all *Sebastes* species. Linear regressions were fitted through all individuals belonging to the same region (North Atlantic: $\log OW = 2.576 \cdot \log OL - 0.547$, $r^2 = 0.875$; North Pacific: $\log OW = 2.501 \cdot \log OL - 0.644$, $r^2 = 0.757$; South Pacific: $\log OW = 3.067 \cdot \log OL - 1.322$, $r^2 = 0.842$).

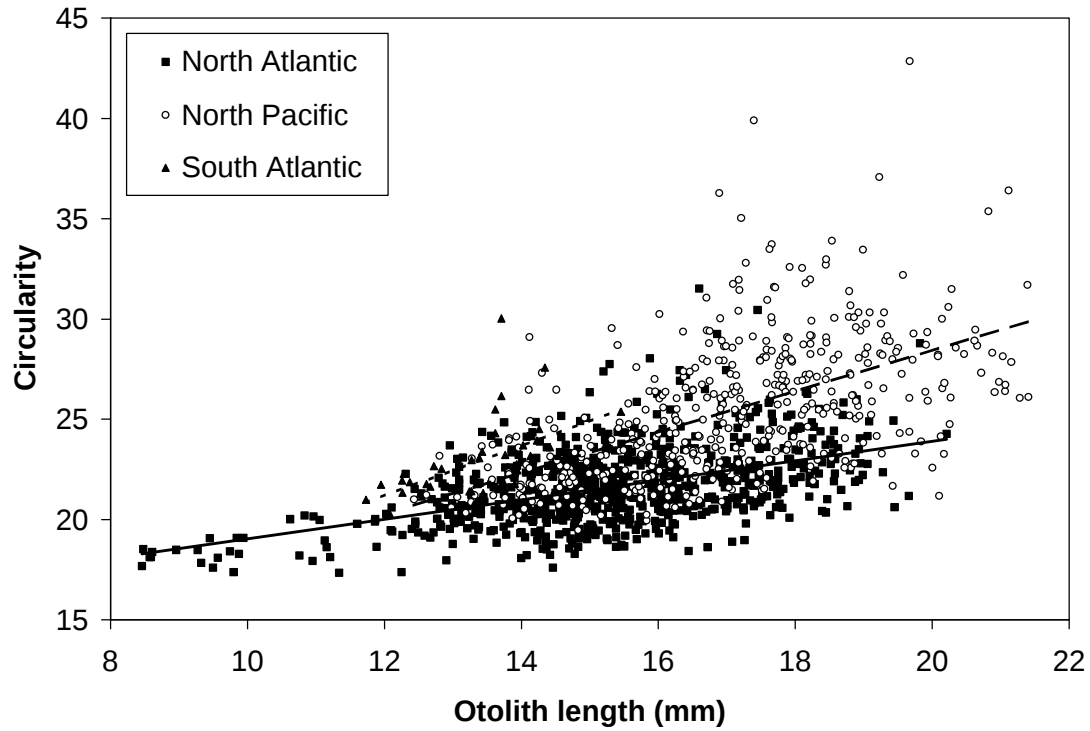


Figure 4. Relationship between otolith length and circularity for North Atlantic, North Pacific and South Atlantic *Sebastes* species. Linear regressions were fitted through all individuals belonging to the same region (North Atlantic: $\text{Circ.} = 0.487 \cdot \text{OL} + 14.178$, $r^2 = 0.234$; North Pacific: $\text{Circ.} = 1.024 \cdot \text{OL} + 7.975$, $r^2 = 0.309$; South Pacific: $\text{Circ.} = 1.247 \cdot \text{OL} + 6.229$, $r^2 = 0.297$). For coding of the regression lines, see Fig. 2.

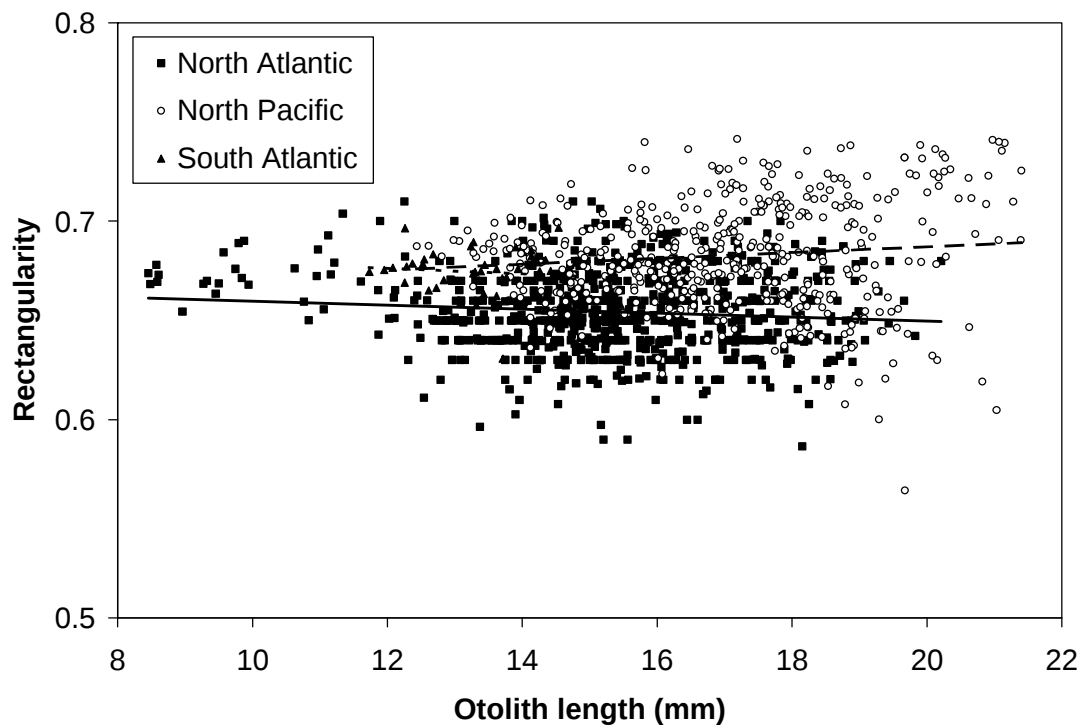


Figure 5. Relationship between otolith length and rectangularity for North Atlantic, North Pacific and South Atlantic *Sebastes* species. Linear regressions were fitted through all individuals belonging to the same region (North Atlantic: $\text{Rect.} = -0.001 \cdot \text{OL} + 0.670$, $r^2 = 0.009$; North Pacific: $\text{Rect.} = 0.002 \cdot \text{OL} + 0.658$, $r^2 = 0.011$; South Pacific: $\text{Rect.} = -0.002 \cdot \text{OL} + 0.6939$, $r^2 = 0.011$). For coding of the regression lines, see Fig. 2.

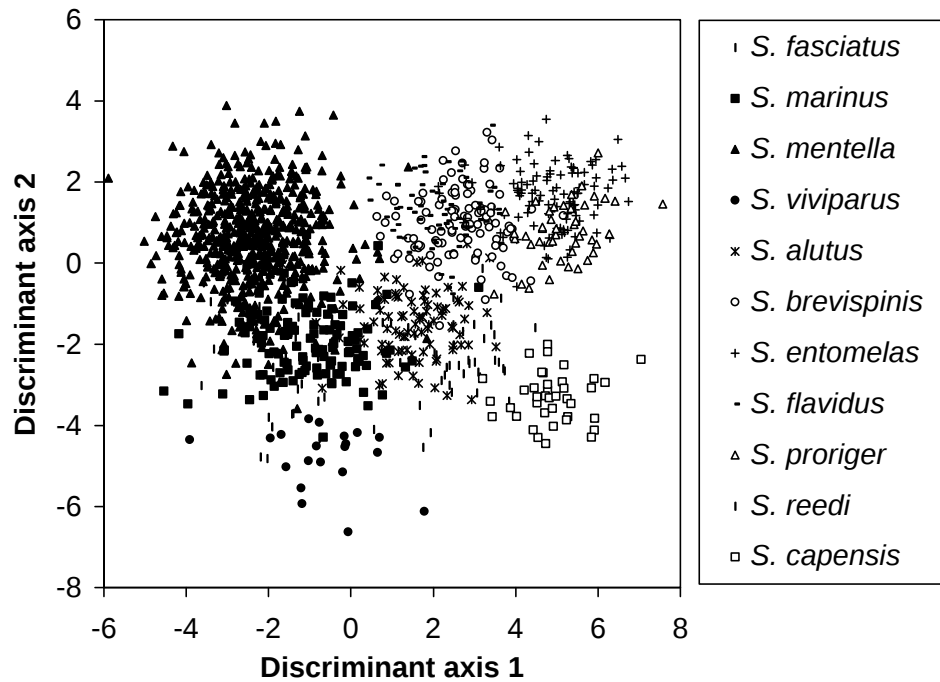


Figure 6. Discriminant function scores for the Fourier descriptors of otoliths from *Sebastes* species in the Atlantic and Pacific Ocean. The first discriminant axis captures 55.8% of the variation, the second axis captures 15.2%. North Atlantic species are marked with filled symbols and appear on the left side of the plot, whereas North Pacific and South Atlantic species, clustered on the right side of the plot, are marked with open and cross/dash symbols.

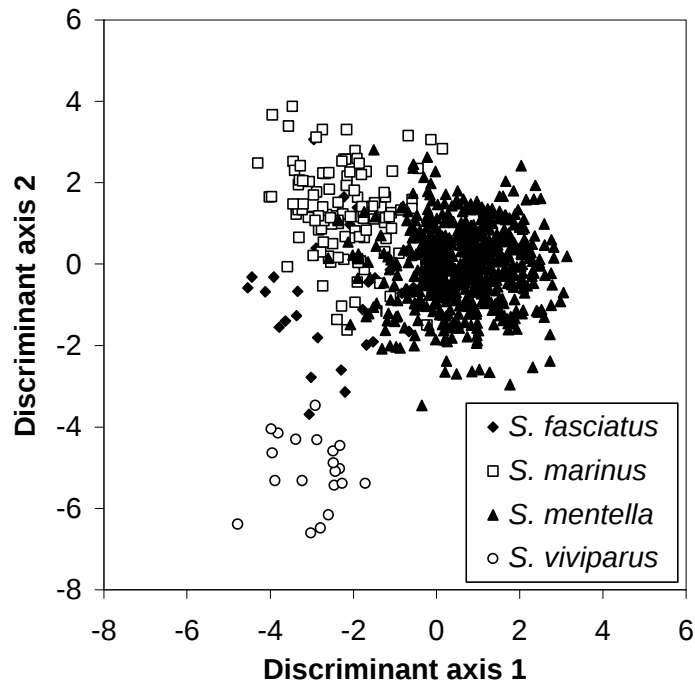


Figure 7. Discriminant function scores for the Fourier descriptors of otoliths from *Sebastes* species in the North Atlantic. The first discriminant axis captures 49.9% of the variation, while the second axis captures 31.6%.

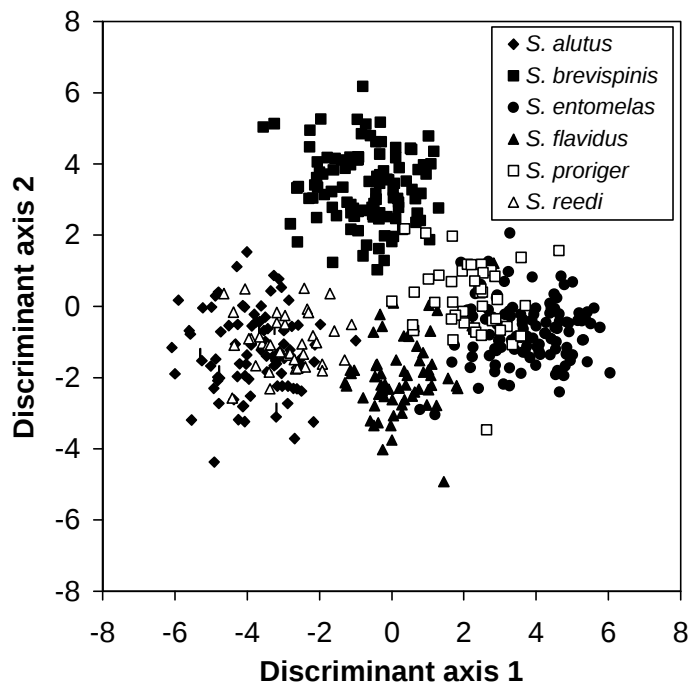


Figure 8. Discriminant function scores for the Fourier descriptors of otoliths from *Sebastes* species in the North Pacific. The first discriminant axis captures 50.7% of the variation, while the second axis captures 24.5%.

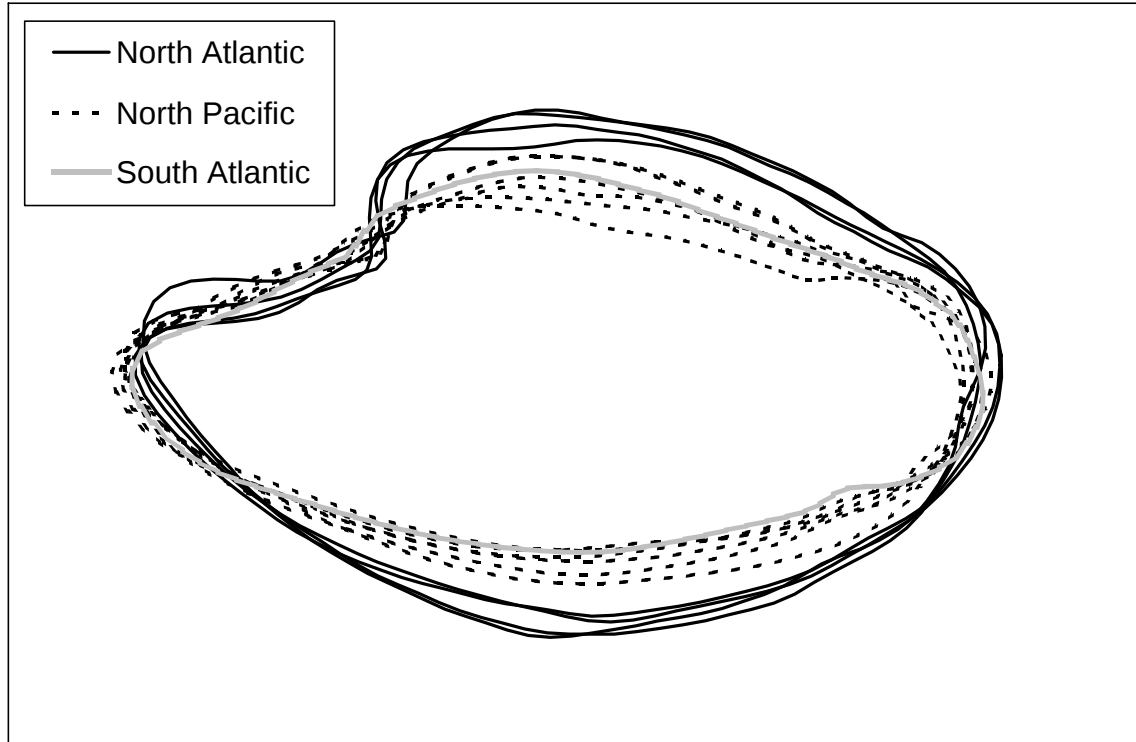


Figure 9. Average shapes of *Sebastes* otoliths from the North Atlantic (*S. fasciatus*, *S. marinus*, *S. mentella*, *S. viviparus*), North Pacific (*S. alutus*, *S. brevispinis*, *S. entomelas*, *S. flavidus*, *S. proriger*, *S. reedi*) and South Atlantic (*S. capensis*).

2.2 Geographic variation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the North Atlantic based on otolith shape analysis

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Abstract

The complex stock structure of North Atlantic redfish (genus *Sebastes*) has raised several problems preventing a stock-adaptive fisheries assessment and management. Within a multidisciplinary project, otolith shapes of golden redfish (*S. marinus*) and deep-sea redfish (*S. mentella*) were analysed for geographic variation within the entire distribution range in the North Atlantic, in order to evaluate this technique for stock separation. Multivariate analysis of Fourier descriptors derived from digitised otolith outlines revealed relatively small differences between sampling sites and high within-area variation. *S. marinus* samples from the central North Atlantic areas (Greenland, Iceland) appeared relatively well separated from samples collected on the Flemish Cap and in the Barents Sea. *S. marinus* from East and West Greenland were closely related and showed high similarity to the Icelandic samples. A separation of central areas from the Flemish Cap and Barents Sea was also observed for *S. mentella*. Otolith shapes of *S. mentella* around Iceland and the Faroe Islands were very similar. The overall jackknifed classification success of the discriminant analysis was poor for both species (< 50%) but increased to 72-74% by combining sampling areas to regions (west, central, east). The observed similarities within the central North Atlantic areas (Greenland, Iceland, Faroe Islands) and weak separation of western and eastern areas are in accordance with current fisheries management units. The lack of clear geographic patterns for *S. mentella* gives no reason for a separation of separate stocks in the Irminger Sea and adjacent waters.

Keywords: redfish, *Sebastes marinus*, *Sebastes mentella*; geographic variation; otolith shape, Fourier analysis

Introduction

Redfish of the genus *Sebastes* provide important fishery resources in the North Atlantic. Among the four species found in the North Atlantic, golden redfish (*S. marinus*) and deep-sea redfish (*S. mentella*), are the most widely distributed and commercially exploited representatives, while Acadian redfish (*S. fasciatus*) is generally limited to the Northwest Atlantic, and small redfish (Norway haddock; *S. viviparus*) is only found in the Northeast Atlantic (Whitehead *et al.* 1986). *S. marinus* grows larger than *S. mentella* and inhabits continental shelves off eastern Canada, Greenland, Iceland, the Faroe Islands, Norwegian waters, the Barents Sea and Svalbard, mainly in depths between 100 and 300 m. *S. mentella* is generally

distributed deeper than *S. marinus* and found in the pelagic zone of the Labrador and Irminger Sea down to 1000 m depth, in addition to the areas where *S. marinus* occurs (e.g. ICES 1998).

Several attempts have been undertaken to investigate the geographic variation of redfish, especially for the highly migratory and widely distributed *S. mentella*. Morphometric and meristic characteristics of the fish body were used by Nagel *et al.* (1991a), Reinert and Lastein (1992), Saborido-Rey (1994) and Saborido-Rey and Nedreaas (2000) to identify sub-units in the distribution of this species. The geographic variation of infestation by the copepod parasite *Sphyrion lumpi* was the subject of several investigations (e.g. Bakay 1988, 2000 and 2001; Nagel *et al.* 1991b; Magnusson 1992, Marcogliese *et al.* 2003), as well as abnormal external coloration and ectolesions (e.g. Bogovski and Bakay, 1989). A series of biochemical and genetic studies were carried out to relate the observed geographic differences to population structure. Gel electrophoresis of haemoglobins and tissue enzymes (Dushchenko 1987, Nedreaas and Nærdal 1989 and 1991, Nedreaas *et al.* 1994, Johansen *et al.* 2000a) was recently followed by microsatellite DNA analyses by Roques *et al.* (2002). The few studies on intraspecific variation of *S. marinus* were also carried out using haemoglobin and enzyme patterns (Nedreaas and Nærdal 1991, Nedreaas *et al.* 1994, Johansen *et al.* 2000b).

In addition to the traditional body morphometry, otolith shape analysis was successfully applied to stock identification of other North Atlantic fish species such as mackerel (Castonguay *et al.* 1991), cod (Campana and Casselman 1993, Cardinale *et al.* 2004), Atlantic salmon (Friedland and Reddin 1994), haddock (Begg and Brown 2000) and herring (Turan 2000). The majority of these studies used Fourier transformations of the outline coordinates (see Lestrel 1997) to quantify differences between proposed stocks. Although most otoliths in general exhibit an ellipsoid shape (Chauvelon and Bach 1993), only very few preliminary applications of elliptic Fourier analysis (Kuhl and Giardina 1982) for the identification of species (Petry 2001) and stocks (Murta 1996, Stransky 2001, 2002 and 2003) were reported to date.

For fisheries management, a clear separation of stocks, ideally resembling self-sustaining populations, would be desirable. The past efforts on clarification of the complex stock structures of redfish, however, all demonstrated high variation within geographic areas or stock units and relatively weak differentiation between these (ICES 1998). The existence of separate stocks of *S. mentella* in the Irminger Sea and adjacent waters has been controversially discussed and raised unsolved problems in the assessment and management of this important fishery resource (Saborido-Rey *et al.* 2001). In a multidisciplinary approach, species and population structure of redfish was investigated on extensive material from all important distribution areas in the North Atlantic. As part of this project, the two-dimensional outline shapes of redfish otoliths were analysed for differences between species (**chapter 2.1**) and proposed stocks, using elliptical Fourier analysis and multivariate techniques. This study investigates geographic

variation in otolith shapes of *S. marinus* and *S. mentella* from the entire distribution area in the North Atlantic in order to provide essential information on the inferred stock structure of these species for an adaptive fisheries management.

Material and methods

Sample collection

Otolith samples were taken from *S. marinus* and *S. mentella*, collected on research vessels and commercial trawlers operating in various redfish fishing areas in the North Atlantic (Table 1, Figure 1). Aiming at a complete coverage of the distribution area of both species, *S. marinus* samples could be obtained from fish caught with bottom trawls on the shelf areas of the Flemish Cap, around Greenland, Iceland and in the Barents Sea, while otoliths from *S. mentella* were additionally taken from demersal catches in the Davis Strait and around the Faroe Islands and from pelagic trawls in the southwestern Irminger Sea. The samples were extracted as sagittal otolith pairs and stored dry in paper envelopes or plastic holders, accompanied by individual fish data (*e.g.* length, weight and sex) recorded on the envelopes or on separate sampling sheets with the corresponding station data (*e.g.* station number, position, date, mean trawl depth). For the Flemish Cap and Davis Strait samples, only fork length data were recorded. These were converted to total length, using regressions between fork and total length for *S. marinus* ($TL = 1.028*FL + 0.075$, $r^2 = 0.999$, $n = 601$) and *S. mentella* ($TL = 1.033*FL - 0.038$, $r^2 = 0.998$, $n = 432$) on the Flemish Cap, respectively (F. Saborido-Rey, unpubl. data). Only otoliths from *S. marinus* measuring over 20 cm and *S. mentella* measuring 30-40 cm in total length were selected, which proved to reduce area*length interactions for the shape descriptors effectively (MANCOVA; $p > 0.05$). Although otoliths were taken as pairs, only samples from the left body side were included into the analysis to ensure that one sample represents one fish. The reduced data set included samples from 399 *S. marinus* and 586 *S. mentella* (Table 1). Before further processing within image analysis, adhering tissue or blood remains were removed from the otolith surface.

Image and shape analysis

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 25x and 50x. 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 OptimasTM 6.51 (Media Cybernetics 1999) image analysis software. 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, OptimasTM macros were applied.

The digitised outline coordinates were forwarded to Elliptical Fourier Analysis (EFA; Kuhl and Giardina 1982, Rohlf and Archie 1984), using C++ modules based on the algorithms of Ferson *et al.* (1985). The methodology of Fourier analysis has been described in detail by several authors (*e.g.* Full and Ehrlich 1982, Bird *et al.* 1986, Lestrel 1997) and will therefore not be presented here. In general, 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. Only the first 15 harmonics were used for multivariate analysis since these were responsible for over 99% of the shape variation (Lestrel 1997). This results in 57 Fourier descriptors (FDs) for each sample, consisting of 3*14 FDs (harmonics 2-15 each) of the sine and cosine parts of the x-direction and sinus part of the y-direction (where FDs of harmonic 1 become constants after the normalisation process, see above) plus 15 FDs (harmonics 1-15) of the cosine part of the y-direction. Before analysing the FDs for differences between areas, the distribution of these data was investigated. All FD amplitudes were normally distributed (Kolmogorov-Smirnov test for normality; $p > 0.05$), so no transformation of the FD data was necessary.

Size correction and multivariate analysis

The correction of morphometric variables by size, *i.e.* uncoupling of otolith shape and fish length in this case, is crucial for further data analysis (Bookstein *et al.* 1985). This was accounted for by using the residuals of the common-within group slopes (Reist 1985, Claytor and MacCrimmon 1987) of the linear regressions of each FD on fish total length. After size-adjustment, the average residuals by area were compared in a set of multivariate analyses. After the calculation of a Euclidean distances matrix, linked by unweighted pair-group average (Sneath and Sokal 1973), multidimensional scaling (MDS) was applied as an ordination technique that offers a detailed comparison of between-group differences (Kruskal and Wish 1978). The so-called stress factor is a measure for the quality of the ordination, values lower than 0.1 being classified as “good”, values lower than 0.05 as “excellent” and values lower than 0.01 as

“perfect” (Clarke and Warwick 1994). As a validation of the grouping patterns, the classification success into these groups was tested within linear discriminant analysis (Klecka 1980, SPSS Inc. 1999). The permuted (jackknifed) classification results also allow a validation of the similarities between groups by listing the misclassification into other areas. Differences between sexes were also tested by discriminant analysis.

Results

The size-correction of the FDs by residuals reduced the correlation between FDs and otolith lengths very effectively. Pearson correlation before size-adjustment was up to $r = 0.42$ with more than two thirds of the FDs being significantly correlated ($p < 0.05$) with otolith length, while the residuals showed no correlation with otolith length ($r < 0.1$, $p > 0.05$). The FD residuals were significantly different between sexes (*S. marinus*: Wilks' $\lambda = 0.772$, $p = 0.004$; *S. mentella*: Wilks' $\lambda = 0.794$, $p < 0.0001$). Since the sex ratio was nearly 1.0 for all areas, this fact was not paid further attention.

MDS ordination of the average size-corrected FDs by area (Figure 2) revealed close relationships between *S. marinus* from the central North Atlantic areas (Iceland, West and East Greenland) and a separation of this group from the Flemish Cap and Barents Sea samples. A strong similarity could be observed between West and East Greenland. These patterns were supported by high misclassification rates (up to 36%) within the central areas in the cross-validation matrix (Table 2). The Barents Sea samples were correctly classified by 67%, representing the highest classification success of the *S. marinus* samples.

For *S. mentella*, the Flemish Cap and Barents Sea samples are also separated from a central group that includes a cluster of very similar areas (Davis Strait, East Greenland, Iceland and Faroe Islands), as well as the Irminger Sea and West Greenland further apart (Figure 3). The separation of the Barents Sea from all other areas is validated by a relatively high classification rate (76%), while misclassification rates of up to 33% (Table 3) within the closely clustered areas of the central group confirm the similarity between these areas apparent in the MDS plot (Figure 3), especially between Iceland and the Faroe Islands. The high misclassification of Flemish Cap samples into the Irminger Sea group and vice versa, however, could not be inferred from the MDS plot. Overall jackknifed classification success into sampling areas was poor for both species (47%).

Combining areas to regions (see Table 1) improved overall correct classification of samples to 74% (*S. marinus*; Table 4) and 72% (*S. mentella*; Table 5). Classification into regions was similar for both

species, with the central and eastern regions being considerably better defined (67-80%) than the western region (< 50%). The western region shows high misclassification into the central region (*S. marinus*: 31%, Table 4; *S. mentella*: 45%, Table 5).

Discussion

This study revealed high within-area variation in otolith shapes of redfish and only weak signals for geographic separation. Geographic variation within redfish species was investigated in several studies, predominantly focusing on the widely distributed *S. mentella*. Morphometric and meristic measurements of the fish body were reported to vary considerably between largely distant areas in the North Atlantic (Nagel *et al.* 1991a, Reinert and Lastein 1992, Saborido-Rey 1994), but also within a relatively limited area in the Northeast Atlantic (Saborido-Rey and Nedreaas 2000). Natural tags such as the infestation by the copepod parasite *Sphyrion lumpi* (Bakay 1988, 2000 and 2001, Nagel *et al.* 1991b, Magnusson 1992), as well as abnormal external coloration and ectolesions (*e.g.* Bogovski and Bakay 1989) pointed to relatively weak sub-structuring of *S. mentella* occurrences. Recently reported parasitological analyses of Marcogliese *et al.* (2003), however, suggested a separation of *S. mentella* on the Flemish Cap, in the Gulf of St. Lawrence and the Laurentian Channel from each other. A pilot study of Reinert *et al.* (1992), using Cs-137 as population marker, indicated a closer relationship of redfish from Faroese waters to samples from the Barents Sea than to Icelandic samples. This was partly confirmed very recently by fatty acid analyses of Joensen and Grahl-Nielsen (2004), dividing the occurrences of *S. mentella* around the Faroe Islands into one group related with the Icelandic shelf and another group connected to the Norwegian coast.

Several biochemical and genetic studies were carried out to investigate redfish population structure. Earlier analyses of haemoglobin and enzyme patterns of Northeast Atlantic *S. mentella* showed low intraspecific variation (Dushchenko 1987, Nedreaas and Nævdal 1989 and 1991, Nedreaas *et al.* 1994), while a more extensive study indicated a structured picture (Johansen *et al.* 2000a). The poor geographic classification by otolith shapes of *S. mentella*, as shown in this study, is in accordance with the earlier genetic studies, as well as recent microsatellite DNA analyses by Roques *et al.* (2002), pointing to a “pan-oceanic” population spanning from the Grand Banks to the Faroe Islands. Although otolith shapes do not necessarily reflect genetic differences, the high individual variation observed in this study prevents differentiation of *S. mentella* population units. Most previous studies pointed to uniformity of *S. mentella* within the Irminger Sea and close relations with the demersal occurrences on the Greenland and Iceland shelves (Saborido-Rey *et al.* 2001). The existence of two proposed stock units of *S. mentella* in the Irminger Sea, as suggested by differences found in haemoglobin and enzyme patterns between “oceanic”

and (pelagic) “deep-sea” *S. mentella* (Johansen *et al.* 2000a), could not be confirmed by any other study to date. No differentiation between these occurrences in the Irminger Sea was found with respect to otolith shapes, neither on a vertical nor on a horizontal scale (Stransky 2002). The Irminger Sea samples in this study appeared separated from the Greenland areas and Iceland, where pelagic redfish are likely to recruit from (*e.g.* Stransky 2000, ICES 2003a) and thus a close relationship would have been expected. On the other hand, *S. mentella* from the Flemish Cap showed considerable similarity with redfish in the southwestern Irminger Sea. The relatively low number of samples from the Flemish Cap, however, does not allow a conclusive statement on the connectivity between these areas.

Although large overlaps between sampling areas were also observed for *S. marinus*, the separation of central North Atlantic areas (Greenland, Iceland) from the Northwest Atlantic (Flemish Cap) and Northeast Atlantic (Barents Sea) is more pronounced than for *S. mentella*. Since *S. marinus* is limited to the shelf areas, a clearer differentiation of geographic units would have been anticipated, though. The considerable similarity between East and West Greenland points to a close relationship between the occurrences on the Greenlandic shelf. Otolith shapes of *S. marinus* from Greenland showed high resemblance to the Icelandic samples, suggesting connectivity between the habitats around Iceland and Greenland. The depth preference of *S. marinus* (usually between 100 m and 300 m) and the lack of a shallow ridge between these shelf areas, however, let a large-scale horizontal migration of *S. marinus* between Greenland and Iceland appear doubtful. This assumption is supported by genetic studies of Nedreaas *et al.* (1994) and Johansen (2003), revealing significant differences between samples from Iceland and Greenland.

For fisheries management purposes, several stock units were established for redfish by the International Council for the Exploration of the Sea (ICES) and the Northwest Atlantic Fisheries Organization (NAFO). *S. marinus* is currently divided into two ICES management units, one comprising the occurrences off Greenland, Iceland and the Faroe Islands (ICES 2003a), and the other one along the Norwegian coast, in the Barents Sea and off Svalbard (ICES 2003b). The same management units apply for demersal *S. mentella*, whereas pelagic *S. mentella* is currently assessed within ICES and managed by the Northeast Atlantic Fisheries Commission (NEAFC). Considering the transboundary distribution of pelagic *S. mentella* within ICES/NEAFC and NAFO areas, a common management of this relatively new resource is currently aimed at (NEAFC 2001, NAFO 2002). In the Northwest Atlantic, demersal redfish, not separated by species, are managed within NAFO in the offshore areas east of Newfoundland, on the Flemish Cap and off West Greenland, while the inshore areas of Canadian and U.S. coastal waters are assessed nationally (NAFO 2004). The geographic variation in otolith shapes of both redfish species investigated in this study gives no reason to deviate from the current fisheries management units. When grouping sampling areas to regions (western, central, eastern North Atlantic), more than 70% of the individuals could be correctly classified, supporting the current division of ICES and NAFO management

regimes. The observed differentiation of the Barents Sea samples from redfish in the central areas is in accordance with the separate management of these redfish resources within ICES (ICES 2003a and 2003b).

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Table 1. Overview of redfish otolith samples used for shape analysis. *S. marinus* samples were restricted to fish total lengths > 20 cm, *S. mentella* samples to 30-40 cm. Only otoliths from the left body side of the fish were included.

Species	Area (Division)	Area code	Region	Sampling date or range	No. of otoliths
<i>S. marinus</i>	Flemish Cap (NAFO 3M)	FC	West	Jul 2001	48
<i>S. marinus</i>	West Greenland (NAFO 1C-1F)	WG	Central	Sep/Oct 2000	61
<i>S. marinus</i>	East Greenland (ICES XIV)	EG	Central	Sep/Oct 2000	96
<i>S. marinus</i>	Iceland (ICES Va)	IC	Central	Feb/Mar 2001	143
<i>S. marinus</i>	Barents Sea (ICES I and II)	BS	East	Jul/Aug 2000	51
<i>S. mentella</i>	Flemish Cap (NAFO 3M)	FC	West	Jul 2001	14
<i>S. mentella</i>	Davis Strait (NAFO 0B)	DS	West	Sep/Oct 2001	15
<i>S. mentella</i>	West Greenland (NAFO 1C-1F)	WG	Central	Sep/Oct 2000	13
<i>S. mentella</i>	East Greenland (ICES XIV)	EG	Central	Sep/Oct 2000	73
<i>S. mentella</i>	Irminger Sea (ICES XII, NAFO 1F)	IR	Central	Jun/Jul 1999	318
<i>S. mentella</i>	Iceland (ICES Va)	IC	Central	Oct 2000-Apr 2001	77
<i>S. mentella</i>	Faroe Islands (ICES Vb)	FA	Central	Sep/Oct 1999	51
<i>S. mentella</i>	Barents Sea (ICES I and II)	BS	East	Jul/Aug 2000	25

Table 2. Jackknifed classification of the discriminant analysis between the size-corrected Fourier descriptors for *S. marinus* otoliths. The percentages in rows represent the classification into the areas given in columns, sample sizes are given in parentheses. Overall classification success is 47%, Wilks' $\lambda = 0.185$.

	Flemish Cap	West Greenland	East Greenland	Iceland	Barents Sea
Flemish Cap	45.8 (22)	8.3 (4)	10.4 (5)	16.7 (8)	18.8 (9)
West Greenland	3.3 (2)	34.4 (21)	36.1 (22)	19.7 (12)	6.6 (4)
East Greenland	8.3 (8)	19.8 (19)	54.2 (52)	13.5 (13)	4.2 (4)
Iceland	13.3 (19)	19.6 (28)	21.7 (31)	40.6 (58)	4.9 (7)
Barents Sea	13.7 (7)	3.9 (2)	7.8 (4)	7.8 (4)	66.7 (34)

Table 3. Jackknifed classification of the discriminant analysis between the size-corrected Fourier descriptors for *S. mentella* otoliths. The percentages in rows represent the classification into the areas given in columns, sample sizes are given in parentheses. Overall classification success is 47%, Wilks' $\lambda = 0.159$.

	Flemish Cap	Davis Strait	West Greenland	East Greenland	Irminger Sea	Iceland	Faroe Islands	Barents Sea
Flemish Cap	21.4 (3)	14.3 (2)	0.0 (0)	0.0 (0)	50.0 (7)	0.0 (0)	14.3 (2)	0.0 (0)
Davis Strait	33.3 (5)	6.7 (1)	6.7 (1)	13.3 (2)	0.0 (0)	33.3 (5)	0.0 (0)	6.7 (1)
West Greenland	0.0 (0)	15.4 (2)	23.1 (3)	15.4 (2)	0.0 (0)	30.8 (4)	15.4 (2)	0.0 (0)
East Greenland	4.1 (3)	11.0 (8)	12.3 (9)	39.7 (29)	5.5 (4)	15.1 (11)	6.8 (5)	5.5 (4)
Irminger Sea	12.6 (40)	4.7 (15)	4.7 (15)	5.7 (18)	59.1 (188)	4.1 (13)	5.0 (16)	4.1 (13)
Iceland	5.2 (4)	13.0 (10)	14.3 (11)	10.4 (8)	10.4 (8)	28.6 (22)	16.9 (13)	1.3 (1)
Faroe Islands	7.8 (4)	7.8 (4)	15.7 (8)	7.8 (4)	9.8 (5)	21.6 (11)	23.5 (12)	5.9 (3)
Barents Sea	0.0 (0)	8.0 (2)	0.0 (0)	4.0 (1)	8.0 (2)	4.0 (1)	0.0 (0)	76.0 (19)

Table 4. Jackknifed classification of the discriminant analysis between the size-corrected Fourier descriptors for *S. marinus* otoliths from three regions in the North Atlantic (see Table 1). The percentages in rows represent the classification into the areas given in columns, sample sizes are given in parentheses. Overall classification success is 74%, Wilks' $\lambda = 0.336$.

	West	Central	East
West	47.9 (23)	31.3 (15)	20.8 (10)
Central	10.7 (32)	80.0 (240)	9.3 (28)
East	15.7 (8)	17.6 (9)	66.7 (34)

Table 5. Jackknifed classification of the discriminant analysis between the size-corrected Fourier descriptors for *S. mentella* otoliths from three regions in the North Atlantic (see Table 1). The percentages in rows represent the classification into the areas given in columns, sample sizes are given in parentheses. Overall classification success is 72%, Wilks' $\lambda = 0.550$.

	West	Central	East
West	48.3 (14)	44.8 (13)	6.9 (2)
Central	21.4 (114)	73.5 (391)	5.1 (27)
East	0.0 (0)	24.0 (6)	76.0 (19)

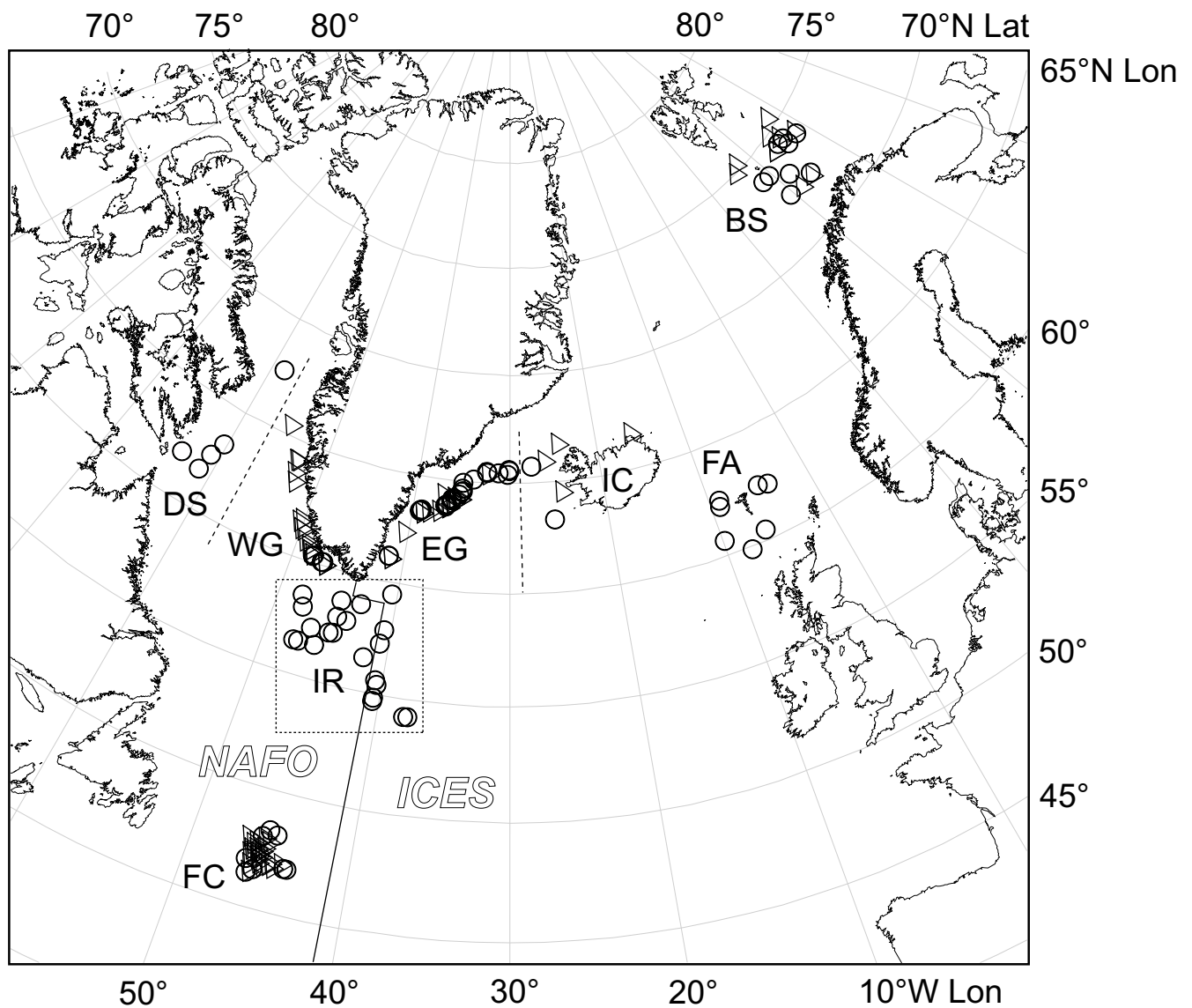


Figure 1. Sampling positions and areas of redfish otoliths (see Table 1 for area codes). *S. marinus* samples are marked by triangles, and *S. mentella* samples by circles. Separation lines between sampling areas are drawn dashed. The division line between NAFO (Northwest Atlantic Fisheries Organization) and ICES (International Council for the Exploration of the Sea) regulatory areas is shown as a solid line at 42° and 44°W, respectively. Map source: GEBCO, polar projection.

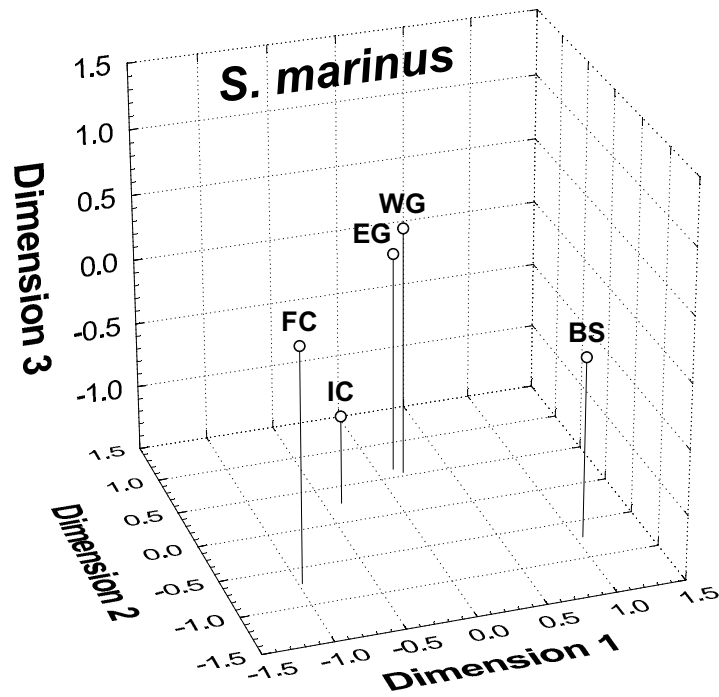


Figure 2. Three-dimensional MDS ordination plot of the Euclidian distances between average size-corrected Fourier descriptors by area (see Table 1 for area codes) for *S. marinus* (stress < 0.00001).

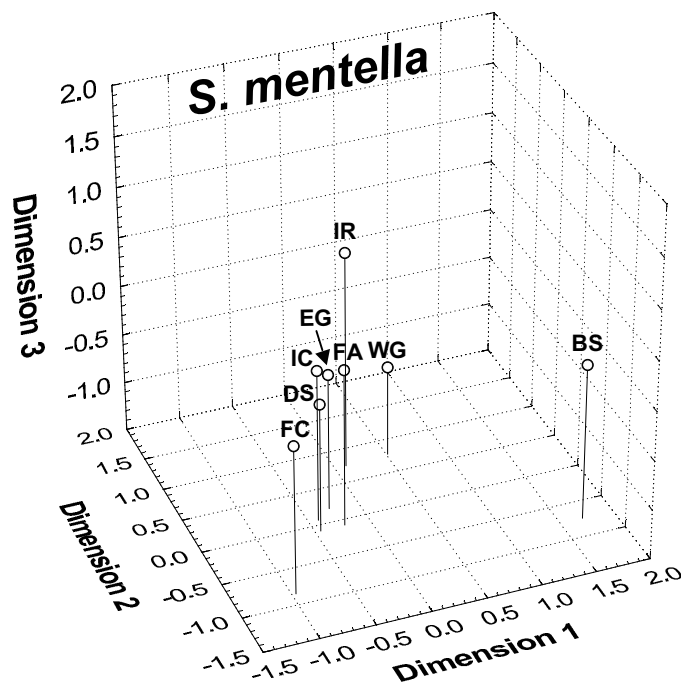


Figure 3. Three-dimensional MDS ordination plot of the Euclidian distances between average size-corrected Fourier descriptors by area (see Table 1 for area codes) for *S. mentella* (stress < 0.001).

2.3 Methodological comparison: Otolith shape analysis as a tool for stock separation of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean

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Abstract

The geographic variability in otolith shape of horse mackerel (*Trachurus trachurus*) was investigated as a tool for stock separation. The outlines of several thousand otoliths, collected in 20 sampling areas during two consecutive years and covering most of the distributional range of the species in the Northeast Atlantic and Mediterranean, were digitised and analysed for shape variation by elliptical Fourier analysis. Only fish of a medium total length (20-35 cm) were included in the data analysis to minimise size effects between areas. The extracted Fourier descriptors were corrected for fish size preceding multivariate analysis. Multidimensional scaling of the average Fourier descriptors by area, combining both sampling years, showed three distinct clusters of areas: a northern, an Ibero-Mauritanian and an eastern Mediterranean group. These patterns were supported by discriminant analysis of the individual Fourier descriptors, with correct classifications ranging from 31-95%. Similar groups could be observed when analysing the two sampling years separately. The discrimination between these groups was 81-88%, while individuals from the Northeast Atlantic and Mediterranean were correctly classified by around 88-91%. Average otolith shapes for these groups showed characteristic differences, especially in the dorsal part of the otolith outline. The results of this study indicate that the separation line between the western and the southern stock should be shifted to the coast of southern Galicia (Cape Finistere). In contrast to previous genetic and morphometric studies, the observed variation in otolith shapes could not confirm a separation of a western horse mackerel stock from the North Sea stock. Further, fish from the areas around the Iberian Peninsula (including the western Mediterranean) appear to be very similar to the northwest African occurrences. By integration of the inferred information on stock structure into a currently pursued multidisciplinary approach to stock identification of horse mackerel, the stock-adaptive assessment and sustainable management of horse mackerel in the Northeast Atlantic and Mediterranean will be significantly improved.

Keywords: horse mackerel, *Trachurus trachurus*; Northeast Atlantic, Mediterranean; geographic variation, stock identification; otolith shape, Fourier analysis

Introduction

Horse mackerel (*Trachurus trachurus*) are widely distributed in the world's oceans and represent a key species for fisheries. In the Northeast Atlantic, horse mackerel commonly occur on the continental shelf from the Norwegian Sea to Senegal, including the North Sea, Mediterranean and Black Sea (*e.g.* Whitehead *et al.* 1986). In European waters, these occurrences have been divided into three stocks that are assumed to form distinct spawning populations (ICES 1992): the western stock in the Norwegian Sea, the northern North Sea, west and south of the British Isles, in the western English Channel and the northern Bay of Biscay; the North Sea stock in the central and eastern parts of the North Sea and in the eastern English Channel; and the southern stock in the Atlantic waters off the Iberian Peninsula. Rückert *et al.* (2002), however, divided the North Sea stock into a distinct northern and southern component. In the Mediterranean and off the west African coast (Saharo-Mauritanian stocks), there is currently no clear separation of horse mackerel stocks.

Earlier stock identification studies have not provided firm evidence for the existence of three horse mackerel stocks (Polonsky and Baydalinov 1964, Nazarov 1976). A significant genetic separation of a northern and southern component was found by Nevedov *et al.* (1978). Borges *et al.* (1993), however, could not confirm these results and reported on the lack of genetic heterogeneity between samples from the North Sea, west of the British Isles, the Bay of Biscay and the Portuguese coast. Further attempts to discriminate horse mackerel stocks have involved measurements of the otoliths at age 1 (Marecos 1986) and body morphometry (Borges 1996, Murta 2000). In addition to body morphometrics and meristic features, otolith shape analysis has become an effective tool for fish species and stock identification. Otolith shapes were shown to be species-specific (*e.g.* Gaemers 1984, L'Abée-Lund 1988), and in many cases, geographic variation in otolith shapes could be related to stock differences (*e.g.* Messieh 1972, Campana and Casselman 1993, Begg and Brown 2000). Small-scale variation in otolith shapes of horse mackerel along the Portuguese coast has been investigated by Murta *et al.* (1996). By precluding confounding effects of sex, age and year-classes on the comparison of otolith shapes between more distant areas, they paved the way for large-scale studies on otolith shape variation and a possible use for stock identification.

In this study, geographical variation in horse mackerel otolith shape was investigated as part of a multidisciplinary approach to horse mackerel stock discrimination using a variety of techniques such as genetics, morphometry, parasite infestation and life-history parameters. Most of the distribution area of the species in the Northeast Atlantic was sampled for the first time, and all methods were applied to the same individual specimens. Considering the highly migratory nature of horse mackerel and the confusion observed during past attempts of stock discrimination, otolith shape analysis is

evaluated as a tool for stock separation of horse mackerel, and the resulting hypothetical stock definitions are compared to the stock delineations currently used for fisheries management.

Material and Methods

Sampling

Horse mackerel were either sampled on research vessels, or from commercial trawlers using bottom trawl gears in a mixed fishery. The sampling areas were situated in the North Sea, off southern Norway, along the shelf edge west of the British Isles and Brittany, in the Bay of Biscay, around the Iberian Peninsula, off the Mauritanian coast and in the Mediterranean (Table 1, Figure 1). The sites were selected after combining information on the boundaries of current stock units, the geographical distance between sites, the distribution pattern of horse mackerel and the oceanographic characteristics of the areas. The collection of samples carried out during 2000 and 2001 was focused on the spawning time, minimising mixing effects between population sub-units. The collected horse mackerel were stored frozen as whole fish. In the frame of multidisciplinary investigations of the identical material, sagittal otoliths were taken as pairs, rinsed with de-ionised water, air-dried and stored in plastic tubes.

Image and shape analysis

For image analysis, 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. 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 30x and 50x. The image analysis system was calibrated in horizontal and vertical direction separately to avoid possible distortion effects of the lens system. The video signal was analysed using OptimasTM 6.51 (Media Cybernetics 1999) image analysis software. 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, OptimasTM macros were applied.

The outline coordinate data were forwarded to Elliptical Fourier Analysis (EFA; Kuhl and Giardina 1982, Rohlf and Archie 1984), using C++ modules based on the algorithms of 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. Only the first 16 harmonics were used for multivariate analysis since these were responsible for over 99% of the shape variation (Lestrel 1997). This results in 61 Fourier descriptors (FDs) for each sample, consisting of 3*15 FDs (harmonics 2-16 each) of the sine and cosine parts of the x-direction and sinus part of the y-direction (where FDs of harmonic 1 become constants after the normalisation process, see above) plus 16 FDs (harmonics 1-16) of the cosine part of the y-direction. Before analysing the FDs for differences between areas, the distribution of these data was investigated. As indications for deviations from normal distribution were found for some areas, multivariate methods were chosen which do not assume normality of data. Fourth root normalisation of the FD data, however, did not reveal significantly different multivariate analysis results.

Size correction and multivariate analyses

From over 5000 processed otoliths, only samples from the left body side were included into the data analysis to ensure that one sample represents one fish and to avoid possible asymmetry effects. Only otoliths from fish of 20-35 cm total length were selected (Table 1), which reduced the amount of FDs with significant ($p < 0.05$) area*length interactions from 42 to 9 (two-way ANOVA, dependent variable: FDs, independent variables: area, fish total length; SYSTAT® 9, SPSS Inc. 1999). Since the FDs are part of a polynomial function describing the otolith shape, the exclusion of FDs with significant area*length interactions would lead to loss of shape information. Thus, the full set of 61 FDs was used for subsequent analysis. Most important for morphometric analysis is the correction of the data by size, *i.e.* uncoupling of otolith shape and fish length in this case. This was accounted for by using residuals (Reist 1985) of the linear regressions of each FD on fish length, adjusted by the common-within group slopes (Reist 1986, Claytor and MacCrimmon 1987). This size-correction reduced the correlation between FDs and fish lengths effectively (from $r^2_{\max.} = 0.45$ to $r^2_{\max.} = 0.21$), although it did not eliminate size-effects completely.

After size-adjustment, the mean residuals by area were compared in a set of multivariate analyses. First, a hierarchical cluster analysis on the basis of Euclidean distances, linked by unweighted pair-group average (Sneath and Sokal 1973), was performed. Other distance and similarity indices were

used for comparison but indicated no significant deviation from the multivariate patterns derived from Euclidian distances. Multidimensional scaling (MDS) was applied to the distance matrices, an ordination technique which offers a much more detailed comparison of between-group differences (Kruskal and Wish 1978), especially in the 3-dimesional representation. The so-called stress factor is a measure for the quality of the ordination, values lower than 0.1 being classified as “good”, values lower than 0.05 as “excellent” and values lower than 0.01 as “perfect” (Clarke and Warwick 1994). As a validation of the grouping patterns, the classification success into these groups was tested within a discriminant analysis (Klecka 1980). The permuted (jackknifed; SPSS Inc. 1999) classification results also allow a validation of the similarities between groups by listing the misclassification into other areas. For a direct comparison of geographical differences between otolith shapes, size-normalised average shapes were calculated from the mean FDs for each area and presented as overlay graph of the reproduced outlines.

Results

In the first series of multivariate analyses of the FD data, the sampling years 2000 and 2001 were pooled to provide an overall picture of the between-area differences in otolith shapes. Figure 2 presents the three-dimensional MDS ordination of the Euclidean distances between the mean FDs of each area, suggesting three main groups of areas: “northern” (areas 1, 2, 3, 5, 7, 21), “Ibero-Mauritanian” (areas 8, 9, 10, 11, 12, 17 and 20) and “eastern Mediterranean” (areas 13, 15, 16, 18, 19). Area 6 (Britanny) appears as an “outlier” between the northern and Ibero-Mauritanian groups. In the Mediterranean, area 14 (Adriatic Sea) represents an outlier separated from the eastern Mediterranean group. The base of the ordination “stick” of area 12 is affiliated with the northern group but links with the Ibero-Mauritanian group in the third dimension (Figure 2). The separate MDS analyses of the sampling years 2000 and 2001 (Figure 3) provide grouping patterns that do not differ significantly from the combined analysis of both sampling years. The northern areas form a relatively dense cluster in both years, while the Mediterranean areas are more widespread. In contrast to the MDS plot for both sampling years combined, area 15 (Ionian Sea) is ordinated further apart from the eastern Mediterranean areas. Area 20 (Gulf of Lions) also shows similarity to area 17 (Alboran Sea) in 2000 (Figure 3, left panel) and to area 10 (Algarve) in 2001 (Figure 3, right panel), but rather in an outlier position.

The classification results of the discriminant analysis (Table 2) of the individual samples validate most of the patterns observed in the MDS analyses (Figures 2 and 3). The high misclassification percentages (up to 21%) within the northern areas as well as within the areas along the Portuguese coast (8, 9, 10) support the separation of these groups from each other and point to high within-group

similarities. Within the Mediterranean areas, the linkages are not as clear-cut and do not always support the similarities found in the MDS analyses. Area 15, for example, misclassifies not only into other Mediterranean areas (up to 7%), but also into the Atlantic areas closest to the Strait of Gibraltar, namely areas 9 (Setúbal; 9%) and 10 (Algarve; 11%). The close relationship of area 21 (Southwest Bay of Biscay) to areas 2 and 3 (west of Ireland; 10% misclassification each), however, is in accordance with the MDS results. The classification matrix also supports the similarity of area 7 (Galicia) to areas 2, 3 and 5 (7-10% misclassification). In contrast, area 6 misclassifies into area 3 to 20% which is not apparent in the MDS analyses. Table 3 presents the classification success of Atlantic (areas 1-11 and 21) and Mediterranean areas (12-20), showing very clear separation (around 90%) and slightly higher values for the Atlantic than for the Mediterranean. To validate the separation of the three area groups inferred from the combined analysis of both sampling years, a discriminant analysis of these groups was carried out, revealing classifications of > 80% in all cases (Table 4). Leaving out the “outlier” areas 6 and 14 only changed this classification slightly (around +2%).

To visualise differences in average shapes, the reproduced outlines of the mean FDs by area group were plotted as overlay picture (Figure 4). Overall, the highest between-area variation clearly occurs in the breadth direction of the otolith, especially in the upper (dorsal) part. From the three clusters of areas, identified by multivariate analyses, the Ibero-Mauritanian areas separate best from the other areas. The eastern Mediterranean areas show a slight tendency of “slimmer” otoliths, only visible in the lower (ventral) part of the otolith.

Discussion

The presented patterns in geographic variation of otolith shapes deviate only partly from current stock definitions (ICES 1992). Area 5, the only representative of the North Sea stock, does not clearly separate from the Western stock (areas 1, 2, 3, 6) and shows similarity to area 7 (Galicia). This may indicate some mixing with the Southern stock through the English Channel and with the Western stock along the Norwegian coast. High within-region similarity was found for the northern areas and the Portuguese coast, markedly separated from each other. Since area 7 off the Spanish Atlantic coast, so far allocated to the Southern stock, was shown to be more connected to the northern Atlantic areas (Western stock) than to the Portuguese coast, these results suggest a boundary of the Western to the Southern stock, re-located between areas 7 and 8, along the coast of southern Galicia off Cape Finistere (Figure 5). Area 11 showed a relatively close relationship to the Portuguese areas, contradicting a boundary between the Southern and the Saharo-Mauritanian stocks at the Strait of Gibraltar. The western Mediterranean areas 12, 17 and 20 exhibited more similarity to the southern Atlantic areas 10 and 11 than to the eastern Mediterranean, suggesting interbreeding between these

areas. Nevertheless, this observation has to be interpreted with care, since the relatively low number of samples from these areas reduces the within-area variation considerably, as indicated by high classification success. A clear differentiation could not be inferred from the classification of the eastern Mediterranean areas, preventing the establishment of sub-units based on the presented data. In contrast, the overall classification of Atlantic and Mediterranean areas revealed an unambiguous discrimination (around 90%) between these two major regions. Relatively high discriminatory success (over 80%) was also reached for the three groups of areas derived from MDS analysis.

Geographical separation of horse mackerel stock components was relatively weak in earlier genetic studies (Polonsky and Baydalinov 1964, Nazarov 1976), while Nevedov *et al.* (1978) could separate a northern and southern component on the basis of muscle enzyme patterns. Borges *et al.* (1993), however, did not find genetic heterogeneity at the plasma transferrin locus between horse mackerel from the North Sea, west of the British Isles, the Bay of Biscay and off the Portuguese coast. Morphometric analyses by Borges (1996) revealed significant differences between horse mackerel west of the Iberian Peninsula and fish from the northern part of the Bay of Biscay, the Celtic Sea and the English Channel. Our results support the lack of significant variation between the North Sea and Western stock components and the separation of the occurrences off the Portuguese coast from these “northern” components. On the basis of 14 morphometric and 5 meristic characters, Murta (2000) found high similarity between samples from the Portuguese coast and considerable differences to samples from the Spanish coast off Cadiz and from the Moroccan coast. The observed variation in otolith shapes does not support a separation of horse mackerel off the Portuguese coast from the Mauritanian area, indicating only low or negligible differences between the Northwest African and southern Iberian occurrences.

Since this study is focusing only on geographic variation in otolith shapes, the results should also be treated only as part of a multidisciplinary study, involving life history data, parasite infestation patterns and genetic markers. Ihssen *et al.* (1981) were among the first to join a variety of fish stock identification methods. More recently, Smith *et al.* (2002) gave an extended example of combining a series of techniques applied to the same material and reported relatively high discriminatory power of otolith shape analysis, besides nuclear DNA markers. Further examples of the “holistic approach” to stock identification were reviewed by Begg and Waldman (1999) and Waldman (1999). By overlaying all available information from a range of techniques, a generalised and consistent pattern of stock structure can be developed and provides the scientific basis for sustainable management of the considered stocks. Preliminary results from body morphometrics and parasite infestation patterns on the same material that was used for the presented otolith shape analyses, are in accordance with the division of stock units inferred from the presented otolith shape patterns. These integrative analyses also include life history parameters such as growth and reproduction (Abaunza *et al.* 2003), adding

important information for the stock-adaptive assessment of horse mackerel in the Northeast Atlantic and Mediterranean.

In conclusion, otolith shape analysis proved to be a powerful tool for stock discrimination of horse mackerel in the North Atlantic and Mediterranean, representing a highly migratory pelagic fish species with high probability of interbreeding. The revised stock definitions of horse mackerel in these regions, based on reproducible geographical patterns of biological features, have immediate impact on the assessment and management of horse mackerel. The applicability of the presented methods to other fish species, such as Atlantic redfish (*Sebastes* spp.; Stransky, unpubl. data) and herring (*Clupea harengus*; Jansen, unpubl. data), is currently evaluated within comparable multidisciplinary approaches to stock identification.

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Table 1. Horse mackerel otoliths used for otolith shape analysis by areas and sampling years. Only otoliths from the left body side from fishes with total lengths of 20-35 cm were included in the analysis. Stock definitions for the Atlantic areas were taken from ICES (1992). Areas where no sampling was possible or where less than 10 samples were available are marked by “-“.

Area code	Area name	Stock / region	No. of samples		
			2000	2001	Total
1	Norway	Western	59	69	128
2	Porcupine	Western	40	146	186
3	Great Sole	Western	46	84	130
5	German Bight	North Sea	66	89	155
6	Britanny	Western	55	-	55
7	Galicia	Southern	51	84	135
8	Aveiro	Southern	73	79	152
9	Setúbal	Southern	74	34	108
10	Algarve	Southern	55	60	115
11	Mauritania	Saharo-Mauritanian	-	76	76
12	Balearic Islands	Mediterranean	-	20	21
13	Tyrrhenian Sea	Mediterranean	-	45	52
14	Adriatic Sea	Mediterranean	38	49	87
15	Ionian Sea	Mediterranean	11	33	44
16	Aegean Sea	Mediterranean	-	78	78
17	Alboran Sea	Mediterranean	14	-	20
18	Sardinia	Mediterranean	-	31	31
19	Sicily	Mediterranean	53	13	66
20	Gulf of Lions	Mediterranean	23	21	44
21	Bay of Biscay	Western	27	85	112
Total			693	1102	1795

Table 2. Jackknifed classification matrix of the discriminant analysis between sampling areas. The percentages in rows represent the classification into the areas given in columns. The sampling years 2000 and 2001 were combined. Overall classification success: 58%, Wilks' $\lambda = 0.0033$.

Area	1	2	3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	50	15	4	6	2	2	4	2	1	0	0	1	2	2	1	0	1	0	0	9
2	17	32	9	7	4	8	3	1	1	1	0	0	0	2	2	1	3	0	0	10
3	7	14	31	7	21	4	2	2	1	0	0	0	0	0	1	0	1	0	0	10
5	3	4	3	66	2	7	3	0	0	3	0	3	1	1	0	1	1	0	0	3
6	2	4	20	4	56	5	4	2	0	0	0	0	0	0	0	0	0	0	0	4
7	2	7	10	7	4	49	5	4	1	0	0	1	0	1	0	0	0	1	0	7
8	1	1	1	1	1	9	53	16	6	3	0	1	0	1	0	1	0	0	3	1
9	1	1	1	1	4	4	15	61	5	3	0	2	1	2	0	0	0	1	0	0
10	0	0	0	1	1	1	3	9	67	3	1	1	1	7	0	1	0	0	6	0
11	0	1	0	4	0	1	3	0	4	83	0	1	0	0	0	1	0	0	0	1
12	0	0	5	0	0	0	0	0	0	0	95	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	2	0	2	2	0	0	83	4	6	0	2	0	0	0	0
14	2	0	0	0	0	1	0	1	1	0	0	6	75	6	5	0	0	1	1	1
15	0	2	0	0	0	2	2	9	11	0	0	2	2	45	7	0	7	5	2	2
16	1	3	0	0	0	0	0	0	0	0	0	0	5	3	79	0	1	6	0	1
17	0	0	0	0	0	0	0	0	5	5	0	0	0	0	0	90	0	0	0	0
18	0	3	0	0	3	0	0	0	0	0	0	3	3	3	10	0	74	0	0	0
19	0	0	0	0	0	2	0	2	2	0	0	3	8	3	6	0	2	74	0	0
20	0	2	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	93	0
21	6	10	10	4	3	7	2	1	0	0	1	1	1	0	2	2	1	0	0	51

Table 3. Jackknifed classification matrix of the discriminant analysis between Atlantic (areas 1-11 and 21) and Mediterranean (areas 12-20) sampling sites. The percentages in rows represent the classification into the areas given in columns. Sampling years 2000 and 2001 were combined. Overall classification success: 90%, Wilks' $\lambda = 0.4513$.

	Atlantic	Mediterranean
Atlantic	91	9
Mediterranean	12	88

Table 4. Jackknifed classification matrix of the discriminant analysis between three groups of sampling areas, as revealed by MDS ordination of the average Fourier descriptors by area. The percentages in rows represent the classification into the areas given in columns. The sampling years 2000 and 2001 were combined. Overall classification success: 85%, Wilks' $\lambda = 0.1942$.

	Northern	Ibero-Mauritanian	Eastern Med.
Northern	85	10	5
Ibero-Mauritanian	15	81	3
Eastern Med.	5	7	88

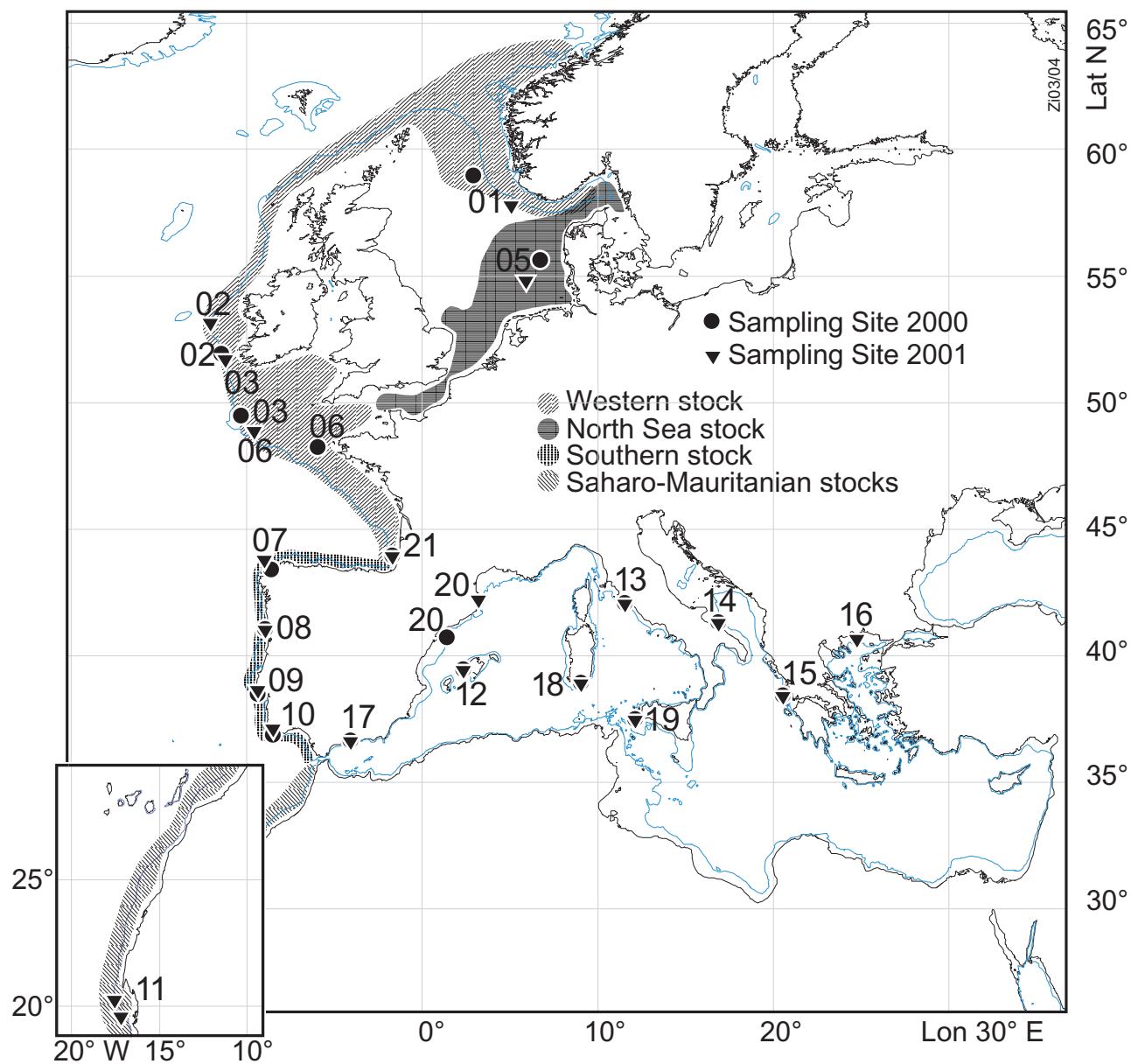


Figure 1. Central positions of the horse mackerel (*Trachurus trachurus*) sampling areas (see Table 1 for area codes) and distribution of the currently defined stocks (ICES 1992). Map source: GEBCO, 200 m depth contour drawn. Cartesian projection, inset in the same scale.

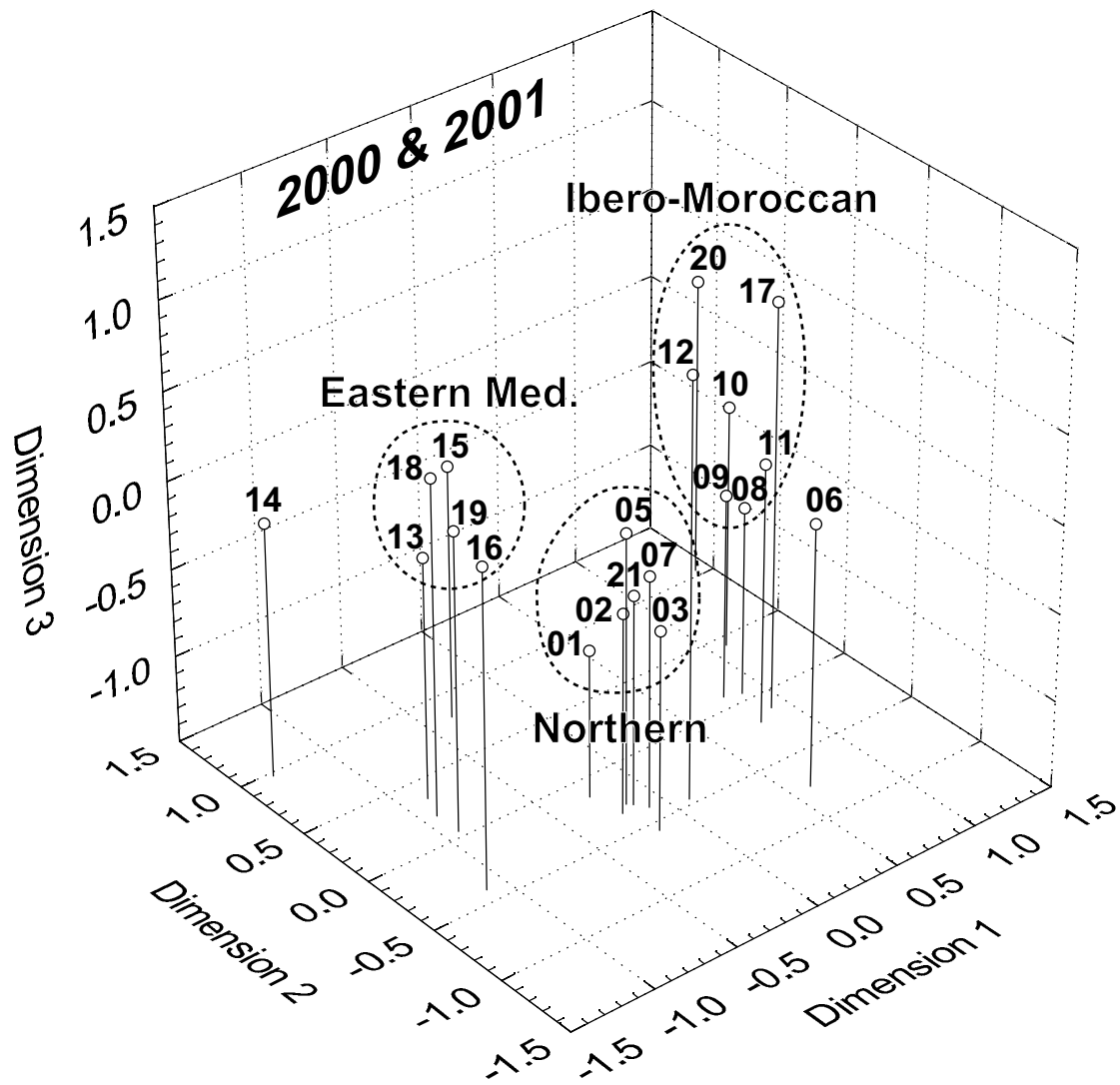


Figure 2. MDS ordination plot of the Euclidean distances between mean size-corrected Fourier descriptors in each sampling area (see Table 1 for area codes). Sampling years 2000 and 2001 were combined. Stress value of the plot = 0.027.

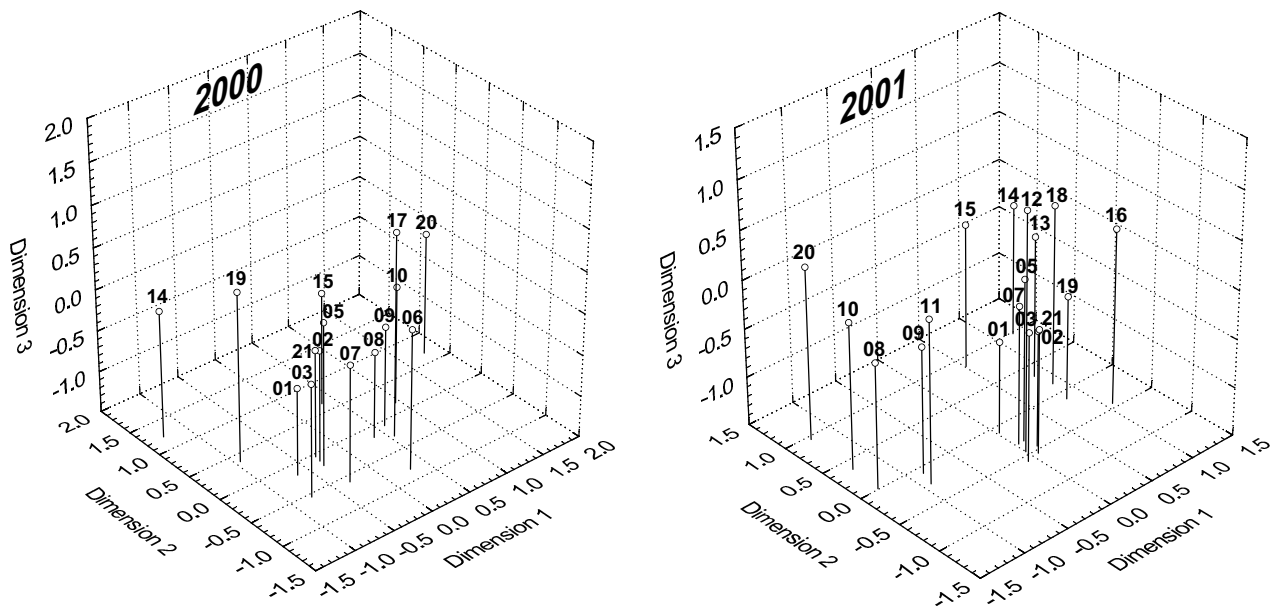


Figure 3. MDS ordination plot of the Euclidean distances between mean size-corrected Fourier descriptors in each sampling area (see Table 1 for area codes). Left panel: only samples taken in 2000 were included (stress = 0.012); right panel: only samples taken in 2001 were included (stress = 0.028).

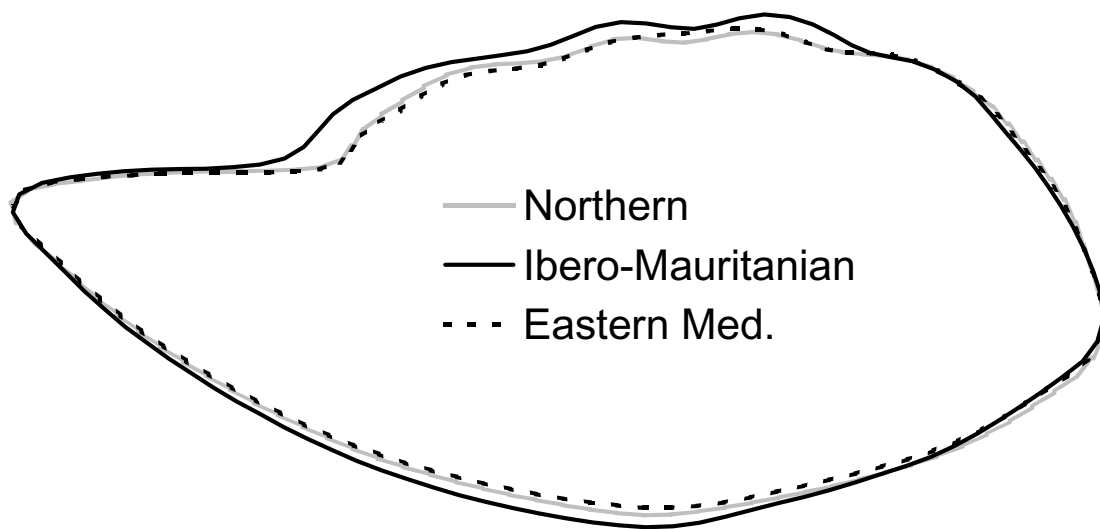


Figure 4. Average shapes of the otoliths in the three groups of sampling areas, as revealed by multivariate analysis of the Fourier descriptors. The sampling years 2000 and 2001 were combined.

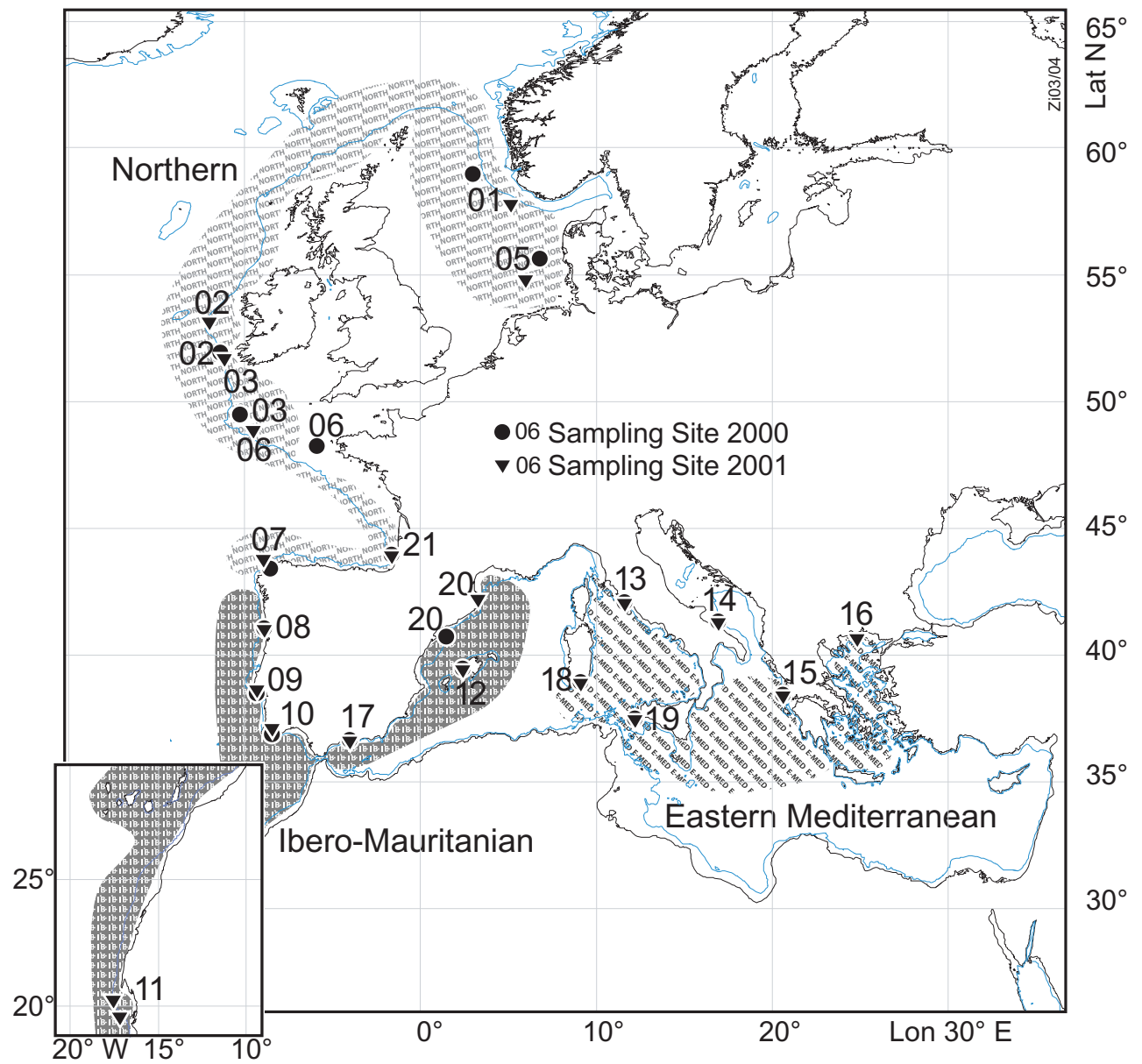


Figure 5. Proposed stock separation of horse mackerel inferred from multivariate analysis of the Fourier descriptors. Map source: GEBCO, 200 m depth contour drawn. Cartesian projection, inset in same scale.

2.4 Microchemistry of Atlantic redfish otoliths: temporal stability, geographic variation and migration

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Abstract

Golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) are heavily exploited fish resources in the North Atlantic, notwithstanding the unresolved delineation of stock units providing the scientific basis for stock-adaptive fisheries management. As part of a multidisciplinary project, this study examined the use of otolith microchemistry as a stock separation tool for redfish. By determining minor and trace elements in different zones of redfish otoliths from various fisheries regions across the North Atlantic, geographic and temporal variation, as well as migration patterns were investigated. Relatively high temporal stability in otolith elemental composition was found for juvenile redfish from a major nursery area off East Greenland, collected during five consecutive years. Elemental concentrations, measured in the nucleus, juvenile and marginal otolith zones, were found to differ significantly between sampling areas and showed consistent longitudinal trends for Li, Mn and Cu. Li concentrations were always higher in the Northwest (Flemish Cap, Davis Strait) and Northeast Atlantic (Barents Sea) than in the central areas (Greenland, Iceland, Irminger Sea, Faroe Islands), while the opposite was found for Mn and Cu. Multivariate analysis of element constituents by area, however, revealed poor geographic separation (< 50% cross-validated classification success) for both species, comparable to recent studies on deep-sea fish in the Northeast Atlantic. Elevated Sr and Ba levels were observed in the otolith edge regions, as compared to the inner growth zones, whereas Li and Mn exhibited opposite patterns. Ontogenetic effects or changes in growth rate are most likely responsible for this phenomenon also reported for other marine fish species. The effect of water chemistry or dietary uptake could not be tested due to insufficient resolution of available trace element and stomach content data. The recently found evidence for migration of juvenile *S. mentella* from the East Greenland shelf into the pelagic habitat of the Irminger Sea could be confirmed by similarity in nucleus chemistry, indicating a common natal origin. The connectivity within the central North Atlantic, inferred from otolith elemental signatures, and the observed weak separation from the Northwest and Northeast Atlantic are in accordance with results from concurrently undertaken body and otolith morphometrics and recent genetic results supporting current fisheries management units.

Keywords: redfish, *Sebastes marinus*, *Sebastes mentella*; North Atlantic; geographic variation, temporal variation, migration; otolith microchemistry, elemental signatures, LA-ICP-MS

Introduction

Golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) are widely distributed in the North Atlantic and provide important fishery resources. *S. marinus* is found on the continental shelves off eastern Canada, Greenland, Iceland, the Faroe Islands, Norwegian waters, the Barents Sea and Svalbard, mainly in depths between 100 and 300 m (Whitehead *et al.* 1986). *S. mentella* is generally distributed deeper than *S. marinus* and additionally inhabits the pelagic zone of the Labrador and Irminger Sea down to 1000 m depth. For fisheries management purposes, several stock units were established for redfish by the International Council for the Exploration of the Sea (ICES) and the Northwest Atlantic Fisheries Organization (NAFO). *S. marinus* is currently divided into two ICES management units, one comprising the occurrences off Greenland, Iceland and the Faroe Islands (ICES 2003a), and the other one along the Norwegian coast, in the Barents Sea and off Svalbard (ICES 2003b). The same management units apply for demersal *S. mentella*, whereas pelagic *S. mentella* is currently assessed within ICES and managed by the Northeast Atlantic Fisheries Commission (NEAFC). Considering the transboundary distribution of pelagic *S. mentella* within ICES/NEAFC and NAFO areas, a common management of this relatively new resource is currently aimed at (NEAFC 2001, NAFO 2002). In the Northwest Atlantic, demersal redfish, not separated by species, are managed within NAFO in the offshore areas east of Newfoundland, on the Flemish Cap and off West Greenland, while the inshore areas of Canadian and U.S. coastal waters are assessed nationally (NAFO 2004).

Only few studies on geographic variation of *S. marinus* have been carried out. Apart from body morphometry (Saborido-Rey 1994), haemoglobin and enzyme patterns were investigated (Nedreaas and Nærdal 1991, Nedreaas *et al.* 1994, Johansen *et al.* 2000a). Due to the highly migratory nature of *S. mentella*, the intraspecific variation and the elucidation of the complex stock structure has gained particular interest, especially with respect to fisheries management of this valuable resource (*e.g.* ICES 1998). Several morphometric studies have attempted to identify sub-units and stocks (Nagel *et al.* 1991a, Reinert and Lastein 1991, Saborido-Rey and Nedreaas 2000), aiming at a quantification of external and internal features of the fish body. Very recently, otolith shape analysis was applied to classify *Sebastes* species (Stransky 2001, Stransky and MacLellan, unpubl. data) and to study intraspecific patterns (Stransky 2002 and 2003). A series of biochemical and genetic studies was carried out to relate the observed geographic differences to population structure. Gel electrophoresis of haemoglobins and tissue enzymes (Dushchenko 1987, Nedreaas and Nærdal 1989, 1991, Nedreaas *et al.* 1994, Johansen *et al.* 2000b) was recently followed by microsatellite DNA analyses by Roques *et al.* (2002). As natural tagging methods, parasite infestation of *S. mentella* (*e.g.* Nagel *et al.* 1991b, Bakay 2000, Marcogliese *et al.* 2003), abnormal external coloration and ectolesions (Bogovski and Bakay 1989) were extensively studied.

Another natural tag that has been widely used in stock separation research during recent years is the elemental composition of calcified structures in the fish body, such as scales and otoliths (see reviews by Campana 1999 and Thresher 1999). Although not always straightforward, 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, Dove and Kingsford 1998, Elsdon and Gillanders 2003). Since otoliths grow continuously, mainly by deposition of calcium carbonate and a variety of minor and trace elements, the environmental history of the investigated fish can be tracked using elemental and stable isotope signatures (*e.g.* Milton *et al.* 2000, Gemperline *et al.* 2002, Hanson *et al.* 2004). From a variety of instrumentation available for the analysis of elemental contents in fish otoliths (see Campana *et al.* 1997), particle-induced X-ray emission (PIXE, *e.g.* Kakuta *et al.* 1999, Babaluk *et al.* 2002, Morris *et al.* 2003) and even more frequently used, inductively coupled plasma mass spectrometry (ICP-MS; *e.g.* Kingsford and Gillanders 2000, Secor *et al.* 2002, Brophy *et al.* 2003, Patterson *et al.* 2004) have prevailed over more traditional electron microprobe techniques.

By coupling a laser ablation system with ICP-MS (LA-ICP-MS), small amounts of material can be analysed from specific regions of the otolith, *e.g.* from the nucleus (core) to trace the origin of the fish, or the marginal growth zones to extract information on the recently inhabited environment. These elemental fingerprints can be compared between geographic areas or habitats across the otolith growth layers to investigate stock origin, mixing and migration (Campana *et al.* 1994). Most recent investigations using these techniques for the separation of marine fish populations have focused on coastal or estuarine areas on different spatial scales (*e.g.* Milton and Chenery 2001a, Gillanders 2002, Patterson *et al.* 2004). Only few LA-ICP-MS studies were carried out in offshore or oceanic areas to facilitate stock discrimination of migratory species, such as bluefin tuna (Rooker *et al.* 2001 and 2003) and Patagonian toothfish (Timmiss and Kalish 2001), as well as deep-water fish (Gordon *et al.* 2001).

This study presents the first attempt to investigate otolith microchemistry of Atlantic redfish across the distributional range of two species with high commercial interest and unresolved stock structure, *S. marinus* and *S. mentella*. The primary aim was to test the use of elemental fingerprints as a stock discrimination tool by analysing the minor and trace element composition in the nucleus region of redfish otoliths, giving information on individual origin. *S. marinus* otoliths were also analysed along the marginal zones, and otoliths of the highly migratory *S. mentella* were assayed in the juvenile and marginal zones to investigate the environmental history of these species. Since temporal stability of elemental signatures is an important prerequisite for the validity of the derived stock patterns (*e.g.* Hamer *et al.* 2003, Swearer *et al.* 2003), otoliths of juvenile *S. marinus* and *S. mentella*, sampled in an important nursery area off East Greenland during five consecutive years, were analysed for between-year variation in element concentrations. Due to the recently found evidence for migration of juvenile *S. mentella* from the East Greenland shelf into the pelagic occurrences in the Irminger Sea during

1998-1999 (Stransky 2000), otoliths collected in these areas during that period were analysed for elemental composition in the nucleus and marginal regions to investigate the possibility of tracking redfish migration routes by otolith microchemistry. In a broader sense, the aim of this study was to substantiate the hypothesis of a common origin and place of juvenile development of the redfish in the Irminger Sea and the distribution of the redfish juveniles into the different areas chiefly by the currents of the North-East Atlantic. A similarity of the trace element composition in or near the nucleus would give proof for this theory.

Material and methods

Sample collection

The otolith samples analysed for microchemistry (Table 1) were divided into three sets. The location of the sampling stations is given in Figure 1. The first sampling set consists of otoliths of juvenile *S. marinus* and *S. mentella* (20-25 cm), caught on the Heimland Ridge off East Greenland (64°N 36°W) during five consecutive years (1998-2002) on an annual groundfish survey in autumn. The second set of otoliths was collected from adult *S. marinus* and *S. mentella* with total lengths of > 30 cm, taken in various locations across the North Atlantic. Since evidence for an extensive migration of juvenile *S. mentella* (25-30 cm total length) from East Greenland into the Irminger Sea was found during 1998/1999 (Stransky 2000), the available material from fish collected during that period in these areas was analysed as third set of otoliths. When only fork lengths were available, total lengths were calculated on the basis of conversion factors for the respective species in the respective area (F. Saborido-Rey, unpubl. data). The Icelandic samples were taken on a commercial vessel, while all other samples were collected on research vessels. Bottom trawls were predominantly used as fishing gear, with the exception of *S. mentella* in the Irminger Sea that were caught with pelagic Gloria-type trawls. Sample sizes of at least 10 otoliths were aimed at but not always achieved due to time and budget constraints. All redfish otoliths were removed from the fish shortly after capture, rinsed in tap water and stored dry in paper envelopes. This storage method was shown to have least effect on elemental concentrations (Milton and Chenery 1998).

LA-ICP-MS analysis

Preceding microchemical analysis, all otoliths were embedded into transparent polyester resin and thin-sectioned transversely through the nucleus (core) region on a diamond-blade saw to about 1.0 mm

thickness. Previous studies using this sample preparation have shown that no contamination was introduced by sectioning the otoliths (*e.g.* Dove *et al.* 1996). The thin sections were mounted onto circular glass microscope slides, rinsed with MilliporeTM filtered Milli-Q water (18 MΩ cm⁻¹) to minimise surface contamination, dried in a laminar flow hood at room temperature and stored in clean sealable plastic bags. The left-sided otolith of an adult *S. marinus* collected off East Greenland (65°29'N 32°31'W, October 2001) was prepared in the same way but not mounted on a slide. This otolith was used as a solid in-house standard (Reference Otolith) for monitoring of long-term reproducibility. The right-sided otolith of the same individual was prepared for solution analysis by grinding the otolith to a core containing the first three growth zones. The concentrations of ⁷Li, ⁶³Cu and ⁸⁵Rb were similar to the median values obtained from nucleus LA-ICP-MS, whereas ⁸⁶Sr and ¹³⁸Ba in the solution were markedly higher than in the nucleus ablations. Solution-based ICP-MS of a certified reference material (CRM) for otoliths (Yoshinaga *et al.* 2000), not concurrently analysed with the solution of the Reference Otolith, confirmed the certified values for ⁶⁶Zn (0.6 µg g⁻¹; CRM: 0.5 µg g⁻¹), ⁸⁶Sr (2460 µg g⁻¹; CRM: 2360 µg g⁻¹) and ¹³⁸Ba (3.0 µg g⁻¹; CRM: 2.9 µg g⁻¹).

The sampling slides, containing 9-20 otolith sections, were analysed for trace elements using LA-ICP-MS (laser-ablation inductively coupled plasma mass spectrometry) with an Agilent 7500s and a Perkin-Elmer Elan 6100 DRC plus. A Geolas M, Microlas (Lamda Physik Compex 110) laser microprobe was mounted to the ICP-MS system to ablate sampling material from the otolith section surface. The laser was operated at a wavelength of 193 nm with a pulse rate of 10 Hz and an energy output of 135 mJ (Günther *et al.* 2003). The produced sample craters had a diameter of about 31 µm. The ablation area was flushed with a 1.2 l min⁻¹ He gas jet and ablated material was transported to the ICP. The He carrier gas stream was mixed with a 0.8 l min⁻¹ Ar gas stream shortly before entrance into the ICP. Analytical blanks were derived from Ar background counts, which were run for 30 s prior to each ablation and subtracted from that ablation. The following 40 s were used for data acquisition during laser ablation of the otolith target area. Another 30 s were used for monitoring of the decaying signal after the ablation was finished. The total acquisition time per spot summarises to 100 s time resolved raw data. The software package LAMTrace (Simon Jackson, Macquarie University, Sydney, Australia) was used for re-evaluation and quantification of the time resolved raw data. To account for instrument drift, the laser was recalibrated every 16 ablations by analysing a NIST 610 (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) glass standard twice at the start and at the end of each run. In addition, the Reference Otolith was re-analysed twice with every batch of 14 unknown sample spot ablations. The location of the sampling spots was viewed with a CCD camera and controlled by moving the sampling chamber on a three-directional stage.

The otolith samples from adult *S. mentella* were analysed in the nucleus region, along the juvenile zones (third-year growth) and along the marginal increments on the Agilent ICP-MS, while all other

samples were analysed in the nucleus region and along the marginal increments only, using the Perkin-Elmer machine. A minimum of two craters was sampled in each otolith region. ^{42}Ca was used as an internal standard to correct for variations in the amount of ablated material, assuming a constant concentration of calcium carbonate in the otolith matrix. In this semiquantitative approach, the concentration of CaO was set to 560000 $\mu\text{g/g}$, and the concentrations of the other elements were calibrated against the NIST 610 glass standard with CaO 11.8 weight %. Limits of detection (LODs) were derived for each run (20 ablations). With the Agilent ICP-MS, nine elements could be measured above the following average LODs ($\mu\text{g g}^{-1}$) throughout the analysis: ^{7}Li : 0.06, ^{25}Mg : 0.52, ^{55}Mn : 0.08, ^{63}Cu : 0.05, ^{66}Zn : 0.11, ^{85}Rb : 0.01, ^{86}Sr : 0.55, ^{120}Sn : 0.06 and ^{138}Ba : 0.01. On the Perkin-Elmer system, the average LODs ($\mu\text{g g}^{-1}$) of the five examined elements were: ^{7}Li : 0.18, ^{25}Mg : 0.98, ^{63}Cu : 0.07, ^{88}Sr : 0.01 and ^{138}Ba : 0.01. LODs for ^{55}Mn (0.28 $\mu\text{g g}^{-1}$) and ^{66}Zn (0.52 $\mu\text{g g}^{-1}$) were not sufficient for otolith analysis.

Statistical analysis

The average elemental concentrations were calculated (as element:Ca ratios) from two or three sampling spots within the same region (nucleus, third-year annulus and edge) on each otolith. Since these values were not normally distributed (Kolmogorov-Smirnov test for normality, $p < 0.05$) for most elements, all concentrations were $\log_{10}$ transformed prior to analysis. This transformation also accounted for the largely different ranges of concentrations (e.g. Cu: 0.1-3.0 $\mu\text{g g}^{-1}$, Sr: 1000-5000 $\mu\text{g g}^{-1}$), decreasing the dominance of Sr in multivariate analysis. Box-and-whisker plots of the elemental concentrations were examined to illustrate differences between sampling years and areas, as well as otolith regions. The significance of these comparisons was tested by multivariate analysis of variance (MANOVA). Individual analyses of covariance (ANCOVA) with fish total length as covariate were performed for each element to test for size effects on elemental concentrations. To investigate possible relationships between elements, bivariate plots of the concentrations of all elemental pairs were examined and tested for significance by Pearson rank correlation.

The discrimination between geographic areas was tested for both *S. marinus* and *S. mentella* by linear discriminant function analysis (LDFA). Scatterplots of the scores of the first two canonical variates were drawn with 95% confidence ellipses to visualise area differences. The classification success between areas was determined by jackknifed cross-validation matrices (SYSTAT 9, SPSS Inc. 1999).

Results

Temporal variation

Before comparing otolith elemental signatures for *S. marinus* and *S. mentella* from various fishing areas in the North Atlantic, the temporal stability of element concentrations of juvenile redfish was investigated within a limited geographic area, namely the Heimland Ridge off East Greenland. For both juvenile *S. marinus* (Figure 2) and *S. mentella* (Figure 3), year-to-year variation in the nucleus region was relatively low, while larger differences between years were observed in the marginal (edge) regions, especially in case of Li and Ba. MANOVA of the elemental concentrations in the nuclei with the sampling year as categorical factor indicated no significant differences between years (*S. marinus*: Pillai's trace = 0.881, $F_{15,36} = 0.89$, $p = 0.581$; *S. mentella*: Pillai's trace = 0.575, $F_{10,42} = 1.69$, $p = 0.114$), but significant year effects for the edge microchemistry (*S. marinus*: Pillai's trace = 1.404, $F_{15,30} = 1.76$, $p = 0.024$; *S. mentella*: Pillai's trace = 1.105, $F_{10,42} = 5.18$, $p < 0.001$). Concentrations of Sr and Ba were consistently higher along the edges than in the nuclei, while Mg concentrations were always lower in the edge than in the nucleus region.

Geographic variation

The Li concentrations measured in the Barents Sea samples of adult *S. marinus* (Figure 4) were considerably higher than in all other areas, especially in the otolith edge region. In the edge region of the samples from the Flemish Cap, Greenland and Iceland, however, the Li values were lower than in the respective nuclei. The distribution patterns of nucleus concentrations of Mg were similar to Ba, with West Greenland and the Barents Sea showing the highest median values (Figure 4). The Cu contents of *S. marinus* otoliths from West and East Greenland were higher than in the samples from the Flemish Cap, Iceland and the Barents Sea. Median Cu values in the nuclei were similar to the corresponding edge concentrations. Little variation between areas was observed for Sr. In all areas, Sr and Ba concentrations were consistently elevated in the edge region, compared to the nucleus values, up to twice-fold in the case of the Flemish Cap samples.

As in the *S. marinus* samples, the *S. mentella* otoliths from the Barents Sea showed higher Li values in the edge than in the nucleus, while samples from all other areas exhibit decreased Li concentrations in the edge region (Figure 5). Further information on elemental concentrations in different otolith regions was introduced by sampling the juvenile (third annulus) region. The Mn concentrations decreased from the nucleus over the third annulus to the edge in all areas, whereas Sr and Ba showed increasing trends from nucleus to edge. Consistent otolith region trends within areas were not observed for the

other detected elements. Mn, Cu and Zn nucleus concentrations were always higher in the central North Atlantic areas (Irminger Sea, East Greenland, Iceland and Faroe Islands) than in the samples from the Flemish Cap, the Davis Strait and the Barents Sea (Figure 5). Only minor differences between areas were found for Rb and Sn.

For both species, elemental concentrations in the otolith nucleus region showed highly significant differences between geographic areas where the fish had been caught (MANOVA, $p < 0.005$, detailed results in Table 2) and repeating patterns for Li and Cu. Li concentrations were highest in the Northwest Atlantic (Flemish Cap) and Northeast Atlantic (Barents Sea), whereas the central North Atlantic areas (Greenland, Iceland etc.) showed lower concentrations, with a consistent U-shaped trend from West to East. The opposite was found for Cu (and Mn in the case of *S. mentella*), exhibiting highest concentrations in the central areas. The observed geographic distribution patterns were also found in the edge regions of the analysed *S. marinus* otoliths but were not present in the juvenile and edge regions of the *S. mentella* samples.

Discriminant analysis of the $\log_{10}$ -transformed concentrations of the elements that were consistently detectable in the otolith nucleus region (*S. marinus*: Li, Mg, Cu, Sr, Ba; *S. mentella*: Li, Mg, Mn, Cu, Zn, Rb, Sr, Sn, Ba), however, revealed only weak separation between geographic areas. Discriminant function scores for the *S. marinus* samples illustrate high similarity in elemental signatures between West and East Greenland (Figure 6), while the *S. mentella* samples show a high connectivity between East Greenland and the Irminger Sea, as well as between Iceland and the Faroe Islands (Figure 7). These patterns are confirmed by the cross-validation matrices (Tables 3 and 4), showing high misclassification rates (*S. marinus*: 11-44%, *S. mentella*: 20-30%) between the central North Atlantic areas. The overall classification success was poor for both species (*S. marinus*: 43%, *S. mentella* 46%), due to the indifferent elemental signatures ($< 50\%$ classification) of *S. marinus* around Greenland (Table 3) and *S. mentella* in the Irminger Sea, around the Faroe Islands and in the Barents Sea (Table 4). When combining the central North Atlantic samples (Greenland, Iceland, Irminger Sea and Faroe Islands) to one area, the overall classification success increased to 64% for *S. marinus* and to 67% for *S. mentella*.

The effect of fish size (total length), tested by individual ANCOVA for each of the five elements detected in the otolith nuclei of both species (Li, Mg, Cu, Sr and Ba), was not significant ($p > 0.05$ in all cases). From the bivariate comparisons of element pairs, Mg and Sr were negatively correlated for both species (Pearson correlation; *S. marinus*: $\text{Sr}[\mu\text{g g}^{-1}] = -17.61 \cdot \text{Mg}[\mu\text{g g}^{-1}] + 2102$, $r^2 = 0.168$, $p = 0.003$; *S. mentella*: $\text{Sr}[\mu\text{g g}^{-1}] = -16.26 \cdot \text{Mg}[\mu\text{g g}^{-1}] + 2128$, $r^2 = 0.089$, $p = 0.029$), while Li and Ba were negatively correlated in *S. mentella* otoliths only ($\text{Ba}[\mu\text{g g}^{-1}] = -0.105 \cdot \text{Li}[\mu\text{g g}^{-1}] + 3.187$, $r^2 = 0.081$, $p = 0.037$).

Migration

Evidence was found during autumn 1998 and summer 1999 for migration of juvenile *S. mentella* with a size range of 25-30 cm total length from the East Greenland shelf into the oceanic occurrences in the central Irminger Sea (Stransky 2000). A subset of otoliths available from this period and areas was analysed for trace elements in the nucleus and in the edge regions, reflecting the first-year and the recently inhabited environment. The nucleus microchemistry was very similar between East Greenland and the Irminger Sea for all five detected elements (Figure 8). No significant differences in elemental signatures were found between areas (MANOVA; Pillai's trace = 0.578, $F_{5,9} = 2.47$, $p = 0.113$). The elemental composition in the edge regions, however, was significantly different between the two geographic areas (MANOVA; Pillai's trace = 0.616, $F_{5,11} = 3.53$, $p = 0.038$), indicated by lower concentrations of Li, Mg and Ba in the Irminger Sea, compared to East Greenland (Figure 8).

Discussion

In contrast to solution-based ICP-MS analyses, the presented LA-ICP-MS assays only provide semiquantitative concentrations relative to Ca with respect to variations in the amount of ablated material. The elemental concentrations found in redfish otoliths, however, are similar to values reported for other North Atlantic fish species, such as cod (Campana and Gagné 1995, Campana *et al.* 2000) and bluefin tuna (Secor *et al.* 2002) in the Northwest Atlantic and deep-water black scabbardfish in the Northeast Atlantic (Swan *et al.* 2003a), using solution-based approaches.

When analysing geographic variation in otolith microchemistry, the estimation of the level of temporal variation of the elemental signatures within an area is crucial (Gillanders 2002, Hamer *et al.* 2003, Swearer *et al.* 2003). The otoliths chosen in this study to test possible year-effects were collected within an important redfish nursery area off East Greenland with consistently high abundance of juvenile *S. marinus* and *S. mentella* (Magnusson *et al.* 1988, Yatsu and Jørgensen 1988, Rätz *et al.* 2004). The investigation shows that the analysed fish are very likely to have originated from the same spawning ground, since no significant differences between sampling years were found for the elements detected in the otolith nuclei. In contrast, the significant year-effects in edge chemistry of these otoliths, mainly caused by largely varying Li, Sr and Ba concentrations, indicate either a differing environment inhabited during the recent few months before capture or between-year variation in habitat chemistry. The low number of samples available for some years and possible edge contamination effects, however, leave some doubt to this interpretation of the edge results.

Considering the markedly higher level of variation in elemental concentrations of Li, Sr and Ba observed for adult *S. marinus* and *S. mentella* from various geographic areas in the North Atlantic, especially in the otolith edge regions, the year-to-year signatures in juvenile redfish otoliths can be regarded as relatively stable.

The most striking geographic patterns in elemental concentrations were found for Li and Cu in otoliths of adult fish of both species and for Mn in *S. mentella* otoliths. Following a longitudinal trend, Li decreased in the otoliths from the Northwest Atlantic to the central areas (Greenland, Irminger Sea, Iceland, Faroe Islands) and increased from the central areas to the Northeast Atlantic, while Mn and Cu showed opposite trends. Despite these strong signals and highly significant area effects for the whole elemental suite in all sampled otolith zones, spatial separation derived from multivariate analysis was relatively poor. Attempts of using various sub-sets of elements in this study to improve discrimination between areas did not reveal higher classification success. A slight improvement of discriminatory power was accomplished by pooling the central Atlantic areas to one group and comparing this group with the Northwest and Northeast Atlantic areas. Similar low overall cross-validated classification rates of 45% were reported for other North Atlantic fish species dwelling deeper habitats (Swan *et al.* 2003a and 2003b). This study, along with preliminary reports of Timmiss and Kalish (2001) and comprehensive studies on deep-sea fish (Gordon *et al.* 2001), represents one of the few investigations carried out in oceanic and deep-sea areas. In these offshore areas, trace element composition only varies marginally, whereas more distinct spatial patterns are to be expected in estuary and coastal areas where the majority of investigations has been carried out so far (*e.g.* Patterson *et al.* 1999, Forrester and Swearer 2002, Geffen *et al.* 2003, Hanson *et al.* 2004).

Swan *et al.* (2003b) observed elevated levels of Li in whole otoliths of the deep-water macrourid *Nezumia aequalis* on the Reykjanes Ridge (SW of Iceland), which they related to relatively high input of trace elements from hydrothermal activity in this region. Several other studies found Li to contribute to spatial separation, *e.g.* Milton *et al.* (1997), Campana *et al.* (2000), Milton and Chenery (2001a), Gillanders (2002) and Rooker *et al.* (2003). In our study, the highest Li concentrations were found in the Barents Sea samples, being confirmed by multiple and independent measurements. Since little is known about variation on Li concentrations in the North Atlantic, no conclusive explanation could be found for the relatively high levels of Li in redfish otoliths from the Barents Sea, especially in the edge regions. Li, as well as Mg and Ba, are conservative elements, directly proportional to salinity (Bruland 1983). However, a relationship between the concentrations of these elements in redfish otoliths with salinity, extracted from the World Ocean Database (Conkright *et al.* 2002), could not be observed.

Relationships between otolith and water chemistry were repeatedly discussed (Campana 1999), but only few studies undertook experimental work on the effect of varying elemental concentrations on otolith composition (Bath *et al.* 2000, Elsdon and Gillanders 2003, Chang *et al.* 2004). The usual Cu and Zn concentrations in the North Atlantic range from 1.2-2.0 nM (75-130 $\mu\text{g g}^{-1}$) and 0.9-1.5 nM (80-130 $\mu\text{g g}^{-1}$), respectively (Danielsson *et al.* 1985, Pohl *et al.* 1993, Saager *et al.* 1997, Cotté-Krief *et al.* 2002). When compared to the average redfish otolith concentrations determined in this study (Cu: 0.7 $\mu\text{g g}^{-1}$, Zn: 1.4 $\mu\text{g g}^{-1}$), element-specific differences in deposition become apparent. The Zn concentration in the otoliths is about twice-fold compared to Cu, while the water concentration of both elements is similar. Interestingly, the difference between Cu and Zn concentrations in muscle tissue of *S. marinus* is about one order of magnitude, with slight variations between Iceland (Cu: 0.2-0.3 $\mu\text{g g}^{-1}$, Zn: 3.5-4.4 $\mu\text{g g}^{-1}$; Stange *et al.* 1996), Norway (Cu: 0.2-0.3 $\mu\text{g g}^{-1}$, Zn: 2.6-2.9 $\mu\text{g g}^{-1}$; Stange *et al.* 1996) and the Barents Sea (Cu: < 0.3 $\mu\text{g g}^{-1}$, Zn: 3.0 $\mu\text{g g}^{-1}$; Zauke *et al.* 1999). Similar values were obtained for *S. mentella* off Greenland, in the Irminger Sea and around the Faroe Islands (Stange *et al.* 1996). Mormede and Davies (2001a and 2001b) measured heavy metals in muscle tissue of deep-sea fish in the Rockall Trough and reported Cu and Zn concentrations (0.1-0.3 and 2.6-3.9 $\mu\text{g g}^{-1}$, respectively) resembling those determined in redfish muscle tissue. Most studies on elemental incorporation into the otolith, however, focused on Sr (Campana 1999). Recent experimental work additionally investigated the uptake of Ba (Bath *et al.* 2000), as well as Ba and Mn (Elsdon and Gillanders 2003). Multi-element comparisons between water and otolith samples have been carried out in rivers, showing significant environmental effects on otolith microchemistry (Thorrold *et al.* 1998, Milton and Chenery 2001b, Wells *et al.* 2003). Unfortunately, no distributional maps of the investigated trace elements in the North Atlantic with a sufficient spatial resolution were available to correlate water and otolith chemistry of redfish directly.

Since the pathways and barriers of elemental uptake and deposition into otoliths are not fully understood (Olsson *et al.* 1998, Campana 1999), experimental work has been carried out to elucidate the principles behind the incorporation of elements from other sources than ambient seawater. In some instances, a significant influence of food items on the elemental composition of fish otoliths could be detected (Limburg 1995, Gallahar and Kingsford 1996, Sanchez-Jerez *et al.* 2002), other studies indicated negligible dietary effects (Hoff and Fuiman 1995, Farrell and Campana 1996, Milton and Chenery 2001b). Buckel *et al.* (2004) reported significant differences in Sr and Ba otolith concentrations between fish and shrimp diet, whereas Na, Mg, K, Ca and Mn were not affected. Demersal redfish (*S. marinus* and *S. mentella*) mainly feed on shrimp, hyperiid and euphausiid crustaceans (Pedersen and Riget 1993, Gorelova 1997), oceanic *S. mentella* additionally feed on amphipod and copepod crustaceans, chaetognaths, small squid and myctophid fishes (Magnusson and Magnusson 1995, González *et al.* 2000). As there were no stomach content analyses performed on the redfish used in this study, the impact from food assimilation could not be assessed. The lack of

consistently high differences between elemental contents of *S. mentella* otoliths from the Irminger Sea and those from demersal redfish, albeit some degree of dietary separation exists, makes imprinting from food sources unlikely.

The observed differences between the concentration of several elements in nucleus, juvenile and edge zones of the analysed redfish otoliths are most probably related to ontogenetic or growth rate changes, commonly found for Sr:Ca ratios (reviewed in Campana 1999; for recent studies, see *e.g.* Markwitz *et al.* 2000, de Pontual *et al.* 2003, Arai *et al.* 2004). Sr as well as Ba concentrations of the investigated redfish otoliths were consistently higher in the edge regions than in the nuclei, which has also been described for other marine fish species, such as Patagonian toothfish (Timmiss and Kalish 2001) and English sole (Brown 2003). The elevated levels of Sr and Ba determined in solution-based analysis of the three-year core of the redfish Reference Otolith provided further indications of ontogenetic increase of these elements in the otolith zones following the first year growth. Contrarily, Li and Mn decreased from the nucleus to the marginal zones. Timmiss and Kalish (2001) and Brown (2003) also observed higher Li values in the cores than in the edges but could not find a conclusive explanation for this phenomenon. Gordon *et al.* (2001) performed line scans across otoliths of deep-sea fish and found a distinct increase in Mn concentration from otolith edge to nucleus for roundnose grenadier in the Rockall Trough. Elevated Mn levels in cores of clupeid otoliths were reported recently by Brophy *et al.* (2004) and were related to embryological development or spatial variation in calcium carbonate crystal structure.

Migration patterns based on otolith microchemistry have primarily been studied for fish moving between freshwater and marine habitats, utilising the effect of changes in ambient salinity on otolith Sr:Ca ratios (*e.g.* Secor *et al.* 1995, Kotake *et al.* 2003, Sanborn and Telmer 2003). Only few studies to date have investigated migration routes of marine fish by multi-element patterns. Campana *et al.* (1999) were able to quantify the degree of mixing and migration of Atlantic cod between the Scotian Shelf and the Gulf of St. Lawrence by elemental fingerprints. Our study represents the first approach to track migration of an important fish species in the Northeast Atlantic. Large quantities of juvenile *S. mentella* of the size range 25-30 cm were observed in groundfish surveys off East Greenland in 1996-1998 but almost disappeared in 1999 (Stransky and Rätz 2000). A considerable proportion of this size group was observed in 1999 as recruits in the Irminger Sea (Stransky 2000). The otoliths from the recruiting fish, collected in these areas during that period, showed strong similarities in age structure (Stransky *et al.* 2003; **chapter 3.1**) and otolith shape (Stransky 2001). The between-area similarity in elemental signatures in the otolith cores, opposed to significant differences in the edges, provides further evidence for a migration from the East Greenland shelf into the pelagic habitat of the Irminger Sea.

The highly migratory behaviour of redfish and unresolved stock delineation, particularly of *S. mentella*, has repeatedly led to problems in fisheries management (ICES 1998, Saborido-Rey *et al.* 2001). As part of a multidisciplinary project, this study investigated the use of otolith microchemistry for stock separation of *S. marinus* and *S. mentella*. The derived weak geographic separation has also been observed in body morphometrics (Saborido-Rey *et al.*, pers. comm.) and otolith shape analysis (Stransky 2003). Although these methods do not necessarily imply genetic differences, the results obtained for *S. mentella* are in accordance with the genetic study of Roques *et al.* (2002), describing a pan-oceanic stock from the Grand Banks to the Faroe Islands that separates from a Western (Gulf of St. Lawrence and offshore Newfoundland) and an Eastern (Norway and Barents Sea) stock unit, based on microsatellite DNA. The moderate separation of Western, central and Eastern areas, as derived in these studies for both redfish species, generally supports the current division of redfish management units of NAFO (NAFO 2004) and ICES (ICES 2003a and 2003b), with the exception that *S. marinus* around Greenland are most likely belonging to the same stock and not divided by West (NAFO) and East Greenland (ICES) and that *S. mentella* in the Irminger Sea are part of the Greenland-Iceland-Faroe Island stock complex. In contrast, previous haemoglobin analyses indicated the existence of several stocks of *S. mentella* in the Irminger Sea and adjacent waters (Johansen *et al.* 2000b). The integration of the outcome of this study with concurrently undertaken genetic analyses and morphometric approaches will further elucidate the degree of stock structure of North Atlantic redfish and will provide a more holistic basis for adaptive management of these valuable resources.

In conclusion, relatively low interannual variation in elemental concentrations in otoliths of juvenile redfish indicated sufficient temporal stability of elemental signatures, providing validity for the investigation of geographic differences in otolith microchemistry. Although consistent geographic patterns were observed for Li and Cu in otoliths of both *S. marinus* and *S. mentella* (and additionally Mn in *S. mentella* otoliths), only weak large-scale separation between the western, central and eastern North Atlantic could be derived from multivariate analysis of the elemental composition of otolith nuclei. Finally, further evidence was found for a common natal origin of *S. mentella* in the Irminger Sea and adjacent waters, inferred from similar trace element composition in the nucleus region.

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Table 1. Overview of otolith samples analysed for microchemistry. ¹samples of juvenile fish used for the investigation of temporal stability, ²samples of juvenile fish used for the investigation of migration from East Greenland into the Irminger Sea.

Species	Area	Area code	Sampling period	Fish total length (cm)	No. of otoliths
<i>S. marinus</i>	East Greenland (Heimland Ridge) ¹		Oct 1998-Oct 2001	21-25	18
<i>S. mentella</i>	East Greenland (Heimland Ridge) ¹		Oct 1998-Oct 2002	20-24	27
<i>S. marinus</i>	Flemish Cap	FC	Jul 2001	31-68	10
<i>S. marinus</i>	West Greenland	WG	Oct 2000	31-58	10
<i>S. marinus</i>	East Greenland	EG	Sep-Oct 2000	31-65	10
<i>S. marinus</i>	Iceland	IS	Feb-Mar 2001	31-45	10
<i>S. marinus</i>	Barents Sea	BS	Jul-Aug 2000	31-40	10
<i>S. mentella</i>	Flemish Cap	FC	Jul 2001	31-43	5
<i>S. mentella</i>	Davis Strait	DS	Sep-Oct 2001	36-46	5
<i>S. mentella</i>	East Greenland	EG	Oct-Nov 2001	31-41	10
<i>S. mentella</i>	Irminger Sea	IR	Jun-Jul 1999	32-47	10
<i>S. mentella</i>	Iceland	IS	Oct 2000-Apr 2001	31-50	10
<i>S. mentella</i>	Faroe Islands	FA	Sep-Oct 1999	33-49	10
<i>S. mentella</i>	Barents Sea	BS	Jul-Aug 2000	32-39	5
<i>S. mentella</i>	East Greenland ²		Oct 1998	25-30	13
<i>S. mentella</i>	Irminger Sea ²		Jun-Jul 1999	26-30	10

Table 2. MANOVA results for area effect in microchemistry of the analysed regions on the redfish otolith sections, based on log₁₀-transformed element concentrations relative to Ca.

Species	Otolith region	Pillai's trace	<i>F</i>	Effect, error df	<i>p</i>
<i>S. marinus</i>	Nucleus	1.034	2.65	20, 152	< 0.0001
<i>S. marinus</i>	Edge	1.543	4.90	20, 156	< 0.0005
<i>S. mentella</i>	Nucleus	1.845	2.07	54, 252	< 0.0001
<i>S. mentella</i>	Juvenile	1.762	1.80	54, 234	< 0.005
<i>S. mentella</i>	Edge	2.024	2.32	54, 246	< 0.00001

Table 3. Jackknifed classification matrix of the discriminant function analysis of the elemental signatures (log₁₀-transformed concentrations of Li, Mg, Cu, Sr and Ba relative to Ca) in the nucleus region of *S. marinus* otoliths from five different areas in the North Atlantic (for area codes, see Table 1). The percentages in rows represent the classification into the areas given in columns. Total classification success is 43%.

	FC	WG	EG	IS	BS
FC	62.5 (5)	12.5 (1)	0.0 (0)	12.5 (1)	12.5 (1)
WG	11.1 (1)	22.2 (2)	44.4 (4)	11.1 (1)	11.1 (1)
EG	10.0 (1)	50.0 (5)	20.0 (2)	0.0 (0)	20.0 (2)
IS	37.5 (3)	12.5 (1)	0.0 (0)	50.0 (4)	0.0 (0)
BS	33.3 (3)	0.0 (0)	0.0 (0)	0.0 (0)	66.7 (6)

Table 4. Jackknifed classification matrix of the discriminant function analysis of the elemental signatures ($\log_{10}$ -transformed concentrations of Li, Mg, Mn, Cu, Zn, Rb, Sr, Sn and Ba relative to Ca) in the nucleus region of *S. mentella* otoliths from seven different areas in the North Atlantic (for area codes, see Table 1). The percentages in rows represent the classification into the areas given in columns. Total classification success is 46%.

	FC	DS	IR	EG	IS	FA	BS
FC	60.0 (3)	0.0 (0)	0.0 (0)	20.0 (1)	0.0 (0)	0.0 (0)	20.0 (1)
DS	0.0 (0)	80.0 (4)	0.0 (0)	0.0 (0)	20.0 (1)	0.0 (0)	0.0 (0)
IR	0.0 (0)	10.0 (1)	20.0 (2)	30.0 (3)	0.0 (0)	30.0 (3)	10.0 (1)
EG	0.0 (0)	0.0 (0)	22.2 (2)	66.7 (6)	0.0 (0)	0.0 (0)	11.1 (1)
IS	0.0 (0)	0.0 (0)	0.0 (0)	20.0 (2)	60.0 (6)	20.0 (2)	0.0 (0)
FA	0.0 (0)	0.0 (0)	0.0 (0)	20.0 (2)	20.0 (2)	40.0 (4)	20.0 (2)
BS	20.0 (1)	0.0 (0)	20.0 (1)	0.0 (0)	20.0 (1)	40.0 (2)	0.0 (0)

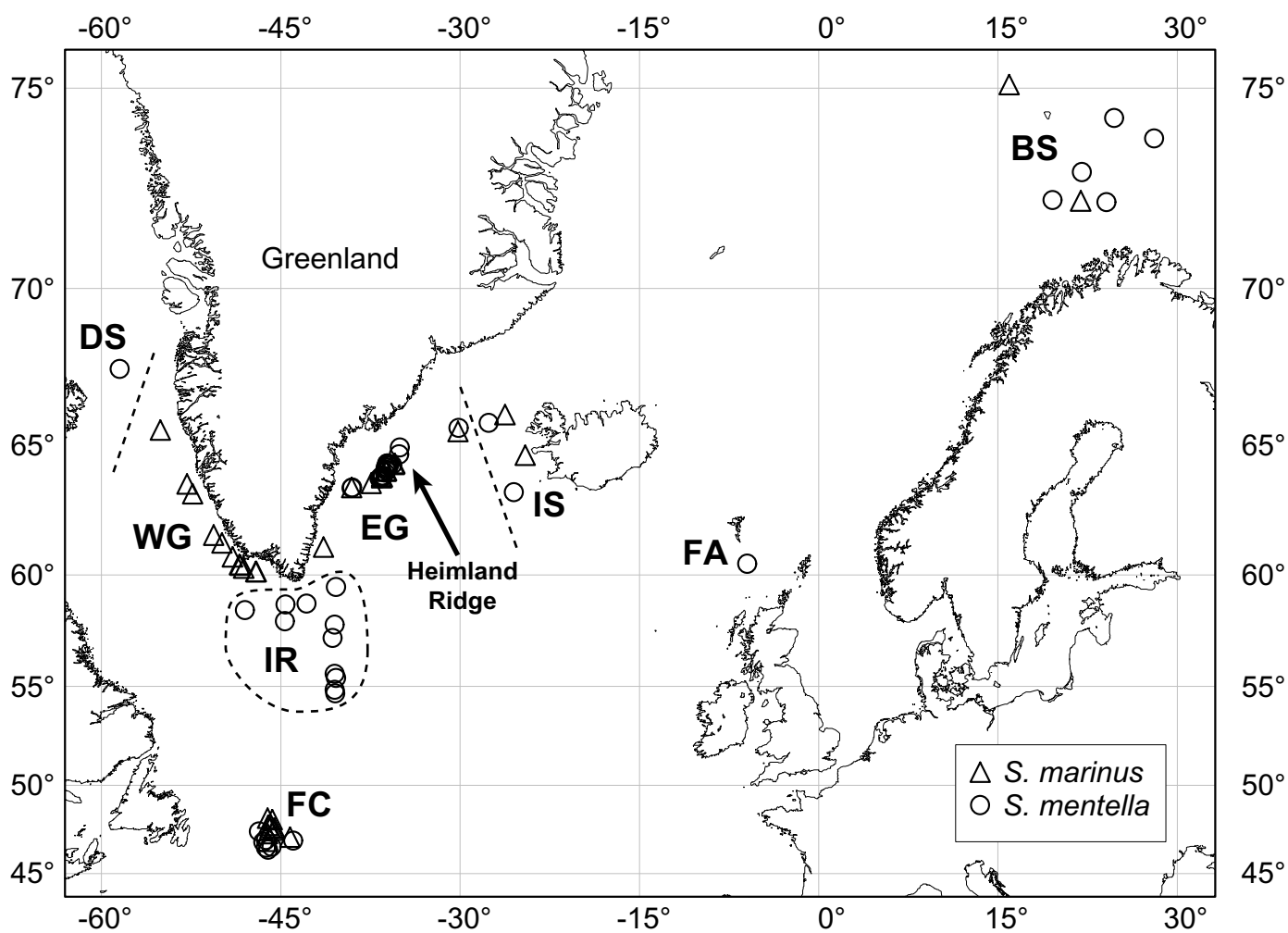


Figure 1. Positions and areas of redfish otolith samples (see Table 1 for area codes). Dashed lines represent boundaries between are definitions. Map source: GEBCO, Mercator projection.

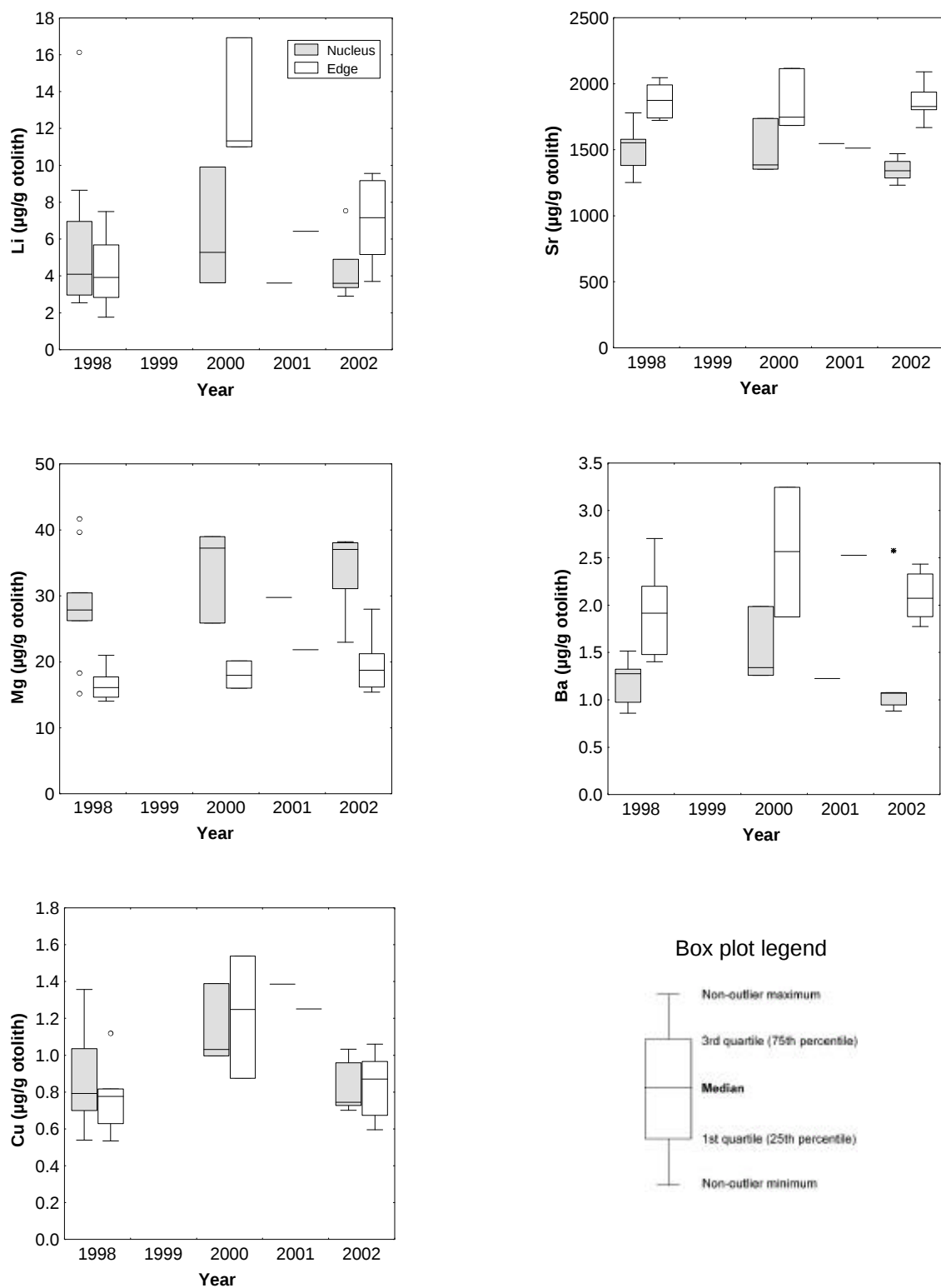


Figure 2. Concentration ($\mu\text{g g}^{-1}$ relative to Ca) of the five elements consistently measured over LOD in the nucleus and edge regions of juvenile *S. marinus* otoliths, collected on the Heimland Ridge/East Greenland during five consecutive years (1998: $n = 9$, 2000: $n = 3$, 2001: $n = 1$, 2002: $n = 5$). Outliers are marked by open circles, extreme values are indicated by stars.

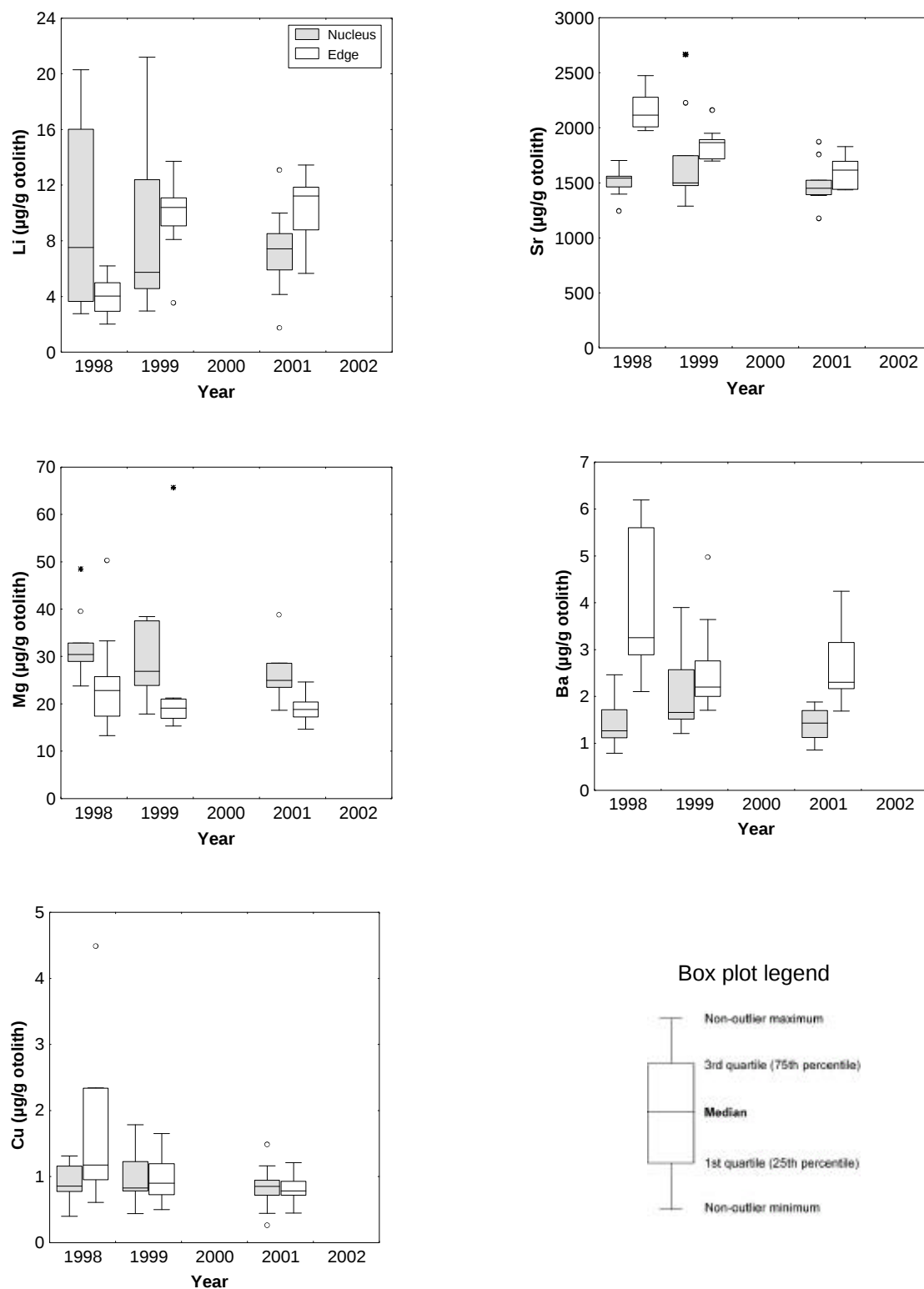


Figure 3. Concentration ($\mu\text{g g}^{-1}$ relative to Ca) of the five elements consistently measured over LOD in the nucleus and edge regions of juvenile *S. mentella* otoliths, collected on the Heimland Ridge/East Greenland during five consecutive years ($n = 9$ in 1998, 1999 and 2001). Outliers are marked by open circles, extreme values are indicated by stars.

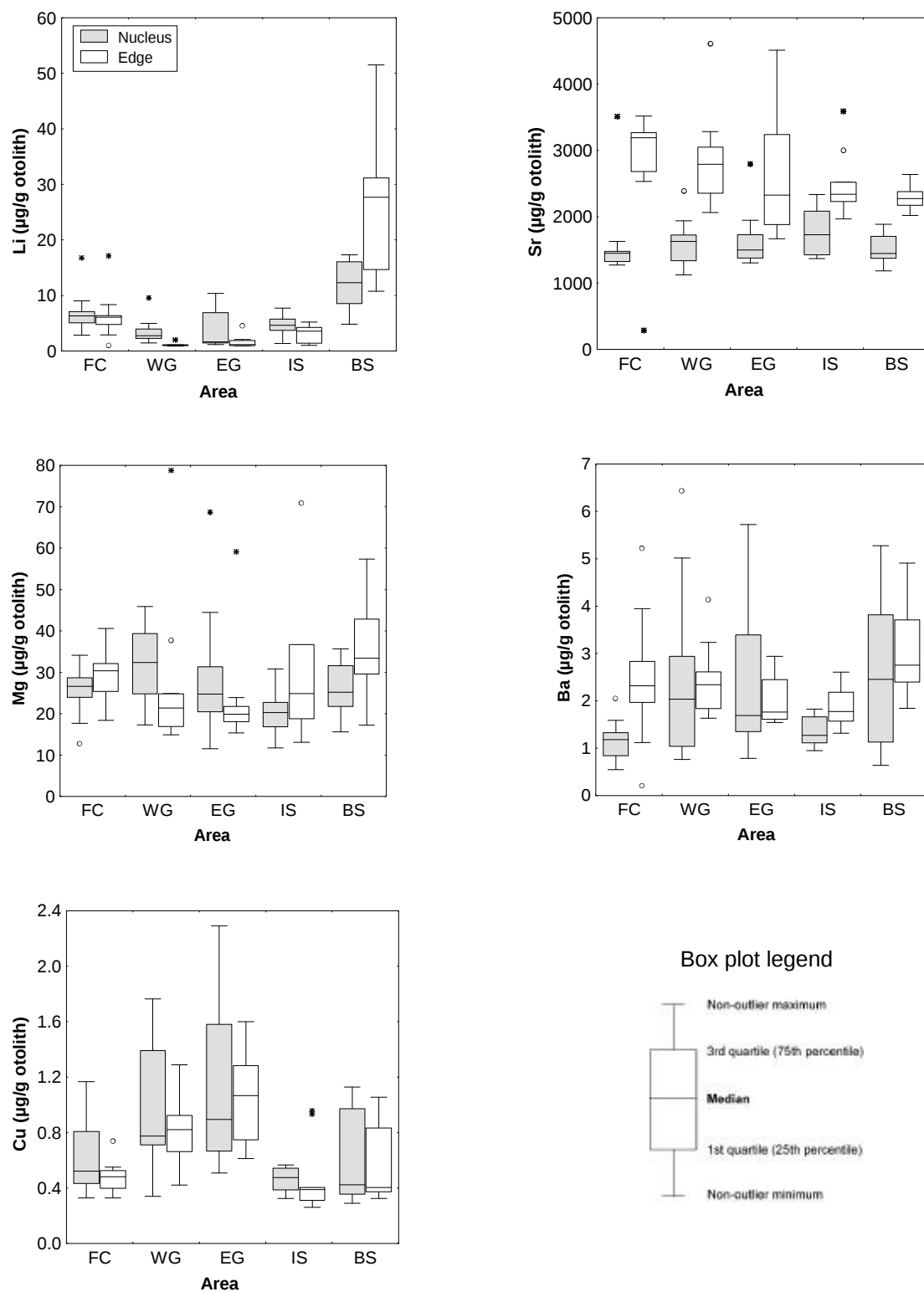


Figure 4. Concentration ($\mu\text{g g}^{-1}$ relative to Ca) of the five elements consistently measured over LOD in the nucleus and edge regions of adult *S. marinus* otoliths, collected in five different areas in the North Atlantic (for area codes, see Table 1). Outliers are marked by open circles, extreme values are indicated by stars.

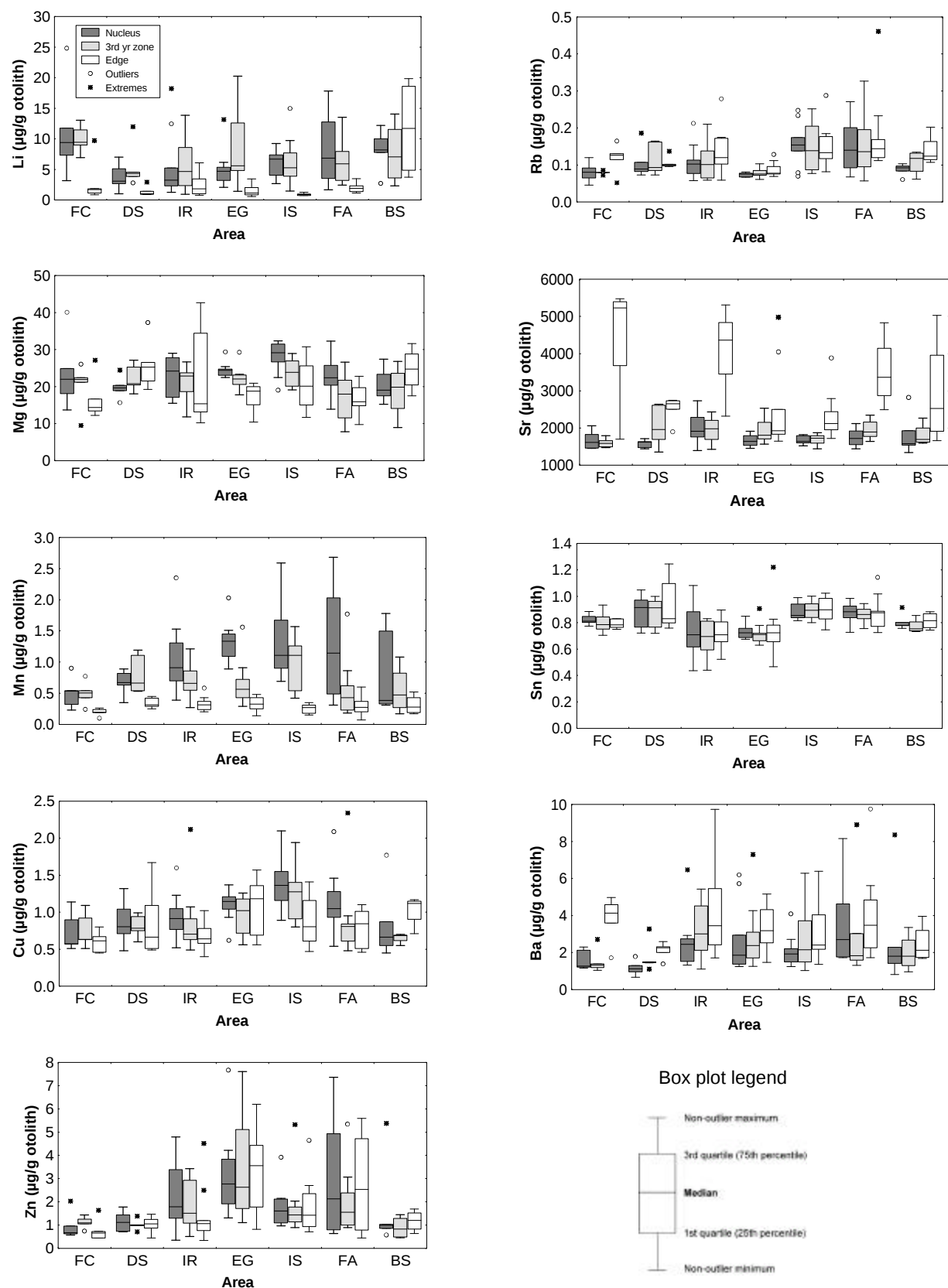


Figure 5. Concentration ($\mu\text{g g}^{-1}$ relative to Ca) of the nine elements consistently measured over LOD in the nucleus, third annulus and edge regions of adult *S. mentella* otoliths, collected in seven different areas in the North Atlantic (for area codes, see Table 1). Outliers are marked by open circles, extreme values are indicated by stars.

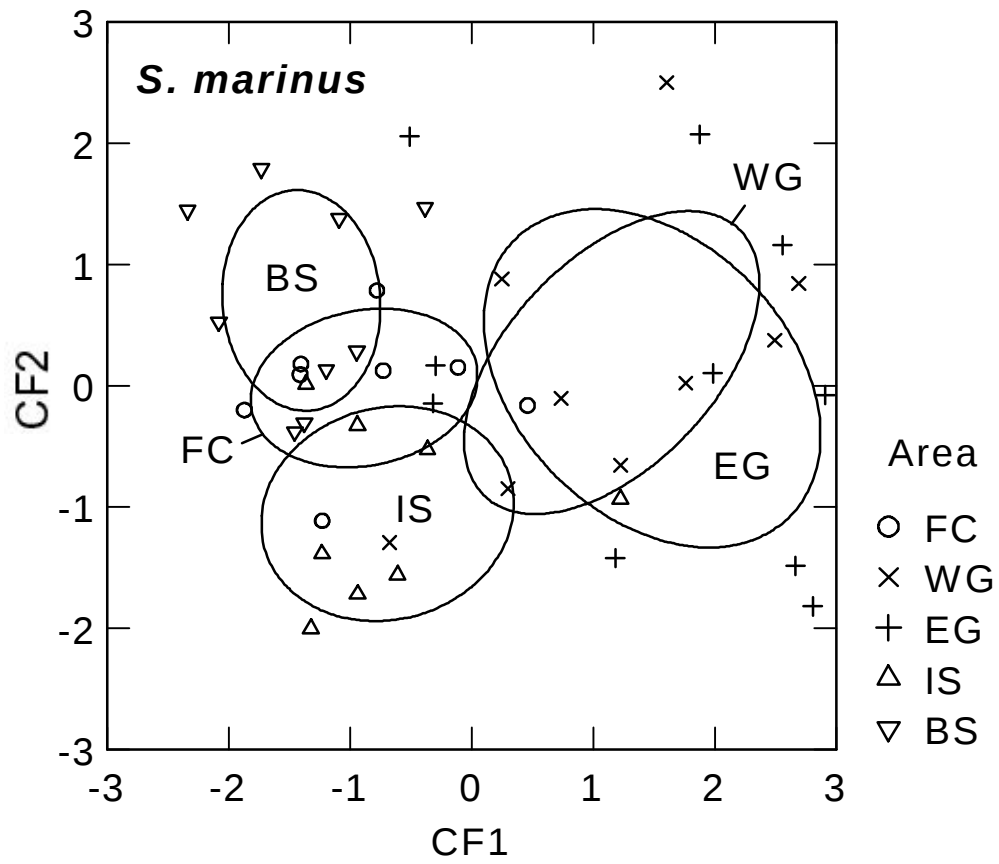


Figure 6. Discriminant function scores and 95% confidence ellipses for *S. marinus* otoliths from five different areas in the North Atlantic (for area codes, see Table 1), based on the $\log_{10}$ -transformed concentrations of five elements (Li, Mg, Cu, Sr, Ba) in the nucleus region.

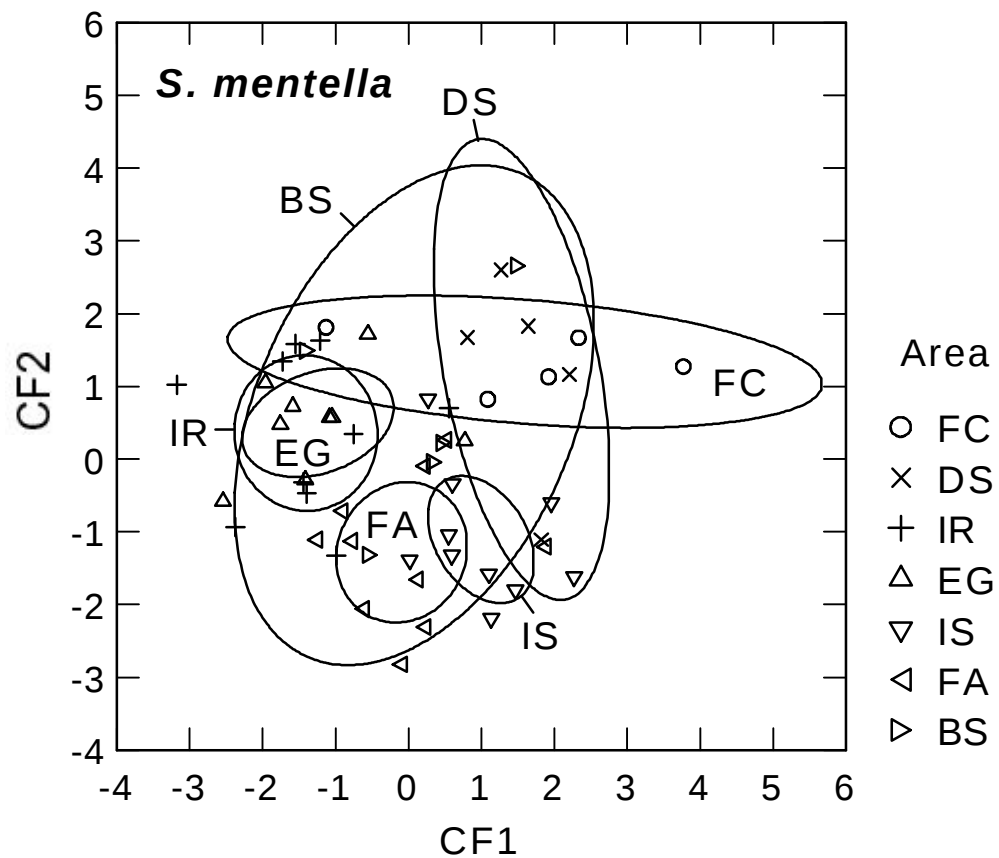


Figure 7. Discriminant function scores and 95% confidence ellipses for *S. mentella* otoliths from seven different areas in the North Atlantic (for area codes, see Table 1), based on the $\log_{10}$ -transformed concentrations of nine elements (Li, Mg, Mn, Cu, Zn, Rb, Sr, Sn, Ba) in the nucleus region.

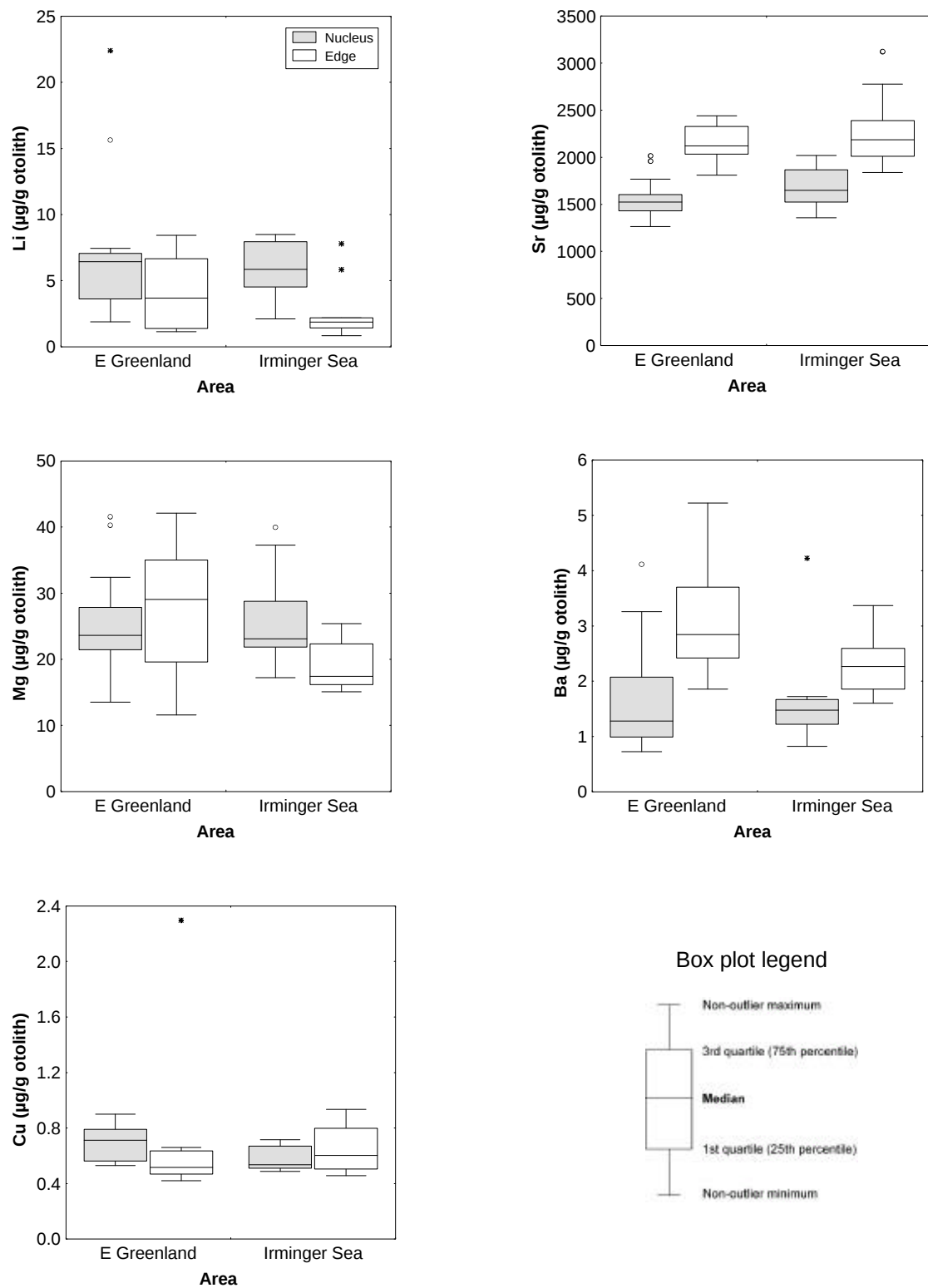


Figure 8. Concentration ($\mu\text{g g}^{-1}$ relative to Ca) of the five elements consistently measured over LOD in the nucleus and edge regions of *S. mentella* otoliths from fish with total lengths of 25-30 cm, collected on the East Greenland shelf in October 1998 and in the Irminger Sea in July 1999 during a large-scale migration observed for these size groups, areas and period. Outliers are marked by open circles, extreme values are indicated by stars.

3.1 Age and growth of Atlantic redfish (*Sebastes marinus*, *S. mentella*): bias and precision of age readers and otolith preparation methods

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Abstract

Age determination of Atlantic redfish (*Sebastes* spp.) has proven to be difficult and led to inconsistent age and growth estimates in the past. Even with consensus on the use of otoliths as preferred structure for ageing, the error observed in redfish age readings has prevented reliable age-based stock assessment. Using otoliths of the two major commercial species, golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*), a series of exchange schemes was carried out to assess bias and precision of age readings between four readers and between two preparation methods, the break-and-burn and the thin-sectioning technique. Considerable bias between readers and moderate precision was observed for the *S. marinus* readings, especially for ages above 20 years, with coefficients of variation (CV) of 7.7-12.0% and average percent error (APE) of 5.4-8.5%. The percent agreement between readers increased from 17-28% to 45-61% when allowing deviations of ± 1 year and to 80-92% with ± 3 years tolerance. *S. marinus* aged from broken and burnt otoliths were estimated to be slightly younger than the same individuals scored from thin-sectioned otoliths. The bias and precision estimates obtained from the *S. mentella* material were generally poorer than for *S. marinus* (CV 8.2-19.1%, APE 5.8-13.5%) but similar to reported values for other long-lived fish species. Above 50% agreement were only achieved with ± 3 years tolerance. Growth functions for both species revealed only minor differences between readers and confirmed slower growth for *S. mentella*. Average ages of around 9-10 years were determined for juvenile *S. mentella* of 24-30 cm length, which were likely to have migrated from East Greenland into the Irminger Sea, based on earlier observations. Since some of the error in the presented age determinations could be attributed to interpretational differences between readers, further intercalibration of redfish ageing is urgently needed in order to provide consistent input data for stock assessment.

Keywords: redfish, *Sebastes marinus*, *Sebastes mentella*; age reading, age determination, ageing methods; bias, precision, percent agreement; growth

Introduction

Age determinations provide essential input data for the assessment of marine fish stocks (Hilborn and Walters 1992). Utilising the periodicity in the formation of growth increments of calcified hard structures of fish, such as scales, otoliths, fin rays or vertebrae, the age of the investigated individuals can usually be estimated by counting annual zones (Campana 2001). Reliable age estimates, however, are difficult to obtain for species found in tropical regions and thus lacking seasonality in growth, and for long-lived species due to the slow growth and narrow increments in the older growth zones. Redfish of the genus *Sebastes* inhabiting the North Atlantic exhibit longevity of up to 75 years (Campana *et al.* 1990), leading to problematic age determination of these commercially important species (*e.g.* ICES 1996). Thus, most laboratories investigating stock dynamics of redfish have not implemented routine age readings since there are concerns about the error and poor reliability. In contrast, regular ageing schemes have been established for Pacific *Sebastes* species (MacLellan 1997, C.A.R.E. 2000), notwithstanding maximum ages of over 100 years (Munk 2001) that were recently confirmed by radiometric ageing (Andrews *et al.* 2002).

The question of reliability of hard body structures of fish for ageing was addressed several times in the past (*e.g.* Bortone and Hollingsworth 1980, Welch *et al.* 1993, Howland *et al.* 2004). Various studies (Chilton and Beamish 1982, Nedreaas 1990, Saborido-Rey 1995) and workshops (ICES 1991, ICES 1996) have shown that the otoliths are the most suitable structure for ageing redfish, considering the underestimation of older ages using scales and difficulties in the interpretation of other structures such as fin rays or vertebrae. But also otolith-based ageing is subject to a certain degree of error, manifesting in two major elements: bias and precision. The bias of age readings is caused by a consistent deviation of reading results between readers and is skewed from the mean to one side or the other, while the precision of age readings measures the closeness of repeated independent age estimates (Wilson *et al.* 1987, ICES 1996). Precision reflects the degree of agreement among readers and is not to be confused with accuracy that relates to the agreement with the true age of the fish (Campana 2001). Although there are routine testing systems and procedures for the assessment of bias and precision of age readings available (Kimura and Lyons 1991, Campana *et al.* 1995, Hoenig *et al.* 1995), a broad-scale application of these methods in the laboratories carrying out redfish age readings is still missing.

The most recent “Workshop on Age Reading of *Sebastes* spp.”, carried out within the ICES (International Council for the Exploration of the Sea) community in 1995, showed considerable bias between readers that was improving after discussion of general interpretation of growth structures on the sectioned otoliths (ICES 1996). Therefore, the need for further exchange of material and knowledge on age reading methods was stressed. Before otoliths can be used for age reading, they

have to be prepared in a way that allows to clearly identify growth structures. While laboratories in Canada, Iceland, Norway and Spain are mainly using the ‘break (and burn)’ method for ageing *Sebastes* species (Chilton and Beamish 1982, Nedreaas 1990, Saborido-Rey 1995, MacLellan 1997), Germany and the USA are using (thin-)sections of otoliths (ICES 1984, Gifford and Crawford 1988). Only few comparisons have been carried out to assess the variability between both methodologies with regard to Pacific rockfish (Boehlert and Yoklavich 1984, Stanley 1987, Andrews *et al.* 2002) but no systematic studies were reported for redfish to elucidate advantages or drawbacks of one or the other technique.

As part of a multidisciplinary research project on the population structure, reproductive strategies and demography of redfish in the Irminger Sea and adjacent waters, several otolith exchanges between four redfish age reading experts of the participating institutions were carried out. The first otolith exchange was based on *S. marinus* from the Icelandic shelf. The obtained ageing scores were compared between readers and preparation methods with respect to bias and precision. The second set of exchanged material consisted of otoliths of pelagic *S. mentella* from the Irminger Sea that were prepared as thin-sections to investigate species-specific differences in the level of error. Considering the expected differences in longevity and growth between *S. marinus* and *S. mentella* (e.g. Nedreaas 1990, Saborido-Rey *et al.* 2004), the age-length relationships and corresponding von-Bertalanffy growth parameters were calculated from the data of both exchange programs. Having estimated ageing errors and growth, the ages of juvenile *S. mentella* involved in a migration from the East Greenland shelf into the Irminger Sea in 1998-1999 (Stransky 2000) were determined with otoliths from fish of the tracked size groups caught during that period.

Materials and methods

The otoliths used within this study were divided into four sets of samples (Table 1), representing the specific tasks of the respective age reading comparisons. Two otolith preparation methods (sections and break-and-burn) were used to age the *S. marinus*, while the *S. mentella* otoliths were only sectioned. Four age readers from different nations were participating in the reader comparisons.

The *S. marinus* otoliths were collected onboard the Icelandic vessel M/V “Brettingur NS” during a groundfish survey in March 1997. The otoliths were taken from five hauls on the Icelandic shelf (ICES Division Va). 212 sagittal otolith pairs were selected for age determination, covering fish of 10-54 cm total length (Table 1). From each pair, one otolith was prepared for age reading using the ‘break-and-burn’ technique (Christensen 1964), while the other otolith was thin-sectioned based on the

technique described by Bedford (1983). The preparation by break-and-burn was carried out at the Marine Research Institute in Reykjavik, Iceland. The annuli visible on the otolith were counted using different microscope magnifications (maximum of 100x). A drop of oil was put on the otolith before counting the rings. The light was coming from above with an angle of about 30-45 degrees to the otolith surface. The thin-sections were produced at the Institute for Sea Fisheries of the Federal Research Centre for Fisheries in Hamburg, Germany. Two diamond-covered saw blades of 0.3 mm thickness and 100 mm diameter, rotating at 6000 rotations/min, were used to cut sections of about 0.5 mm thickness. These thin-sections were mounted onto glass plates with translucent polyester resin and read at a magnification of 20-40x using transmitted light. For the comparison of preparation methods, only the age readings of the Icelandic reader were compared. Pelagic *S. mentella* were sampled from twelve trawls in the Irminger Sea (ICES Sub-area XII) within a commercial sampling scheme onboard the German F/V “Fornax” in July 1999 (Table 1). From the sampled individuals, 213 otolith pairs were selected for thin-sectioning and subsequent age determination. *S. mentella* otoliths from fish of a size range of 24-30 cm were taken onboard the German FRV “Walther Herwig III” off East Greenland (ICES Division XIVb, bottom trawls) in October 1998 and in the Irminger Sea in June/July 1999 (ICES Sub-area XII, pelagic trawls).

For the comparison of bias and precision between readers and methods, a suite of statistical tests and graphical methods was applied. Bias estimates were based on simple linear regression analysis, the parametric paired t-test and the nonparametric Wilcoxon matched-pairs rank test (Conover 1998, Hollander and Wolfe 1999). The slope and intercept of simple linear regressions were tested for significant differences ($\alpha = 0.05$) from 1.0 and 0, respectively. The parametric paired t-test and the nonparametric Wilcoxon matched pairs rank test were used to detect significant differences from a paired difference of 0. As error terms, 95% confidence limits were calculated. Age bias plots (Campana *et al.* 1995) were produced to visualise the deviation of the age scores of two readers or methods from the 1:1 equivalence line. These plots also allowed the detection of non-linear bias patterns, *e.g.* the relative underestimation of ages by one reader in one part of the age range and relative overestimation in another part of the age range.

Various estimators of precision were suggested for comparisons of age readings. One of the more common indices is the percent agreement, comparing the percentage of age determinations that are in agreement within a specified number of years. This index, however, does not evaluate the degree of precision equally for all species. If, for example, 95% of the age readings agree within a range of ± 1 year for cod (*Gadus morhua*), this could be a very poor precision since there are just few year-classes in the fishery. For *S. mentella*, a 95% agreement within a tolerance range of ± 5 years would represent a good precision, given 75-year longevity and 30-40 age groups present in the fishery. Beamish and

Fournier (1981), therefore, suggested an average percent error (APE), which is dependent on the average age of the fish species investigated:

$$APE_j(\%) = 100 \times \frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} - X_j|}{X_j}$$

where R is the number of times each fish is aged, X_{ij} is the i th age determination of the j th fish, and X_j is the mean age calculated for the j th fish.

Chang (1982) modified this index to a coefficient of variation (CV), substituting the absolute deviation by the standard deviation from the mean age:

$$CV_j(\%) = 100 \times \frac{\sqrt{\sum_{i=1}^R \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j}$$

In addition to these indices, the correlation coefficient r^2 was calculated to evaluate the fraction of variation explained by the linear relationship between readers or otolith preparation methods.

For both the *S. marinus* and the *S. mentella* age readings, the age-length-relationships were plotted and fitted with the von-Bertalanffy growth function:

$$L_t = L_{\text{inf}} (1 - e^{-k(t-t_0)})$$

with L_t : fish length (cm) at age t (years), L_{inf} : asymptotic maximum fish length (cm), and t_0 : theoretical age (years) when the fish was at length zero. L_{inf} , the growth coefficient k and t_0 were calculated within an iterative process. To estimate the reader effect on the growth functions, individual sets of growth parameters were calculated for each reader and compared to literature data.

Results

S. marinus: comparison of readers

As indicated by the age bias plots for the *S. marinus* readings (Figure 1), all between-reader comparisons exhibit a certain degree of bias, particularly for ages greater than 20 years. In all six cases, the deviation from the 1:1 equivalence line is non-linear, most pronounced in the comparisons between reader 4 and all other readers (Figure 1, right side). Reader 4 generally allocated higher ages in the range 2-12 years and lower ages in the range 13-30 years, compared to the other readers. But

also in the comparisons of reader 1 and 2 and reader 2 and 3, the mean ages assigned by one reader deviate considerably from the age assignments of the second reader, particularly in the age range 17-30 years. Table 2 presents the statistical tests applied to the comparison of readers in terms of bias. Regression analysis as well as the Wilcoxon test and the paired t-test show high significance levels in most of the cases, generally indicating bias between readers. The scores of reader pairs 2 vs. 4 and 3 vs. 4, however, did not differ significantly with regard to the Wilcoxon test, and the comparison between reader 3 and 4 resulted in a non-significant mean paired difference (-0.2 years, $p = 0.327$). The overestimation of ages assigned by reader 2 compared to reader 1 in the older ages (deviation up to 10 years), as shown in Figure 1, results in a slope > 1 and negative intercept of the linear regression. The highest overall bias was observed between readers 1 and 2, exhibiting a mean paired difference of about 1 year. Slopes of < 1 and positive intercepts are present in all other comparisons. The largest deviation from the 1:1 equivalence line could be detected for reader 2 vs. reader 4, with a slope of < 0.6 and an intercept of > 5 (Table 2). In all six age bias plots (Figure 1), a general trend in increasing standard deviations around the mean with increasing age is visible.

From the precision estimates between readers (Table 3), the correlation coefficient, the CV and APE of the first three comparisons (reader 1 vs. reader 2, reader 1 vs. reader 3, reader 2 vs. reader 3) show relatively good agreement, whereas all comparisons with reader 4 resulted in considerably lower precision. The agreement between readers was 24-28% in the first three cases, and it was markedly below 20% in the latter cases. If the tolerance level of agreement between readers is raised, as illustrated in Figure 2, a level of around 80% and higher is reached with a tolerance of ± 3 years, considering the whole age range. For *S. marinus* aged 0-10 years, this tolerance leads to over 95% agreement in the first three reader pairs, while all pairs with reader 4 only agree to around 80%. In the medium age range (11-20 years), around 90% of the readings agree in all cases, while ageing of the oldest *S. marinus* (21-30 years) revealed poor agreement between readers, in the worst case (reader 2 vs. reader 4) below 60% even with a tolerance of ± 5 years (Figure 2).

S. marinus: comparison of otolith preparation methods

The age bias plot for the comparison of otolith preparation methods (Figure 3a) shows a slight relative underestimation of ages from 12 years onwards, using the break-and-burn technique. This observation is also indicated by a regression slope of < 1 and a positive intercept (Table 2). The mean paired difference between both methods was about 0.8 years. In contrast to the reader comparisons, the variation around the mean of the break-and-burn age scores does not increase steadily with higher ages. All precision indices for the comparison between methods were better than between readers (Table 3). The regression explains about 93% of the observed variation, and the coefficient of

variation and average percent error are comparably low. The agreement between otolith preparation methods is about 29%, which represents a markedly better value than achieved in the reader comparisons. The percent agreement plot for the comparison of methods (Figure 3b) shows a relatively poor agreement in the age range 21-30 years but considerably high correspondence in the younger age ranges (> 90% agreement with ± 3 years tolerance).

S. mentella: comparison of readers

An even higher degree in bias was obvious from the comparisons of *S. mentella* age readings (Figure 4). The deviation from the 1:1 equivalence line is non-linear for all reader pairs, and the most pronounced bias again occurred in all comparisons involving reader 4 (Figure 4, right side). In the age range 15 years and older, reader 4 is considerably underestimating most of the ages relative to the other readers, resulting in regression slopes markedly below 1 and mean paired differences of up to 5 years (Table 4). Readers 2 and 3 generally assigned higher ages than reader 1 (Figure 4), with mean paired differences of -2.2 and -0.8 years, respectively (Table 4). The nonparametric Wilcoxon test and the parametric paired t-test show high significance levels in all six comparisons.

A relatively high correlation between reader scores (87-95%), but slightly higher CVs and APE values as in the *S. marinus* readings indicated medium precision for most of the reader pairs, apart from the comparison of readers 2 and 3 with reader 4 that revealed considerably larger error terms (Table 5). The percent agreement between *S. mentella* readers was very variable (4-19%). When dividing the percent agreement plots into age ranges of 10 years (Figure 5), the fractions of the life span of *S. mentella* where most of the ageing error occurs, become visible. The curves for all reader pairs are changing from asymptotic to linear with increasing age range, showing that for ages of over 20 years, tolerance levels of $\pm 1-2$ years only lead to moderate improvements in the percent agreement. The agreement between readers increased to 62-87% when allowing ± 5 years tolerance (Figure 5). In the younger age groups (≤ 20 years), 73-100% agreement were achieved with a tolerance of ± 3 years, whereas in the age ranges 31 years and above, the agreement was mostly below 50% on this tolerance level. In the medium age range (21-30 years), a clear separation of reader pairs was obvious (Figure 5). The three comparisons with reader 4 revealed percent agreement values of 24-42%, applying ± 5 years tolerance, while the other reader pairs reached 79-89% on this level.

Age-length relationships and growth parameters

The calculated growth parameters for Icelandic *S. marinus* varied considerably between readers and only slightly between methods (Table 6). The age-length data of reader 2, both from section and break-and-burn readings, led to a relatively low L_{inf} (< 48 cm) and relatively high k values (0.12-0.13). Most of the other studies on *S. marinus* reported L_{inf} values of about 50 cm and k values of 0.09-0.12, being similar to the parameters obtained from the combination of all readings. The overall growth function derived from the *S. marinus* readings (Figure 6) also shows an asymptotic maximum length of about 50 cm and high variation in ageing scores, particularly for reader 4.

For *S. mentella* from the Irminger Sea, an asymptotic length of about 40 cm was observed (Figure 7). A markedly slower growth than that of *S. marinus* is clearly visible, also indicated by a lower k value (0.08 for all readers combined, Table 6). In concordance with the relative underestimation of ages by reader 4, the k value calculated from the ageing results of this reader (0.12) is by far exceeding the k values obtained for the other readers (0.07). The t_0 values for Irminger Sea redfish are largely varying between readers and indicate erratic estimates of down to -9.6 years in the worst case (Table 6).

Juvenile *S. mentella*

Comparative readings carried out on *S. mentella* otoliths from fish of a selected size range of 24-30 cm, collected off East Greenland and in the Irminger Sea during a period with migration signs, revealed largely differing estimates of involved age groups. Most of the scores of readers 1 and 2 were in the range 9-11 years, while reader 3 assigned older ages (10-13 years), and reader 4 allocated 8-9 years to the same material (Figure 8). Readers 2 and 3 generally scored pelagic *S. mentella* from the Irminger Sea 2-3 years older than *S. mentella* of the same length from the East Greenland shelf, while only a minor shift in age distribution between areas was found by readers 1 and 4. Reader 3 contributed the broadest age range (5-20 years), and reader 4 aged these fish within a narrow range of 5-11 years. From the combination of all readers' results, the mean age of the East Greenland samples was 9.1 years, and that of the Irminger Sea redfish was 10.6 years.

Discussion

All between-reader comparisons in the *S. marinus* otolith exchange showed a considerable bias, caused by relative over- or underestimation of up to 1 year mean paired difference. In the age range over 20 years, individual ageing score pairs differed by up to 10 years. Since maximum ages of over 40 years were reported for this species in the Northeast Atlantic (Nedreaas 1990), the ageing bias for ages beyond 30 years could be even higher. But also when similar age scores are produced by different readers on the same individual, this does not imply similar interpretation of growth structures. As illustrated in an example overlay of reading marks (Figure 9a), reader 1 had a different perception of the nucleus zone than the other readers and used a different reading axis from the eighth reading mark onwards, but came to the identical age estimate as reader 4. Differences in the interpretation of marginal zones also become apparent but were more pronounced for fish aged above 20 years.

The ranges of the precision estimates calculated for the *S. marinus* reader comparisons (CV 7.7-12.0%, APE 5.4-8.5%), are slightly above the average values in the literature (CV 7.6%, APE 5.5%; Campana 2001). CVs of 12.9% and 14.8%, however, were reported for fish species with similar longevity, such as sablefish (*Anoplopoma fimbria*; Kimura and Lyons 1991) and Atlantic sturgeon (*Acipenser oxyrinchus*; Stevenson and Secor 1999), respectively. Laidig *et al.* (2003) compared age readings carried out on blue rockfish (*Sebastes mystinus*) and obtained an APE of 5.6% between readers. The percent agreement of *S. marinus* readings within a tolerance of ± 0 years does not exceed 30%, being relatively poor compared to age reading results for *e.g.* herring (*Clupea harengus*; Corten 1993), mackerel (*Scomber scombrus*; Villamor and Meixide 1995) or horse mackerel (*Trachurus trachurus*; Eltink 1997). Since the percent agreement index does not account for the high number of age groups, *i.e.* life-span of the investigated species, comparisons with short-living species can only be approached by applying higher tolerance of ageing deviations between readers. At a tolerance of ± 1 year, more than 60% agreement was reached for the best reader pair, which recently motivated an exploratory analytical assessment of *S. marinus* on the basis of three-year intervals (Rätz *et al.* 2004a). The resulting stock projection estimates were similar to those obtained from production models such as BORMICON (Björnsson and Sigurdsson 2003).

Although the precision of the comparison between otolith preparation methods was generally higher than that for the readers, a significant bias between methods was observed. Age readings based on the 'broken and burnt' otoliths showed a slightly lower estimate of age, relative to the results obtained from thin-section readings. Since the primary aim of this study was the comparison between readers by exchange of thin-sectioned otoliths and, in the case of breakage in one of the otoliths of a pair, the other one was kept for sectioning, the number of otoliths available for the break-and-burn preparation was relatively low. One disadvantage of the break-and-burn method is the reading variability

introduced by different angles of the light applied to the broken surface. Several laboratories ageing Pacific *Sebastes* species, however, have harmonised their age reading protocols (MacLellan 1997, C.A.R.E. 2000) in order to reduce reading error caused by systematic differences in interpretation. Within an age validation study, Andrews *et al.* (2002) have recently compared thin-section and break-and-burn readings on yelloweye rockfish (*Sebastes ruberrimus*) with ages of 15-117 years and noted slightly higher correspondence between methods ($r^2 = 0.971$) than in our study ($r^2 = 0.931$). In contrast to our results, they found minor relative overestimation of ages using the break-and-burn method.

The bias of the *S. mentella* readings was particularly apparent in the comparisons of reader 4 with the other readers, manifesting in up to 5 years mean paired difference, with individual deviations reaching 20 years. These inconsistencies between readers can be partly attributed to a different interpretation of the nucleus zone (Figure 9b). Considering expected longevity of *S. mentella* of 75 years (Campana *et al.* 1990), elevated reading bias in data obtained for older individuals of this species is common and often caused by the difficult differentiation of marginal increments. These interpretational differences also affect the precision of readings to a large extent, which was markedly poorer in the *S. mentella* reader comparisons than in the *S. marinus* readings. Regular otolith exchange schemes between Canadian and US ageing labs for Pacific *Sebastes* species (C.A.R.E. 2000) of similar longevity as *S. mentella* have revealed only slightly better CVs of 8.2-12.2% and APE of 5.7-9.1% (C.A.R.E. 2002). Andrews *et al.* (2002), however, noted a CV of 4.5% and an APE of 2.6% for section readings of *S. ruberrimus*. Among reader intercalibration studies for other long-lived species, APEs of 4.3-10.6% were reported for Patagonian toothfish (*Dissostichus eleginoides*) with ages of 2-53 years (Horn 2002). Bergstad *et al.* (1998) found a significant improvement of CVs for tusk (*Brosme brosme*) age readings after consensus on a common interpretation principle, decreasing from 11.6% to 7.6% and resulting in non-significant differences between three readers in the final exchange. Keeping the commercial importance of pelagic *S. mentella* in the Irminger Sea in mind, a comprehensive reader intercalibration and standardised ageing protocols (*e.g.* Beanlands 1997, Walsh and Burnett 2002) are urgently needed.

Although a relatively high degree of bias was observed between *S. marinus* readers, the age-length relationships and growth parameters only varied moderately. Interestingly, the von-Bertalanffy parameters derived for reader 1 came closest to those from the largest dataset available (almost 13000 readings of *S. marinus* around Iceland). An asymptotic maximum length of over 50 cm and a growth coefficient k of < 0.1 were calculated for both datasets, while the relative underestimation of ages by reader 4 did not lead to an extraordinarily high k . The break-and-burn results of reader 2, however, indicated faster growth ($k = 0.12$) than suggested by the all thin-section readings combined ($k = 0.11$) but slower growth than derived from the section readings of reader 2 only ($k = 0.13$). A more pronounced difference in k was observed in the *S. mentella* readings, with the relative underestimation

in the ageing scores of reader 4 leading to a k of 0.12, opposed to 0.07 inferred from the data of the other readers. Most other studies suggested k values below 0.10, but the narrow length range of the *S. mentella* investigated in this study (22-41 cm) makes a comparison with other studies based on material from an extended length range problematic. Since the smaller juveniles of 20 cm length and below inhabit demersal nursery areas on the shelf (Magnússon *et al.* 1988), the lack of younger age groups in the Irminger Sea (Magnússon and Magnússon 1995) contributes largely to the remarkably low t_0 values found for Irminger Sea redfish. The *S. mentella* aged in this study were caught at comparatively shallow depths where larger fish are underrepresented, probably causing the relatively low L_{inf} of 39 cm. Pelagic redfish in the Irminger Sea are found down to 1000 m, with maximum lengths of over 50 cm (Sigurdsson *et al.* 1999), suggesting higher L_{inf} values when including fish from deeper layers where the bigger specimens usually occur.

An additional error might be introduced by the presence of relatively large fast-growing juveniles that have been recruiting from the highly productive shelf areas off East Greenland and Iceland. Due to our results, a 25 cm pelagic *S. mentella* would be around 6 years old, while in shelf areas of the Flemish Cap (NAFO Division 3M), in the Northeast Arctic (ICES Sub-areas I and II) and off East Greenland (ICES Division XIVb), demersal *S. mentella* of the same size would be 7, 8 and 8.5 years old, respectively (Nedreaas 1990, Kosswig and Rätz 1995, Saborido-Rey *et al.* 2004). Since the L_{inf} and k values were reported to be higher for the shelf areas, faster growth of demersal *S. mentella* compared to pelagic *S. mentella* was observed for ages greater than 10 years. By tracking strong cohorts in the length frequency distributions derived from regular monitoring programs, an indirect validation of age reading results is possible (*e.g.* Mayo *et al.* 1981, Beamish and MacFarlane 1983, Nedreaas 1990). The investigated *S. marinus* material includes two strong year classes, most probably 1985 and 1990, dominating the length distributions in regular surveys and commercial sampling (ICES 2003). After 10 years, the length peaks had reached 28-29 cm, which corresponds well with the age-length-relationship inferred from the data of this study and recently undertaken radiometric ageing (**chapter 3.2**).

Considerable deviations between readers were also present for *S. mentella* of the length range 24-30 cm, selected with regard to evidence of migration found for these length groups from East Greenland into the Irminger Sea during 1998-1999 (Stransky 2000). The extraordinarily strong cohort that could be tracked in the length distributions of *S. mentella* below 20cm length off East Greenland (Rätz *et al.* 2004b), however, is most likely comprised of fish from the 1991 year-class, so the ageing results of reader 4 are closest to this estimate (age 7 in 1998, age 8 in 1999). In contrast, 0-group indices from the Icelandic surveys (Magnússon and Jóhannesson 1997) did not match the observed strong cohorts.

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Table 1. Redfish otolith samples exchanged between four age readers.

Species	Sampling area (ICES Sub-area or Division)	Sampling date or period	Depth range (m)	Length range (cm)	n	Preparation methods
<i>S. marinus</i>	Iceland (Va)	March 1997	247-421	10-54	212	Sections, break & burn
<i>S. mentella</i>	Irminger Sea (XII)	July 1999	200-350	22-41	213	Sections
<i>S. mentella</i>	East Greenland (XIVb)	October 1998	246-389	24-30	60	Sections
<i>S. mentella</i>	Irminger Sea (XII)	June-July 1999	200-650	25-30	86	Sections

Table 2. Statistical tests for the detection of bias for age readings of *S. marinus* between readers and methods.

Statistic	Age reader						
	Reader 1 vs. Reader 2 (n = 199)	Reader 1 vs. Reader 3 (n = 212)	Reader 2 vs. Reader 3 (n = 199)	Reader 1 vs. Reader 4 (n = 212)	Reader 2 vs. Reader 4 (n = 199)	Reader 3 vs. Reader 4 (n = 212)	Sections vs. break & burn (n = 105)
Regression							
Slope	1.157 ± 0.038	0.950 ± 0.034	0.783 ± 0.024	0.693 ± 0.040	0.588 ± 0.030	0.688 ± 0.035	0.877 ± 0.024
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Intercept	-0.993 ± 0.510	1.221 ± 0.460	2.615 ± 0.357	4.345 ± 0.536	5.153 ± 0.435	4.267 ± 0.497	0.987 ± 0.368
P	0.053	0.009	0.000	0.000	0.000	0.000	0.009
Wilcoxon test							
P	0.000	0.000	0.012	0.006	0.134	0.762	0.000
Paired t-test							
Mean paired difference	-1.005 ± 0.320	-0.585 ± 0.274	0.372 ± 0.296	-0.406 ± 0.360	0.513 ± 0.401	0.179 ± 0.360	0.771 ± 0.315
P	0.000	0.000	0.014	0.028	0.019	0.327	0.000

Table 3. Measures of precision for age readings on *S. marinus* between readers and methods.

Statistic or index	Age reader						
	Reader 1 vs. Reader 2 (N = 199)	Reader 1 vs. Reader 3 (N = 212)	Reader 2 vs. Reader 3 (N = 199)	Reader 1 vs. Reader 4 (n = 212)	Reader 2 vs. Reader 4 (n = 199)	Reader 3 vs. Reader 4 (n = 212)	Sections vs. break & burn (n = 105)
Correlation coefficient (r^2)	0.824	0.787	0.840	0.590	0.667	0.631	0.930
Coefficient of variation (%)	8.79	8.19	7.66	11.96	11.22	10.61	6.69
Average percent error	6.21	5.79	5.42	8.45	7.93	7.50	2.49
Percent agreement	24.12	25.00	27.64	16.51	18.59	18.87	28.57

Table 4. Statistical tests for the detection of bias for age readings of *S. mentella* between readers.

Statistic	Age reader					
	Reader 1 vs. Reader 2 (n = 191)	Reader 1 vs. Reader 3 (n = 213)	Reader 2 vs. Reader 3 (n = 191)	Reader 1 vs. Reader 4 (n = 207)	Reader 2 vs. Reader 4 (n = 188)	Reader 3 vs. Reader 4 (n = 207)
Regression						
Slope	1.016 ± 0.017	1.055 ± 0.017	1.006 ± 0.017	0.853 ± 0.021	0.787 ± 0.021	0.768 ± 0.021
<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000
Intercept	1.903 ± 0.396	-0.348 ± 0.408	-1.632 ± 0.448	0.244 ± 0.495	-0.405 ± 0.540	1.310 ± 0.524
<i>P</i>	0.000	0.395	0.000	0.622	0.455	0.013
Wilcoxon test						
<i>P</i>	0.000	0.001	0.000	0.000	0.000	0.000
Paired t-test						
Mean paired difference	-2.215 ± 0.412	-0.808 ± 0.431	1.492 ± 0.443	2.729 ± 0.566	5.074 ± 0.660	3.580 ± 0.706
<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000

Table 5. Measures of precision for age readings on *S. mentella* between readers.

Statistic or index	Age reader					
	Reader 1 vs. Reader 2 (n = 191)	Reader 1 vs. Reader 3 (n = 213)	Reader 2 vs. Reader 3 (n = 191)	Reader 1 vs. Reader 4 (n = 213)	Reader 2 vs. Reader 4 (n = 191)	Reader 3 vs. Reader 4 (n = 213)
Correlation coefficient (r^2)	0.951	0.950	0.947	0.891	0.880	0.869
Coefficient of variation (%)	11.22	8.16	11.06	12.31	19.10	14.22
Average percent error	7.93	5.77	7.82	8.70	13.51	10.06
Percent agreement	6.81	19.25	11.52	15.46	3.72	16.91

Table 6. Von Bertalanffy growth parameters, derived from this study and other studies (A: T. Sigurdsson, Marine Research Institute, Reykjavík, Iceland, pers. comm.; B: Nedreaas 1990; C: Saborido-Rey *et al.* 2004; D: K. Nedreaas, Institute of Marine Research, Bergen, Norway, pers. comm.).

Species	Material	Reference	Reader/ Lab	Method	Length range (cm)	n	von Bertalanffy parameters		
							L _{inf}	k	t ₀
<i>S. marinus</i>	Iceland 1997	(this study)	1	sections	10-54	212	52.66	0.095	0.470
<i>S. marinus</i>	Iceland 1997	(this study)	2	sections	10-54	199	46.36	0.131	1.587
<i>S. marinus</i>	Iceland 1997	(this study)	3	sections	10-54	212	49.55	0.113	1.233
<i>S. marinus</i>	Iceland 1997	(this study)	4	sections	10-54	212	49.00	0.121	1.427
<i>S. marinus</i>	Iceland 1997	(this study)	all four	sections	10-54	835	50.50	0.105	0.935
<i>S. marinus</i>	Iceland 1997	(this study)	2	break & burn	10-54	108	47.80	0.124	0.913
<i>S. marinus</i>	Iceland 1995-2002	A	2	break & burn	9-82	12974	50.33	0.088	-1.427
<i>S. marinus</i>	Norway/Barents Sea 1985-88	B	3	break & burn	8-82	488	50.2	0.11	0.08
<i>S. marinus</i>	Flemish Cap 1990-2000 (males)	C	4	break & burn	8-51 ¹	3215	46.40 ¹	0.104	-0.79
<i>S. marinus</i>	Flemish Cap 1990-2000 (females)	C	4	break & burn	11-57 ¹	2823	58.15 ¹	0.069	-1.49
<i>S. mentella</i>	Irminger Sea 1999	(this study)	1	sections	22-41	213	40.08	0.066	-8.531
<i>S. mentella</i>	Irminger Sea 1999	(this study)	2	sections	22-41	191	39.23	0.073	-6.379
<i>S. mentella</i>	Irminger Sea 1999	(this study)	3	sections	22-41	213	39.27	0.069	-9.635
<i>S. mentella</i>	Irminger Sea 1999	(this study)	4	sections	22-41	204	38.82	0.117	-2.293
<i>S. mentella</i>	Irminger Sea 1999	(this study)	all four	sections	22-41	824	39.31	0.078	-6.797
<i>S. mentella</i>	Irminger Sea 1999	A	2	break & burn	20-52	920	44.27	0.087	-3.492
<i>S. mentella</i>	Irminger Sea 1999	D	3	break & burn	21-50	426	43.06	0.107	-0.894
<i>S. mentella</i>	Irminger Sea 2001	D	3	break & burn	25-49	690	43.69	0.093	-2.463
<i>S. mentella</i>	Irminger Sea 2001	D	3	sections	31-48	115	46.76	0.063	-6.577
<i>S. mentella</i>	Norway/Barents Sea 1985-88	B	3	break & burn	8-58	142	49.0	0.06	-2.47
<i>S. mentella</i>	Flemish Cap 1990-2000 (males)	C	4	break & burn	9-48 ¹	3588	43.24 ¹	0.107	-1.07
<i>S. mentella</i>	Flemish Cap 1990-2000 (females)	C	4	break & burn	11-46 ¹	3454	45.82 ¹	0.096	-1.28

¹fork length

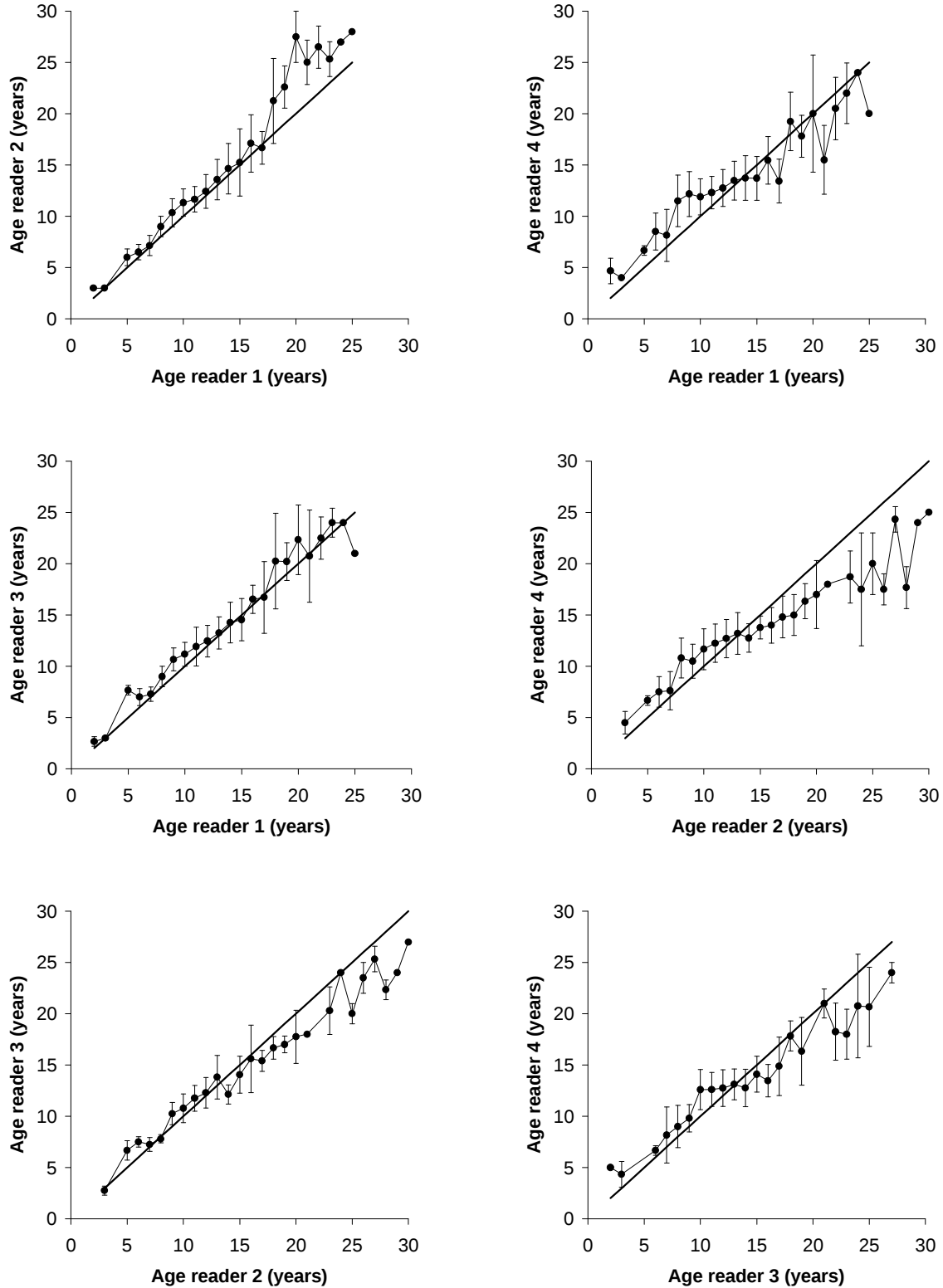


Figure 1. Age bias plots for the reader comparisons based on the *S. marinus* otoliths from the Icelandic shelf. Each error bar represents the standard deviation around the mean age assigned by one reader for all fish assigned a given age by the second reader. The 1:1 equivalence (straight line) is also indicated.

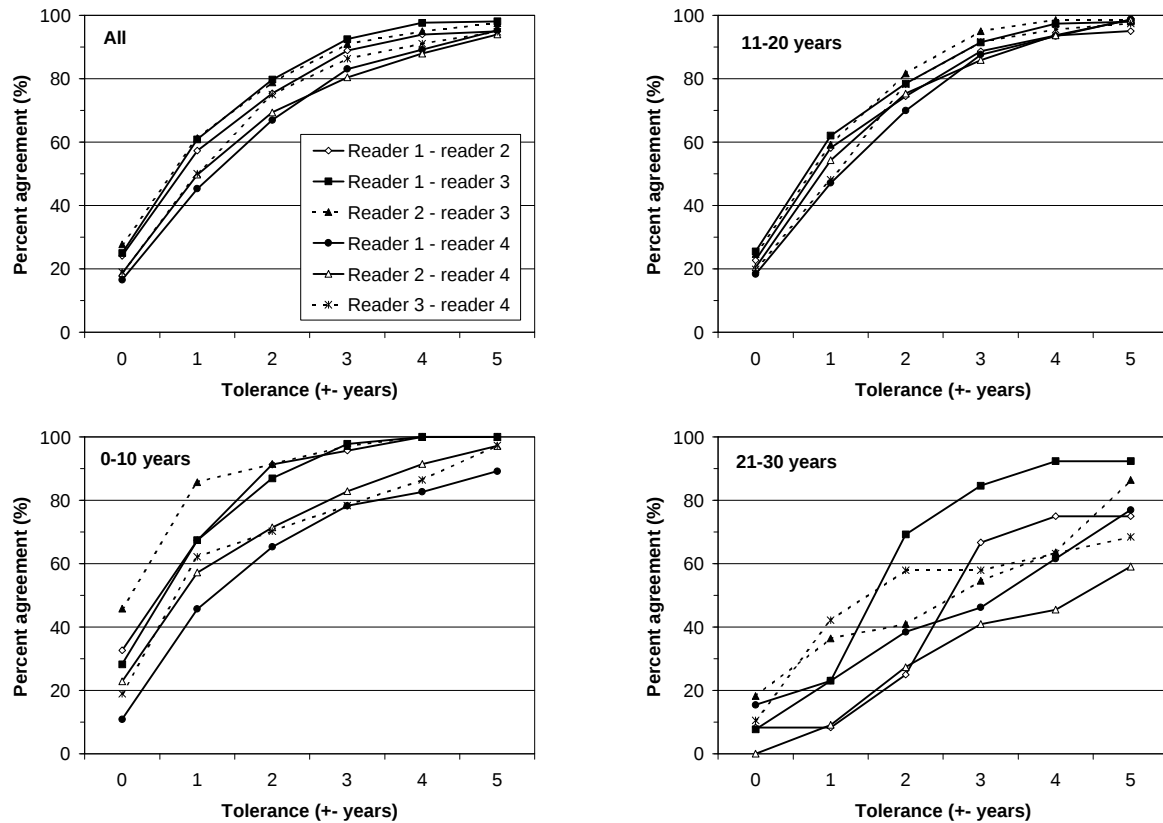


Figure 2. Percent agreement plots for the reader comparisons based on the *S. marinus* otoliths from the Icelandic shelf for a tolerance level (deviation of assigned ages between both readers) of ± 0 (total agreement) to ± 5 years. These were applied to all age groups and sub-sets of age ranges assigned by the first reader.

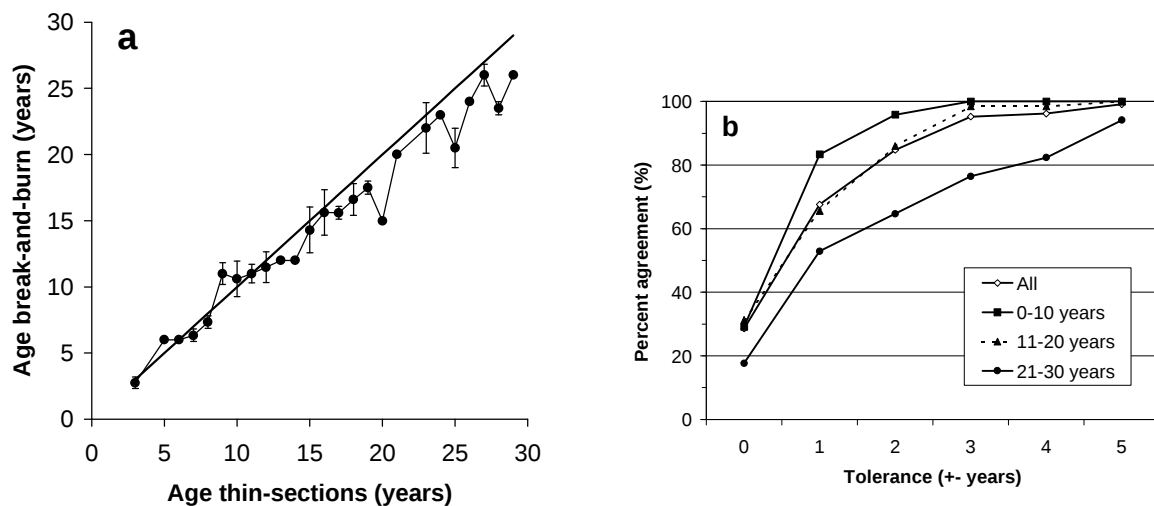


Figure 3. Age bias plot (a) for the comparison of otolith preparation methods based on *S. marinus* from the Icelandic shelf. Each error bar represents the standard deviation around the mean age assigned in the break-and-burn readings for all fish assigned a given age in the thin-section readings. The 1:1 equivalence (straight line) is also indicated. In the percent agreement plot (b), tolerance levels (deviation of assigned ages between methods) of ± 0 (total agreement) to ± 5 years are applied to all age groups and sub-sets of age ranges assigned in the thin-section readings.

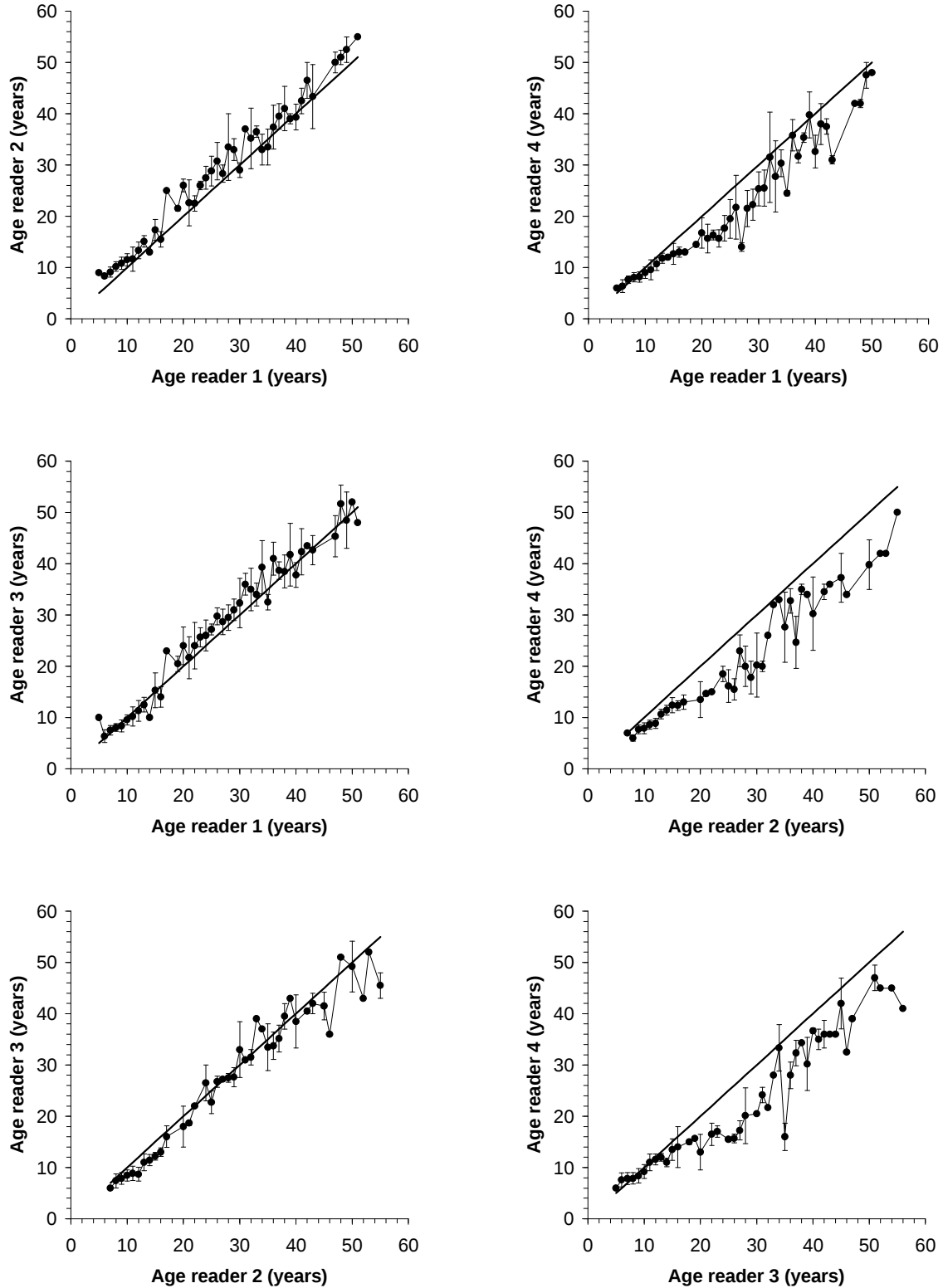


Figure 4. Age bias plots for the reader comparisons based on the pelagic *S. mentella* otoliths from the Irminger Sea. Each error bar represents the standard deviation around the mean age assigned by one reader for all fish assigned a given age by the second reader. The 1:1 equivalence (straight line) is also indicated.

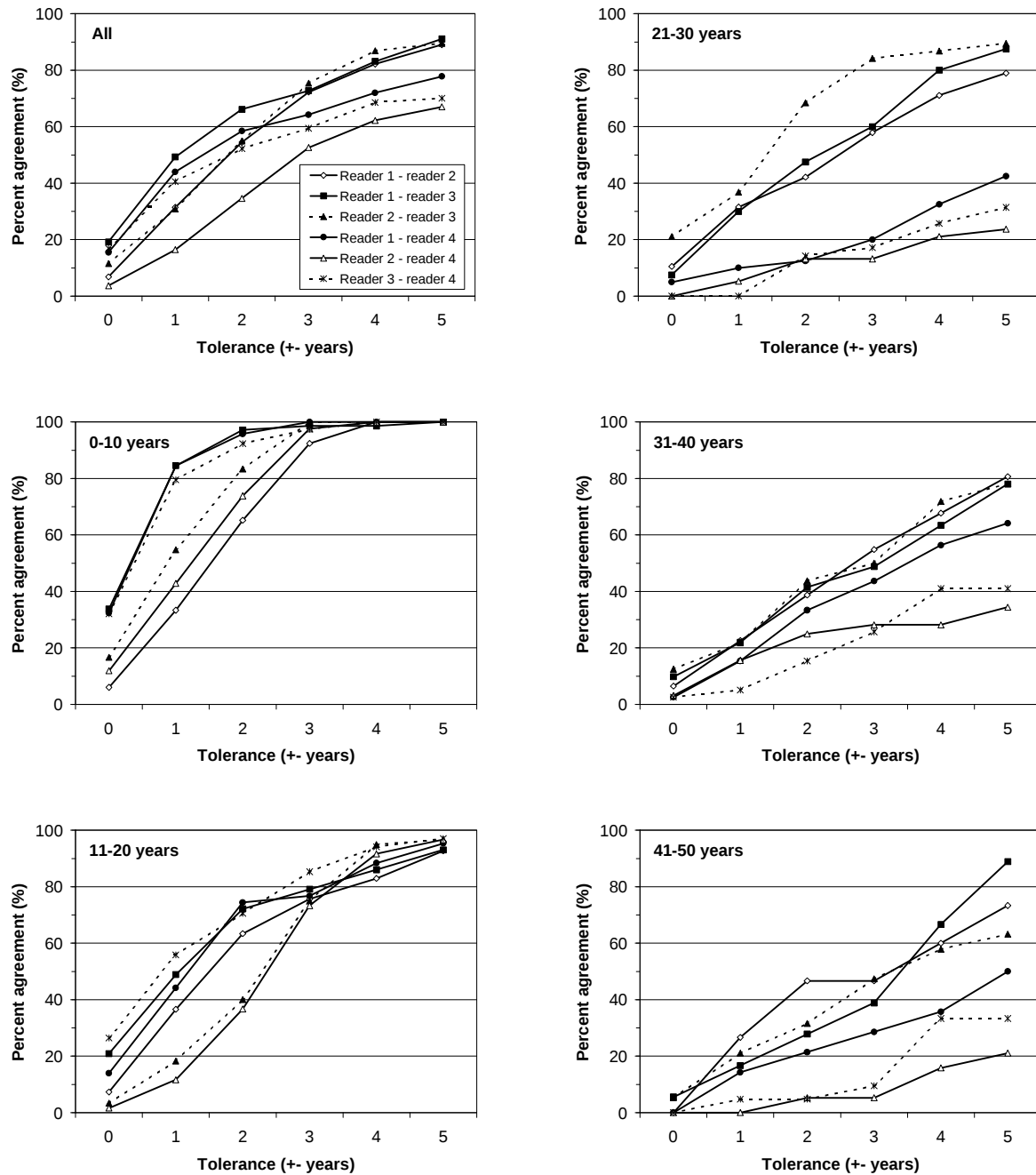


Figure 5. Percent agreement plots for the reader comparisons based on the pelagic *S. mentella* otoliths from the Irminger Sea for a tolerance level (deviation of assigned ages between both readers) of ± 0 (total agreement) to ± 5 years. These were applied to all age groups and sub-sets of age ranges assigned by the first reader.

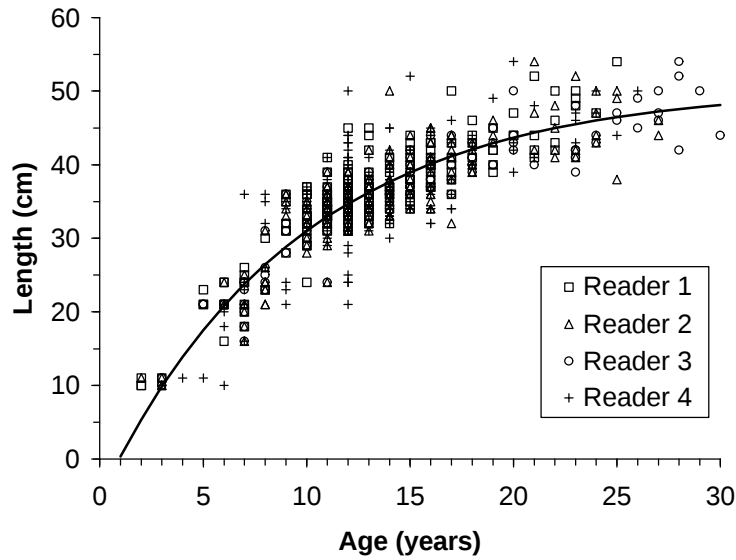


Figure 6. Age-length relationship and fitted von-Bertalanffy growth curve of the reading comparison based on *S. marinus* otoliths from the Icelandic shelf. For growth parameters, see Table 6.

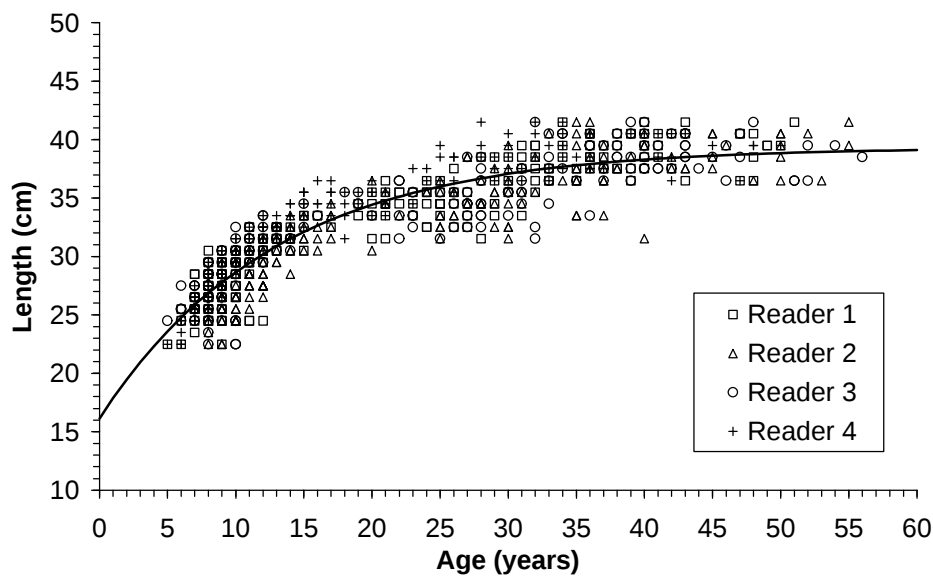


Figure 7. Age-length relationship and fitted von-Bertalanffy growth curve of the reading comparison based on *S. mentella* otoliths from the Irminger Sea. For growth parameters, see Table 6.

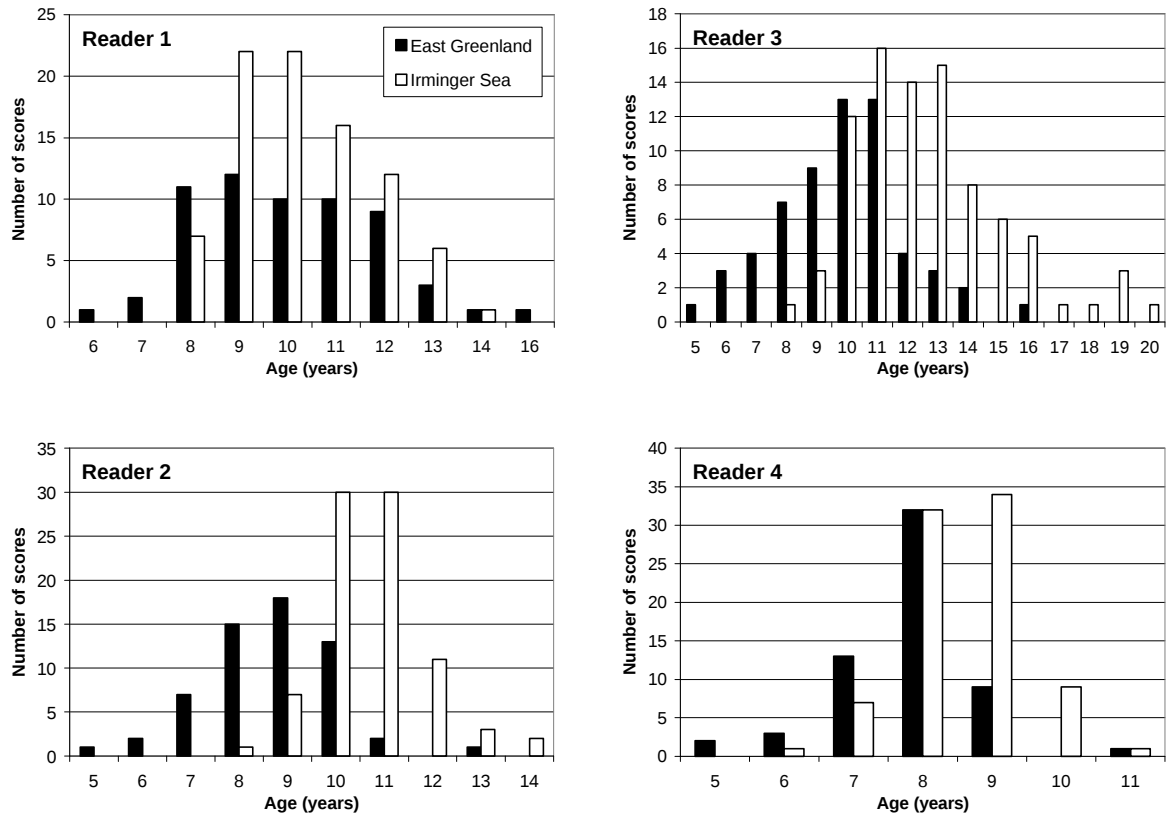


Figure 8. Age distribution of demersal *S. mentella* from the East Greenland shelf (24-30 cm total length) and pelagic *S. mentella* from the Irminger Sea (25-30 cm total length).

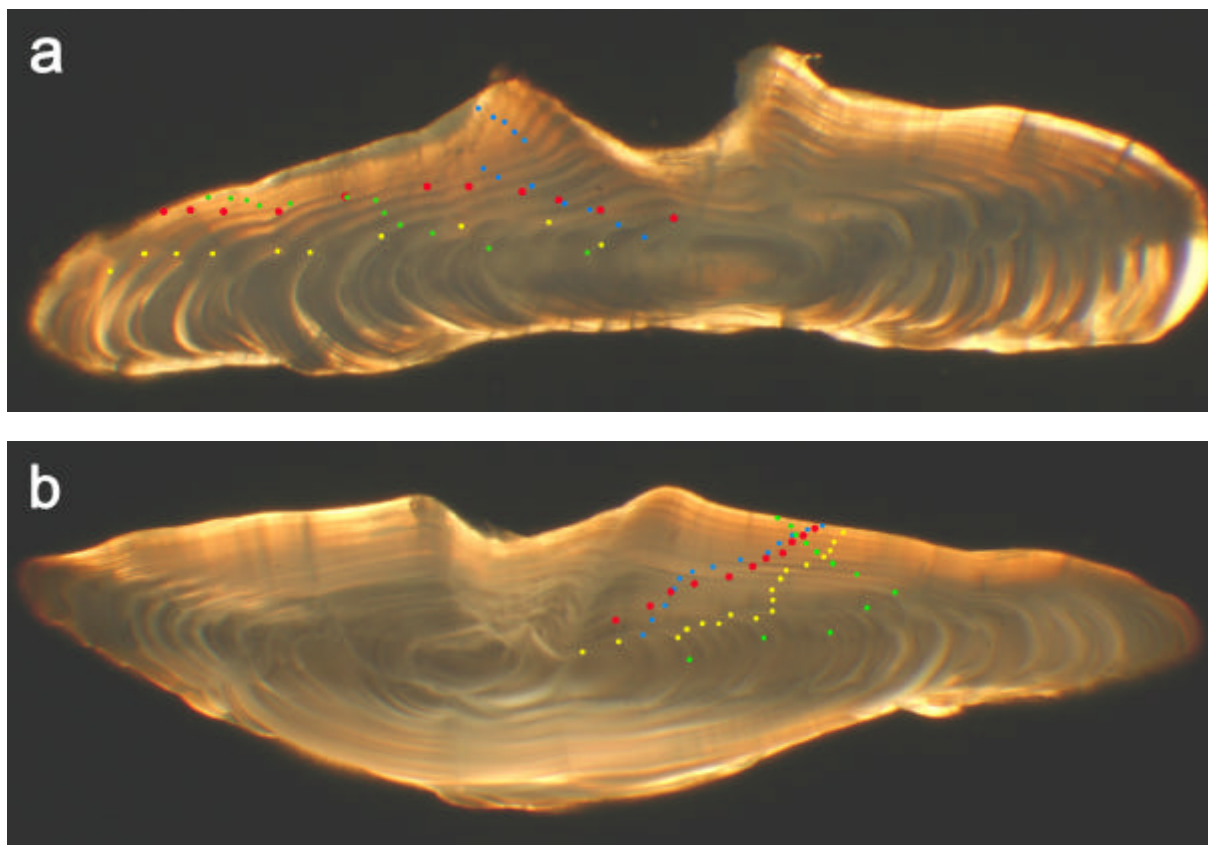


Figure 9. Example pictures of a *S. marinus* (a) and a *S. mentella* (b) otolith thin-section, including age reading marks of readers 1 (blue), 2 (red), 3 (yellow) and 4 (green). The reading scores are (a) 12, 11, 10, 12 years, and (b) 13, 11, 18, 12 years, respectively.

3.2 Radiometric age validation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*)

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Abstract

Age determination of redfish (genus *Sebastes*) has been inconsistent in the past, preventing age-based stock assessment. Validation studies for *Sebastes* species were predominantly focused on Pacific rockfish, whereas only few verification attempts have been undertaken for North Atlantic redfish. Using a radiometric ageing technique based on $^{210}\text{Pb}/^{226}\text{Ra}$ isotope ratios, ages of golden redfish (*S. marinus*) around Iceland as well as deep-sea redfish (*S. mentella*) off East Greenland and in the Irminger Sea were determined by alpha-spectrometric measurement of these radioisotopes in otolith core samples, pooled by length group. In general, the measured isotope ratios corresponded well with the expected radioactive ingrowth curves and with traditional age estimates for fish of the same length group. A slight tendency towards an underestimation of age by traditional annulus counts could be inferred from the comparison with the derived radiometric ages. Considerable differences between ageing methods were found for *S. marinus* over 40 cm length and *S. mentella* from the deeper layers of the Irminger Sea. Irminger Sea redfish of the biggest investigated length group (41-45 cm) exhibited the maximum radiometric age recorded (41.3 years). This study confirms slow growth and high longevity of North Atlantic redfish.

Keywords: redfish, *Sebastes marinus*, *Sebastes mentella*; Northeast Atlantic; radiometric ageing, age validation; radioisotopes, polonium, lead, radium

Introduction

Age determinations provide essential input data for any age-based assessment of marine fish stocks (Hilborn and Walters 1992). An optimum harvesting strategy takes differences in productivity inferred from species- and stock-specific growth rates into consideration. For some fish species, however, reliable age data are missing due to difficulties in the ageing of the assessed species. These difficulties are either arising from low seasonality in growth, *e.g.* in tropical waters (Milton *et al.* 1995), or from different interpretations of growth structures that particularly lead to high age reading error for species

with high longevity (e.g. Beamish 1979, Smith *et al.* 1995). For several species of the genus *Sebastes*, maximum ages of over 100 years were reported (Munk 2001) and recently validated (Andrews *et al.* 2002). For representatives of this genus inhabiting the North Atlantic, golden redfish *Sebastes marinus* and deep-sea redfish *S. mentella*, estimates of longevity varied largely due to high bias and a lack of precision in age readings (ICES 1996, Stransky *et al.* 2003; **chapter 3.1**). Thus, the demand for age validation studies for these commercially important species was stressed repeatedly.

Apart from recent tagging experiments, traditional age validation of redfish involved the tracking of strong cohorts in the length distributions (Mayo 1981, Nedreaas 1990, Saborido-Rey *et al.* 2004). Campana *et al.* (1990) reported maximum ages for *S. mentella* on the Scotian Shelf (Northwest Atlantic) of up to 75 years and were the first to validate ages up to 63 years by radiometric ageing based on $^{210}\text{Pb}/^{226}\text{Ra}$ disequilibria. The basis for radiometric age determination is the incorporation of the calcium analogue ^{226}Ra into the otolith and its subsequent radioactive decay to ^{210}Pb . The ratio of these two isotopes serves as an index of elapsed time since ^{226}Ra incorporation. During recent years, several radiometric age validation studies were carried out, based on bomb radiocarbon (e.g. Kalish 1993, Campana 1997, Baker and Wilson 2001, Kerr *et al.* 2004), $^{228}\text{Th}/^{228}\text{Ra}$ (Campana *et al.* 1993) or $^{210}\text{Pb}/^{226}\text{Ra}$ ratios (see Burton *et al.* 1999 for a review) in the otoliths. The latter technique has also been successfully applied to Pacific *Sebastes* species (Bennett *et al.* 1982, Watters 1993, Kestelle *et al.* 2000, Andrews *et al.* 2002).

This study represents the first radiometric ageing approach to *S. marinus* and to *S. mentella* from commercially important fishing grounds in the Northeast Atlantic, the East Greenland shelf and in the Irminger Sea. Using standard procedures for the determination of ^{210}Pb and a recently improved radiochemical method for alpha-spectrometric measurement of Ra isotopes, ^{210}Pb and ^{226}Ra activities were recorded in 20 pooled otolith core samples. From the obtained $^{210}\text{Pb}/^{226}\text{Ra}$ ratios, radiometric ages were calculated and compared to traditional ages derived from annulus counts.

Material and methods

Sample collection

The investigated otoliths were taken from *S. marinus* caught with demersal gear around Iceland (ICES Sub-area Va) on Icelandic commercial trawlers in March 2000 and from *S. mentella* taken onboard the German FRV “Walther Herwig III” within a regular groundfish survey off East Greenland (ICES Sub-area XIVb) in September 2000 and during a pelagic survey in the Irminger Sea (ICES Division XII, NAFO Divisions 1F/2H/2J) in June/July 2001 (Table 1). A total of 20 samples, divided by species,

area and fish length groups, was analysed, aiming at the coverage of the demographic range of each species. For the East Greenland samples, however, only otoliths from *S. mentella* measuring ≥ 17 cm in total length were included due to difficulties in the separation from *S. marinus* below this size. Additional otoliths from fish showing lengths above or below the selected ranges did not occur or were available but amounted to insufficient numbers to form a sample. Sample S1 contained otoliths from 0-group redfish caught off West Greenland on FRV “Walther Herwig III” in October/November 1999 to investigate the initial uptake of the two radioisotopes measured in this study. Catch depths ranged from about 100 to 400 m for the samples taken with bottom trawls (*S. marinus* around Iceland, *S. mentella* off East Greenland) and from 200 to 700 m for *S. mentella* caught with a pelagic GLORIA 1024 net in the Irminger (and Labrador) Sea.

Otolith core extraction

To avoid assumptions about otolith mass growth when analysing radiometric measurements of fish otoliths (Campana *et al.* 1990, Kimura and Kesteven 1995), only the first 3 years of juvenile growth were analysed, with the exception of sample S1 (see above) and sample MA1 (which was assumed to contain otoliths from average 3-year old fish) that were comprised of whole otoliths. The first 3 years of growth were chosen in a compromise between the time available for core extraction and the number of cores necessary for a sample of 1 gram, providing enough material for the detection of radioisotopes. 1-year cores would have required about 3500-4000 cores instead of roughly 500 cores analysed in this study. The extraction of the 3-year cores required the removal of the outer (later deposited) parts of the adult otoliths. Otolith core sizes were determined by measuring the dimensions of 14 otoliths of juvenile redfish, assumed to be on average 3 years old, and the measurement of the dimensions of the first 3 years of growth on 10 thin-sections of otoliths taken from adult redfish. The required dimensions were achieved by grinding the otoliths down to 6.1 x 3.9 x 1.3 mm with a purpose-built grinding wheel coated with 46-grit diamond-grain surface. During coring, the grinding wheel and cored samples were repeatedly washed with MilliporeTM filtered Milli-Q water ($18 \text{ M}\Omega \text{ cm}^{-1}$) to minimise surface contamination of the cores. The cores were stored in screw-cap polyethylene vials. The cleaning of the cores involved a multi-step process with a 3-times Milli-Q water wash between the following cleaning steps: 1) soaking in Milli-Q water for at least 5 minutes, 2) ultrasonic bath in Milli-Q water for at least 10 minutes, 3) decontamination with 0.05N Na_4EDTA (pH 10.5) in an ultrasonic bath for at least 5 minutes, 4) washing with 0.15N HNO_3 in an ultrasonic bath for 60 seconds, and 5) storage in 30% H_2O_2 at $+50^\circ\text{C}$ for 16-20 hours (Fenton *et al.* 1991). The cleaned cores were placed into teflon vials and dried at $+70^\circ\text{C}$ for at least 24 hours. After drying and cooling in an exsiccator for at least 1 hour, the core samples were weighed to the nearest 0.1 mg.

Radiochemical analysis

Because of the extremely low levels of ^{210}Pb and ^{226}Ra expected from a pilot analysis and literature values for *Sebastes* otoliths (attograms [10^{-18} g] to femtograms [10^{-15} g]; *e.g.* Andrews *et al.* 2002), lab handling and cleaning procedures were carried out with trace metal precautions. All lab equipment that was in direct contact with the samples was decontaminated with 0.1N EDTA (adjusted with NaOH to pH10) in an ultrasonic cleaning bath for at least 1 hour, acid-washed (silicon, rubber and plastics at least 16 hours in 1N HNO_3 ; glassware and teflon at least 1 hour in boiling concentrated HNO_3), stored in Milli-Q water for at least 16 hours and rinsed three times with Milli-Q water. When available, trace metal grade (Merck SuprapurTM) chemicals were used, dilutions were made with Milli-Q water only. Where possible, teflon and quartz glass lab equipment was used throughout the radiochemical analyses to minimise contamination of the samples from these surfaces. These rigorous cleaning procedures and careful sample handling contributed to considerably lower reagent blanks than reported in previous studies (Table 2).

Before analysing the inorganic matrix of the otolith cores, the organic constituents (such as the high-fibre protein otolin; Degens *et al.* 1969) must be denaturised. This was attempted by dissolving the samples in five consecutive 1 ml portions of concentrated HNO_3 and heating the solution in a closed container to $+200^\circ\text{C}$ in a lab microwave oven for 30 minutes. Complete destruction of the organics, however, was not achievable, indicated by a yellowish residue after vaporising the solution almost to dryness before radiochemical separation.

The analysis of ^{210}Pb in the samples was accomplished by detecting its alpha-emitting daughter ^{210}Po . ^{209}Po and ^{229}Th - ^{225}Ra were added as tracers before radiochemical analysis for the determination of the chemical yield of ^{210}Po and ^{226}Ra , respectively. Both tracer solutions were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, USA). The ^{210}Po was autodeposited onto cleaned silver planchets (99.97% purity, Allgemeine Gold- und Silberscheideanstalt AG) at $+90$ - 95°C for 3 hours (Flynn 1968, Fenton *et al.* 1990) while stirred with a flat-end teflon stick at about 80 rotations per minute. After ^{210}Po deposition, the remaining solution was evaporated to dryness on a hot plate ($\sim +150^\circ\text{C}$) and re-dissolved with 1 ml of concentrated HNO_3 for five times to destroy any remaining organic compounds. The whitish residue was re-dissolved in 100 ml 2N HNO_3 and stored in teflon bottles for ^{226}Ra analysis.

Since the ^{226}Ra separation technique for alpha-spectrometric analysis reported by Hancock and Martin (1991) revealed insufficiently low chemical yields, an improved method for the determination of Ra isotopes by Purkl and Eisenhauer (2003a) was applied to the samples. Major improvements of the chemical yield were accomplished by selective extraction of Ra with 3M EmporeTM Radium Rad

Disks (Ľ urecová 1997, Smith *et al.* 1997, Purkl and Eisenhauer 2003b), purification of the sample using low-pressure column chromatography and electrodeposition in vials coated by copper cells placed onto Peltier elements for effective cooling of the electrolyte solution (Purkl and Eisenhauer 2003a).

Alpha-spectrometric measurement of ^{210}Po and ^{226}Ra

The alpha-spectrometric measurements of ^{210}Po and ^{226}Ra activity were performed within three long-term runs in one Ortec SoloistTM and eight Ortec 576 alpha-spectrometry systems. These encompassed 17 detectors (surface barrier and ion-implanted silicon detectors), connected to two Canberra multi-channel analysers (model CI 556 AIM interfaced with eight-channel analog multi-plexer, model CI 8224) and one Fast ComTec system (ADC model 7074 interfaced with MCA model MCD4LAP). Alpha spectra were acquired on IBM-compatible PCs with Canberra Basic Genie2000TM and Fast ComTec MCDWINTM software. Counting times varied between 56-78 d. The distances from source to detector were always the smallest possible for the respective alpha chambers.

For the analysis of the spectra, Microsoft WindowsTM-based in-house software was used. Peak areas were determined by Poisson maximum likelihood estimation peak fitting, especially needed for the very complex radium alpha spectra. Three (short, medium and long-range) alpha peak tailing contributions were considered in the analytical description of the peak shape. It included also the calculation of the massic activities and their complete uncertainty budgets considering all known parameter uncertainties (ISO 1995, EURACHEM/CITAC 2000). Due to the alpha-recoil effect, which at least for close sample-detector distances increase the measured count rates of the members within a decay chain, it was decided to use only the ^{225}Ac peak multiplet for the Ra yield determination. Reagent blanks, determined by three blank samples for the entire radiochemical analysis (^{210}Po and ^{226}Ra) and four separate reagent blanks for ^{226}Ra only, as well as regularly recorded instrument background values were subtracted from the measured activities (Table 2).

It was assumed that the Ra/Ac separation would not be complete, leaving some initial ^{225}Ac excess in the sample at the time where the ^{225}Ac ingrowth from ^{225}Ra started (Crespo 2000, Blanco *et al.* 2002). Therefore, but also to prevent loss of data, all alpha spectra were saved and evaluated weekly, especially to follow and detect the expected small decreases of the apparent Ra yield estimated from the ^{225}Ac daughter of the ^{225}Ra tracer. Statistical procedures for correlated input data were used to quantify the initial ^{225}Ac excess at the Ra/Ac separation from these series of intermediate spectrum evaluations. This allowed the correction of the apparent yields and activities of ^{226}Ra of the final (full counting time) Ra spectra. The corrections were estimated in the range 0.1%-4.7% (average 2.5%) for

the final Ra spectra. For some Ra spectra, $^{225}\text{Ra}/^{225}\text{Ac}$ build-up factors, needed for the ^{226}Ra activity calculation, had to be corrected for a few maintenance breaks throughout the very long counting period.

Calculation of fish age

The age t of the otolith cores was determined by solving first the following basic formula (Fenton and Short 1995) by iteration (method of bisection) for the physical core age t_p :

$$\frac{A_{\text{Pb-210}}}{A_{\text{Ra-226}}} = \left(1 - e^{-\lambda(t_p - T)}\right) + \left[1 - (1 - R) \cdot \left(\frac{1 - e^{-\lambda T}}{\lambda T}\right)\right] \cdot e^{-\lambda(t_p - T)} \quad (1)$$

where A = specific activity of ^{210}Pb or ^{226}Ra , respectively (dpm g^{-1})

λ = decay constant of ^{210}Pb (year^{-1})

t_p = physical core age at the start of the radiochemical analysis (years)

T = period of core formation (years)

R = initial uptake activity ratio $^{210}\text{Pb}/^{226}\text{Ra}$

The desired age t of the otolith (fish age) is finally obtained by simply subtracting the storage duration (sampling to the start of radiochemical analysis) from t_p .

The specific activity of ^{210}Pb in equation (1) was calculated from the ^{210}Po activity, which was actually measured:

$$A_{\text{Pb-210}} = A_{\text{Po-210}} \cdot d(t_p) \quad (2)$$

The dependence of the decay correction $d(t_p)$ on the physical core age t_p required further iteration of equation (1) to solve for t_p . $d(t_p)$ was derived by using a numerical algorithm for the solution of the Bateman equations for radioactive decay chains (Bateman 1910, Skrabble *et al.* 1974). For this purpose, it was necessary to consider the development of the complete decay chain from ^{226}Ra via ^{210}Pb to ^{210}Po , starting from $t_p = 0$ to the begin of the analysis, rather than treating only the sub-chain ^{210}Pb to ^{210}Po for the short period between sampling and radiochemical analysis. However, it is emphasised that the initial uptake activity ratio R was always interpreted as $^{210}\text{Pb}/^{226}\text{Ra}$ ratio.

Average traditional ageing data for the corresponding radiometric samples were calculated on the basis of 12903 age determinations for *S. marinus* around Iceland (T. Sigurdsson, Marine Research Institute, Reykavík, Iceland, pers. comm.; **chapter 3.1**), 1367 age determinations for *S. mentella* from

the Irminger Sea (Stransky *et al.* 2002, T. Sigurdsson, pers. comm.; **chapter 3.1**) and 157 age determinations for *S. mentella* with total lengths of 26-30 cm from the East Greenland shelf (Stransky *et al.* 2003). The mean traditional ages of the remaining samples from East Greenland, namely length groups 17-20, 21-25 and 31-35 cm, were estimated from tracking strong cohorts in the annual length frequencies (Rätz *et al.* 2004).

Results

A total of 20 pooled otolith core samples were analysed for ^{210}Po and ^{226}Ra (Table 3). The individual weight of the samples ranged from 0.646 to 1.316 g (excluding sample S1). The expected tendency of increasing ^{210}Po activities with increasing fish length (age) is generally visible. Minimum ^{210}Po activities of 0.004 dpm g⁻¹ were recorded for *S. marinus* around Iceland with 16-20 cm fish length (sample MA2), exhibiting the highest uncertainty (21.2%) of all measurements (with exception of sample S1). For samples with higher ^{210}Po activities, however, standard deviations of 5-10% were determined. The ^{210}Po activity measured in sample MI4 (0.034 dpm g⁻¹) was the highest in the data series. Average chemical yields of 100.1% indicated quantitative radiochemical separation of ^{210}Po . Since the ^{226}Ra activities showed strong individual variation, the activity ratios were calculated on the basis of individual radioisotope measurements instead of averaging them by species/area groups. The highest ^{226}Ra activities (up to 0.053 dpm g⁻¹ in sample MI4) were determined for the *S. mentella* samples from the Irminger Sea. Standard deviations for ^{226}Ra activities ranged from 4.8 to 14.4%. Chemical yields for ^{226}Ra were much more variable than the ^{210}Po recoveries, ranging from 37.9 to 91.5% (average 67.1%). As expected, the calculated $^{210}\text{Po}/^{226}\text{Ra}$ activity ratios varied between 0 and 1 (minimum 0.110, maximum 0.727). Unfortunately, the ^{210}Po activity of sample S1, containing otoliths from 0-group redfish, was not measurable above detector background, preventing the direct determination of the initial uptake ratio R . The ^{226}Ra activity in this sample was about two-fold the average levels detected in all other samples.

The radiometric ages calculated from the $^{210}\text{Po}/^{226}\text{Ra}$ ratios for each sample were as low as 2.9 years for juvenile *S. marinus* and as high as 41.3 years for the largest adult *S. mentella* in the Irminger Sea (Table 3). For Icelandic *S. marinus*, a continuous increase in age (and standard deviations) with elevating fish lengths was observed in traditional ageing. The radioisotope-derived ages also showed this trend but deviated by up to +9.3 years from the average traditional ages in the case of sample MA7. Radiometric ages, however, generally overlapped with the annulus-derived age determinations. In the *S. mentella* material from East Greenland, a shift of +1.0-3.6 years was obtained from radiometric ageing relative to the traditional and length cohort data. Radiometric ages for *S. mentella* of 26-30 cm length were about 4 years lower than determined for Irminger Sea redfish of the same

sizes, while more than -7 years difference between areas was observed for the samples within the 31-35 cm length group. Within the Irminger Sea samples, radiometric ages were predominantly higher than the corresponding traditional values, with the highest deviation occurring in samples MI3 (+6.9 years) and MI5 (+12.2 years). Sample MI3 exhibited only a slightly lower radiometric age than sample MI2 that was composed of otolith cores from the same length group (31-35 cm), but originating from fish caught in a shallower depth layer. In contrast, the traditional estimates for fish of this size group from the shallower layer were on average almost 7 years higher than for *S. mentella* of equal lengths from the deeper layer. The same difference between depth layers was found in traditional ageing of *S. mentella* with lengths of 36-40 cm, whereas the radiometric age for this length group was 5.6 years lower in the shallower depth zone.

The determined $^{210}\text{Po}/^{226}\text{Ra}$ activity ratios generally coincide with the expected ^{210}Po ingrowth functions (Figure 1, left column). From a set of assumed initial uptake ratios R , most samples are closest related with R values around 0.1. For samples with $^{210}\text{Po}/^{226}\text{Ra}$ ratios greater than 0.4, elevated variation and higher deviations from the ingrowth curves were observed, suggesting R values between 0.0 and 0.1 or higher than 0.2. Radiometric ages for *S. marinus* overall correspond well with the traditionally determined ages (Figure 1, right column), with the exception of two samples from larger fish (MA7 and MA9). Although the regression of the radiometric ages on the traditional estimates for the *S. marinus* samples deviates considerably from the line of agreement, the mean values are relatively evenly distributed around the equivalence line. The slope of the regression was found to be significantly different (t -test; $\text{df } 8$, $t = 6.46$, $p < 0.001$) from a hypothetical slope of 1. A paired-sample t -test, however, indicated that there was no difference between the individual age estimates ($\text{df } 8$, $t = -0.752$, $p = 0.473$). The radiometric ages of *S. mentella* from East Greenland were consistently higher than the traditional ages, confirmed by a significant slope ($\text{df } 3$, $t = 4.95$, $p < 0.05$) and paired t -test statistic ($\text{df } 3$, $t = -4.22$, $p < 0.05$). In contrast, the fit of the regression line was very good ($R^2 = 0.925$). In the Irminger Sea material, the large differences between radiometric and traditional values occurring in *S. mentella* of medium sizes/ages groups from depths of greater than 500 m (MI3 and MI5) led to considerable deviations from the line of agreement, which resulted in a significantly differing slope ($\text{df } 5$, $t = 3.96$, $p < 0.05$) but non-significant paired differences ($\text{df } 5$, $t = -2.53$, $p = 0.052$).

Discussion

The average ^{226}Ra activities of (0.031 ± 0.007) dpm g^{-1} calculated for the samples of the bottom-dwelling *S. marinus* (Iceland; samples MA1-MA9) and *S. mentella* (East Greenland; samples MG1-

MG4) coincide with the average value of $(0.033 \pm 0.002) \text{ dpm g}^{-1}$ for *S. mentella* on the Scotian shelf, reported by Campana *et al.* (1990). Other studies determining ^{226}Ra activities in otolith cores of *Sebastes* species found varying values ranging from $(0.032 \pm 0.002) \text{ dpm g}^{-1}$ (*S. ruberrimus*; Andrews *et al.* 2002) to $(0.122 \pm 0.002) \text{ dpm g}^{-1}$ (*S. polyspinis*; Kestelle *et al.* 2000). Kestelle *et al.* (2000) also investigated *S. alutus*, *S. aleutianus* and *S. borealis* and measured average core ^{226}Ra activities of (0.075 ± 0.002) , (0.065 ± 0.003) and $(0.077 \pm 0.003) \text{ dpm g}^{-1}$, respectively. The pelagic *S. mentella* samples from the Irminger Sea (samples MI1-MI6) investigated in this study showed average ^{226}Ra activities of $(0.042 \pm 0.010) \text{ dpm g}^{-1}$. These variations in ^{226}Ra activities between species and areas could be due to differences in sources of ^{226}Ra . A part of the Irminger Sea redfish were caught near the Reykjanes Ridge where elevated elemental and radioisotope levels are expected from hydrothermal activity.

The mechanisms of Ra uptake by marine fishes were examined by Porntepkasemsan and Nevissi (1990), noting that ^{226}Ra levels previously found in Pacific salmon were five times higher than the values they found in several demersal species in Puget Sound off the Eastern US coast. Since salmon are pelagic, a coincidence of their findings with the elevated ^{226}Ra activities in pelagic redfish is plausible. However, they excluded bottom sediments as direct source of Ra to fish due to the fact that the demersal fish, being closer to the sediment, contained markedly less ^{226}Ra than salmon. They attributed higher Ra levels in salmon to higher metabolic rate of salmon opposed to the demersal species, increasing the uptake of elements from water and/or food. As metabolic rates of pelagic redfish are not known, this effect could not be evaluated in this study.

Due to the expected ingrowth of ^{210}Pb into the otolith with increasing age, ^{210}Po activities previously determined in fish otoliths vary with the age of the fish and thus with the longevity of the respective species. As a possible source of error, contamination of samples from other ^{210}Pb sources has to be excluded. This was approached by removing the outer layers of the otoliths in the coring process, rigorous cleaning of the cores and trace metal lab precautions throughout the sample handling and radiochemical analyses. In the sample containing whole otoliths from the smallest juvenile redfish in this study (MA1), however, external uptake of ^{210}Pb via the unprocessed otolith surface could have caused the elevated level of ^{210}Po in comparison to the samples from the next two bigger length groups (MA2, MA3).

Since a relatively good fit of the estimated traditional age and measured $^{210}\text{Po}/^{226}\text{Ra}$ activity ratios with the expected ingrowth functions could be observed, the presented radiometric technique was successfully applied to validate ages of *S. marinus* and *S. mentella* in the central North Atlantic. The initial uptake ratio R could unfortunately not be determined by direct measurement due to the low sample weight and resulting low ^{210}Po activity of sample S1. The samples from juvenile *S. marinus*,

however, indicated R values of about 0.1, and most of the other samples also varied around this value. We used a R of 0.0 for the calculation of radiometric ages but also investigated R values of 0.05 and 0.1. Assuming $R = 0.1$ reduced the radiometric ages by 3.0-3.4 years and thus improved the correspondence with traditional ages for the bigger length groups of the *S. mentella* samples, but also led to radiometric ages of below 1 year for juvenile *S. marinus* of 16-20 cm and 21-25 cm length. Kastle et al. (2000) reported quasi-empirical R values of -0.01 to 0.06 for four *Sebastes* species, determined from $^{210}\text{Pb}/^{226}\text{Ra}$ in otoliths of the youngest fish in their samples (3-4 years traditional age).

For *S. marinus* around Iceland, ages up to 12 years could be tracked by the length frequencies of the strong year-classes 1985 and 1990 (Björnsson and Sigurdsson 2003). The radiometric age for *S. marinus* of 31-35 cm length coincides very closely with the average traditional age and with the year-to-year length frequency distribution, showing a peak 32-34 cm in 1997 (cohort 1985) and in 2002 (cohort 1990) (ICES 2003). *S. marinus* with lengths above 50 cm may be underestimated by traditional ageing, since the radiometric age is 6 years higher than the average traditional age. This might be due to the problematic differentiation of narrow growth increments in the marginal zones of the older *S. marinus* (Stransky et al. 2001; **chapter 3.1**). Radiometric ages for *S. mentella* exceeded those estimated from traditional ageing, especially for the Irminger Sea samples collected in depths greater than 500 m. A possible reason for this discrepancy could also be a relative underestimation in traditional ageing, caused by converging growth zones in otoliths of redfish from deeper layers. As the otoliths used in this study were not aged directly by annulus counts, however, some error could be introduced by interannual or year-class differences in growth when comparing radiometric ages with the average ages of material collected within a larger temporal range.

Having faced severe difficulties and high bias in traditional ageing of redfish (e.g. ICES 1996, Stransky et al. 2001, 2002 and 2003; **chapter 3.1**), this study provides further proof of slow growth and confirmation of high longevity in redfish, with the highest ages of over 40 years found for *S. mentella* in the Irminger Sea. Maximum reported ages for *S. marinus* range from 42 to 44 years, and from 34 to 49 for *S. mentella* (Sandemann 1969, Nedreaas 1990, Saborido-Rey et al. 2004). Campana et al. (1990) noted an age of 75 years for the oldest *S. mentella* individuals in their samples. These extraordinary high maximum ages were not found in our study, which may be attributed to the pooling of the samples which may mask the occurrence of single ‘methusalems’. In addition, a general decline of maximum ages has been observed for *Sebastes* species since the 1980s (Andrews et al. 2002), likely due to increased fishing pressure inducing faster growth (Leaman and Beamish 1984).

Clearly, the presented radiochemical procedures and alpha-spectrometric measurements are too time-consuming and expensive for routine ageing. The $^{210}\text{Pb}/^{226}\text{Ra}$ technique usually serves as validation tool for fish species where ageing by growth structure observation is prohibited, difficult or in doubt

(Campana 2001). While the ^{210}Po analyses reported here can be accomplished relatively easy and reveal consistently high chemical yields, ^{226}Ra analyses of the otolith matrix are far from straightforward and demand high material and handling effort to achieve sufficient recovery rates. Alternative measurement of ^{226}Ra by isotope-dilution thermal ionisation mass spectrometry (TIMS; e.g. Andrews *et al.* 1999) delivers high yields and improved analytical uncertainties but requires extended sample preparation and purification procedures. Bearing the technological developments in the field of mass spectrometry in mind, further alternative measurement techniques might simplify radioisotope analyses of fish otoliths in future.

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Table 1. Capture date, catch depths and length ranges of *S. marinus* and *S. mentella* that were aged by radioisotope determination of ^{210}Pb and ^{226}Ra in pooled otolith core samples. Note that samples MI2 and MI3, as well as samples MI4 and MI5, included otoliths from *S. mentella* of the same length range, but different catch depth zones.

Sample	Species	Area	Capture date or range	Catch depth (m)	Total fish length (cm)	Sample weight (g)	No. of cores
MA1	<i>S. marinus</i>	Iceland	11-20 Mar 2000	97-258	10-15	0.646	29
MA2	<i>S. marinus</i>	Iceland	11-12 Mar 2000	97-233	16-20	0.859	27
MA3	<i>S. marinus</i>	Iceland	9-23 Mar 2000	97-310	21-25	1.316	30
MA4	<i>S. marinus</i>	Iceland	9-12 Mar 2000	97-262	26-30	0.994	24
MA5	<i>S. marinus</i>	Iceland	9-12 Mar 2000	149-262	31-35	0.986	24
MA6	<i>S. marinus</i>	Iceland	9-14 Mar 2000	129-262	36-40	1.045	25
MA7	<i>S. marinus</i>	Iceland	9-22 Mar 2000	135-262	41-45	0.976	24
MA8	<i>S. marinus</i>	Iceland	9-23 Mar 2000	119-386	46-50	0.954	24
MA9	<i>S. marinus</i>	Iceland	9-23 Mar 2000	186-386	51-55	0.872	25
MG1	<i>S. mentella</i>	East Greenland	22 Sep 2000	373-384	17-20	0.828	29
MG2	<i>S. mentella</i>	East Greenland	22 Sep 2000	373-384	21-25	0.937	28
MG3	<i>S. mentella</i>	East Greenland	22 Sep 2000	373-384	26-30	0.928	25
MG4	<i>S. mentella</i>	East Greenland	22-26 Sep 2000	323-392	31-35	0.865	25
MI1	<i>S. mentella</i>	Irminger Sea	24 Jun - 2 Jul 2001	202-691	26-30	0.776	23
MI2	<i>S. mentella</i>	Irminger Sea	26-29 Jun 2001	234-249	31-35	0.975	28
MI3	<i>S. mentella</i>	Irminger Sea	26 Jun - 2 Jul 2001	652-691	31-35	0.928	27
MI4	<i>S. mentella</i>	Irminger Sea	26-29 Jun 2001	234-249	36-40	0.904	25
MI5	<i>S. mentella</i>	Irminger Sea	25 Jun - 2 Jul 2001	656-691	36-40	0.902	25
MI6	<i>S. mentella</i>	Irminger Sea	23-25 Jun 2001	682-700	41-45	0.875	25
S1	<i>S. spec.</i>	West Greenland	31 Oct - 5 Nov 1999	45-297	5-6	0.052	36

Table 2. Reagent blanks for ^{210}Po and ^{226}Ra and values reported in other radiometric ageing studies. sd = standard deviation, n.r. = not reported.

Reference	^{210}Po (dpm)	± 1 sd	^{226}Ra (dpm)	± 1 sd	Ra determination
This study ¹	0.0021	0.0005	0.0101	0.0013	alpha spectrometry
Fenton <i>et al.</i> 1990	0.0103	0.0027	0.0125	0.0043	alpha spectrometry
Fenton <i>et al.</i> 1991	0.0103	0.0027	0.0255	0.0023	alpha spectrometry
Fenton and Short 1995	0.0071	0.0012	0.0174	0.002	alpha spectrometry
Milton <i>et al.</i> 1995	0.0071	0.0012	0.0174	0.0026	alpha spectrometry
Stewart <i>et al.</i> 1995	0.0082	0.0015	0.0192	0.0019	alpha spectrometry
Bennett <i>et al.</i> 1982	below detector background		0.039	ca. 0.003	Rn emanation
Campana <i>et al.</i> 1990	0.0005	n.r.	n.r.	n.r.	Rn emanation
Kline 1996	0.0038	0.0006	0.077	0.008	Rn emanation
Baker <i>et al.</i> 2001	0.056	0.011	0.049	0.019	alpha liquid scintillation

¹ including detector background

Table 3. Radiometric results for each sample. Uncertainties are one standard deviation (sd). n.d. not determined.

Sample	Length group (cm)	Sample weight (g)	^{210}Po activity ($\pm$ % sd) (dpm g $^{-1}$)	^{210}Po yield (%)	^{226}Ra activity ($\pm$ % sd) (dpm g $^{-1}$)	^{226}Ra yield (%)	$^{210}\text{Po}/^{226}\text{Ra}$ ratio	Radiometric age (yrs)	Traditional age (yrs)
MA1	10-15	0.646	0.0061 ($\pm$ 13.1)	95.0	0.0379 ($\pm$ 7.3)	77.6	0.162 $\pm$ 0.024	4.8 $\pm$ 0.9	2.6 $\pm$ 0.7
MA2	16-20	0.859	0.0040 ($\pm$ 21.2)	99.0	0.0364 ($\pm$ 6.6)	69.1	0.110 $\pm$ 0.024	2.9 $\pm$ 0.9	4.4 $\pm$ 1.2
MA3	21-25	1.316	0.0057 ($\pm$ 8.3)	97.0	0.0420 ($\pm$ 6.1)	43.2	0.137 $\pm$ 0.014	3.9 $\pm$ 0.5	6.8 $\pm$ 1.9
MA4	26-30	0.994	0.0065 ($\pm$ 13.0)	99.0	0.0239 ($\pm$ 12.1)	37.9	0.273 $\pm$ 0.049	9.4 $\pm$ 2.1	9.0 $\pm$ 2.0
MA5	31-35	0.986	0.0095 ($\pm$ 6.8)	101.6	0.0280 ($\pm$ 11.9)	39.0	0.341 $\pm$ 0.047	12.5 $\pm$ 2.3	11.6 $\pm$ 2.1
MA6	36-40	1.045	0.0111 ($\pm$ 6.2)	101.0	0.0334 ($\pm$ 6.7)	72.1	0.333 $\pm$ 0.030	12.1 $\pm$ 1.5	14.3 $\pm$ 2.9
MA7	41-45	0.976	0.0148 ($\pm$ 5.5)	98.0	0.0251 ($\pm$ 10.9)	41.8	0.587 $\pm$ 0.072	27.3 $\pm$ 5.5	18.0 $\pm$ 4.6
MA8	46-50	0.954	0.0197 ($\pm$ 5.5)	101.3	0.0418 ($\pm$ 6.0)	76.5	0.471 $\pm$ 0.038	19.5 $\pm$ 2.3	22.1 $\pm$ 6.0
MA9	51-55	0.872	0.0176 ($\pm$ 5.9)	102.5	0.0278 ($\pm$ 9.3)	62.9	0.634 $\pm$ 0.070	31.1 $\pm$ 6.0	25.1 $\pm$ 6.0
MG1	17-20	0.828	0.0048 ($\pm$ 18.1)	102.9	0.0209 ($\pm$ 11.5)	66.3	0.230 $\pm$ 0.049	8.1 $\pm$ 2.1	4.5¹
MG2	21-25	0.937	0.0076 ($\pm$ 9.6)	101.8	0.0305 ($\pm$ 6.6)	84.6	0.250 $\pm$ 0.029	8.9 $\pm$ 1.2	6.5¹
MG3	26-30	0.928	0.0079 ($\pm$ 9.5)	98.9	0.0274 ($\pm$ 8.2)	72.0	0.290 $\pm$ 0.036	10.7 $\pm$ 1.6	9.7 $\pm$ 1.7
MG4	31-35	0.865	0.0083 ($\pm$ 9.8)	102.3	0.0224 ($\pm$ 14.4)	46.9	0.373 $\pm$ 0.065	14.6 $\pm$ 3.3	12.5¹
MI1	26-30	0.776	0.0094 ($\pm$ 11.2)	98.6	0.0262 ($\pm$ 10.7)	66.9	0.360 $\pm$ 0.056	14.8 $\pm$ 2.8	9.2 $\pm$ 1.3
MI2	31-35	0.975	0.0238 ($\pm$ 5.7)	98.8	0.0487 ($\pm$ 5.1)	89.3	0.488 $\pm$ 0.037	21.6 $\pm$ 2.3	22.2 $\pm$ 9.2
MI3	31-35	0.928	0.0217 ($\pm$ 5.4)	99.4	0.0435 ($\pm$ 5.6)	84.5	0.498 $\pm$ 0.039	22.3 $\pm$ 2.5	15.4 $\pm$ 6.6
MI4	36-40	0.904	0.0341 ($\pm$ 4.6)	100.7	0.0529 ($\pm$ 4.8)	91.5	0.646 $\pm$ 0.043	33.3 $\pm$ 3.8	33.7 $\pm$ 9.0
MI5	36-40	0.902	0.0326 ($\pm$ 4.6)	102.6	0.0464 ($\pm$ 5.1)	86.8	0.704 $\pm$ 0.049	38.9 $\pm$ 5.1	26.7 $\pm$ 9.0
MI6	41-45	0.875	0.0265 ($\pm$ 5.0)	102.0	0.0364 ($\pm$ 7.7)	56.8	0.727 $\pm$ 0.067	41.3 $\pm$ 7.6	34.8 $\pm$ 9.6
S1	5-6	0.052	< 0.0498	100.3	0.0991 ($\pm$ 24.6)	76.1	n.d.	n.d.	n.d.

¹average traditional age estimated from strong cohorts in length distributions of regular surveys (Rätz *et al.* 2004)

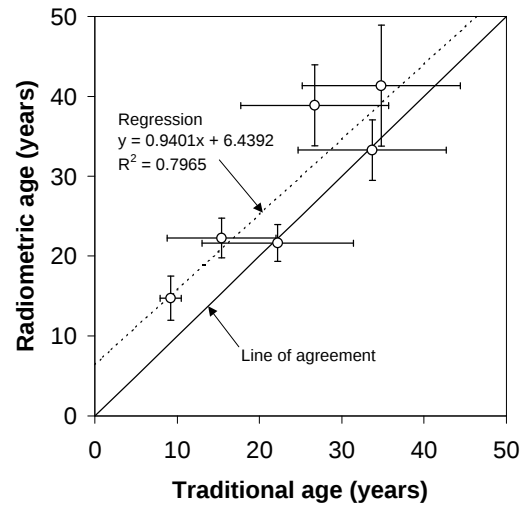
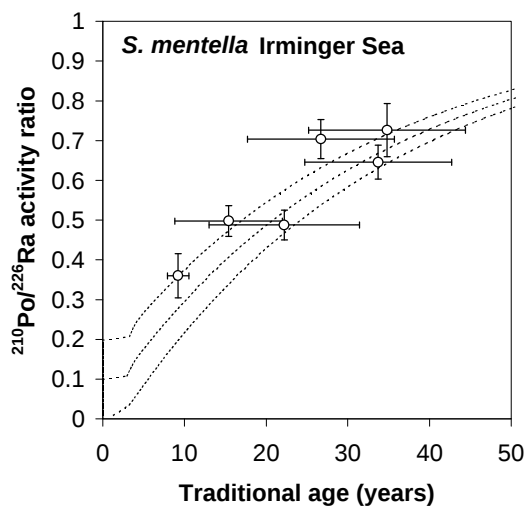
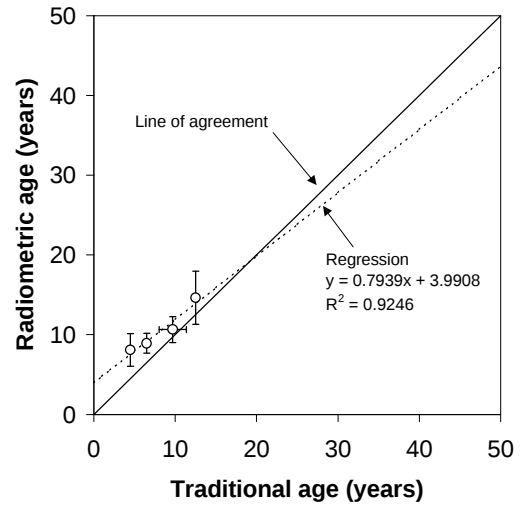
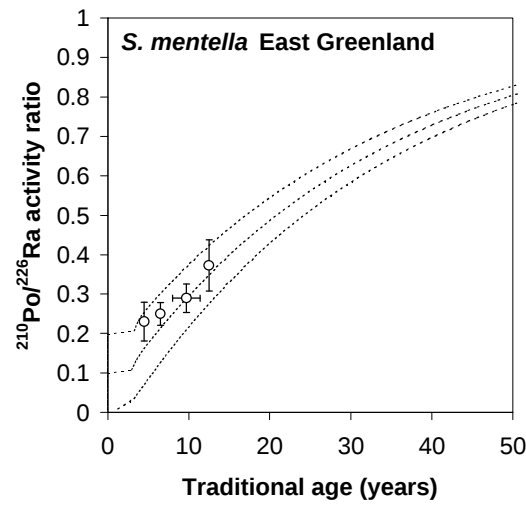
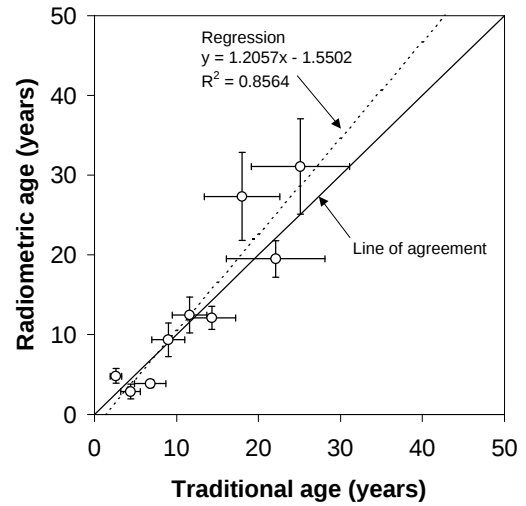
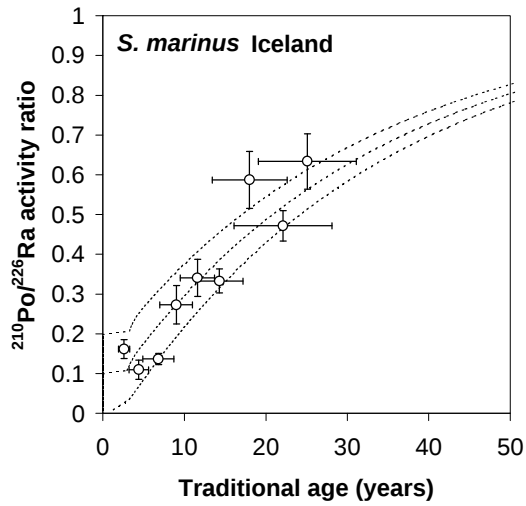


Figure 1. Left column: Observed $^{210}\text{Po}/^{226}\text{Ra}$ activity ratios for otolith core samples of a certain fish length group with expected activity ratio ingrowth curves (for initial uptake ratios of 0.0, 0.1 and 0.2) in relation to the mean traditional (annulus-derived) age estimates. Right column: Traditional age estimates in relation to radiometric ages calculated from the activity ratios. Error bars represent ± 1 standard deviation.

4. Conclusions and perspectives

Several aspects of the biology of redfish could be addressed in this thesis, providing new information on the species and stock structure as well as age and growth. The otolith shape and elemental analyses employed in the first section of this work point to good species differentiation and weak geographic or stock separation of redfish, whereas otolith shape analysis could be successfully applied as stock identification tool for horse mackerel. Species separation within the genus *Sebastes* was achieved on a level of over 90%, with slightly above 88% classification obtained for the four North Atlantic species (**chapter 2.1**). The observed differences between North Pacific and North Atlantic species and the similarity of the North Pacific *Sebastes* (rockfish) with *S. capensis* from the South Atlantic are in accordance with current zoogeographic theories and recent genetic analyses (Rocha-Olivares *et al.* 1999). On an evolutionary scale, the North Atlantic *Sebastes* species have speciated relatively recently, which might explain the moderate differences between species and high intraspecific variation. Hybrids between *S. fasciatus* and *S. mentella* were found in the Northwest Atlantic (Roques *et al.* 2001), and intermediate forms of *S. marinus* and *S. mentella* regularly occur off Greenland, whereas most studies on redfish stock separation point to weak differences between areas and high phenotypic and genetic variation within the areas. Concurrently performed body morphometric and genetic studies, additionally involving *S. fasciatus* and *S. viviparus*, will complement the picture of species separation of redfish in the North Atlantic.

Otolith shapes (**chapter 2.2**) and elemental contents (**chapter 2.4**) of *S. marinus* and *S. mentella* were found to vary only marginally between areas. The inferred geographic patterns could rather be allocated to large-scale differences between western, central and eastern regions of the North Atlantic than to certain shelf or oceanic areas. This is in accordance with microsatellite DNA analyses of Roques *et al.* (2002), describing a “pan-oceanic” population of *S. mentella* ranging from the Grand Banks to the Faroe Islands, which was found to differ from western (Gulf of St. Lawrence and off Newfoundland) and eastern units (Norway and Barents Sea). Considerably clearer small-scale geographic patterns were, for comparative purposes, found for otolith shapes of horse mackerel (**chapter 2.3**), providing new information on stock boundaries that will have immediate impact for fisheries management (ICES 2004). Geographic segregation between the shelf-based *S. marinus* occurrences would imply higher stock differentiation than expected for the highly migratory *S. mentella*. The classification success of multivariate otolith shape and elemental patterns, however, was similarly low for both species (< 50%). Strikingly, *S. marinus* off West and East Greenland exhibited considerable similarity in both otolith shapes and microchemistry, whereas *S. mentella* did not show this connectivity. Recently found evidence for a migration of juvenile *S. mentella* from East Greenland into the Irminger Sea (Stransky 2000) was supported by elemental signatures in otolith nuclei that showed no significant differences between both areas and thus indicated a common natal origin

(**chapter 2.4**). These results should be regarded as a pilot study due to the small sample size in some of the elemental analyses and a variety of confounding effects on otolith elemental composition. The field of otolith microchemistry is expanding incredibly fast, with over 60 papers being published since Campana's (1999) and Thresher's (1999) reviews, demonstrating high potential for further studies on migratory species such as redfish. Earlier work on otolith shapes of the same *S. mentella* material also found no differences between East Greenland and the Irminger Sea (Stransky 2001) and no sub-structure within the Irminger Sea, neither on a horizontal nor on a vertical scale (Stransky 2002). As the common ground of all these analyses, strong support was provided for the single-stock hypothesis, allocating all *S. mentella* in the central North Atlantic to one stock.

The large confidence intervals observed in age determination of redfish (**chapter 3.1**) could certainly be improved by carrying out further age reading workshops aiming at intercalibration of growth structure interpretation, by introducing standard procedures for quality control and building up otolith reference collections (Campana 2001). However, even if the bias between readers could be decreased to a negligible level and precision would be close to perfect, age-length-keys and growth curves for redfish repeatedly confront the scientific community with high natural variation in individual growth performance. Following the most extreme examples of this study, a *S. marinus* of 40 cm length could be 11 or 28 years old, and a *S. mentella* of 35 cm length could have an age of 15 or 53 years. Compared with long-lived Pacific *Sebastes* species, this variation is not uncommon: Andrews *et al.* (2002) showed even higher variation in age determination of *S. ruberrimus*, with ages determined for fish of 60 cm length ranging from 19 to 85 years. This sort of confounding error, leading to major uncertainties in the estimation of productivity of species or stock, is rarely discussed when calculating age-disaggregated input data for fisheries stock assessment.

Improved reliability of age data would be desirable for several other reasons, for example enabling the evaluation of density-dependent growth of redfish or environmental impacts on growth rates and inferred chronology of individual fish (similar to dendrochronology). Also the effect of growth rates on otolith shape (Campana and Casselman 1993; **chapters 2.1** and **2.2**), as well as the relationship between otolith weight and age as alternative ageing technique (*e.g.* Pawson 1990, McDougall 2004) could be explored further if more certainty in age determination could be achieved in future. Another interesting aspect deserving further attention would be the relationship of depth and growth, keeping the observed discrepancy between radiometric and traditional ages of *S. mentella* from the deeper layers in the Irminger Sea (**chapter 3.2**) in mind. Cailliet *et al.* (2001) indicated an exponential relationship between depth and longevity within the genus *Sebastes*. In this respect, especially the expected high maximum ages of so-called 'giant' redfish with lengths of up to 90 cm found on the Reykjanes Ridge down to 2000 m depth (Hareide *et al.* 2001) would add knowledge to this issue.

The high longevity observed for some representatives of the genus *Sebastes* has caught worldwide interest. Only recently, Munk (2001) reported a maximum age of 205 years for rougheye rockfish (*S. aleutianus*) from offshore Southeast Alaska, as well as six other rockfish species reaching close to or more than 100 years. Most of these species occurred north of 48°N, supporting Gerking's (1957) and Pauly's (1980) finding of increased longevity in fishes inhabiting cold deepwater environments. Andrews *et al.* (2002) confirmed ages of yelloweye rockfish (*S. ruberrimus*) exceeding 100 years by radiometric age validation (see **chapter 3.2**). Fascination over the fact that rockfish grow older than the people who investigate or eat them (Capiello 1999) and the mechanisms behind this phenomenon certainly motivated a review article by Cailliet *et al.* (2001), outlining several possible reasons for the extraordinary high longevity of *Sebastes* species. Their observation of deep-dwelling rockfish living longer than their relatives from shallower waters was explained by altered physiological processes relative to environmental parameters like low temperature, high pressures, low light levels, low oxygen, and poor food resources. Beverton (1987) stated that, generally, higher longevity is characteristic of living representatives of those taxa that evolved early in the lineage of fishes. In a comprehensive but little known review on age determination and longevity in fishes, Das (1994) took up Beverton's theory and noted that some elasmobranchs have a life span of 70 years and that sturgeons aged 152 years were reported, both groups having evolved early. Since the *Sebastes* species are relatively young on an evolutionary scale (**chapter 2.1**; Seeb 1986, Rocha-Olivares *et al.* 1999), their high longevity obviously represents an exception in the fish realm. With many deep-sea fish generally regarded as slow-growing (Bergstad 1995, Morales-Nin 2001) but probably several species still unknown, new accounts of longevity will allow further insights into the age and growth of deep-dwelling fish.

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