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Suitability of otolith microchemistry for stock separation of Baltic cod

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ABSTRACT: Microchemical otolith analyses have been shown to provide valuable information on the life history, dispersal and stock characteristics of teleost fish. In the present study, the suitability of this technique for identifying the origin and distribution of Atlantic cod *Gadus morhua* L. from the Baltic Sea was examined using laser ablation-ICPMS. The capacity to distinguish individuals from different Baltic Sea stocks and from the adjacent North Sea stock based on incoporation of stock-specific elemental fingerprints along otolith growth axes was investigated. It was further tested if different origins led to spawning-site specific element concentrations in otolith cores. The results indicate that microchemical analyses of Baltic cod otoliths are applicable for differentiating individuals of different stocks. Analyses of similarities including 12 element/calcium ratios resulted in significant differences between individuals from the eastern and the western Baltic Sea and between North Sea and Baltic Sea samples. Sr/Ca, Ba/Ca, Y/Ca, Mg/Ca, Zr/Ca and Mn/Ca ratios had the strongest discriminatory power. A further separation of individuals caught in 3 different spawning grounds of the eastern Baltic, however, was not possible. Elemental compositions from the core regions of otoliths from young of the year cod caught in eastern and western Baltic Sea spawning grounds showed significant differences in Sr/Ca, Ba/Ca and Mg/Ca concentrations. Analyses of similarities again showed significant differences between these areas for juveniles. This study demonstrates the potential of otolith microchemical analyses to provide important information about the stock structure and connectivity of *G. morhua* in the Baltic Sea.

KEY WORDS: *Gadus morhua* · Stock discrimination · Baltic Sea · Otolith microchemistry

INTRODUCTION

The Atlantic cod *Gadus morhua* is distributed over vast areas of the northern Atlantic, including the North Sea and almost the entire Baltic Sea. Since the Baltic Sea is a brackish water system, Baltic cod face a range in salinity from fully marine levels in the northern Kattegat to almost freshwater conditions in the north-eastern inner parts of the Baltic Sea. Genotypic and phenotypic characteristics indicate a separation into a western (International Council for the Exploration of the Sea [ICES] subdivisions SD 22-24) and an eastern Baltic cod stock (SD 25-32). The separation into 2 Baltic cod stocks is made for management reasons based on the assumption that these stocks are spatially separated. However, overlapping

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between the stocks has been reported in a relatively narrow zone around the island of Bornholm (Bagge et al. 1994, Nielsen et al. 2003).

Both Baltic stocks are subject to large fluctuations in spawning stock biomass and recruitment (Eero et al. 2011, Hüssy 2011). Besides the influence of fisheries, the eastern Baltic stock dynamics are mainly driven by changes in salinity and oxygen (Köster et al. 2005). This is particularly due to a special set of environmental conditions required for successful reproduction in the eastern parts of the Baltic Sea (MacKenzie et al. 2000). Historically, there have been 3 main spawning areas for the eastern Baltic stock: the Bornholm Basin (BB), the Gdansk Deep (GD) and the Gotland Basin (GB). Analyses of the spatial and temporal heterogeneity of the spawning environment have revealed that beneficial conditions for egg survival are most likely to be found in the BB (MacKenzie et al. 2000). Due to a lack of oxygen combined with low salinities, the more eastern spawning grounds in the GD and GB do not often provide adequate environmental conditions for egg survival, especially since the early 1980s (Nissling et al. 1994, Köster et al. 2005). For the western Baltic cod, the number of potential spawning grounds is less limited, and good spawning conditions are found in several areas from the Kattegat to the Arkona Basin (Hüssy 2011).

The degree of connectivity between the 2 Baltic cod stocks is still under debate. In the Baltic Sea, *Gadus morhua* is distributed over a large area and may perform wide and variable feeding and spawning migrations through different environments (Otterlind 1985). The younger age groups are usually found in coastal areas (Bagge & Steffensen 1989), while horizontal movements of adult individuals in the distribution area were found to not be clearly directed and were therefore described as random migrations (Bagge et al. 1974). In the transition zone between the eastern and the western stocks, some exchange may occur. Tagging studies revealed that individuals from the Arkona Basin can undertake long eastward migrations (Otterlind 1985), and genetic studies suggest that even interbreeding between both stocks occurs in that area (Nielsen et al. 2003). However, the extent that migrations and interbreeding contribute to the mixing between stocks remains to be quantified. Successful management of Baltic cod stocks requires knowledge about the connectivity among stocks within the Baltic Sea as well as between the North Sea and the western Baltic Sea. Furthermore, identification of the natal origins of individuals would allow quantification of the contribution of each spawning ground to the spawning stock biomass and therefore significantly improve the understanding of stock dynamics.

Among other uses, otolith microchemistry analysis provides a promising tool for fish stock discrimination, as shown for pink snapper *Chrysophrys auratus* (Edmonds et al. 1989), orange roughy *Hoplostethus atlanticus* (Edmonds et al. 1991), jackass morwong *Nemadactylus macropterus* (Thresher et al. 1994), Spanish mackerels *Scomberomorus* spp. (Begg et al. 1998) and Atlantic cod (Campana et al. 2000, Higgins et al. 2010, Limburg et al. 2010, Svedäng et al. 2010). In these studies, several elements in otoliths differed among sampling sites. Svedäng et al. (2010) even found better distinction among populations by otolith chemistry than by genetic investigation. In most of the studies on fish otoliths, Mg or Na had the highest discrimination potential, but the results from different fish species are not consistent. For Atlantic cod, particularly Mg, Mn, Ba and Sr as well as Rb (Higgins et al. 2010) and Br (Limburg et al. 2010) could distinguish among populations. The differing element levels in otoliths can be explained either by individual physiology constraints (Kalish 1989), ambient environmental conditions, like temperature and salinity (Fowler et al. 1995), or a combination of both, but genetic or ontogenetic influences are also discussed (Thresher et al. 1994, Begg et al. 1998).

It seems promising that the east-west salinity gradient within the Baltic Sea and the strong regional influence of river discharge on the water composition (e.g. Andersson et al. 1992, Maksymowska et al. 2000, Wachniew 2006) provide appropriate conditions for a discrimination of spawning-site specific elemental fingerprints in the otoliths of discrete Baltic cod stocks. The objective of the present study was to assess the potential of otolith microchemistry to differentiate between the cod stocks through comparative analyses of the multi-elemental composition of otoliths of adult specimens caught in the North Sea (NS), the western Baltic Sea (WB) as well as the 3 eastern Baltic spawning grounds BB, GD and GB using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). In addition, it was tested whether individuals of different origins are characterized by distinguishable elemental compositions in the core regions of their otoliths. Therefore, multi-element analyses were conducted in the core area of juvenile cod otoliths from the eastern and the western Baltic Sea.

MATERIALS AND METHODS

Sample material and preparation

Adult Atlantic cod *Gadus morhua* were caught in winter to spring of 1998 during the spawning season in the Baltic and North Sea (Fig. 1, Table 1). Juveniles were collected later in the same year from WB and BB (Fig. 1). A total of 89 sagittal otoliths were removed from adult Atlantic cod and 20 sagittal otoliths from juvenile specimens (Table 1). All otoliths collected were stored in paper bags until preparation.

Adult cod otoliths were embedded in a mixture of GTS polyester casting resin and MEKP-hardener (both from Voss Chemie) and thin-sectioned across the center of the otolith using a semi-automated mineralogy sawing machine (Conrad). These cross sections as well as the juvenile otoliths were mounted on glass slides with thermoplastic glue (Crystalbond Type 509; Kager) and subsequently polished with lapping film (30, 12 and 3 µm; 3M).

Otolith analyses

Concentrations of trace elements were determined by LA-ICPMS using a NewWave UP193 solid-state laser coupled to a ThermoFinnigan Element2™ at the Department of Geosciences, University of Bremen. Elemental composition of adult cod otoliths was measured along transects from the core to the dorsal edge of the otolith sections (Fig. 2). Otoliths were ablated with an irradiance of ca. 1 GW cm^{-2}, a pulse rate of 10 Hz, a spot size of 75 µm and a line scan speed of 4 µm s^{-1}. Sixteen isotopes (^{7}Li, ^{23}Na, ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{111}Cd, ^{138}Ba, ^{208}Pb and ^{238}U) were analyzed. Element/calcium (El/Ca) ratios were averaged over the entire measuring transects for every element. Otoliths of juveniles were ablated as single points in the core region for 60 s with a pulse rate of 5 Hz and a spot size of 50 µm, producing shallow (<25 µm) craters. Mean El/Ca ratios were determined for every juvenile specimen, measuring the same 16 elements as for transect analyses. A pre-ablation was carried out prior to every measurement in order to clean the

Table 1. *Gadus morhua* sampling data. NS: North Sea, WB: Western Baltic, BB: Bornholm Basin, GD: Gdansk Deep, GB: Gotland Basin, SD: ICES Subdivision, N: number of fish, TL: total length

Region	Catch date	N	TL (cm)	
			Range	Mean
Adults				
NS	Feb 1998	16	30–49	35.7
WB	Jan–Mar 1998	19	51–60	55
BB	May 1998	24	46–76	53
GD	May 1998	9	50–61	54.2
GB	May 1998	21	48–67	56
Juveniles				
SD22 (WB)	Jun 98	11	2.6–4.4	3.5
SD25 (BB)	Nov 98	9	3.4–4.5	3.8

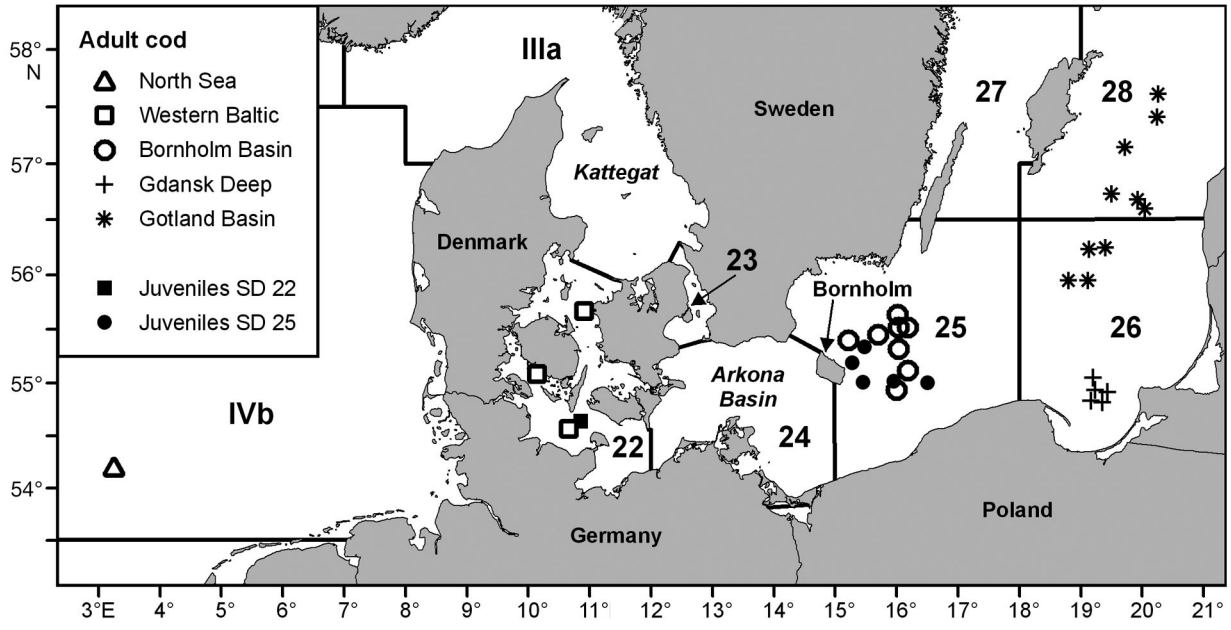

Fig. 1. Sampling areas of Atlantic cod *Gadus morhua*. Numbers represent ICES subdivisions (SD)

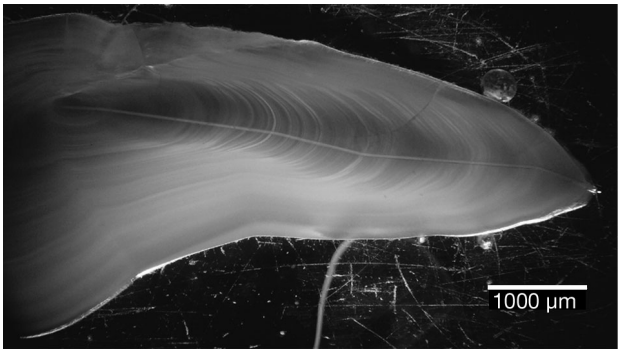

Fig. 2. Ablation line after LA-ICPMS measurements along the growth axis of an adult cod otolith

surface. Helium was used (0.4 l min^{-1}) as sample gas and argon (0.8 l min^{-1}) as make-up gas. Plasma power was 1200 W. Isotopes were analyzed at low resolution with 5 samples in a 20% mass window and a total dwell time of 50 ms per isotope. Blanks were measured for 20 s prior to ablation.

For external calibration, the NIST612 standard reference material, a sodium silicate glass, was analyzed after each transect (standard bracketing). Calcium was used as an internal standard with an assumed concentration of 38.8 wt% for the otoliths (similar to the NIES22 otolith material; Yoshinaga et al. 2000). For data quantification, the Cetac GeoPro™ software was used with the concentrations for NIST612 of Pearce et al. (1997). The Mg concentration provided by these authors (77.4 µg g^{-1}), however, significantly differed from the newly determined value of 68 µg g^{-1} (Jochum et al. 2011).

The data quality was assessed by repeated analyses of a pressed pellet of NIES22 otolith powder (Table 2) and of BCR2G basaltic glass (United States Geological Survey). For Na, Mg, Cu, Zn, Sr and Ba, there is good to excellent agreement with the certified values, which indicates that NIST612 is well suited as a calibration standard for carbonate analyses. The accuracy for Mg improves significantly if the Jochum et al. (2011) value rather than the Pearce et al. (1997) value for NIST612 is used for calibration. Because the variations of our NIES22 analyses include heterogeneities within the pellet, the actual analytical precision of the laboratory setup is better than the relative standard deviations shown in Table 2; based on our BCR2G data, the overall precision is better than 5% for most elements at concentrations above 0.5 to 1 µg g^{-1}.

Table 2. Average concentration and relative standard deviation (RSD) of 9 analyses of a pressed pellet from NIES22 otolith powder. The Mg concentration in parentheses is based on the calibration value from Jochum et al. (2011)

Element	Concentration (µg g^{-1})	RSD (%)	Reference value (µg g^{-1})
Na	2270	5.1	2230
Mg	25.8 (22.7)	14	21
Cu	0.796	30	0.74
Zn	0.531	23	0.47
Sr	2302	2.8	2360
Ba	2.7	2.6	2.89
Pb	0.042	46	0.023

Statistical analysis

Statistical evaluation was conducted using the software STATISTICA (Version 6.1, StatSoft 2003) and PRIMER 6 (Version 6.1.9, PRIMER-E) (Clarke & Gorley 2001).

For statistical analysis, transect data for each adult cod otolith were averaged for single elements. Means were grouped according to the sampling region (NS, WB, BB, GD or GB) and tested in univariate analyses of variance (ANOVA) for each element. In order to assign differences among sampling regions, ANOVA was followed by Tukey's honestly significant difference (HSD) multiple comparison test. Where variances were not distributed homogeneously among factor levels, a Kruskal-Wallis *H* test was performed. The ANOVA and Kruskal-Wallis *H* test were conducted using a Bonferroni corrected level of significance ($p = 0.004$).

The combination of transect means of all analyzed elements forms an elemental fingerprint of the otolith of the respective individual. To test if these elemental fingerprints differ significantly among sampling areas, 4th-root transformed data were used to perform an analysis of similarities (ANOSIM; significance level $p = 0.05$). In addition, a discriminant analysis was conducted using the transformed data to evaluate the contribution of single elements to the differentiation among sampling areas (significance level $p = 0.05$). For graphical representation, a non-metric multidimensional scaling (MDS) plot was created on the basis of a Bray-Curtis-similarity matrix.

Differences of element concentrations of juvenile cod from the western and eastern Baltic Sea were analyzed using *t*-tests (Bonferroni corrected level of significance $p = 0.004$). Differences in otolith elemental fingerprints of juveniles were visualized using an MDS plot.

RESULTS

Elemental fingerprints of adult cod

An overview of the mean values of measured otolith El/Ca ratios for each sampling area is presented in Table 3. The elements Cd, Nb and U were excluded from statistical evaluation because their concentrations were close to the detection limits.

Highly significant differences between the sampling areas were found for all 12 remaining elements (Table 4). Individual elemental fingerprints including the 12 elements (Li, Na, Mg, Mn, Cu, Zn, Rb, Sr, Y, Zr, Ba and Pb) are visualized by the MDS plot in Fig. 3. Samples from the North Sea (NS) are isolated from all other samples, and the Western Baltic (WB)

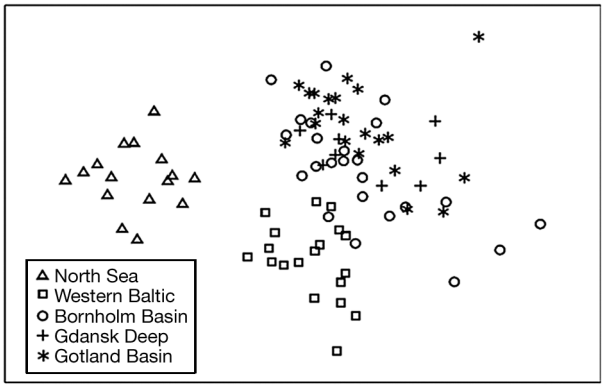

Fig. 3. Multidimensional scaling plot of otolith elemental fingerprints of adult Atlantic cod. Each data point represents one elemental fingerprint, including 12 element/calcium ratios of the respective otolith

Table 3. Mean (± SD) element/calcium ratios of adult Atlantic cod otoliths for different sampling areas. NS: North Sea, WB: Western Baltic, BB: Bornholm Basin, GD: Gdansk Deep, GB: Gotland Basin

	NS	WB	BB	GD	GB
Li/Ca (µmol mol^{-1})	8.40 (±5.00)	2.57 (±0.97)	1.31 (±0.88)	1.50 (±0.52)	1.67 (±0.84)
Na/Ca (mmol mol^{-1})	16.98 (±0.58)	17.44 (±1.01)	16.52 (±0.75)	16.10 (±0.44)	16.13 (±0.67)
Mg/Ca (µmol mol^{-1})	161.89 (±22.40)	130.53 (±13.62)	129.34 (±21.45)	129.28 (±16.01)	123.35 (±14.65)
Mn/Ca (µmol mol^{-1})	13.65 (±7.89)	37.56 (±22.52)	24.00 (±7.94)	21.37 (±6.45)	27.10 (±12.75)
Cu/Ca (µmol mol^{-1})	0.53 (±0.20)	2.19 (±0.81)	1.48 (±1.27)	1.99 (±1.36)	0.88 (±0.60)
Zn/Ca (µmol mol^{-1})	1.21 (±0.30)	2.72 (±0.91)	2.00 (±0.97)	2.01 (±0.78)	1.41 (±0.61)
Rb/Ca (µmol mol^{-1})	0.23 (±0.03)	0.41 (±0.08)	0.34 (±0.12)	0.44 (±0.15)	0.29 (±0.07)
Sr/Ca (mmol mol^{-1})	2.38 (±0.28)	2.18 (±0.22)	1.70 (±0.21)	1.54 (±0.11)	1.45 (±0.14)
Y/Ca (nmol mol^{-1})	29.41 (±3.74)	20.64 (±2.47)	16.98 (±3.52)	20.47 (±1.70)	18.43 (±1.93)
Zr/Ca (nmol mol^{-1})	15.93 (±6.70)	42.23 (±29.01)	112.94 (±96.60)	59.20 (±88.55)	95.06 (±88.79)
Nb/Ca (nmol mol^{-1})	2.40 (±0.47)	0.50 (±0.96)	0.22 (±0.84)	1.43 (±0.29)	0.70 (±0.66)
Cd/Ca (nmol mol^{-1})	13.29 (±12.15)	14.73 (±14.70)	20.73 (±18.52)	21.07 (±21.43)	19.43 (±11.22)
Ba/Ca (µmol mol^{-1})	2.21 (±0.60)	4.88 (±1.14)	6.80 (±1.56)	6.70 (±1.67)	6.60 (±1.16)
Pb/Ca (nmol mol^{-1})	6.43 (±3.46)	46.20 (±30.57)	36.34 (±29.77)	24.42 (±17.14)	22.12 (±17.93)
U/Ca (nmol mol^{-1})	0.22 (±0.24)	0.10 (±0.04)	−0.01 (±0.25)	0.08 (±0.07)	0.07 (±0.02)

Table 4. Results of analysis of variance (ANOVA, *F*-test) and Kruskal-Wallis *H*-test, summary of discriminant analysis in otoliths of adult Atlantic cod among sampling areas and results of *t*-tests for element/calcium ratios in the core region of juvenile cod otoliths

	ANOVA & Kruskal-Wallis *H*-test				Discriminant analysis		*t*-test juvenile cod		
	F	*H*	p	df	*F*	p		*t*-value	p
Sr/Ca	–	66.61	0.000	4	40.09	0.000	Sr/Ca	3.60	0.002
Ba/Ca	–	52.69	0.000	4	27.66	0.000	Ba/Ca	−4.49	0.000
Y/Ca	–	51.78	0.000	4	14.86	0.000	Y/Ca	1.98	0.063
Mg/Ca	11.92	–	0.000	4	6.98	0.000	Mg/Ca	3.83	0.001
Zr/Ca	–	37.30	0.000	4	6.28	0.000	Zr/Ca	−0.77	0.452
Mn/Ca	–	32.14	0.000	4	5.66	0.001	Mn/Ca	2.24	0.038
Na/Ca	9.94	–	0.000	4	4.09	0.005	Na/Ca	2.22	0.039
Rb/Ca	–	41.72	0.000	4	2.16	0.082	Rb/Ca	1.28	0.217
Cu/Ca	–	40.00	0.000	4	1.57	0.193	Cu/Ca	−1.36	0.192
Li/Ca	–	53.57	0.000	4	1.31	0.273	Li/Ca	−1.01	0.325
Pb/Ca	–	36.58	0.000	4	0.86	0.490	Cd/Ca	1.81	0.088
Zn/Ca	–	31.65	0.000	4	0.34	0.851	Zn/Ca	−0.24	0.812

samples are separated from the cluster of eastern Baltic samples (BB, GD and GB). Non-parametric ANOSIM revealed significant differences among sampling areas (global R = 0.574, p < 0.001). Pairwise *a posteriori* tests showed significant differences based on elemental composition between the NS and all other areas (Table 5) as well as between WB and the 3 different eastern Baltic areas. Within the eastern Baltic, only BB and GB differed significantly.

Discriminant analysis showed that Sr, Ba, Y, Mg, Zr and Mn had the strongest influence on the differentiation among the sampling areas (Table 4). Concentrations of these individual elements differed significantly between North Sea samples and most samples from the different Baltic Sea areas (Tukey's HSD multiple comparison test) (Fig. 4). Furthermore, Sr and Ba concentrations diverged between western and eastern Baltic samples, and Sr and Y differed between at least 2 of the 3 eastern Baltic areas.

Elemental fingerprints of juvenile cod

With respect to core concentrations of juvenile cod otoliths, Pb, Nb and U fluctuated strongly around zero. These 3 elements were therefore excluded from statistical analyses. Results of *t*-tests between otoliths from WB (SD 22) and those from BB (SD 25) are listed in Table 4 for each element. The core concentrations of juvenile cod otoliths differed significantly among sampling areas for Sr, Ba and Mg (Table 4, Fig. 5). Other elements showed no significant differences when a Bonferroni corrected level of significance was applied. Elemental fingerprints of juvenile cod, including all 12 El/Ca ratios, were sufficient to distinguish samples according to their sampling areas (p = 0.002; Fig. 6). Test statistics of a conducted ANOSIM were significant (p = 0.002, global R = 0.506).

Table 5. Results (R statistic) of pairwise tests of similarity analysis (ANOSIM). NS: North Sea, WB: Western Baltic, BB: Bornholm Basin, GD: Gdansk Deep, GB: Gotland Basin. $^{**}p \leq 0.01$; $^{***}p \leq 0.001$

	NS	WB	BB	GD
WB	0.960**	–	–	–
BB	0.881**	0.453***	–	–
GD	0.985***	0.736***	−0.036	–
GB	0.946***	0.781***	0.090**	0.133

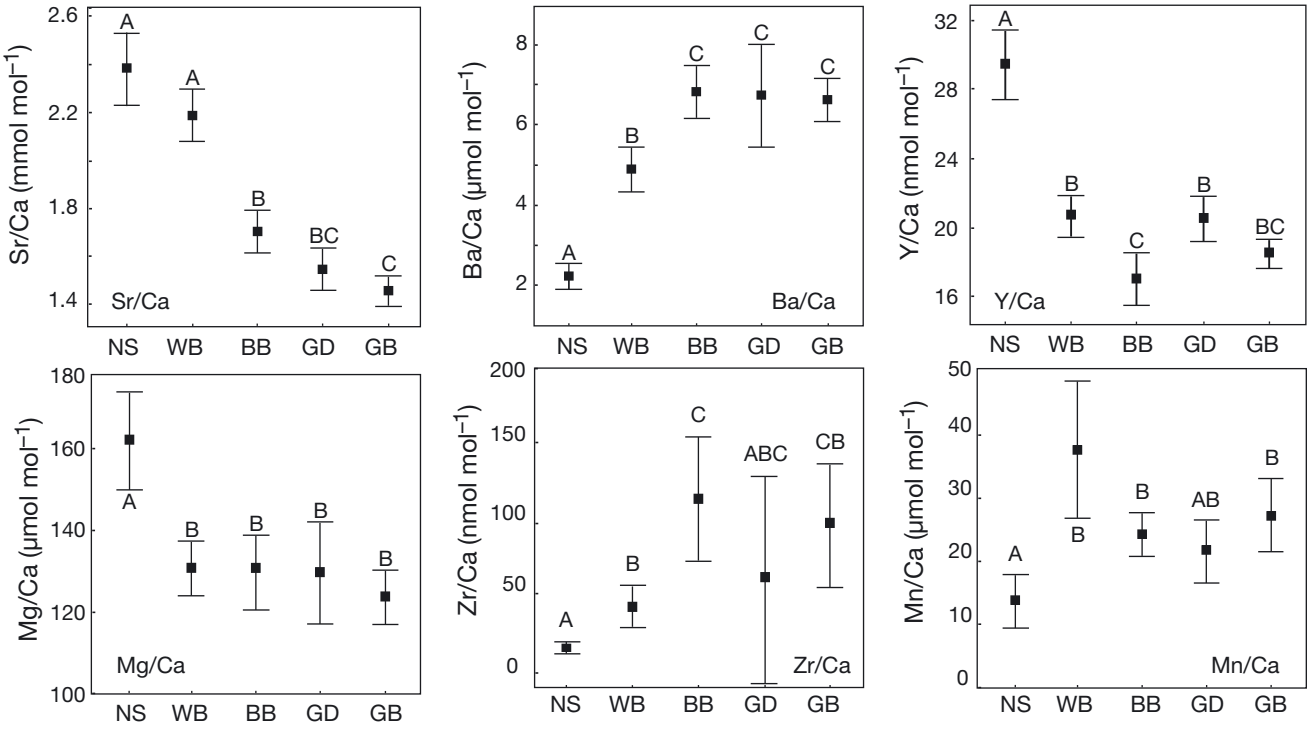

Fig. 4. Element/calcium ratios of elements with highest potential to distinguish among sampling areas of Atlantic cod. (■) Overall mean values, error bars: 95% confidence interval. Groups without significant difference are labeled with the same letters. NS: North Sea, WB: Western Baltic, BB: Bornholm Basin, GD: Gdansk Deep, GB: Gotland Basin

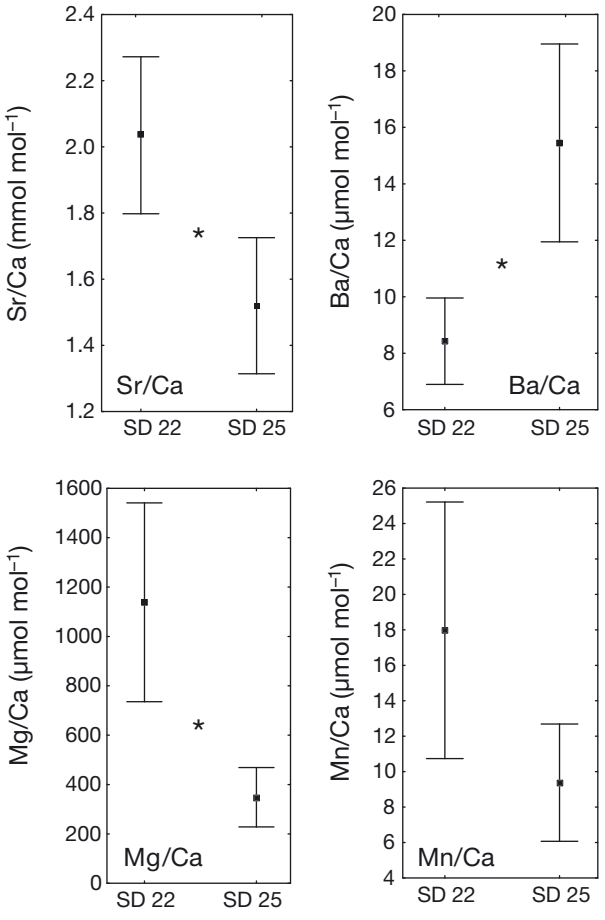

Fig. 5. Element/calcium ratios in core of juvenile cod otoliths for Sr, Ba, Mg and Mn. (■) Mean values. Error bars show the 95% confidence interval. *Significant differences between the sampling areas based on *t*-tests. SD 22: Western Baltic, SD 25: Bornholm Basin

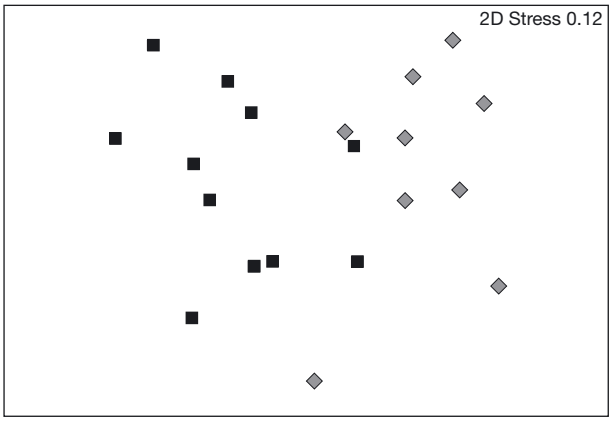

Fig. 6. Multidimensional scaling plot of elemental fingerprints in cores of juvenile cod otoliths, grouped according to sampling areas. ■: Western Baltic (SD 22), ◆: Bornholm Basin (SD 25)

DISCUSSION

The present study demonstrates that the unique hydrographic conditions of the Baltic Sea, which change over comparably short distances, provide suitable conditions for the use of otolith microchemistry as a potential discriminator of Baltic cod stocks. Water salinity ranges from almost fully marine in the Kattegat to almost freshwater in the innermost part of the Gulf of Bothnia, and the local influence of river discharge is strong (Andersson et al. 1992). It can be assumed that most of the discrimination among areas detected in the present study is caused by differences in water salinity, which was shown to be positively correlated with otolith Sr/Ca ratios for several fish species (e.g. Secor et al. 1995, Secor & Rooker 2000). However, the Sr/Ca ratio alone does not explain the separation of Atlantic cod between the North Sea and the Western Baltic. Hence, consistent variations in additional elements are responsible for the significant differentiation of the 2 stocks. Besides Sr, Ba incorporation is known to vary with water salinity (de Vries et al. 2005). In our results, Ba was the second strongest discriminator between sampling locations for adult Atlantic cod, with concentrations that increased eastwards inversely to water salinity. In juvenile cod, Ba had the largest effect on separation among sampling areas, supporting the assumption that water salinity has the main influence on the element incorporation into cod otoliths in the Baltic Sea.

Although differences in elements with high discriminatory power (Sr, Ba, Y, Mg, Zr and Mn) were generally most pronounced between the North Sea and the Western Baltic, some elements (Sr and Ba) also differed between western and eastern Baltic samples. Beside these comparably highly concentrated elements, some heavy metals (Zn, Cu, Pb and Cd) also showed elevated concentrations in Baltic cod otoliths, potentially reflecting higher water concentrations caused by pollution (Pohl et al. 1999, BLMP 2002). Although values fluctuated largely among individuals from the same sampling areas, heavy metals might provide potential candidate elements in future studies. In contrast, Y concentrations remained very stable for each sampling area. Although Y could only be used to distinguish among sampling areas for adult cod and showed no differences between areas for juveniles, it seems promising to determine its discrimination potential in future studies.

The results support earlier studies that suggested a limited exchange of individuals between western

and eastern Baltic Sea stocks. Morphometric and meristic analyses (e.g. Berner & Vaske 1985, Müller 2002) as well as genetic studies (Nielsen et al. 2003) revealed a separation of the Baltic Sea cod into a western and an eastern stock. Mixing apparently only occurs to a limited extent and can be caused either by individual migration or by egg and larval drift. Our results concur with these findings and indicate that otolith microchemistry provides a powerful tool to further understand stock structure in the Baltic Sea, particularly because this method is moderately time-consuming and involves low costs compared to e.g. genetic methods, if the technology is available. Since adult Atlantic cod in this study were caught during the spawning season, differences in otolith element concentrations as detected among spawning areas indicate that spawning communities are separated for a considerable time of the year and suggest spawning site fidelity and a limited range of feeding migrations.

Despite the good discrimination potential of many of the elements analyzed, it was not possible to determine characteristic elemental fingerprints that clearly differ among the 3 eastern Baltic spawning grounds. Although Sr and Y contents differed significantly among BB, GD and GB, the combination of all analyzed elements did not lead to clear discriminating patterns. This might be caused by very similar water composition within the eastern Baltic Sea or by high connectivity among spawning areas, as reported for cod from GB that leave endemic spawning areas to migrate westward when spawning conditions suffer from a lack of oxygen or when cod densities in this area are high (Baranova 1989, 1995).

Our analysis revealed that otoliths of adult Atlantic cod individuals from different areas differed in their elemental composition, which may indicate that the exchange among stocks is limited. However, definite conclusions on the connectivity among stocks and spawning areas could not be drawn by this approach. The averaging of measurement data over the entire transect did not allow resolution of the elemental composition in the otolith core area, which corresponds to the early life stage and thus to the area where an individual was born, because an adequate exposure of the otolith core was precluded by the preparation of the entire otolith. Therefore, a second approach was necessary to evaluate the suitability of otolith microchemistry to assess the connectivity among Baltic Sea cod stocks. To address this question, core regions of young of the year cod from 2 spawning grounds in the Baltic Sea were analyzed. Statistically significant differences between juveniles originating from the western and the eastern Baltic stocks demonstrate the feasibility of chemical otolith tags to trace the individual origin of Baltic Sea cod. Our findings strengthen the assumption that core regions of Baltic cod otoliths store the specific hydrographic conditions of their respective natal origin. Presuming these conditions to be stable, site specific otolith core element concentrations could be used as markers for stock affiliation and deliver invaluable information on stock separation and stock connectivity. However, it has to be considered that water elemental composition may vary in time, and differences among regions might change among years (e.g. Gillanders & Kingsford 2000). Campana et al. (2000) reported that long term stability of fingerprints for a certain spawning group was not given but that chemical signatures incorporated at a certain location are very stable for up to 1 yr. Whether this is true for the Baltic Sea or if conditions are stable over longer time periods has to be clarified in future studies.

In summary, elemental fingerprints of Atlantic cod otoliths are well suited to gain additional knowledge on the stock structure within the Baltic Sea. Even though the method is apparently not appropriate for the small-scale separation within the eastern Baltic cod stock, it can provide valuable information to improve stock management. The combination of biological markers with otolith microchemistry as done by Higgins et al. (2010) and the inclusion of water composition analyses could further improve the separation of Baltic cod stocks. Since the inter-relationships between the western and the eastern Baltic cod stocks are presently poorly understood, the utilization of otolith microchemistry for adult and juvenile fish may be helpful as a management tool for quantifying the importance of east-westward-oriented migrations. This could be particularly useful for investigating the mixing of the 2 Baltic cod stocks, especially in ICES-Subdivision 24, a topic which is presently still under debate.

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