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Stock structure of Icelandic cod *Gadus morhua* L. based on otolith chemistry

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Otolith chemistry was used to study the stock structure of Icelandic cod *Gadus morhua*. Otoliths were collected from spawning cod at 12 and 17 different spawning locations around Iceland during the spawning season in 2002 and 2003, respectively. The otolith elemental composition of cod spawning north and south of Iceland differed substantially from each other, as did spawning cod below and above 125 m depth at the main spawning ground in the south of Iceland. Otolith chemistry differed more between locations than among years within a location, indicating temporal stability between the 2 years. The Icelandic cod stock is managed as a single stock. These results suggest, however, that optimal fisheries management may require different management units than are currently present.

Key words: cod; *Gadus morhua*; otolith chemistry; stock structure.

INTRODUCTION

A major concern for fisheries management is the ability to recognize the stock structure of the targeted fish species so that each stock can be managed optimally (Begg *et al.*, 1999; Stephenson, 1999). Populations expressing different life histories are particularly at risk, as less productive populations may be more vulnerable to overexploitation than more productive populations (Jennings *et al.*, 1998). Information on stock structure is especially important for fish species that are under high exploitation pressure and are being managed as a homogeneous stock. Proper knowledge of the stock structure will promote measures that are likely to prevent overfishing, preserve genetic diversity and help ensure sustainable exploitation of the fish stock (Booke, 1981; Begg *et al.*, 1999; Stephenson, 1999).

The Icelandic cod *Gadus morhua* L. stock is currently managed as a single stock. An increasing number of studies, however, indicate that the Icelandic cod stock may be composed of more than one population expressing different life-history strategies (Marteinsdottir *et al.*, 2000*a*, *b*; Jónsdóttir *et al.*, 2002,

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2006; Petursdottir *et al.*, 2006; Pampoulie *et al.*, in press). Most of these studies have concentrated on a limited study area, although some recent studies have focused on cod spawning all around Iceland (Jónsdóttir *et al.*, 2006; Pampoulie *et al.*, in press). On the basis of otolith shape and genetics (both microsatellite DNA and *Pan* I) it has been possible to discriminate between two major groups of cod residing north and south of Iceland (Jónsdóttir *et al.*, 2006; Pampoulie *et al.*, in press). Furthermore, with both methods it was possible to discriminate between cod spawning at different depths at the main spawning area south of Iceland (Jónsdóttir *et al.*, 2006; Petursdottir *et al.*, 2006; Pampoulie *et al.*, in press).

Relatively little gene flow is needed in order to maintain a genetically homogeneous stock (Edmonds *et al.*, 1989). By using phenotypic methods, however, it may be possible to detect differences among genetically homogeneous groups (Booke, 1981). Various analyses of phenotypic differences have been used to study fish stock structure, including otolith shape (Campana & Casselman, 1993; Begg & Brown, 2000; Jónsdóttir *et al.*, 2006) and otolith chemistry (Campana *et al.*, 1995; Patterson *et al.*, 1999; Gillanders *et al.*, 2001). These methods indicate whether groups of fishes have inhabited different environments during the preceding several months or years (Begg & Waldman, 1999). Elemental analyses of otoliths are now being used frequently to distinguish among groups of fishes (Campana *et al.*, 1995, 2000; Patterson *et al.*, 2004). This technique has become popular because it is possible to use the elemental composition as a natural tag even without knowledge of the source of the elements (Campana, 1999). Combining results from more than one stock discrimination technique will provide more reliable information on the most likely stock structuring (Begg & Waldman, 1999; Waldman, 1999).

In this study, chemical analysis of otoliths was used to discriminate between spawning groups of Icelandic cod. Specifically, the hypotheses that otolith chemistry differed between spawning groups of cod around Iceland but not among years within a location were tested. Otoliths from cod collected at 22 different spawning locations around Iceland in the studies of Jónsdóttir *et al.* (2006) and Pampoulie *et al.*, (in press) were used for the elemental analysis. The temporal stability of elemental composition was tested by comparing seven spawning locations in two consecutive years.

METHODS

SAMPLING

Spawning cod of both sexes were sampled during the peak of the spawning season in April 2002 and April to May 2003. Samples were collected from 12 and 17 different spawning locations around Iceland in 2002 and 2003, respectively (Fig. 1). Each spawning location was identified with a three digit number, where the first digit represented one of nine regions, the second digit the depth interval (1, <75 m; 2; 75–125 m; 3, >125 m) and the last digit the station number. Sampling was carried out from fishing boats, using gillnets, hand-lines or Danish seines. A total of 35–121 mature or spawning cod were sampled at each spawning location. At sea, all cod were measured for total length (L_T) to the nearest cm, ungutted (M) and gutted mass and gonad mass (M_G) recorded, and sex and maturity stage determined macroscopically. The gonado-somatic

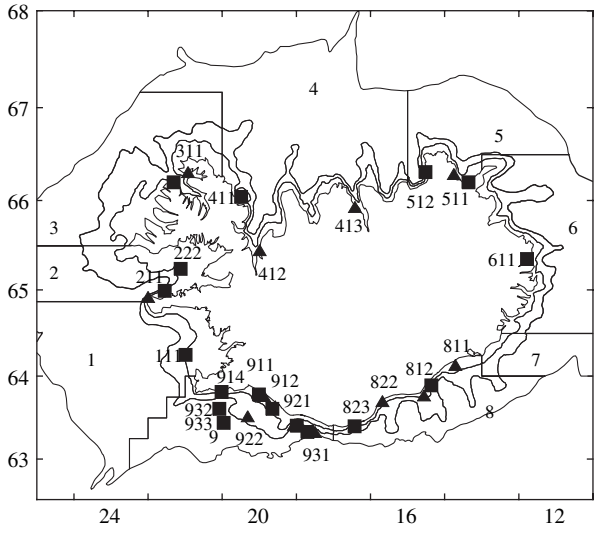

FIG. 1. Sampling locations in spring 2002 (▲) and 2003 (■). Depth contours at 75, 125 and 500 m.

index (I_G) was calculated as $I_G = 100\ M_G M^{-1}$. Sagittal otoliths were removed from each fish with non-metallic forceps, cleaned of adhering tissue and stored dry in paper envelopes until further analysis. The left otolith from each pair was used in the elemental analysis while the right otolith was sectioned and aged.

ELEMENTAL ANALYSIS

Otoliths were decontaminated with a 5 min sonification in an acid-washed vial and Milli-Q water, followed by a 1 min scrubbing of the otolith, triple rinse in Milli-Q water, two 5 min sonifications, and a final triple rinse in Milli-Q water. The otoliths were then dried under a laminar flow hood and weighed to the nearest 0·1 mg. The decontaminated otoliths were stored dry in sealed, acid-washed polypropylene vials until analysis. All otoliths were exposed only to acid-washed plastic materials during decontamination and all steps other than sonification and brushing were carried out under a laminar flow hood.

The decontaminated otoliths were dissolved in 0·6 ml of 70% (v v^{-1}) high purity nitric acid (Fluka, Traceselect, Sigma Aldrich Co., St Louis, MO, U.S.A.) per 0·1 g otolith. All otoliths were dissolved in an acid volume proportional to the otolith mass to ensure that the solutions were of similar concentration to minimize possible instrumental drift. The acid-otolith solution was heated in a microwave oven for 5 min until it reached 120° C and kept at that temperature for 25 min to complete digestion. When the solution had cooled, the volume was brought up to 50 ml with Milli-Q water. Solutions were further diluted prior to analysis, ×5000 compared to otolith dry mass. Internal standards (Ga, In and Ce) were then added to the samples. Five trace elements were measured (Ba, Mg, Li, Mn and Sr) using inductively coupled plasma mass spectrometry (LECO Renaissance mass spectrometer). A standard was run for every four samples, while a blank and a laboratory reference sample were run every eight samples. The laboratory reference sample, consisting of a batch solution of digested otolith material, was used to monitor measurement precision across sample batches, and was subsequently used to normalize sample batches to a constant reference value. Detection limits for each element (in μg g^{-1} for all elements except Sr, which was in mg g^{-1}) were calculated as three times the S.D. of the blank: Ba 0·1, Li 0·3, Mg 1·4, Mn 0·2 and Sr 0·01.

The relative S.D. of the laboratory reference sample concentrations (five in each run) was used as a measure of precision. The precision was quite good for Ba (3%), Mg (3%), Mn (4%) and Sr (1%) but lower for Li (13%).

DATA ANALYSIS

Discriminant analysis was used to identify the elements that contributed most to discrimination among the spawning samples. Li, Mg and Mn were ln-transformed to correct for non-normality and inequality of variance. ANCOVA was used to determine the effect of otolith mass on the concentration of each element, with otolith mass as a covariate and spawning location as a main factor. Li, Mg and Mn had significant effects of otolith mass and were standardized by removing the product of the common, within-group slope (b) and otolith mass from the ln-transformed Li ($b = -0{\cdot}122$), ln-transformed Mn ($b = -0{\cdot}456$) and ln-transformed Mg ($b = -0{\cdot}140$). Multivariate analysis of variance (MANOVA) was used to test for mean differences in elemental composition between spawning locations. One-way ANOVA and Tukey's HSD were then used to identify the individual elements contributing to any significant difference in the MANOVAs. Two-way ANOVA was used to test for temporal and spatial differences among locations and years. Forward stepwise discriminant analysis of the standardized data was used to discriminate between the different spawning groups. Age distribution varied among the different spawning locations (Jónsdóttir *et al.*, 2006). Since the common age at all spawning locations in both 2002 and 2003 was 6–8 years old, all analyses were restricted to those age groups (see total number of individuals in Table I).

RESULTS

Most individuals at all spawning locations were spawning (classified by the presence of hydrated oocytes or freely running sperm; Table I). Out of the 29 spawning locations sampled, 90–100% of the cod were spawning at 22 locations and 76–90% were spawning at the remaining seven locations. Those individuals that had not started to spawn were all close to spawning (characterized by high I_G values as well as large but not fully hydrated oocytes). At 16 out of 29 spawning locations a slightly higher proportion of the sampled cod was male (50–70%). At only five spawning locations the proportion of either males or females was over 70%. Out of the 145 comparisons (five elements and 29 locations) only six showed significant difference among sex [ANOVA: Mn, location 111 in 2003 (d.f. = 1,41, $P < 0{\cdot}05$); Sr, location 511 in 2002 (d.f. = 1,74, $P < 0{\cdot}05$); Sr, location 912 in 2003 (d.f. = 1,23, $P < 0{\cdot}05$); Sr, location 921 in 2003 (d.f. = 1,45, $P < 0{\cdot}05$); Ba, location 931 in 2002 (d.f. = 1,58, $P < 0{\cdot}05$); Li, location 931 in 2002 (d.f. = 1,58, $P < 0{\cdot}05$)]. Sex is therefore unlikely to have influenced the difference in elemental concentrations of the otoliths found in this study.

The concentration of each of the five trace elements (Ba, Li, Mg, Mn and Sr) differed significantly among spawning locations (ANOVA, d.f = 21,1322, all $P < 0{\cdot}001$) (Appendix). In general, higher values of Ba, Mn and Sr were detected in otoliths north of Iceland compared to those from the south (Appendix). At the main spawning ground south of Iceland, higher values of Ba and Li were detected among cod spawning below 125 m compared to those above 125 m (Appendix).

To determine temporal stability in elemental concentrations among locations and years, seven spawning locations sampled in both 2002 and 2003 were

TABLE I. Number of cod 6, 7 and 8 years old at each spawning location (see Fig. 1) in 2002 and 2003. Spawning is proportion of spawning individuals at the age of 6–8 years old

	2002				2003			
	Age (years)				Age (years)			
Location	6	7	8	Spawning	6	7	8	Spawning
111					25	14	4	0·99
211	24	29	13	0·99	28	10	5	1·00
222					15	12	18	1·00
311	12	49	12	1·00	31	7	4	0·96
411					8	5	35	0·98
412	8	47	14	0·88				
413	45	14	4	0·84				
511	43	30	3	0·90	14	9	22	0·76
512					13	26	7	1·00
611					20	6	5	0·97
811	13	16	9	1·00				
812	6	16	17	1·00	14	7	17	0·98
822	2	14	22	1·00				
823					4	11	27	1·00
911	6	13	6	0·96	9	5	2	1·00
912					10	6	9	0·82
914					26	12	5	0·99
921	8	19	21	1·00	20	13	14	1·00
922	13	20	23	1·00				
931	19	30	11	0·87	24	18	4	0·90
932					28	17	1	0·84
933					35	9	3	0·83

compared. Ba was temporally stable between years at all seven of the spawning locations [ANOVA: 211 (d.f. = 1,107, $P > 0{\cdot}05$); 311 (d.f. = 1,113, $P > 0{\cdot}05$); 511 (d.f. = 1,119, $P > 0{\cdot}05$); 812 (d.f. = 1,75, $P > 0{\cdot}05$); 911 (d.f. = 1,38, $P > 0{\cdot}05$); 921 (d.f. = 1,93, $P > 0{\cdot}05$); 931 (d.f. = 1,104, $P > 0{\cdot}05$; Fig. 2]. Li was significantly different among years at spawning locations 211 (ANOVA, d.f. = 1,107, $P < 0{\cdot}05$) and 931 (ANOVA, d.f. = 1,104, $P < 0{\cdot}05$) (Fig. 2), Mg was significantly different among years at locations 511 (ANOVA, d.f. = 1,119, $P < 0{\cdot}01$) and 931 (ANOVA, d.f. = 1,104, $P < 0{\cdot}05$) (Fig. 2) and Mn was significantly different among years at locations 511 (ANOVA, d.f. = 1,119, $P < 0{\cdot}05$) and 812 (ANOVA, d.f. = 1,75, $P < 0{\cdot}05$) (Fig. 2). Sr was significantly lower in 2003 than in 2002 at spawning locations 211 (ANOVA, d.f. = 1,107, $P < 0{\cdot}05$), 311 (ANOVA, d.f. = 1,113, $P < 0{\cdot}001$), 511 (ANOVA, d.f. = 1,119, $P = 0{\cdot}001$) and 931 (ANOVA, d.f. = 1,104, $P < 0{\cdot}001$) (Fig. 2). All elements, however, were significantly different among the seven spawning locations (ANOVA, d.f. = 6,657, $P < 0{\cdot}001$). For all elements (except Li), the location explained a greater part of the total variation (Ba 7·2%, Li 0%, Mg 1·4%, Mn 1·2% and Sr 12·2%) than year alone (Ba 0·1%, Li 1·5%, Mg 0·3%, Mn 0% and Sr 6·2%).

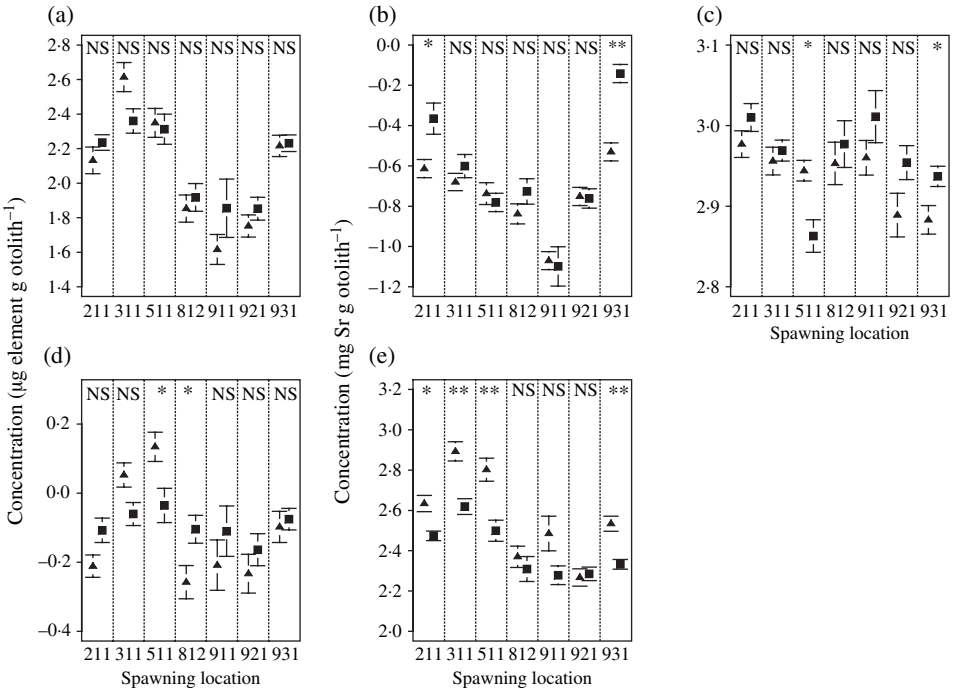

FIG. 2. Mean ± S.E. elemental concentration: (a) Ba, (b) ln Li, (c) ln Mg, (d) ln Mn and (e) Sr of cod otoliths at seven spawning locations (see Fig. 1) in two consecutive years, 2002 (▲) and 2003 (■). For locations see Fig. 1. Significance levels testing for difference among years within each location: NS, not significant; *, $0{\cdot}05 > P > 0{\cdot}001$; **, $P \leq 0{\cdot}001$.

Discriminant analysis using otolith elemental composition for both 2002 and 2003 showed clear separation between two groups of spawning cod around Iceland (Fig. 3). The first discriminant function explained 60% of the variance and discriminated between cod spawning in the north (regions 4 and 5) and the south (regions 8 and 9) (Fig. 3). Furthermore, cod spawning at regions 1 and 2 south-west and west of Iceland (except 211 in 2002) were separated from region 3 north-west of Iceland (Fig. 3). Four spawning locations overlapped with locations from different regions. Spawning locations 512 and 611 north and east of Iceland overlapped with some southern spawning locations. Furthermore, spawning locations in region 2 overlapped with the deep southern locations (Fig. 3). The second discriminant function explained 27% of the variance and separated between cod spawning above and below 125 m in region 9 south of Iceland (Fig. 3). Other regions around Iceland were not clearly discriminated from each other by the second discriminant function (Fig. 3). The variables explaining the variance of the first and second discriminant function were Ba, Li and Sr (Table II). The nine regions explained 20 and 9% of the difference of the first and second discriminant function, respectively. Eight and 6% of the variance, however, was explained by differences among locations within regions of the first and second discriminant function, respectively. Therefore, the differences among the nine regions were greater than the differences within regions.

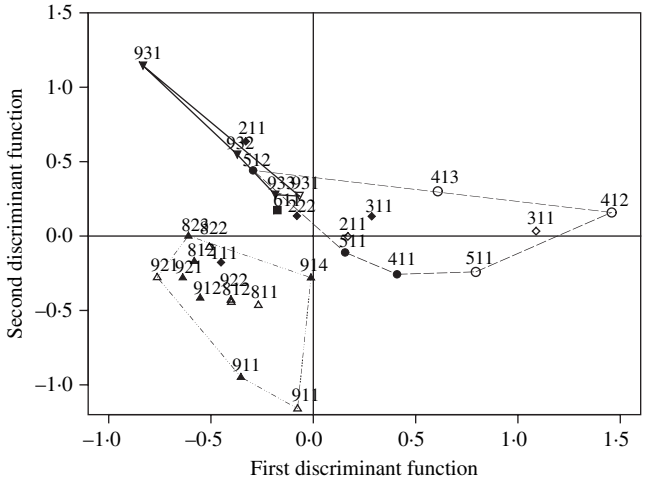

FIG. 3. Discrimination of spawning groups of Icelandic cod in 2002 (open symbols) and 2003 (closed symbols) using otolith chemistry at sampling locations (see Fig. 1) north of Iceland (○, ●), south of Iceland below 125 m (▽, ▼), south of Iceland above 125 m (△, ▲), west of Iceland (◇, ◆) and east of Iceland (□, ■). The lines surround spawning locations above 125 m south of Iceland (–··–; shallow), below 125 m south of Iceland (——; deep) and northern (– – –) spawning location. The first and second discriminant functions explained 60 and 27% of the variance, respectively.

When only the seven spawning locations common to both 2002 and 2003 were included in the discriminant analysis, the same general discrimination was obtained as when all spawning locations were used (Fig. 4). The first discriminant function separated between spawning locations north and south of Iceland and the second function discriminated between cod spawning in shallow waters and those spawning below 125 m in region 9 (Fig. 4). As when all spawning locations were combined, one western location (211 in 2003) and a deep south location (931 in 2002) were not discriminated from each other. The first two discriminant functions explained 52 and 36% of the variance, respectively. The variables explaining the variance were Ba, Li, Mg and Sr (Table II). Using the scores from the first discriminant function, a significant

TABLE II. The first and second standardized function coefficients from the discriminant analysis using otolith chemistry for both 2002 and 2003 combined, the temporal study and the first discriminant function using only a single cohort (1995 and 1996)

	Standardized canonical discriminant function coefficients					
	All locations		Temporal		Cohort	
	DFA 1	DFA 2	DFA 1	DFA 2	1995 DFA 1	1996 DFA 1
Ba	0·37	0·48	0·48	0·11	0·98	—
Li	−0·36	0·82	−0·02	0·92	0·09	—
Mg	—	—	−0·06	−0·27	—	—
Mn	—	—	—	—	—	—
Sr	0·77	−0·21	0·66	−0·30	—	1·00

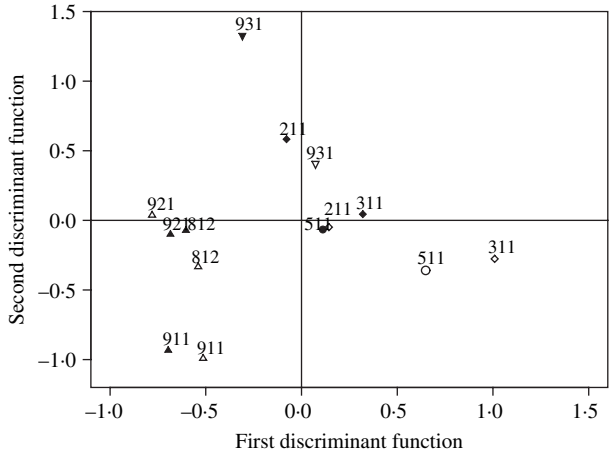

FIG. 4. Mean values of the first and second canonical variates based on otolith chemistry from seven spawning locations (see Fig. 1) in 2 consecutive years, 2002 (open symbols) and 2003 (closed symbols) at sampling locations north of Iceland (○, ●), south of Iceland below 125 m (▽, ▼), south of Iceland above 125 m (△, ▲) and west of Iceland (◇, ◆). The first and second discriminant functions explained 52 and 36% of the variance, respectively.

difference was found among years within a location at location 311 (Tukey's HSD, $P < 0{\cdot}05$). Furthermore, a significant difference was found between years at spawning location 931 using the scores from the second discriminant function (Tukey's HSD, $P < 0{\cdot}001$). Significant differences, however, were found between spawning locations north and north-west of Iceland (311 and 511, except 511 in 2003) and south of Iceland (911, 921 and 931) using the scores of the first discriminant function (Tukey's HSD, $P < 0{\cdot}05$). Therefore, the difference was greater between spawning locations than among years within a spawning location.

To determine if year class influenced the discrimination between cod north and south of Iceland, discriminant analyses were carried out using only a single cohort at a time; the 1995 cohort (186 individuals of 7 year-old cod in 2002 and 68 individuals of 8 year-old cod in 2003) and the 1996 cohort (118 individuals of 6 year-old cod in 2002 and 69 individuals of 7 year-old cod in 2003). The first discriminant function separated between spawning locations north and south of Iceland for both cohorts (Fig. 5). The first discriminant function also separated between cod spawning below and above 125 m south of Iceland for the 1995 cohort but not as clearly for the 1996 cohort (Fig. 5). The first discriminant function explained 71 and 100% of the variance for the 1995 and 1996 cohort, respectively. The variables explaining the variance were Ba and Li for the 1995 cohort and Sr for the 1996 cohort (Table II). This was the same general discrimination as when all year classes were used.

DISCUSSION

In this study, significant and consistent differences in the elemental composition of otoliths among spawning groups of Icelandic cod were established.

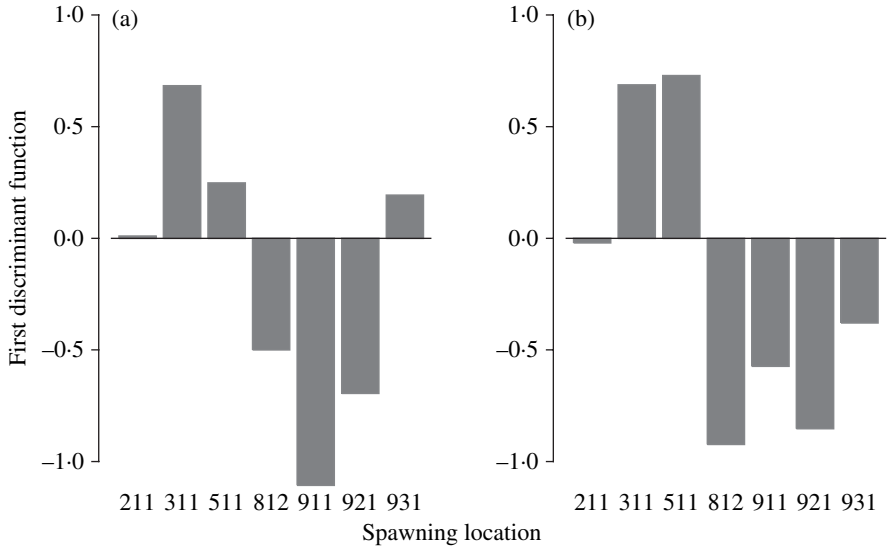

FIG. 5. Mean values of the first discriminant function based on otolith chemistry of the (a) 1995 (combined 7 year old in 2002 and 8 year old in 2003) and (b) 1996 (combined 6 year old in 2002 and 7 year old in 2003) cohorts from seven spawning locations (see Fig. 1). The first function explained 71 and 100% of the variance for the 1995 and 1996 cohort, respectively.

Three major groups of cod were identified: northern Iceland, southern Iceland spawning below 125 m, and southern Iceland spawning above 125 m. Differences in the elemental composition of whole otoliths indicate that the groups must have spent part of their life history in different locations, but cannot be used to determine the length of the separation. The magnitude of the difference between the spawning stocks suggests a prolonged separation period. Otolith chemistry has been used successfully to discriminate among groups of various fish species (Campana *et al.*, 1995, 2000; Begg *et al.*, 1998; Patterson *et al.*, 2004) but has not been applied previously on any fish species in Icelandic waters.

The greatest difference in otolith elemental composition was found between cod to the north and south of Iceland. Only small differences were found among adjacent spawning locations within each area. Temperature, and to a lesser degree salinity, have been found to influence the elemental composition of otoliths (Fowler *et al.*, 1995). These environmental factors differ markedly between the areas north and south of Iceland. Two major currents greatly influence these two regions. The southern regions are dominated by the warm and saline waters of the northward flowing Atlantic Current (6–8° C). The northern regions are influenced by the southward flow of cold and low saline waters of the East Greenland and East Iceland Current (<0–2° C; Valdimarsson & Malmberg, 1999). Furthermore, a branch of the Irminger Current, flowing northwards around the north-west peninsula into the North Icelandic shelf areas greatly influences the northern regions (Stefansson, 1962; Malmberg & Valdimarsson, 2003). West of Iceland a front is created where the Irminger Current and the East Greenland Current meet. In addition, a front is created

east of Iceland where the cold water from the north meets the warm water from the south. It is likely that these two fronts create a barrier between cod spawning north-west, north and east of Iceland (regions 3–6), and cod spawning south-west and south of Iceland (regions 1, 2, 8 and 9).

In addition to the separation among cod spawning north and south of Iceland, cod spawning above and below 125 m at the main spawning ground south of Iceland were distinguished from each other. Li and Ba were in higher concentration in cod spawning below 125 m than above. The differences in otolith chemistry among cod spawning at different depths south of Iceland are most likely to be explained by the different water masses at depth. Differences in otolith chemistry due to depth have previously been noted at depth intervals as small as 3–16 m (Kingsford & Gillanders, 2000). Cod spawning at shallower depths south of Iceland may be more influenced by river runoff than deeper spawning sites. Two major rivers transport fresh water [(mean water transport 1996–2003: 340 $m^3 s^{-1}$ (Ölfusá at Selfoss) and 333 $m^3 s^{-1}$ (Þjórsá at Urriðafoss)] into the ocean on the south-west coast of Iceland (Gíslason *et al.*, 2003). In early spring these rivers play a large role in stratifying the water column near the shore (Thordardottir, 1986). The coastal current, which is fed by freshwater runoff, is characterized by salinities <35 while Atlantic water is ≥ 35 (Stefansson, 1962). Due to flow variations in freshwater runoff, seasonal variations are greater in the coastal current than in the Atlantic water (Malmberg, 1978). If cod freely migrated between these two water masses the difference in elemental signature between the two groups would not be present, as they would inhabit similar environments. The differences in elemental signatures do exist, however, indicating that the deep- and shallow-spawning cod must remain largely separate. Indeed, recent tagging studies indicate that cod spawning at different depths on the main spawning grounds off southern Iceland are unlikely to intermix during the spawning or feeding seasons (Pálsson & Thorsteinsson, 2003).

The western spawning locations were not clearly discriminated from spawning locations below 125 m south of Iceland. Tagging studies have shown that Icelandic cod follow many types of migration patterns and that some groups practise either shallow or deep-water migrations (Pálsson & Thorsteinsson, 2003). The deep-water migrators (locations 932 and 933) migrate to feeding locations both east and north-west of Iceland (V. Thorsteinsson, unpubl. data). Cod spawning in region 2 also migrate to feeding locations north-west of Iceland (V. Thorsteinsson, unpubl. data). During the autumn, cod from these two regions may therefore inhabit similar environments and in terms of otolith chemistry they may be similar. Cod spawning above 125 m south of Iceland also migrate to the north-west feeding locations. The migration route, however, differs between cod spawning below and above 125 m. Tagging returns of cod spawning above 125 m show that most of these cod migrate closer to shore (V. Thorsteinsson, unpubl. data) and may therefore be more influenced by the coastal current than cod spawning below 125 m. This could explain why separation occurs between the deep and shallow locations but not between the western and deep locations.

Not all of the elements assayed contributed noticeably to the discrimination among the three groups of cod nor did the same element have the most discriminating power for all samples. The reasons for these variations in elemental

composition are not clear. Complex interactions between environmental factors, genetics and the physiology of the fish influence elemental uptake into otoliths (Campana, 1999; Thresher, 1999), including such factors as diet (Sanchez-Jerez *et al.*, 2002), growth rate (Thresher *et al.*, 1994), elemental concentration in the sea water (de Vries *et al.*, 2005), temperature (Fowler *et al.*, 1995) and salinity (Fowler *et al.*, 1995). Due to the large variation in the environment experienced by cod that spawn in the areas north and south of Iceland, all of these factors may have contributed to the differences in otolith chemistry.

When different year classes are combined in a discriminant analysis it is possible that age and year-class effects explain a part of the discrimination among the spawning groups. Large variations in elemental concentration have been detected among year classes of other fish species (Kalish, 1989; Begg *et al.*, 1998; Hamer *et al.*, 2003). Despite the variation among the year classes in these other studies, this variation was found to be consistent among locations and therefore did not confound the discrimination among locations (Hamer *et al.*, 2003). The same principle may also apply to Icelandic cod; despite some differences in elemental concentration among year classes, the discrimination between the northern and southern spawning locations was maintained when only a single year class (both the 1995 and 1996 cohorts) was used for the analysis. Therefore it is unlikely that year class played a great role in the discrimination among spawning cod aggregations in the present study.

As expected, the whole-otolith elemental composition varied only slightly between years within a spawning location. The differences detected within any given spawning location were most likely to be due to the different exposure histories experienced by the fish, which would have been exposed to different combinations of year-specific environments. Differences in otolith chemistry have been detected over short periods (two consecutive months; Hamer *et al.*, 2003), but are typically much longer. In the present study, the magnitude of the inter-year difference varied both among locations and elements. No element was significantly different among years at all spawning locations. Sr showed the greatest difference and was significantly different among years at four out of seven locations. The other four elements, however, tended to remain the same across years at most of the locations. Despite the small interannual variability, differences in elemental composition among locations were consistently greater than that between years. Therefore, cod inhabiting these different areas are likely to remain largely separate and show fidelity to their spawning location.

By using the elemental composition of the otolith, it was possible to discriminate among three groups of Icelandic cod. Furthermore, at least at the seven locations studied, it seems the groups were temporally stable in time. The results from this study provide more support for the view that the spawning groups of the Icelandic cod do not represent a single homogeneous stock. To this point, four different methods: otolith chemistry (present study), otolith shape (Jónsdóttir *et al.*, 2006), *Pan* I and microsatellite (Pampoulie *et al.*, in press), have been applied to the same large set of fish samples, and all have identified the same three regional groups of cod. Furthermore, these groups appear to remain stable between years. Today, the Icelandic cod stock is managed as a single unit. The results of the present study as well as those from Jónsdóttir *et al.* (2006) and Pampoulie *et al.*, (in press), strongly suggest that

the management of this stock needs to be redesigned taking into consideration the existence of these different life-history units.

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APPENDIX. Mean ± S.E. concentration of Ba, Li, Mg, Mn and Sr in otoliths sampled at different spawning locations (see Fig. 1) in 2002 and 2003

	Mean ± S.E. concentration (μg element g otolith^{-1}, except Sr: mg Sr g otolith^{-1})											
	2002						2003					
Location	*n*	Ba	Li	Mg	Mn	Sr	*n*	Ba	Li	Mg	Mn	Sr
111							43	2·0 ± 0·1	0·5 ± 0·0	20·6 ± 0·4	0·9 ± 0·0	2·4 ± 0·1
211	66	2·1 ± 0·1	0·6 ± 0·0	19·8 ± 0·4	0·8 ± 0·0	2·6 ± 0·0	43	2·2 ± 0·1	0·9 ± 0·1	20·6 ± 0·6	1·0 ± 0·1	2·5 ± 0·0
222							45	2·2 ± 0·1	0·6 ± 0·0	19·5 ± 0·4	0·9 ± 0·1	2·5 ± 0·1
311	73	2·6 ± 0·1	0·5 ± 0·0	19·4 ± 0·4	1·1 ± 0·1	2·9 ± 0·1	42	2·4 ± 0·1	0·6 ± 0·1	19·6 ± 0·4	1·0 ± 0·0	2·6 ± 0·1
411							48	2·4 ± 0·1	0·6 ± 0·1	18·6 ± 0·5	0·9 ± 0·0	2·6 ± 0·1
412	69	3·1 ± 0·1	0·5 ± 0·0	19·7 ± 0·4	1·1 ± 0·1	2·9 ± 0·1						
413	63	2·4 ± 0·1	0·7 ± 0·0	18·8 ± 0·3	1·4 ± 0·1	2·8 ± 0·1						
511	76	2·3 ± 0·1	0·5 ± 0·0	19·1 ± 0·3	1·2 ± 0·1	2·8 ± 0·1	45	2·3 ± 0·1	0·5 ± 0·0	17·8 ± 0·5	1·1 ± 0·1	2·5 ± 0·1
512							46	2·4 ± 0·1	0·7 ± 0·1	19·3 ± 0·3	1·0 ± 0·1	2·4 ± 0·0
611							31	2·2 ± 0·1	0·6 ± 0·0	20·4 ± 0·6	1·1 ± 0·1	2·5 ± 0·1
811	38	2·0 ± 0·1	0·4 ± 0·0	18·9 ± 0·4	0·9 ± 0·4	2·4 ± 0·1						
812	39	1·9 ± 0·1	0·5 ± 0·0	19·4 ± 0·6	0·8 ± 0·0	2·4 ± 0·1	38	1·9 ± 0·1	0·5 ± 0·0	20·0 ± 0·7	0·9 ± 0·1	2·3 ± 0·1
822	38	1·9 ± 0·1	0·5 ± 0·0	19·0 ± 0·5	0·8 ± 0·0	2·4 ± 0·0						
823							42	2·0 ± 0·1	0·5 ± 0·0	19·4 ± 0·6	0·9 ± 0·1	2·3 ± 0·0
911	25	1·6 ± 0·1	0·4 ± 0·0	19·4 ± 0·5	0·9 ± 0·1	2·3 ± 0·1	16	1·9 ± 0·2	0·4 ± 0·0	20·5 ± 0·7	0·9 ± 0·1	2·3 ± 0·1
912							25	1·8 ± 0·1	0·5 ± 0·0	20·5 ± 0·5	0·8 ± 0·1	2·3 ± 0·1
914							43	2·1 ± 0·1	0·5 ± 0·0	21·0 ± 0·4	0·9 ± 0·0	2·5 ± 0·0
921	48	1·8 ± 0·1	0·5 ± 0·0	18·3 ± 0·5	0·9 ± 0·0	2·3 ± 0·0	47	1·9 ± 0·1	0·5 ± 0·0	19·4 ± 0·4	0·9 ± 0·1	2·3 ± 0·0
922	56	1·8 ± 0·1	0·5 ± 0·0	18·6 ± 0·5	0·7 ± 0·0	2·4 ± 0·0						
931	60	2·2 ± 0·0	0·6 ± 0·0	18·0 ± 0·3	1·0 ± 0·0	2·5 ± 0·0	46	2·2 ± 0·1	0·9 ± 0·1	18·9 ± 0·3	1·0 ± 0·0	2·3 ± 0·0
932							46	2·3 ± 0·1	0·7 ± 0·0	20·2 ± 0·5	1·0 ± 0·0	2·4 ± 0·0
933							47	2·2 ± 0·1	0·6 ± 0·0	19·1 ± 0·4	0·9 ± 0·1	2·5 ± 0·1