The use of otolith chemistry to determine the juvenile source of spawning cod in Icelandic waters

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Chemical fingerprinting was used to discriminate spatial groups of juvenile cod (*Gadus morhua*) and to backtrack spawning cod in Icelandic waters to their area of origin as 0-group juveniles. Juvenile 0-group cod were collected around Iceland in August 1996 and 1997 to establish the spatial distribution of otolith chemistry at the juvenile stage. Spawning cod from the same year classes were sampled in the same areas in April 2002 and April/May 2003. The core, corresponding to the juvenile otolith, was extracted from the adult otolith and its content of Ba, Mn, and Sr compared with the chemistry of whole otoliths of juveniles of the same year class. High Atlantic inflow into the shelf area north of Iceland in 1997 mixed the juveniles from different areas, and the spawners of that year class were not backtraceable to their origin. For the 1996 year class, however, mixed-stock analysis indicated that most of the spawning cod northwest, north, and northeast of Iceland originated from juveniles off the central part of the north coast. Using otolith chemistry to backtrace the origin of spawners appears well suited for areas with limited mixing, but less well suited for areas or years of high current velocity.

Keywords: 0-group, chemical fingerprinting, cod, Iceland, otoliths, spawners.

Introduction

Many commercially important fish stocks consist of smaller subunits, some genetically distinct (Ruzzante *et al.*, 1999, 2000; Knutsen *et al.*, 2003; Pampoulie *et al.*, 2006). To maintain genetic differentiation between the subunits, mature fish need to have a strong tendency to return to spawn in their natal area (Wootton, 1998). With information on dispersal and connectivity, the rate of movement between populations can be estimated and characterized as populations or metapopulations (McQuinn, 1997; Smedbol and Wroblewski, 2002). Connectivity between larvae/early juveniles and adult fish populations is not easily established using conventional tagging methods, however, owing to the small size of larvae and early juveniles and the high mortality rate of those early life stages.

Otolith elemental fingerprinting is a recently developed technique that can be a powerful tool in discriminating populations or aggregations of fish species (Campana *et al.*, 1995; Patterson *et al.*, 1999). Moreover, elemental composition of otoliths is well suited to determining the eventual fate of juveniles using a forward-faced analysis, i.e. by comparing the composition of juvenile otoliths with the corresponding otolith cores of the future adults when they mature and arrive at the spawning grounds several years later (Thorrold *et al.*, 2001; Gillanders, 2002; Wright *et al.*, 2006). Elemental composition of whole otoliths has already been used successfully to discriminate between groups of spawning cod (*Gadus morhua*) around Iceland (Jónsdóttir *et al.*, 2006) as well as to estimate the contribution of different spawning groups to the harvested stock (Jónsdóttir *et al.*, 2007). Otolith chemistry may, therefore, be ideal for studying the connectivity between juvenile and adult populations of the Icelandic cod.

The cod fisheries in Iceland have been managed historically using a single-stock model (Schopka, 1994; Anon., 2006). Cod, however, spawn at a multitude of locations all around the island (Saemundsson, 1926; Marteinsdottir *et al.*, 2000), and the contribution of different spawning grounds to the surviving population of juvenile cod varies both spatially and temporally (Begg and Marteinsdottir, 2000, 2002; Marteinsdottir *et al.*, 2000). Recent studies have discriminated between populations spawning north and south of Iceland, as well as between shallow and deep spawning components in the main spawning area off the south coast (Jónsdóttir *et al.*, 2006; Pampoulie *et al.*, 2006; Petursdottir *et al.*, 2006). The main cod spawning areas are located south and southwest of Iceland, and the eggs and larvae drift with currents clockwise around the island towards the main nursery grounds north of the country (Schmidt, 1904; Saemundsson, 1926). A freshwater-induced, clockwise coastal current encircles Iceland close to the shore. Farther out, the Irminger Current, carrying warm Atlantic water from the south, flows along the continental shelf west of Iceland, where a part of it branches off into Greenland waters and the rest flows onto the shelf north of Iceland (Stefansson, 1962; Jonsson and Valdimarsson, 2005). There are strong year-to-year variations in the inflow of Atlantic

water into the main nursery area north of Iceland. The main driving force of this inflow is the eastern branch of the Irminger Current, the strength of which varies widely with the north–south movements of the Polar Front northwest of the Vestfirdir Peninsula. In a given year, the magnitude of this branch of the Irminger Current may be of the order of 1 Sverdrup (10^6 m^3 s^{-1}), but the next year, it may be almost non-existent (Jonsson and Valdimarsson, 2005). The strength of the inflow greatly influences the transport of cod larvae and juveniles towards the nursery grounds, and local spawning in fjords along the north coast is likely to be proportionally more important in years with limited inflow, compared with years when Atlantic inflow is strong (Begg and Marteinsdottir, 2002).

In the present study, elemental fingerprinting analysis was used to discriminate between spatial groups of cod juveniles and as a forward-facing analysis to determine the spawning group to which these juveniles later recruited. The chemical analysis of whole juvenile otoliths was used to explore the spatial variation in elemental fingerprints among different areas within the nursery ground. In the forward-facing analysis, the chemical composition of juvenile otoliths was used to classify the origin of adult spawners of the same year classes by comparing it with the elemental fingerprints of the otolith cores obtained from spawning cod 5–7 years later. Two year classes were chosen for this study: 1996 and 1997. These two year classes are likely to represent contrasting spatial origin of juveniles owing to distinct differences between the two years in the inflow of Atlantic water to the nursery areas north of Iceland. In 1996, the inflow was very weak, but in 1997, it was much stronger (Jonsson and Valdimarsson, 2005). Strong inflow of Atlantic water onto shelf areas north of Iceland is beneficial to the survival of a cod year class (Begg and Marteinsdottir, 2002). At the 0-group stage, the 1996 cod year class ranked among the smallest, but the 1997 year class among the largest in a 30-year time-series (Sveinbjornsson, 1996; Sveinbjornsson and Jonsson, 1997). When recruiting to the fishable stock (at 3 years old), the 1996 cod year class was estimated to be ~50 million fish, whereas a comparable estimate of the 1997 year class was ~180 million fish (Anon., 2002).

The hypothesis to be tested was that mature cod would home to their origin to spawn. However, because of the known clockwise drift of eggs and larvae from their spawning grounds around Iceland, 0-group juveniles may be caught some distance clockwise from their areas of origin. The natal areas of mature cod (of the same year class, but caught 5–7 years later) may therefore be some distance anticlockwise from the area inhabited by their corresponding 0-group counterparts.

Material and methods

Juvenile cod were collected during the annual 0-group survey performed by Iceland's Marine Research Institute in August 1996 and 1997. Collections were made in shallow water close to known spawning sites around Iceland (Figure 1) using a pelagic trawl (16 × 16 m opening, 5 mm codend). In the southwest and southeast, where fewer than ten juveniles were caught at all stations combined, no elemental analysis was attempted. The juveniles were frozen at −20°C in sealed plastic bags. In the laboratory, they were thawed and measured to the nearest 0.1 mm standard length. The sagittal otoliths were extracted, cleaned, and stored for further analysis. Spawning cod from the same two year classes as the juveniles (i.e. 1996 and 1997) were collected during

the peak of the spawning season in April 2002 and again in April/May 2003. Those samples were collected from seven different spawning locations around Iceland (Figure 1). Sampling was carried out from fishing boats using gillnets, handlines, or Danish seines. Sagittal otoliths were removed from each fish and stored dry in paper envelopes until further analysis. The right otolith from each pair was used in the elemental analysis.

Elemental analysis

Whole otoliths from 0-group juvenile cod were used for elemental analysis. From the adult otolith, a 1-mm thick slice was cut through the core. From that slice, the zone representing the first summer and autumn of growth was removed using a MicroMill® (computerized micromilling machine) fitted with a 0.5-mm diameter drill bit. The milling was programmed to follow the manually traced inner side of the first annual winter ring. Care was taken to avoid any material laid down during the first winter, but because of the tapering form of the otolith, the annual rings inside the slice may not be exactly vertical, so some unwanted material can be included or some wanted material lost. The milling of this outline down through the otolith slice was done in 26–28 steps each 35-μm deep, or until the core could be removed. The width, and hence the weight, of the cores varied both locally and especially between areas, similar to the variable size and weight of the 0-group juveniles of the same year class caught some years earlier. Otoliths and cores were weighed to the nearest microgramme.

Juvenile otoliths were decontaminated with a 5-min sonification in Milli-Q water (© Millipore Corporation) in an acid-washed vial, followed by scrubbing with a nylon brush, triple rinsing in Milli-Q water, sonification for 3 min, and finally rinsing in Milli-Q water. The same method was used for the cores from the adult cod otoliths, except for the brushing. All otoliths were exposed only to acid-washed plastic material during decontamination, and all steps other than sonification and brushing were carried out under a laminar-flow fume hood.

The decontaminated otoliths were dissolved in 25 μl of 70% (w/v) high purity nitric acid (Fluka, Traceselect) per milligramme of otolith. All otoliths were therefore dissolved in an acid volume proportional to the otolith mass to ensure that the solutions were of similar concentration to minimize possible instrumental effects. The acid–otolith solution was left overnight to complete digestion, then the volume was brought up to 1000 μl with Milli-Q water. Solutions were further diluted before analysis: 1:1 with solution containing Milli-Q water, 1% high purity nitric acid, 0.1% HAc (acetic acid), and internal standards (Ga, In, and Ce). Because of the limited size of the samples, the analysis was carried out using transient signal analysis, with a double 30-μl sample injection. Four trace elements were measured (Ba, Mg, Mn, and Sr) using inductively coupled plasma mass spectrometry (LECO Renaissance mass spectrometer). A standard was run for every four samples, and 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}) were calculated as three times the standard deviation of the blank: Ba, 0.3; Mg, 3.9; Mn, 0.5; and Sr, 4. Owing to the small sample sizes used (0.1–3 mg) and the low concentrations of some of the elements analysed, some of the analyses were close to their limits
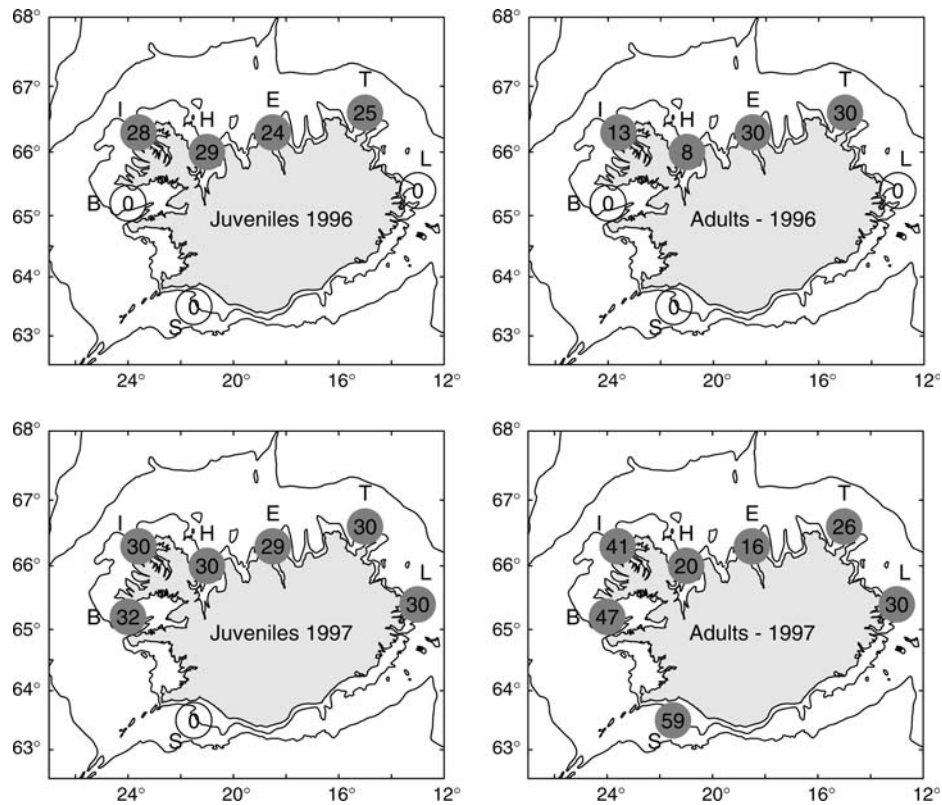
Figure 1. Sampling locations and total number of juvenile otoliths (left column) and adult otoliths (right column) collected from the 1996 and 1997 year classes in each area. Depth contours at 100 and 500 m. S, Selvogsbanki; B, Breidafjordur; I, Isafjordur; H, Hunafloi; E, Eyjafjordur/Skagafjordur; T, Thistilfjordur; L, Lodmundarfjordur.

of detection. The relative standard deviation of the laboratory reference sample concentrations (five in each run) was used as a measure of precision. The precision was quite good for Sr (2%), Mg (4%), and Ba (6%), but less good for Mn (9%).

Data analysis

All otolith variables of the juvenile samples were tested for normality and homogeneity of variance and were transformed if necessary. Ba was transformed by natural-log transformation. Analysis of covariance was used to determine the effect of otolith weight on the concentration of each element, with otolith weight as a covariate and area as a factor. The Mn content was significantly affected by otolith weight and was standardized by removing the product of otolith weight and the common within-group slope (b) from ln-transformed Mn ($b = -0.502$). Multivariate analysis of variance (MANOVA) for all variables combined was used to test for overall difference among juvenile samples. One-way ANOVA and Tukey's HSD were used to examine mean differences between individual elements and discriminant scores among juvenile samples. Forward-stepwise discriminant analysis was used to discriminate between the different juvenile samples and to identify the elements that contributed most to discrimination. Classification accuracy was estimated with "leave-one-out" cross validation.

The timing of juvenile settlement caused some practical problems. Because of the inaccessibility of the juveniles after settlement, they had to be caught in August, while still pelagic. Unfortunately, the month of September corresponds to a period of rapid growth, a month during which the weight of 0-group

juveniles can double (Vilhjálmsson and Fridgeirsson, 1976; Palsson, 1980). Accompanied by fast growth is not only a rapid deposition of otolith material, but also in this new environment (demersal), its chemical composition is probably also different. The only practical way of delimiting the core of the adult otoliths, however, is between the summer and winter growth zones. The unavoidable inclusion of the September growth period in the adult cores caused appreciable differences in the Mg, Mn, and Sr concentrations between the whole juvenile otoliths and the adult otolith cores. To minimize the difference between juvenile otoliths and adult cores, the elements were standardized to a common juvenile otolith weight (explained below). Otolith weight was correlated with both Mg and Mn in the juvenile otolith. However, no correlation was found between the weight of otolith cores from spawning cod and Mg or Mn, indicating that the core weight was not a good (backfacing) indicator of the size or otolith weight of the corresponding juvenile. More importantly, the size-corrected Mg and Mn values could not be calculated for the adult cores, because the actual weight of the core during August could not be measured. Rather than remove Mg and Mn from the statistical analysis, we attempted to calculate an equivalent juvenile otolith weight for each adult core. Using juvenile otoliths from all areas and years, the size-specific juvenile otolith Mn concentration was modelled with a non-linear (power) curve as a function of juvenile otolith weight. By assuming that this one functional relationship applied equally to all areas (an assumption which is probably not true, but not grossly in error), we could then use the observed Mn values in the adult cores to estimate what the equivalent juvenile otolith weight must have been. This estimation

was made by noting the modelled otolith weight that best corresponded to the mean Mn of the adult cores. The resulting estimated core weight of 1.302 mg was used to standardize the cores by subtracting the product of the estimated weight and the common within-group slope estimated for the juveniles ($b = -0.502$) from the ln-transformed Mn. The mean Mg concentration of the adult cores, however, never intersected the non-linear concentration–weight curve for the Mg of the juvenile otoliths (i.e. there was no single otolith weight in which Mg concentration was comparable for both groups). Estimation of the equivalent juvenile otolith weight of the spawning fish was, therefore, not possible for Mg, so this element was eliminated from further analysis. No relationship was found between otolith weight and Sr. Therefore, Sr was standardized by dividing the mean value of Sr of the juvenile otoliths into the juvenile values and the mean Sr value of the adult cores into the core values. After careful consideration, these unconventional standardization methods seemed the only logical way to make the widely different elemental concentrations of the juvenile otoliths and adult cores comparable. The above adjustments/standardizations of the chemical concentrations of the adult cores were used throughout the subsequent analysis.

Mixed-stock analysis

The origin of the spawners was estimated with maximum-likelihood-based integrated stock mixture analysis (Campana *et al.*, 1999, 2000). The reference data (known stock) were the juveniles sampled in autumn 1996 and 1997, whereas the unknown samples were the otolith cores of the spawning stocks from spring 5–7 years later (the same year classes). The product of the analysis was the proportion of each juvenile reference group contributing to each of the spawning stocks. Three elements (Ba, Mn, and Sr) contributed to the discrimination of the juveniles in 1996 and they were used for the mixed-stock analyses. In 1997, mixed-stock analysis was not possible, because discrimination between spatial groups of juveniles of that year class was only partly successful, and some baseline reference groups may have been missing.

Results

Size of 0-group cod

In 1996, the mean length of juveniles ranged from 39.8 to 44.5 mm in all areas except in Thistilfjordur, where the mean length was lower (29.3 mm; Figure 2). Significant differences in mean length were detected among all locations in 1996 (Tukey's HSD: $p < 0.05$) except Isafjordur and Eyjafjordur. In 1997, the mean juvenile length, ranging from 41.2 to 51.8 mm, was generally greater than in 1996, but was more similar among locations than in 1996 (Figure 2). The only significant difference detected in 1997 was the lower mean length in Hunafloi than in Breidafjordur, Isafjordur, and Thistilfjordur (Tukey's HSD: $p < 0.05$).

Elemental concentration of 0-group cod otoliths

Figure 3 shows the standardized Mn and ln-transformed Ba concentrations of the juvenile otoliths at different locations in 1996 and 1997. In 1996, all the areas were separated, at least partly, from each other by the different content of these two elements, but in 1997, only the bay areas Breidafjordur and Hunafloi were clearly separated by the Mn content. The concentration of each

of the three trace elements Ba, Mn, and Sr differed significantly among 0-group cod at different locations in 1996 (ANOVA: $p < 0.001$). In Figure 4, significant differences between individual areas are indicated (by broken lines) for each element separately (Tukey's HSD: $p < 0.001$, except for I–T and I–E: $p < 0.01$). For each element, only 1–2 pairs of areas were chemically similar in 1996, i.e. not significantly different and shown with a solid line, so only Hunafloi and Eyjafjordur/Skagafjordur are, in this way, potentially linked for two elements (Figure 4). In 1997, however, about half the area pairs were chemically similar with respect to Mn, and no areas were significantly different in their Ba and Sr content.

Discrimination among 0-group cod

The overall otolith chemistry, using all standardized elements, differed significantly between 0-group cod at different locations around Iceland in 1996 and 1997 (MANOVA: $p < 0.001$). The discriminant analyses provided further evidence for a separation between 0-group juveniles (Figure 5). In 1996, the first dimension explained 82.1% of the variance, and the discriminant scores from the first function were significantly different between all three areas, i.e. Isafjordur, Hunafloi + Eyjafjordur/Skagafjordur, and Thistilfjordur (Tukey's HSD: $p < 0.001$). The second dimension explained 17.9% of the variance and separated Hunafloi + Eyjafjordur/Skagafjordur from the other 0-group juveniles (Tukey's HSD: $p < 0.001$). The classification accuracy ranged between 64 and 76% (Table 1). This still leaves 24–36% of the measurements ungrouped, a finding that has to be borne in mind below when mixed-stock analytical results are interpreted.

In 1997, discrimination between groups of 0-group cod was also successful (Figure 5). The first dimension explained 100% of the variance and separated Breidafjordur and Hunafloi from the other locations. Discriminant scores were significantly different between Breidafjordur and all other locations (Tukey's HSD: $p < 0.001$), except Isafjordur. The discriminant scores were also significantly different between Hunafloi and all other locations (Tukey's HSD: $p < 0.001$). Classification accuracy was more variable in 1997 than in 1996, and ranged between 3 and 80% (Table 2).

Area-specific elemental concentration was studied with discriminant scores where the two year classes of four juvenile locations were combined in a single discriminant analysis, with area used as discriminating factor. The two year classes were different at all four locations (Figure 6). For both year classes, there was almost no overlap of the discriminant scores of the 0-group juveniles in Isafjordur (northwest), and limited overlap at Eyjafjordur/Skagafjordur (north). The difference was least in Thistilfjordur northeast of Iceland (Figure 6).

To infer whether the adults (cores) might have originated from a juvenile group that had not been sampled and characterized (a missing baseline reference group), discriminant analysis of the juveniles (only) was used to calculate discriminant scores for the adults. The majority of the discriminant scores of the adults fitted into the discriminant space defined by the juveniles in 1996 (Figure 7), suggesting that the baseline sampling of the juveniles contained most, if not all, of the reference groups of importance for the mixed-stock analysis. A small number of the adult otoliths (7%) did not fit into the discriminant space of the juveniles, possibly suggesting a missing baseline group. Alternatively, the discrepancy may be the result of less-than-perfect adjustment/standardization of the adult otolith cores. As the first
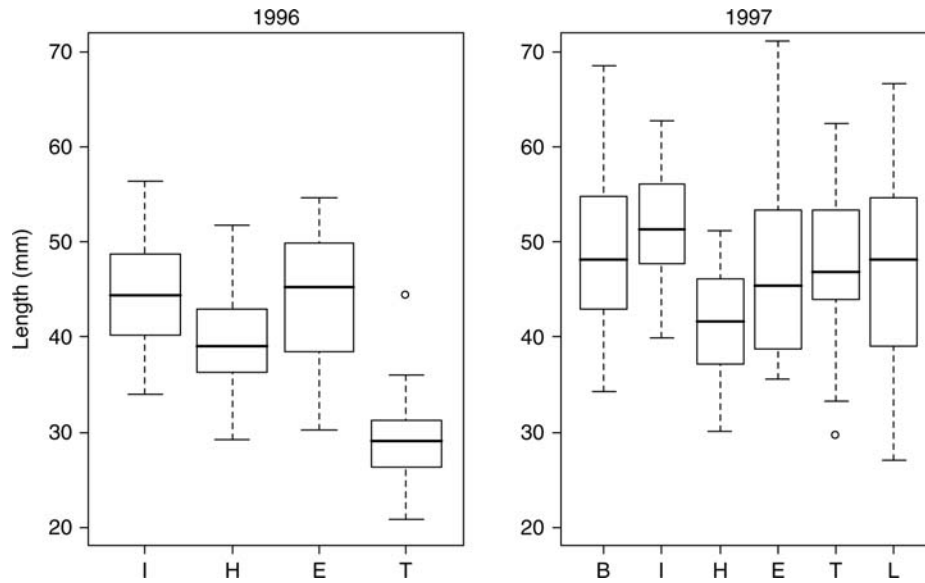
Figure 2. Medians, quartiles, and ranges of juvenile length for the different locations in 1996 and 1997. B, Breidafjordur; I, Isafjordur; H, Hunafloi; E, Eyjafjordur/Skagafjordur; T, Thistilfjordur; L, Lodmundarfjordur. For locations, see Figure 1.
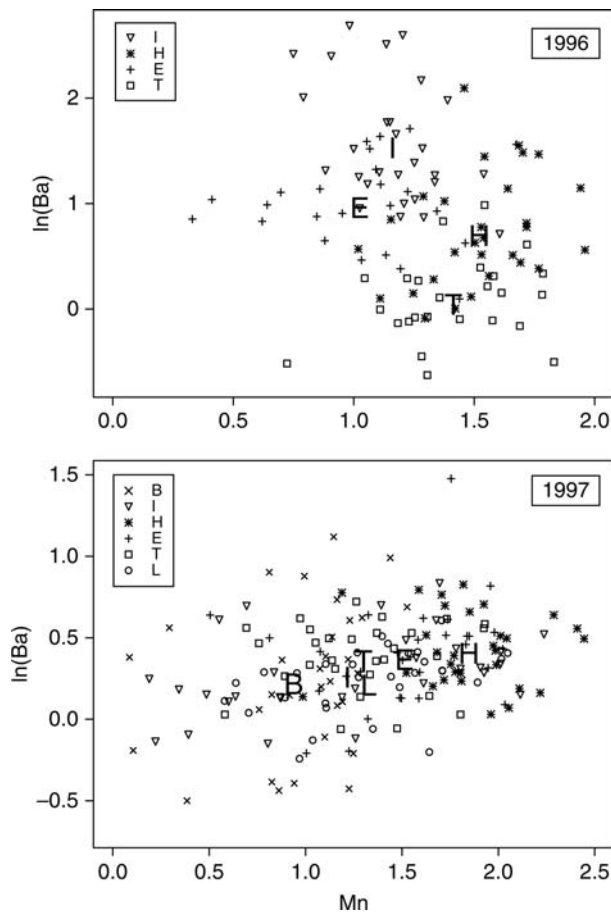

Figure 3. Standardized Mn and ln-transformed Ba concentration of otolith 0-group juveniles at four and six different locations in 1996 and 1997, respectively. B, Breidafjordur; I, Isafjordur; H, Hunafloi; E, Eyjafjordur/Skagafjordur; T, Thistilfjordur; L, Lodmundarfjordur. The average for each area is also shown with respective capital letters. For locations, see Figure 1.

discriminant function (Mn content) explained all the variance in the 1997 data, the data do not allow for this type of analysis for 1997. The high average Mn content (and large variance) of the adult cores (average 2.8 ± 1.4) relative to that of the juvenile otoliths (average 2.2 ± 0.3) does, however, strongly suggest that some baseline groups may have been missing in 1997.

Mixed-stock analysis

Because of the small number of adult cod (eight) caught in Hunafloi, that area was combined with the Eyjafjordur/Skagafjordur area in the following analysis. Mixed-stock analysis based on otolith chemistry indicated that most of the spawning cod of the 1996 year class northwest, north, and northeast of Iceland originated from juveniles from the central part of the north coast (Figure 8). Moreover, 30–34% of the cod spawning northwest and northeast of Iceland were estimated to originate from juveniles northwest of Iceland (Figure 8). Only some 9–14% of the spawners in each of the three areas were estimated to have originated from juveniles located northeast of Iceland (Figure 8). Although confidence intervals around each of the mixed-stock estimates were not available, the classification accuracy estimates from Table 1 suggest that the mixed-stock classifications provided an accurate view of the overall spatial pattern, although not precisely estimated. The 1997 material could not be analysed in the mixed-stock analysis.

Discussion

For 1996, when inflow of Atlantic water onto the shelf area north of Iceland was minimal, groups of juveniles from different sampling locations could be successfully discriminated from each other based on otolith chemistry. Using mixed-stock analysis, spawning cod of that year class were largely backtraced to juveniles off the central part of the north coast. The increased mixing in 1997, caused by the strong Atlantic inflow, is probably responsible for the difficulty in separating the juvenile groups in 1997, and the spawners of that year class were not traceable back to their juvenile locations.
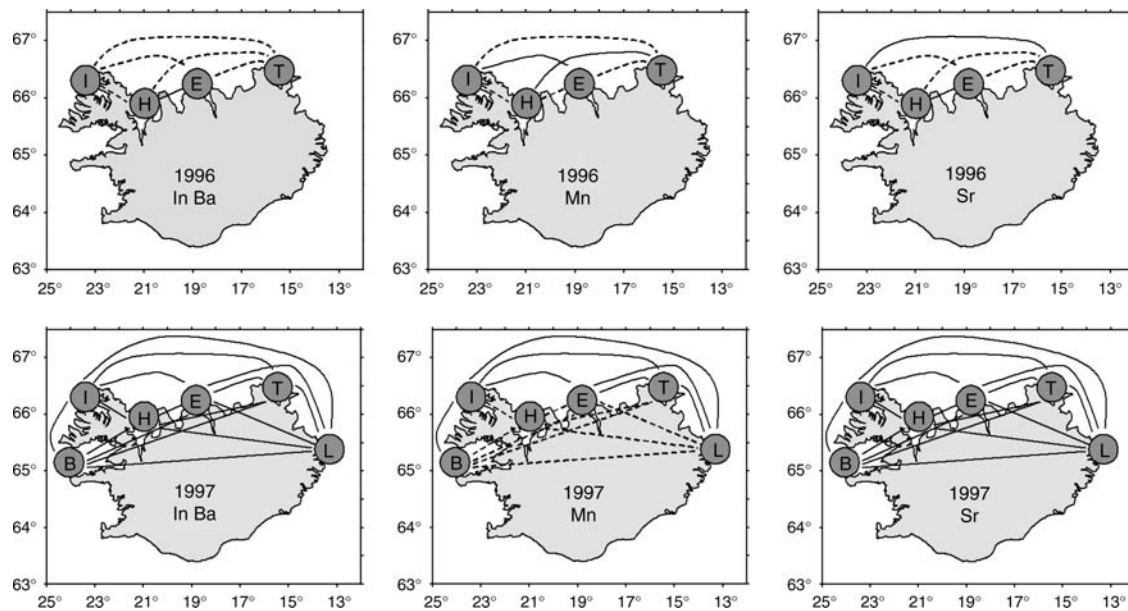
Figure 4. Chemical similarity between sampling areas based on the concentration of Ba (ln-transformed), Mn, and Sr in the juvenile otoliths. Dashed lines indicate significant differences between areas, and solid lines indicate the chemical similarity based on a single element (Tukey's HSD test).

The strength of the Atlantic inflow onto shelf areas north of Iceland varies greatly between years (Stefansson, 1962; Jonsson and Valdimarsson, 2005). In 1996, 0-group juveniles from different areas were easily discriminated based on otolith elemental composition, indicating limited mixing of juveniles. This finding is in accord with the limited inflow and low current velocities reported for that year (Jonsson and Valdimarsson, 2005). In 1997, on the other hand, discrimination between juveniles from different areas was not possible, except for the areas inside the bays of Breidafjordur in the west and Hunafloi in the north. Both those areas differed significantly from the other areas, consistent with increased mixing caused by the strong inflow of Atlantic water into the areas north of Iceland during 1997 (Jonsson and Valdimarsson, 2005).

Based on the positive results from 1996, it is evident that sufficient background variability exists in north Icelandic waters for chemical discrimination of juvenile otoliths, given the right environmental conditions. Why the juvenile groups of the 1997 year class were not separable from each other, based on otolith chemistry, is most probably inflow-related, but other explanations are possible:

(i) large-scale mixing of the seawater may have been thorough enough to homogenize the initial differences in background concentration and hence prevent discrimination;

(ii) juvenile groups of different origin may have been brought together early enough to make their environmental background too similar for discrimination to be effective;

(iii) currents and eddies may have moved the juveniles successively through different water masses, resulting in a mixed chemical background that was inseparable from that of other juvenile groups with different, but also mixed, chemical background.

Those possibilities may not be mutually exclusive, but they all involve increased mixing compared with the 1996 condition. Using otolith chemistry to backtrace the origin of spawners seems well suited to areas with limited mixing, but less well suited to areas (or years) of high current velocity resulting in extensive mixing.

The successful discrimination of juveniles from different sampling locations does not, by itself, guarantee that the juveniles came from spatially separated spawning groups. Supplementary information suggests that spawning groups are separated, however. Otolith cores from the spawners of the 1996 year class were successfully traced back to spatially separated groups of 1996 juveniles, indicating some homing tendency, because the adults mix at the feeding grounds west and east of Iceland (Jonsson, 1996; Jónsdóttir *et al.*, 2007). The pooled Hunafloi + Eyjafjordur/Skagafjordur (mid-north) location is, however, the only location dominated by locally produced spawners. No spawners caught off the mid-north coast originated from the northwest area. A considerable portion (30%) of the spawners caught in the northeast, however, originated in the northwest, perhaps indicative of some cod eggs/larvae drifting from the northwest to be caught later as 0-group juveniles in the northeast. This hypothetical drift route seems to have passed (probably in deeper water) the sampling areas off the mid-north coast. Separate spawning groups south and north of Iceland have been discriminated by various methods, including otolith chemistry (Jónsdóttir *et al.*, 2006) and genetics (Pampoulie *et al.*, 2006). The present results further suggest that the spawning stock on the shelf north of Iceland may be spatially structured.

A considerable difference in the contribution of the main spawning area southwest of Iceland was indicated in the two years studied. In 1996, very few juvenile cod were found during the annual 0-group survey, and all of them were caught northwest, north, and northeast of Iceland (Marteinsdottir *et al.*, 2000). Those juvenile cod probably originated from areas relatively close to
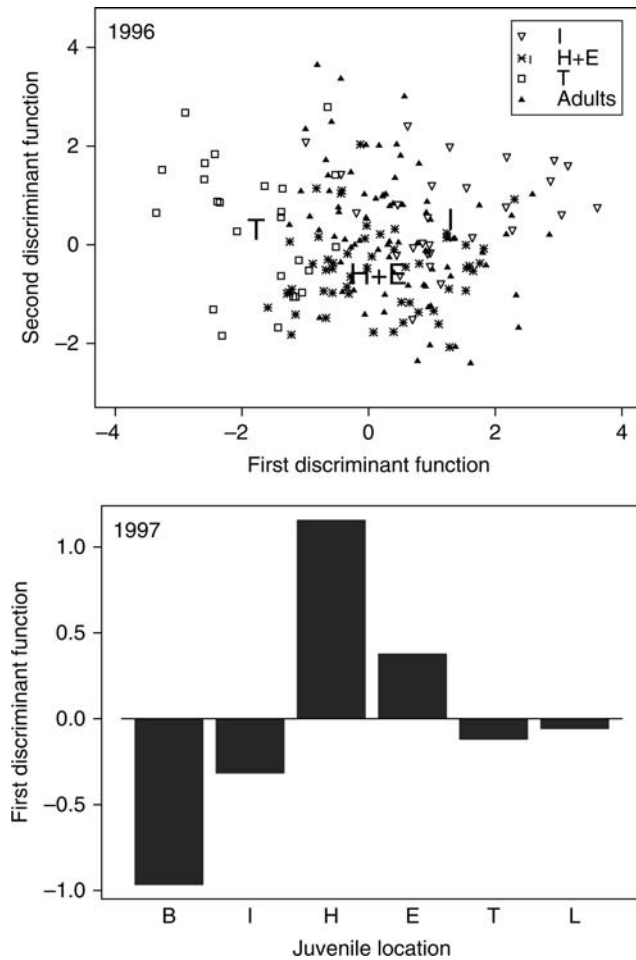
Figure 5. Discriminant scores of juvenile 0-group cod in 1996 using otolith trace elements (Ba, Mn, and Sr). The average for each area is shown with capital letters. For 1997, the mean value for the first discriminant function is shown equivalent to the average Mn concentration. B, Breidafjordur; I, Isafjordur; H + E, Hunafloi + Eyjafjordur/Skagafjordur; T, Thistilfjordur; and L, Lodmundarfjordur. Because of the small sample size (eight) in Hunafloi in 1996, H and E are combined into one area in 1996. For locations, see Figure 1.

Table 1. Classification accuracy from discriminant analyses between juvenile cod in 1996.

	I	H + E	T
I	**64**	29	7
H + E	19	**64**	17
T	0	24	**76**

Because of the small sample size in Hunafloi (eight), H and E are combined into one area. Emboldened numbers indicate classification accuracy for correctly classified juveniles. I, Isafjordur; H + E, Hunafloi + Eyjafjordur/Skagafjordur; T, Thistilfjordur. For locations, see Figure 1.

where they were sampled, because weak inflow of Atlantic water suggested limited mixing and transport of juveniles from the southwest and west coasts (Marteinsdottir *et al.*, 2000; Jonsson and Valdimarsson, 2005). Results on hatch-date distribution of the juveniles in 1996 also indicated that those taken north of Iceland hatched in June and July, considerably later than the recorded hatching dates in April and May in the southern

Table 2. Classification accuracy from discriminant analyses between juvenile cod in 1997.

	B	I	H	E	T	L
B	**56**	31	0	3	6	3
I	43	**3**	33	10	7	3
H	3	3	**80**	13	0	0
E	17	7	38	**28**	3	7
T	27	17	17	17	**7**	17
L	37	3	20	20	7	**13**

Emboldened numbers indicate classification accuracy for juveniles classified correctly. B, Breidafjordur; I, Isafjordur; H, Hunafloi; E, Eyjafjordur/Skagafjordur; T, Thistilfjordur; L, Lodmundarfjordur. For locations, see Figure 1.

spawning areas (Marteinsdottir *et al.*, 2000). The 1996 year class, therefore, probably originated largely from spawning sites north of Iceland (Begg and Marteinsdottir, 2000; Marteinsdottir *et al.*, 2000). Regrettably, no otolith cores from spawners were analysed from the southwestern spawning areas that year. Juveniles caught west of Iceland (which may also originate from the southwest spawning area) are usually spawned earlier and grow faster than juveniles taken north of Iceland. This was also the case in 1996 (Marteinsdottir *et al.*, 2000). The greater mean length of juveniles north of Iceland in 1997 and the limited spatial difference in length distributions, compared with 1996, may indicate a substantial contribution from the main spawning area southwest of Iceland in 1997. Moreover, if juveniles of different origin were transported into the northern areas in 1997, but not in 1996, one would assume that the chemical fingerprints in the two years would be more different close to the inflow (with a large proportion of imported juveniles in 1997) and more similar in the easternmost areas (and probably inside bays), where there would be fewer imported juveniles. This line of reasoning is supported by the discriminant scores for the two years (Figure 6).

Juveniles originating from known spawning areas west and even southwest of Iceland may, therefore, have been carried into many of the sampling sites north of Iceland in 1997. In a study on damselfish (*Stegastes beebei*) juveniles around the Galápagos Islands, discrimination was unsuccessful over large spatial scales, indicating that the drift of larvae over long distances is likely to lead to diminished discrimination power (Ruttenberg and Warner, 2006). When transport is limited, however, many studies have reported clear separation between groups of juveniles (Thorrold *et al.*, 2001; Gillanders, 2002; Warner *et al.*, 2005; Gibb *et al.*, 2006; Wright *et al.*, 2006), similar to the results of the 1996 year class of the present study.

Fisheries management based on a substock spatial scale, especially during spawning, may be necessary to reduce the risk of recruitment failure for stocks with complex stock structure (Stephenson, 1999), especially in areas of extremely variable environmental conditions. In attempting to understand better the dynamics of the life history of such complex stocks, one important link, which has been missing in the past, is the connectivity between juveniles and adults. In contrast to some other studies with good separation during the juvenile phase (e.g. the studies of Thorrold *et al.*, 2001; Gibb *et al.*, 2006; Wright *et al.*, 2006), spawning stock origin can be difficult to estimate in turbulent areas. Nevertheless, the results of the present study, for the 1996 year class in particular, underscore the importance of the stock-structure scale for fish stocks such as the Icelandic cod which
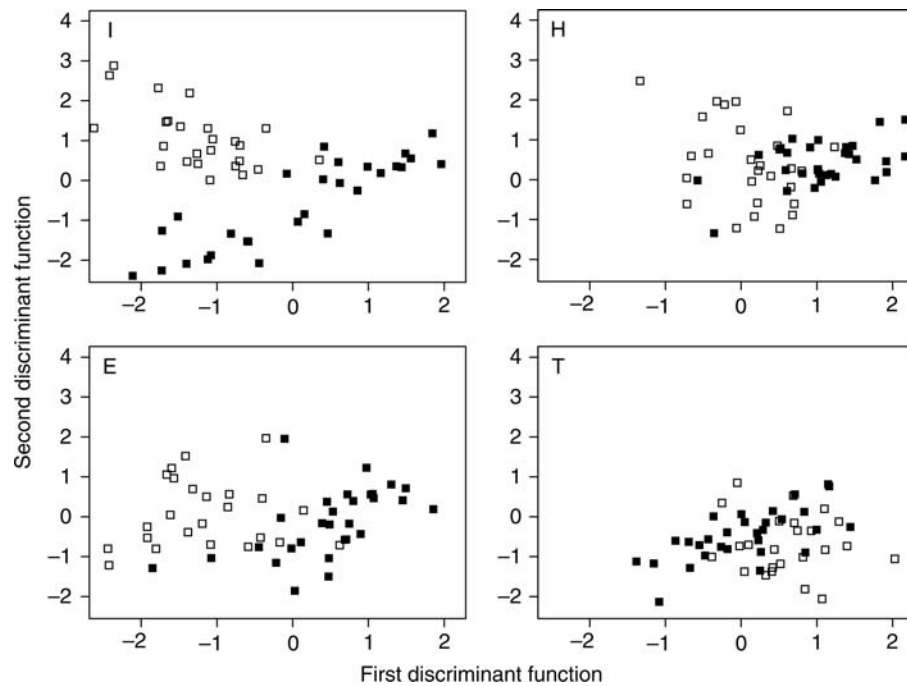
Figure 6. Discriminant scores of juvenile 0-group cod at four different locations in 1996 (open symbols) and 1997 (filled symbols) based on otolith elemental composition (Ba, Mn, and Sr). Discriminant analysis is based on the four locations independent of year. I, Isafjordur; H, Hunafloi; E, Eyjafjordur/Skagafjordur; T, Thistilfjordur. For locations, see Figure 1.
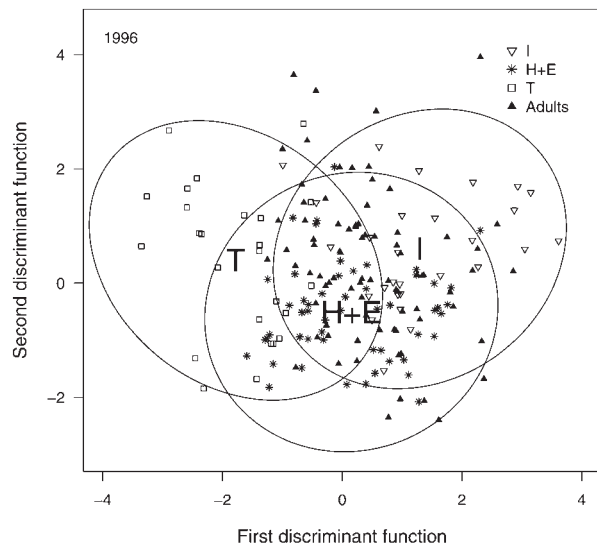

Figure 7. Discrimination between different groups of 0-group cod in 1996 using the trace elements Ba and Sr. For each area, bold letters denote the mean, surrounded by 95% confidence ellipses. Because of the small sample size (eight) in Hunafloi, H and E are combined into one area. I, Isafjordur; H + E, Hunafloi + Eyjafjordur/Skagafjordur; and T, Thistilfjordur (see Figure 1 for locations). Discriminant scores from the adult otolith cores (filled symbols) were calculated from the 0-group discriminant functions. Adjusted/standardized values for the otolith cores of adults (see text) were used.

Figure 8. Results of the mixed-stock analysis for the 1996 year class. Pies indicate locations of spawning groups, and small circles indicate sampling locations of juveniles. The area of a colour in a pie corresponds to the proportion of a juvenile group from a sampling location with the same colour. Unfilled circles represent null samples. Depth contours at 100 and 500 m.

contain spatially separated spawning populations and are exposed to variable environmental conditions. The methods used in our study may therefore prove useful in assessing the structure of heterogeneous fish stocks, a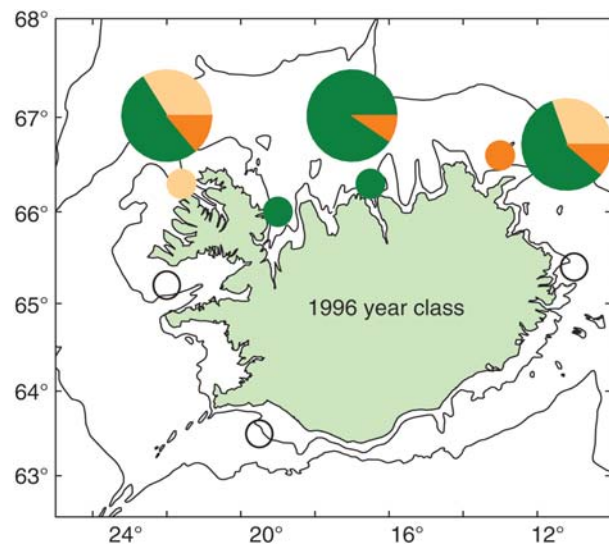t least where turbulence is limited, although to map the true complexity of the Icelandic cod stock, a finer spatial scale than currently applied is needed.

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