

Otolith shape and temporal stability of spawning groups of Icelandic cod (*Gadus morhua* L.)

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During the past 5–10 years, studies exploring small-scale structure of cod populations have provided accumulating evidence for the existence of local populations. In Iceland, all cod have been thought to belong to a single management unit. Recent studies on genetic and life history variation have, however, indicated the existence of local populations. The main objective of this study is to explore the potential existence of local populations by use of otolith shape to discriminate between spawning groups of Icelandic cod. Otoliths were sampled from mature and spawning cod at spawning locations around Iceland in 2002 and 2003, and otolith shape was described using variables correlated with size (otolith area, length, width, perimeter, and weight) as well as shape (rectangularity, circularity, and 10 Fourier coefficients). Only standardized otolith variables were used so as to remove the effect of otolith size on the shape variables. Cod were on average larger and older south of Iceland, where ambient temperature was higher than northwest, north, and east of Iceland. Otolith shape effectively discriminated between cod north and south of Iceland, and it was also possible to discriminate among cod spawning below and above 125 m at spawning locations south of Iceland. Recent genetic and tagging studies have indicated differences between cod at these two depths. Correct classification of groups of cod at the different spawning locations ranged between 0% and 44%. Incorrectly classified cod were in most cases classified to adjacent spawning locations, and a high percentage of cod south of Iceland was classified to other southern locations and cod north of Iceland to other northern locations. The temporal stability of otolith shape was studied at seven spawning locations in two consecutive years. Otolith shape differences were greater between locations than among years within a location. The spawning groups are therefore likely to have remained separate during much of their lifetime.

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Introduction

Atlantic cod (*Gadus morhua* L.) are widely distributed in the North Atlantic and have traditionally been divided into separate stocks on the basis of their major spawning areas (Garrod, 1977). Studies on genetic variation among these major cod stocks have demonstrated distinct differences between the Northeast and Northwest Atlantic (Pogson *et al.*, 1995; Bentzen *et al.*, 1996; Árnason, 2004). Evidence for differentiation within these regions as well

as the existence of local populations has also been established on the basis of variation in life history (Olsen *et al.*, 2004; Salvanes *et al.*, 2004; Neat *et al.*, 2006) and genetics (Ruzzante *et al.*, 1996; Nielsen *et al.*, 2001; Knutsen *et al.*, 2003). Understanding stock structure is important when managing multi-stock commercial fisheries because different stocks may respond differently to exploitation and rebuilding. Much effort has been put into studying the stock structure of cod using methods such as otolith shape (Campana and Casselman, 1993; Cardinale *et al.*,

2004), otolith chemistry (Campana *et al.*, 1995), and genetics (Ruzzante *et al.*, 1996; Knutsen *et al.*, 2003; Sarvas and Fevolden, 2005).

Historically, all cod in Icelandic waters have been thought to belong to a single stock (Schopka, 1994), and research on mitochondrial DNA indicates a homogeneous cod stock in Icelandic waters (Árnason and Rand, 1992; Árnason *et al.*, 1992). The main spawning areas are located southwest of Iceland, and spawning outside the main spawning grounds has been considered limited and of little significance (Jónsson, 1954, 1982). However, cod spawn at numerous locations all around Iceland (Saemundsson, 1926; Marteinsdóttir *et al.*, 2000a), and recent studies indicate that the structure of the stock may be composed of multiple units that have limited interaction (Thorsteinsson and Marteinsdóttir, 1993; Marteinsdóttir *et al.*, 2000a, b; Jónsdóttir *et al.*, 2002; Petursdóttir *et al.*, 2006). Age and hatch date analysis showed that 0-group cod north and east of Iceland were younger and smaller than 0-group cod south of Iceland, and that hatching was later than would have been expected based on the recorded spawning time southwest of Iceland (Marteinsdóttir *et al.*, 2000a). Therefore, a large proportion of the 0-group cod was unlikely to have originated from the main spawning area southwest of Iceland (Marteinsdóttir *et al.*, 2000a). Tag-recapture studies of cod from the east and west coast have shown that cod from these areas display high fidelity to their native spawning ground (Thorsteinsson and Marteinsdóttir, 1993; Thorsteinsson *et al.*, 1998; Saemundsson, 2005). Moreover, otolith shape differed among cod spawning at three adjacent spawning locations of the main spawning area south of Iceland (Petursdóttir *et al.*, 2006), and significant differences in the *Pan I* genotype were found between two groups (deep and shallow) within the same main spawning area (Jónsdóttir *et al.*, 2002). However, the *Pan I* genotype has been shown to be under selection (Fevolden and Pogson, 1997; Karlsson and Mork, 2003), so variation at the *Pan I* locus does not necessarily demonstrate genetic divergence, although it may indicate the existence of different life history groups.

Phenotypic characters such as meristics and morphometrics have commonly been used for stock identification, but they indicate prolonged separation of fish inhabiting different environments, not necessarily genetic differentiation (Begg and Waldman, 1999). The shape of calcified structures such as scales and otoliths has been used successfully to discriminate between fish stocks. Otolith shape has been used to distinguish among stocks of species such as Atlantic cod (Campana and Casselman, 1993; Cardinale *et al.*, 2004), herring (*Clupea harengus*; Bird *et al.*, 1986), king mackerel (*Scomberomorus cavalla*; DeVries *et al.*, 2002), and haddock (*Melanogrammus aeglefinus*; Begg and Brown, 2000). Otoliths have been considered ideal for stock discrimination because they grow throughout the life of the fish and are metabolically inert (Campana and Neilson, 1985). Both environmental and genetic factors

influence the shape of otoliths (Cardinale *et al.*, 2004). Although it is often difficult to distinguish between the effects of the two factors, environmental effects are generally thought to be more influential (Campana and Casselman, 1993; Begg and Brown, 2000).

Although otolith shape has been used successfully to discriminate between stocks, its use for the purposes of stock discrimination has been questioned (Castonguay *et al.*, 1991; Begg and Brown, 2000). Castonguay *et al.* (1991) emphasized the need for carefully drawn conclusions about stock structure from Fourier analysis, because the discrimination might have been caused by age- and year-class effects and would therefore highlight sample differences rather than stock discrimination. Therefore, it has been suggested that for stock discrimination, otolith shape characteristics have to be recalculated each year for each major age group (Begg and Brown, 2000).

Here we use otolith shape analysis to study the differences between 22 spawning groups of cod sampled at different locations around Iceland. The temporal stability of otolith shape was studied by comparing seven different spawning locations around Iceland in two consecutive years.

Methods

Sampling

Female and male spawning cod were sampled during the peak of the spawning season in April 2002 and April/May 2003. Samples were collected from 12 and 17 spawning locations around Iceland in 2002 and 2003, respectively (Figure 1). Each spawning location was identified with a three digit number, the first digit representing one of

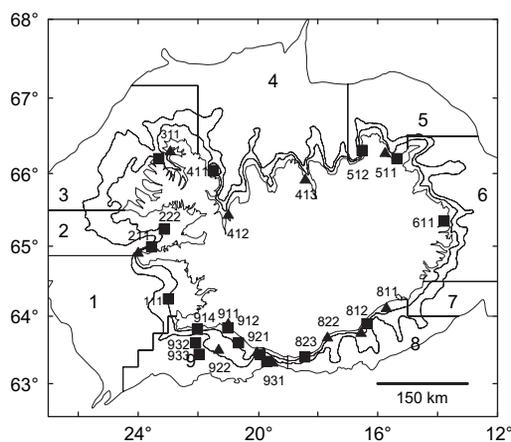


Figure 1. Sampling locations in spring 2002 (triangles) and 2003 (squares). Depth contours at 75, 125, and 500 m. Each spawning location was identified with a three digit number, the first representing one of the nine areas around Iceland, the second the depth interval (1, <75 m; 2, 75–125 m; 3, >125 m), and the last the station number.

nine regions, the second the depth interval (1, <75 m; 2, 75–125 m; and 3, >125 m), and the last the station number. Sampling was carried out from fishing boats using gillnets, handlines, or Danish seines. In all, 35–121 mature or spawning cod were sampled at each spawning location. At sea, the total length of all sampled cod was measured to the nearest cm, gutted and ungutted weights of the fish were recorded, and sex and maturity stage were determined macroscopically. Sagittal otoliths were carefully removed from each fish, cleaned of adhering tissue, and stored dry in paper envelopes until further analysis. The mean growth rate of each fish was calculated as the total length divided by the age.

Shape analysis

Otoliths from the left side of the fish were digitized using a microscope attached to an image analyser. Otoliths were orientated in a consistent manner, with the sulcus side up (magnification 3.6–4.8× depending on the size of the otolith). The area, length, width, perimeter, circularity, rectangularity, and 64 Fourier coefficients (based on an angle of 5.625°) of each otolith were measured using Optimas version 6.51. Circularity was defined as the perimeter of the otolith squared, divided by its area. Rectangularity was defined as the otolith area divided by the area of its minimum enclosing rectangle (a value of 1 would be a perfect square). The Fourier coefficients were calculated based on the centroid of the otolith instead of the nucleus, because [Campana and Casselman \(1993\)](#) found it to reduce the variability of the amplitudes. All otoliths were weighed to the nearest 0.1 mg. The remaining right otolith from each pair was sectioned and the age was determined.

Statistical analysis

All otolith variables and the first 10 Fourier amplitudes were used in the discriminant analyses. To remove the effect of otolith length, the amplitudes were standardized by dividing each by its mean radius. The mean radius of the otoliths was based on 64 radii measured for each otolith. All variables were tested for normality and homogeneity of variance, and transformed if necessary. Otolith weight was standardized by natural-log transformation, and circularity was transformed using $1/x$. Analysis of covariance (ANCOVA) was used to determine the effect of fish length on the magnitude of the otolith variables, with length as a covariate, and spawning location as a factor. Where the effect of fish length was significant, the product of fish length and the common within-group slope (b) from the ANCOVA for a given variable was subtracted from the variable to create a standardized variable. The standardized variables were natural-log-transformed otolith weight ($b = 1.518$), length ($b = 7.311$), width ($b = 0.0627$), perimeter ($b = 23.937$), and natural-log-transformed area ($b = 0.936$). Two-way analysis of variance (two-way ANOVA) with an interaction term (age × location) was used to investigate the effect of age and location on

length-at-age. Multivariate analysis of variance (MANOVA) for all variables combined was used to test for overall difference among spawning locations. When differences were detected, mean differences between spawning locations for individual variables were tested with ANOVA. Tukey HSD was then used to examine individual shape variables in order to explain any significant differences detected by the ANOVA. Forward stepwise canonical discriminant analysis of the standardized data was used to discriminate between the different spawning groups. Classification accuracy was estimated with “leave-one-out” cross-validation. Discriminant function analyses were restricted to common age groups for all spawning locations. As such, the common age distribution ranged from 5 to 10 years and 6 to 8 years in 2002 and 2003, respectively ([Figure 2](#)). For the temporal stability study, discriminant function analysis was carried out for seven spawning locations in two consecutive years. The common age distribution for these seven spawning locations ranged between 6 and 9 years.

Results

Length, weight, growth, length-at-age, and maturity

Total length of cod south and southwest of Iceland was in general greater than that of cod northwest, north, and east of Iceland ([Figure 2](#)). Cod south and southwest of Iceland were also generally heavier than cod northwest, north, and east of Iceland ([Figure 2](#)). Mean growth rate was generally greatest south and southwest of Iceland ([Figures 2, 3](#)). However, cod spawning at depths >125 m south of Iceland were smaller, lighter, and grew more slowly than cod spawning <125 m south of Iceland ([Figure 2](#)). The mean length-at-age of cod south of Iceland was greater than that of cod caught north of Iceland ([Figure 3](#)), and was greater than the overall mean length-at-age across all spawning locations. Mean length-at-age north and east of Iceland was less than the overall mean length-at-age ([Figure 3](#)). Differences in length-at-age were tested for age groups 6–8 years in both 2002 and 2003. The mean length-at-age for all three age groups was significantly different between spawning locations in both years (ANOVA, $p < 0.001$). At spawning locations south of Iceland, the age distribution was broader, and older cod contributed more to the spawning than at spawning locations west and north of Iceland ([Figure 2](#)). Most sampled cod were in spawning condition. At 23 spawning locations 90–100% of the cod were spawning, and at the other six locations, 77–89% were spawning. The remaining cod were maturing.

Discrimination among spawning groups

The overall otolith shape, using all standardized otolith variables, differed significantly between spawning locations (MANOVA, $p < 0.001$). The discriminant analyses

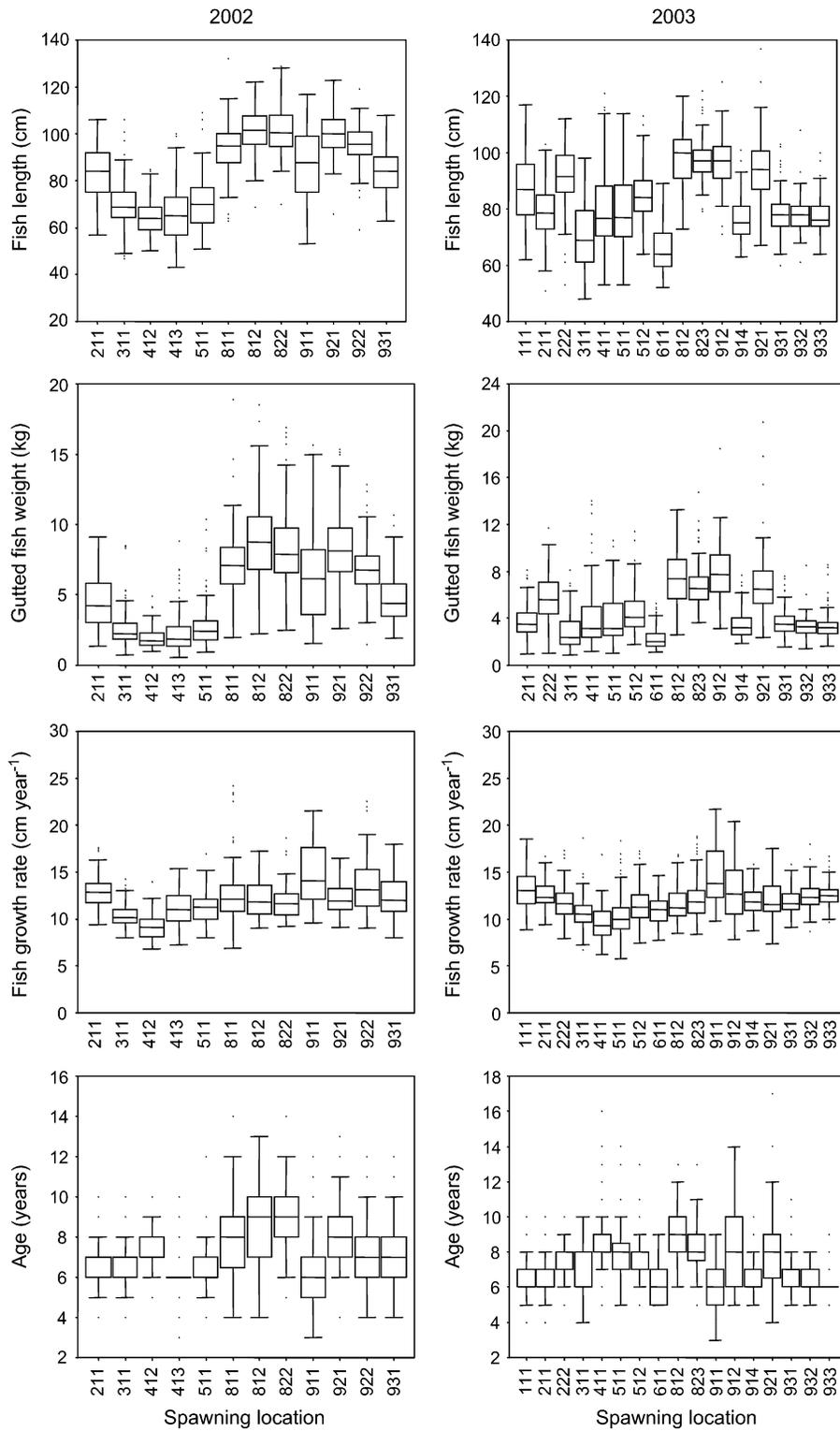


Figure 2. Medians, quartiles, and ranges of fish parameters (length, weight, growth rate, and age) for the different spawning locations in spring of 2002 and 2003. For locations see Figure 1.

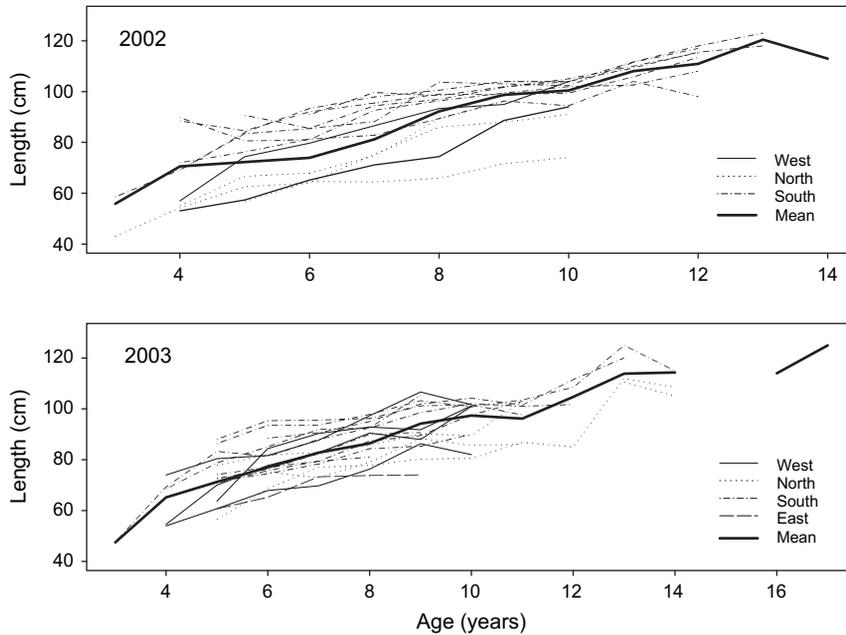


Figure 3. Mean length-at-age of cod at different spawning locations in spring of 2002 and 2003.

provided further evidence for a separation between spawning locations (Figure 4). The first discriminant function explained 78% and 48% of the variance in 2002 and 2003, respectively. In both years otolith area and length explained most of the variation in the first discriminant function (Table 1). The first function separated between cod northwest, north, and east of Iceland (regions 3, 4, 5, and 6) and cod spawning south and southwest of Iceland (regions 1, 2, 8, and 9; Figure 4). Discriminant scores of the first function were not significantly different among spawning locations shallower than 125 m south of Iceland (Tukey HSD, $p > 0.05$). Similarly, significant differences were not detected among the spawning locations north of Iceland (Tukey HSD, $p > 0.05$). The exceptions were locations 914 in 2003, which was not significantly different from the northern spawning

locations (Tukey HSD, $p > 0.05$), and 512, which was not significantly different from spawning locations 911 and 914 (Tukey HSD, $p > 0.05$). However, discriminant scores of the first function were significantly different between spawning locations north and south of Iceland (i.e. those that were < 125 m; Tukey HSD, $p < 0.001$). Significant differences were also detected between spawning locations deeper and shallower than 125 m south of Iceland (except between 911 and 914 in 2003; Tukey HSD, $p < 0.05$). The second discriminant function explained 17% and 34% of the variance for 2002 and 2003, respectively. In both years the variables explaining most of the variation in the second discriminant function were otolith weight and area (Table 1). The second function discriminated spawning locations south of Iceland > 125 m deep (locations 931, 932, and 933) from spawning

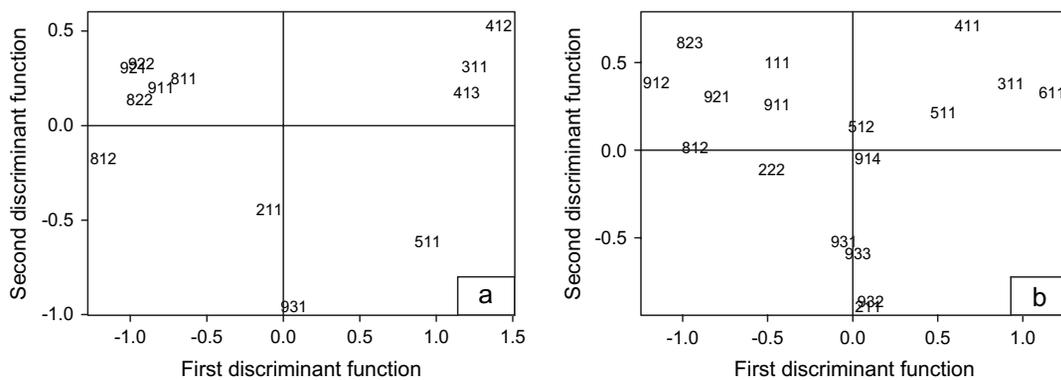


Figure 4. Mean values of the first two canonical variants based on otolith shape from (a) 12 spawning locations in spring 2002, and (b) 17 spawning locations in spring 2003. For locations see Figure 1.

Table 1. The first and second standardized function coefficients from the discriminant analysis for spawning groups in spring 2002, spring 2003, the 1995 cohort, and the temporal study (seven spawning locations in two consecutive years).

Parameter	Standardized canonical discriminant function coefficients							
	2002		2003		1995 cohort		Temporal study	
	I	II	I	II	I	II	I	II
Otolith weight	0.28	1.74	0.23	2.10	—	—	0.10	1.70
Otolith area	1.61	-1.33	1.82	-2.90	2.18	0.79	1.92	-1.47
Otolith length	-2.17	-0.04	-2.10	0.29	-2.40	-0.33	-2.35	0.23
Otolith perimeter	—	—	-0.26	1.14	—	—	—	—
Amplitude 1	-0.49	0.47	-0.39	-0.003	-0.36	0.56	-0.48	0.35
Amplitude 4	1.26	-0.03	1.10	-0.21	1.38	-0.47	1.52	-0.10
Amplitude 6	0.39	-0.02	0.55	0.002	0.50	0.79	0.30	0.18

locations <125 m (locations 911, 912, 914, 921, and 922; Figure 4). Significant differences were also detected between spawning locations west of Iceland. Cod spawning in regions 1 and 2 were significantly different from those spawning in region 3 (Tukey HSD, $p < 0.001$; Figure 4). Spawning location 211 was classified together with spawning locations >125 m south of Iceland (locations 931, 932, and 933). However, location 311 was grouped with the northern and eastern locations (Figure 4).

In 2002, the classification accuracy ranged between 0% and 44% (Table 2). The incorrectly classified cod at spawning locations south of Iceland were in most cases classified to other spawning locations south of Iceland. Fewer than 8% of these cod were classified to spawning locations north of Iceland, except for location 931, where 20% were classified

to spawning locations north of Iceland (Table 2). A slightly higher percentage (8–33%) of cod north of Iceland were classified to spawning locations south of Iceland (Table 2).

In 2003, the classification accuracy for the spawning locations ranged between 0% and 33% (Table 3). More than half the incorrectly classified cod were classified to adjacent spawning locations. As such, fewer than 39% of the cod from spawning locations north and northwest of Iceland (regions 3, 4, 5, and 6) were classified to spawning locations south of Iceland (regions 8 and 9). Similarly, fewer than 34% of cod from spawning locations south of Iceland (regions 8 and 9) were classified to spawning locations north of Iceland (regions 3, 4, 5, and 6).

To determine if year class was influencing the discrimination between cod north and south of Iceland,

Table 2. Classification success (%) from discriminant analysis between spawning locations in spring 2002. Emboldened numbers indicate classification success for correctly classified cod at each spawning location. n is the total number of cod at each location used in the discriminant analyses. Maturity is the proportion (%) of spawning cod at each location. Age groups 5–10 years. For locations see Figure 1.

Location	n	Maturity	Classification success (%)											
			West of Iceland		North of Iceland			South of Iceland						
			211	311	412	413	511	811	812	822	911	921	922	931
211	91	99	13	8	4	1	12	2	8	1	4	12	9	25
311	93	100	5	26	28	8	16	2	3	0	0	3	1	8
412	87	90	7	36	36	7	7	2	0	0	0	1	0	5
413	83	82	5	24	29	6	20	2	1	0	0	1	5	6
511	86	91	5	13	10	8	31	0	5	1	0	2	1	23
811	72	99	17	3	4	0	1	8	8	0	7	25	11	15
812	79	99	11	1	0	0	1	5	27	3	5	19	11	16
822	79	98	15	1	1	0	6	6	18	0	6	24	14	8
911	49	99	16	6	2	0	2	6	14	4	8	16	16	8
921	89	100	6	3	3	0	1	8	12	1	7	34	15	10
922	86	100	15	2	2	0	0	8	14	1	9	33	9	6
931	94	91	15	1	2	0	18	1	10	1	2	3	3	44

Table 3. Classification success (%) of discriminant analysis between spawning locations around Iceland in spring 2003. Emboldened numbers indicate classification success for correctly classified cod at each spawning location. *n* is the total number of cod at each location used in the discriminant analyses. Maturity is the proportion (%) of spawning cod at each location. Age groups 6–8 years. For locations see Figure 1.

Location	<i>n</i>	Maturity	Classification success (%)																
			West of Iceland				North of Iceland			East of Iceland			South of Iceland						
			111	211	222	311	411	511	512	611	812	823	911	912	914	921	931	932	933
111	73	99	15	1	7	7	7	3	8	1	0	15	1	5	3	3	16	5	1
211	80	100	0	21	13	3	3	0	1	1	0	3	0	1	5	0	20	29	1
222	69	100	6	9	17	7	4	6	6	0	1	6	0	7	6	4	10	9	1
311	72	94	6	11	6	15	21	1	7	14	0	0	0	0	1	0	10	8	0
411	60	99	8	5	5	20	20	5	8	8	2	0	0	2	8	0	7	2	0
511	65	77	9	12	8	12	6	3	3	8	2	3	0	2	8	0	12	3	0
512	77	100	10	6	10	9	12	4	6	3	0	5	1	3	6	1	12	9	1
611	57	100	4	4	4	25	16	5	9	19	0	0	0	0	0	0	5	11	0
812	42	99	10	5	21	2	2	2	5	2	7	14	0	10	0	2	2	14	0
823	53	100	17	2	9	4	2	0	4	0	4	25	0	9	2	4	15	4	0
911	15	94	27	7	13	0	0	0	0	0	0	13	0	0	7	0	20	0	13
912	26	81	27	8	19	4	0	0	8	0	8	8	0	15	4	0	0	0	0
914	67	99	3	12	16	10	6	4	10	3	1	7	1	3	6	0	6	6	3
921	54	100	7	4	15	0	6	0	9	0	2	22	0	6	9	0	11	7	2
931	81	91	6	7	6	5	0	2	7	6	2	1	0	0	2	1	33	19	0
932	84	85	2	23	5	0	0	2	6	4	0	2	0	0	8	0	25	20	2
933	54	84	4	22	17	0	4	4	4	4	0	2	0	4	7	0	9	19	2

a discriminant analysis using only the 1995 cohort (176 × 7-year-old cod in 2002, 137 × 8-year-old cod in 2003) was carried out. The first discriminant function separated between spawning locations south and north of Iceland, and the second function discriminated between cod spawning in shallow waters and those spawning deeper than 125 m (Figure 5). The first and second discriminant

functions explained 85% and 11% of the variance, respectively, and the variables explaining most of the variance were otolith area, length, and amplitude 7.

Temporal stability in otolith shape

All standardized otolith variables were temporally stable among years within locations 511, 812, and 921 (Figure 6). Standardized perimeter did not show significant differences among years at any location (ANOVA, $p > 0.05$). Standardized weight was significantly different at location 911, standardized area and standardized width were significantly different at location 211, standardized weight and standardized width at location 311, and standardized length at location 931 (ANOVA, $p < 0.05$; Figure 6).

As with the single-year discriminant analysis, the discriminant tests for the seven spawning locations in the two consecutive years effectively discriminated among the northern and the southern locations (Figure 7). The first discriminant function explained 63% of the variance, and the variable explaining most of the variance was otolith length. The discriminant scores from the first discriminant function were significantly different among locations (ANOVA, $p < 0.001$; Figure 7). However, there was no significant difference among years using scores from the first discriminant function (Tukey HSD, $p > 0.05$). The second

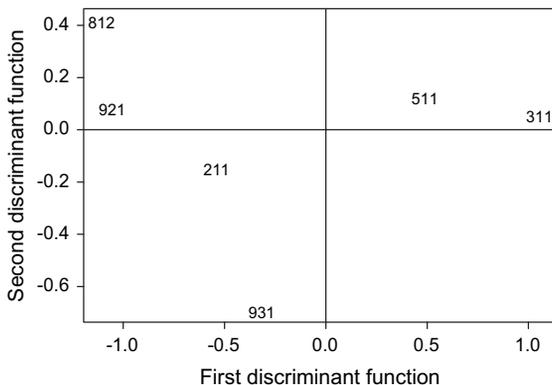


Figure 5. Mean values of the first two canonical variants based on otolith shape of the 1995 year class (combined 7-year-olds in 2002, and 8-year-olds in 2003) from six spawning locations. For locations see Figure 1.

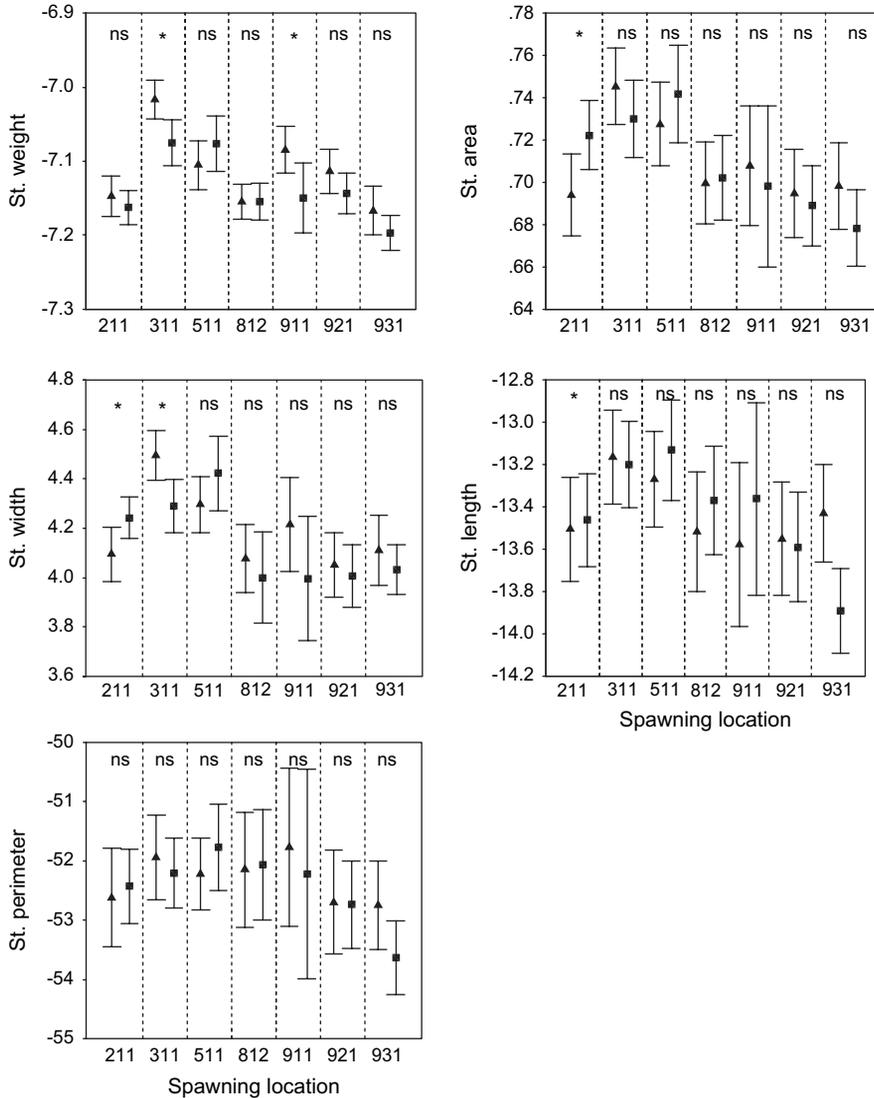


Figure 6. Means, 95% confidence limits, and significance level for standardized otolith weight, area, length, width, and perimeter at seven spawning locations around Iceland in spring 2002 (triangles) and 2003 (squares). Age groups 6–9 years. For locations see Figure 1. The significance test indicates the level of difference among years within a spawning location (n.s., not significant; $0.05 > p > 0.001$).

discriminant function explained 28% of the variance, and the variable explaining most of the variance was otolith weight. One location was significantly different among years, location 211 (Tukey HSD, $p < 0.001$), using scores from the second discriminant function.

Discussion

Increasing numbers of studies exploring small-scale structure of cod populations have provided evidence for the existence of local populations (Ruzzante et al., 1999, 2000; Chouinard and Swain, 2002; Knutsen et al., 2003; Olsen

et al., 2004; Salvanes et al., 2004; Neat et al., 2006). In Iceland, all cod were thought to belong to a single management unit (Schopka, 1994). However, recent studies on genetic (Jónsdóttir et al., 2002) and life history variations (Marteinsdottir et al., 2000a, b; Begg and Marteinsdottir, 2000) have indicated the existence of local cod populations. In this study, cod were successfully discriminated into distinct spawning groups residing north and south of Iceland. Although the spawning locations were seemingly well separated in discriminant space, correct classification to spawning locations was low, only once exceeding 40%. Nevertheless, incorrectly classified cod were usually classified to a nearby spawning location, and a high percentage of

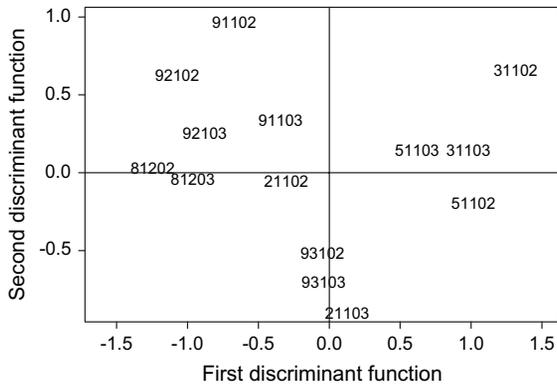


Figure 7. Mean values of the first two canonical variants based on otolith shape variables from seven spawning locations in two consecutive years, 2002 and 2003. The first three digits indicate location and the last two years (02 = 2002, 03 = 2003). Age groups 6–9 years. For locations see Figure 1.

cod south of Iceland were classified to spawning locations south of Iceland; similarly, a high percentage of cod north of Iceland were classified to spawning locations north of Iceland. Even though there have been past suggestions of substructure within the Icelandic cod stock (Marteinsdottir *et al.*, 2000a; Jónsdóttir *et al.*, 2002; Petursdottir *et al.*, 2006), the present study is one of the first to study spawning cod throughout Icelandic waters and to suggest the presence of at least two separate populations.

In addition to the two major groups of cod north and south of Iceland, depth may separate Icelandic cod into additional groups (Figure 6). There was a clear separation between cod spawning shallower and deeper than 125 m at locations south of Iceland. Offshore and inshore populations have been reported for cod off Norway (Sarvas and Fevolden, 2005), Greenland (Storr-Paulsen *et al.*, 2004), and Newfoundland (Ruzzante *et al.*, 1996). In the present study, cod spawning at different depths south of Iceland may undergo different migration patterns. A recent tagging study indicated that, when feeding, cod spend most of their time either shallower or deeper than 200 m, and therefore followed either deep-water or shallow-water migration patterns (Pálsson and Thorsteinsson, 2003). Cod spawning deeper than 125 m are likely to follow the deep-water migration pattern, and cod spawning shallower than this follow the shallow-water migration (V. Thorsteinsson, pers. comm.). Moreover, genetic differences (*Pan I*) have been found among spawning cod at locations 931 and 911 (Jónsdóttir *et al.*, 2002), so these two depth groups are unlikely to intermix during spawning seasons or feeding seasons.

Spawning locations at the two adjacent regions west of Iceland (regions 2 and 3) did not group together in discriminant space. These locations are characterized by large variations in the environment. A flux of Atlantic water flows as the Irminger Current up towards the south coast of Iceland, follows the bottom contours along the west coast,

and splits into two branches west of Iceland's Northwest Peninsula (Stefansson, 1962). The largest part of the Irminger Current flows west towards Greenland, but a smaller branch continues around the Northwest Peninsula onto the shelf north of Iceland. The East Greenland Current, which originates in the Arctic Ocean, transports cold water south along the Greenland coast. Northwest of Iceland it splits into two branches, one flowing onto the shelf north of Iceland, the other flowing south between Iceland and Greenland. There the latter branch meets the warm Atlantic water flowing north and creates a frontal zone west of Iceland. The frontal zone is determined by the strength of the Irminger Current (Stefansson, 1962; Jónsson and Valdimarsson, 2005). Living conditions for cod west of Iceland are greatly influenced by these two currents and the frontal zone created where the two currents meet. The frontal zone is located west of Iceland's Northwest Peninsula at latitudes between regions 2 and 3. As a result, conditions at location 311 are more similar to those of the northern area, whereas locations 211 and 222 are more similar to those of the southern area, so explaining the discriminant analysis results.

Most studies using otolith shape to discriminate among fish stocks have dealt with groups of fish of similar size (Campana and Casselman, 1993; DeVries *et al.*, 2002; Smith *et al.*, 2002). The extensive growth and size differences among spawning groups seen in the present study are unusual. As a result, the size of the spawning cod varied substantially among the different areas. Most of the discrimination among groups was based on otolith size parameters (weight, area, and length), and the Fourier amplitudes explained a considerably smaller part of the variation. However, the amplitudes by themselves did provide acceptable discrimination between the two areas. As several year classes were used for the discrimination in the present study, the presence of these different year classes might conceivably have influenced the discrimination among the spawning groups. Although age- and year-class effects have been suggested to influence discrimination among stocks (Castonguay *et al.*, 1991), this is not always accounted for in recent studies, sometimes through lack of information on fish age (Stransky and MacLellan, 2005). In the present study, discrimination between the northern and the southern areas was also successful when only a single year class was included in the analysis. Moreover, when year class was used as a variable in the discriminant analysis together with otolith variables, year class explained the smallest part of the variation of the first discriminant function in 2002, and was among the least important variables in 2003. Therefore, year class is not likely to be the main factor responsible for the otolith-based discrimination among spawning groups north and south of Iceland.

The small fish length and weight and slow growth rate of cod north of Iceland compared with cod in the south can to some extent be explained by the lower seawater temperature north of Iceland (Malmberg and Valdimarsson, 2003). Temperature's influence on fish growth rate is well

documented (Brander, 2000; Björnsson *et al.*, 2001). The discrimination between cod at different spawning locations is also likely to be influenced by environmental factors experienced outside the spawning locations. Most Icelandic cod only remain on the spawning grounds while spawning, after which they migrate to the main feeding grounds north-west and east of Iceland (Jónsson, 1996; Thorsteinsson and Marteinsdóttir, 1998). Tag-recapture studies from the middle of the 20th century indicated that most Icelandic cod migrated to the main spawning grounds south of Iceland to spawn (Jónsson, 1954). Since then it has generally been believed that most Icelandic cod spawn in waters south and southwest of Iceland. The tag-recapture studies also indicated that after spawning, cod migrated back to the feeding areas in the north and northwest, taking a route either west or east of the country (Jónsson, 1950). However, the studies reported by Jónsson (1954, 1996) indicated that only a few cod from the main spawning grounds appeared to migrate all the way into northern waters (i.e. north of the Horn located at latitude 66°30'N). Jónsson's results showed that of the 2000 cod tagged on the southern spawning grounds, only six were recaptured in the northern waters, while most (or 297 individuals, 95% of the recaptures) were recaptured on the feeding grounds west and northwest of the country (Jónsson, 1996). The tagging studies also suggested some local spawning north of Iceland, as no cod from some of the spawning locations north of Iceland were recaptured south of Iceland (Jónsson, 1996). Therefore, although the results of Jónsson (1950, 1954, 1996) have not been previously interpreted in such a way, they do indeed indicate a certain level of separation between spawners in the north and the south.

More recent results based on tagging experiments focusing on spawning cod in areas around Iceland from 1992 to 2004 have also shown that cod tagged in the south do not migrate the whole way to the northern feeding areas, instead either staying in the warm southern waters or migrating towards the frontal zones west and east of Iceland (V. Thorsteinsson, unpublished). Similarly, cod that spawn in the north have a greater tendency to stay in the northern waters throughout the year, although some of them do migrate into southern waters (V. Thorsteinsson, unpublished). Consequently, the cod that spawn north and south of the country appear to display different migration patterns and may belong to different life history groups. The distinct differences between cod from the southern and northern spawning grounds based on both fish and otolith shape parameters in the present study support this view. Therefore, it is possible that cod spawning north and south of Iceland do not intermix outside the spawning season, or if they do they would only intermix at the feeding sites west and east of Iceland, i.e. where cod from both areas have been recaptured (Jónsson, 1996). As such, it is likely that cod in Icelandic waters may consist of more than one stock or management unit, separated by geographical distance as well as different

environmental conditions shaped by the warm southern and cold northern currents that meet in the areas west and east of Iceland. If this is the case, cod in region 2, located in the frontal zone, may be representative of a mixing zone, but cod from this region did not group well with either northern or southern spawners.

Although only two sampling years were available, the results indicate temporal stability of otolith shape within a location in the two consecutive years. Therefore, random mixing across spawning locations is unlikely. Cod spawning at one spawning location are therefore likely to return to that same spawning location in subsequent years. In the temporal stability study, a significant difference was found among spawning locations, but only one spawning location (211) showed significant differences between the two years. Nonetheless, some differences in individual otolith parameters were found among years at four of the locations (211, 311, 911, and 931). Environmental factors are known to influence otolith shape (Campana and Casselman, 1993; Cardinale *et al.*, 2004). Seawater temperature can vary substantially among years within the same region (Malmberg and Valdimarsson, 2003). As described earlier, two of the locations (211 and 311) are located at a frontal zone west of Iceland characterized by variation in environmental conditions attributable to the variable strength of the Irminger Current (Stefansson, 1962; Jónsson and Valdimarsson, 2005). In 1997 there was a stronger flow of the Irminger Current onto the shelf north of Iceland than in 1994 and 1995 (Jónsson and Valdimarsson, 2005). The age groups (6–9 years) chosen for the analysis here include different year classes (1993–1996 and 1994–1997 for 2002 and 2003, respectively). Temperature differences experienced by the different year classes may have caused the minor variation in both fish and otolith parameters among years at these spawning locations. Nevertheless, as the difference among locations was greater than the variation observed within a location, it is likely that cod spawning in the different areas remained separate during their lifetime.

The results of this study provide evidence for the existence of a local population structure within the Icelandic cod stock. Although otolith shape does not confirm the existence of genetically distinct populations, it does reflect life history and phenotypic difference among spawning components. For the purpose of management, concise information on the population structure of Icelandic cod is vital. Today, the Icelandic cod stock is managed as a single unit. Results of this study indicate that this management procedure should be reconsidered, and that further studies on the population structure employing multidisciplinary tools of discrimination (genetics, elemental fingerprints, parasites, tagging) should be commenced.

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References

- Árnason, E. 2004. Mitochondrial cytochrome *b* DNA variation in the high-fecundity Atlantic cod: trans-Atlantic clines and shallow gene genealogy. *Genetics*, 166: 1871–1885.
- Árnason, E., Pálsson, S., and Arason, A. 1992. Gene flow and lack of population differentiation in Atlantic cod, *Gadus morhua* L., from Iceland, and comparison of cod from Norway and Newfoundland. *Journal of Fish Biology*, 40: 751–770.
- Árnason, E., and Rand, D. M. 1992. Heteroplasmy of short tandem repeats in mitochondrial DNA of Atlantic cod, *Gadus morhua*. *Genetics*, 132: 211–220.
- Begg, G. A., and Brown, R. W. 2000. Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. *Transactions of the American Fisheries Society*, 129: 935–945.
- Begg, G. A., and Marteinsdottir, G. 2000. Spawning origins of pelagic juvenile cod *Gadus morhua* inferred from spatially explicit age distributions: potential influences on year-class strength and recruitment. *Marine Ecology Progress Series*, 202: 193–217.
- Begg, G. A., and Waldman, J. R. 1999. An holistic approach to fish stock identification. *Fisheries Research*, 43: 35–44.
- Bentzen, P., Taggart, C. T., Ruzzante, D. E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences*, 53: 2706–2721.
- Bird, J. L., Eppler, D. T., and Checkley, D. M. 1986. Comparisons of herring otoliths using Fourier series shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 43: 1228–1234.
- Björnsson, B., Steinarsson, A., and Oddgeirsson, M. 2001. Optimal temperature for growth and feed conversion of immature cod (*Gadus morhua* L.). *ICES Journal of Marine Science*, 58: 29–38.
- Brander, K. 2000. Effects on environmental variability on growth and recruitment in cod (*Gadus morhua*) using a comparative approach. *Oceanologica Acta*, 23: 485–496.
- Campana, S. E., and Casselman, J. M. 1993. Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 50: 1062–1083.
- Campana, S. E., Gagné, J. A., and McLaren, J. W. 1995. Elemental fingerprinting of fish otoliths using ID–ICPMS. *Marine Ecology Progress Series*, 122: 115–120.
- Campana, S. E., and Neilson, J. D. 1985. Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, 42: 1014–1032.
- Cardinale, M., Doering-Arjes, P., Kastowsky, M., and Mosegaard, H. 2004. Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, 61: 158–167.
- Castonguay, M., Simard, P., and Gagnon, P. 1991. Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. *Canadian Journal of Fisheries and Aquatic Sciences*, 48: 296–302.
- Chouinard, G. A., and Swain, D. P. 2002. Depth-dependent variation in condition and length-at-age of Atlantic cod (*Gadus morhua*) in the southern Gulf of St Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, 59: 1451–1459.
- DeVries, D. A., Grimes, C. B., and Prager, M. H. 2002. Using otolith shape analysis to distinguish eastern Gulf of Mexico and Atlantic Ocean stocks of king mackerel. *Fisheries Research*, 57: 51–62.
- Fevolden, S. E., and Pogson, G. H. 1997. Genetic divergence at the synaptophysin (*Syp* I) locus among Norwegian coastal and northeast Arctic populations of Atlantic cod. *Journal of Fish Biology*, 51: 895–908.
- Garrod, D. J. 1977. The North Atlantic cod. *In* *Fish Population Dynamics*, pp. 216–242. Ed. by J. A. Gulland. John Wiley, London.
- Jónsdóttir, Ó. D. B., Imsland, A. K., Daniélsdóttir, A. K., and Marteinsdottir, G. 2002. Genetic heterogeneity and growth properties of different genotypes of Atlantic cod (*Gadus morhua* L.) at two spawning sites off south Iceland. *Fisheries Research*, 55: 37–47.
- Jónsson, E. 1982. A survey of spawning and reproduction of the Icelandic cod. *Rit Fiskideildar*, 6(2): 1–45.
- Jónsson, J. 1950. Enn um þorskermerkingar 1948. *Ægir*, 43: 27–31 (in Icelandic).
- Jónsson, J. 1954. Göngur íslenska þorskins. *Ægir*, 47: 2–9 (in Icelandic).
- Jónsson, J. 1996. Tagging of cod (*Gadus morhua*) in Icelandic waters 1948–1986. *Rit Fiskideildar*, 14: 1–82.
- Jónsson, S., and Valdimarsson, H. 2005. Flow of Atlantic water to the North Icelandic shelf in relation to drift of cod larvae. *ICES Journal of Marine Science*, 62: 1350–1359.
- Karlsson, S., and Mork, J. 2003. Selection-induced variation at the pantophysin locus (*Pan* I) in a Norwegian fjord population of cod (*Gadus morhua* L.). *Molecular Ecology*, 12: 3265–3274.
- Knutsen, H., Jorde, P. E., André, C., and Stenseth, N. C. 2003. Fine-scaled geographical population structuring in highly mobile marine species: the Atlantic cod. *Molecular Ecology*, 12: 385–394.
- Malmberg, S.-A., and Valdimarsson, H. 2003. Hydrographic conditions in Icelandic waters, 1990–1999. *ICES Marine Science Symposia*, 219: 50–60.
- Marteinsdottir, G., Gunnarsson, B., and Suthers, I. M. 2000a. Spatial variation in hatch date distributions and origin of pelagic juvenile cod in Icelandic waters. *ICES Journal of Marine Science*, 57: 1182–1195.
- Marteinsdottir, G., Guðmundsdóttir, A., Þorsteinsson, V., and Stefánsson, G. 2000b. Spatial variation in abundance, size composition and viable egg production of spawning cod (*Gadus morhua* L.) in Icelandic waters. *ICES Journal of Marine Science*, 57: 824–883.
- Neat, F. C., Wright, P. J., Zuur, A. F., Gibb, I. M., Gibb, F. M., Tulett, D., Righton, D. A., and Turner, R. J. 2006. Residency and depth movements of a coastal group of Atlantic cod (*Gadus morhua* L.). *Marine Biology*, 148: 643–654.
- Nielsen, E. E., Hansen, M. M., Schmidt, C., Meldrup, D., and Gronkjaer, P. 2001. Fisheries – population of origin of Atlantic cod. *Nature*, 413: 272.
- Olsen, E. M., Knutsen, H., Gjosaeter, J., Jorde, P. E., Knutsen, J. A., and Stenseth, N. C. 2004. Life-history variation among local populations of Atlantic cod from the Norwegian Skagerrak coast. *Journal of Fish Biology*, 64: 1725–1730.
- Pálsson, Ó. K., and Þorsteinsson, V. 2003. Migration patterns, ambient temperature, and growth of Icelandic cod (*Gadus morhua*): evidence from storage tag data. *Canadian Journal of Fisheries and Aquatic Sciences*, 60: 1409–1423.
- Petursdottir, G., Begg, G. A., and Marteinsdottir, G. 2006. Discrimination between Icelandic cod (*Gadus morhua* L.) populations from adjacent spawning areas based on otolith growth and shape. *Fisheries Research*, doi: 10.1016/j.fishres.2006.05.002.

- Pogson, G. H., Mesa, K. A., and Boutilier, R. G. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. *Genetics*, 139: 375–385.
- Ruzzante, D. E., Taggart, C. T., and Cook, D. 1999. A review of the evidence for genetic structure of cod (*Gadus morhua*) in the Northwest Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St Lawrence. *Fisheries Research*, 43: 79–97.
- Ruzzante, D. E., Taggart, C. T., Cook, D., and Goddard, S. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. *Canadian Journal of Fisheries and Aquatic Sciences*, 53: 634–645.
- Ruzzante, D. E., Wroblewski, J. S., Taggart, C. T., Smedbol, R. K., Cook, D., and Goddard, S. V. 2000. Bay-scale population structure in coastal Atlantic cod in Labrador and Newfoundland, Canada. *Journal of Fish Biology*, 56: 431–447.
- Saemundsson, B. 1926. *Fiskarnir (Pisces Islandiae)*. Bókaverslun Sigfúsar Eymundssonar, Reykjavík, Iceland. 579 pp.
- Saemundsson, K. 2005. Geographical distribution and dispersal of juvenile Icelandic cod (*Gadus morhua*). MSc thesis, University of Iceland. 118 pp.
- Salvanes, A. G. V., Skjaeraasen, J. E., and Nilsen, T. 2004. Subpopulations of coastal cod with different behaviour and life-history strategies. *Marine Ecology Progress Series*, 267: 241–251.
- Sarvas, T. H., and Fevolden, S. E. 2005. The scnDNA locus *Pan I* reveals concurrent presence of different populations of Atlantic cod (*Gadus morhua* L.) within a single fjord. *Fisheries Research*, 76: 307–316.
- Schopka, S. A. 1994. Fluctuations in the cod stock off Iceland during the twentieth century in relation to changes in the fisheries and environment. *ICES Marine Science Symposia*, 198: 175–193.
- Smith, P. J., Robertson, S. G., Horn, P. L., Bull, B., Anderson, O. F., Stanton, B. R., and Oke, C. S. 2002. Multiple techniques for determining stock relationships between orange roughy, *Hoplostethus atlanticus*, fisheries in the eastern Tasman Sea. *Fisheries Research*, 58: 119–140.
- Stefansson, U. 1962. North Icelandic waters. *Rit Fiskideildar*, 3: 1–269.
- Storr-Paulsen, M., Wieland, K., Hovgård, H., and Rätz, H-J. 2004. Stock structure of Atlantic cod (*Gadus morhua*) in West Greenland waters: implications of transport and migration. *ICES Journal of Marine Science*, 61: 972–982.
- Stransky, C., and MacLellan, S. E. 2005. Species separation and zoogeography of redfish and rockfish (genus *Sebastes*) by otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 62: 2265–2276.
- Thorsteinsson, V., Björnsson, H., Sólmundsson, J., and Eggertsson, G. I. 1998. Þorskurinn í Breiðafirði. *Ægir*, 91: 22–30 (in Icelandic).
- Thorsteinsson, V., and Marteinsdóttir, G. 1993. Þorskerkingar. *Ægir*, 86: 92–100 (in Icelandic).
- Thorsteinsson, V., and Marteinsdóttir, G. 1998. Size specific time and duration of spawning cod (*Gadus morhua*) in Icelandic waters. *ICES Document CM 1998/DD: 5*. 18 pp.