Otolith shape differences between ecotypes of Icelandic cod (*Gadus morhua*) with known migratory behaviour inferred from Data Storage Tags.

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1 Introduction

Knowledge of structure and dynamics of populations is essential for successful management of harvested stocks. Mismatches between simple management units (e.g. assuming panmixia) and the actual ecological complexity including population richness's of harvested stocks can have serious consequences and lead to unequal exploitation of stock sub-units as well as erroneous estimations of stock sizes (Stephenson, 1999; Sterner, 2007; Marteinsdóttir and Pardoe, 2008; Reiss *et al.*, 2009). Indeed, many commercially harvested fish stocks consist of smaller sub-units, with more complex, and diverse life history strategies than their management scheme implies (Begg and Marteinsdottir, 2000; Smedbol and Stephenson, 2001; Wright *et al.*, 2006; Kell *et al.*, 2009). Important life history parameters that may differ between sub-units include properties such as age-at-maturation, growth rate, condition, survival, abundance and distribution. All of which are important for accurate stock assessment and successful management (Begg *et al.*, 1999).

Otolith shape has proven to be an efficacious discrimination tool between stock units. Fish otoliths are species-specific in shape and their metabolically inert nature makes them robust to short-term changes, thus especially useful in morphometric analyses (Campana and Casselman, 1993; Begg *et al.*, 2005). Both genetic and environmental factors affect otolith shape, but the latter is thought to have more effects (Campana and Casselman, 1993; Lombarte and Lleonart, 1993). That both genes and the environment act on otolith shape has been confirmed on Atlantic cod in the Faroes where otolith shape has been used to separate cods at the Faroe Bank from cod on the Faroe Plateau (Cardinale *et al.*, 2004). Furthermore, otolith shape differs between dispersed and resident components of winter-spawning Celtic stock of Atlantic herring (*Clupea harengus*) (Burke *et al.*, 2008), between stock components of the Horse mackerel (*Trachurus trachurus L.*) in the North-East Atlantic and Mediterranean (Stransky *et al.*, 2008b), and between spawning populations of the Atlantic cod in the North-Sea, and West of Scotland (Galley *et al.*, 2006) as well as between the Northeast Arctic cod and Norwegian Coastal cod in Norway (Stransky *et al.*, 2008a).

Atlantic cod is characterized by a great population richness, with distinct sub-stocks exhibiting varying degrees of migratory behavior (Robichaud and Rose, 2004, and references therein). Evidence of different migratory behaviors among the Icelandic cod population were first revealed by conventional tagging (Jónsson, 1996), and later confirmed by Data Storage Tags (DSTs) which record the ambient temperature and depth (pressure) history of the fish (Pálsson and Thorsteinsson, 2003; Thorsteinsson and Saemundsson, 2006). The results from the DSTs revealed that the Icelandic cod stock consists of two ecotypes including individuals that stay at shallow depths all year round, identified as coastal cod, and also fish that migrate during winter feeding season towards deeper areas of the continental shelf. The thermal profiles from the DSTs showed that these latter individuals migrate towards areas characterized by temperature fronts where mixing of the cold Arctic and warmer Atlantic water masses occurs (Malmberg and Kristmannsson, 1992; Valdimarsson and Malmberg, 1999). The deep migrating type has been named frontal cod due to this behavior.

The main objective of this study was to relate otolith shape to the variation in the migrating behavior of cod in Iceland. Some success of using otolith shape as a discriminator between spawning groups of cod in Iceland has already been shown (Jonsdottir *et al.*, 2006; Petursdottir *et al.*, 2006). Genetic differences at the pantophysin I locus (*Pan* I) have also been revealed with individuals carrying the *Pan* I^{AA} genotype being more often shallow and fish carrying the *Pan* I^{BB} genotype being mostly of the deep migratory type (Pampoulie *et al.*, 2006; Pampoulie *et al.*, 2008; Arnason *et al.*, 2009; Grabowski *et al.*, 2011). The genetic difference was revealed by analysis on the same DSTs cod as is used in this study and here the otoliths will be used for shape analysis, applying two-dimensional shape characteristics as well as Fast Fourier Transforms of the otolith outline. The differentiating power of the otolith shape for the behavior types will be examined with a discriminant analysis applying Generalized Linear Models (GLM).

2 Materials and methods

2.1 Data Storage Tags

The electronic data storage tags (DSTs) used in the present analysis come from annual spring tagging experiments, conducted in Icelandic coastal waters from 2002 to 2005 by the Marine Research Institute (MRI) of Iceland, with tags manufactured by Star-Oddi, Reykjavík, Iceland (Pálsson and Thorsteinsson, 2003; Thorsteinsson and Saemundsson, 2006). Two different types of tags, differing mainly in their storage capacity, were employed. DST-Milli had a capacity of 10,000 to 21,000 records of depth and temperature and DST-Centi 130,000 records. The tags were 13mm x 39.4mm and weigh 9.2g (5g in water). All DST-Centi were programmed to measure depth and temperature every 10 min while the DST-Milli were set to record either at 10 min or 6 hour interval with the aim of extending the time at liberty. The DSTs were surgically attached in the peritoneal cavity (for information on method see Website: http://www.hafro.is/catag/b-fish_tags_tagging/b13-methods_section/b1302-conv-double-cod.html). V. Thorsteinsson with license No. 0304-1901, issued by the Icelandic Committee for Welfare of Experimental Animals from the Chief Veterinary Office at Ministry of Agriculture, Reykjavík, Iceland, carried out all surgical operations required for tagging.

2.2 Behavior assignment

Of all recaptured DSTs, 423 tags included successfully recorded profiles containing recordings of three parameters: temperature (°C), depth (meters), and time (date and time of day). From these we calculated three values that were used to classify the cod according to behavior. These were daily range of temperature, daily range of depth, and daily mean depth. Temperature daily means were not exploited as that might produce undesired separation of fish that resided in the northern cold versus southern warm waters rather than by behavior. According to prior analysis of the DST profiles, the main difference in behavior found within the cod stock in Iceland takes place during the feeding season (June-January) (Pálsson and Thorsteinsson, 2003; Thorsteinsson and Saemundsson, 2006). Fish with DST profiles that lasted at least from June to September (203 out of the initial 423) were used in the analyses. This was done to ensure that the migrating behavior would be captured in the DST data assuming that all migration towards feeding areas is finished before the end of this time frame. To simplify the large datasets, twelve 5.0m bins for range in daily depth (<1m, 1-5m, 5-10m, ... 45-50m, >50m), nine 0.5°C bins for the range in daily temperature (<0.5°C, 0.5-1.0°C ... 3.5-4.0°C, >4.0°C), and twelve mean depth bins (<20m, 20-40m, 40-80m, … 360-400m, >400m), were assigned. Each bin is given a number from 1-12 for the depth bins, and 1-9 for the temperature bins. Based on these the proportion of days spent at each bin was calculated. As a result, each individual gets assigned three numbers, one for temperature and two for depth; e.g. if the daily temperature range of an individual is 30% at <0.5°C, 30% at 0.5-1.0°C and 40% >4.0°C than the temperature number is calculated as: $0.3\times1+0.3\times2+0.4\times9 = 4.5$. Calculation of these numbers was carried out for each month, yielding a total of 33 estimates per year (12+12+9). The values were there after used in a Cluster Analysis (CA) with Euclidian distance measures and the Ward clustering method (Legendre and Legendre, 2012). The choice of bins for the data follows the one applied by Grabowski et al (2011), however, here all visual, *a priori* assignment of behavior was avoided, therefore removing all subjectivity in the approach. The difference between groups based on the CA was tested with ANOVA, with groups as the categorical variable, and absolute depth, and depth and temperature range as the response variable. Tukey HSD test was then applied to get *a posteriori* comparison between the groups, as the resulting groups from the CA were more than two.

2.3 Otolith morphology

High contrast, calibrated otolith images were captured with a microscope at 0.63x magnification linked to an 8 bit MediaCybernetics® PL-A662 digital camera powered by PixeLINK™ resulting in a dark two-dimensional otolith shape on a bright background. The right otolith was chosen and placed with the rostrum in the lower left corner. When only the left otolith was available, its image was flipped and transformed using standard image editing tools. Out of the 203 individuals that were at liberty long enough for their behavior to be analyzed, only 56 individuals had whole otoliths fit for the analysis. In an effort to increase the sample size, a method was developed by the authors, which involved gluing otoliths that had been broken by hand for aging (Bardarson *et al.*, 2014). Using this method the sample size was increased to 86 individuals. Four measurements were taken on each otolith from the two-dimensional photos using ImageJ™ software: length, width, area, and perimeter. From these measurements additional four shape variables (roundness, aspect ratio, circularity and rectangularity) were calculated using previously described methods (Russ, 1990; Tuset *et al.*, 2003, Table I). Finally, the outline of each otolith was automatically traced by ImageJ™ software using pixel gradient of the otolith silhouette. Two hundred and fifty six equidistant points, given as (x,y) coordinates, were extracted from the outline of each otolith using the tip of the rostrum as a common starting point. These were treated as complex numbers x+iy and subjected to a Cartesian fast Fourier Transformation to obtain 256 Fourier Descriptors (FDs) (Lestrel, 2008). The FDs were standardized for rotation and size of the otolith by setting the 0th FD to zero and by dividing all the FDs with the first FD, respectively. The FDs were then transformed by converting the complex numbers into absolute values (modulus of the complex numbers), resulting in 256 harmonic numbers (Smith *et al.*, 2002). The FDs have the quality that the more descriptors used to describe the shape, the more closely it resembles the original shape. However, in a discriminant analysis it is appropriate to reduce the total number of FDs to an adequate amount that can explain enough of the variation. The minimum number of FDs needed to capture at least 90% of the shape variation in our study was calculated with the same procedure as described in Smith *et al.* (2002)

2.4 Discriminant analyses

All otolith parameters were tested for normality before included in the analyses. Since not all fish were recorded for length, and in some cases length measurements by fishermen could be contested, all variables were tested for otolith length correlation instead of the more commonly applied fish length correlation. Using otolith length might even be more appropriate given the fact that otolith length is unaffected by inter-sample differences in preservation, shrinkage and distortion (Campana and Casselman, 1993). Normalization was conducted with a procedure that uses a theoretically derived method based on the equations of allometric growth described by Lleonart *et al.* (2000). The method is based on the allometric power equation: $Y = aX^b$, where a and b are both constants, and X is the size measure (here otolith length). The important difference between using this equation and the widely used approach of linear equation, Y = A +BX, is that in the latter case the independent term A has no sense in morphometrics where at X = 0, Y must be 0 (Lleonart *et al.*, 2000). Since otolith length is used here as a normalizing agent it will not be used in the discriminant analysis. The effect of age on the shape parameters was also analyzed using ANCOVA. Generalized Linear models (GLMs) were used to explore the difference between the two extreme groups from the behavior assignment results (Cluster Analyses) with binomial errors and a logit link function where behavior was treated as a binary response variable i.e. either coastal or frontal (Quinn and Keough., 2002). The significance of variables was evaluated by comparing all subsets of models and the best fitting one chosen with the Akaike Information Criterion (AIC). The best fitting GLM was then used to predict the behavior of all individuals based on their otolith shape, and the success estimated with correct classifications.

3 Results

3.1 Assignment of Ecotypes

Two main groups of behavior were identified with the CA (Figure 1). The main difference between these groups is linked to the depth measurements (depth range and mean depth) with one group being in shallow inshore waters, the other in deep offshore waters (Figure 2). Both of these groups could also be further divided into two intermediate subgroups (Figure 1). These groups will be identified hereafter as coastal, intermediate coastal, intermediate frontal, and frontal, respectively based on their placement along a depth gradient from the shallowest to the deepest depth occupied, as well as to keep up with the nomenclature of former analysis of these DST data (Figure 2). The inshore group was divided into subgroups that differed mainly in the depth they resided, but not the temperature range (Figure 2). The coastal type rarely migrated deeper than 100m while the intermediate coastal occupied depths between 100 and 150m. The temperature profiles of both inshore types display a graduate rise in temperature reaching a maximum in late summer before cooling off again reaching a minimum late winter (Figure 3, red lines). The frontal group differed from the others by both temperature and depth. The intermediate frontal type stayed between 150 and 200m, while the frontal type spent most of the time below 200m (Figure 2). The temperature profile of the frontal type contained a high fluctuation in daily range of temperature during most of the feeding season, while the intermediate frontal type had slightly less fluctuation. This is presented by a large standard deviation (Figure 3, blue vertical lines). The depth at spawning did also differ between the ecotypes where the deep migrating individuals spawn deeper in the water column (Figure 3).

3.2 Discriminant Analyses

The outcome of the Fourier reconstruction indicated that more than 95% of the original shape could be described by the eight largest Fourier Descriptors. As a result, of all the 256 Fourier descriptors, only these eight were used in the discriminant analysis. Otolith area, perimeter and minimum length were all significantly correlated with otolith size and were consequently rescaled before further analysis. The same three parameters tested significantly as covariates with age (ANCOVA, $p < 0.05$). However, after standardizing the otoliths for length, the effect of age was no longer significant. Therefore, all of the eight shape parameters and the first eight Fourier Descriptors were used in the complete GLM. The best model (AIC = 37.696) consisted of two Fourier Descriptors, FD3 and FD8, along with three shape variables, Roundness, Aspect Ratio and Circularity (Table II).

The GLM was very good at predicting the ecotypes of the coastal and frontal cod (91% and 90% correct respectively, Table III). The four individuals that were wrongly classified, (two frontal and two coastal) were not obviously different from the rest, e.g. they were not older or younger, not exclusively from one part of Iceland, and represented both sexes. The intermediate behaving cod, according to the CA of the DST, were classified by otolith morphology to either of the main behavior types with a success of only 64% (Table III). Correct classification of intermediate behavior types was defined such that all intermediate frontal with a predicted value below 0.5 were considered correctly classified while the rest as incorrectly classified, and vice versa for the intermediate coastal. The overall success rate for all four behavior types is, therefore, 77%. However, since the behavior of the intermediate groups is, as the name implies, intermediate compared to the frontal and coastal groups it might be incorrect or at least inaccurate to use the 0.5 prediction value to estimate correct assignments for these individuals.

The otolith shape was reconstructed using the inverse value of the Fourier Descriptors (Figure 4). The average shape of the otoliths from deep migrating cod (frontal and intermediate frontal) was more elongated or flatter. In contrast, the otoliths of the shallow individuals (coastal and intermediate coastal) were more round (Figure 4). There is an apparent gradient in the otolith shape with the intermediate types having a shape that is on average in-between the shape observed by the two extreme ecotypes, with the intermediate frontal closer to the frontal shape and the intermediate coastal closer to coastal in shape (Figure 4).

4 Discussion

4.1 Successful Discriminant Analysis

In this study we demonstrated how the different ecotypes of cod in Iceland can be distinguished by otolith shape. The success rate of correctly classified individuals of the two extreme behavior types, coastal and frontal was 90% and 77% when the intermediate types were also included. This is the first time that a method has been developed that can be used to discriminate between ecotypes of Icelandic cod for all individuals (Pampoulie *et al*., 2008). Until now the Pantophysin I locus could be applied but only for homozygotes leaving almost half of the population, the heterozygotes, unidentified. It must be pointed out that the current results are based on a small sample size and the success of the discriminant function is based on the testing it on the same sample that was used to develop it. This is not ideal and there is a need to re-validate the function with a new, independent sample. The best option would be to get a new sample of DST tagged cod with known migratory behavior. Unfortunately such sample is currently not available. The second best option is to analyze the correlation between the two markers of ecotypes that has now been established, that is the otolith shape and the *Pan* I locus (Pampoulie *et al*., 2008).

This is also the first time that full attention has been placed on the individuals displaying intermediate behavior. The assignment of individuals into ecotypes has been based on duration of time spent below the 200m depth level, and it has not been consistent how long the duration needed to last. Palsson and Thorsteinsson (2003) based their categorization on describing shallow-water cod as individuals that spent most of their time, >90%, at depth less than 200m. In a study looking at the relationship between migration behavior and the Pantophysin locus, Pampoulie et al

(2008), classified the shallow water coastal as an individual spending >70% of the time at depths less than 200m. In another study describing vertical separation of ecotypes during spawning, Grabowski et al (2011), used the >90% time limit criterion. Finally in Thorsteinsson et al (2012) the definition of a coastal behavior was based on criterion where the cod spent at least 70% of their time in shallow waters while the cod displaying frontal behavior were those that had migrated during feeding season to depths between 250 – 600m. In that study 12% (5 out of 41) of individuals were classified as coastal but were recorded at depth > 200m and therefore termed intermediate but still it was stated that real intermediate characters were not found (Thorsteinsson *et al.*, 2012). In the current study, we used Cluster Analysis based on pair-wise comparisons to avoid the application of-a-priori cut-off values. Better resolution of the overall behavior of cod was accomplished by comparing the individual behavior to all other individuals. This method revealed the relative placement of each individual on the gradient seen in the behavior pattern (Figure 2). This approach established the existence of the two corresponding ecotypes of cod, coastal and frontal, having intermediately behaving subgroups.

4.2 Environmental effect

The purpose of this study was not to analyze the underlying factors that affect otolith shape. However, it is interesting to see that the otolith shape seems to follow a gradient that is somewhat similar to body morphology. In the study on body shape *Pan* I^{BB} cod were more streamlined in shape, and with lower aspect ratio, while *Pan* I^{AA} cod had deeper bodies and higher aspect ratio (McAdam *et al.*, 2012). The *Pan* I^{AB} individuals expressed an intermediate body shape. Here the otoliths of the Frontal type, which is more commonly *Pan* I^{BB} are also more streamlined than the more round otoliths of the coastal ecotypes (Figure 4). As already mentioned the *Pan* I^{BB} types have been shown to be associated with the deep migration behavior, while *Pan* I^{AA} types are coastal (Pampoulie *et al.*, 2008). The slim body shape of *Pan* I^{BB} might indicate that some factor is acting on the deep migrating cod that makes it an advantage to be streamlined. The oceanography of Iceland is influenced by cold Arctic water flowing from the north and warmer waters transported with strong currents from the south, and the thermal fronts that form where these two water masses meet. The velocity of the currents in the deep ocean, especially in the frontal zone formed north-west of Iceland in the Denmark strait where many of the frontal types migrate, can be high (Aagaard and Malmberg, 1977; Våge *et al.*, 2013). This environment in the deep sea might be acting as a shaping force on the cod in such a way that they need to be streamlined to overcome these currents. Shape differences linked to slow and rapid water currents was found in Atlantic salmon which made Pez *et al.* (2008) conclude that streamlined and robust shapes might be important for individuals exploiting resources within rapid water current conditions. Another possibility is that the shallow coastal types are more sedentary than the deep migrating types and that longer distance and more active swimming behavior might be acting as a shaping force (Webb, 1982; Boily and Magnan, 2002). This difference in activity of migration cannot, however, be verified with the raw data from the DST profiles alone, since the tags only record temperature and depth, not actual location. We have an example from a DST tagged cod that exhibited a coastal behavior in the DST profiles, but the tagging location was in the south while the recaptured location was considerable distance away north of Iceland. This indicates that coastal cod may undertake long distance migrations, in a similar manner to the frontal types. The DST data can, however, be analyzed in relation to tidal wave patterns to try to locate the swimming path of the fish and therefore estimate the distance of the migrations. This has been done successfully for DST tagged cod in the North Sea (Wright *et al.*, 2006; Pedersen *et al.*, 2008), as well as in the Gulf of Maine (Gröger *et al.*, 2007). This has also been applied to DST tagged cod in Iceland with results that seem to indicate that most of the coastal cods are sedentary in nature, but also that some indications of considerable travel range along the coast were observed among coastal cod (Thorsteinsson *et al.*, 2012). It might also explain why some of the otoliths are wrongly classified, i.e. as mentioned before it might be that the otoliths from the coastal cod that were identified as frontal originate from cod traveling long distances, and vice versa.

4.3 Studies using otolith shape

The application of otolith shape in stock discrimination has been demonstrated successfully in many other cases, although it hasn't been widely used as a routine part of stock assessments. For the purpose of stock assessment, the Norwegian Coastal Cod (NCC) and Northeast Arctic cod (NEAC) are identified visually by the shape of the first two annuli. This difference was first discovered by Rollefsen (1933) and is routinely applied by otolith age-readers to discriminate between the NEAC and the NCC (Berg et al., 2005). Stransky et al (2008a) recently compared the identification of stock components by the age-readers using the method described above with the outer shape of the otoliths, and found a good consensus between the two methods. Other species, stock components have also been identified by otolith shape. The resident and migration component of the Celtic Sea herring was distinguished based on otolith shape with a high level of classification success (97%) (Burke *et al.*, 2008). European and North American stocks of Atlantic salmon were discriminated with a 88% success, while stocks within these two were classified with less success (Friedland and Reddin, 1994). Stransky *et al.* (2008b) applied elliptical Fourier Transforms on otolith shape of horse mackerel and found a good separation between stocks in the Northeast Atlantic and Mediterranean.

Furthermore this is not the first time that otolith shape has been used to discriminate among groups of cod in Iceland. Jonsdottir et al (2006) looked at the differences in otolith shape between spawning groups of cod in Iceland. The highest reported success rate was 44%. However, they rightly pointed out that most of the cod that was wrongly identified were mostly classified to a nearby spawning location, meaning that otoliths could be used to identify between cod from different spawning regions such as north and south of Iceland or between areas of different depths such as the shallow and deep water spawning sites at the SW coast of Iceland. The major part of the discrimination between the north and the south spawning cod was explained by otolith size parameters, i.e. weight, area and length, while shape parameters contributed little to the separation (Jonsdottir *et al.*, 2006). This is contrary to our results where most of the variation is explained by Fourier

Descriptors and shape parameters, i.e. Aspect ratio, Roundness and Circularity (Table II). What needs to be understood is that Jonsdottir et al (2006) did not have access to the DST tags used in this study or any other information on the behavior of the cod outside the spawning areas. Therefore, it is quite likely that their observations were based on groups of mixed ecotypes as it appears that both shallow and the deep water ecotypes home into similar spawning locations even though they spawn at different depths in the water column (Grabowski *et al.*, 2011; Thorsteinsson *et al.*, 2012). The only exception to this may be the cod that spawn at the deepest areas along the slopes, i.e. the cod that were discriminated from the shallow spawning cod in Jónsdottir et al (2006). These cod do likely represent the frontal ecotype as most of those have been shown to be *Pan* I^{BB} (Pampoulie et al, 2008). Similarly, Petursdottir *et al.* (2006) did also separate these deep water spawning cod from those in more shallow waters, using similar techniques in otolith shape analysis as used in this paper.

4.4 Conclusion

The present study reveals that otolith shape can be successfully used to distinguish between different ecotypes of cod. Otolith shape analysis is a straight forward method that can be applied to samples form the harvested stock either in the laboratory or onboard research vessels. As different ecotypes of cod do clearly utilize the ecosystem in a different way and may represent sub-populations that vary in size and abundance independently of each other, this discrimination technique may assist in further exploration in to the nature and origin of the different ecotypes.

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Iceland. The recaptured fish are the foundation of one of the world largest database on cod behavior. For this and many other reasons, Thorsteinsson will not be forgotten.

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Tables

Table I. The eight shape variables extracted from the otoliths, as defined by ImageJ software.

Shape variables	**Definition (from ImageJ)**
Area	Area of otolith, counted in pixels.
Perimeter	The length of the outside boundary of the otolith.
Feret (length)	The longest distance between any two points along the otolith boundary.
Minimum Feret (width)	The minimum caliper diameter.
Aspect Ratio	Feret / Minimum Feret
Circularity	Calculated with the formula: 4π*Area / Perimeter^2.
Roundness	Calculated with the formula: 4*Area / (π*Feret^2).
Rectangularity	The Area of the smallest rectangle enclosing the otolith / Otolith Area.

Table II. Model comparison results for the different otolith shape variables, evaluated with the significance of parameter addition (p-values) and by fit estimations with the Akaike Information Criterion (AIC).

Model	df	Deviance	*P*	AIC
*4: Behavior = FD3 + FD8 + Circ + AR + Round	36	4.0908	0.129	37.69
3: Behavior = FD3 + FD8 + Circ	38	4.4143	0.036	37.78
2: Behavior = FD3 + FD8	39	11.256	7.94 e-4	40.20
1: Behavior = FD3 + FD8	40	12.386	4.33 e-4	49.56

* No parameter addition to the third model measured significantly ($P < 0.05$), however out of all possible combinations, model four had the lowest AIC.

Table III. Classification s**uccess (%) of** the GLM predicted ecotypes for the 86 individuals based on otolith shape.

	Ecotypes based on DST profiles			
Predicted ecotypes	frontal	int. frontal	int. coastal	coastal
(GLM results)	(n=19)	(n=23)	(n=21)	(n=23)
frontal	**90**	**57**	48	9
coastal	10	43	**52**	**91**

Figure Captions

Figure 1. Dendrogram of the Cluster Analysis results depicting pair-wise dissimilarity (y-axis) between individuals. The results indicate that cod in Iceland could be described as having a gradient in behavior according to the depth and temperature profiles of the DST tagged recaptures. There can be identified two main groups which differ mainly in the depth regime they reside in during feeding season, with one group (1 and 2) staying at shallow inshore areas, while the other (3 and 4) migrates offshore to deeper areas. The results also show a gradient to this behavior, represented by the secondary division in the dendrogram which separates the main inshore and offshore groups into four subgroups. These groups are named coastal, intermediate coastal, intermediate frontal, and frontal, to maintain consistency in naming of the first analyses of the DST recaptured cod (Palsson & Thorsteinsson, 2003; Thorsteinsson & Saemundsson, 2006). For more details of the difference between the groups see Figure 2.

Figure 2. The difference of variance in daily temperature (A), and depth (B) range, and mean daily depth (C), between the four groups which were formed in the Cluster Analysis in Figure 1. ANOVA and a Tukey's Honestly Significant Difference test was used to test for difference between the four groups. There is a significant difference ($p < 0.05$, df = 3, 198) in all pairwise comparisons, except in temperature range per day, where only the frontal behavior measures different from the rest.

Figure 3. The annual, weekly mean depth (left panel) and temperature (right panel) profiles of the four groups identified by the Cluster Analysis in Figure 1. The dots are the mean values for each week and the vertical lines indicate the Standard Deviation (SD). The four groups are plotted with a slight shift so that they do not overlap, therefore it might seem that the lines start and end at different weeks, which is not the case. Spawning season is between week 10 and 20 the rest is considered as feeding season. Frontal cods (dark blue lines) undertake deep migration during feeding season and are well below 200m on average, while the intermediate frontal cod (light blue lines) can be found at around 200m on average. Coastal cod and intermediate coastal cod do not show vertical migration during feeding season and can both be found below or at around 100m depth. The temperature profiles of the frontal cods have larger SD and also stay more level on average than for the other types. Although not used in the Cluster Analysis, the four types due experience different mean temperature during feeding season.

Figure 4. The inverse Fast Fourier Transformation reconstruction of the otolith shape, averaged over each of the four behavior groups. In the upper-left panel, all four groups are compared and it can be seen that there is gradient from being more round (coastal) to flatter and more elongated shape (frontal). On average the frontal otoliths have smaller values when calculated for Roundness than the coastal. The other three panels are plotted to ease the visual comparison between the groups.