Otolith-based discrimination of cod ecotypes and the effect of growth rate

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2020**

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90 ECTS thesis submitted in partial fulfillment of a
Magister Scientiarum degree in Biology

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Cod ecotypes, otolith shape and growth rate
90 ECTS thesis submitted in partial fulfillment of a *Magister Scientiarum* degree in
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Bibliographic information:

Einar Pétur Jónsson, 2020, *Otolith-based discrimination of cod ecotypes and the effect of growth rate*, Master's thesis, Faculty of Life and Environmental Sciences, University of Iceland.

Printing: Háskólaprent, Fálkagata 2, 107 Reykjavík
Reykjavík, Iceland, September 2020

Abstract

Otolith shape has previously been used to identify ecotypes within the Icelandic cod (*Gadus morhua*) stock and growth rate has repeatedly been found to be a strong contributing factor to otolith shape variation in fish. To examine the effect of growth rate on the relationship between otolith shape and cod ecotypes (using *Pan I* genotypes as proxies for ecotypes), 826 archived sagittal otoliths from a 52 year sampling period were retrieved, the individual growth rate calculated and otolith shape described, using both Normalized Elliptic Fourier transform and Discrete Wavelet transform. Discriminant functions of otolith shape yielded low ecotype classification success, both when using Fourier and Wavelet descriptors. High classification success was significantly lowered by the shape variability of an intermediate ecotype, while otolith shape was significantly affected by growth rate and cohort. Growth rate differences commonly reported for the ecotypes were present, but were less marked than expected and indeed, growth rate variance attributable to ecotype identity was dwarfed by cohort- and location-related variance in growth. This suggests that cod ecotype discrimination based on otolith shape is sensitive to both temporal and spatial variations in growth, as these can mask the effect of ecotype-related growth rate differences on otolith shape.

Útdráttur

Kvarnalögun hefur áður verið notuð til greiningar vistgerða innan íslenska þorsstofnsins og sterkra áhrifa vaxtarhraða á kvarnalögun ítrekað gætt í ýmsum tegundum fiska. Með það markmið að rannsaka áhrif vaxtarhraða á tengsl kvarnalögunar og vistgerða þorsks (skilgreindar með breytileika á *Pan I* genastæðinu), var sótt í safn 826 kvarna frá 52 ára tímabili, vaxtarhraði hverrar kvarnar reiknaður og kvarnalögun lýst, og til þess notuð bæði *Normalized Elliptic Fourier transform* og *Discrete Wavelet transform*. Lítil árangur fékkst af flokkun vistgerðanna með aðgreiningarföllum á kvarnalögun, hvort heldur með *Fourier* eða *Wavelet* stuðlum. Fjölbreytt kvarnalögun mið-vistgerðar olli lágrí flokkun, og bæði vaxtarhraði og árgangur höfðu marktæk áhrif á kvarnalögun. Vaxtarhraðamynstur vistgerðanna var ekki eins skýrt og í fyrri rannsóknum, en í raun féll breytileiki í vaxtarhraða vegna vistgerða í skugga breytileika af völdum árganga og svæða. Þetta bendir til þess að aðgreining vistgerða þorsks út frá kvarnalögun sé viðkvæm fyrir breytileika á vaxtarhraða í bæði tíma og rúmi, þar sem sá breytileiki getur hulið mun á vaxtarhraða milli vistgerða, og þar með mun á kvarnalögun milli vistgerða.

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Acknowledgements

This work builds upon previous research conducted by Klara Jakobsdóttir (2011) and Hlynur Bárðarson (2016), on whose shoulders I now gratefully stand.

Staff of the Marine and Freshwater Research Institute (MFRI) collected and archived the samples used in the study, as well as supporting the current research with equipment, facilities and a helping hand.

Statistical support was given by Denis Warshan, Bjarki Elvarsson and Sölvi Rögnvaldsson.

Many thanks to Jón Sólmundsson of the MFRI for his guidance and introduction to the current field of research.

Special thanks to Professor Steven E. Campana, his supervision and valuable mentorship have formed and motivated a young scientist.

1 Introduction

Ecotypes are sub-groups of populations differing in allele frequency across loci, formed through their multiple trait adaptations to environmental variables (Lowry, 2012). The ecological separation that is entailed in the formation of ecotypes has been linked to niche partitioning, where a single species may occupy a wider environmental gradient through a number of distinct ecotypes within the species, as exemplified by the cyanobacterium *Prochlorococcus* (Johnson *et al.*, 2006) and lake whitefish (*Coregonus clupeaformis*) (Rogers *et al.*, 2002). The ecotype stage has been suggested to be precursive to evolutionary processes such as speciation and this divergence has been studied extensively (see Lowry, 2012) since Turesson's (1922) studies on saltbushes in Sweden, where he coined the ecotype term. Studies on ecotypes and the divergence they entail are now common in fish, such as in Arctic char (*Salvelinus alpinus*) (Kapralova *et al.*, 2013) and Atlantic cod (Grabowski *et al.*, 2011). Increased importance is now given to maintaining the diversity that ecotypes confer to species and ecosystems, especially in the context of sustainable fisheries management (Wood *et al.*, 2008; Knutsen *et al.*, 2018).

Two distinct ecotypes of Atlantic cod (*Gadus morhua*) have been repeatedly reported from throughout the trans-oceanic range of the species (Pálsson and Thorsteinsson, 2003; Robichaud and Rose, 2004; Pampoulie *et al.*, 2008). Off the coast of Iceland, Data Storage Tag (DST) profiles have identified a coastal ecotype and a more migratory (frontal) ecotype associated with thermal fronts (Thorsteinsson *et al.*, 2012; Grabowski *et al.*, 2011). In addition, there is evidence of fine-scale differences in spawning habitat selection between the ecotypes (Grabowski *et al.*, 2011). The ecotypes present morphological differences (Jónsdóttir *et al.*, 2008; Pardoe and Marteinsdóttir, 2009), as well as genetic divergence at the pantophysin I locus (*Pan I*). The *Pan I*^{AA} genotype is associated with the coastal ecotype and the *Pan I*^{BB} genotype with the frontal ecotype (Pampoulie *et al.* 2006, 2008; Árnason *et al.* 2009), while the *Pan I*^{AB} presents intermediate behaviour (Bardarson *et al.*, 2016).

Discrimination of stock components is important in fisheries science, where otoliths have proven to be a convenient tool (Campana and Casselman, 1993; Begg and Brown, 2011). Otoliths are metabolically inert structures with many varied uses, such as age reading and the discriminations of stocks (Campana, 2005). The otolith's shape is unique to each species and both internal and external otolith shape have been used to discriminate between stock components (Campana and Casselman, 1993; Cardinale *et al.*, 2004; Stransky *et al.*, 2008). The external otolith shape has recently been used to predict the ecotype identity of cod around Iceland, with DST profiles and *Pan I* genotyping used to validate the results (Bardarson *et al.*, 2016).

Otolith shape variation has been associated with both intrinsic and extrinsic factors, but some of these have been suggested to be ultimately acting on otolith shape through growth variability among individuals. Genetic regulation of otolith shape has been detected, but these studies have also identified environmental determinants of otolith shape, suggesting dual shape regulation (Vignon and Morat, 2010; Cardinale *et al.*, 2004). Despite the influence of environmental factors such as diet (Hüssy, 2008) and temperature (Lombarte and Lleonart, 1993) on otolith shape, a stronger link has been discovered between otolith

shape and fish growth rate (Campana and Casselman, 1993). Environmental factors affecting otolith shape have also been proposed to be acting via individual growth rate (Cardinale *et al.*, 2004; Denechaud, 2020). The coastal cod ecotype around Iceland has been found to grow faster than its frontal counterpart (Jónsdóttir *et al.*, 2002; Pálsson and Thorsteinsson, 2003; Jakobsdóttir *et al.*, 2011), although this finding is not universal (Jónsdóttir *et al.*, 2008). Although growth rate's effect on otolith shape differences between cod ecotypes has not been confirmed, it is reasonable to assume the relationship exists as it does in the case of separate cod stocks, where otolith shape differences were driven by growth rate (Campana & Casselman, 1993).

The objective of this study was to investigate the effect of growth rate on the relationship between otolith shape and the cod ecotypes off Iceland. The study examined cod from three separate spawning grounds collected over a period of half a century. Archived otoliths were retrieved and their shape described using Normalized Elliptic Fourier transform and Discrete Wavelet transform. These had already been genotyped for the *Pan I* locus (by Jakobsdóttir *et al.*, 2011), which was used as a proxy for ecotype identity. Ecotype discrimination using otolith shape was tested and the relative contributions of cohort, location and growth rate to the shape variation were evaluated. We hypothesized that ecotype discrimination based on otolith shape would be sensitive to spatial and temporal variations in fish growth rate.

2 Methods

2.1 Sampling and measurements

Samples of Icelandic cod were collected both at port and on surveys by the Icelandic Marine Research Institute (MRI) using three types of commercial fishing gear between 1948 and 2000 (Table 1). Cod were caught from late March through April (season of spawning) at three known spawning grounds (Marteinsdottir *et al.*, 2000) west and southwest of Iceland: Breiðafjörður (BRE), Faxaflói (FAX) and the southwest (SW) (Figure 1).

The total length of each cod was measured and their stage of sexual maturity determined. Sagittal otoliths were removed from the fish and their age estimated by experienced otolith age-readers. The otolith growth increments were counted from the core to the edge of the otolith in a transverse section through the otolith core, perpendicular to the otolith's longest axis. The age range was from 6 to 14 years.

Otolith shape analysis was based on the intact (unaged) sagittal otolith of each pair, which was photographed and measured following the methods detailed in Bardarson *et al.* (2016). The right sagittal otolith, with the *sulcus acusticus* facing up and the dorsal side pointing towards the top of the image, was photographed under a Leica MZ6 stereomicroscope at 0.63x magnification using a Plan Apo 0.4x objective. In cases where the right otolith had been sectioned for ageing, the left otolith was flipped horizontally and used instead. Strong backlighting of the otolith resulted in a contrasting dark shape on a light background. The images were then converted to 8-bit binary images, creating a black shape on a white background, followed by measurement of the otolith's maximum Feret diameter (the maximum distance between any two points on the otolith's edge). All image manipulation and measurements were carried out with the Fiji software (Schindelin *et al.*, 2012). In order to maintain sample size, broken otoliths were glued together when possible, following Bardarson *et al.*, (2014).

The shape of the otolith perimeter was analysed using the ShapeR package (Libungan and Pálsson, 2015) for the R software (R Core Team, 2020). In this procedure, the dark otolith was distinguished from the white background and the otolith's outline detected. Two independent methods were used to generate shape coefficients from the otolith outline: (1) Normalized Elliptic Fourier transform generated 45 Normalized Fourier coefficients that describe and quantify the shape with sines and cosines (Libungan and Pálsson, 2015). The Fourier coefficients were normalized with regards to otolith length (maximum Feret diameter), as part of the Normalized Elliptic Fourier analysis. (2) Discrete Wavelet transform was better suited for the description of localized changes in shape, such as sharp edges, as the 64 Wavelet coefficients detect shape variations at specific regions of the otolith (Graps, 1995; Libungan and Pálsson, 2015).

All shape coefficients were used in the analyses. The accuracy of shape description generally improves as the number of shape coefficients are increased, although the first 10 Fourier coefficients commonly describe most of the shape (Campana and Casselman, 1993; Bardarson *et al.*, 2016).

The otolith shape coefficients were detrended to remove any correlation with fish length. For each shape coefficient significantly affected by fish length, a general linear model (GLM) was fitted with the shape coefficient as a function of fish length and location. The shape coefficient was then detrended by subtracting from it the product of the fish length and the within-group slope (β) acquired from the GLM. The resulting detrended shape coefficient was thus comparable among fish of different lengths from different locations.

The *Pan I* locus genotype was used as the exclusive indicator for ecotype identity, as the AA genotype is associated with the coastal ecotype, the BB genotype with the frontal ecotype, while the heterozygote AB is intermediate between frontal and coastal (Bardarson *et al.*, 2016). This genetic information was obtained from tissue remaining on the otoliths as reported by Jakobsdóttir *et al.* (2011).

2.2 Data analysis

To analyse all age groups simultaneously and still retain a relative measure of fish growth rate, a growth rate index was created. This was done by first fitting a von Bertalanffy growth equation to length-at-age data using nonlinear least squares (Figure 2).

$$E[L|t] = L_1 + (L_3 - L_1) * \frac{1 - r^{2 * \frac{t-t_1}{t_3-t_1}}}{1 - r^2}$$

where

$$r = \frac{L_3 - L_2}{L_2 - L_1}$$

Equation 1. The von Bertalanffy growth function as re-parametrized by Francis (1988).

L_1 , L_2 and L_3 represent length at ages t_1 , t_2 and t_3 , respectively.

The von Bertalanffy function was fitted to the data using the function *vbfr* from the fishmethods package (Nelson, 2018) for R. A growth rate index (G) was defined as the

residual (res_i) for a given individual fish i at age j from the length predicted by the von Bertalanffy growth function at that age ($\hat{y}_j$). Calculation of G allowed comparison of relative growth rates across age groups. An age-independent growth rate index has seldom been reported, so its validity was confirmed through comparison with length-at-age presentation of the data.

Discriminant function analysis (DFA) was used to identify the shape coefficients most effective in discriminating among the *Pan* I groups. Classification accuracy was assessed using cross validation where the prior probabilities were computed from the group sizes.

Analyses of Variance (ANOVAs) were used to evaluate the variance of growth rate among ecotypes, locations and cohorts. A Shapiro-Wilks test indicated non-normality of the distribution of residuals for the ANOVAs, but as the analyses are robust to modest deviations such as these, they were carried out nonetheless.

SPSS version 26 (IBM Corp., 2019) and the R software (version 3.6.1) were used for all statistical analyses in the study.

3 Results

Of the three *Pan* I genotypes, AB was generally the most numerous, accounting for a mean proportion of 0.60 across cohorts and locations, while the mean proportion of AA was 0.24 and that of BB 0.16. There were clear changes in *Pan* I proportions through time, although not among locations (Figure 3). There were significant differences in *Pan* I proportions across cohorts (Chi-squared test, $p < 0.01$, $n = 826$). The most prominent cohort-related variations were the inversions in the proportions of the AA and AB genotypes. However, these cohort-specific *Pan* I proportions did not vary significantly between the three locations (Chi-squared test, $p > 0.05$, $n = 826$).

Otolith shape analysis allowed the *Pan* I genotypes to be identified, but with relatively low accuracy. Stepwise Discriminant function analyses (DFAs) of *Pan* I genotypes using either Fourier descriptors or Wavelet coefficients resulted in two significant discriminant functions incorporating up to 10 Fourier coefficients in one analysis and 15 Wavelet coefficients in the other. Cohort and *G* were selected in both DFAs, while location was not selected at any step in either analysis ($p < 0.01$, $n = 826$). Classification success using these discriminant functions was 68%, which was similar to DFA functions without cohort and *G*. The classification success was in all cases highest for AB (92-95%), the most numerous of the *Pan* I genotypes, while AA and BB were classified with 17-40% and 2-5% success, respectively (Table 2), and both were most commonly (60-90%) misclassified as AB.

Discriminant function analyses including only the *Pan* I^{AA} and *Pan* I^{BB} genotypes showed markedly higher classification success for these genotypes than the DFAs including all three genotypes. Stepwise DFAs incorporated 10 Fourier and 9 Wavelet descriptors (as well as cohort and the growth rate index (*G*)) and classified the *Pan* I^{AA} and *Pan* I^{BB} genotypes with over 85 and 72% success, respectively (Table 3). The *Pan* I^{AA} and *Pan* I^{BB} genotypes separated along the discriminant scores from the functions of the two DFAs, while the AB genotype was distributed over the whole range of these (Figure 4).

Although the *Pan* I genotype could be predicted in part by otolith shape, otolith shape in turn was significantly influenced by the growth rate of the fish. Multivariate General linear models indicated that *G* significantly affected 18 of the 42 Fourier descriptors and 16 of the 63 Wavelets. Adding location, cohort and the *Pan* I identity to the analysis revealed that neither location nor *Pan* I identity had a significant effect on shape, while both *G* and cohort had a significant effect on these shape descriptors, with cohort and its interaction with *G* contributing most to the model ($p < 0.01$, $n = 826$ for all analyses).

The growth rate of the three genotypes differed at the gross scale, but these differences disappeared once account was taken of growth rate differences across location and cohort. A one-way Analysis of Variance (ANOVA) revealed statistically significant differences in *G* among the *Pan* I genotypes when all data were pooled ($F(2, 826)$, $p < 0.01$, Figure 5). A post-hoc Tukey's test indicated that *G* for the *Pan* I^{AA} genotype differed significantly from the AB and BB genotypes ($p < 0.05$) and that the latter two did not differ between themselves. However, when accounting for location and cohort, there were significant variations in *G* among locations ($F(2, 826)$, $p < 0.001$) and cohorts ($F(55, 826)$, $p < 0.001$, Figure 6), but not

among the *Pan* I genotypes ($F(2, 826)$, $p=0.638$) (3-way ANOVA, see Table 4). The only significant interaction in the analysis was that between location and cohort ($F(19, 826)$, $p<0.01$).

Differences in fish growth rate between genotypes were not consistent when accounting for location. One-way ANOVAs revealed significant differences in G between the *Pan* I genotypes in both Faxaflói ($F(2, 207)$, $p<0.01$) and the Southwest ($F(2, 473)$, $p<0.01$), and post-hoc Tukey's tests showed this to be the AA genotype separating from the other two genotypes in both cases ($p<0.05$, see Figure 5). However, no significant differences in G were found between the genotypes in Breiðafjörður ($F(2, 146)$, $p=0.9$). When accounting for cohort as well as location, no consistent differences could be identified in fish growth rate between the genotypes in any of the three locations (see Figure 7 for example).

4 Discussion

There were clear cohort-related variations in ecotype proportions in all three locations, with inversions in genotype dominance occurring between cohorts. The cod ecotypes have been shown to use similar spawning grounds (Jónsdóttir *et al.*, 2008), with the separation in depth they inhabit during spawning season being no more than 50 meters (Bardarson *et al.*, 2016), so it was to be expected that random sampling of these spawning grounds would yield proportions similar to those in the spawning population. No significant variation in proportions were detected between locations, despite the sampling in Breiðafjörður taking place at greater depth (Table 1) and the frequency of the B allele being positively correlated with depth (Pampoulie *et al.*, 2006; Árnason *et al.*, 2009). Overall, the intermediate ecotype (represented by the *Pan* I^{AB} genotype) was most frequent in all three locations, as has been pointed out in other studies (Jónsdóttir *et al.*, 2006), but there were also strong changes in genotype proportions among cohorts, both at shorter and longer time scales. As no such marked changes in proportions were found in genotype proportions for each sampling year, regardless of the cohort (Table 1), we conclude that the cohort-related variations were not due to biased sampling of the genotypes. If the changes in genotype proportions between cohorts were not the product of random variation, then the year-class strength of each ecotype would seem to vary. Similar temporal and spatial variations in ecotype proportions have also been noted along the Norwegian Skagerrak coast for the Fjord and North Sea ecotypes present there (Knutsen *et al.*, 2018).

Discriminating ecotype identity using otolith shape yielded lower success than expected, both when using the Fourier and Wavelet coefficients. The low classification success of 68% contrasted with the higher overall classification success of 77% presented by Bardarson *et al.* (2016). They had most success classifying the frontal (90%) and coastal (91%) ecotypes, while the classification success in the present study was highest for the intermediate ecotype; the coastal and frontal ecotypes were usually misclassified as the intermediate ecotype in our study. It is therefore apparent that the otolith shape differences between the ecotypes were not strong enough to allow for successful discrimination. The distorting effect of the intermediate ecotype in the DFAs was evidenced by excluding this ecotype from the analyses, which increased the classification success for the *Pan* I^{AA} and *Pan* I^{BB} genotypes to levels close to those reported by Bardarson *et al.* (2016) (Table 3). This is further supported by the distribution of the discriminant scores from the two-group DFA, along which the *Pan* I^{AA} and *Pan* I^{BB} genotypes clearly separated. In contrast, the *Pan* I^{AB} genotype discriminant scores from this analysis were distributed along the entire gradient between the *Pan* I^{AA} and *Pan* I^{BB} endpoints, indicating that the otolith shape of *Pan* I^{AB} can range from a “*Pan* I^{AA} shape” to a “*Pan* I^{BB} shape” (Figure 4). This is consistent with DST data showing the AB genotype to present behaviour evenly distributed across the behaviour patterns (Pampoulie *et al.*, 2008). It seems likely that *Pan* I^{AB} individuals are subject to greater variation in environmental parameters such as temperature and food availability, which in turn affect otolith shape through fish growth (Hüssy, 2008; Irgens *et al.*, 2017; Denechaud *et al.*, 2020). Furthermore, the large temporal and spatial scale of our study almost certainly includes shape variation independent of the genotype which can affect the classification success for all genotypes.

Both the Fourier and Wavelet descriptions of otolith shape were affected by fish growth. Calculating the growth rate index (G) allows relative growth rate to be compared across different ages. Evaluating G 's effect on otolith shape indicated that growth rate, as well as cohort, had a significant effect on the otolith shape. While otolith shape is determined both by environmental and genetic influences (Vignon and Morat, 2010), a link between individual growth rate and otolith shape has repeatedly been found (Campana and Casselman, 1993; Oozeki and Watanabe, 2000; Simoneau *et al.*, 2000; Strelcheck *et al.*, 2003) and environmental variables have indeed been suggested to influence otolith shape through growth rate (Cardinale *et al.*, 2004; Denechaud *et al.*, 2020). The relationship between growth and shape presented here is concordant with other studies which have found that fish growth is an important factor to consider in stock discrimination based on otolith shape, as stocks with similar growth rates tend to have similar otolith shapes (Campana and Casselman, 1993; Begg and Brown, 2000). Thus, it is reasonable to extend that logic to the cod ecotypes – that their otolith shapes become more distinguishable with increasing differences in growth rate.

Variation in G was greater between locations and among cohorts than between the ecotypes, which did not consistently present the growth rate differences previously suggested by literature (Jónsdóttir *et al.*, 2002; Pálsson and Thorsteinsson, 2003; Jakobsdóttir *et al.*, 2011). Spatial and temporal separation has not been considered in previous studies on the growth rate of the ecotypes, except in one case (Jónsdóttir *et al.*, 2008), where the finding interestingly suggested that the relationship between growth rate and the *Pan I* genotypes was more complicated than previously thought and that growth rate varied more among spawning areas than among the *Pan I* genotypes within each area. Indeed, when comparing the variance in G among locations, cohorts and *Pan I* identity, the identity did not have a significant effect on G , while both location and cohort were significant factors (Table 4). Although the spawning grounds sampled are only inhabited by these cod during a small part of the year, the fish still retain some spatial separation during the foraging season (Sólmundsson *et al.*, 2015), and are thus exposed to different environments. A recent study on Northeast Arctic cod found environmental factors to exert a joint, indirect influence on otolith shape through changes in fish growth rate (Denechaud *et al.*, 2020). Growth rate variations among locations and cohorts are common in fish and are strongly driven by temperature and food supply (Brandt *et al.*, 1992; De Angelis *et al.*, 1993), as is most likely the case here.

Growth rate's effect on discrimination analyses based on otolith shape has also been noted in other species, such as haddock (*Melanogrammus aeglefinus*) spawning components on Georges Bank (Begg and Brown, 2011), blue whiting (*Micromesistius poutassou*) morphotypes in the Northeast Atlantic (Keating *et al.*, 2014) and herring (*Clupea harengus*) stock components in the Celtic and Irish seas (Burke *et al.*, 2008). Otolith shape varied more with growth rate and cohort than with ecotype identity in the present study. This, and the greater variation in G between locations and among cohorts than between ecotypes, suggests that ecotype-related variation in shape was masked by growth-driven variations in shape. As cod spawn in a number of areas around Iceland and each ecotype can be found in widely separate spawning grounds, individuals of the same ecotype are likely exposed to different environments, which can then influence the fish growth (Jónsdóttir *et al.*, 2008). We conclude that any discrimination of ecotypes based on shape will have to take account of the spatial scale it encompasses, as the discrimination of individuals inhabiting different environments will be confounded by the variation in growth rate.

Cod ecotypes have been reported elsewhere, with the Norwegian coastal and Northeast Arctic cod ecotypes being the most extensively studied. These are genetically distinct (Knutsen *et al.*, 2018) and the ecotypes are readily distinguished using either internal or external otolith morphology (Stransky *et al.*, 2008). Although such discrimination based on otoliths has not been successfully carried out on residential and migratory cod ecotypes in the Northwest Atlantic, cod ecotypes differing in body shape and coloration are readily distinguished in the Gulf of Maine (Sherwood and Grabowski, 2010) and genetic differentiation between spawning groups has been detected in the area (Clucas *et al.*, 2019). Although not yet described as ecotypes, genetic differences indicating strong ecological adaptation have also been found between the western and eastern Baltic cod stocks (Weist *et al.*, 2019).

The European anchovy (*Engraulis encrasicolus*) is another widely distributed marine fish that presents ecotypes, with genetic differentiation between coastal and offshore population pairs of the species consistently being found through its range, rendering genetic support to the previously identified phenotypic divergence between coastal and marine anchovies (Le Moan, 2016 and references therein). The most striking ecotype examples in fish are evidently found in freshwater, where impressive phenotypic and genetic divergence have taken place since the end of the last glacial period. Phenotypically and genetically divergent benthic and limnetic ecotypes have repeatedly been found for the threespine stickleback (*Gasterosteus aculeatus*) within the water systems they inhabit (Cresko and Baker, 1996; Smith *et al.*, 2015). Such intra-lake divergence has also been noted in lake trout (*Salvelinus namaychus*), where ecotypes have developed independently in separate lake populations (Harris *et al.*, 2015). Perhaps the clearest example of ecotype development is found in Arctic char (*Salvelinus alpinus*) in Þingvallavatn (Iceland), where significantly divergent ecotypes have developed rapidly in the last 10,000 years, offering an opportunity to study speciation (Skúlason *et al.*, 1989; Kapralova *et al.*, 2011, 2013; Guðbrandsson *et al.*, 2019)). While the clear morphological differences between the Arctic char ecotypes make them readily distinguishable, the identification of ecotypes in other species can be more complicated, as is the case for cod in the present study.

Although discrimination of the Icelandic cod ecotypes by their otolith shape has been shown to yield some success here and in Bardarson *et al.* (2016), the method seems to be sensitive to both the broad distribution of the intermediate *Pan* I^{AB} genotype as well as the effect of growth rate on shape, and therefore to temporal and spatial variations in growth. Combining this with the finding that the relationship between growth rate and ecotype is not as straightforward as previously suggested indicates that care must be taken to consider fish growth rate variations when otolith shape is studied. Otolith shape analysis is a promising morphological identification method for the Icelandic frontal and coastal cod ecotypes, but any such discrimination should be adjusted to account for temporal or spatial changes in growth rate, as these affect otolith shape.

5 Tables and Figures

Table 1. Overview of the Pan I genetically identified samples originally presented in Jakobsdóttir et al. (2011). Depth distributions (10-90% quantiles) within location are presented where the exact depth of catch was unavailable (entries with asterisks). These samples were analyzed for their otolith shape and their growth rate index (G) calculated.

Sampling year	n	Location	Depth (m)	Gear type	AA	AB	BB	Nr. of cohorts
1948	68	BRE	100	Longline	16%	61%	26%	9
1957	84	SW	37	Gill net	15%	70%	15%	8
1959	61	SW	37-74*	Gill net	11%	47%	45%	7
1966	67	SW	37-74*	Bottom trawl	4%	87%	9%	7
1972	69	FAX	38-83*	Gill net	4%	70%	26%	6
1973	73	SW	70	Gill net	3%	81%	16%	6
1976	52	FAX	55	Gill net	35%	65%	0%	6
1976	47	SW	45	Bottom trawl	70%	28%	2%	6
1979	61	FAX	50	Gill net	0%	98%	2%	5
1985	73	SW	37-74*	Gill net	19%	55%	26%	8
1996	40	BRE	63	Gill net	28%	72%	0%	5
1996	25	FAX	55	Gill net	92%	8%	0%	5
1996	68	SW	56	Gill net	57%	43%	0%	8
2000	38	BRE	150	Gill net	21%	66%	13%	5

Table 2. Classification success (%) of the *Pan I* genotypes by Discriminant function analyses of otolith shape. Overall classification success was between 64 and 68% for all analyses. N=826 for each analysis.

Model	<i>Pan I</i>	<i>Pan I</i> ^{AA}	<i>Pan I</i> ^{AB}	<i>Pan I</i> ^{BB}
<i>Pan I</i> ~ Wavelet		17.0	94.5	5.0
<i>Pan I</i> ~ Fourier		29.1	92.6	5.0
<i>Pan I</i> ~ Wavelet + cohort + G		34.1	93.1	2.5
<i>Pan I</i> ~ Fourier + cohort + G		39.0	91.8	1.7

Table 3. Classification success (%) of the *Pan I*^{AA} and *Pan I*^{BB} genotypes by Discriminant function analyses of otolith shape. N=826 for each analysis.

Model	<i>Pan I</i>	<i>Pan I</i> ^{AA}	<i>Pan I</i> ^{BB}
<i>Pan I</i> ~ Wavelet + cohort + G		87.9	72.3
<i>Pan I</i> ~ Fourier + cohort + G		85.2	74.8

Table 4. Results of a three-way ANOVA evaluating the relative effect of location, cohort and Pan I identity on the growth rate index (G), as well as their two-way interactions.

Source	Type III Sum of Squares	df	Mean Square	F	<i>p</i>
Corrected Model	37523	146	257	5.3	<0.000
Intercept	3	1	3.0	0.062	<0.804
<i>Pan I</i>	43	2	21.9	0.45	<0.638
Location	1965	2	982.7	20.2	<0.000
Cohort	11728	55	213.3	4.4	<0.000
Location * Cohort	2195	19	115.6	2.4	<0.001
<i>Pan I</i> * Cohort	3945	64	61.6	1.3	<0.085
<i>Pan I</i> * Location	28	3	9.5	0.19	<0.900
Error	33037	679	48.7		
Total	70561	826			
Corrected Total	70561	825

Figure 1. Map of the sampling locations. Colour indicates sampling year and shape indicates gear type.

Figure 2. A von Bertalanffy growth curve fitted to the length-at-age data. Each dot represents an individual and its residual from the curve is the individual's relative growth rate (G). $N=826$. Data are jittered within age-groups.

Figure 3. Relative proportions of the three Pan I genotypes across cohorts in the three sampling locations: **a)** Breiðafjörður ($n=146$), **b)** Faxaflói ($n=207$) and **c)** Southwest ($n=473$). Note different scales on the x-axes.

Figures 4a-c. Distributions of the three *Pan I* genotypes along the discriminant scores from a Discriminant function analysis of the $Pan I^{AA}$ and $Pan I^{BB}$ genotypes using Wavelet coefficients, cohort and the growth rate index (*G*).

Figure 5. Growth rate index (G) for the ecotypes represented by the *Pan I* genotypes. Letters indicate significant differences among *Pan I* genotypes pooled across locations while shape and fill indicate significant differences among *Pan I* genotypes within each location. Error bars represent 95% confidence interval.

Figure 6. Variation in the growth rate index (G) across cohorts and among locations. Error bars indicate one standard error.

Figure 7. Growth rate (G) of the Pan I genotypes across cohorts in the Southwest location. Error bars indicate one standard error.

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