

Mixed origin of juvenile Atlantic cod (*Gadus morhua*) along the Swedish west coast

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Cryptic population structure in exploited fishes poses a major challenge for fisheries management. In Atlantic cod, sympatric ecotypes are well-known, for instance off the coast of northern Norway. Recently, two sympatric cod ecotypes have also been documented in the Skagerrak and Kattegat, where adult cod has declined dramatically. One ecotype has an apparent offshore origin and displays a more migratory lifestyle than the other, which is generally resident at the coast throughout its life. To investigate their relative abundances along the Swedish west coast, juvenile cod were collected at high spatial resolution along the coast and genetically assigned to ecotypes. The results reveal large proportions of the coastal ecotype in the southern Kattegat, Öresund, and inshore Swedish Skagerrak, whereas the offshore ecotype dominates in offshore areas. A regression model comparison suggests that differences in bottom depth, rather than distance from the open sea, explain the heterogeneous spatial distribution of the two ecotypes. In addition, the two ecotypes display genetic differences suggesting adaptation to different environments. The results underline that successful fisheries management requires identification and consideration of locally adapted sympatric ecotypes.

Keywords: ecology, ecotype divergence, fisheries management, genomics, population genetics, recruitment.

Introduction

Atlantic cod has been an important commercial fish species since the Middle Ages (Star *et al.*, 2017). In recent times, however, stocks on both sides of the North Atlantic have been severely depleted and have shown little recovery, even in areas with cod-fishing moratoria (COSEWIC, 2010; ICES, 2021c, d). In the Skagerrak and Kattegat, current fisheries-independent surveys catch almost no adult cod (Andersson *et al.*, 2020; Bland and Börjesson, 2020). Juvenile cod has also decreased in this area, but the trend is less clear as juvenile abundances show large interannual fluctuations (Svedäng, 2003; Cardinale and Svedäng, 2004).

Tagging and genetic studies have identified two sympatric cod ecotypes in the North Sea–Skagerrak–Kattegat region (Knutsen *et al.*, 2011; André *et al.*, 2016; Barth *et al.*, 2017). One ecotype genetically resembles North Sea cod and dominates in the offshore and outer coastal regions (Knutsen *et al.*, 2018). This “offshore” ecotype is believed to consist, at least in part, of juvenile cod from the North Sea that are transported into the area by ocean currents (Stenseth *et al.*, 2006; Jonsson *et al.*, 2016). The influx of offshore juveniles varies between years, and large juvenile abundances have been correlated with large proportions of the offshore ecotype (Stenseth *et al.*, 2006). The offshore cod appear to use the Skagerrak–Kattegat coast as a nursery for 2–4 years before migrating offshore to spawn (André *et al.*, 2016; Hüseyin *et al.*, 2022). To date, it is unclear whether all offshore cod migrate to the North Sea, or if some fraction completes its whole life cycle in offshore

Skagerrak (Knutsen *et al.*, 2018). The second ecotype, referred to as “fjord cod” in Norway and “coastal cod” in Sweden, genetically resembles cod in the southern Kattegat and Öresund and is more common in inshore Skagerrak (Knutsen *et al.*, 2018). The coastal ecotype displays resident behaviour and does not appear to undertake long-range spawning migrations (Knutsen *et al.*, 2011; Kristensen *et al.*, 2021). The two ecotypes coexist on multiple spatial scales but show differences in growth rate (Jørgensen *et al.*, 2020), behaviour (Kristensen *et al.*, 2021), and geographical distribution (Knutsen *et al.*, 2018). In addition, the ecotypes are genetically differentiated at both neutral (Knutsen *et al.*, 2011) and potentially adaptive loci (Barth *et al.*, 2019). Thus, increased knowledge on the life-history strategies and relative abundances of these ecotypes is essential to the successful management of Skagerrak–Kattegat cod (Knutsen *et al.*, 2018). Despite this, cod fisheries management does not presently account for the presence of two sympatric ecotypes in the Skagerrak and Kattegat (ICES, 2020).

The low abundance of adult cod, coupled with the variable juvenile abundance, in Swedish waters has led to hypotheses that most cod presently found along the Swedish Skagerrak coast have an offshore origin (Svedäng, 2003; Cardinale and Svedäng, 2004). However, the relative proportions of offshore and coastal ecotype cod have not been assessed explicitly in Swedish waters. In addition, recent declines in the offshore stocks in the North Sea (ICES, 2021a, c) have raised questions about whether the Skagerrak–Kattegat coastal zone has

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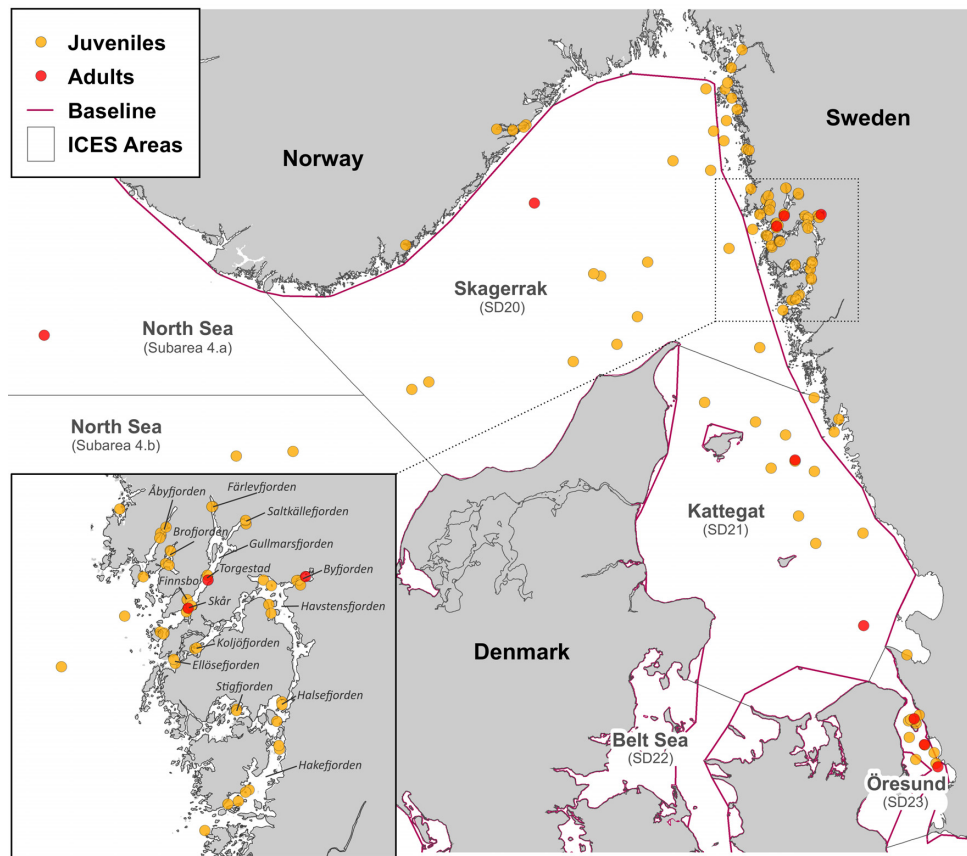


Figure 1. Map of the study area. Sampling stations are indicated with points, with colours indicating the type of sample (juvenile/adult). The national baselines of Norway, Sweden, and Denmark, which define the outer coastline, are indicated with purple lines. ICES subareas and subdivisions (SDs) are indicated with narrow black lines.

lost some of its function as a nursery habitat for juvenile cod, regardless of ecotype.

In this study, we aim to (1) examine whether there are juvenile cod of both offshore and coastal origins along the Swedish west coast; (2) if so, whether the relative proportions of the two ecotypes vary geographically and between years with large and small juvenile abundances; (3) determine whether the ecotypes are genetically differentiated at candidate loci associated with environmental adaptation; and (4) explore further potential genetic substructure within the coastal ecotype. To do this, we have genotyped juvenile cod collected in the Skagerrak, Kattegat, and Öresund during two years with different juvenile abundances. We used targeted single nucleotide polymorphism (SNP) loci to assign individuals to ecotype and sex and to analyse differences at loci under putative selection. We also used genome-wide SNP loci obtained through 2b-RAD sequencing to explore genetic differences within and between ecotypes at a high genomic resolution.

Methods

Sampling

Juvenile cod were collected along the Swedish west coast in 2019 and 2020, and along the Norwegian Skagerrak coast in 2020. Adult cod from the North Sea, Skagerrak, Kattegat, and Öresund, collected during spawning season in 2012, 2015, 2013, and 2020, respectively, were included as references for known and putative spawning populations (Figure 1;

Supplementary Table S1). DNA was extracted from fin clips with a Qiagen DNeasy® Blood and Tissue kit. Juveniles were aged from otolith readings or assigned to cohorts based on their total length (Supplementary Figure S1).

Targeted loci

A total of 1002 juvenile and adult individuals were genotyped at 65 SNP loci, including ecotype- and sex-diagnostic loci (Star *et al.*, 2016), as well as candidate loci located within chromosomal (chr) inversions and haemoglobin (Hb) genes (Supplementary Table S2). SNP genotyping was performed on an Agena MassARRAY platform. After quality control and filtering (Supplementary Note S1), 52 SNP loci remained: 33 ecotype-diagnostic loci, 5 sex-linked loci, 10 loci located within inversions (two each on chrs 1 and 2, and three each on chrs 7 and 12), and 4 Hb loci. The final dataset consisted of 406 and 488 juveniles collected in Swedish waters in 2019 and 2020, respectively, 25 juveniles from the Norwegian Risør fjord, along with 11 adult reference cod from the North Sea: six from the Norwegian Skagerrak, 18 from Gullmarsfjorden, three from Byfjorden, 17 from Kattegat, and 13 from Öresund (Figure 1).

Ecotype assignment

We assigned all individuals to ecotypes through *K*-means clustering using ADEGENET 2.1.3 (Jombart, 2008). Given that our panel was developed to distinguish the offshore and coastal ecotypes, we performed the clustering assuming two groups

($K = 2$). Individuals were assigned to the offshore or coastal ecotype according to whether they were assigned to the same group as adult reference individuals from the North Sea or from Kattegat/Öresund, respectively. Ecotype assignments thus resulted in 491 offshore and 496 coastal ecotype individuals. Assignments were evaluated using four methods (Supplementary Note S2): discriminant analysis of principal components (DAPC; adegenet); principal component analysis (PCA; ade4 1.7–16; Dray and Dufour, 2007); sparse non-negative matrix factorization (sNMF; lea 3.1.2; Frichot and François, 2015); and population assignment (assignPOP 1.2.2; Chen *et al.*, 2020). Lastly, we estimated the genetic divergence between the ecotypes and its significance (wcF_{ST} ; 10000 permutations) in strataG 2.5.01 (Archer *et al.*, 2016).

Assignment of sex and inversion state

We used PCA to assign sex and inversion state for each individual (Supplementary Figure S2). To determine which inversion state was the ancestral and which was the derived, we compared the genotypes to those reported by Matschiner *et al.* (2022). Note that different studies have used different nomenclature for the inversion states (see Supplementary Table S3). Individuals with one homozygous and one heterozygous SNP locus within the same inversion were removed from downstream analyses.

Interannual differences in ecotype proportions

We tested for differences in ecotype proportions between years using weighted analyses of variance (ANOVA) in stats 4.1.2 (R Core Team, 2021). To analyse inter-cohort differences, we only included stations where 0-group cod were collected in both years. For intra-cohort analysis, we included stations where juveniles from the 2019 cohort were collected in both 2019 and 2020 (as 0- and 1-group cod, respectively). Station-wise estimates of ecotype proportions were weighted against the number of individuals included from each station and year, and ANOVAs were performed separately for offshore and inshore stations. Öresund stations were excluded from this analysis, as sample sizes in 2020 were small.

Genetic differences between ecotypes at candidate loci

We tested for differences in allele frequencies between ecotypes for the inversion- and Hb loci by calculating locus-wise F_{ST} between the ecotypes using strataG (10000 permutations). The assigned inversion state of each individual was recoded as a single superlocus genotype to avoid biasing the F_{ST} estimates by including multiple non-independent markers. To test for differences in sex ratio, we performed χ^2 tests using stats 4.1.2.

Geographical predictors of ecotype and candidate locus genotype

We used regression modelling and model comparison to explore potential geographical correlates with station-wise ecotype proportions and sex ratios and with individual genotypes at candidate loci. We included only 0-group juveniles from 2019 to 2020 collected in Skagerrak (ICES Subdivision 20; SD20), Kattegat (SD21), and Öresund (SD23), to avoid including age classes with small sample sizes per station and SDs sampled at only a few stations.

For the ecotype proportions and sex ratios, we fitted weighted linear regression models using stats 4.1.2. We included year and SD as categorical explanatory variables;

bottom depth and distance inshore from the coastal baseline for each station as linear explanatory variables; and the number of individuals per station as weights. SD was included as an explanatory variable, as the SDs correlate with the current ICES advisory units for cod in the area: the “North Sea, eastern English Channel, Skagerrak” advisory unit includes SD20; the “Kattegat” advisory unit corresponds to SD21; and the “western Baltic Sea” advisory unit includes SD23 (ICES, 2020). For individual chromosomal inversion states and Hb genotypes, we fitted ordinal logistic regression models using MASS 7.3–54 (Venables and Ripley, 2002). As these models were fitted against individual-level data, we included ecotype and sex as categorical explanatory variables, in addition to year, SD, depth, and distance from the baseline. Depth was log-transformed to address the skewed distribution of this variable and to approach a linear relationship with the response variables. For more details, see Supplementary Note S3.

We applied a model comparison approach to the full suite of models to identify the most informative predictors. Model quality was estimated with the sample-size-corrected Akaike’s information criterion (AIC_c), using $AIC_{cmodavg}$ 2.3–1 (Mazerolle, 2020). Models with $\Delta AIC_c < 2$ compared to the top model were treated as having equal quality, following Burnham and Anderson (2004), and are referred to as the “top-ranked” models. We considered our analyses of geographical correlates exploratory (see Tredennick *et al.*, 2021), and thus, we opted against selecting a single model to make statistical inferences. Instead, we identified the most important explanatory variables across the top-ranked models and their respective effects to indicate the main geographical correlations.

Genome-wide loci

A subset of 268 individuals was selected for genome-wide genotyping using 2b-RAD sequencing. The subset included juveniles from Swedish ($n = 181$) and Norwegian ($n = 12$) Skagerrak fjords, the Kattegat ($n = 18$), and the Öresund ($n = 16$), together with adult reference cod from the North Sea ($n = 10$), Gullmarsfjorden ($n = 9$), Byfjorden ($n = 3$), the Kattegat ($n = 10$), and the Öresund ($n = 9$). The aim of this approach was to identify potential further substructure within the coastal ecotype, and specifically whether juveniles collected in fjords were distinct from cod in the Kattegat and Öresund. We also included offshore ecotype cod, thus enabling independent evaluation of the ecotype assignment using random genome-wide loci (Supplementary Note S4).

Library preparation and sequencing

DNA was purified using a Zymo DNA Clean and Concentrator™-25 Kit, and the quality was assessed with agarose gels and a NanoDrop® ND-1000 spectrophotometer. The 2b-RAD libraries (Wang *et al.*, 2012) were prepared following a modified protocol by Mikhail Matz (https://github.com/z0on/2bRAD_GATK/blob/master/2bRAD_protocol_june1_2018.pdf) as described in Kinnby *et al.* (2020).

Mapping and filtering

Trimming, filtering, and genotype calling followed Pereyra *et al.* (2022), and sequences were mapped to the cod genome assembly gadMor3.0 (NCBI BioProject accession no. PRJEB33455). Raw 2b-RAD sequences were deposited in the

Sequence Read Archive Repository at the National Centre for Biotechnology and Information (NCBI BioProject PRJNA850268).

Genotype calling

Individual RAD libraries produced 0.3–14.8 (median: 4.4) million reads per individual. Mapping to the gadMor3.0 genome assembly resulted in alignment rates ranging from 74.9 to 95.9% (median: 94.0%). Technical replicates rendered 15320 SNPs, used as a “true” SNP dataset for variant recalibration. A total of 55449 SNPs were called, and a set of 23364 SNPs and 235 individuals was obtained after filtering. Subsequent removal of loci with a minor allele frequency <5% resulted in 9956 SNPs for downstream analyses (Supplementary Table S4). We used *inveRision* 1.40.0 (Cáceres, 2021) to identify loci located within inverted chromosomal regions (Supplementary Note S5).

Local coastal populations

We performed PCA in *adeget* to visualize the genetic distances among coastal ecotype individuals at genome-wide loci located outside inversions. We also calculated pairwise multi-locus F_{ST} between sampling sites with *strataG* (10000 permutations), pooling nearby stations with small sample sizes (Supplementary Table S5). Due to low 2b-RAD genotyping success, adults from Gullmarsfjorden and Byfjorden were also pooled (“Fjord adult”). The dimensions of the pairwise F_{ST} matrix were then reduced using multi-dimensional scaling (MDS) in *stats* 4.1.2.

Outlier analyses

Pairwise outlier tests were performed using *BayeScan* 2.1 (Foll and Gaggiotti, 2008) and *OutFLANK* 0.2 (Whitlock and Lotterhos, 2014) with default settings, to identify loci displaying patterns of selection between the ecotypes. Annotation of outlier loci was performed by running 2.5 kb flanking regions of each outlier locus through *blastx* to match with non-redundant GenBank CDS translations + PDB + SwissProt + PIR + PRF databases. Loci with annotated hits were subsequently searched for Gene Ontology (GO) terms using PANTHER DB (Mi *et al.*, 2021) and PANTHER’s tool (Thomas *et al.*, 2006) to access the list of GO annotations (Gene Ontology Consortium, 2021).

Unless otherwise stated, data filtering and statistical analyses were performed in R 4.1.2 (R Core Team, 2021), using RStudio 1.4.1717 (RStudio Team, 2021). All *p*-values were corrected for multiple testing with *stats* 4.1.2, using the false discovery rate (FDR) method with the threshold for significance set at $q < 0.05$.

Results

Ecotype assignment

DAPC (Figure 2a) and PCA (Figure 2b) with the ecotype-diagnostic SNPs separated the ecotypes into two distinct clusters. The same pattern was found for the genome-wide loci located outside chromosomal inversions (Figure 2c), even when outlier loci (Figure 3) were removed (Figure 2d–e). Three individuals, assigned to the offshore ecotype with the ecotype-diagnostic SNP panel, clustered together with the coastal ecotype in the PCA using genome-wide loci. Hence, we estimated a misassignment rate of 1.3% (3/232), which is smaller than that reported by Jorde *et al.* (2018a) for a similar panel. The

assignment was further corroborated by DAPC, sNMF, and assignPOP (Supplementary Note S2, Supplementary Figure S3, and Supplementary Table S6). Probabilities from these alternative assignment methods suggested that individuals positioned intermediately between the two main clusters in Figures 2b–e represented first-generation hybrids. The multi-locus F_{ST} between the ecotypes was 0.14 ($p < 0.01$) at ecotype-diagnostic loci and 0.006 ($p < 0.01$) at genome-wide loci outside inversions.

Geographical distribution of ecotypes

Juveniles (0- and 1-group) of the two ecotypes co-occurred both offshore and inshore across the study area (Figure 4), but the ecotypes differed in their geographical distributions, which were stable between the years. Juveniles in offshore areas and the northern Skagerrak predominantly assigned to the offshore ecotype, whereas juveniles in inshore areas, southern Kattegat, and Öresund predominantly assigned to the coastal ecotype. With few exceptions, the proportion of coastal ecotype juveniles increased toward the innermost parts of the fjords, although the offshore ecotype was also found in this environment (Figure 4, inset maps).

The ecotype proportions for 0-group cod were similar in 2019 and 2020 (inter-cohort: Figure 4a versus b), at both offshore ($F_{1,17} = 3.04$, $q = 0.20$) and coastal stations ($F_{1,67} = 0.48$, $q = 0.49$). Ecotype proportions for 0-group in 2019 and 1-group cod in 2020 (intra-cohort) were also similar at offshore stations ($F_{1,12} = 0.91$, $q = 0.48$), but differed at inshore stations ($F_{1,30} = 7.33$, $q = 0.04$) due to an increase in coastal ecotype proportions in the outer part of the archipelago between 2019 (0-group, Figure 4a) and 2020 (1-group, Figure 4c). However, 1-group cod were absent at many stations in 2020, especially in inshore Skagerrak.

Differentiation at candidate loci

The two ecotypes showed differentiation in several genomic regions previously linked with environmental adaptation (Figure 5). These included inversions on chr 2 ($F_{ST} = 0.08$, $q < 0.01$), chr 7 ($F_{ST} = 0.03$, $q < 0.01$), and chr 12 ($F_{ST} = 0.21$, $q < 0.01$), as well as Hb- $\beta 1$ ($F_{ST} = 0.01$, $q < 0.01$) and Hb- $\beta 5$ ($F_{ST} = 0.02$, $q < 0.01$). However, the ancestral chr 1 inversion state appeared to be fixed in both ecotypes, and the ecotypes did not differ in genotype frequencies at Hb- $\alpha 1$ ($F_{ST} = 0.00$, $q = 1.00$), Hb- $\alpha 4$ ($F_{ST} = 0.00$, $q = 0.11$), or sex ratio ($\chi^2 = 0.37$, $df = 1$, $q = 0.62$).

Geographical predictors

Ecotype

Our exploration of potential drivers behind the distribution of ecotypes using model comparison returned 23 models (Table 1a), with three top-ranked models ($\Delta AICc < 2$). The simplest of the three models, Model 1, included only SD and depth as predictors, and both had significant effects (Supplementary Table S7). Models 2 and 3 also included these variables, and additional explanatory variables were non-significant. According to all three models, the proportion of coastal ecotype was similar between Skagerrak and Kattegat, but larger in Öresund, and decreased with depth in all SDs.

Candidate locus genotype

Ecotype was the single most important explanatory variable for most loci, but the chromosomal inversions on chrs 2, 7,

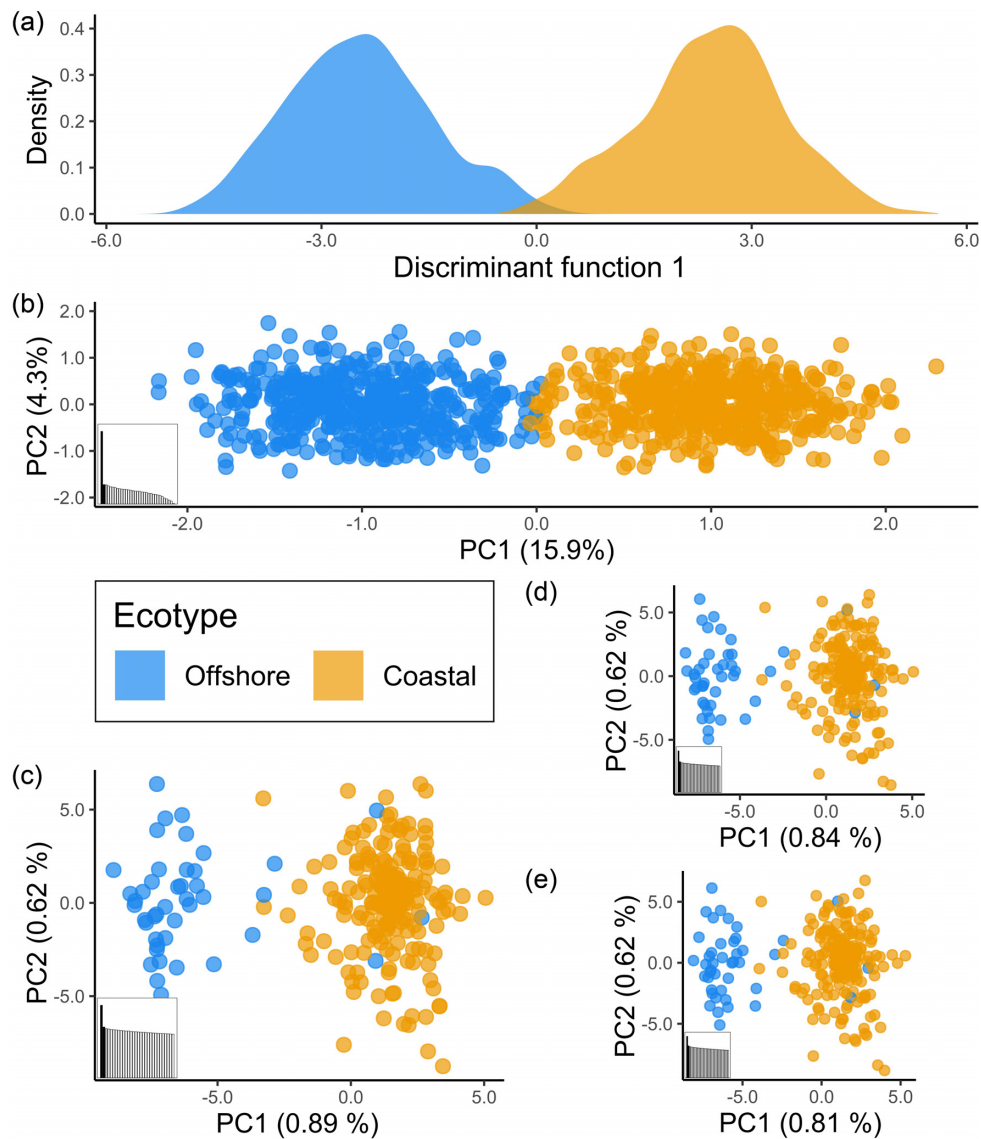


Figure 2. Ecotype assignment. **(a)** DAPC scores along discriminant function 1, and **(b)** PCA score plots for 987 individuals, based on genotypes at 33 ecotype-diagnostic loci. **c–e** show PCA score plots based on genome-wide loci outside of inversions, with **c** using all these loci, **d** excluding BayeScan outlier loci, and **e** excluding OutFLANK outliers. Colour corresponds to the assigned ecotype of individuals, according to the ecotype-diagnostic SNP panel. The aspect ratio between PC1 and 2 is scaled against the relative proportions of variance explained by each PC (in parentheses).

and 12 were also correlated with geographical variables. For the chr 2 inversion, both distance inshore from the baseline and ecotype were important explanatory variables (Table 1b). Both variables were included in all four top-ranked models and had significant effects, but additional explanatory variables were non-significant (Supplementary Table S9). In all top-ranked models, the ancestral chr 2 inversion state was more common in the coastal ecotype and increased in frequency with distance inshore. Model comparison for the chr 7 inversion returned six top-ranked models, for which the only common explanatory variable was bottom depth, either alone or as a depth \times ecotype interaction (Table 1c). Thus, depth was the most important explanatory variable for the chr 7 inversion, but its effect may be moderated by ecotype, SD, and distance from the baseline. Overall, the frequency of the derived inversion state increased with depth, but this effect was stronger in the offshore ecotype (Supplementary Table S10). For the chr 12 inversion, all eight top-ranked models included various combinations of ecotype, depth, and distance from

the baseline as predictors (Table 1d). The top-ranked model with the fewest explanatory variables (model 5) included distance and the depth \times ecotype interaction, the latter having the strongest effect. A distance \times depth interaction was also frequent across the top-ranked models. Shared among the top-ranked models was the finding that the ancestral allele increased sharply with depth in the offshore ecotype but not in the coastal ecotype, whereas distance had a small effect (Supplementary Table S11).

We excluded the chr 1 inversion and Hb- α 1 loci from the regression analyses as the total genetic variance was too small. For Hb- α 4, Hb- β 1, and Hb- β 5 genotypes, ecotype was the only important variable (Supplementary Tables S12–S14), whereas the sex ratio did not correlate with any of the included explanatory variables (Supplementary Table S8).

Outlier analyses

From the genome-wide SNP panel, BayeScan and OutFLANK identified 57 and 156 outlier loci between the ecotypes,

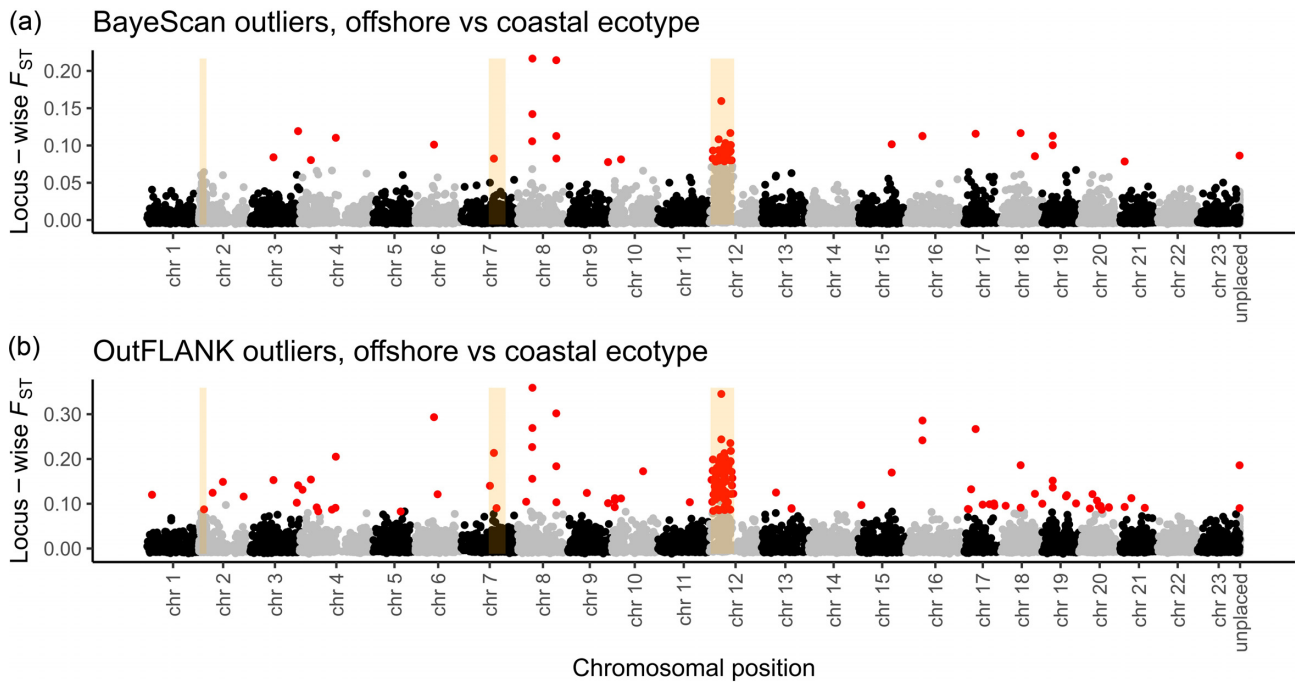


Figure 3. Manhattan plots of pairwise F_{ST} between the two ecotypes for all 2b-RAD SNP loci, with outlier loci detected by (a) BayeScan and (b) OutFLANK indicated as red points. Note that the OutFLANK F_{ST} values shown in b are not corrected for sample size. The inverted regions are highlighted in orange.

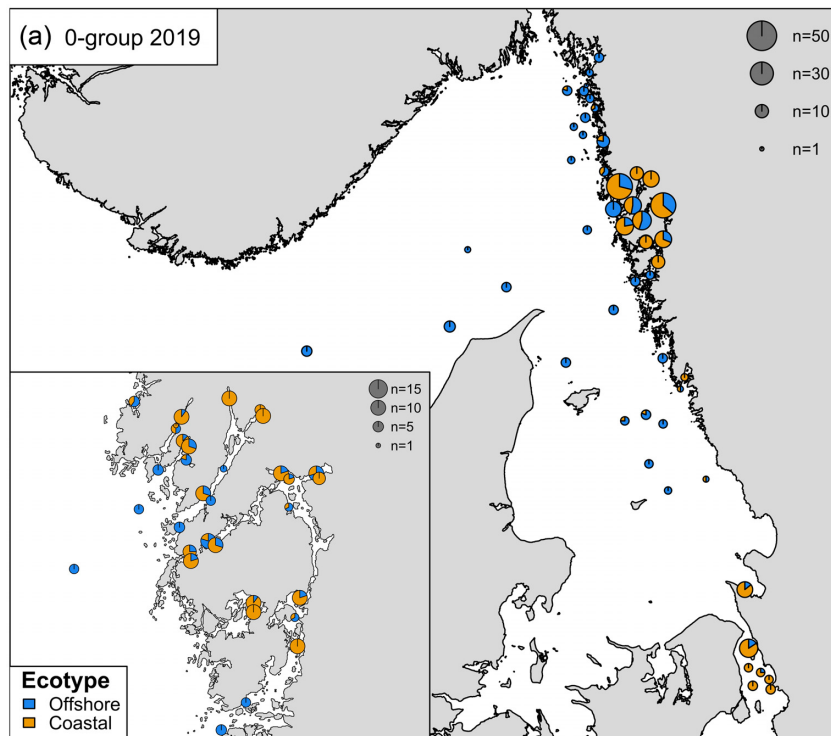


Figure 4. Maps of ecotype distributions for (a) 0-group cod in 2019, (b) 0-group cod in 2020, and (c) 1-group cod in 2020. The pie chart area indicates the number of individuals per station, and the size scale is indicated with grey pie charts. Note that different size scales are used for full maps and inset maps but that the same scales are used in a, b, and c. The size of each pie slice indicates the proportion of the offshore (blue) or coastal ecotype (orange). Sampled stations at which no 1-group cod were caught in 2020 are indicated with red crosses in c.

respectively (Figure 3). All outliers detected by BayeScan were also detected by OutFLANK, and more than half of the outlier loci were located within the chr 12 inversion (33/57 for BayeScan and 80/156 for OutFLANK). Gene

annotation of all 156 outlier loci resulted in 80 unique gene hits obtained for 85 loci (see Supplementary Table S15), and GO terms were available for 71 of these genes (see Supplementary Table S16). However, no enrichment analysis was

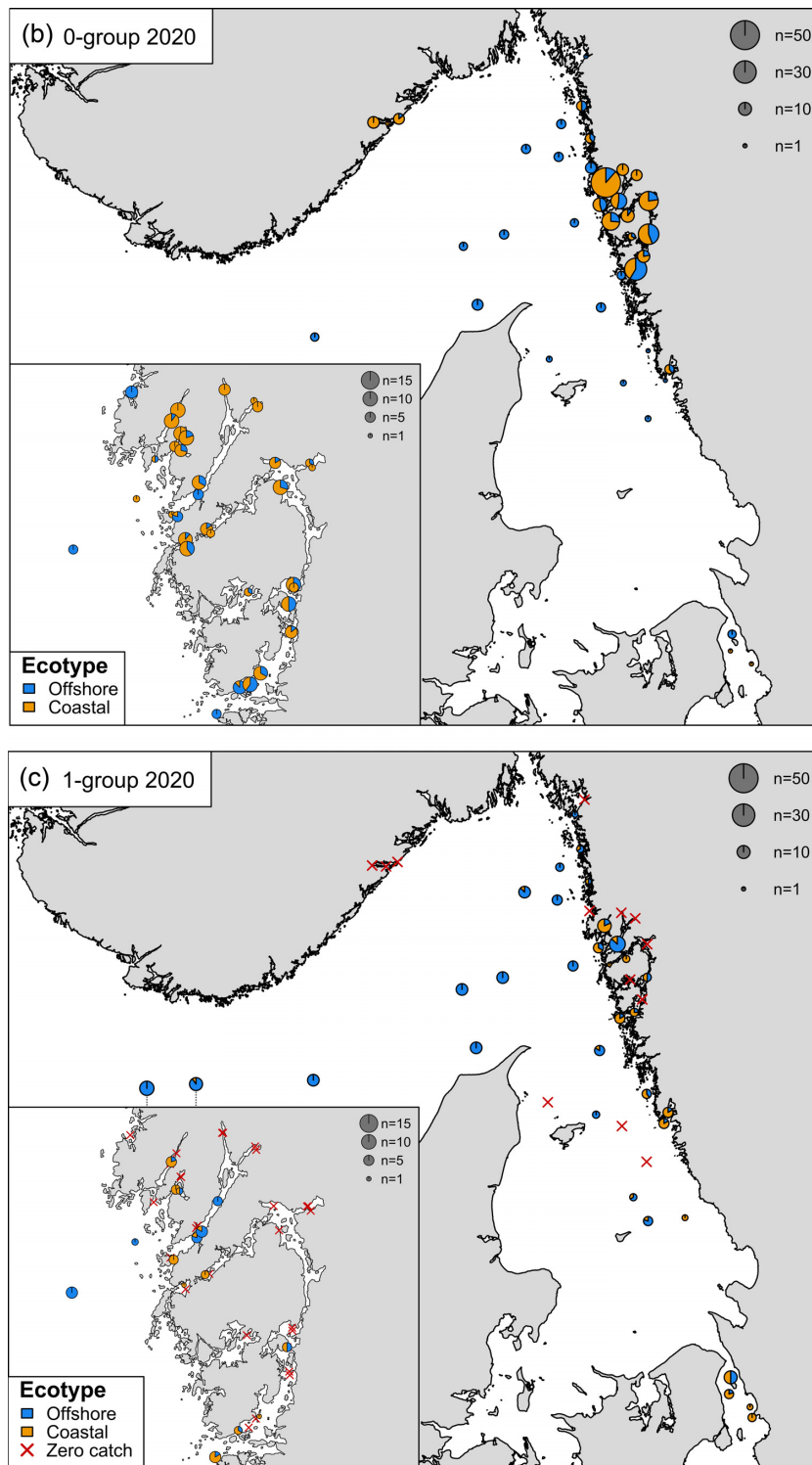


Figure 4. Continued.

performed on these GO terms, as the number of genes was too small.

Local coastal populations

The PCA based on 9418 genome-wide loci outside inversions revealed no distinct clusters among samples of the coastal ecotype (Figure 6a). Pairwise F_{ST} (Table 2) was significant

between the North Sea offshore adults and all coastal samples ($F_{ST} = 0.006\text{--}0.010$, $q = 0.001\text{--}0.036$). Within the coastal ecotype, F_{ST} was significantly different between juveniles from Norwegian fjords (Risør) and most Swedish fjords ($F_{ST} = 0.003\text{--}0.004$, $q = 0.001\text{--}0.045$). However, F_{ST} was non-significant for all coastal ecotype samples compared to both the Kattegat and Öresund spawning adults. The MDS

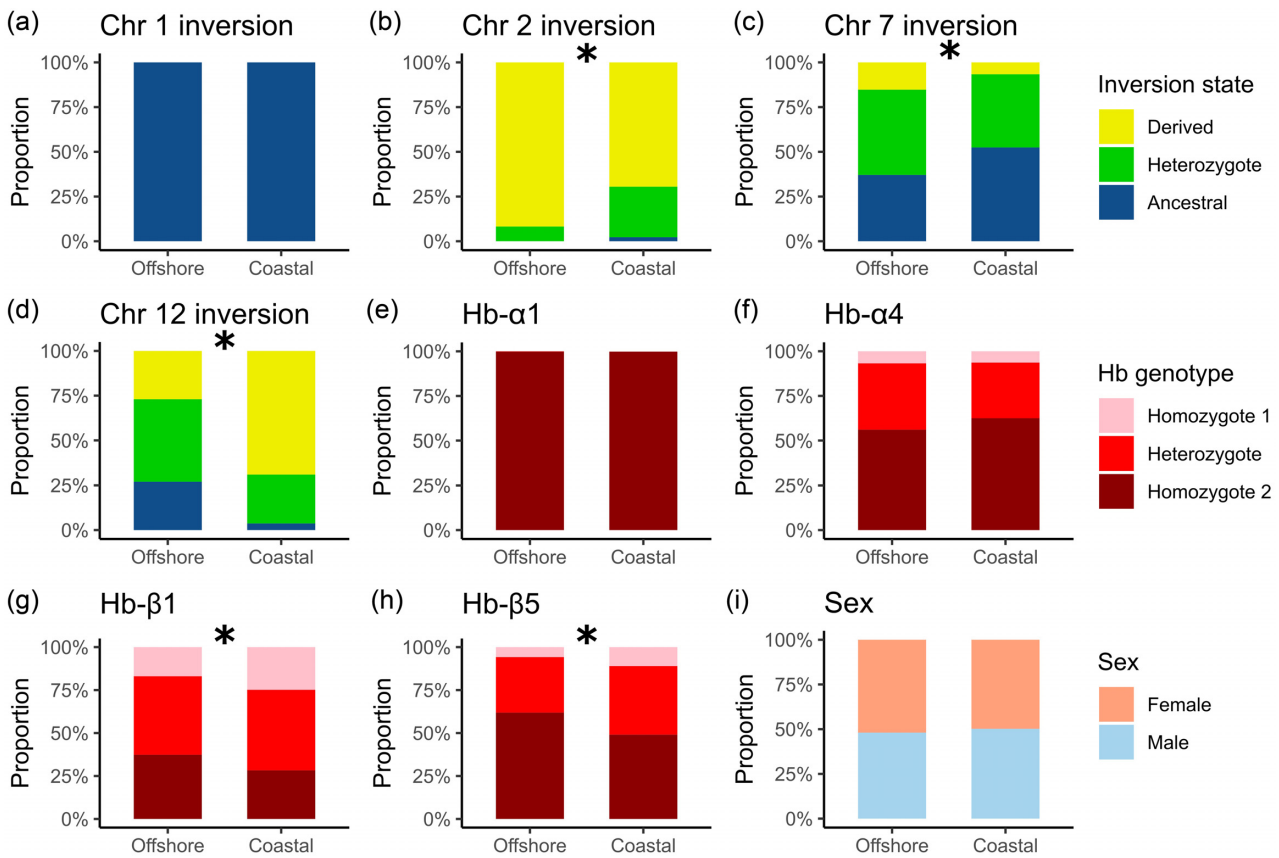


Figure 5. Bar plots showing the overall (a–d) inversion state frequencies, (e–h) genotype frequencies at haemoglobin (Hb) loci, and (i) sex ratio for each ecotype. Asterisks indicate significant F_{ST} between the ecotypes after FDR correction for multiple testing.

plot based on these pairwise F_{ST} values (Figure 6b) showed, similarly to the PCA, a large cluster clearly separated from the North Sea adults. Within this cluster, coastal ecotype juveniles from the Skagerrak, Kattegat, and Öresund clustered together with spawning adults from both the Kattegat and Öresund. In contrast to the PCA, coastal ecotype juveniles from Risør, in Norway, were separated from the main coastal cluster, indicating genetic divergence from the Swedish samples.

Discussion

Our results show that the 2019 and 2020 cohorts of Atlantic cod collected along the Swedish west coast were mechanical mixtures of offshore and coastal ecotype juveniles. The coastal ecotype was dominant in many locations inshore and in the south, even in the stronger 2019 cohort. Thus, our results shed new light on the previous postulation that juvenile cod found along the Swedish west coast mainly originate from offshore spawning areas (Svedäng, 2003; Cardinale and Svedäng, 2004). In line with the heterogeneous geographical distribution, the two ecotypes were differentiated at multiple SNP loci that may be involved in adaptation to local environmental conditions.

Ecotype distribution

The large-scale geographical distribution of the two ecotypes in Swedish waters is consistent with previous studies, showing a dominance of the offshore ecotype in offshore Skagerrak,

and a dominance of the coastal ecotype at inshore localities in the Skagerrak, southern Kattegat, and Öresund (Barth *et al.*, 2017; Knutsen *et al.*, 2018; Hüsey *et al.*, 2022). In particular, the present study provides the first fine-scaled overview of the geographical distribution of both ecotypes in Swedish waters, showing that juveniles from both ecotypes coexist in coastal areas at small spatial scales, as has been described in Norwegian fjords (Jorde *et al.*, 2018b; Knutsen *et al.*, 2018).

Model comparison suggests that the ecotypes occupy habitats with different bottom depths and that this effect is not the result of differences in the distance inshore or offshore. The correlation with depth could reflect niche partitioning between the ecotypes in coastal areas. Depth-related niche partitioning (Michalsen *et al.*, 2014) and differences in juvenile settling depths (Fevolden *et al.*, 2012) have been observed between the migratory northeast Arctic cod (NEAC) and stationary Norwegian coastal cod (NCC). The apparent utilization of different depth strata is also in line with differences in prey choice between the two cod ecotypes, which have been suggested for cod in Norwegian Skagerrak fjords (Kristensen *et al.*, 2021). Ecotype divergences associated with differences in depth distribution are well-documented in both marine and freshwater fishes, for instance in beaked redfish (*Sebastes mentella*; Cadrin *et al.*, 2010) and lake whitefish (*Coregonus sp.*; Vonlanthen *et al.*, 2009).

While the absolute abundances of each ecotype are yet to be quantified with appropriate sampling designs not covered here, our study provides estimates of the relative abundances

Table 1. AIC table for the five highest-ranked **(a)** linear regression models fitted against station-wise coastal ecotype proportions and **(b–d)** ordinal logistic regression models fitted against individual inversion state genotypes on **(b)** chr 2, **(c)** chr 7, and **(d)** chr 12 for 0-group juveniles. The top-ranked models ($\Delta AIC_c < 2$) are indicated in bold. “Accuracy” refers to the proportion of correctly assigned genotypes for each model.

| | Model | R ² | BIC | AIC | AIC _c | ΔAIC_c |
|-----|--|----------------|----------|----------|------------------|----------------|
| (a) | 1. prop_coast ~ Subdiv + log(Depth_m) | 0.59 | 55.52 | 40.71 | 41.15 | - |
| | 2. prop_coast ~ Subdiv + Dist_baseline_km + log(Depth_m) | 0.59 | 59.32 | 41.54 | 42.16 | 1.01 |
| | 3. prop_coast ~ Subdiv + log(Depth_m) + Dist_baseline_km:log(Depth_m) | 0.59 | 59.63 | 41.86 | 42.47 | 1.33 |
| | 4. prop_coast ~ Year + Subdiv + log(Depth_m) | 0.59 | 60.42 | 42.64 | 43.26 | 2.11 |
| | 5. prop_coast ~ Year + Subdiv + log(Depth_m) + Dist_baseline_km:log(Depth_m) | 0.59 | 64.57 | 43.83 | 44.66 | 3.51 |
| | Model | Accuracy | BIC | AIC | AIC _c | ΔAIC_c |
| (b) | 1. INV02 ~ Dist_baseline_km + Eco | 0.80 | 741.48 | 723.22 | 723.24 | - |
| | 2. INV02 ~ Dist_baseline_km + Eco + Sex | 0.80 | 746.18 | 723.36 | 723.39 | 0.14 |
| | 3. INV02 ~ Year + Dist_baseline_km + Eco | 0.80 | 747.12 | 724.30 | 724.33 | 1.09 |
| | 4. INV02 ~ Year + Dist_baseline_km + Eco + Sex | 0.80 | 751.86 | 724.48 | 724.52 | 1.27 |
| | 5. INV02 ~ log.Depth_m + Eco | 0.80 | 743.62 | 725.36 | 725.38 | 2.14 |
| (c) | 1. INV07 ~ Subdiv:Dist_baseline_km + log.Depth_m:Eco | 0.54 | 1 342.54 | 1 310.57 | 1 310.62 | - |
| | 2. INV07 ~ log.Depth_m + Subdiv:Dist_baseline_km | 0.53 | 1 338.56 | 1 311.15 | 1 311.19 | 0.57 |
| | 3. INV07 ~ log.Depth_m + Dist_baseline_km:Eco | 0.52 | 1 334.91 | 1 312.07 | 1 312.10 | 1.48 |
| | 4. INV07 ~ log.Depth_m:Eco | 0.53 | 1 330.55 | 1 312.28 | 1 312.30 | 1.68 |
| | 5. INV07 ~ Year + Dist_baseline_km + Subdiv:Dist_baseline_km + log.Depth_m:Eco | 0.53 | 1 348.94 | 1 312.39 | 1 312.46 | 1.84 |
| (d) | 1. INV12 ~ log.Depth_m + Dist_baseline_km:log.Depth_m + log.Depth_m:Eco | 0.61 | 1 225.39 | 1 202.65 | 1 202.68 | - |
| | 2. INV12 ~ log.Depth_m + Eco + Dist_baseline_km:log.Depth_m | 0.60 | 1 225.89 | 1 203.15 | 1 203.18 | 0.51 |
| | 3. INV12 ~ log.Depth_m + Dist_baseline_km:log.Depth_m + Dist_baseline_km:Eco + log.Depth_m:Eco | 0.61 | 1 231.17 | 1 203.88 | 1 203.92 | 1.24 |
| | 4. INV12 ~ Year + log.Depth_m + Dist_baseline_km:log.Depth_m + log.Depth_m:Eco | 0.61 | 1 231.29 | 1 204.01 | 1 204.05 | 1.37 |
| | 5. INV12 ~ Dist_baseline_km + log.Depth_m:Eco | 0.61 | 1 226.86 | 1 204.12 | 1 204.14 | 1.47 |

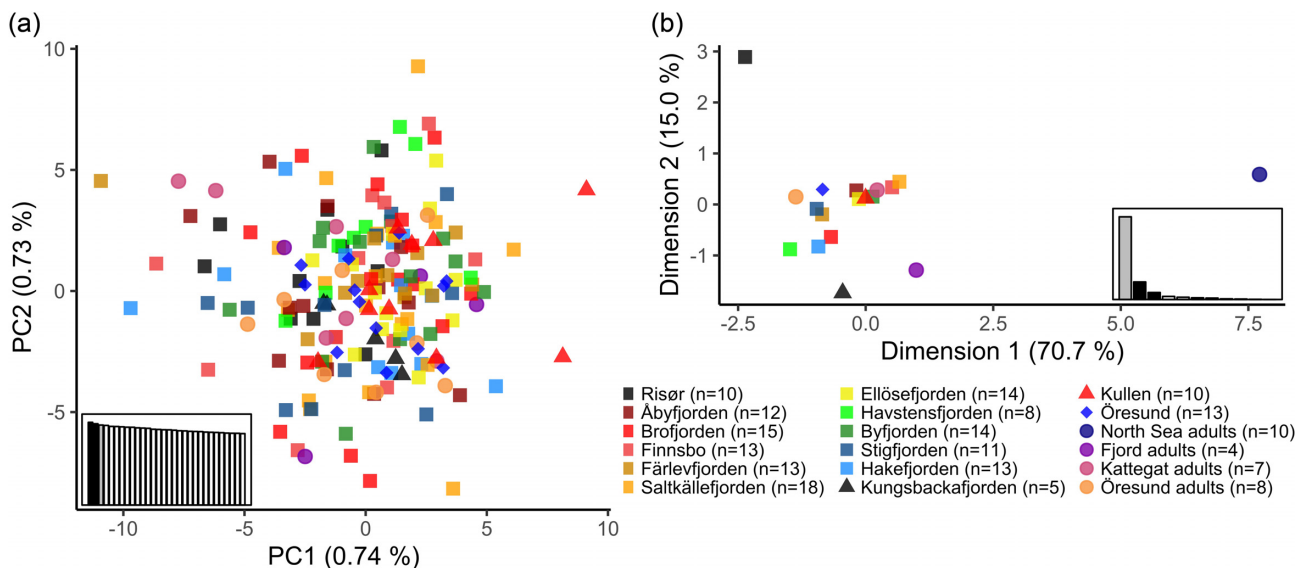


Figure 6. **(a)** PCA score plot showing the genotypic distances among individuals assigned to the coastal ecotype; and **(b)** MDS plot based on the F_{ST} values in Table 2. Note that **a** includes only individuals assigned to the coastal ecotype, whereas **b** also includes the North Sea adults as an outgroup. Genetic distances in both plots are based on the genome-wide loci located outside of inverted regions. The scree plots show the relative eigenvalues of **(a)** the first 30 PCs or **(b)** all MDS dimensions. Squares = Skagerrak juveniles, triangles = Kattegat juveniles, diamonds = Öresund juveniles, and circles = adults.

Table 2. Pairwise F_{ST} among sampling stations at genome-wide loci outside of inversions. Only coastal ecotype individuals are included, with North Sea adults as an outgroup. Significant values after FDR correction ($q < 0.05$) are indicated in bold font. Individuals are grouped based on sampling station, irrespective of survey and year, with sample sizes within parentheses. Adult samples are located at the bottom and right-hand sides of the table.

| | | | | | | | | | | | | | | | | | | |
|--------------|-------------------|-------------------|----------------|----------------------|-------------------------|----------------------|-----------------------|------------------|--------------------|--------------------|-------------------------|---------------|----------------|-------------------------|--------------------|-----------------------|----------------------|-------------------------|
| | 0.003 | 0.004 | 0.003 | 0.003 | 0.003 | 0.003 | 0.004 | 0.003 | 0.003 | 0.004 | 0.005 | 0.003 | 0.002 | 0.010 | 0.005 | 0.003 | 0.002 | Risør (n=10) |
| 0.003 | | 0.002 | 0.000 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.002 | 0.000 | 0.001 | 0.008 | 0.001 | 0.001 | -0.000 | Åbyfjorden (n=12) |
| 0.004 | 0.002 | | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.001 | 0.002 | 0.001 | 0.000 | 0.002 | 0.008 | 0.001 | 0.001 | -0.001 | Brofjorden (n=15) |
| 0.003 | 0.000 | 0.001 | | -0.000 | 0.001 | 0.000 | 0.001 | 0.000 | 0.001 | -0.000 | -0.001 | 0.000 | -0.001 | 0.007 | -0.001 | -0.000 | 0.000 | Finnsbo (n=13) |
| 0.003 | 0.001 | 0.001 | -0.000 | | -0.000 | -0.001 | 0.001 | -0.001 | 0.001 | 0.000 | 0.002 | 0.001 | -0.000 | 0.009 | 0.002 | 0.000 | 0.000 | Färlevfjorden (n=13) |
| 0.003 | 0.001 | 0.001 | 0.001 | -0.000 | | -0.001 | 0.001 | -0.000 | 0.001 | 0.000 | 0.001 | 0.000 | -0.001 | 0.007 | 0.000 | 0.001 | -0.001 | Saltkällefjorden (n=18) |
| 0.003 | 0.001 | 0.001 | 0.000 | -0.001 | -0.001 | | 0.001 | 0.000 | 0.001 | 0.000 | 0.002 | -0.000 | -0.000 | 0.008 | 0.001 | 0.002 | 0.000 | Ellösefjorden (n=14) |
| 0.004 | 0.002 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | | 0.003 | 0.003 | 0.001 | 0.002 | -0.000 | 0.001 | 0.009 | 0.002 | 0.003 | 0.002 | Havstensfjorden (n=8) |
| 0.003 | 0.001 | 0.001 | 0.000 | -0.001 | -0.000 | 0.000 | 0.003 | | 0.001 | 0.001 | 0.002 | 0.000 | -0.000 | 0.007 | 0.002 | 0.001 | 0.000 | Byfjorden (n=14) |
| 0.003 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.003 | 0.001 | | 0.000 | 0.000 | 0.001 | 0.001 | 0.009 | -0.002 | 0.001 | 0.001 | Stigfjorden (n=11) |
| 0.004 | 0.001 | 0.002 | -0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | | 0.000 | 0.000 | 0.000 | 0.009 | -0.001 | 0.002 | 0.000 | Hakefjorden (n=13) |
| 0.005 | 0.002 | 0.001 | -0.001 | 0.002 | 0.001 | 0.002 | 0.002 | 0.002 | 0.000 | 0.000 | | -0.001 | 0.001 | 0.009 | -0.002 | 0.002 | 0.001 | Kungsbackafjorden (n=5) |
| 0.003 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | -0.000 | -0.000 | 0.000 | 0.001 | 0.000 | -0.001 | | -0.001 | 0.007 | -0.001 | 0.001 | -0.000 | Kullen (n=10) |
| 0.002 | 0.001 | 0.002 | -0.001 | -0.000 | -0.001 | -0.000 | 0.001 | -0.000 | 0.001 | 0.000 | 0.001 | -0.001 | | 0.009 | -0.001 | 0.000 | -0.001 | Öresund (n=13) |
| 0.010 | 0.008 | 0.008 | 0.007 | 0.009 | 0.007 | 0.008 | 0.009 | 0.007 | 0.009 | 0.009 | 0.009 | 0.007 | 0.009 | | 0.006 | 0.007 | 0.009 | North Sea adults (n=10) |
| 0.005 | 0.001 | 0.001 | -0.001 | 0.002 | 0.000 | 0.001 | 0.002 | 0.002 | -0.002 | -0.001 | -0.002 | -0.001 | -0.001 | 0.006 | 0.001 | 0.001 | -0.001 | Fjord adults (n=4) |
| 0.003 | 0.001 | 0.001 | -0.000 | 0.000 | 0.001 | 0.002 | 0.003 | 0.001 | 0.001 | 0.002 | 0.002 | 0.001 | 0.000 | 0.007 | 0.001 | | -0.001 | Kattegat adults (n=7) |
| 0.002 | -0.000 | -0.001 | 0.000 | 0.000 | -0.001 | 0.000 | 0.002 | 0.000 | 0.001 | 0.000 | 0.001 | -0.000 | -0.001 | 0.009 | -0.001 | -0.001 | | Öresund adults (n=8) |
| Risør (n=10) | Åbyfjorden (n=12) | Brofjorden (n=15) | Finnsbo (n=13) | Färlevfjorden (n=13) | Saltkällefjorden (n=18) | Ellösefjorden (n=14) | Havstensfjorden (n=8) | Byfjorden (n=14) | Stigfjorden (n=11) | Hakefjorden (n=13) | Kungsbackafjorden (n=5) | Kullen (n=10) | Öresund (n=13) | North Sea adults (n=10) | Fjord adults (n=4) | Kattegat adults (n=7) | Öresund adults (n=8) | |

of the ecotypes at each sampling station. We found no differences in 0-group ecotype proportions between 2019 and 2020, despite a generally larger 0-group abundance in 2019 (Andersson *et al.*, 2019, 2020; Bland and Börjesson, 2020). This contrasts with what would be expected if strong cohorts were dominated by offshore juveniles (Stenseth *et al.*, 2006). Recent evidence from the Norwegian Skagerrak shows that natural selection may favour the coastal ecotype in fjords (Barth *et al.*, 2019) and that years of large cod abundance can also result from the reproductive success of the coastal ecotype (Knutsen *et al.*, 2018). Both these findings may explain the lack of differences in ecotype proportions between the years. Regardless of the mechanisms involved, the inshore ecotype composition was unaffected by cohort strength in our study, motivating further investigation of the influence of offshore spawning on the inshore cod assemblage.

Within the 2019 cohort, the coastal ecotype proportions increased from 0- to 1-group in the inshore region. This may have resulted from natural selection favouring the coastal ecotype in shallow, coastal environments (Barth *et al.*, 2019). On the other hand, the lack of 1-group cod in several of the innermost fjord locations suggests that overall survival was low in the inshore Skagerrak. Indeed, annual mortality rates as high as 75% have been suggested on the Norwegian Skagerrak coast (Olsen and Moland, 2011). In the severely depleted Skagerrak and Kattegat cod stocks, where recruitment is already reduced (ICES, 2021a, b, c, d), such large mortality rates would most likely result in reduced juvenile abundances. Alternatively, the apparent “offshore shift” within the 2019 cohort could have resulted from a net migration of 1-group cod toward the outer coastal zone. Different age classes of cod may well utilise different habitats (Pihl *et al.*, 2006), but the habitat preference, feeding ecology, and

behaviour of juvenile cod in this region have yet to be explored in detail.

Environmental adaptation

The variation at candidate loci suggests that the ecotypes may be genetically adapted to different environments. For instance, the valine allele (“homozygote 1” in Figure 5g) at the Hb- β 1 locus increases tolerance for hypoxia and low temperatures (Petersen and Steffensen, 2003). This allele is close to fixation in the migratory NEAC and the brackish-adapted eastern Baltic cod (Andersen *et al.*, 2009), likely reflecting environmental adaptation. The ecotype differences at Hb- β 1 in the present study were small but consistent with those between North Sea and Kattegat cod (Andersen *et al.*, 2009). Similar to Hb, the chromosomal inversions have been associated with salinity, temperature, and oxygen conditions (Berg *et al.*, 2015; Kess *et al.*, 2020). The most striking difference between ecotypes was in the chr 12 inversion state, as indicated both by the large genotype-frequency differences and by most genome-wide outlier loci being located within this inversion. The ancestral chr 12 inversion state was rare in the coastal ecotype, in line with evidence that homozygotes for this rearrangement have lower survival in the fjord environment (“inverted” in Barth *et al.*, 2019; see Supplementary Table S3).

Model comparison indicated that the inversion states on chrs 2, 7, and 12 were different between ecotypes but also correlated with SD, bottom depth, and/or distance from the baseline. According to the top-ranked models, the inversion state that was more frequent in the coastal ecotype also increased in frequency in the offshore ecotype with distance inshore or towards shallower depths, suggesting that coastal ecotype-like inversion genotypes are selectively favoured in coastal

habitats. Indeed, recent research suggests that the mode of natural selection (balancing or directional) on the alternative inversion states depends on both ecotype and location (Sode-land *et al.*, 2022). This motivates more research efforts aimed at linking inversion genotypes to phenotypes and environmental variables.

The outlier analysis provides additional evidence that differential environmental adaptation may underlie the offshore-coastal ecotype divergence. Multiple outlier genes involved in regulation of egg buoyancy, cold adaptation, migratory, social, and feeding behaviour, as well as growth, were identified (Supplementary Note S6). Together, the outlier gene functions may provide mechanistic insights into how the divergence has evolved and persisted between these sympatric ecotypes.

Local coastal populations

In Norwegian fjords, local populations genetically similar to, but distinct from, cod in the Kattegat and Öresund have been identified (Barth *et al.*, 2019), and both local spawning (Jorde *et al.*, 2018b) and strong resident behaviour (Knutson *et al.*, 2011; Kristensen *et al.*, 2021) have been documented. Recently, locally spawned cod eggs that assign genetically to local adults were documented also in Swedish fjords (Svedäng *et al.*, 2019). Moreover, models of pelagic egg drift on a local scale in Gullmarsfjorden and Brofjorden suggest that, if local spawning occurs, a large proportion of eggs are likely retained within fjords (P. Jonsson, pers. comm.).

The analysis of 9956 genome-wide SNP loci, however, provided no evidence that coastal ecotype juveniles inside Swedish fjords are genetically distinct from adult spawning populations in Kattegat and Öresund. Sequencing efforts with greater genomic resolution (such as whole-genome sequencing), more individuals per location, and more reference spawning adults would provide more power to detect cryptic population structure. Nevertheless, if there are reproductively isolated local populations in the Swedish coastal Skagerrak and Kattegat, genetic differentiation between them is likely minimal and potentially restricted to genomic regions not covered by our 2b-RAD sequencing. In addition, even small migration rates with limited demographic importance in marine populations can contribute to sufficient gene flow to erode any genetic population structure (Allendorf *et al.*, 2022). Hence, while the presence of genetic population structure is a strong indication of demographic independence, its absence is not evidence of the opposite. As genetic differentiation is apparently weak, future investigation of whether local spawning aggregations along the Swedish west coast represent demographically independent populations may benefit from interdisciplinary approaches combining genomics, otolith microchemistry, and tagging data.

Conclusions and implications for management

There are considerable proportions of coastal ecotype juveniles in inshore Skagerrak, which should be accounted for in fisheries management and in efforts to explain why cod abundances are declining. The dominance of the coastal ecotype in many locations along the Swedish west coast, even in a relatively strong cohort, indicates that the lack of adult cod cannot only be attributed to the offshore migration of offshore ecotype individuals. We suggest that future studies look for alternative explanations connected to the population dynamics of the coastal ecotype. Altogether, this study provides an

overview of the genetic population structure of juvenile Atlantic cod off the Swedish west coast. Our findings highlight that the cod stock of the Skagerrak, Kattegat, and Öresund is a mechanical mixture of two or more genetically distinct ecotypes. It is essential to consider this population structure and the local genetic adaptation for the conservation of Atlantic cod in this region. If genetic diversity is not preserved in severely depleted cod stocks, it may negatively affect the potential for recovery of this ecologically and (once) economically important species.

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Supplementary material

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Author contributions

SH, RP, MS, HW, and CA conceived the study. SH and OOM performed the laboratory work. SH and RP performed the data analysis. SH and CA drafted the manuscript, with RP, MS, HK, and HW contributing with revisions. All authors read and approved the manuscript.

Data availability statement

All the data underlying the results presented in this study are available at the data repository Zenodo (<https://doi.org/10.5281/zenodo.7334387>).

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