

# Genetic assignment predicts depth of benthic settlement for 0-group Atlantic cod

Ólafsdóttir, Guðbjörg Ásta; Turnbull, Shaun; Jónsdóttir, Ingibjörg G.; Nickel, Anja; Karlsson, Hjalti; Henke, Theresa; Nielsen, Einar Eg; Pálsson, Snæbjörn

Published in: PLOS ONE

Link to article, DOI: 10.1371/journal.pone.0292495

Publication date: 2023

Document Version Publisher's PDF, also known as Version of record

#### Link back to DTU Orbit

*Citation (APA):* Ólafsdóttir, G. Á., Turnbull, S., Jónsdóttir, I. G., Nickel, A., Karlsson, H., Henke, T., Nielsen, E. E., & Pálsson, S. (2023). Genetic assignment predicts depth of benthic settlement for 0-group Atlantic cod. PLOS ONE, 18(10), Article e0292495. https://doi.org/10.1371/journal.pone.0292495

#### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.

- · You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



# GOPEN ACCESS

**Citation:** Ólafsdóttir GÁ, Turnbull S, Jónsdóttir IG, Nickel A, Karlsson H, Henke T, et al. (2023) Genetic assignment predicts depth of benthic settlement for 0-group Atlantic cod. PLoS ONE 18(10): e0292495. https://doi.org/10.1371/journal. pone.0292495

Editor: Even Moland, Havforskningsinstituttet, NORWAY

Received: April 15, 2023

Accepted: September 21, 2023

Published: October 4, 2023

**Copyright:** © 2023 Ólafsdóttir et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the manuscript and can be found in  $\underline{S1}$  Table.

**Funding:** GAO. The Icelandic research fund. <u>www.</u> <u>rannis.is</u>. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

**RESEARCH ARTICLE** 

# Genetic assignment predicts depth of benthic settlement for 0-group Atlantic cod

Guðbjörg Ásta Ólafsdóttir<sup>1,2</sup>\*, Shaun Turnbull<sup>1</sup>, Ingibjörg G. Jónsdóttir<sup>2</sup>, Anja Nickel<sup>1</sup>, Hjalti Karlsson<sup>2</sup>, Theresa Henke<sup>1</sup>, Einar Eg Nielsen<sup>3©</sup>, Snæbjörn Pálsson<sup>4©</sup>

1 University of Iceland, Research Centre of the Westfjords, Bolungarvík, Iceland, 2 Marine and Freshwater Research Institute, Hafnarfjörður, Iceland, 3 DTU Aqua, National Institute of Aquatic Resources, Silkeborg, Denmark, 4 University of Iceland, Faculty of Life and Environmental Sciences, Reykjavík, Iceland

These authors contributed equally to this work.
\* gaol@hi.is

# Abstract

Atlantic cod is a keystone species that remains among the most economically important demersal fish in the North Atlantic. Throughout its distribution range, Atlantic cod is composed of populations with varying environmental preferences and migratory propensities. This life-history variation is likely to have contributed to the niche width and large population sizes of Atlantic cod, and its relative resilience to environmental change and exploitation. The lcelandic cod stock is currently managed as a single unit, but early research indicates population variation by depth and temperature and distinct offshore and inshore spawning components. Pelagic 0-group juveniles from different spawning grounds coexist in nursery areas around Iceland, but their genetic composition or habitat partitioning had not been examined post benthic settlement. In the current study we examine the genetic composition of Atlantic cod juvenile aggregations at nearshore nursery grounds in NW-Iceland and report distinct segregation by the depth of offshore and inshore juvenile cod. The physiological mechanism of this segregation is not known, but the pattern demonstrates the need to consider population structure at nursery grounds in the application of marine spatial planning and other area-based conservation tools.

# Introduction

Atlantic cod is a keystone species that remains among the most economically important demersal fish in the North Atlantic despite severe overexploitation in many of the stocks [1]. Throughout its distribution range, Atlantic cod populations vary genetically across environmental gradients, such as temperature and salinity [2, 3]. Population variation in migratory propensity is also common [4], and was early on linked to genetic markers, notably the Pan I locus [5]. The Pan I locus has since been located on linkage group 1 (LG1) on the Atlantic cod genome, a supergene divergent between cod with different migratory propensity and life history [6]. Other linkage groups, or supergenes, have also been identified as important for the Atlantic cod population structure [6–9]. The origin of these supergenes is ancient [10], and variation in cod migratory behavior has been noted throughout historical times [11]. This life-

history variation is likely to have contributed to the niche width and large population sizes of Atlantic cod, and its relative resilience to environmental change and exploitation.

The Icelandic cod has recovered in recent years, with reference stock biomass estimates exceeding 900 thousand tons since 2012 [12]. Currently managed as a single unit, stock structure of Atlantic cod in Icelandic waters has been examined for over two decades, first by identifying depth gradients in Pantophysin I (Pan I) and hemoglobin (HBI) frequencies [13] and soon after by confirming segregation by depth and geographic stock structure at the Pan I locus, microsatellites and by otolith shape and chemistry [14–16]. Later studies using genome examination of single-nucleotide polymorphism (SNPs) confirmed this differentiation and defined offshore and coastal populations based on a clustering analysis of spawning components around Iceland and Greenland [17].

Results from data storage tags (DSTs) show markedly different environmental profiles of Atlantic cod in Icelandic waters, part of the stock inhabits deeper and cooler waters both during foraging migrations and at spawning grounds [18, 19]. This environmental divergence is associated with genetic divergence on LG1 [20, 21]. The differentiation of stocks by foraging grounds [18], prompted the terms frontal and coastal cod [22], but the association of genetic stock structure and migratory behavior is likely complex [21] and there is no recent or geographically detailed genomic analysis of spawning populations of Atlantic cod around Iceland. In a study including contemporary and historical samples from Greenland, Iceland and Canada, Therkildsen et al. [17] sampled Icelandic cod from north and south coastal populations as well as at different depths at spawning grounds in south Iceland. Those results supported two genetic clusters associated with depth [17]. Further research is needed to resolve population structure of Icelandic cod and to understand how genetic structure relates to migratory behavior. However, in the current paper we make use of these previous population genetic clusters and refer to offshore or inshore populations as the identified groups by Therkildsen et al. [17]. We also use Pan I genotypes and refer to; Pan I<sup>AA</sup>, associated with cod in shallower, warmer waters, Pan I<sup>BB</sup>, associated with cod in offshore, cooler waters, and heterozygotes Pan I<sup>AB</sup> [14]. When discussing Atlantic cod grouping based on depth or temperature profiles recorded by DSTs, we refer to migratory types [18, 19].

Pelagic juveniles from different spawning grounds coexist in nursery regions around Iceland [23, 24], but there is limited knowledge on juvenile distribution following benthic settlement. The main spawning area of Atlantic cod around Iceland is off the south-west coast, where migratory types have spawned at divergent depths [25]. Eggs and larvae from the main spawning ground are carried along the west and north coast with the Irminger current and then transported with coastal currents into fjords and nearshore waters, were they settle to benthic habitats and may mix with juveniles from local spawning components [23, 24, 26]. The abundance of pelagic juveniles found in these ocean currents varies year to year [27] but in any given year, more than half of the pelagic 0-group juveniles found in the northern area were likely to have originated from the main spawning area [24, 26, 28]. Tagging and recapture of age 1 and age 2 juvenile cod in the area has further shown that they remain mostly resident in their first two or three years [29].

Many questions remain unanswered on the stock complexities and population connectivity of Atlantic cod around Iceland. Resolving population structure and dynamics in the early life stages is a critical issue in stock management, for example, understanding how the influx of juveniles into nursery grounds and recruitment out of nursery grounds varies between different stock components, and how area-based conservation tool could be used to manage genetic variation. Moreover, smaller-scale patterns, caused by behavioral or physiological mechanisms post-settlement, can be important in determining growth and survival. Atlantic cod juveniles of different spawning stock origins co-occur in specific regions [30–32] but may use different

habitats within those nursery grounds. A good example is the depth segregation of juvenile Norwegian coastal cod (NCC) and juvenile Northeast Arctic cod (NEAC) in near-shore waters off northern Norway, as the coastal cod juveniles were found in much shallower waters following benthic settlement [33]. Similar depth segregation of juveniles by ecotype was recently reported along the west coast of Sweden [34]. Conversely, in Skagerrak, coastal cod juveniles and juveniles from North Sea spawning populations inhabit nearshore nursery grounds without clear habitat or depth segregation [35, 36], although juvenile aggregations have differed temporally in genetic composition [37]. Different spawning time of ecotypes and spawning components could contribute to the segregation of juvenile cod ecotypes on nursery grounds.

The current study determined the genetic origin of Atlantic cod juvenile aggregations at nearshore nursery grounds in the Westfjords, NW-Iceland. The area is known as an important nursery area for cod with mixing of pelagic juveniles from different spawning grounds and genotypes [23, 28]. However, prior studies have not sampled 0-group juveniles post settlement or shallow tidal waters. For the current study, we repeatedly sampled juvenile cod in both shallow tidal waters and deep fjords around the Westfjords, analyzed variation in juvenile length and assigned juveniles to inshore and offshore populations using the specifically developed SNP panel described above [17]. We also determined size and Pan I alleles for a larger sample of individuals available as a measure of intra-annual variation. We ask if juvenile origin differs by sampling depth, indicating habitat segregation, or by time, indicating an influx of juveniles with different genetic makeup from different spatial or temporal spawning grounds.

#### Materials and methods

#### **Ethics statement**

The fish used for this research were either by-catch in a fishery survey conducted by the Marine and Freshwater Research Institute of Iceland (MFRI) or caught by beach seine for ecological field sampling. Neither is subject to licensing by an animal welfare or ethics committee by Icelandic law. The fish were anesthetized, immediately upon capture, by overexposure to phenoxyethanol to minimize stress or suffering.

#### Study area and samples

The juvenile cod used in this study were sampled around the Westfjords, a large peninsula in NW Iceland (Fig 1). Iceland is situated on the Greenland-Scotland ridge were the warm Irminger current from the south meets the cold currents from the north, resulting in a distinct temperature gradient between southern and northern Icelandic waters [38, 39]. The ocean front is located off the west of the Westfjords, making the peninsula an ideal system to examine biological variation across these ocean currents and the resulting temperature gradient. Juvenile cod were sampled with a beach seine (1.5 m x 20 m, mesh size = 6 mm) in tidal waters (depth < 1.5m) in four areas (fjord systems), Breiðafjörður being the southernmost area, then Arnarfjörður, Ísafjarðardjúp and Strandir being the furthest north. The beach seining occurred at the time of benthic settlement of pelagic cod juveniles, in late August to early September, and again a few weeks post-settlement in 2017 and again in 2019, at all sites except for Breiðafjörður (Table 1). In October 2019, juvenile cod were specifically sampled for this study from by-catch in shrimp trawl (40 mm mesh size cod end) in an annual shrimp survey conducted by the Marine and Freshwater Research Institute (MFRI) in İsafjarðardjúp and Arnarfjörður. As the numbers of juveniles caught as by-catch is highly variable among sampling stations juveniles were pooled from nearby stations to form four groups that represented distinct geographical variation, outer Arnarfjörður (Outer\_ARN), inner Arnarfjörður (Inner\_ARN), Skötufjörður (SKÖ) and Ísafjörður (ISA) (Fig 1). Additionally, samples from several trawl stations



**Fig 1. Map of sample sites.** The map shows the four areas and the sample sites within each area (areas and site code matching <u>Table 1</u>). Beach seining sites are indicated by a black dot and trawl sites by a black triangle. The coastline presented in the map is based on the National Land Survey of Iceland (Landmælingar Íslands) IS 50V database, made available to the authors by CC BY 4.0, downloaded 12/2018 (www.lmi.is).

of the same survey carried out in Ísafjarðardjúp in November 2017 could be used and were pooled to represent two sample groups, Ísafjörður (ISA) and Mjófjörður (MJO). The trawl sites were at depths ranging from 33–93 meters. Catch from all sampling was frozen as soon as possible pending further analysis. Subsequently, the juveniles were defrosted, weighted, their standard length (SL) measured, and a fin clip taken for genetic analysis. Note that the juveniles caught in 2017 represent a post-hoc addition to core sampling of 2019 that was specifically for this study. This resulted in a somewhat unbalanced study design, as is reflected in the number of samples per group, number of samples per depth and the different timing of the trawl survey between the two years. Moreover, the SNP analysis could only be done on the 2019 samples. Nevertheless, we concluded that the inclusion of a second year was beneficial as it allowed examination of inter-annual variation.

After examining the length/frequency distributions, juveniles < 14 cm (SL) were assigned to age as 0-group juveniles and juveniles > 14 cm as 1-year-old juveniles. This cut-off may not fully differentiate between age classes in 2017 because the trawl survey was conducted in November. This makes differentiating age classes solely on size difficult and may result in some 0-group juveniles in 2017 being misclassified as 1-year-olds. However, this is preferable to false positive assignment of 1-year-olds as 0-groups, as the current analysis is focused on the

Area	Site code	Age group	Month	Year	Gear	n SNPs	Inshore	Offshore	H <sub>E</sub>	Ho	FIS	n Pan I	AA	AB	BB
Ísafjarðardjúp	ARG	Age 0	10	2017	Seine	-	-	-	-	-	-	32	7	21	4
Ísafjarðardjúp	ARG	Age 0	8	2017	Seine	-	-	-	-	-	-	31	24	7	0
Ísafjarðardjúp	ARG	Age 0	8	2019	Seine	14	14	0	0.23	0.21	0.11	14	9	5	0
Arnarfjörður	AUD	Age 0	10	2017	Seine	-	-	-	-	-	-	18	16	2	0
Arnarfjörður	AUD	Age 0	9	2017	Seine	-	-	-	-	-	-	22	21	1	0
Arnarfjörður	AUD	Age 0	9	2019	Seine	38	38	0	0.25	0.23	0.10	38	31	7	0
Arnarfjörður	AUD	Age 0	10	2019	Seine	28	28	0	0.24	0.23	0.04	27	23	4	0
Strandir	BAS	Age 0	10	2017	Seine	-	-	-	-	-	-	19	10	7	2
Strandir	BAS	Age 0	10	2019	Seine	35	31	4	0.24	0.22	0.14	34	28	5	1
Strandir	BAS	Age 0	8	2017	Seine	-	-	-	-	-	-	26	20	4	2
Strandir	BAS	Age 0	8	2019	Seine	35	32	3	0.25	0.22	0.10	62	52	9	1
Strandir	EYJ	Age 0	8	2017	Seine	-	-	-	-	-	-	11	2	8	1
Strandir	EYJ	Age 0	8	2019	Seine	-	-	-	-	-	-	42	39	3	0
Arnarfjörður	Inner_ARN	Age 0	10	2019	Trawl	31	18	13	0.22	0.19	0.16	49	2	13	34
Ísafjarðardjúp	ISA	Age 0	10	2019	Trawl	55	14	41	0.23	0.20	0.14	35	1	11	23
Ísafjarðardjúp	ISA	Age 0	11	2017	Trawl	-	-	-	-	-	-	127	9	45	73
Ísafjarðardjúp	ISA	Age 1	10	2019	Trawl	-	-	-	-	-	-	26	8	11	7
Ísafjarðardjúp	ISA	Age 1	11	2017	Trawl	-	-	-	-	-	-	13	0	6	7
Ísafjarðardjúp	MJO	Age 0	11	2017	Trawl	-	-	-	-	-	-	23	0	16	7
Ísafjarðardjúp	MJO	Age 1	11	2017	Trawl	-	-	-	-	-	-	15	1	10	4
Arnarfjörður	Outer_ARN	Age 0	10	2019	Trawl	39	7	32	0.19	0.16	0.18	39	1	9	29
Arnarfjörður	Outer_ARN	Age 1	10	2019	Trawl	-	-	-	-	-	-	21	6	13	2
Ísafjarðardjúp	SEY	Age 0	10	2019	Seine	34	31	3	0.24	0.22	0.10	36	27	6	3
Ísafjarðardjúp	SEY	Age 0	9	2019	Seine	45	40	5	0.25	0.23	0.11	34	28	5	1
Ísafjarðardjúp	SKO	Age 0	10	2019	Trawl	40	18	22	0.19	0.17	0.142	37	2	14	21
Breiðafjörður	VAT	Age 0	9	2017	Seine	-	-	-	-	-	-	9	2	6	1
Breiðafjörður	ÞOR	Age 0	10	2017	Seine	-	-	-	-	-	-	3	2	1	0
Breiðafjörður	ÞOR	Age 0	9	2017	Seine	-	-	-	-	-	-	40	26	12	2

Table 1. Overview of the juvenile cod samples used in the current study. The table shows the four sample areas as well as each sample site within that area, separated by time of sampling. The number of juvenile cod used for assignment to inshore / offshore populations is indicated as well as Pan I allele frequencies in each sample group and summary statistics for the SNP data.

early settling 0-group juveniles, and most of the sampling (before November) assigns well two the two size groups (S2 Fig). However, the size distributions presented should be interpreted keeping this uncertainty in mind. Only three putative 1-year-old juveniles were caught with the beach seine (and excluded from any further analysis), but the size (SL) and Pan I genotypes of the 77 putative 1-year-old juvenile cod from the trawl survey were used in the current study (Table 1). Note that these sample numbers do not reflect the relative frequencies of age classes in the catch as the collection of by-catch in the shrimp survey focused primarily on the 0-group. No fish larger than 23 cm was used in this study. The geographical areas, and sample groups within areas, as they are referred to in the statistical analysis can be found in Table 1 and are depicted in Fig 1.

#### Genotyping

DNA was extracted using a Genomic DNA Purification Kit (Thermo Scientific) following the manufacturer's protocol. Representative samples of geographical and depth variation from 2019 (n = 393), were analyzed using a 96 SNP panel previously developed to resolve the

population structure of Atlantic cod around Iceland and Greenland (see Christensen et al. [40] for details of methods). A larger sample of 883 juvenile cod, from 2017 and 2019 was analyzed for the allele variation at the Pan I loci. Not all individuals genotyped at the Pan I locus could be analyzed using the 96 SNP panel and vice versa (see S1 Table for details). Pan I alleles A and B are distinguished by a single-nucleotide polymorphism (SNP) located on LG1, making the Pan locus potentially useful to differentiate between Atlantic cod inshore and offshore types [6]. Concurrence between SNP assignment and Pan I genotypes was examined when both were available for the same individuals (n = 346).

Samples were SNP genotyped using allele specific primers on a Fluidigms 96.96 Dynamic Array<sup>™</sup> IFC. Individuals genotyped for less than 60 SNPs were discarded from further analysis (a total of 13 individuals). The Pan I alleles were determined by targeting variation of the specific Pan I SNP [6] using a KASP (Kompetitive Allele-Specific PCR) assay following the manufacturer's protocol (LGC Genomics). The qPCR runs were replicated at least twice with a negative control for each eleven samples. The PCR reactions were run on a QuantStudio 3 Real-Time PCR system (Applied Biosystems), and the alleles called manually using the cloud-based Applied Biosystems analysis modules for genotyping.

#### Statistical analysis

The data used for analysis is available in <u>S1</u> Table. Data handling, statistics, and graphs were done in R 4.1.0 software [41] and by using the R package tidyverse [42] in addition to the packages and software cited below. All models were checked using the R package DHARMa [43], and the examination revealed no significant deviations from the expected distribution of residuals. All the statistical analysis was done separately for the 0-group and 1-year-old juveniles, as well as for the inshore/offshore and Pan I dataset. To examine if juveniles of different origins (inshore/offshore assignment or Pan I genotypes) from different areas, sampling times or depths (as represented by beach seine and trawl) differed in size, we used a general linear model (GLM) with log-transformed standard length as the response variable, either inshore/offshore assignment or Pan I genotype (Pan I<sup>AA</sup>, Pan I<sup>AB</sup> and Pan I<sup>BB</sup>), area (Breiðafjörður, Arnarfjörður, Ísafjarðardjúp, and Strandir), gear (trawl or beach seine), year and month as fixed effects. The model included an interaction effect between assignment/Pan I genotype and gear (acknowledging the obvious size difference between juveniles by gear) as well as the sampling area and month of sampling to examine temporal changes. The standard length of 0-group juveniles is depicted in Fig 2 and putative 1-year-old juveniles is S2 Fig.

Descriptive statistics,  $H_E$ ,  $H_O$  and  $F_{IS}$  were calculated for each sample group of the 96-SNP dataset using the Genetix 4.05 software [44]. Differentiation between samples representing sampling event or time was estimated by first calculating the pairwise  $F_{ST}$  values of all sample groups using the R package hierfstat version 0.5–10 [45]. Second, we used a discriminant analysis of principal components (DAPC), a non-model-based clustering method implemented in the R package adegenet 1.3–1 [46], to visualize the genetic composition of sample groups. We used the find clusters function in adegenet to estimate the number of clusters in our sample and find clusters used K-means clustering to maximize between group variance and minimizes within group variance. To select the optimal k, we applied the Bayesian Information Criterion (BIC) as suggested by the authors [46]. We then used DAPC on the pre-defined clusters with the dapc function in adegenet. Third, we identified the loci that had the highest loading on the axis of divergence between the identified clusters also in adegenet. Finally, we assigned the juveniles to the previously identified inshore/offshore populations originated from the SNP study of Atlantic cod population structure in Greenland and Iceland [17]. In that study 847 contemporary and historical cod tissue samples were analysed with 935 SNPs and individual



**Fig 2. Standard length of juvenile Atlantic cod populations and genotypes across fishing gear and sample sites.** The violin plots show the distribution of juvenile size in the areas sampled and by different fishing gear. The SNP assignment to inshore and offshore populations (a) and Pan I genotypes (b & c) are depicted separately and note that although the two sample years are depicted for the Pan I genotypes (b & c) the figure does not reflect variation in sampling time post benthic settlement.

genotypes clustered with DAPC [46] into four groups were two included the Icelandic samples [17].

Two binomial generalized linear models were used to examine how the likelihood of inshore vs offshore assignment and Pan I<sup>AA</sup> vs PanI<sup>BB</sup> genotypes varied across gear (trawl or beach seine), depth, area (Breiðafjörður, Arnarfjörður, Ísafjarðardjúp, and Strandir), and month of sampling. Note that gear and depth are highly correlated, but both are included in the model to examine if the juvenile genotype distribution would be better reflected by gear (indicating an abrupt shift) or depth (indicating a more gradual shift). For both models, Arnarfjordur was used as the reference (area) for the categorical factor of area.

# Results

The 0-group juveniles caught in trawls were much larger than the juveniles caught with a beach seine (Fig 2, Table 2). Moreover, 0-group juveniles were significantly smallest in Strandir, the sites furthest north. The 0-group juveniles were smaller in 2019 than in 2017 (Table 2). Juvenile standard length increased slightly by month of sampling. The standard length of juveniles assigned as offshore differed between trawl and seine samples (Table 2) but inshore/offshore assignment or Pan I genotype did not affect the standard length in other comparisons. The 1-year-old juveniles were larger in Arnarfjörður than in Ísafjarðardjúp and smaller in 2019 than in 2017 (Table 2). It should be noted that the difference in the time of the trawl survey between years could confound any between year as well as overall size comparison (S1 Fig).

Only 0-group juveniles were genotyped with the 96-SNP panel. There were no instances of heterozygote deficiency or significant  $F_{IS}$  values within sample groups (Table 1). Pairwise  $F_{ST}$  values calculated using the 96-SNP dataset were most often significant between juvenile groups sampled with a trawl at more depth and juvenile groups sampled with a beach seine (S2 Table).

The largest drop in BIC values was observed between one and two clusters, and the values then plateaued. The clear clusters (Fig 3) support that juveniles of two population components were present and that the previously identified differentiation between inshore and offshore populations [17] explained much of the variation in the SNP data. Three SNPs were found to have the highest loadings on the axis discriminating between groups: Rhodopsin, cgpGmo-S1166, and cgpGmo-S523. These loci are all on LG1.

Table 2. Results from the three generalized linear models examining variation in juvenile cod standard length (SL). The analysis was done separately for 0-group and putative 1-year-old juveniles as well as for the SNP and Pan I dataset. The results indicate differences in size by area, such as a north-south size gradient, as well as differences in sizes between years. The most notable result relating to genotype or population is that juveniles assigned to the offshore population are slightly (but significantly) larger that inshore juvenile within the trawled samples.

Predictors	0-grou	p by Pan I geno	otypes	0-group by inshore /offshore populations			1-year-old		
	Estimate	CI	p-value	Estimate	CI	p-value	Estimate	CI	p-value
(Intercept)	62.62	46.27-78.97	<0.001	0.70	0.69-0.72	<0.001	-70.42	-100.2140.62	<0.001
Pan genotype [AB]	0.01	-0.01-0.03	0.384	-	-	-	0.02	-0.01-0.05	0.186
Pan genotype [BB]	0.01	-0.04-0.05	0.787	-	-	-	0.03	-0.01-0.06	0.176
Gear [Trawl]	0.20	0.15-0.25	<0.001	0.13	0.10-0.16	<0.001	-	-	-
Area [Breiðafjörður]	-1.07	-2.14-0.00	0.051	-	-	-	-	-	-
Area [Ísafjarðardjúp]	-0.08	-0.35-0.20	0.595	0.03	0.01-0.05	-0.05 0.001		-0.100.03	<0.001
Area [Strandir]	-0.61	-0.950.27	<0.001	-0.09	-0.120.05	<0.001	-0.01	-0.09-0.07	0.777
Month	0.01	-0.02-0.04	0.538	-	-	-	-	-	-
Year	-0.03	-0.040.02	<0.001	-	-	-	0.04	0.02-0.05	<0.001
Pan genotype [AB] × Gear[Trawl]	-0.04	-0.08-0.01	0.128	-	-	-	-	-	-
Pan genotype [BB] × Gear[Trawl]	-0.02	-0.08 - 0.04	0.571	-	-	-	-	-	-
Area [Breiðafjörður] ×Month	0.12	0.00-0.24	0.042	-	-	-	-	-	-
Area [Ísafjarðardjúp] × Month	0.01	-0.02-0.04	0.457	-	-	-	-	-	-
Area [Strandir] × Month	0.07	0.03-0.10	<0.001	-	-	-	-	-	-
SNP assignment [Offshore]	-	-	-	-0.03	-0.09-0.02	0.230	-	-	-
SNP assignment [Offshore]× Gear [Trawl]	-	-	-	0.08	0.02-0.14	0.009	-	-	-
Observations	692			253			77		
R <sup>2</sup> / R <sup>2</sup> adjusted	0.751 / 0.74	5		0.683 / 0.677			0.522 / 0.488		

https://doi.org/10.1371/journal.pone.0292495.t002



Fig 3. Results from the DAPC analysis. The figure shows how the juveniles separated to the two identified clusters based on the SNP data and how the identified groups association to fishing gear.

Most juveniles (> 95%) were assigned to the previously defined offshore or inshore populations with over 90% probability. Juveniles assigned to the inshore population were much more common in seined samples (Figs <u>3</u> and <u>4</u>), but depth was the only significant predictor of the likelihood of inshore vs. offshore assignment (<u>Table 3</u>), conversely sampling gear predicted Pan I<sup>AA</sup> vs. Pan I<sup>BB</sup> genotypes (<u>Table 3</u>). It should be noted that since gear and depth were highly correlated these differing results most likely reflects the unbalances sample distribution. This is particularly relevant for the Pan I genotype dataset when sampling by gear and depth differed notably by year of sampling. Pan I<sup>BB</sup> genotypes were more likely in the later months of sampling but this likely reflects that the trawling survey samples were more frequent in later months and the shrimp survey that was conducted in November in 2017 rather than in October 2019. No other geographical or temporal variations were significant.

Examining agreement between the inshore/offshore assignment and Pan I genotypes there was a general agreement of assignment by the 96-SNP panel and the Pan I genotypes by site (Fig 4). However, there was considerable percentage of inshore individuals that were Pan I<sup>BB</sup> genotypes (7.9%), but this was much less common for offshore juveniles that rarely had a Pan I<sup>AA</sup> genotype (2.9%). Pan I heterozygotes were assigned as offshore in 33.9% of the cases.

#### Discussion

The current study shows that juveniles of both inshore and offshore Atlantic cod populations, as defined by Therkildsen et al. [17], co-occur in nursery habitats around the Westfjords in NW Iceland. However, 0-group juveniles classed to the offshore population (and Pan I<sup>BB</sup> genotypes) were rarely sampled in shallow tidal waters, and juveniles of inshore population origin (and Pan I<sup>AA</sup> genotypes) make up most of the juvenile groups sampled with the beach seine and were rare at deeper stations. This pattern was consistent between years and time after ben-thic settlement. However, for 1-year-old cod, there was a mix of Pan I genotypes in the trawled samples with Pan I heterozygotes being the most common (Table 1).

The inshore / offshore assignment by the SNP panel and the Pan I allele frequencies showed similar differentiation by depth/gear, as is to be expected as the differentiation of Icelandic cod is most notably on LG1 [21]. Heterozygotes were common in this present study as in previous studies of the Icelandic cod stock, perhaps indicating incomplete differentiation, admixture, or





a role of selection [21]. The Pan I<sup>AA</sup> individuals in the current study were most often classed as inshore cod using the SNP panel. However, the classification of Pan I<sup>BB</sup> and Pan I<sup>AB</sup> individuals was more erratic, and almost 20% of Pan I<sup>BB</sup> juveniles were assigned to the inshore population. This may highlight the relatively recent origin of Icelandic inshore populations [10] reflected in the plasticity of the Pan I<sup>BB</sup> genotypes. This was previously suggested by examining the variation in Pan I genotypes with depth and temperature profiles of Atlantic cod around Iceland [21]. Although the genetic segregation by depth in the current study is clear we suggest that a comprehensive genetic analysis including pelagic and benthic juveniles as well as individuals from spawning grounds across regions is important to further understand the dynamics of demersal juvenile Atlantic cod around Iceland.

Predictors	In	shore vs offs	shore assignment		Pan I <sup>AA</sup> vs Pan I <sup>BB</sup> genotypes					
	Odds Ratios	SE	CI	p-value	Odds Ratios	SE	CI	p-value		
(Intercept)	0.01	0.03	0.00-3.59	0.129	0.00	0.00	0.00-0.03	0.001		
Gear [Trawl]	1.48	1.60	0.17-12.07	0.716	87.92	86.85	12.92-631.99	<0.001		
Depth	1.05	0.02	1.02-1.08	0.002	1.00	0.01	0.98-1.03	0.998		
Area [Ísafjarðardjúp]	1.26	0.39	0.69-2.32	0.452	1.24	0.46	0.60-2.58	0.562		
Area [Strandir]	1.56	1.48	0.81-8.03	0.103	1.72	0.99	0.54-5.40	0.347		
Month	1.20	0.40	0.63-2.37	0.589	1.79	0.51	1.03-3.19	0.040		
Area [Breiðafjörður]					3.11	2.30	0.62-12.35	0.124		
Observations	400		·	·	624					
R <sup>2</sup> Tjur	0.432				0.702					

Table 3. Results from binomial models examining the likelihood of inshore/offshore and Pan I genotypes in different samples. Both results show the clear differentiation by depth / gear (likely to be highly correlated). Other factors explain little of the variation and the slightly higher likelihood in later sampling may simply result from the trawling survey samples being more frequent in later months.

https://doi.org/10.1371/journal.pone.0292495.t003

Any comparison of beach seine and trawl samples should consider different mesh sizes and gear selectivity. In the current study, the catchability of cod juveniles with the shrimp trawl depends on many factors, importantly other catches, and the retention of very small juveniles may be lower than with the beach seine. This could affect the size distribution reported by the different gear, but also genotype/population assignment frequencies, if genotypes differ by size. Juveniles and subadults of Icelandic cod Pan I genotypes have differed in growth rate [47, 48] and variation in growth rate has been found for Norwegian coastal and Northeast Arctic cod reared under identical conditions [49]. However, juvenile growth rate is highly affected by the environment [50-52], and environmentally induced variation in growth may mask any ecotype effect [48]. Consequently, disentangling genetic and environmental effects on juvenile cod size and growth can be difficult across very different life-time temperature regimes. For example, cod originating from offshore and inshore spawning grounds around Iceland did not differ in growth rate when measured at standardized aquaculture conditions [53]. In the current study, 0-group juvenile cod caught by beach seining were much smaller than juveniles caught in trawls, although size is more associated with geographical and temporal variation (Table 2, Fig 2). Nevertheless, the current results could partly reflect coastal juveniles being equally likely to settle at a range of depths but less likely to be caught in the shrimp trawl because of their smaller size. However, it is unlikely that fishing gear selectivity explains the lack of offshore/Pan I<sup>BB</sup> genotypes in tidal waters.

Segregation by depth of migratory and coastal ecotypes was described in northern Norway a decade ago [33]. The authors concluded that environmental preferences, perhaps based on the divergent adaptations of cod ecotypes to different salinity levels during glacial epochs, could explain the depth segregation [33]. Moreover, a recent analysis of juvenile Atlantic cod aggregations along the west coast of Sweden shows that the North Sea (offshore) and coastal juveniles segregate by depth and suggests that the divergence is associated with environmental adaptations, such as, to temperature, hypoxia, and salinity [34]. The current study cannot be used to identify specific drivers of depth divergence, and many environmental factors co-vary with depth, notably temperature and light. Icelandic fjords are not very stratified and salinity at the beach seining sites in the current study was always above 32.5 PSU. In Arnarfjörður, the only fjord were vertical salinity profiles were available, salinity varies from c.a. 32.5 PSU at the surface to 35 or 36 PSU at benthic depths [54]. Although the difference in salinity is not as pronounced as reported in Norway, the variation of 2-3 PSU in Iceland could result in habitat selection. However, temperature [55] and light regime [56] have also been associated with divergence on LG1, the most divergent genomic region between Icelandic cod migratory types [21]. Rhodopsin, a gene that encodes variant visual pigments differs by depth profiles in Icelandic cod [56] and was also an outlier in the current analysis. Still, several other stressors or impacts vary by depth. Young adult cod infested by ectoparasites have, for example, been shown to inhabit deeper waters [57], and parasite load has been suggested as a biomarker for cod ecotypes as they experience very different infestation levels in their lifetime [58]. Juveniles from inshore populations may merely be more physiologically adept to tolerate the stressors associated with the tidal habitat, for example, fluctuations in environmental factors. The benefits of residing in the shallow habitat are likely to include low predation as well as high and diverse food availability [59]. A recent study from Norway found high growth of coastal cod 0-group juveniles in very shallow water [60]. Finally, a recent study indicates a relationship between individual tendency for exploration and Pan I genotypes in 0-group juvenile cod [61] supporting that innate behavioral differences could also influence habitat choice.

It should also be considered that juvenile aggregations can differ in genetic composition because of the temporal variation in the arrival and settlement of juveniles from geographically, or temporally, different spawning components. The prevalence of juvenile cod aggregations from the North Sea vs. local groups in Skagerrak, for example, differ temporally [30, 31, 37]. Atlantic cod at the large spawning grounds off SW Iceland spawn earlier and may grow initially faster, than smaller spawning components along the west coast [23, 25]. A possible explanation for the current pattern would be that the offshore juveniles inhabited the shallow tidal habitat earlier than juveniles from inshore populations that spawn later in the season. However, no evidence was found for larger aggregations of offshore juvenile cod in tidal waters earlier in the season, within the temporal range of the current sampling, and there were no differences in the genetic composition of November 2017 and October 2019 Isafjörður (ISA) trawling sites. Therefore, we infer that the temporal variation in benthic settlement of juveniles from different spawning grounds is unlikely to fully explain the current results but recommend that a more comprehensive study, including repeated within-year sampling at both shallow and deeper sites and including otolith analysis for inference of hatch date, growth and age.

To conclude, we show that genotypes of 0-group Atlantic cod juveniles in NW- Iceland differ by depth at the time of benthic settlement, and in the following weeks. Possible environmental drivers of the divergence cannot be determined but such selection mechanisms could be experimentally examined. The current results add significantly to the two previous studies of similar segregation and highlight that physiological divergence is already clear in 0-group juvenile cod. With the clear climate-driven northward shift of Atlantic cod and the establishment of local northern populations, understanding how genotypes and environmental factors shape distribution is a critical issue. From a national management perspective, the results highlight the need for juvenile surveys to include a range of depths and times and suggest that the anthropogenic impacts in coastal waters disproportionately affect inshore juveniles. Conservation efforts should focus on protecting the total within species biodiversity, and population segregation at nursery grounds should be considered in the application of future marine spatial planning and other area-based conservation tools.

## Supporting information

**S1 Table. Dataset.** (DOCX)

S2 Table. Pairwise  $\mathrm{F}_{\mathrm{ST}}$  values between sample groups. (PDF)

**S1 Fig. Juvenile standard length distributions.** (PDF)

**S2 Fig. 1-year-old juvenile standard length.** (PDF)

## Acknowledgments

We would like to thank Chris Pampoulie for useful discussions on this manuscript. We thank Anna Ragnheiður Jónsdóttir, Rosanne Beukeboom, Michelle Valliant, and Ragnar Edvardsson for help with juvenile sampling. Moreover, we would like to thank two anonymous reviewers for many useful suggestions to improve this manuscript.

## **Author Contributions**

Conceptualization: Guðbjörg Ásta Ólafsdóttir, Shaun Turnbull, Anja Nickel, Hjalti Karlsson.

Data curation: Guðbjörg Ásta Ólafsdóttir, Shaun Turnbull, Anja Nickel, Theresa Henke.

Formal analysis: Guðbjörg Ásta Ólafsdóttir, Shaun Turnbull, Theresa Henke, Einar Eg Nielsen, Snæbjörn Pálsson.

Funding acquisition: Guðbjörg Ásta Ólafsdóttir, Einar Eg Nielsen, Snæbjörn Pálsson.

- Investigation: Shaun Turnbull, Ingibjörg G. Jónsdóttir, Anja Nickel, Hjalti Karlsson.
- Methodology: Shaun Turnbull, Ingibjörg G. Jónsdóttir, Hjalti Karlsson, Einar Eg Nielsen.

Project administration: Guðbjörg Ásta Ólafsdóttir.

Resources: Ingibjörg G. Jónsdóttir, Snæbjörn Pálsson.

Supervision: Guðbjörg Ásta Ólafsdóttir, Ingibjörg G. Jónsdóttir, Einar Eg Nielsen, Snæbjörn Pálsson.

Validation: Theresa Henke.

Writing - original draft: Guðbjörg Ásta Ólafsdóttir.

Writing – review & editing: Shaun Turnbull, Ingibjörg G. Jónsdóttir, Anja Nickel, Hjalti Karlsson, Theresa Henke, Einar Eg Nielsen, Snæbjörn Pálsson.

#### References

- Hutchings JA. Collapse and recovery of marine fishes. Nature. 2000; 406: 882–885. <u>https://doi.org/10.1038/35022565 PMID: 10972288</u>
- Bradbury IR, Hubert S, Higgins B, Borza T, Bowman S, Paterson IG, et al. Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. Proceedings of the Royal Society B: Biological Sciences. 2010; 277: 3725–3734. https://doi.org/10.1098/rspb.2010.0985 PMID: 20591865
- 3. Berg PR, Star B, Pampoulie C, Sodeland M, Barth JM, Knutsen H, et al. Three chromosomal rearrangements promote genomic divergence between migratory and stationary ecotypes of Atlantic cod. Scientific Reports. 2016; 6:1–12.
- Robichaud D, Rose GA. Migratory behaviour and range in Atlantic cod: inference from a century of tagging. Fish and Fisheries. 2004; 5: 185–214.
- Pogson GH, Mesa KA, Boutilier RG. Genetic population structure and gene flow in the Atlantic cod Gadus morhua: a comparison of allozyme and nuclear RFLP loci. Genetics. 1995; 139: 375–385. https://doi.org/10.1093/genetics/139.1.375 PMID: 7705638
- Hemmer-Hansen J, Therkildsen NO, Meldrup D, Nielsen EE. Conserving marine biodiversity: insights from life-history trait candidate genes in Atlantic cod (Gadus morhua). Conservation Genetics. 2014; 15: 213–228.
- Karlsen BO, Klingan K, Emblem Å, Jørgensen TE, Jueterbock A, Furmanek T, et al. Genomic divergence between the migratory and stationary ecotypes of Atlantic cod. Molecular Ecology. 2013; 22: 5098–5111. https://doi.org/10.1111/mec.12454 PMID: 23998762
- Kirubakaran TG, Grove H, Kent MP, Sandve SR, Baranski M, Nome T, et al. Two adjacent inversions maintain genomic differentiation between migratory and stationary ecotypes of Atlantic cod. Molecular Ecology. 2016; 25: 2130–2143. https://doi.org/10.1111/mec.13592 PMID: 26923504
- Sinclair-Waters M, Bradbury IR, Morris CJ, Lien S, Kent MP, Bentzen P. Ancient chromosomal rearrangement associated with local adaptation of a postglacially colonized population of Atlantic Cod in the northwest Atlantic. Molecular Ecology. 2018; 27: 339–351. <u>https://doi.org/10.1111/mec.14442</u> PMID: 29193392
- Matschiner M, Barth JMI, Tørresen OK, Star B, Baalsrud HT, Brieuc MSO, et al. Supergene origin and maintenance in Atlantic cod. Nature Ecology & Evolution. 2022; 6: 469–481. <u>https://doi.org/10.1038/</u> s41559-022-01661-x PMID: 35177802
- 11. Edvardsson R, Patterson WP, Bárðarson H, Timsic S, Ólafsdóttir GÁ. Change in Atlantic cod migrations and adaptability of early land-based fishers to severe climate variation in the North Atlantic. Quaternary Research. 2022; 108: 81–91.
- 12. MFRI Assessment Reports. Atlantic cod. Marine and Freshwater Research Institute, 15 June 2022.

- Jónsdóttir Ó. D. B., Imsland A. K., Daníelsdóttir A. K., Thorsteinsson V., & Nævdal G. (1999). Genetic differentiation among Atlantic cod in south and south-east Icelandic waters: Synaptophysin (Syp I) and haemoglobin (HBI) variation. Journal of Fish Biology, 54, 1259–1274.
- 14. Jónsdóttir IG, Campana SE, Marteinsdottir G. Otolith shape and temporal stability of spawning groups of Icelandic cod (Gadus morhua L.). ICES Journal of Marine Science. 2006; 63: 1501–1512.
- Pampoulie C, Berg PR, Jentoft S. Hidden but revealed: After years of genetic studies behavioural monitoring combined with genomics uncover new insight into the population dynamics of Atlantic cod in Icelandic waters. Evolutionary Applications. 2022; 16: 223–233. https://doi.org/10.1111/eva.13471 PMID: 36793686
- Petursdottir G, Begg GA, Marteinsdottir G. Discrimination between Icelandic cod (Gadus morhua L.) populations from adjacent spawning areas based on otolith growth and shape. Fisheries Research. 2006; 80: 182–189.
- Therkildsen NO, Hemmer-Hansen J, Hedeholm RB, Wisz MS, Pampoulie C, Meldrup D, et al. Spatiotemporal SNP analysis reveals pronounced biocomplexity at the northern range margin of Atlantic cod Gadus morhua. Evolutionary Applications. 2013; 6: 690–705. https://doi.org/10.1111/eva.12055 PMID: 23789034
- Pálsson ÓK, Thorsteinsson V. Migration patterns, ambient temperature, and growth of Icelandic cod (Gadus morhua): evidence from storage tag data. Canadian Journal of Fisheries and Aquatic Sciences. 2003; 60: 1409–1423.
- Thorsteinsson V, Pálsson ÓK, Tómasson GG, Jónsdóttir IG, Pampoulie C. Consistency in the behaviour types of the Atlantic cod: repeatability, timing of migration and geo-location. Marine Ecology Progress Series. 2012; 462: 251–260.
- 20. Pampoulie C, Daníelsdóttir AK, Thorsteinsson V, Hjörleifsson E, Marteinsdóttir G, Ruzzante DE. The composition of adult overwintering and juvenile aggregations of Atlantic cod (Gadus morhua) around lceland using neutral and functional markers: a statistical challenge. Canadian Journal of Fisheries and Aquatic Sciences. 2012; 69: 307–320.
- Pampoulie C, Skirnisdottir S, Star B, Jentoft S, Jónsdóttir IG, Hjörleifsson E, et al. Rhodopsin gene polymorphism associated with divergent light environments in Atlantic cod. Behavior Genetics. 2015; 45: 236–244. https://doi.org/10.1007/s10519-014-9701-7 PMID: 25577394
- Pampoulie C, Jakobsdóttir KB, Marteinsdóttir G, Thorsteinsson V. Are vertical behaviour patterns related to the pantophysin locus in the Atlantic cod (Gadus morhua L.)? Behavior Genetics. 2008; 38: 76–81. https://doi.org/10.1007/s10519-007-9175-y PMID: 17978867
- Astthorsson OS, Gislason A, Gudmundsdottir A. Distribution, abundance and length of pelagic juvenile cod in Icelandic waters in relation to environmental conditions. In: ICES Marine Science Symposia. Copenhagen, Denmark: International Council for the Exploration of the Sea. 1994; 198: 529–541.
- 24. Marteinsdottir G, Gunnarsson B, Suthers IM. Spatial variation in hatch date distributions and origin of pelagic juvenile cod in Icelandic waters. ICES Journal of Marine Science. 2000; 57: 1182–1195.
- Grabowski TB, Thorsteinsson V, McAdam BJ, Marteinsdóttir G. Evidence of segregated spawning in a single marine fish stock: sympatric divergence of ecotypes in Icelandic cod? PLoS One. 2011; 6: 17528.
- 26. Begg GA, Marteinsdottir G. Environmental and stock effects on spatial distribution and abundance of mature cod Gadus morhua. Marine Ecology Progress Series. 2002; 229: 245–262.
- 27. Jonasson JP, Gunnarsson B, Marteinsdottir G. Abundance and growth of larval and early juvenile cod (Gadus morhua) in relation to variable environmental conditions west of Iceland. Deep Sea Research Part II: Topical Studies in Oceanography. 2009; 56: 1992–2000.
- Begg GA, Marteinsdottir G. Spawning origins of pelagic juvenile cod Gadus morhua inferred from spatially explicit age distributions: potential influences on year-class strength and recruitment. Marine Ecology Progress Series. 2000; 202: 193–217.
- Saemundsson K, Jonasson JP, Begg GA, Karlsson H, Marteinsdottir G, Jónsdóttir IG. Dispersal of juvenile cod (Gadus morhua L.) in Icelandic waters. Fisheries Research. 2020; 232: 105721.
- Knutsen H, André C, Jorde PE, Skogen MD, Thuróczy E, Stenseth NC. Transport of North Sea cod larvae into the Skagerrak coastal populations. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2004; 271: 1337–1344. https://doi.org/10.1098/rspb.2004.2721 PMID: 15306331
- Stenseth NC, Jorde PE, Chan K-S, Hansen E, Knutsen H, André C, et al. Ecological and genetic impact of Atlantic cod larval drift in the Skagerrak. Proceedings of the Royal Society B: Biological Sciences. 2006; 273: 1085–1092.
- 32. Puncher GN, Rowe S, Rose GA, Parent GJ, Wang Y, Pavey SA. Life-stage-dependent supergene haplotype frequencies and metapopulation neutral genetic patterns of Atlantic cod, Gadus morhua, from Canada's Northern cod stock region and adjacent areas. Journal of Fish Biology. 2021; 98: 817–828. https://doi.org/10.1111/jfb.14632 PMID: 33244791

- Fevolden SE, Westgaard JI, Pedersen T, Præbel K. Settling-depth vs. genotype and size vs. genotype correlations at the Pan I locus in 0-group Atlantic cod Gadus morhua. Marine Ecology Progress Series. 2012; 468: 267–278.
- Henriksson S, Pereyra RT, Sodeland M, Ortega-Martinez O, Knutsen H, Wennhage H, et al. Mixed origin of juvenile Atlantic cod (Gadus morhua) along the Swedish west coast, ICES Journal of Marine Science. 2023; 80: 145–157, https://doi.org/10.1093/icesjms/fsac220
- André C, Svedäng H, Knutsen H, Dahle G, Jonsson P, Ring AK, et al. Population structure in Atlantic cod in the eastern North Sea-Skagerrak-Kattegat: early life stage dispersal and adult migration. BMC Research Notes. 2016; 9: 1–11.
- Knutsen H, Jorde PE, Hutchings JA, Hemmer-Hansen J, Grønkjær P, Jørgensen KEM, et al. Stable coexistence of genetically divergent Atlantic cod ecotypes at multiple spatial scales. Evolutionary Applications. 2018; 11: 1527–1539. https://doi.org/10.1111/eva.12640 PMID: 30344625
- Synnes AEW, Huserbråten M, Knutsen H, Jorde PE, Sodeland M, Moland E. Local recruitment of Atlantic cod and putative source spawning areas in a coastal seascape. ICES Journal of Marine Science. 2021; 78: 3767–3779.
- Astthorsson OS, Gislason A, Jonsson S. Climate variability and the Icelandic marine ecosystem. Deep Sea Research Part II: Topical Studies in Oceanography. 2007; 54: 2456–2477.
- Semper S, Våge K, Pickart RS, Jónsson S, Valdimarsson H. Evolution and Transformation of the North Icelandic Irminger Current Along the North Iceland Shelf. Journal of Geophysical Research: Oceans. 2022; 127: p.e2021JC017700.
- Christensen HT, Rigét F, Retzel A, Nielsen EH, Nielsen EE, Hedeholm RB. Year-round genetic monitoring of mixed-stock fishery of Atlantic cod (Gadus morhua); implications for management. ICES Journal of Marine Science. 2022; 79: 1515–1529.
- R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2021. URL <u>https://www.R-project.org/</u>.
- 42. Wickham H, Averick M, Bryan J, Chang W, McGowan LDA, François R, et al. Welcome to the Tidyverse. Journal of Open Source Software. 2019; 4: 1686.
- 43. Hartig F, Hartig MF. Package 'DHARMa'. R package. 2017.
- 44. Belkhir K. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. <u>http://www.genetix.univ-montp2.fr/genetix/genetix.htm</u>. 2004.
- Goudet J, Jombart T, Goudet MJ. Package 'hierfstat'. R package version 0.04-22. <u>http://www.r-project.org, http://github.com/jgx65/hierfstat</u>. 2015.
- Jombart T, Ahmed I. adegenet 1.3–1: new tools for the analysis of genome-wide SNP data. Bioinformatics. 2011; 27: 3070–3071. https://doi.org/10.1093/bioinformatics/btr521 PMID: 21926124
- 47. Jónsdóttir IG, Marteinsdóttir G, Pampoulie C. Relation of growth and condition with the Pan I locus in Atlantic cod (Gadus morhua L.) around Iceland. Marine Biology. 2008; 154: 867–874.
- Jónsson EP, Campana SE, Sólmundsson J, Jakobsdóttir KB, Bárðarson H. The effect of growth rate on otolith-based discrimination of cod (Gadus morhua) ecotypes. PLoS One. 2021; 16: p.e0247630. https://doi.org/10.1371/journal.pone.0247630 PMID: 34587180
- Svåsand T, Jørstad KE, Otterå H, Kjesbu OS. Differences in growth performance between Arcto-Norwegian and Norwegian coastal cod reared under identical conditions. Journal of Fish Biology. 1996; 49 (1): 108–119.
- Campana SE, Mohn RK, Smith SJ, Chouinard GA. Spatial implications of a temperature-based growth model for Atlantic cod (Gadus morhua) off the eastern coast of Canada. Canadian Journal of Fisheries and Aquatic Sciences. 1995; 52: 2445–2456.
- 51. Lekve K, Enersen K, Enersen SE, Gjøsæter J, Stenseth NC. Interannual variability in abundance and length of young coastal cod in the subtidal zone. Journal of Fish Biology. 2006; 68: 734–746.
- Jørgensen KEM, Neuheimer AB, Jorde PE, Knutsen H, Grønkjær P. Settlement processes induce differences in daily growth rates between two co-existing ecotypes of juvenile cod Gadus morhua. Marine Ecology Progress Series. 2020; 650: 175–189.
- Kristjánsson T. Comparison of growth in Atlantic cod (Gadus morhua) originating from the northern and southern coast of Iceland reared under common conditions. Fisheries Research. 2013; 139: 105–109.
- 54. Macrander A, Ólafsdóttir SR, Danielsen M, Karlsson H, Kristjánsson AB, Sliwinski J. Arnarfjörður: Ástand sjávar, straumar og endurnýjun botnlags / Arnarfjörður: Hydrographic conditions, currents and renewal of bottom layer. Marine and Freshwater Research Institute; 2021. Report No.: HV 2021–38.
- 55. Hemmer-Hansen JA, Nielsen EE, Meldrup D, Mittelholzer C. Identification of single nucleotide polymorphisms in candidate genes for growth and reproduction in a nonmodel organism; the Atlantic cod,

Gadus morhua. Molecular Ecology Resources. 2011; 11: 71–80. https://doi.org/10.1111/j.1755-0998. 2010.02940.x PMID: 21429164

- 56. Pampoulie C, Ruzzante DE, Chosson V, Jörundsdóttir TD, Taylor L, Thorsteinsson V, et al. The genetic structure of Atlantic cod (Gadus morhua) around Iceland: insight from microsatellites, the Pan I locus, and tagging experiments. Canadian Journal of Fisheries and Aquatic Sciences. 2006; 63: 2660–2674.
- 57. Aalvik IM, Moland E, Olsen EM, Stenseth NC. Spatial ecology of coastal Atlantic cod Gadus morhua associated with parasite load. Journal of Fish Biology. 2015; 87: 449–464. https://doi.org/10.1111/jfb. 12731 PMID: 26177748
- Hemmingsen W, Lombardo I, MacKenzie K. Parasites as biological tags for cod, Gadus morhua L., in northern Norway: a pilot study. Fisheries Research. 1991; 12: 365–373.
- 59. Juanes F. Role of habitat in mediating mortality during the post-settlement transition phase of temperate marine fishes. Journal of Fish Biology. 2007; 70: 661–677.
- 60. Dunlop K, Staby A, van der Meeren T, Keeley N, Olsen EM, Bannister R, et al. Habitat associations of juvenile Atlantic cod (Gadus morhua L.) and sympatric demersal fish communities within shallow inshore nursery grounds. Estuarine, Coastal and Shelf Science. 2022; 279: 108111.
- Beukeboom R. Phillips J. Ólafsdóttir G. Benhaïm D. Personality in juvenile Atlantic cod ecotypes and implications for fisheries management. Ecology and Evolution. 2023; 13(4): e9952. <u>https://doi.org/10.1002/ece3.9952</u> PMID: 37091554