

Where do you come from, where do you go: early life stage drift and migrations of cod inferred from otolith microchemistry and genetic population assignment

Hüssy, Karin; Albertsen, Christoffer Moesgaard; Hemmer-Hansen, Jakob; Vinther, Morten; Serre, Simon Hansen; Thomsen, Tonny Bernt; Eero, Margit

Published in: Canadian Journal of Fisheries and Aquatic Sciences

Link to article, DOI: 10.1139/cjfas-2020-0409

Publication date: 2022

Document Version Peer reviewed version

Link back to DTU Orbit

Citation (APA):

Hüssy, K., Albertsen, C. M., Hemmer-Hansen, J., Vinther, M., Serre, S. H., Thomsen, T. B., & Eero, M. (2022). Where do you come from, where do you go: early life stage drift and migrations of cod inferred from otolith microchemistry and genetic population assignment. *Canadian Journal of Fisheries and Aquatic Sciences*, *79*(2), 300-313. https://doi.org/10.1139/cjfas-2020-0409

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.



Canadian Journal of Fisheries and Aquatic Sciences

Where do you come from, where do you go: Early life stage drift and migrations of cod inferred from otolith microchemistry and genetic population assignment

Journal:	Canadian Journal of Fisheries and Aquatic Sciences	
Manuscript ID	cjfas-2020-0409.R2	
Manuscript Type:	Article	
Date Submitted by the Author:	20-May-2021	
Complete List of Authors:	Hüssy, Karin; Technical University of Denmark, National Institute of Aquatic Sciences Albertsen, Christoffer; Technical University of Denmark, National Institute of Aquatic Sciences Hemmer-Hansen, Jakob; Technical University of Denmark, Vinther, Morten; Technical University of Denmark, National Institute of Aquatic Sciences Serre, Simon Hansen; Geological Survey of Denmark and Greenland Thomsen, Tonny Bernt; Technical University of Denmark Eero, Margit; Technical University of Denmark, National Institute of Aquatic Sciences	
Keyword:	GENETICS < General, migrations, otolith microchemistry, stock mixing, spawning stock origin	
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)	

SCHOLARONE[™] Manuscripts

1	Where do you come from, where do you go: Early life stage drift and migrations of cod inferred
2	from otolith microchemistry and genetic population assignment
3	
4	
5	Karin Hüssy ^{1*} , Christoffer Moesgaard Albertsen ¹ , Jakob Hemmer-Hansen ² , Morten Vinther ¹ ,
6	Simon Hansen Serre ³ , Tonny Bernt Thomsen ³ , Margit Eero ¹
7	
8	
9	Affiliations
10	¹ National Institute of Aquatic Resources, Technical University of Denmark, Kemitorvet,
11	building 201, Lyngby Campus, 2800 Kgs. Lyngby, Denmark
12	² National Institute of Aquatic Resources, Technical University of Denmark, Vejlsøvej 39,
13	8600 Silkeborg, Denmark
14	³ Geological Survey of Denmark and Greenland, Øster Voldgade 10, DK-1350 Copenhagen
15	K., Denmark
16	
17	* Corresponding author: kh@aqua.dtu.dk, Mobile +45 93511840
18	
19	Competing interests: The authors declare there are no competing interests.
20	
21	

22 Abstract

This study investigates stock mixing of genetically distinct Atlantic cod (Gadus morhua) stocks 23 in the Kattegat, an area geographically located between the North Sea and the Baltic Sea, by 24 combining genetic population identification with habitat assignments from hatch to capture 25 from otolith microchemistry. Cod captured in Kattegat were genetically assigned to either the 26 North Sea or the endemic Kattegat population. Otolith chemical fingerprints differed 27 28 significantly between populations during the larval and pelagic juvenile stage with higher strontium and lower barium and manganese concentrations in the North Sea population than 29 30 the Kattegat population, indicating that North Sea cod are spawned in the North Sea or Skagerrak and drift into the Kattegat during the early life stages. Individual cod of both 31 populations undertook frequent, but predominantly short term, migrations to other areas than 32 the Kattegat, with < 25 % of individuals remaining resident within the Kattegat throughout 33 their life. Across seasons and age classes, the two populations were both most frequently 34 distributed in the Kattegat (67 %), with approximately 25 % of both population distributed in 35 the western Baltic Sea and less than 10 % in the Skagerrak/North Sea. This study demonstrates 36 the usefulness of this approach to infer population-specific connectivity and migration 37 trajectories for individual fish and its potential applications in basic and applied fields of fish 38 ecology and fisheries sciences. 39

40

41 Key words: genetics, migrations, otolith microchemistry, spawning stock origin, stock mixing

42

43 Introduction

Fish migrations and stock mixing are major issues for assessments of stock status and for 44 fisheries management, which are usually constrained to geographical management areas that 45 do not always correspond to the population's distribution boundaries (e.g. Kerr et al. 2017). 46 Stock assessment approaches are being developed to account for spatial stock structure (Cadrin 47 and Secor 2009; Goethel et al. 2011; Albertsen et al. 2018). However, an understanding of 48 49 migration patterns and stock mixing must be gained before the potential of these assessment models can be fully realized. The Kattegat is a good case for testing different approaches to 50 51 investigate migration and mixing, since it geographically is located between the North Sea and the Baltic Sea (Fig. 1). Atlantic cod (Gadus morhua) populations inhabit the entire area and 52 area currently managed as four stocks: the North Sea/Skagerrak, the Kattegat, the western 53 Baltic Sea/Sound and the eastern Baltic Sea, with distinct spawning areas and seasons (Brander 54 1994; Fox et al. 2008; Vitale et al. 2008; Munk et al. 2009; Hüssy, 2011; Börjesson et al. 2013). 55 Cod from these areas are clearly distinct genetically (Nielsen et al. 2003; Berg et al, 2015; 56 57 Barth et al. 2019), and we refer to these populations as the "North Sea", "Kattegat" and eastern and western "Baltic Sea" throught this study. In the Kattegat, mixing of the local cod population 58 (the Kattegat population) with adjacent populations (primarily the North Sea population) takes 59 place (Knutsen et al. 2004; ICES 2015; André et al. 2016). However, the mechanisms and 60 61 dynamics of the mixing and migrations patterns of cod in this area are currently not well 62 understood. It is believed that connectivity between these populations exists through both drift of early life stages (Eero et al. 2016; Jonsson et al. 2016; André et al. 2016) as well as migration 63 of adult individuals (Svedäng et al. 2007). There is no targeted cod fishery in Kattegat at 64 present, and cod is mainly taken as bycatch in the trawl fishery for Norway lobster (*Nephrops* 65 norvegicus) (ICES 2020). Lack of knowledge on population mixing hampers stock assessment 66

as well as limits the understanding of the recovery potential of the presently severely depletedKattegat cod population (ICES 2019).

69

As an environmental transition zone between the North Sea and the Baltic Sea, Kattegat is well 70 suited for the application of methods for inferring stock mixing. The bathymetry becomes 71 successively shallower from > 120 m in the North Sea and Skagerrak, to 40 - 80 m in the 72 northern Kattegat, to depths of 20 - 40 m in the southern Kattegat. The hydrography is 73 characterized by deep-water inflow of saline water from the North Sea and outflow of 74 75 freshwater from river-runoff in the surface. These topographic and hydrographic conditions lead to a salinity gradient ranging from fully marine saltwater in the North Sea to brackish 76 water of very low salinity in the eastern Baltic Sea (Fig. S1). This salinity gradient provides an 77 ideal setup for studying fish migrations based on chronological analyses of the chemical 78 79 composition of their otoliths.

Otoliths consist of calcium carbonate (\sim 98 %) and organic matrix (\sim 2 %) and small quantities 80 of trace elements. The biomineralization of the otolith is regulated by physiological processes 81 resulting in both daily growth increments and distinct annual growth zones reflecting 82 seasonally fluctuating conditions in temperature and food availability (Beckman and Wilson 83 1995; Weidman and Millner 2000; Høie and Folkvord 2006). Trace elements are absorbed 84 85 primarily from the water across the gill surface and therefore provide a record of environmental 86 conditions experienced by the fish (Watanabe et al. 1997; Campana 1999; Milton and Chenery 2001). The chemical composition of the water depends on the geo-chemistry of the surrounding 87 catchment and therefore provides an area-specific chemical "fingerprint" which is reflected in 88 89 the fish's otoliths (Walther and Limburg 2012). The most prominent example of an element's applicability for reconstruction of migration patterns is strontium (Sr), which since the mid 90 1990s has been considered a useful proxy for environmental salinity owing to its ability to track 91

92 fish's migrations across salinity gradients (Limburg 1995; Secor et al. 1995). Otolith chemistry has over the last three decades gained increasing attention as a tool for analysing fish stock 93 dynamics, migration patterns, pollution exposure, connectivity between areas, and plays an 94 increasingly important role as a fisheries management tool (Campana 1999; Campana and 95 Thorrold 2001; Elsdon et al. 2008; Carlson et al. 2017). Statistically significant differences in 96 element concentrations have previously been observed between cod stocks from the North Sea, 97 98 eastern and western Baltic Sea, where concentrations of strontium (Sr) and magnesium (Mg) decreased continually from North Sea to Baltic Sea, while concentrations of barium (Ba), 99 100 zirconium (Zr) and manganese (Mn) increased (Heidemann et al. 2012). The strongest differences occurred between the North Sea and the western Baltic (Mg, Mn) or between these 101 two areas and the eastern Baltic (Sr, Ba, Zr). Within the eastern Baltic Sea, no strong 102 103 differences in element compositions were observed between different spawning areas. Chemical fingerprints even suggested indications for small-scale stock-structuring and natal 104 homing as important stock separating mechanisms over short distances of < 100 km within the 105 Kattegat/Sound area (Svedäng et al. 2010). 106

107

We combined genetic population identification of cod originating from two different 108 populations (North Sea and Kattegat), but caught together in the Kattegat, with chemical 109 composition of otoliths analysed with a new state-of-the art regime-switching state-space 110 111 migration model, to examine drift and migration patterns of each individual cod. In this study we demonstrate the applicability of this approach to provide new insights into the drift and 112 migration patterns between geographically adjacent areas. More specifically, we first tested 113 whether cod caught in the Kattegat consist of a genetic mixture of populations (North Sea and 114 Kattegat). Then we tested whether North Sea cod are spawned in the same spawning areas as 115 Kattegat cod by comparing the elemental composition of the otolith core, corresponding to the 116

117 chemical signature at the time of hatching, between the two populations. Subsequently, we 118 tested if adult cod in the Kattegat perform return-migrations to the Skagerrak/North Sea or the 119 Baltic Sea by assigning area occupation throughout the lifetime of each cod using the 120 chronological record of otolith strontium concentration.

121

122

123 Materials and methods

124 Sample collection

125 Atlantic cod were sampled during research surveys in December 2016 in the Kattegat (Fig. 1). From each cod a dorsal muscle tissue sample was stored in an Eppendorph vial with 98 % 126 ethanol for genotyping. Prior to the selection of otolith samples for this study, all cod were 127 genetically assigned to either the North Sea or the Kattegat population (for details on genetic 128 population assignment, see below) and their age determined by expert readers from the 129 sectioned otoliths following routine procedures (Vitale et al. 2019). The bathymetry of the 130 Kattegat is somewhat heterogeneous, where the area south of 57 degree north is shallower than 131 the northern area. From the genotyped individuals, 306 otoliths were therefore selected to 132 ensure adequate sample sizes aiming at a uniform geographic coverage (northern and southern 133 Kattegat) within the size ranges < 35 cm, 40 - 55 cm, > 60 cm (corresponding age ranges: 0 - 1134 years, 2-3 years, > 3 years), as well as of the two populations (North Sea and Kattegat). Equal 135 136 sample distribution was not possible owing to limited catches in some of the groups. The spatial distribution and number of samples within groups are summarized in Fig. 1 and Table 1. 137 Otoliths were extracted, cleaned of adhering tissue, air dried and stored in individually labelled 138 paper bags. 139

140

141 *Genetic assignment to population of origin*

Genetic assignment to population of origin was conducted through the analyses of 192 genetic 142 143 markers (single nucleotide polymorphism, SNPs) with high levels of population differentiation in comparisons involving North Sea, Kattegat and Eastern Baltic samples. The markers were selected 144 145 from published studies (Heath et al. 2014, Barth et al. 2018; Nielsen et al. 2012) and re-analyzed in new baseline samples analyzed for the specific purpose in this study (586 fish in total baseline; 146 see Supplementary information). DNA was extracted by Chelex resin (Estoup et al. 1996) and SNPs 147 were genotyped on a Fluidigm Biomark HD system. After initial screening of the 192 markers, 5 148 markers were excluded because they did not provide reliable genotype information, resulting in a 149 final data set composed on 187 SNPs. Since the markers originated from different studies, we re-150 assessed marker independence through analyses of linkage disequilibrium in new baseline samples 151 from the North Sea, Kattegat and the eastern Baltic Sea, using the R package LDheatmap (Shin et 152 al. 2006). These analyses found low levels of LD between the markers, confirming that they would 153 provide independent information for population assignment (see Supplementary information, Fig. 154 S2). Assignment to the most likely baseline sample ("North Sea", "Kattegat", "Eastern Baltic") 155 was conducted by calculating genotype likelihoods (following Rannala and Mountain (1997)) using 156 the programme GeneClass2 (Piry et al. 2004). Population assignment to baseline samples was 157 158 based on the highest assignment score (ranging between 0 and 100; a measure of the likelihood of 159 the most likely population divided by the sum of all likelihoods) calculated for each fish. Re-160 analyses of the new panel of markers in baseline samples confirmed the high power for population assignment, as few baseline fish were mis-assigned and likelihood ratio distributions in pairwise 161 comparisons were well separated. For details, see Supplementary information (Fig. S3). 162

163

164 Otolith preparation and chemical analysis

In the laboratory, otoliths were soaked in deionized water, cleaned for 10 minutes in an ultrasonic bath of deionized water, rinsed under deionized water and left to dry overnight under a laminar flow hood in acid-washed trays. Otoliths were embedded in Epoxy resin (Struers®)

and sectioned through the core using an Accutom-100 multi-cut sectioning machine to obtain 168 a 1 cm wide block containing the rostral part of the otolith with the nucleus exposed at the 169 sectioned surface. The surface of each section was polished with 3 µm abrasive paper mounted 170 on rotating disks (Buehler®) to obtain a smooth surface and cleaned in the ultrasonic bath again 171 as described above. Trace element analyses were carried out by Laser Ablation Inductively 172 Coupled Plasma Mass Spectrometry (LA ICP-MS) at the Geological Survey of Denmark and 173 Greenland (GEUS), employing a NWR213 frequency-quintupled Nd:YAG solid state laser 174 system from Elemental Scientific Lasers (ESI) that was coupled to an ELEMENT 2 double-175 focusing, single-collector magnetic sector field ICP-MS from Thermo-Fisher Scientific. Each 176 transect line analysis used a beam diameter of 40 µm and a laser fluence of ~9.5 J/cm², a 177 repetition rate of 10 Hz, and a travelling speed of 5 µm sec⁻¹. This study focused on the 178 measurement of magnesium (²⁵Mg), calcium (⁴³Ca), manganese (⁵⁵Mn), copper (⁶⁵Cu), zink 179 (⁶⁶Zn), strontium (⁸⁸Sr) and barium (¹³⁷Ba), which all are elements known to have 180 discriminatory power in cod from this area (Heidemann et al. 2012). Concentrations are 181 reported in element: Ca ratios in ppm, using Ca as an internal standard element to account for 182 any variable sample introduction parameters affecting the ablation yield such as variation in 183 the amount of ablated material, laser energy, and ablation rate. Further details on operating 184 conditions, data acquisition parameters, analytical protocols and data processing techniques are 185 described in Serre et al. (2018) which describes the general procedure for analysing otoliths at 186 Geological Survey of Denmark and Greenland. Additional details of the analytical settings are 187 presented in the Supplementary information (Table S1), including analytical precision and 188 accuracy of the LA ICP-MS data (Fig. S4). The otoliths were analysed along a transect from 189 the nucleus to the dorsal edge of the otolith following the axis of maximum growth. The data 190 thus represent elemental signatures spanning from hatch to death of each individual. Values > 191

4x standard deviations from the mean were treated as outliers and discarded (percentage of data
discarded, Mg: 0.6 %, P: 0.3 %, Mn: 0.7, Cu: 1.9 %, Zn: 1.7 %, Sr: 0.4 %, Ba: 0.7 %).

Otolith sections were digitized using a Leica DCF290 camera at a magnification of 380 µm 194 pixel⁻¹ with a standard setup (8 bit/channel, 2048 x 1536 pixel frame). Otolith growth 195 chronologies were obtained for each individual by measuring the widths of successive opaque 196 and translucent growth bands along the laser track, from nucleus to edge using ImageJ (Rueden 197 et al. 2017). Opaque zones were divided into three equally spaced sections in order to obtain a 198 measure of sub-seasonal time of formation (spring, summer, fall). LA ICP-MS data were 199 thereafter assigned to the corresponding zones of the otolith and element concentration values 200 averaged by zone, thereby allowing to assess the data on a temporal scale representing different 201 periods in the fish's life (Fig. 2). 202

203

204 *Statistical analyses*

205 *Otolith size and growth patterns*

Linear trends in the element – fish size relationship, if present, are usually removed by subtracting the common, within-group slope of the regression. Since otolith size and fish size are strongly correlated in cod (Li et al. 2008), we tested whether the otolith size at the end of the first year of life differed between year classes and populations using ANOVA, with subsequent Tukey's Honest Significant Difference test for post-hoc pairwise comparison.

Since some of the elements analysed here are under physiological regulation, leading to the possibility of growth-related differences in element concentration (Hüssy et al. 2020), we tested whether there were differences in growth rate between the two populations. Growth patterns were compared by testing the linearity of the relationship between fish size on fish age using population as group covariate with an ANCOVA. All analyses were carried out in "R" ver. 3.4.2 (R Core Team 2020). 217

218 Spawning origin

Identification of spawning origin: Unfortunately, no larval otolith samples were available to be 219 used as baseline, therefore an accurate assignment of the early life stages is not possible. The 220 conclusions here are therefore based on the approach that if chemical fingerprints of the otolith 221 core are not statistically different between populations, all individuals have been spawned in 222 223 the same area (presumably the Kattegat). On the other hand, if the chemical fingerprints are significantly different between populations, individuals from the two populations have been 224 225 spawned in different areas, implying that individuals of the North Sea genotype were spawned in either Skagerrak or the North Sea and had been advected into the Kattegat (Eero et al. 2016), 226

while those of the Kattegat genotype were spawned in the Kattegat (Jonsson et al. 2016).

Year class effect on chemical fingerprint: The samples used in this study contain 8 different age classes, spanning the year classes 2008 - 2016 (Table 1). To rule out bias introduced by combining samples from different year classes, we tested whether there were differences in chemical fingerprints in between year classes using ANOVA, with subsequent Tukey's Honest Significant Difference test for post-hoc pairwise comparison in "R" ver. 3.4.2 (R Core Team 2020) prior to all subsequent analyses.

Chemical fingerprints: Analyses of variance are based on the assumptions that elemental concentrations are normally distributed and that variance is homogenous between groups, in the present case the two populations. Comparison of elemental concentrations between populations also requires that there is no size effect on element concentration. Element concentrations were tested for normality of distribution using Shapiro's test, and homogeneity of variance using Bartlett's test. Non-normally distributed data were (log+1)-transformed.

Univariate (ANOVA) tests were used to test for differences in individual elements and
 multivariate tests (MANOVA) on all elements combined to test for overall differences between

the two populations using the packages "MVA" (Everitt and Hothorn 2011) and "MASS" 242 (Venables and Ripley 2002) in "R" ver. 3.4.2 (R Core Team 2020). Stepwise Linear 243 Discriminant Analysis (LDA) using centred and scaled coefficients was used to identify the 244 elements that drive differences between populations and estimate their ability to correctly 245 classify individuals into the correct group using the "Ifda" package (Tang and Li 2016). 246 Differences between populations were visualized using biplots of the first two discriminant 247 248 functions against each other. In all three analyses, each specific period in the fish's life was analysed separately. To rule out bias introduced by combining samples from different year 249 250 classes, the elemental composition in the pelagic juvenile stage was compared between year classes within each population separately using ANOVA and MANOVA, prior to testing for 251 differences in elemental concentration between populations. 252

253

254 *Migration analyses*

Because otolith Sr concentration is strongly correlated with ambient concentrations, with 255 correlations generally > 0.95 found throughout the literature (review of studies in Hüssy et al. 256 (2020)) the analyses of migration patterns presented in this study rely on otolith Sr 257 concentration exclusively. Some studies indicate a potential impact of seasonal temperature 258 and growth on otolith Sr concentration (Sturrock et al. 2015; Walther et al. 2010), while others 259 found that otolith Sr patterns were unrelated to water temperature and somatic growth (Clarke 260 261 and Friedland 2004). To avoid introducing bias into the following analyses related to these potential seasonal effects of temperature and growth, otolith Sr concentrations in consecutive 262 seasons and ages were tested using an ANOVA with subsequent Tukey's Honest Significant 263 Difference test for post-hoc pairwise comparison in in "R" ver. 3.4.2 (R Core Team 2020). 264 Migration patterns were analysed based on otolith strontium concentrations using a newly 265

developed regime-switching state-space model. Specific details of this approach may be found

in Albertsen et al. (*accepted*). Here, only a brief summary of the approach is given. Throughout
this paper, "habitat preference" is referring to geographic area (see Fig. 1) and "habitat
assignment" means the geographic area each otolith Sr measurement along the otolith
chronologies is assigned to.

Since LA ICP-MS transect data have inherent measurement variability, a regime-switching state-space model was developed to filter the signal from the noise and infer habitat preference and occupancy for each measurement along a transect. The model consisted of three layers: 1. A discrete, unobserved Markov chain to describe habitat preference, 2. Estimation of the true, unobserved element level in the otolith, related to habitat occupancy, given a specific habitat preference and temporal correlation in the element concentration in addition to habitat preference, and 3. Measurement variability.

Layer 1: A discrete, unobserved Markov chain is used to describe habitat preference at any given time of the fish's life, similar to Fablet et al. (2007). For the Markov chain, a predetermined number of habitats must be given. For this application, the model included four habitats representing the four biological populations and management areas in the region. The only other restriction on habitat areas was that strontium levels increased from habitat 1 to 2 to 3 to 4 (mirroring geographic trends in salinity levels from the eastern Baltic Sea to the North Sea – see Fig. S1), to ensure areas to be identifiable.

Layer 2: Given the habitat preference at any given time of the fish's life, the true, unobserved log-strontium concentration in the otolith was modelled by a first order autoregressive process where the mean depends on the current habitat preference. The true unobserved strontium concentration is interpreted as the average concentration that would be observed for a fish at the same geographical position throughout its life. Consequently, the habitat preference in layer l determines where an individual is moving towards while the true, unobserved strontium concentration in layer 2 reflects habitat occupancy. With this interpretation, measurement noise

may be observed in measured strontium concentrations, even for an immobile fish, masking 292 the signal from the true unobserved concentration. Finally, the autoregressive process in layer 293 2 models temporal correlation in the element levels besides the habitat type to reflect that fish 294 may move between habitats at time scales that are longer than the time between measurements. 295 Layer 3: The signal-to-noise ratio will depend on inherent background spectral interferences 296 and sample volume determined by the laser beam diameter (e.g. Lear et al. 2012). Further, 297 variability at small time scales may occur from variability in unmodelled factors such as 298 temperature and food rations. Therefore, observed log-strontium was modelled by a normal 299 300 distribution where the mean was the true log-strontium level.

Model parameters were estimated simultaneously for all individuals by maximum likelihood 301 using an approximate filter (Albertsen 2018) implemented in "R" ver. r75965 (R Core Team 302 2019) using the package "TMB" (version 1.7.15; Kristensen et al. 2016). For the estimated 303 parameters, most likely habitat preference and true strontium level transects were obtained 304 through an approximate smoother (Albertsen 2018) for each individual. Subsequently, most 305 likely true strontium (Sr) levels at the edge of the otoliths, corresponding to the final 306 observation, were combined with salinity (S) levels at capture locations to estimate the 307 calibration curve (Fig. S5): 308

$$\log(Sr) = 6.241 (\pm 0.099 \text{ se}) + 0.442 (\pm 0.030 \text{ se}) \cdot \log(S)$$
(1)

This calibration curve was derived from cod otolith samples with associated water salinity levels from all four habitat areas, but are not presented in this study, and through comparison with experimentally derived values from the literature (Albertsen et al. *accepted*).

While the state-space migration model estimates four levels of habitat-specific salinity values, it needs to be informed of the threshold salinities that characterize the geographic boundaries from one habitat area to its neighbour. Salinity cut-off values between habitat areas of interest

(North Sea, Kattegat, western Baltic Sea and eastern Baltic Sea) were determined based on 316 annual mean salinities for the years 2013-2016 from ICES Hydrographic database (covering 317 the entire lifespan of all fish in the study) (Available at https://ices.dk/data/data-318 portals/Pages/ocean.aspx) and known depth distribution ranges of cod: 30 - 80 m in the 319 Skagerrak and Kattegat (Casini et al. 2005), 10 - 40 m in the western Baltic Sea and 30 - 60320 m in the eastern Baltic Sea (Oeberst 2008). Cut-off salinities selected at the boundaries between 321 322 areas were 10 psu between eastern and western Baltic Sea, 17.5 psu between western Baltic Sea and Kattegat, and 34 psu between Kattegat and Skagerrak/North Sea. An overview over 323 324 geographic areas with associated salinity levels and data selected is given in Fig. S1. Using the strontium – salinity calibration curve, the strontium levels corresponding to these salinities was 325 calculated. 326

In summary, with this approach each Sr measurement along the individual chronological otolith transects is translated into a corresponding salinity value and be assigned to one of the four habitat preference areas (example in Fig. 2). In order to simplify the visualisation of habitat preferences, preferences were grouped by time interval (spring, summer, fall, and winter of each year of life) and the most frequently occurring assignment chosen to represent a single habitat preference per individual and time interval.

Habitat assignment precision: One of the key features of the regime-switching state-space 333 model used for assigning habitat association is a strontium - salinity calibration curve, 334 described in model (1). Here, we use the assignments of individuals to the Skagerrak/North 335 Sea for demonstration, since this is the area where habitat assignments are most uncertain as 336 the area-specific differences in salinity are less pronounced than between other areas and the 337 logarithmic relationship of the calibration curve at the same time is flattening. The confidence 338 intervals of the salinity - strontium calibration curve at the salinity cut-off between the Kattegat 339 340 and the Skagerrak/North Sea (34 psu) were used to estimate the proportion of individuals

assigned to the Skagerrak/North Sea. Habitat assignments were carried out for salinities estimated from the mean parameter values of model (1) as well as from mean \pm standard error of the mean.

344

345 **Results**

346 *Characterisation of the two populations: Genetics, otolith size and growth*

Genetic population identification: Among the 306 fish analysed for population assignment, 141 assigned to the Kattegat while 165 assigned to the North Sea (Table 1). No fish assigned to the Eastern Baltic Sea population. The assignment scores were generally very high (average score of 97.97, range: 61.35-100.00), indicating high support for population assignment for the majority of the individuals. A few individuals had scores below 75 (4 from the North Sea and 4 from the Kattegat).

Otolith size: Otolith size at the end of the first year of life was significantly different between 353 populations but did not differ between year classes within populations (ANOVA, df = 10 and 354 191, $p_{population} < 0.05$, $p_{age} > 0.05$). Pairwise comparison of all groups showed that the 355 population- related difference was caused by North Sea individuals of the age groups 1 and 2 356 having larger otoliths (Tukey's HSD, all p < 0.05), while there were no differences among all 357 other groups (Tukey's HSD, all p > 0.05) (Fig. 3). Since otolith size and fish size are strongly 358 correlated in cod (Li et al. 2008), this suggests that there were only limited differences in fish 359 size at first winter between year classes and populations. For the analyses of elemental 360 composition during the first three life stages (larval, pelagic and demersal juvenile) it is 361 therefore not necessary to remove any size-related trend as this is not present. 362

363 *Growth patterns*: Growth patterns were compared by testing the linearity of the relationship 364 between fish size and age using population as group covariate. Fish size was linearly related to 365 the age of the fish (Fig. 4) but with significant differences in the intercept and slopes between populations with a smaller intercept and higher slope in the North Sea population (ANCOVA, F = 2944, df = 4 and 296, p < 0.05, $r^2 = 0.98$). A higher slope in the North Sea population indicates faster growth than the Kattegat population, and needs to be considered in the discussion of growth-related differences in element concentration.

370

371 Spawning origin

372 Year class effect on chemical fingerprint: For both the North Sea and the Kattegat population, significant differences in chemical fingerprint during the pelagic juvenile stage were detected 373 between the 8 year classes (North Sea: MANOVA, df = 7 on 292, p < 0.05; Kattegat: 374 MANOVA, df = 7 on 130, p < 0.05). For both populations, these differences between year 375 classes were attributable to Mg, Mn, Cu, and Zn (ANOVA, df = 1, p < 0.05 for all). No 376 significant differences were found for P, Sr and Ba (ANOVA, df = 1, p > 0.05 for all). Even 377 though there were significant differences between year classes within each population, these 378 were restricted to elements that are under physiological control (Campana 1999; Hüssy et al. 379 2020), and not in Sr and Ba, which are the element of key interest for inferring migration 380 history. Year class effects were therefore not considered relevant in the subsequent analyses. 381

382

Pelagic juvenile stage: The chemical fingerprints in the pelagic juvenile stage differed 383 significantly between the two populations (MANOVA, df = 7 on 292, p < 0.05). Significant 384 385 differences occurred in all elements except for P (ANOVA, df = 1, p < 0.05 for all except P). Of particular interest are Sr, Ba and Mn, where Sr was higher in the North Sea population, 386 while Ba and Mn were higher in the Kattegat population (Table 2). Differences between year-387 classes within the two populations were not significant (MANOVA, df = 7 on 292, p > 0.05). 388 The LDA showed that 83 % of the North Sea population and 68 % of Kattegat population were 389 correctly classified to their respective population with an overall classification success of 76 % 390

based on the first four discriminant functions. The biplot of the first two discriminant functions 391 against each other with loadings (representing individual elements) showed that in particular 392 Sr, Ba and Mn drive population-specific fingerprints in the pelagic juvenile stage (Fig. 5). In 393 this plot, LD1 explains 41.4 % of the variation in chemical fingerprint between populations 394 and LD2 21.1 %. A considerable part of the variation (37.5 %) is thus explained by dimensions 395 that are orthogonal to the first two dimensions, and thereby difficult to represent visually. 396 Together, the MANOVA and LDA results of the chemical fingerprint in the pelagic juvenile 397 stage show that cod of different populations captured at the same locations in the Kattegat have 398 399 different spawning origins.

400

Juvenile demersal life stages: In order to address during which life stage (pelagic juvenile, 401 402 demersal juvenile, first winter, first, second etc. year of life) the North Sea cod enter Kattegat, the ANOVA, MANOVA and LDA analyses were repeated for each of the progressively older 403 life stages. The results for the demersal juvenile stage mirrored those for the pelagic stage, with 404 significant differences between populations (MANOVA, df = 7 on 292, p < 0.05), where 405 differences were primarily driven by Sr, Ba and Mn (Fig. 5, Table S2). There were no statistical 406 differences in concentration of any elements during the subsequent first winter (MANOVA, df 407 = 7 on 292, p > 0.05) (Fig. 5, Table S3). The classification success decreased correspondingly 408 from 83 % to 72 % and 50 % in the North Sea population and from 68 % to 62 % and 51 % in 409 410 the Kattegat population (total classification success decreasing from 76 % to 67 % and 51 %), which is illustrated by the increasing overlap between groups in the LDA biplot (Fig. 5). From 411 age 2 onward, differences between populations were no longer significant (MANOVA, df = 7412 on 292, p > 0.05). 413

414

415 *Adult migrations*

The comparison of otolith Sr concentration between consecutive seasons and ages found 416 significant differences overall (ANOVA, df = 19, p < 0.05), no significant differences were 417 evident between any of the consecutive seasons and ages in the pairwise comparisons (Tukey 418 HSD, all p > 0.05). This shows that there is no consistent seasonal effect on otolith Sr 419 concentration. It was therefore assumed, that any change in otolith Sr was associated with a 420 change in habitat occupation and not seasonally varying temperature or growth. From the 421 422 regime-switching state-space migration model habitat area assignments (Fig. 1) were obtained for all time periods of each individual's life. Examination of individual cod's habitat area 423 424 occupation patterns of the North Sea population (Fig. 6a) and the Kattegat population (Fig. 6b) showed that the majority of individuals from both populations showed migrations in and out 425 of the Kattegat, with 25 % (n = 21) of the North Sea individuals and only 8 % (n = 9) of the 426 Kattegat individuals remaining resident within the Kattegat from the first winter and until 427 capture (Fig. 6, green). The majority of individuals from both populations showed evidence of 428 migration to other areas at some point between the first winter and time of capture. By far the 429 most frequent migration signature was alternating distribution in the Kattegat and western 430 Baltic Sea (55 % (n=47) of North Sea cod; 68 % (n = 78) of Kattegat cod) (Fig. 6, blue green), 431 while a proportion of fish from both populations also displayed signatures consistent with 432 migration to the Skagerrak/North Sea and back to the Kattegat (31 % (n=27) of North Sea cod; 433 45 % (n = 52) of Kattegat cod) (Fig. 6, lime). Ten individuals (8.5 %) from the Kattegat 434 population appeared to have visited the eastern Baltic Sea for shorter periods of time (Fig. 6, 435 purple). In general, the migration patterns were not consistent between seasons, suggesting the 436 absence of synchronized migrations between areas. 437

438

While the habitat area assignments of individuals (Fig. 6) provide insight into individual fish's
movements between areas, they do not provide an easily interpretable picture of how much

time the two populations on average spend in the different areas. To get an estimate of how 441 many individuals had been assigned to the four habitats at any given fish age and time of the 442 year, percentages of habitat area assignments were calculated across all individuals for each 443 age and season separately. The resulting percentages thus represent a population's distribution 444 pattern in space (North Sea/Skagerrak, Kattegat, western and eastern Baltic Sea) and time (age 445 and season). The most notable observation is that habitat distribution patterns are remarkably 446 447 similar in the two populations (Fig. 7). Across all four seasons and all four age classes, the two populations were both most frequently distributed in the Kattegat, with 66.8% (range = 44 - 81 448 %) of North Sea population and 67.1 % (range = 53 - 79 %) of the Kattegat population (Fig. 449 8, green bars), making the Kattegat the primary habitat of distribution. Across seasons and age 450 classes, approximately 25 % of both population was distributed in the western Baltic Sea (North 451 Sea: mean = 24.9, range = 0 - 50 %; Kattegat: mean = 23.5, range = 8 - 37 %) (Fig. 7, blue 452 green bars). Less than 10 % of both populations were distributed within the Skagerrak/North 453 Sea (North Sea: mean = 8.3, range = 5 - 12 %; Kattegat: mean = 7.9, range = 3 - 13 %) across 454 all seasons for the age classes 1 - 3 years (Fig. 7, lime bars). An apparently higher degree of 455 migrations between the Skagerrak/North Sea and back to the Kattegat seemed to occur during 456 age class 4 in the North Sea population, in that increasing percentages (mean = 26.6, range = 457 13 -50 %) were distributed within the Skagerrak/North Sea. However, it is important to note 458 that the sample sizes are relatively low for these age groups and inferred distribution patterns 459 therefore somewhat uncertain. Surprisingly, in the Kattegat population 1.4 ± 1.3 % of 460 individuals were assigned to the eastern Baltic Sea for restricted periods of time around fall 461 and winter (Fig. 7, purple bars). Migration patterns of fish older than four years could not be 462 analysed for the North Sea population, as no samples of this population were available. 463

464

Habitat assignment precision: Correct habitat area assignment relies heavily on the strontium 465 - salinity calibration curve used as well as the salinity cut-off values used for defining the 466 boundary between areas. The impact of salinity cut-off values selected for defining the 467 boundary between areas on estimated habitat assignments was evaluated with a simple 468 sensitivity analysis. Since this selection is more related to the study area, but is less relevant 469 for the methodological approach as such, these result are not presented here, but may be found 470 471 in the Supplementary information (Fig. S6) together with details on the hydrography in the study area (Fig. S1). The impact of uncertainty in the strontium – salinity calibration curve on 472 473 the other hand is a generic issue. Here, we demonstrate the impact thereof on the number of individuals assigned to the Skagerrak/North Sea, because the environmental salinity at the 474 boundary between this area and the Kattegat is at the upper end of the salinity range, where the 475 calibration curve flattens. This exercise shows, that the uncertainty in the calibration curve on 476 proportion of individuals assigned to the Skagerrak/North Sea results in an average deviation 477 of 3.4% for both populations (North Sea: range 0 to 12.5%; Kattegat: range 0.9 to 6.5%) 478 between the highest (mean - se) and lowest (mean + se) estimates (Fig. 8). 479

480

481 **Discussion**

Spawning origin: In this study, we found population-specific chemical fingerprints in the early 482 life stages of cod in Kattegat. The elements driving this differentiation were Sr, Ba and Mn. 483 484 These elements are under strong environmental control. Strontium has for over two decades been known to reflect ambient salinity, owing to the fact that the Sr content in marine habitats 485 is fairly constant worldwide, and mixing with freshwater not only dilutes salinity but also the 486 Sr concentration (Kraus and Secor 2004; Walther and Limburg 2012). Because otolith Sr 487 concentration is strongly correlated with ambient concentrations, with correlations generally > 488 0.95 found throughout the literature (review of studies in Hüssy et al. (2020)), otolith Sr is a 489

useful proxy for environmental salinity and is globally used to track movements of fish between 490 marine and freshwater and within estuaries (Bath et al. 2000; Elsdon and Gillanders 2003; 491 Miller 2011; Sturrock et al. 2012). While several studies report a significant impact of 492 temperature on otolith Sr, the results are largely conflicting, ranging from a positive influence 493 to a negative (Bath et al. 2000) – or no influence at all (Clarke and Friedland 2004; Walther et 494 al. 2010; Sturrock et al. 2015), explaining at best a few percent of the variation in otolith Sr 495 496 (Hüssy et al. 2020). Also food (Walther et al. 2010) and other physiological factors (Sturrock et al. 2015) have been suggested to regulate otolith Sr concentrations. However, similar to other 497 498 studies on Atlantic cod from the North Sea to the Baltic Sea (Hüssy et al. 2016; Heimbrand et al. 2020; Hüssy et al. 2021), the samples used in the present study showed no apparent 499 seasonality in otolith Sr patterns, suggesting that changes in otolith Sr are associated with a 500 501 change in habitat occupation rather than seasonally varying temperature or growth.

502 The higher Sr concentrations in the North Sea population are thus consistent with a spawning area in more saline water than the Kattegat. Barium shows a nutrient-like distribution in the 503 aquatic environment that is strongly related to environmental salinity with depletion in surface 504 waters, with higher concentrations in freshwater and nearshore areas (Elsdon and Gillanders 505 2005; Walther and Limburg 2012). Highest Ba concentrations generally occur at salinities 506 between 5 and 20 psu (Walther and Limburg 2012). The Ba concentration in otoliths almost 507 exclusively reflects ambient concentrations (Bath et al. 2000; Elsdon and Gillanders 2003; 508 509 Hicks et al. 2010; Miller 2011; Reis-Santos et al. 2013). Consistent with the hypothesis that the North Sea population is spawned in a more offshore environment, Ba concentrations were 510 higher in the Kattegat population. Manganese concentrations on the other hand are known to 511 increase in hypoxic areas owing to the reduction of manganese oxides from the sediment with 512 decreasing ambient oxygen content. Otolith Mn concentrations have proven useful for tracking 513 hypoxia exposure in e.g. Baltic cod (Limburg et al. 2011, 2015). Prolonged seasonal hypoxia 514

21

is known to occur in the Kattegat (Rosenberg et al. 1992, 1996), which is reflected in the much
higher Mn concentration during the early life stages of the Kattegat population.

Significant differences in particularly Mg, P, Cu and Zn were also detected between a few year 517 classes within the two populations. These elements are under strong physiological control 518 (Campana 1999; Sturrock et al. 2015; Limburg et al. 2018; Hüssy et al. 2020) and have been 519 shown to reflect fish growth (Heimbrand et al. 2020; Hüssy et al. 2021). Otolith element 520 521 concentrations of these elements were consistently higher in the North Sea populations. This corresponds with the larger size at age observed between populations and suggests a genetic 522 523 component in growth regulation, and a mechanism promoting early settlement of North Sea cod. No differences between year classes in all other elements shows that the classification to 524 population based on Sr, Ba and Mn is not influenced by inter-annual growth rate differences. 525

526 These results provide consistent and biologically meaningful evidence for the hypothesis that 527 the North Sea population was spawned in a more marine and offshore environment compared 528 to the Kattegat population, presumably the Skagerrak or northern North Sea.

529

Early life stage drift: The rapidly decreasing classification success from the pelagic juvenile 530 stage to the beginning of the first winter and the lack of significant differences in 531 environmentally related elements (Sr, Ba, Mn) in subsequent years, suggests that the North Sea 532 fish arrived in the Kattegat during the early life stages. Kattegat cod spawn within a restricted 533 534 area of the Kattegat (Vitale et al. 2008; Börjesson et al. 2013), and early life stages of this population have in recent years largely been retained in the area (Jonsson et al. 2016). North 535 Sea cod spawn along the southern and eastern edges of the Dogger Bank, in the German Bight, 536 537 the Moray Firth and to the east of the Shetlands (Brander 1994; Fox et al. 2008; Munk et al. 2009). Drift simulations have shown that cod eggs, larvae and early juveniles spawned in the 538 North Sea may be advected for more than 600 km during their drift period (Eero et al. 2016). 539

Particularly individuals spawned in the German Bight may frequently be transported into the 540 Skagerrak (Eero et al. 2016). The early lifestages are entrained by the Jutland current running 541 north along the west coast of Denmark into the eastern part of Skagerrak (Svansson 1975; 542 Dyrssen 1993; Jakobsen 1997). During westerly winds, strong currents prevail from the 543 Skagerrak towards the Baltic Sea along the coast of Sweden. An inflow of North Sea cod into 544 the Kattegat during their early life stages is thus highly likely, where drift durations from the 545 North Sea to the Kattegat may range from days to months (Eero et al. 2016). This inter-annual 546 and inter-individual variability in drift duration explains the lack of a stronger separation of 547 548 individuals in the pelagic juvenile stage and the gradually decreasing classification success between the two populations with age. Genetic studies of juvenile cod from the fjords from the 549 Norwegian Skagerrak coast to the central Kattegat support this, in that a large proportion can 550 be genetically similar to cod from offshore spawning areas in the eastern North Sea, with 551 substantial temporal variation in advection (Knutsen et al. 2004; Stenseth et al. 2006; André et 552 al. 2016). Owing to the low stock size of Kattegat cod and the influx of individuals from other 553 areas, Jonsson et al. (2016) estimated that currently only approximately 34 % of the juveniles 554 in Kattegat are from locally retained spawning areas compared to 83 % in the 1970s when the 555 stock was larger. 556

557

Migrations between Kattegat and Skagerrak/North Sea: The present study showed that even though the majority of individuals undertook predominantly short-term migrations to other areas, they were most frequently distributed within the Kattegat, regardless of their population of origin. These results suggest the absence of strong synchronised migrations between areas. Only a few individuals (< 10 %) were distributed within the Skagerrak/North Sea with subsequent return to the Kattegat. The absence of North Sea cod individuals older than four years old suggests that the North Sea cod leave the Kattegat, presumably returning to the North Sea once mature in support of natal homing as suggested by Svedäng et al. (2007). From our
data it is not possible to deduce whether the migratory cod did in fact spawn during their stay
in the Skagerrak/North Sea.

The habitat assignments largely depend on the salinity cut-off value selected as defining the 568 boundary between areas and the accuracy of the strontium – salinity calibration curve. Since 569 both salinity and strontium are subject to measurement errors, the calibration curve is subject 570 571 to some uncertainty, in particular at the Kattegat/Skagerrak boundary, where area-specific differences in salinity are less pronounced than between other areas and the logarithmic 572 573 relationship of the calibration curve at the same time is flattening. Although a rigorous crossvalidation would be needed to estimate the power of our method, we did estimate effects from 574 calibration curve uncertainty and different cut-off salinity values used for categorizing areas 575 and found only minor variations in these proportions, suggesting the presented approach to be 576 relatively robust. Consequently, our results suggest some migration between the Kattegat and 577 Skagerrak/North Sea. We also found some evidence for inter-individual variability in 578 migratory behavior with some fish displaying a more stationary signature while other fish 579 seemed to display signatures in agreement with a pattern of recurrent migrations and longer 580 time occupancy in the North Sea. 581

Information of juvenile and adult cod migrations is available from tagging experiments from 582 the 1950s, 1990s and 2000s and have documented migration patterns similar to the ones 583 584 inferred from the present study. These studies found that cod tagged as 1-year old juveniles in Kattegat only undertook limited migrations until the age of 2 years and 30 - 50 cm in length, 585 when they moved offshore towards the south and west – towards the spawning grounds in the 586 southern Kattegat and the eastern North Sea (Pihl and Ulmestrand 1993). Adult cod tagged at 587 offshore and in coastal locations in the Skagerrak undertook long-distance migrations ranging 588 from the southern North Sea to the central Kattegat (Danielssen 1969). Migrations of adult cod 589

tagged along the Swedish Kattegat coast were directed both south and north towards the Sound 590 and the eastern North Sea, with some coherence between tagging location and migration 591 direction (Righton et al. 2010; Svedäng et al. 2010). Of the cod tagged in the northern Kattegat, 592 12 % were recaptured in the Skagerrak or eastern North Sea, while cod tagged in the Sound 593 and the southern/central Kattegat were primarily resident (Svedäng et al. 2010), suggesting 594 migration patterns quite similar to the distribution patterns observed in the present study. 595 596 Geolocation of individuals based on electronic tags (DST's) documented that the migrations of cod tagged in the northern Kattegat and Skagerrak are directional towards the North Sea and 597 598 coincide with known spawning times (Svedäng et al. 2007). Subsequent returns to the areas where the cod were tagged also occurred, but quantification thereof was not possible (Svedäng 599 et al. 2007). Linking genetics with tagging-based migration patterns, André et al. (2016) 600 601 suggested a strong correspondence between population and philopatric migrations towards 602 natal spawning grounds in the Skagerrak/Kattegat area. One may argue that these tagging studies represent migration patterns at a time when the cod stocks were not yet depleted and 603 may therefore not apply to today's scenarios. However, given the similar conclusions from 604 widely different analytical approaches and a large range of years, it seems that the migratory 605 exchange between Kattegat and Skagerrak/North Sea is - and has always been - limited in 606 immature cod, but extensive in mature cod and most likely linked to spawning migrations. 607

608

Migration between Kattegat and western Baltic Sea: This study also found that a large proportion (approximately 25 %) of individuals from both populations were distributed within the western Baltic Sea during all seasons and across all age classes. The salinity regimes in the western Baltic and Kattegat differ considerably from each other with mean bottom salinities of 10 - 15 psu and 30 - 33 psu respectively. This salinity gradient lies within the ideal range for discriminating habitat use (Kraus and Secor 2004; Walther and Limburg 2012), and the results

are therefore considered reliable. The migration patterns of tagged cod discussed above do not 615 suggest a pronounced migration further south than the Sound. However, the dynamics in stock 616 size have changed considerably over the last decades, with unknown impact on migratory 617 behaviour. Considerable numbers of eggs and larvae spawned in the western Baltic Sea, more 618 specifically the Great Belt and the Kiel Bay, may drift into the Kattegat and Skagerrak 619 depending on wind conditions prevailing during the spawning season (Huwer et al. 2016). 620 621 Provided that these individuals also show philopatric migration behaviour, one would expect to find seasons with high assignments to the western Baltic Sea. A clear seasonal pattern with 622 623 higher proportions of western Baltic Sea signals in spring (the main spawning season of western Baltic cod) was, however, not evident. The occurrence of 2 % cod with a clear western 624 Baltic/Sound otolith chemistry signal over most of their lives prior to being captured in the 625 Kattegat further suggests considerable connectivity between these areas. Cod spawning in the 626 Kattegat and western Baltic Sea likely belong to the same biological population or population-627 complex which has been found to display a hybrid zone genetic signature between the clearly 628 differentiated North Sea and Baltic Sea populations (Nielsen et al. 2003). Consequently, some 629 migration between areas is also well aligned with the overall patterns of genetic similarity 630 observed in the geographical region in the populations. Further work will be needed to 631 investigate potential patterns of minor genetic sub-structuring of spawning components within 632 the Kattegat and western Baltic Sea. However, our results clearly indicate that current 633 634 assumptions of closed units used for stock assessments of cod in the North Sea, Kattegat and the western Baltic Sea are likely violated by the dynamic patterns of exchange between areas. 635

636

Evaluation of the interdisciplinary approach: With this study we have demonstrated that an
interdisciplinary approach combining information from genetics and otolith microchemistry
coupled with advanced modelling tools may provide unprecedented new insights into complex

biological questions. Genotyping to identify population of origin and chemical fingerprinting 640 of the otolith core to identify hatch area are well established methodologies. Combined, 641 however, they provide a powerful tool to study connectivity in area use of early life stages. The 642 most innovative aspect of this study is the demonstration of how time-series models of 643 chronological microchemistry data from hatch to death can be combined with genetic 644 information and used to infer migration patterns of individual fish as well as stock mixing 645 646 proportions at different life stages and ages. While we used Atlantic cod in the geographic area between the North Sea and the Baltic Sea as a case study here, the approach is generally 647 648 applicable where environmental gradients in water chemistry are present and sampling designs cover all areas/environments through which the fish can be expected to move. The approach 649 outlined here therefore has wider applicability in both basic and applied research in fish and 650 fisheries biology and could provide valuable data as direct input for conservation and 651 management of fish stocks. 652

653

654 Acknowledgements

Thanks go to all technical staff of DTU Aqua and GEUS involved in the collection and processing of samples used in this study. Additional thanks to Kristian Ege Nielsen for sample preparation and testing of setup for the chemical analyses of samples. We thank Benjamin Heredia Dominguez, GEUS, for providing the figure on LA ICP-MS data accuracy and precision. We would also like to thank two anonymous reviewers for their valuable feedback and input on an earlier version of this paper.

661

662 **Competing interests**

663 The authors declare there are no competing interests.

664

665 Contributors' statement

- 666 KH: Conceptualization, Formal analysis, Methodology, Writing Original Draft, Funding 667 acquisition
- 668 CMA: Conceptualization, Formal analysis, Methodology, Writing Review & Editing
- 669 JHH: Conceptualization, Methodology, Writing Review & Editing, Investigation, Data
- 670 curation, Funding acquisition, Project administration
- 671 MV: Conceptualization, Writing Review & Editing
- 672 SHS: Methodology, Data curation, Writing Review & Editing
- 673 TBT: Methodology, Data curation, Writing Review & Editing
- 674 ME: Conceptualization, Writing Review & Editing

675

676 Funding statement

- The study was supported by the Danish Ministry for Environment and Food and the European
 Maritime Fisheries Fond through the projects *Sustainable management of Kattegat cod; better*
- 679 know-ledge of stock components and migration [grant number 33113-B-16-034] and
- 680 Management of mixed cod stocks in the transition zone between the North Sea and the Baltic
- 681 *Sea: How can this be achieved most efficiently?* [grant number 33113-B-19-140].

682

683 Data availability statement

Access to the data upon which this study is based may be obtained by contacting the corresponding author.

686

687 **References**

Albertsen, C.M. 2018. An approximate filter and smoother for general regime-switching statespace models *In* State-space modelling in marine science. PhD thesis, National Institute

690 of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby, Denmark.

- Albertsen, C.M, Hüssy, K., Serre, S.H., Hemmer Hansen, J., and Thomsen, T.B. (xxxx)
 Estimating migration patterns of fish from otolith chemical composition time-series,
 submitted to Can. J. Fish. Aquat. Sci. (*accepted*).
- Albertsen, C.M., Nielsen, A., and Thygesen, U.H. 2018. Connecting single-stock assessment
 models through correlated survival. ICES J. Mar. Sci. 75(1): 235-244. doi:
 10.1093/icesjms/fsx114.
- 697 André, C., Svedäng, H., Knutsen, H., Dahle, G., Jonsson, P., Ring, A.-K., Sköld, M., and Jorde,
- P.E. 2016. Population structure in Atlantic cod in the eastern North Sea-SkagerrakKattegat: early life stage dispersal and adult migration. BMC Res. Notes 9(1): 63.
 doi:10.1186/s13104-016-1878-9.
- Barth, J.M.I., Berg, P.R., Jonsson, P., Bonanomi, S., Corell, H., Hemmer-Hansen, J., Jakobsen,
- K.S., Johannesson, K., Jorde, P.E., Knutsen, H., Moksnes, P.O., Star, B., Stenseth, N.C.,
 Svedäng, H., Jentoft, S., and André, C. 2018. Genome architecture enables local
 adaptation of Atlantic cod despite high connectivity. Mol. Ecol. 26: 4452-4466. doi:
 10.1111/mec.14207.
- Barth, J.M., Villegas-Ríos, D., Freitas, C., Mpland, E., Star, B., André, C., Knutsen, H.,
 Bradbury, I., Dierking, J., Petereit, C., Righton, C., Metcalfe, J., Jakobsen, K.S., Olsen,
 E.M., and Jentoft, S. 2019. Disentangling structural genomic and behavioural barriers in
 a sea of connectivity. Mol. Ecol. 28: 1394-1411. doi: 10.1111/mec.15010.
- 710 Bath, G.E., Thorrold, S.R., Jones, C.M., Campana, S.E., McLaren, J.W., and Lam, J.W. 2000.
- 711 Strontium and barium uptake in aragonitic otoliths of marine fish. Geochim. Cosmochim.
- 712 Acta **64**(10): 1705–1714. doi:10.1016/S0016-7037(99)00419-6.
- 713 Beckman, D., and Wilson, C.A. 1995. Seasonal timing of opaque zone formation in fish
- otoliths. *In* Recent Developments in Fish Otolith Research. *Edited by* D.H. Secor, J.M.

715 Dean, and S.E. Campana. University of South Carolina Press, Columbia, SC. pp. 27–44.

- 716 Berg, P.R., Jentoft, S., Star, B., Ring, K.H., Knutsen, H., Lien, S., Jakobsen, K.S., and André,
- C. 2015. Adaptation to Low Salinity Promotes Genomic Divergence in Atlantic Cod
 (*Gadus morhua* L.). Gen. Biol. Evol. 7: 1644-1663. doi:10.1093/gbe/evv093.
- 719 Becker, R.A., and Wilks A.R. 1993. Maps in S, AT&T Bell Laboratories Statistics Research
- 720 Report [93.2]. Available at: http://ect.bell-labs.com/sl/doc/93.2.ps
- Börjesson, P., Jonsson, P., Pacariz, S., Björk, G., Taylor, M.I., and Svedäng, H. 2013.
 Spawning of Kattegat cod (*Gadus morhua*)—Mapping spatial distribution by egg surveys.

Fish. Res. **147**: 63–71. doi:10.1016/J.FISHRES.2013.04.012.

- Brander, K.M. 1994. The location and timing of cod spawning around the British Isles. ICES
 J. Mar. Sci. 51: 71–89. doi: 10.1006/jmsc.1994.1007.
- Cadrin, S.X., and Secor, D.H. 2009. Accounting for Spatial Population Structure in Stock
 Assessment: Past, Present, and Future. *In* The Future of Fisheries Science in North
 America. Springer Netherlands, Dordrecht. pp. 405–426. doi:10.1007/978-1-4020-9210729 7 22.
- Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and
 applications. Mar. Ecol. Prog. Ser. 188: 263–297. doi: 10.3354/meps188263.
- Campana, S.E., and Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a
 comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. 58(1): 30–38.
 doi:10.1139/f00-177.
- Carlson, A.K., Phelps, Q.E., and Graeb, B.D.S. 2017. Chemistry to conservation: using otoliths
 to advance recreational and commercial fisheries management. J. Fish Biol. 90(2): 505–
 527. doi:10.1111/jfb.13155.
- 738 Casini, M., Cardinale, M., Hjelm, J., and Vitale, F. 2005. Trends in cpue and related changes in
- spatial distribution of demersal fish species in the Kattegat and Skagerrak, eastern North

- 740 Sea, between 1981 and 2003. ICES J. Mar. Sci. **62**:671e682.
- Clarke ,L.M., and Friedland, K.D. 2004. Influence of growth and temperature on strontium
 deposition in the otoliths of Atlantic salmon. J Fish Biol. 65(3):744–759.
 doi:10.1111/j.0022-1112.2004.00480.x.
- Danielssen, D. 1969. On the migrations of the cod in the Skagerrak shown by tagging
 experiments in the period 1954-1965. Fisk. Skr. Ser. Havundersøkelser 15: 331–338.
 Available
- https://brage.bibsys.no/xmlui/bitstream/handle/11250/114468/sh_vol15_03(21)_1969.pd
 f?sequence=1 [accessed 25 March 2019].
- Dyrssen, D. 1993. The Baltic-Kattegat-Skagerrak Estuarine System. Estuaries 16(3): 446.
 doi:10.2307/1352592.
- Eero, M., Hemmer-Hansen, J., Hüssy, K., Huwer, B., Berg, C., Mariani, P., Mosegaard, H.,

752 Nielsen, A., Nielsen, E.E., Rindorf, A., Ulrich, C., Vinther, M., and Worsøe Clausen, C.

2016. Optimal bæredygtig udnyttelse af tilgængelige torskebestande for dansk fiskeri.

DTU Aqua scientific report (EMFF J.nr. 33010-13-k-0269). Charlottenlund, Denmark.

- Elsdon, T., and Gillanders, B. 2003. Relationship between water and otolith elemental
 concentrations in juvenile black bream *Acanthopagrus butcheri*. Mar. Ecol. Prog. Ser.
 260: 263–272. doi:10.3354/meps260263.
- Elsdon, T.S., and Gillanders, B.M. 2005. Alternative life-history patterns of estuarine fish:
 barium in otoliths elucidates freshwater residency. Can. J. Fish. Aquat. Sci. 62(5): 1143–
 1152. doi:10.1139/f05-029.
- Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E.,
 Secor, D.H., Thorrold, S.R., and Walther, B.D. 2008. Otolith chemistry to describe
 movements and life-history parameters of fishes—hypotheses, assumptions, limitations
- and inferences. *In* Oceanography and Marine Biology: An Annual Review. *Edited by* R.N.

- Gibson, A.R. J, and J.D.M. Gordon. CRC Press, Boca Raton, London, New York. pp.297–330.
- Estoup, A., Largiader, C.R., Perrot, E., and Chourrout, D. 1996. Rapid one tube DNA
 extraction for reliable PCR detection of fish polymorphic markers and transgenes. Mol.
 Mar. Biol. Biotech. 5: 295–298.
- Everitt, B., and Hothorn, T. 2011. An Introduction to Applied Multivariate Analysis with R.
 Springer New York, New York, NY. doi:10.1007/978-1-4419-9650-3.
- Fablet, R., Daverat, F., and De Pontual, H. (2007). Unsupervised bayesian reconstruction of

individual life histories from otolith signatures: case study of sr:ca transects of european

- eel (anguilla anguilla) otoliths. Can. J. Fish. Aquat. Sci. **64**(1): 152–165. doi: 10.1139/f06-
- 775 173.

773

- Fox, C.J., Taylor, M., Dickey-Collas, M., Fossum, P., Kraus, G., Rohlf, N., Munk, P., van
 Damme, C.J.G., Bolle, L.J., Maxwell, D.L., and Wright, P.J. 2008. Mapping the spawning
 grounds of North Sea cod (*Gadus morhua*) by direct and indirect means. Proc. R. Soc. B
 275(1642): 1543–1548. doi:10.1098/rspb.2008.0201.
- Goethel, D.R., Quinn, T.J., and Cadrin, S.X. 2011. Incorporating Spatial Structure in Stock
 Assessment: Movement Modeling in Marine Fish Population Dynamics. Rev. Fish. Sci.
 19(2): 119–136. doi:10.1080/10641262.2011.557451.
- Heath, M.R., Culling, M.A., Crozier, W.W., Fox, C.J., Gurney, W.S.C., Hutchinson, W.F.,
- Nielsen, E.E., O'Sullivan, M., Preedy, K.F., Righton, D.A., Speirs, D.C., Taylor, M.I.,
- 785 Wright, P.J., and Carvalho, G.R. 2014. Combination of genetics and spatial modelling
- highlights the sensitivity of cod (*Gadus morhua*) population diversity in the North Sea to
- distributions of fishing. ICES J. Mar. Sci. 71: 794-807. doi: 10.1093/icesjms/fst185.
- Heidemann, F., Marohn, L., Hinrichsen, H.H., Huwer, B., Hüssy, K., Klügel, A., Böttcher, U.,
- and Hanel, R. 2012. Suitability of otolith microchemistry for stock separation of Baltic

cod. Mar. Ecol. Prog. Ser. **465**: 217-226. doi:10.3354/meps09922.

- 791 Heimbrand, Y., Limburg, K., Hüssy, K., Casini, M., Sjöberg, R., Palmén Bratt, A.-M.,
- Levinsky, S.-E., Karpushevskaia, A., Radtke, K., and Öhlund, J. 2020. Seeking the true
- time: Exploring otolith chemistry as an age-determination tool. J. Fish Biol.doi:10.1111/jfb.14422.
- Hemmer-Hansen, J., Hüssy, K., Vinther, M., Albertsen, C. M., Storr-Paulsen, M., and Eero, M.
- 796 2020. Sustainable management of Kattegat cod; better knowledge of stock components
- and migration. DTU Aqua Report no. 357-2020. National Institute of Aquatic Resources,
- Technical University of Denmark. 42 pp. ISBN: 978-87-7481-281-4.
- Hicks, A.S., Closs, G.P., and Swearer, S.E. 2010. Otolith microchemistry of two amphidromous 799 galaxiids across an experimental salinity gradient: A multi-element approach for tracking 800 801 diadromous migrations. J. Exp. Mar. Bio. Ecol. **394**(1): 86-97. doi: 10.1016/j.jembe.2010.07.018. 802
- Høie, H., and Folkvord, A. 2006. Estimating the timing of growth rings in Atlantic cod otoliths
 using stable oxygen isotopes. J. Fish Biol. 68(3): 826–837. doi:10.1111/j.00221112.2006.00957.x.
- Huwer, B., Hinrichsen, H.-H., Hüssy, K., and Eero, M. 2016. Connectivity of larval cod in the
 transition area between North Sea and Baltic Sea and potential implications for fisheries
 management. ICES J. Mar. Sci. 73(7): 1815-1824. doi:10.1093/icesims/fsw043.
- 809 Hüssy, K. 2011. Review of western Baltic cod (Gadus morhua) recruitment dynamics. ICES
- 810 J. Mar. Sci., 68: 1459–1471. doi:10.1093/icesjms/fsr088.
- 811 Hüssy, K., Gröger, J., Heidemann, F., Hinrichsen, H.-H., & Marohn, L. (2016). Slave to the
- 812 rhythm: Seasonal signals in otolith microchemistry reveal age of eastern Baltic cod
- 813 (*Gadus morhua*). *ICES J. Mar. Sci.* **73**(4): 1019–1032. doi:10.1093/icesjms/fsv247.
- Hüssy, K., Krüger-Johnsen, M., Thomsen, T.B., Heredia, B.D., Naeraa, T., Limburg, K.E.,

- McQueen, K., Haase, S., Krumme, U., Casini, M., Mion, M., Radtker, K. (2021) It's
 elemental, my dear Watson: Validating seasonal patterns in otolith chemical
 chronologies. Can. J. Fish. Aquat. Sci. doi: 10.1139/cjfas-2020-0388.
- 818 Hüssy, Karin, Limburg, K.E., de Pontual, H., Thomas, O.R.B., Cook, P.K., Heimbrand, Y.,
- Blass, M., and Sturrock, A.M. 2020. Trace Element Patterns in Otoliths: The Role of
- Biomineralization. Rev. Fish. Sci. Aquacult. 1–33. doi:10.1080/23308249.2020.1760204.
- 821 ICES. 2015. Report of the Benchmark Workshop on Baltic Cod Stocks (WKBALTCOD).

822 ICES C. 2015/ACOM35 (2–6 March 2015, Rostock, Germany): pp 172.

- ICES. 2019. Baltic Fisheries Assessment Working Group (WGBFAS). ICES Sci. Rep. 1:20:
 pp 653. doi:10.17895/ices.pub.5256.
- ICES. 2020. Cod (*Gadus morhua*) in Subdivision 21 (Kattegat). *In* Report of the ICES
 Advisory Committee, 2020. ICES Advice 2020, cod.27.21.
 https://doi.org/10.17895/ices.advice.5903.
- Jakobsen, F. 1997. Hydrographic investigation of the Northern Kattegat front. Cont. Shelf Res.
 17(5): 533–554. doi:10.1016/S0278-4343(96)00044-1.
- Jonsson, P.R., Corell, H., André, C., Svedäng, H., and Moksnes, P.-O. 2016. Recent decline in
- cod stocks in the North Sea-Skagerrak-Kattegat shifts the sources of larval supply. Fish.
 Oceanogr. 25(3): 210–228. doi:10.1111/fog.12146.
- 833 Kerr, L., Hintzen, N., Cadrin, S., Worsøe Clausen, L., Dickey-Collas, M., Goethel, D.R.,

Hatfield, E.M.C., Kritzer, J., and Nash, R.D.M. 2017. Lessons learned from practical

- approaches to reconcile mismatches between biological population structure and stock
- units of marine fish. ICES J. Mar. Sci. 74(6): 1708–1722. doi:10.1093/icesjms/fsw188.
- 837 Knutsen, H., André, C., Jorde, P.E., Skogen, M.D., Thuróczy, E., and Stenseth, N.C. 2004.
- 838 Transport of North Sea cod larvae into the Skagerrak coastal populations. Proc. R. Soc.
- London. Ser. B Biol. Sci. **271**(1546): 1337–1344. doi:10.1098/rspb.2004.2721.

- 840 Kraus, R.T., and Secor, D.H. 2004. Incorporation of strontium into otoliths of an estuarine fish.
- 841 J. Exp. Mar. Bio. Ecol. **302**(1): 85–106. doi:10.1016/J.JEMBE.2003.10.004.
- 842 Kristensen, K., Nielsen, A., Berg, C. W., Skaug, H., Bell, B. M. (2016). TMB: Automatic
- Differentiation and Laplace Approximation. Journal of Statistical Software, 70(5), 1-21.
- doi:10.18637/jss.v070.i05.
- Lear, J., Hare, D. J., Fryer, F., Adlard, P. A., Finkelstein, D. I., Doble, P. A. (2012). High-
- 846 Resolution Elemental Bioimaging of Ca, Mn, Fe, Co, Cu, and Zn Employing LA-ICP-MS
- and Hydrogen Reaction Gas. Analytical Chemistry. 84(15), 6707-6714.
 doi:10.1021/ac301156f.
- Li, L., Høie, H., Geffen, A. J., Heegaard, E., Skadal, J., Folkvord, A. (2008). Back-calculation
 of previous fish size using individually tagged and marked Atlantic cod (*Gadus morhua*).
 Can. J. Fish. Aquat. Sci. 65, 2496–2508. doi:10.1139/F08-157.
- Limburg, K. E. 1995. Otolith strontium traces environmental history of subyearling American
- 853 shad *Alosa sapidissima*. Mar. Ecol. Prog. Ser. 119(1/3), 25-35.
 854 http://www.istor.org/stable/24849794.
- Limburg, K.E., Olson, C., Walther, Y., Dale, D., Slomo, C.P., and Høie, H. 2011. Tracking
 Baltic hypoxia and cod migration over millennia with natural tags. Proc. Natl. Acad. Sci.
 U.S.A. 108(22): E177–E182. doi: 10.1073/pnas.1100684108.
- Limburg, K.E., Walther, B.D., Lu, Z., Jackman, G., Mohan, J., Walther, Y., Nissling, A., 858 Weber, P.K., and Schmitt, A.K. 2015. In search of the dead zone: Use of otoliths for 859 J. tracking fish exposure to hypoxia. Mar. Syst. 141: 167–178. 860 doi:10.1016/J.JMARSYS.2014.02.014. 861
- Limburg, K.E., Wuenschel, M.J., Hüssy, K., Heimbrand, Y., and Samson, M. 2018. Making the Otolith Magnesium Chemical Calendar-Clock Tick: Plausible Mechanism and Empirical Evidence. Rev. Fish. Sci. Aquac. **26**(4): 479–493.

doi:10.1080/23308249.2018.1458817.

- Miller, J.A. 2011. Effects of water temperature and barium concentration on otolith
 composition along a salinity gradient: implications for migratory reconstructions. J. Exp.
- 868 Mar. Bio. Ecol. **405**(1): 42–52. doi:10.1016/j.jembe.2011.05.017.
- Milton, D.A., and Chenery, S.R. 2001. Sources and uptake of trace metals in otoliths of juvenile
 barramundi (*Lates calcarifer*). J. Exp. Mar. Bio. Ecol. 264(1): 47–65. doi:10.1016/S00220981(01)00301-X.
- Munk, P., Fox, C.J., Bolle, L.J., van Damme, C.J.G., Fossum, P., and Kraus, G. 2009.
 Spawning of North Sea fishes linked to hydrographic features. Fish. Oceanogr. 18(6):
 458–469. doi:10.1111/j.1365-2419.2009.00525.x
- Nielsen, E.E., Cariani, A., Mac Aoidh, E., Maes, G.E., Milano, I., Ogden, R., Taylor, M.,
 Hemmer-Hansen, J., Babbucci, M., Bargelloni, L., Bekkevold, D., Diopere, E., Grenfell,
- 877 L., Helyar, S., Limborg, M.T., Martinsohn, J.T., McEwing, R., Panitz, F., Patarenello, T.,
- Tinti, F., van Houndt, J.K.J., Volckaert, F.A.M., Waples, R.S., and Carvalho, G.R. 2012.
- 879 Gene-associated markers provide tools for tackling illegal fishing and false eco880 certification. Nat. Com. 3: 851. doi: 10.1038/ncomms2975.
- Nielsen, E.E., Hansen, M.M., Ruzzante, D.E., Meldrup, D., and Grønkjær, P. 2003. Evidence
 of a hybrid-zone in Atlantic cod *(Gadus morhua)* in the Baltic and the Danish Belt Sea
 revealed by individual admixture analysis. Mol. Ecol. 12: 1497–1508. doi:
 10.1046/j.1365-294X.2003.01819.x.
- Oeberst, R. 2008. Distribution pattern of cod and flounder in the Baltic Sea based on
 international coordinated trawl surveys. ICES CM 2008/J:09.
- Pihl, L., and Ulmestrand, M. 1993. Migration pattern of juvenile cod (*Gadus morhua*) on the
 Swedish west coast. ICES J. Mar. Sci. 50(1): 63–70. doi: 10.1006/jmsc.1993.1007.
- Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L., and Estoup, A. 2004.

- 891 J. Hered. **95**: 536-539. doi:10.1093/jhered/esh074.
- 892 R Core Team (2020). R: A language and environment for statistical computing. R Foundation
- for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. Venables,
- W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer,
- 895 New York. ISBN 0-387-95457-0.
- Rannala, B., and Mountain, J.L. 1997. Detecting immigration by using multilocus genotypes.
 Proc. Nat. Acad. Sci. USA 94: 9197-9221. doi:10.1073/pnas.94.17.9197.
- 898 Reis-Santos, P., Tanner, S.E., Elsdon, T.S., Cabral, H.N., and Gillanders, B.M. 2013. Effects of temperature, salinity and water composition on otolith elemental incorporation of 899 Dicentrarchus labrax. J. Mar. Bio. Ecol. **446**: 245-252. 900 Exp. 901 doi:10.1016/J.JEMBE.2013.05.027.
- 902 Righton, D.A., Andersen, K.H., Neat, F., Thorsteinsson, V., Steingrund, P., Svedäng, H.,
- 903 Michalsen, K., Hinrichsen, H.H., Bendall, V., Neuenfeldt, S., Wright, P., Jonsson, P.,
- Huse, G., Van Der Kooij, J., Mosegaard, H., Hüssy, K., and Metcalfe, J. 2010. Thermal
- niche of Atlantic cod *Gadus morhua*: Limits, tolerance and optima. Mar. Ecol. Prog. Ser.
- **420**: 1–13. doi:10.3354/meps08889.
- Rosenberg, R., Cato, I., Förlin, L., Grip, K., and Rodhe, J. 1996. Marine environment quality
 assessment of the Skagerrak Kattegat. J. Sea Res. 35(1–3): 1–8. doi:10.1016/S13851101(96)90730-3.
- 910 Rosenberg, R., Loo, L.-O., and Möller, P. 1992. Hypoxia, salinity and temperature as
- 911 structuring factors for marine benthic communities in a eutrophic area. Netherlands J. Sea
 912 Res. 30: 121–129. doi:10.1016/0077-7579(92)90051-F.
- 913 Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T., and
- Eliceiri, K.W. 2017. ImageJ2: ImageJ for the next generation of scientific image data.

⁸⁹⁰ GeneClass2: A Software for Genetic Assignment and First-Generation Migrant Detection.

915 BMC Bioinformatics **18**(1): 529. doi:10.1186/s12859-017-1934-z.

- 916 Secor, D. H., Henderson-Arzapalo, A., and Piccoli, P. M. 1995. Can otolith microchemistry
- 917 chart patterns of migration and habitat utilization in anadromous fishes? J. Exp. Mar. Biol.
- 918
 Ecol.
 192:
 15–33.
 Available
 from
- 919 https://www.sciencedirect.com/science/article/pii/002209819500054U.
- 920 Serre, S.H., Nielsen, K.E., Fink-Jensen, P., Thomsen, T., and Hüssy, K. 2018. Analysis of cod
- otolith microchemistry by continuous line transects using la-icp-ms. Geological Survey
 of Denmark and Greenland Bulletin, 41:91–94. doi:1604-8156.
- 923 Shin, J.-H., Blay, S., McNeney, B., and Graham J. 2006. LDheatmap: An R Function for graphical display of pairwise linkage disequilibria between single nucleotide 924 Stat. Soft. **16**: Code Snippet 3. Available polymorphisms. J. from 925 https://www.jstatsoft.org/article/view/v016c03. 926
- Stenseth, N.C., Jorde, P.E., Chan, K.-S., Hansen, E., Knutsen, H., André, C., Skogen, M.D., 927 and Lekve, K. 2006. Ecological and genetic impact of Atlantic cod larval drift in the 928 Skagerrak. Proc. R. Soc. В Biol. Sci. **273**(1590): 1085-1092. 929 doi:10.1098/rspb.2005.3290. 930
- 931 Sturrock, A.M., Hunter, E., Milton, J.A., EIMF, Johnson, R.C., Waring, C.P., and Trueman,
- 932 C.N. 2015. Quantifying physiological influences on otolith microchemistry. Methods
 933 Ecol. Evol. 6(7): 806–816. doi:10.1111/2041-210X.12381.
- Sturrock, A.M., Trueman, C.N., Darnaude, A.M., and Hunter, E. 2012. Can otolith elemental
 chemistry retrospectively track migrations in fully marine fishes? J. Fish Biol. 81(2): 766–
 795. doi:10.1111/j.1095-8649.2012.03372.x.
- 937 Svansson, A. 1975. Physical and chemical oceanography of the Skagerrak and the Kattegat.
 938 Fishery Board of Sweden, Uddevalla. p. 93. Available from
 939 https://gupea.ub.gu.se/bitstream/2077/49089/1/gupea_2077_49089_1.pdf.

940	Svedäng, H., André, C., Jonsson, P., Elfman, M., and Limburg, K.E. 2010. Migratory
941	behaviour and otolith chemistry suggest fine-scale sub-population structure within a
942	genetically homogenous Atlantic Cod population. Environ. Biol. Fishes 89(3-4): 383-
943	397. doi:10.1007/s10641-010-9669-y.
944	Svedäng, H., Righton, D., and Jonsson, P. 2007. Migratory behaviour of Atlantic cod Gadus
945	morhua: natal homing is the prime stock-separating mechanism. Mar. Ecol. Prog. Ser.
946	345 : 1–12. doi:10.3354/meps07140.
947	Tang, Y., and Li, W. 2016. Ifda: An R Package for Local Fisher Discriminant Analysis and
948	Visualization. Available from https://arxiv.org/abs/1612.09219.

- Venables, W.N., and Ripley, B.D. 2002. Modern Applied Statistics with S, Fourth edition.
 Springer, New York. ISBN 0-387-95457-0, https://www.stats.ox.ac.uk/pub/MASS4/.
- Vitale, F., Börjesson, P., Svedäng, H., and Casini, M. 2008. The spatial distribution of cod
 (*Gadus morhua* L.) spawning grounds in the Kattegat, eastern North Sea. Fish. Res. 90(1–

953 3): 36–44. doi:10.1016/j.fishres.2007.09.023.

- 954 Vitale, F., Worsøe Clausen, L., and Ní Chonchúir, G. (Eds.) 2019. Handbook of fish age
- estimation protocols and validation methods. ICES Cooperative Research Report No. 346.
- 956 180 pp. doi:10.17895/ices.pub.5221.
- Walther, B.D., Kingsford, M.J., O'Callaghan, M.D., and McCulloch, M.T. 2010. Interactive
 effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of
 a coral reef fish. Environ. Biol. Fish. 89(3–4):441–451. doi:10.1007/s10641-010-9661-6.
- Walther, B.D., and Limburg, K.E. 2012. The use of otolith chemistry to characterize
 diadromous migrations. J. Fish Biol. 81(2): 796–825. doi:10.1111/j.10958649.2012.03371.x.
- Watanabe, T., Kiron, V., and Satoh, S. 1997. Trace minerals in fish nutrition. Aquaculture
 151(1-4): 185-207. doi:10.1016/S0044-8486(96)01503-7.

- 965 Weidman, C.R., and Millner, R. 2000. High-resolution stable isotope records from North
- 966 Atlantic cod. Fish. Res. **46**(1–3): 327–342. doi:10.1016/S0165-7836(00)00157-0.

967

968 Figure captions

969

Figure 1. Map of the sampling area, where the management areas of the cod populations are 970 1. North Sea stock: North Sea and Skagerrak, 2. Kattegat stock: Kattegat, and 3. western Baltic 971 Sea stock: Belt Sea, Sound and Arkona Sea. Pie charts indicate sampling locations, where the 972 size of the symbols represents number of fish, and the colour represents populations North Sea 973 974 (bright blue) and Kattegat (red). The colouring of the geographic areas indicate the habitat occupation areas used: North Sea/Skagerrak (lime), Kattegat (green), western Baltic Sea (blue 975 976 green), and eastern Baltic Sea (purple). Map created using the packages "maps", "mapdata" and "maptools" (Becker and Wilks 1993) in "R" (R Core Team 2020). The samples used in this 977 study were collected during scientific surveys by the Technical University of Denmark in 2016. 978

979

Figure 2. Image of otolith cross-section, viewed under reflected light, with laser track indicated 980 with a solid black line, and the corresponding otolith Sr profile where colours indicate the 981 habitat area assignments (North Sea = lime, Kattegat = green, western Baltic = blue green). 982 Translucent winter growth zones are outlined by vertical shaded bars linking the visual image 983 984 with the corresponding sections of the Sr profile. This individual is 4 years old Kattegat population cod captured in the northern Kattegat had spent most of its life in the western Baltic/ 985 986 Kattegat and performed two migrations into the Skagerrak or North Sea during fall/winter of its second and third year of life. 987

988

Figure 3. Otolith size at the end of first year of life for the 6 age groups of cod captured in Kattegat, representing the year classes 2010 - 2015, where colours represent different populations: Kattegat (red) and North Sea (blue). Otolith sizes of age 0 individuals (year class 2016) are not included in this graph, because they were captured before the end of their first winter. Horizontal lines indicate mean, box upper and lower limits the 25% and 75%
percentiles, whiskers represent the highest and lowest values within 1.5 interquartile range and
dots represent outliers.

996

997 Figure 4. Fish size in relation to age of cod captured in Kattegat with linear regression lines 998 and confidence interval bands, where colours represent different populations: Kattegat (red) 999 and North Sea (blue). The slope of the North Sea population is significantly higher than that of 1000 the Kattegat population, suggesting faster growth in North Sea cod.

1001

Figure 5. Biplot of the first two discriminant functions of the LDA analysing otolith chemical fingerprints in the larval and pelagic juvenile stage (0 to ca. 4 months old), the demersal juvenile stage until the first winter zone (ca. 4 to 8 months old), and the first winter, with life stage indicated above each biplot. Colours represent the two populations: Kattegat (red) and North Sea (blue), and arrows the direction and strength of the loadings (= elements).

1007

1008 Figure 6. Area assignment for each individual fish and each time interval of its entire lifespan 1009 from the first winter to catch for cod from the Kattegat population and the North Sea population (population indicated above graph). The time from hatch to the first winter is not included in 1010 1011 this plot, as the area assignment for this age group is based on a different habitat area levels and provides less precise assignments. Colours indicate the habitat area assignments: North 1012 Sea = lime, Kattegat = green, western Baltic = blue green, eastern Baltic = purple. Lifetime 1013 area use is shown for the two populations separately, where individuals are ordered according 1014 to latitude of their capture location in the Kattegat. 1015

1016

42

Figure 7. Percentage distributions of habitat area assignments by age and season, representing
a population's distribution pattern in space and time, for the Kattegat population and the North
Sea population separately (population indicated above graph). Colours indicate percentage of
individuals with an area use assigned to the North Sea (lime), the Kattegat (green), the western
Baltic Sea (blue green), and the eastern Baltic Sea (purple).

1022

Figure 8. Percentage of individuals assigned to the Skagerrak/North Sea (lime coloured area in Fig. 1) by age and season, for the Kattegat population and the North Sea population (population indicated above graph). Colour shading represents the percentage estimate based on the mean values of the otolith strontium – salinity calibration curve (dark lime), for mean se values (no colour), and mean + se (transparent lime).



© The Author(s) or their Institution(s)



Distance from core

Image of otolith cross-section, viewed under reflected light, with laser track indicated with a solid black line, and the corresponding otolith Sr profile where colours indicate the habitat area assignments (North Sea = lime, Kattegat = green, western Baltic = blue green). Translucent winter growth zones are outlined by vertical shaded bars linking the visual image with the corresponding sections of the Sr profile. This individual is 4 years old Kattegat population cod captured in the northern Kattegat had spent most of its life in the western Baltic/ Kattegat and performed two migrations into the Skagerrak or North Sea during fall/winter of its second and third year of life.

290x179mm (150 x 150 DPI)



Otolith size at the end of first year of life for the 6 age groups of cod captured in Kattegat, representing the year classes 2010 – 2015, where colours represent different populations: Kattegat (red) and North Sea (blue). Otolith sizes of age 0 individuals (year class 2016) are not included in this graph, because they were captured before the end of their first winter. Horizontal lines indicate mean, box upper and lower limits the 25% and 75% percentiles, whiskers represent the highest and lowest values within 1.5 interquartile range and dots represent outliers.

85x101mm (300 x 300 DPI)



Fish size in relation to age of cod captured in Kattegat with linear regression lines and confidence interval bands, where colours represent different populations: Kattegat (red) and North Sea (blue). The slope of the North Sea population is significantly higher than that of the Kattegat population, suggesting faster growth in North Sea cod.

85x101mm (300 x 300 DPI)



Biplot of the first two discriminant functions of the LDA analysing otolith chemical fingerprints in the larval and pelagic juvenile stage (0 to ca. 4 months old), the demersal juvenile stage until the first winter zone (ca. 4 to 8 months old), and the first winter, with life stage indicated above each biplot. Colours represent the two populations: Kattegat (red) and North Sea (blue), and arrows the direction and strength of the loadings (= elements).

266x86mm (300 x 300 DPI)



Season



Season





	Population						
Age	Kattegat	North Sea	Total				
0	23	79	102				
1	7	28	35				
2	32	42	74				
3	14	8	22				
4	52	7	59				
5	5	1	6				
6	7		7				
8	1		1				
Total	141	165	306				

Table 1. Overview over samples used in this study by population and age.

Table 2. Concentration of element/Ca ratios (ppm), in the pelagic juvenile stage by population (mean+/- standard deviation), together with ANOVA statistics indicating differences between populations(ns = not significant, *** < 0.001)</td>

	Po				
Element	Kattegat	North Sea	df	F	р
Sr	1458.1 (128.9)	1604.4 (181.1)	1	12.34	***
Ba	24.3 (7.9)	17.1 (7.3)	1	7.56	***
Mn	12.4 (5.7)	8.2 (3.7)	1	101.18	***
Mg	61.1 (9.9)	73.0 (39.1)	1	13.38	***
Р	276.9 (55.7)	312.7 (90.1)	1	0.001	ns
Cu	0.7 (0.1)	0.4 (0.2)	1	11.90	***
Zn	5.5 (2.6)	3.2 (1.5)	1	22.82	***

