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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

Karin Hüssy[1*], Christoffer Moesgaard Albertsen[1], Jakob Hemmer-Hansen[2], Morten Vinther[1], Simon Hansen Serre[3], Tonny Bernt Thomsen[3], Margit Eero[1]

Affiliations

[1] National Institute of Aquatic Resources, Technical University of Denmark, Kemitorvet, building 201, Lyngby Campus, 2800 Kgs. Lyngby, Denmark

[2] National Institute of Aquatic Resources, Technical University of Denmark, Vejlsøvej 39, 8600 Silkeborg, Denmark

[3] Geological Survey of Denmark and Greenland, Øster Voldgade 10, DK-1350 Copenhagen K., Denmark

* Corresponding author: kh@aqua.dtu.dk, Mobile +45 93511840

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Abstract

This study investigates stock mixing of genetically distinct Atlantic cod (*Gadus morhua*) stocks in the Kattegat, an area geographically located between the North Sea and the Baltic Sea, by combining genetic population identification with habitat assignments from hatch to capture from otolith microchemistry. Cod captured in Kattegat were genetically assigned to either the North Sea or the endemic Kattegat population. Otolith chemical fingerprints differed 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 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 populations undertook frequent, but predominantly short term, migrations to other areas than the Kattegat, with < 25 % of individuals remaining resident within the Kattegat throughout their life. Across seasons and age classes, the two populations were both most frequently distributed in the Kattegat (67 %), with approximately 25 % of both population distributed in the western Baltic Sea and less than 10 % in the Skagerrak/North Sea. This study demonstrates the usefulness of this approach to infer population-specific connectivity and migration trajectories for individual fish and its potential applications in basic and applied fields of fish ecology and fisheries sciences.

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

Introduction

Fish migrations and stock mixing are major issues for assessments of stock status and for fisheries management, which are usually constrained to geographical management areas that do not always correspond to the population's distribution boundaries (e.g. Kerr et al. 2017). Stock assessment approaches are being developed to account for spatial stock structure (Cadrin and Secor 2009; Goethel et al. 2011; Albertsen et al. 2018). However, an understanding of 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 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 area currently managed as four stocks: the North Sea/Skagerrak, the Kattegat, the western Baltic Sea/Sound and the eastern Baltic Sea, with distinct spawning areas and seasons (Brander 1994; Fox et al. 2008; Vitale et al. 2008; Munk et al. 2009; Hüssy, 2011; Börjesson et al. 2013). Cod from these areas are clearly distinct genetically (Nielsen et al. 2003; Berg et al, 2015; 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 (the Kattegat population) with adjacent populations (primarily the North Sea population) takes place (Knutsen et al. 2004; ICES 2015; André et al. 2016). However, the mechanisms and dynamics of the mixing and migrations patterns of cod in this area are currently not well 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 of adult individuals (Svedäng et al. 2007). There is no targeted cod fishery in Kattegat at present, and cod is mainly taken as bycatch in the trawl fishery for Norway lobster (*Nephrops norvegicus*) (ICES 2020). Lack of knowledge on population mixing hampers stock assessment

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

As an environmental transition zone between the North Sea and the Baltic Sea, Kattegat is well suited for the application of methods for inferring stock mixing. The bathymetry becomes successively shallower from > 120 m in the North Sea and Skagerrak, to 40 – 80 m in the northern Kattegat, to depths of 20 – 40 m in the southern Kattegat. The hydrography is characterized by deep-water inflow of saline water from the North Sea and outflow of 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 water of very low salinity in the eastern Baltic Sea (Fig. S1). This salinity gradient provides an ideal setup for studying fish migrations based on chronological analyses of the chemical composition of their otoliths.

Otoliths consist of calcium carbonate (~98 %) and organic matrix (~2 %) and small quantities of trace elements. The biomineralization of the otolith is regulated by physiological processes resulting in both daily growth increments and distinct annual growth zones reflecting seasonally fluctuating conditions in temperature and food availability (Beckman and Wilson 1995; Weidman and Millner 2000; Høie and Folkvord 2006). Trace elements are absorbed primarily from the water across the gill surface and therefore provide a record of environmental 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 catchment and therefore provides an area-specific chemical “fingerprint” which is reflected in 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 1990s has been considered a useful proxy for environmental salinity owing to its ability to track

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 dynamics, migration patterns, pollution exposure, connectivity between areas, and plays an increasingly important role as a fisheries management tool (Campana 1999; Campana and Thorrold 2001; Elsdon et al. 2008; Carlson et al. 2017). Statistically significant differences in element concentrations have previously been observed between cod stocks from the North Sea, 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), 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 two areas and the eastern Baltic (Sr, Ba, Zr). Within the eastern Baltic Sea, no strong differences in element compositions were observed between different spawning areas. Chemical fingerprints even suggested indications for small-scale stock-structuring and natal homing as important stock separating mechanisms over short distances of < 100 km within the Kattegat/Sound area (Svedäng et al. 2010).

We combined genetic population identification of cod originating from two different populations (North Sea and Kattegat), but caught together in the Kattegat, with chemical composition of otoliths analysed with a new state-of-the art regime-switching state-space 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 migration patterns between geographically adjacent areas. More specifically, we first tested whether cod caught in the Kattegat consist of a genetic mixture of populations (North Sea and Kattegat). Then we tested whether North Sea cod are spawned in the same spawning areas as Kattegat cod by comparing the elemental composition of the otolith core, corresponding to the

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

Materials and methods

Sample collection

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 % ethanol for genotyping. Prior to the selection of otolith samples for this study, all cod were genetically assigned to either the North Sea or the Kattegat population (for details on genetic population assignment, see below) and their age determined by expert readers from the sectioned otoliths following routine procedures (Vitale et al. 2019). The bathymetry of the Kattegat is somewhat heterogeneous, where the area south of 57 degree north is shallower than the northern area. From the genotyped individuals, 306 otoliths were therefore selected to ensure adequate sample sizes aiming at a uniform geographic coverage (northern and southern Kattegat) within the size ranges < 35cm, 40 – 55 cm, > 60cm (corresponding age ranges: 0 – 1 years, 2 – 3 years, > 3 years), as well as of the two populations (North Sea and Kattegat). Equal 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. Otoliths were extracted, cleaned of adhering tissue, air dried and stored in individually labelled paper bags.

Genetic assignment to population of origin

Genetic assignment to population of origin was conducted through the analyses of 192 genetic 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 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; see Supplementary information). DNA was extracted by Chelex resin (Estoup et al. 1996) and SNPs were genotyped on a Fluidigm Biomark HD system. After initial screening of the 192 markers, 5 markers were excluded because they did not provide reliable genotype information, resulting in a final data set composed on 187 SNPs. Since the markers originated from different studies, we re-assessed marker independence through analyses of linkage disequilibrium in new baseline samples from the North Sea, Kattegat and the eastern Baltic Sea, using the R package LDheatmap (Shin et al. 2006). These analyses found low levels of LD between the markers, confirming that they would provide independent information for population assignment (see Supplementary information, Fig. S2). Assignment to the most likely baseline sample ("North Sea", "Kattegat", "Eastern Baltic") was conducted by calculating genotype likelihoods (following Rannala and Mountain (1997)) using the programme GeneClass2 (Piry et al. 2004). Population assignment to baseline samples was based on the highest assignment score (ranging between 0 and 100; a measure of the likelihood of the most likely population divided by the sum of all likelihoods) calculated for each fish. Re-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 comparisons were well separated. For details, see Supplementary information (Fig. S3).

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

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

Statistical analyses

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).

Spawning origin

Identification of spawning origin: Unfortunately, no larval otolith samples were available to be used as baseline, therefore an accurate assignment of the early life stages is not possible. The conclusions here are therefore based on the approach that if chemical fingerprints of the otolith core are not statistically different between populations, all individuals have been spawned in 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 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), 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" (Venables and Ripley 2002) in "R" ver. 3.4.2 (R Core Team 2020). Stepwise Linear Discriminant Analysis (LDA) using centred and scaled coefficients was used to identify the elements that drive differences between populations and estimate their ability to correctly classify individuals into the correct group using the "lfda" package (Tang and Li 2016). Differences between populations were visualized using biplots of the first two discriminant 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 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 differences in elemental concentration between populations.

Migration analyses

Because otolith Sr concentration is strongly correlated with ambient concentrations, with correlations generally > 0.95 found throughout the literature (review of studies in Hüssy et al. (2020)) the analyses of migration patterns presented in this study rely on otolith Sr concentration exclusively. Some studies indicate a potential impact of seasonal temperature and growth on otolith Sr concentration (Sturrock et al. 2015; Walther et al. 2010), while others found that otolith Sr patterns were unrelated to water temperature and somatic growth (Clarke 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 seasons and ages were tested using an ANOVA with subsequent Tukey's Honest Significant Difference test for post-hoc pairwise comparison in in "R" ver. 3.4.2 (R Core Team 2020).

Migration patterns were analysed based on otolith strontium concentrations using a newly 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 1 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 the signal from the true unobserved concentration. Finally, the autoregressive process in layer 2 models temporal correlation in the element levels besides the habitat type to reflect that fish may move between habitats at time scales that are longer than the time between measurements. *Layer 3*: The signal-to-noise ratio will depend on inherent background spectral interferences and sample volume determined by the laser beam diameter (e.g. Lear et al. 2012). Further, variability at small time scales may occur from variability in unmodelled factors such as temperature and food rations. Therefore, observed log-strontium was modelled by a normal distribution where the mean was the true log-strontium level.

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

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

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

316 (North Sea, Kattegat, western Baltic Sea and eastern Baltic Sea) were determined based on
317 annual mean salinities for the years 2013-2016 from ICES Hydrographic database (covering
318 the entire lifespan of all fish in the study) (Available at https://ices.dk/data/data-
319 portals/Pages/ocean.aspx) and known depth distribution ranges of cod: 30 – 80 m in the
320 Skagerrak and Kattegat (Casini et al. 2005), 10 – 40 m in the western Baltic Sea and 30 – 60
321 m in the eastern Baltic Sea (Oeberst 2008). Cut-off salinities selected at the boundaries between
322 areas were 10 psu between eastern and western Baltic Sea, 17.5 psu between western Baltic
323 Sea and Kattegat, and 34 psu between Kattegat and Skagerrak/North Sea. An overview over
324 geographic areas with associated salinity levels and data selected is given in Fig. S1. Using the
325 strontium – salinity calibration curve, the strontium levels corresponding to these salinities was
326 calculated.

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

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

Results

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

Growth patterns: Growth patterns were compared by testing the linearity of the relationship between fish size and age using population as group covariate. Fish size was linearly related to the age of the fish (Fig. 4) but with significant differences in the intercept and slopes between

366 populations with a smaller intercept and higher slope in the North Sea population (ANCOVA,
367 $F = 2944$, $df = 4$ and 296, $p < 0.05$, $r^2 = 0.98$). A higher slope in the North Sea population
368 indicates faster growth than the Kattegat population, and needs to be considered in the
369 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,
373 significant differences in chemical fingerprint during the pelagic juvenile stage were detected
374 between the 8 year classes (North Sea: MANOVA, $df = 7$ on 292, $p < 0.05$; Kattegat:
375 MANOVA, $df = 7$ on 130, $p < 0.05$). For both populations, these differences between year
376 classes were attributable to Mg, Mn, Cu, and Zn (ANOVA, $df = 1$, $p < 0.05$ for all). No
377 significant differences were found for P, Sr and Ba (ANOVA, $df = 1$, $p > 0.05$ for all). Even
378 though there were significant differences between year classes within each population, these
379 were restricted to elements that are under physiological control (Campana 1999; Hüssy et al.
380 2020), and not in Sr and Ba, which are the element of key interest for inferring migration
381 history. Year class effects were therefore not considered relevant in the subsequent analyses.
382
383 *Pelagic juvenile stage:* The chemical fingerprints in the pelagic juvenile stage differed
384 significantly between the two populations (MANOVA, $df = 7$ on 292, $p < 0.05$). Significant
385 differences occurred in all elements except for P (ANOVA, $df = 1$, $p < 0.05$ for all except P).
386 Of particular interest are Sr, Ba and Mn, where Sr was higher in the North Sea population,
387 while Ba and Mn were higher in the Kattegat population (Table 2). Differences between year-
388 classes within the two populations were not significant (MANOVA, $df = 7$ on 292, $p > 0.05$).
389 The LDA showed that 83 % of the North Sea population and 68 % of Kattegat population were
390 correctly classified to their respective population with an overall classification success of 76 %

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

Juvenile demersal life stages: In order to address during which life stage (pelagic juvenile, 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 life stages. The results for the demersal juvenile stage mirrored those for the pelagic stage, with significant differences between populations (MANOVA, df = 7 on 292, $p < 0.05$), where differences were primarily driven by Sr, Ba and Mn (Fig. 5, Table S2). There were no statistical differences in concentration of any elements during the subsequent first winter (MANOVA, df = 7 on 292, $p > 0.05$) (Fig. 5, Table S3).The classification success decreased correspondingly from 83 % to 72 % and 50 % in the North Sea population and from 68 % to 62 % and 51 % in 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 age 2 onward, differences between populations were no longer significant (MANOVA, df = 7 on 292, $p > 0.05$).

Adult migrations

The comparison of otolith Sr concentration between consecutive seasons and ages found significant differences overall (ANOVA, df = 19, $p < 0.05$), no significant differences were evident between any of the consecutive seasons and ages in the pairwise comparisons (Tukey HSD, all $p > 0.05$). This shows that there is no consistent seasonal effect on otolith Sr concentration. It was therefore assumed, that any change in otolith Sr was associated with a change in habitat occupation and not seasonally varying temperature or growth. From the 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 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 of the Kattegat, with 25 % (n = 21) of the North Sea individuals and only 8 % (n = 9) of the Kattegat individuals remaining resident within the Kattegat from the first winter and until capture (Fig. 6, green). The majority of individuals from both populations showed evidence of migration to other areas at some point between the first winter and time of capture. By far the most frequent migration signature was alternating distribution in the Kattegat and western Baltic Sea (55 % (n=47) of North Sea cod; 68 % (n = 78) of Kattegat cod) (Fig. 6, blue green), while a proportion of fish from both populations also displayed signatures consistent with migration to the Skagerrak/North Sea and back to the Kattegat (31 % (n=27) of North Sea cod; 45 % (n = 52) of Kattegat cod) (Fig. 6, lime). Ten individuals (8.5 %) from the Kattegat population appeared to have visited the eastern Baltic Sea for shorter periods of time (Fig. 6, purple). In general, the migration patterns were not consistent between seasons, suggesting the absence of synchronized migrations between areas.

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 many individuals had been assigned to the four habitats at any given fish age and time of the year, percentages of habitat area assignments were calculated across all individuals for each age and season separately. The resulting percentages thus represent a population's distribution pattern in space (North Sea/Skagerrak, Kattegat, western and eastern Baltic Sea) and time (age and season). The most notable observation is that habitat distribution patterns are remarkably 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 %) of North Sea population and 67.1 % (range = 53 – 79 %) of the Kattegat population (Fig. 8, green bars), making the Kattegat the primary habitat of distribution. Across seasons and age classes, approximately 25 % of both population was distributed in the western Baltic Sea (North Sea: mean = 24.9, range = 0 – 50 %; Kattegat: mean = 23.5, range = 8 – 37 %) (Fig. 7, blue green bars). Less than 10 % of both populations were distributed within the Skagerrak/North Sea (North Sea: mean = 8.3, range = 5 – 12 %; Kattegat: mean = 7.9, range = 3 - 13 %) across all seasons for the age classes 1 – 3 years (Fig. 7, lime bars). An apparently higher degree of migrations between the Skagerrak/North Sea and back to the Kattegat seemed to occur during age class 4 in the North Sea population, in that increasing percentages (mean = 26.6, range = 13 -50 %) were distributed within the Skagerrak/North Sea. However, it is important to note that the sample sizes are relatively low for these age groups and inferred distribution patterns therefore somewhat uncertain. Surprisingly, in the Kattegat population 1.4 ± 1.3 % of individuals were assigned to the eastern Baltic Sea for restricted periods of time around fall and winter (Fig. 7, purple bars). Migration patterns of fish older than four years could not be analysed for the North Sea population, as no samples of this population were available.

Habitat assignment precision: Correct habitat area assignment relies heavily on the strontium – salinity calibration curve used as well as the salinity cut-off values used for defining the boundary between areas. The impact of salinity cut-off values selected for defining the boundary between areas on estimated habitat assignments was evaluated with a simple sensitivity analysis. Since this selection is more related to the study area, but is less relevant for the methodological approach as such, these result are not presented here, but may be found 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 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 boundary between this area and the Kattegat is at the upper end of the salinity range, where the calibration curve flattens. This exercise shows, that the uncertainty in the calibration curve on proportion of individuals assigned to the Skagerrak/North Sea results in an average deviation of 3.4% for both populations (North Sea: range 0 to 12.5%; Kattegat: range 0.9 to 6.5%) between the highest (mean - se) and lowest (mean + se) estimates (Fig. 8).

Discussion

Spawning origin: In this study, we found population-specific chemical fingerprints in the early life stages of cod in Kattegat. The elements driving this differentiation were Sr, Ba and Mn. 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 is fairly constant worldwide, and mixing with freshwater not only dilutes salinity but also the Sr concentration (Kraus and Secor 2004; Walther and Limburg 2012). Because otolith Sr concentration is strongly correlated with ambient concentrations, with correlations generally > 0.95 found throughout the literature (review of studies in Hüssy et al. (2020)), otolith Sr is a

useful proxy for environmental salinity and is globally used to track movements of fish between marine and freshwater and within estuaries (Bath et al. 2000; Elsdon and Gillanders 2003; Miller 2011; Sturrock et al. 2012). While several studies report a significant impact of temperature on otolith Sr, the results are largely conflicting, ranging from a positive influence to a negative (Bath et al. 2000) – or no influence at all (Clarke and Friedland 2004; Walther et al. 2010; Sturrock et al. 2015), explaining at best a few percent of the variation in otolith Sr (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 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 seasonality in otolith Sr patterns, suggesting that changes in otolith Sr are associated with a change in habitat occupation rather than seasonally varying temperature or growth.

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 aquatic environment that is strongly related to environmental salinity with depletion in surface waters, with higher concentrations in freshwater and nearshore areas (Elsdon and Gillanders 2005; Walther and Limburg 2012). Highest Ba concentrations generally occur at salinities between 5 and 20 psu (Walther and Limburg 2012). The Ba concentration in otoliths almost exclusively reflects ambient concentrations (Bath et al. 2000; Elsdon and Gillanders 2003; 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 higher in the Kattegat population. Manganese concentrations on the other hand are known to increase in hypoxic areas owing to the reduction of manganese oxides from the sediment with decreasing ambient oxygen content. Otolith Mn concentrations have proven useful for tracking hypoxia exposure in e.g. Baltic cod (Limburg et al. 2011, 2015). Prolonged seasonal hypoxia

515 is known to occur in the Kattegat (Rosenberg et al. 1992, 1996), which is reflected in the much
516 higher Mn concentration during the early life stages of the Kattegat population.
517 Significant differences in particularly Mg, P, Cu and Zn were also detected between a few year
518 classes within the two populations. These elements are under strong physiological control
519 (Campana 1999; Sturrock et al. 2015; Limburg et al. 2018; Hüssy et al. 2020) and have been
520 shown to reflect fish growth (Heimbrand et al. 2020; Hüssy et al. 2021). Otolith element
521 concentrations of these elements were consistently higher in the North Sea populations. This
522 corresponds with the larger size at age observed between populations and suggests a genetic
523 component in growth regulation, and a mechanism promoting early settlement of North Sea
524 cod. No differences between year classes in all other elements shows that the classification to
525 population based on Sr, Ba and Mn is not influenced by inter-annual growth rate differences.
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
530 *Early life stage drift*: The rapidly decreasing classification success from the pelagic juvenile
531 stage to the beginning of the first winter and the lack of significant differences in
532 environmentally related elements (Sr, Ba, Mn) in subsequent years, suggests that the North Sea
533 fish arrived in the Kattegat during the early life stages. Kattegat cod spawn within a restricted
534 area of the Kattegat (Vitale et al. 2008; Börjesson et al. 2013), and early life stages of this
535 population have in recent years largely been retained in the area (Jonsson et al. 2016). North
536 Sea cod spawn along the southern and eastern edges of the Dogger Bank, in the German Bight,
537 the Moray Firth and to the east of the Shetlands (Brander 1994; Fox et al. 2008; Munk et al.
538 2009). Drift simulations have shown that cod eggs, larvae and early juveniles spawned in the
539 North Sea may be advected for more than 600 km during their drift period (Eero et al. 2016).

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

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

565 Sea once mature in support of natal homing as suggested by Svedäng et al. (2007). From our
566 data it is not possible to deduce whether the migratory cod did in fact spawn during their stay
567 in the Skagerrak/North Sea.
568 The habitat assignments largely depend on the salinity cut-off value selected as defining the
569 boundary between areas and the accuracy of the strontium – salinity calibration curve. Since
570 both salinity and strontium are subject to measurement errors, the calibration curve is subject
571 to some uncertainty, in particular at the Kattegat/Skagerrak boundary, where area-specific
572 differences in salinity are less pronounced than between other areas and the logarithmic
573 relationship of the calibration curve at the same time is flattening. Although a rigorous cross-
574 validation would be needed to estimate the power of our method, we did estimate effects from
575 calibration curve uncertainty and different cut-off salinity values used for categorizing areas
576 and found only minor variations in these proportions, suggesting the presented approach to be
577 relatively robust. Consequently, our results suggest some migration between the Kattegat and
578 Skagerrak/North Sea. We also found some evidence for inter-individual variability in
579 migratory behavior with some fish displaying a more stationary signature while other fish
580 seemed to display signatures in agreement with a pattern of recurrent migrations and longer
581 time occupancy in the North Sea.
582 Information of juvenile and adult cod migrations is available from tagging experiments from
583 the 1950s, 1990s and 2000s and have documented migration patterns similar to the ones
584 inferred from the present study. These studies found that cod tagged as 1-year old juveniles in
585 Kattegat only undertook limited migrations until the age of 2 years and 30 - 50 cm in length,
586 when they moved offshore towards the south and west – towards the spawning grounds in the
587 southern Kattegat and the eastern North Sea (Pihl and Ulmestrand 1993). Adult cod tagged at
588 offshore and in coastal locations in the Skagerrak undertook long-distance migrations ranging
589 from the southern North Sea to the central Kattegat (Danielssen 1969). Migrations of adult cod

590 tagged along the Swedish Kattegat coast were directed both south and north towards the Sound
591 and the eastern North Sea, with some coherence between tagging location and migration
592 direction (Righton et al. 2010; Svedäng et al. 2010). Of the cod tagged in the northern Kattegat,
593 12 % were recaptured in the Skagerrak or eastern North Sea, while cod tagged in the Sound
594 and the southern/central Kattegat were primarily resident (Svedäng et al. 2010), suggesting
595 migration patterns quite similar to the distribution patterns observed in the present study.
596 Geolocation of individuals based on electronic tags (DST's) documented that the migrations of
597 cod tagged in the northern Kattegat and Skagerrak are directional towards the North Sea and
598 coincide with known spawning times (Svedäng et al. 2007). Subsequent returns to the areas
599 where the cod were tagged also occurred, but quantification thereof was not possible (Svedäng
600 et al. 2007). Linking genetics with tagging-based migration patterns, André et al. (2016)
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
603 studies represent migration patterns at a time when the cod stocks were not yet depleted and
604 may therefore not apply to today's scenarios. However, given the similar conclusions from
605 widely different analytical approaches and a large range of years, it seems that the migratory
606 exchange between Kattegat and Skagerrak/North Sea is – and has always been - limited in
607 immature cod, but extensive in mature cod and most likely linked to spawning migrations.
608
609 *Migration between Kattegat and western Baltic Sea*: This study also found that a large
610 proportion (approximately 25 %) of individuals from both populations were distributed within
611 the western Baltic Sea during all seasons and across all age classes. The salinity regimes in the
612 western Baltic and Kattegat differ considerably from each other with mean bottom salinities of
613 10 – 15 psu and 30 - 33 psu respectively. This salinity gradient lies within the ideal range for
614 discriminating habitat use (Kraus and Secor 2004; Walther and Limburg 2012), and the results

615 are therefore considered reliable. The migration patterns of tagged cod discussed above do not
616 suggest a pronounced migration further south than the Sound. However, the dynamics in stock
617 size have changed considerably over the last decades, with unknown impact on migratory
618 behaviour. Considerable numbers of eggs and larvae spawned in the western Baltic Sea, more
619 specifically the Great Belt and the Kiel Bay, may drift into the Kattegat and Skagerrak
620 depending on wind conditions prevailing during the spawning season (Huwer et al. 2016).
621 Provided that these individuals also show philopatric migration behaviour, one would expect
622 to find seasons with high assignments to the western Baltic Sea. A clear seasonal pattern with
623 higher proportions of western Baltic Sea signals in spring (the main spawning season of
624 western Baltic cod) was, however, not evident. The occurrence of 2 % cod with a clear western
625 Baltic/Sound otolith chemistry signal over most of their lives prior to being captured in the
626 Kattegat further suggests considerable connectivity between these areas. Cod spawning in the
627 Kattegat and western Baltic Sea likely belong to the same biological population or population-
628 complex which has been found to display a hybrid zone genetic signature between the clearly
629 differentiated North Sea and Baltic Sea populations (Nielsen et al. 2003). Consequently, some
630 migration between areas is also well aligned with the overall patterns of genetic similarity
631 observed in the geographical region in the populations. Further work will be needed to
632 investigate potential patterns of minor genetic sub-structuring of spawning components within
633 the Kattegat and western Baltic Sea. However, our results clearly indicate that current
634 assumptions of closed units used for stock assessments of cod in the North Sea, Kattegat and
635 the western Baltic Sea are likely violated by the dynamic patterns of exchange between areas.
636
637 *Evaluation of the interdisciplinary approach*: With this study we have demonstrated that an
638 interdisciplinary approach combining information from genetics and otolith microchemistry
639 coupled with advanced modelling tools may provide unprecedented new insights into complex

biological questions. Genotyping to identify population of origin and chemical fingerprinting of the otolith core to identify hatch area are well established methodologies. Combined, however, they provide a powerful tool to study connectivity in area use of early life stages. The most innovative aspect of this study is the demonstration of how time-series models of chronological microchemistry data from hatch to death can be combined with genetic information and used to infer migration patterns of individual fish as well as stock mixing 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 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 outlined here therefore has wider applicability in both basic and applied research in fish and fisheries biology and could provide valuable data as direct input for conservation and management of fish stocks.

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Competing interests

The authors declare there are no competing interests.

Contributors' statement

KH: Conceptualization, Formal analysis, Methodology, Writing - Original Draft, Funding acquisition

CMA: Conceptualization, Formal analysis, Methodology, Writing - Review & Editing

JHH: Conceptualization, Methodology, Writing - Review & Editing, Investigation, Data curation, Funding acquisition, Project administration

MV: Conceptualization, Writing - Review & Editing

SHS: Methodology, Data curation, Writing - Review & Editing

TBT: Methodology, Data curation, Writing - Review & Editing

ME: Conceptualization, Writing - Review & Editing

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Data availability statement

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

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967

968 **Figure captions**

969

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

979

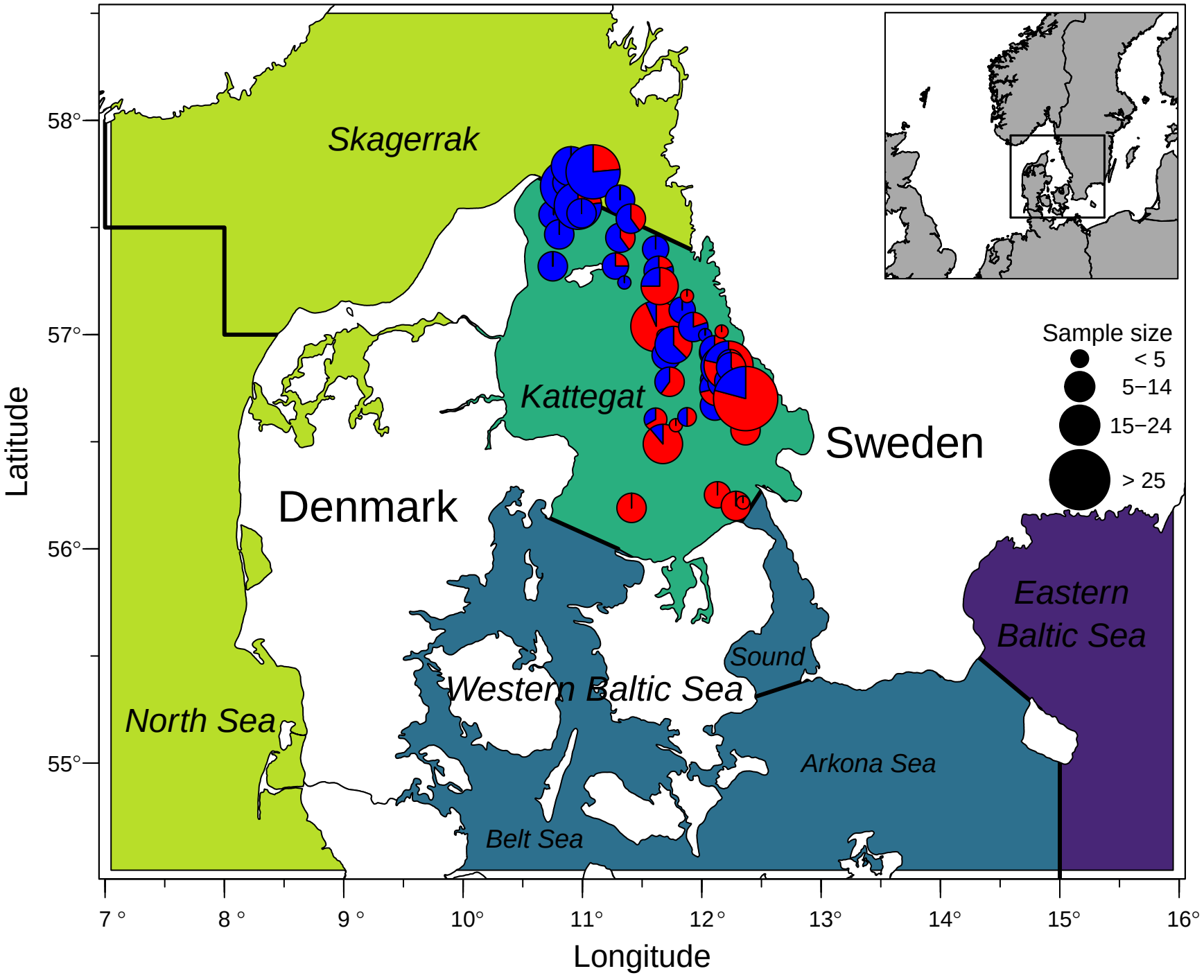
980 **Figure 2.** Image of otolith cross-section, viewed under reflected light, with laser track indicated
981 with a solid black line, and the corresponding otolith Sr profile where colours indicate the
982 habitat area assignments (North Sea = lime, Kattegat = green, western Baltic = blue green).
983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

988

989 **Figure 3.** Otolith size at the end of first year of life for the 6 age groups of cod captured in
990 Kattegat, representing the year classes 2010 – 2015, where colours represent different
991 populations: Kattegat (red) and North Sea (blue). Otolith sizes of age 0 individuals (year class
992 2016) are not included in this graph, because they were captured before the end of their first

993 winter. Horizontal lines indicate mean, box upper and lower limits the 25% and 75%
994 percentiles, whiskers represent the highest and lowest values within 1.5 interquartile range and
995 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
1002 **Figure 5.** Biplot of the first two discriminant functions of the LDA analysing otolith chemical
1003 fingerprints in the larval and pelagic juvenile stage (0 to ca. 4 months old), the demersal
1004 juvenile stage until the first winter zone (ca. 4 to 8 months old), and the first winter, with life
1005 stage indicated above each biplot. Colours represent the two populations: Kattegat (red) and
1006 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
1010 (population indicated above graph). The time from hatch to the first winter is not included in
1011 this plot, as the area assignment for this age group is based on a different habitat area levels
1012 and provides less precise assignments. Colours indicate the habitat area assignments: North
1013 Sea = lime, Kattegat = green, western Baltic = blue green, eastern Baltic = purple. Lifetime
1014 area use is shown for the two populations separately, where individuals are ordered according
1015 to latitude of their capture location in the Kattegat.
1016

1017 **Figure 7.** Percentage distributions of habitat area assignments by age and season, representing
1018 a population's distribution pattern in space and time, for the Kattegat population and the North
1019 Sea population separately (population indicated above graph). Colours indicate percentage of
1020 individuals with an area use assigned to the North Sea (lime), the Kattegat (green), the western
1021 Baltic Sea (blue green), and the eastern Baltic Sea (purple).
1022
1023 **Figure 8**. Percentage of individuals assigned to the Skagerrak/North Sea (lime coloured area
1024 in Fig. 1) by age and season, for the Kattegat population and the North Sea population
1025 (population indicated above graph). Colour shading represents the percentage estimate based
1026 on the mean values of the otolith strontium – salinity calibration curve (dark lime), for mean -
1027 se values (no colour), and mean + se (transparent lime).



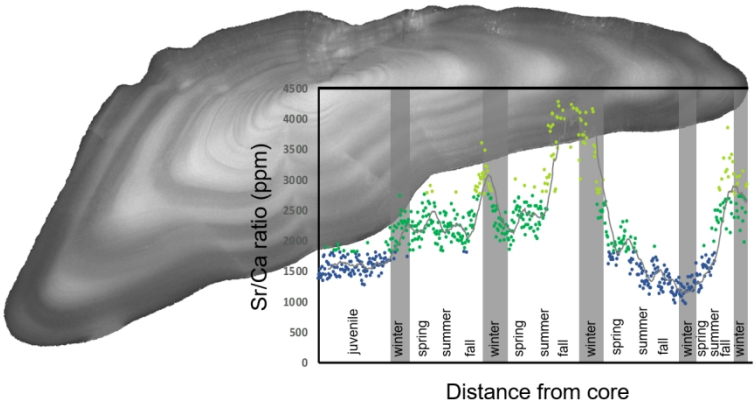


Image of otolith cross-section, viewed under reflected light, with laser track indicated with a solid black line, and the corresponding otolith Sr profile where colours indicate the habitat area assignments (North Sea = lime, Kattegat = green, western Baltic = blue green). Translucent winter growth zones 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.

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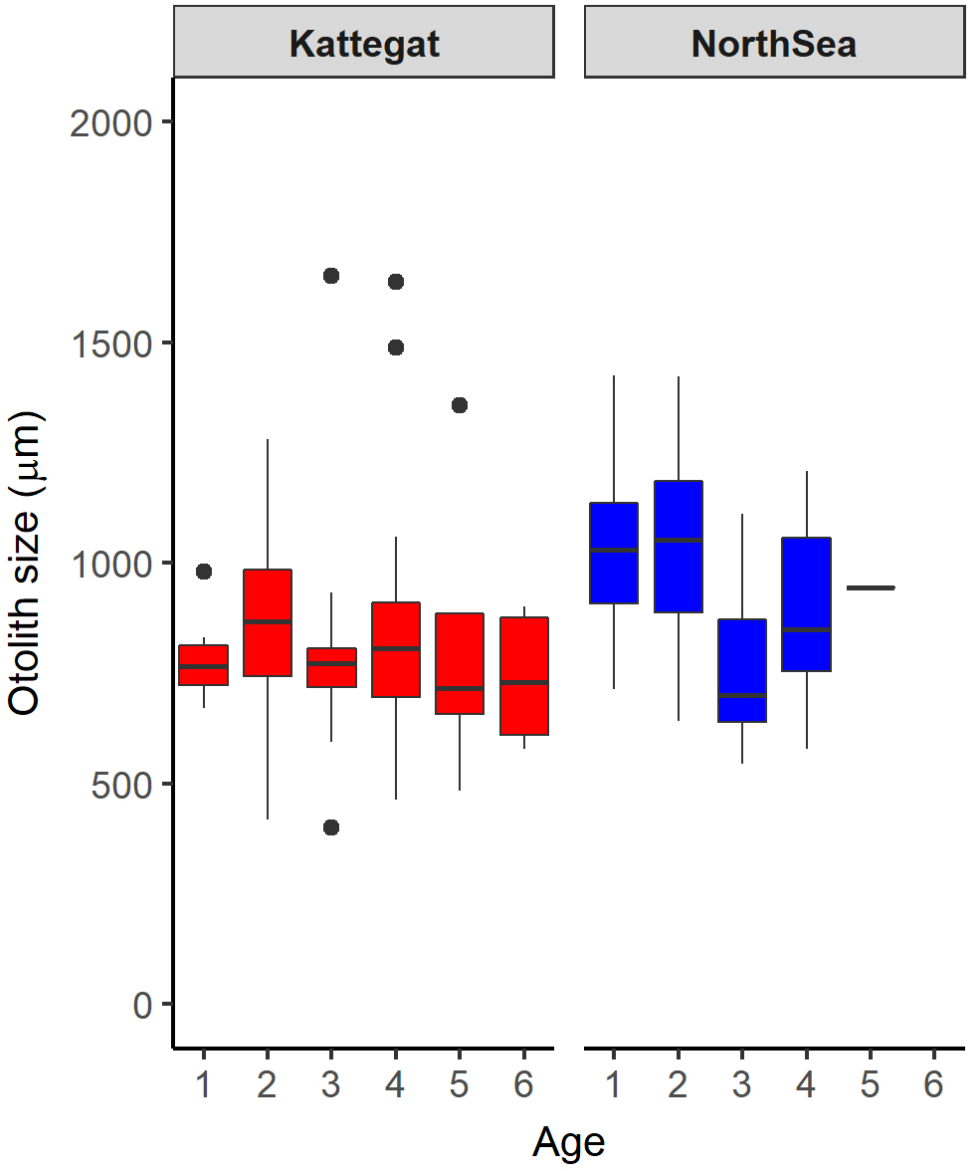
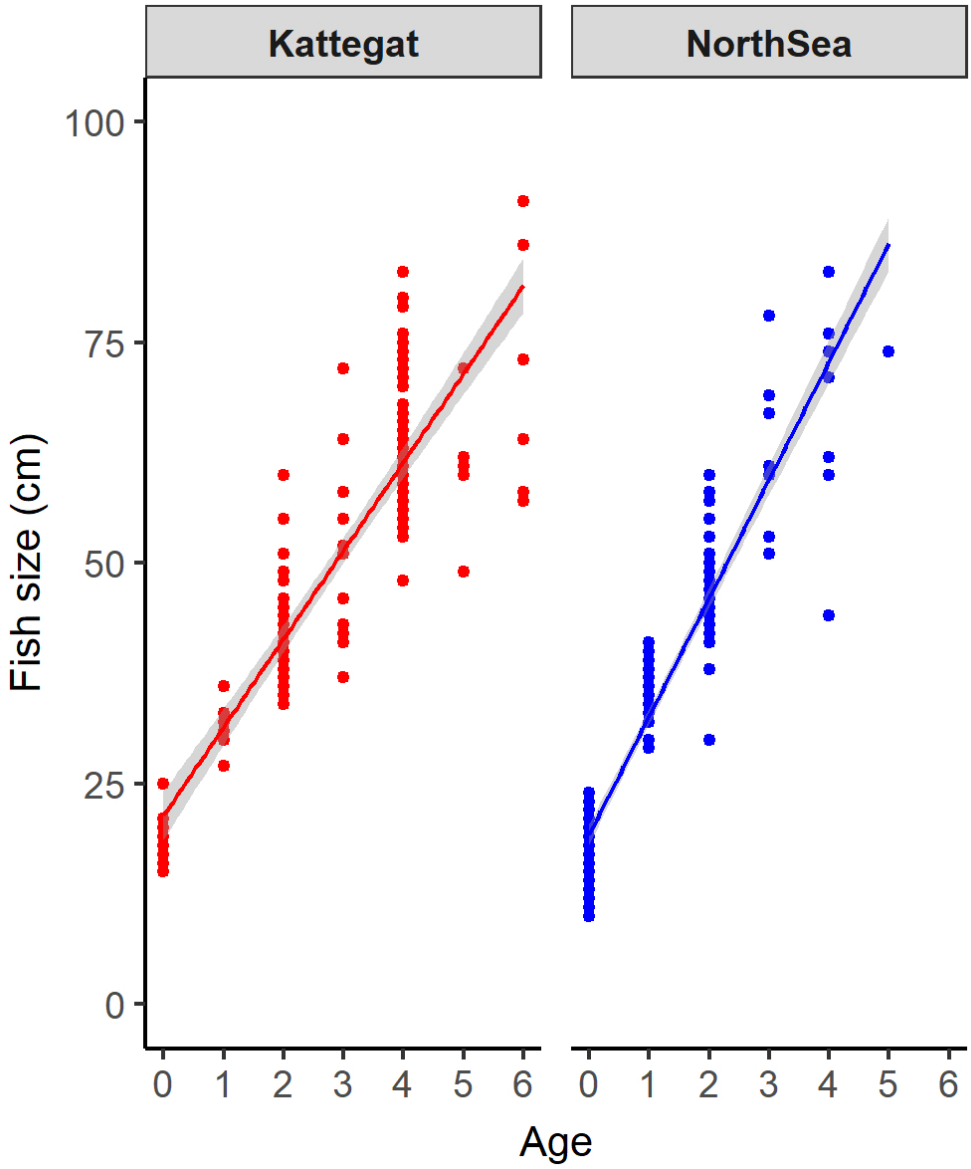


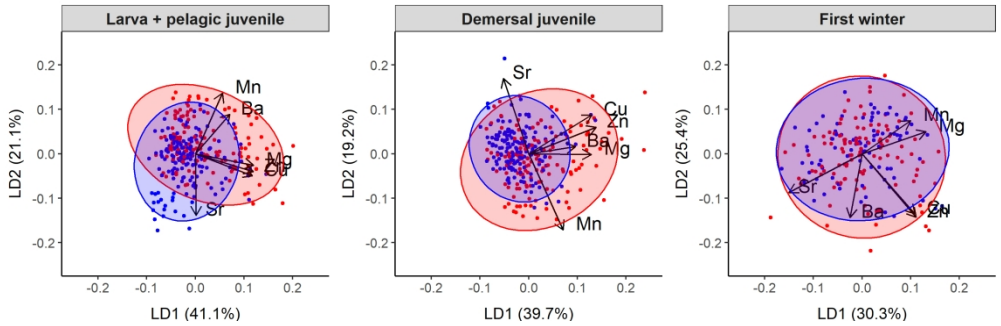
Otolith size at the end of first year of life for the 6 age groups of cod captured in Kattegat, representing the year classes 2010 – 2015, where colours represent different populations: Kattegat (red) and North Sea (blue). Otolith sizes of age 0 individuals (year class 2016) are not included in this 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).

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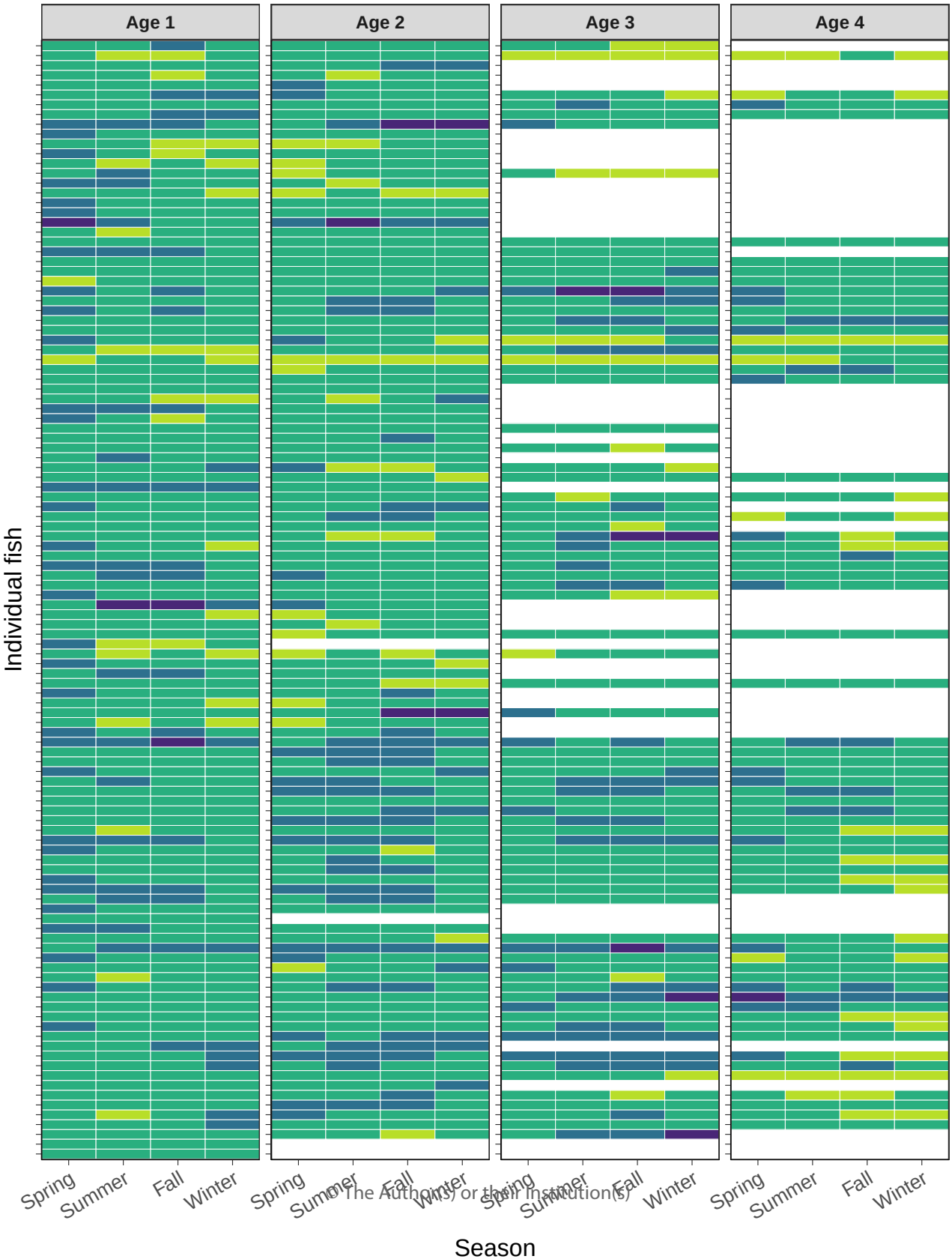

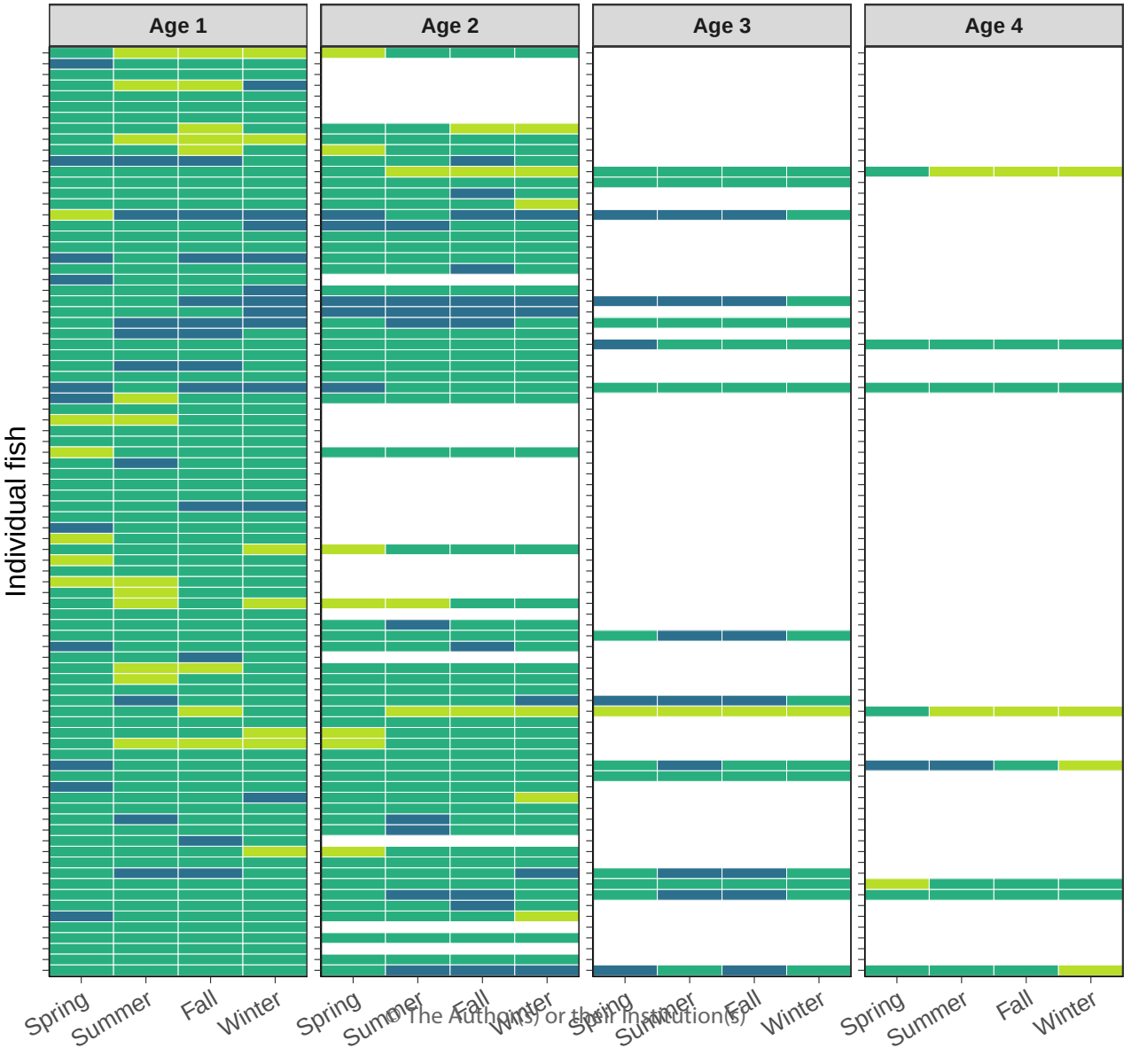

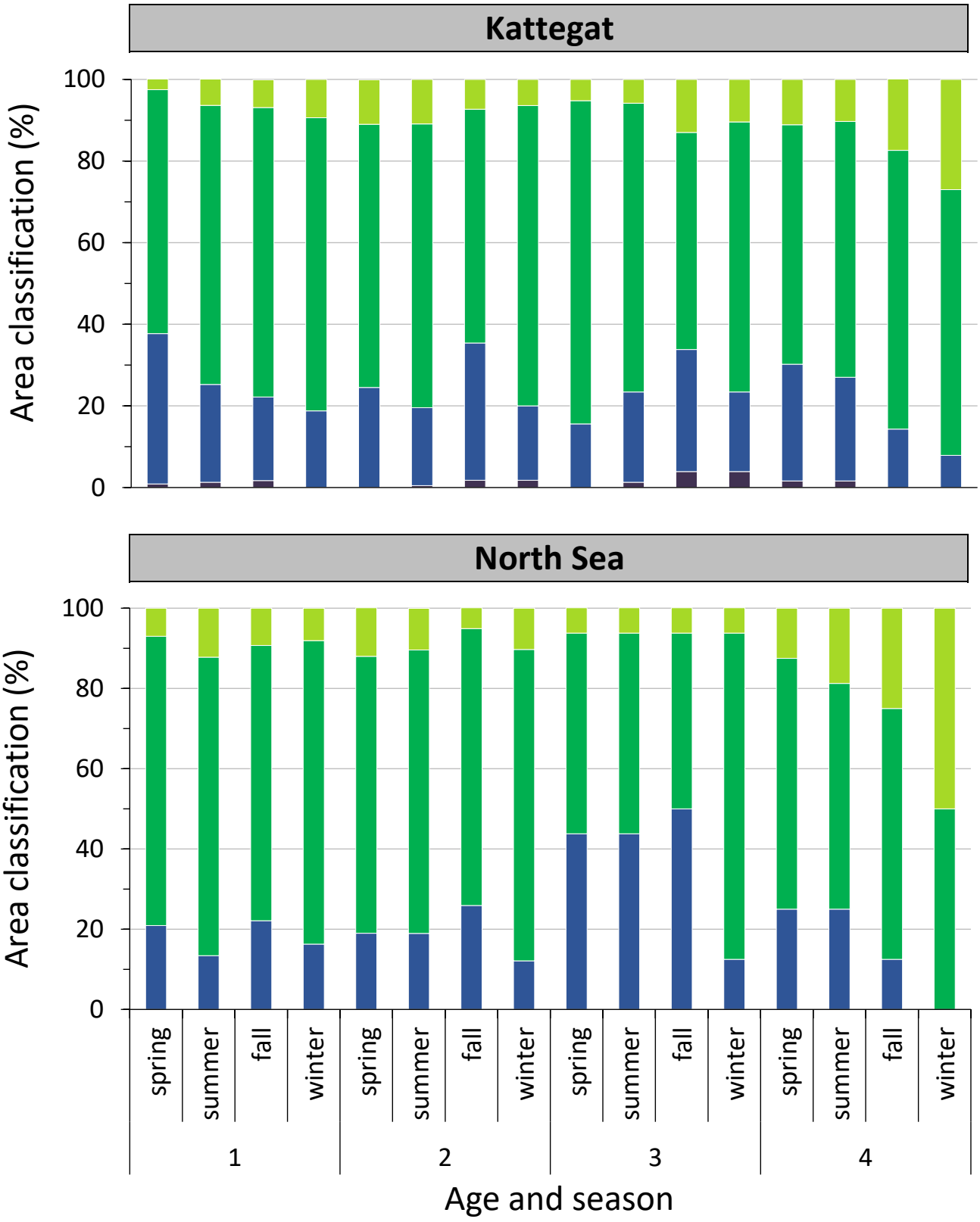

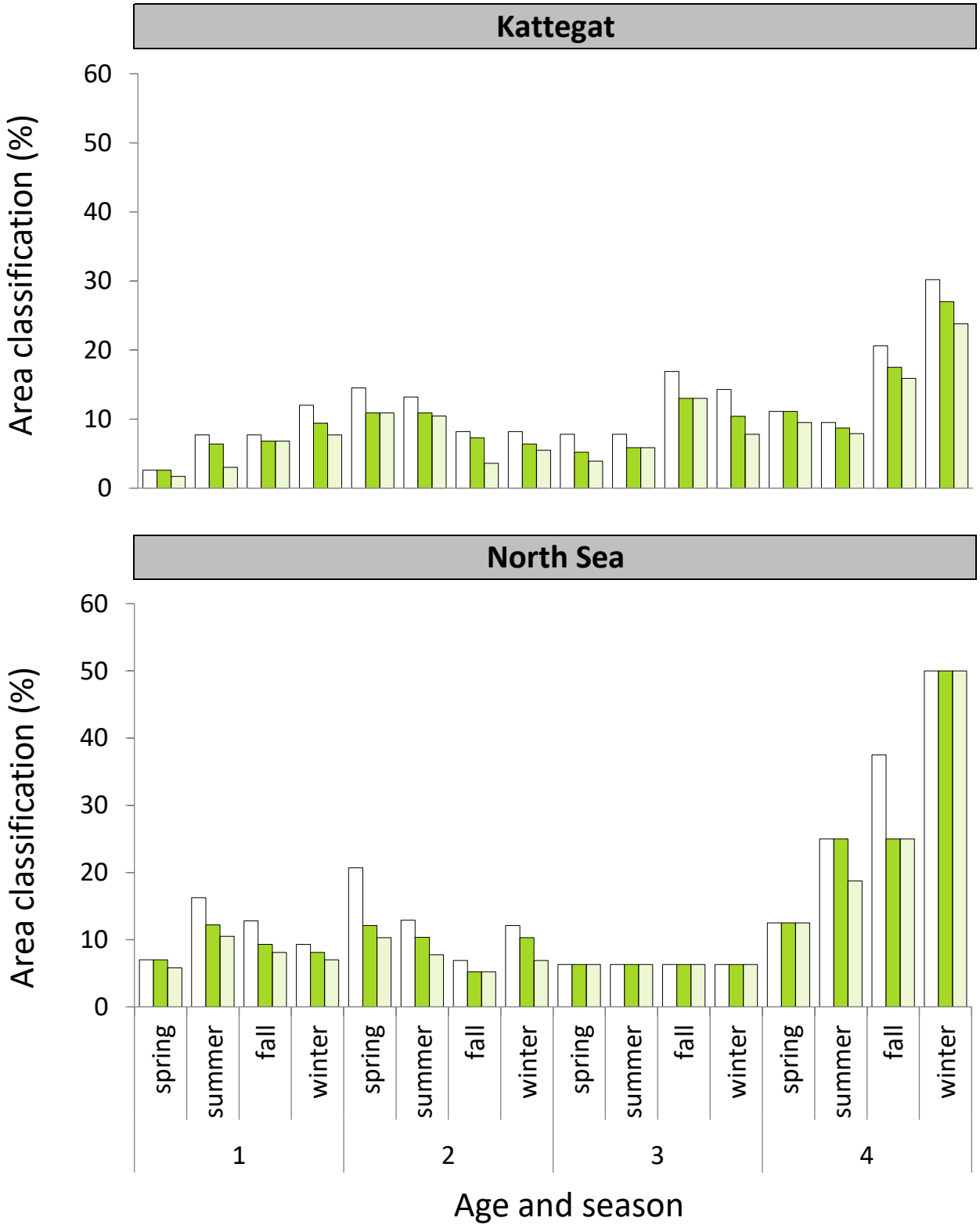

Table 1. Overview over samples used in this study by population and age.

	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 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)

	Population				
Element	Kattegat	North Sea	df	F	*p*
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	***
P	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	***