Management of mixed cod stocks in the transition zone between the North Sea and the Baltic Sea: How can this be achieved efficiently? (FABBIO)

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Colophon

Preface

The present report is based on the project "Management of mixed cod stocks in the transition zone between the North Sea and the Baltic Sea: How can this be achieved most efficiently? (FABBIO). The project had the journal no 33113-B-19-140 and was funded with 2.75 million DKK by the European Maritime and Fisheries Fund and the Danish Fisheries Agency. The project leader was Karin Hüssy and the project period was from June 2019 to May 2023.

In Danish the project title is "Forvaltning af blandede torskebestande i overgangszonen mellem Nordsø og Østersø: Hvordan kan det gøres mere effektivt? (FABBIO).
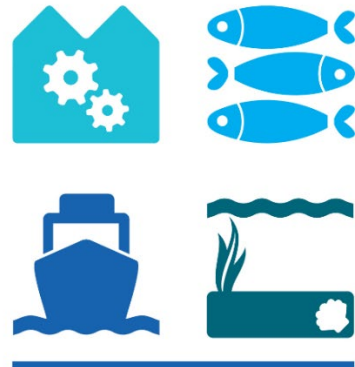
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Content

Summary

The project "*Management of mixed cod stocks in the transition zone between the North Sea and the Baltic Sea: How can this be achieved most efficiently?*" focused on one of the key challenges for assessment and management of fish stocks: Movement of individuals and stocks mixing. The project focused on resolving issues of stock mixing in cod stocks between the North Sea (Subarea 4), Skagerrak (SD 20), Kattegat (SD 20), the western Baltic (Belt Sea (SD 22), and Sound (SD 23)), and the eastern Baltic Sea (SDs 24 – 32). The project was funded by European Maritime and Fisheries Fund and the Danish Fisheries Agency. The activities in the project involved providing new biological knowledge on genetic and ecological connectivity, advancing methods, and providing a tool to evaluate the impact of stock mixing on stock assessment.

Genetic identification of populations
Full genome resequencing data revealed marked genetic differences between samples of fish at spawning time collected in the North Sea, eastern Baltic Sea and the transition zone between the two areas (i.e. SD 21-SD 24). In contrast, we found limited divergence between spawning fish collected in the southern Kattegat, Belt Sea, Sound and Arkona Sea (SD 24, spring), suggesting that these fish belong to the same biological population, identifying a total of three major biological units for management in this system. Weaker gradient signals within the transition zone may be related to introgression in the hybrid zone from parental populations in the North Sea and eastern Baltic Sea, and they are much weaker than any difference observed between the three populations. Future sampling may refine these analyses, in particular regarding the relationship between spawning fish in the transition zone. However, they are not expected to change the overall relationship and magnitude of differentiation between the three populations presented in the current report.

Genetic identification of population mixing
Through the use of a panel of genetic markers specifically designed to identify population of origin in a scenario with three baselines (North Sea, transition zone, eastern Baltic Sea, as supported by the genomic analyses above) we analyzed samples collected across the full transition zone to estimate proportions from the three baseline populations. The results confirmed previous findings of substantial mixing in the Kattegat and Arkona Sea, with North Sea fish and transition zone fish mixing in Kattegat and eastern Baltic Sea fish and transition zone fish mixing in the Arkona Sea. We also confirmed the gradient of mixing in the Kattegat, with higher proportions of North Sea fish in the northern parts of the area. In contrast, we found limited mixing in the Belt Sea and the Sound where fish appeared to be primarily of transition zone origin. While data for older age classes were limited, we also found evidence for higher proportions of North Sea fish among younger age classes, as also reported in previous studies.

Natal origin and movements of adults
This project investigated the natal origin and adult movements of cod in the transition zone, focusing on spatial differences in the chemical composition (the chemical "fingerprint") recorded in the cod's otoliths. Examining the elements analyzed in this study, it becomes apparent that they reflect gradients in environmental conditions and physiological processes, aligning with known mechanisms of otolith biomineralization. Therefore, otolith chemistry proves to be a suitable method for assessing the movement patterns of fish in the transition zone.

The analyses of the otolith cores, representing the natal origin of cod, revealed spatial variations in their chemical fingerprint, indicating at least three different clusters (= spawning areas). Without baseline samples from cod larvae, it is not possible to identify where these spawning areas are, but combined with knowledge of known spawning areas, the results suggested that the cod originated primarily from the North Sea (concentrated in the Skagerrak and northern Kattegat), the Southern Kattegat (spread throughout Kattegat and partly in the Sound and Belt Sea), and the Belt Sea (predominantly found in the Belt Sea and the Sound). The contribution of the spawning area clusters varies significantly among different year classes of cod, indicating fluctuations in the relative contribution of cod originating from each spawning area.

Cod inhabiting the *Skagerrak* and *northern Kattegat* regions either exhibit a similar chemical fingerprint throughout their lives or exhibit extensive mixing. Generally, these cod populations do not migrate south into the western Baltic Sea. However, some cod in the southernmost Kattegat display a Belt Sea/Sound signal throughout their lives, which is likely not due to movements but rather due to the incomplete alignment of environmental gradients with the boundaries of management areas. In contrast, the *Belt Sea* region predominantly hosts cod that remain resident throughout their lives, with minimal movements observed. In contrast, cod in the *Sound* region show a connection with the Belt Sea. The majority of cod immigrate from the Belt Sea to the Sound before the age of 3 and tend to remain resident there. However, occasional Belt Sea signals detected at irregular intervals suggest some movement in and out of the Sound.

The combined analysis of natal origin and adult movements provided valuable insights into the dynamics of the cod population in the transition zone. While cod in this zone may originate from different spawning areas, they are largely resident within the respective management areas as adults. Consequently, the project results suggested the presence of an ecological stock separation into two distinct stocks: The Skagerrak/Kattegat and the Belt Sea/Sound.

Stock mixing: Combining genetics and otolith chemistry
Collectively, the compilation of knowledge gained from historical data and new samples, from genome sequencing and otolith chemistry, indicate that there is considerable genetic and ecological structuring of cod between the North Sea and the Baltic Sea, with three genetically distinct populations: 1. North Sea, 2. eastern Baltic Sea and 3. transition zone (Kattegat, Belt Sea, Sound and Arkona Sea). The geographic distribution of these populations overlap in the Kattegat and Arkona Sea, respectively. While it was not possible to detect genetic differentiation in the transition zone, otolith chemistry revealed considerable ecological stock structuring. This scenario is consistent with considerable exchange of individuals between areas, presumably as a result of drift of early life stages. While cod in the transition zone may originate from different spawning areas (two apparently distinct areas were identified), they are largely resident within the geographical areas they settle into as adults. This leads to stock structuring in the transition zone with an ecological separation into two distinct components: 1. the (eastern) Skagerrak and the Kattegat, 2. the Belt Sea and the Sound.

For cod in Belt Sea, the Sound and the spring-spawners in the Arkona Sea, the combined genetic and otolith chemistry results thus indicate that the current management area for western Baltic is appropriate. In the Kattegat, the spatially and temporally variable mixing dynamics with the North Sea need to be considered for a sustainable management of the stock, similarly to the mixing scenario with the eastern Baltic cod in the Arkona Sea. Recommendations as to what type of

stock assessment approach is most suitable for this complex system of genetic and ecological stock structuring is not within the scope of this project.

Impact of fish movements and stock mixing on stock assessment

This project implemented the prototype of a simulation tool to evaluate migration impacts on stock assessment and propose mitigation strategies. While functional, the tool can be improved with documentation, user-friendly implementations, and inclusion of additional factors. Simulations highlighted challenges in specifying complex scenarios and limited data availability. Migration patterns affected assessment data. Closing parts of the norther Kattegat, for example, showed potential for stock recovery. A cost-benefit analysis compared genetic samples and otolith shape analysis for stock composition estimation. Precision and cost influenced the choice between methods. Note that the analysis did not consider sample collection or baseline costs. Given the new biological knowledge on genetic and ecological stock structure, movement patterns in the transition zone from the North Sea to the eastern Baltic, this will be the next focus area for the application of this tool.

Potential management scenarios

Based on the collective genetics, otolith chemistry and modelling simulation results from this project, we have identified three different scenarios for how stock mixing could be implemented in stock assessment and management for cod stocks in the transition zone:

- Area-based assessment and management – current scenario. This is a *status quo* scenario, where current practices are continued with two distinct stocks in the transition zone: Kattegat and the western Baltic Sea (Belt Sea, Sound and Arkona Sea), with separate stock assessments and TACs, irrespective of genetic population. Stock mixing of eastern/wester Baltic cod in the Arkona Sea is already implemented in stock assessment. By not addressing stock mixing of North Sea/Kattegat populations, the severely declined Kattegat population is at risk of local depletion.

- Area-based assessment and management – updated scenario. This scenario implies continuing with current practices of separate stock assessments for the current management areas Kattegat and western Baltic Sea (Belt Sea, Sound and Arkona Sea). In addition to the stock mixing of eastern/wester Baltic cod in the Arkona Sea the mixing of North Sea and Kattegat cod in the Kattegat should be addressed in a similar approach. This scenario disregards the fact that cod in the transition zone are genetically the same population, but would on the other hand reflect the ecological stock structuring, and thereby minimize the risk of local depletion of population components.

- Population-based stock assessment and management. A population-based approach to stock assessment would require cod from the Kattegat, Belt Sea, Sound and Arkona Sea to be combined into a single stock representing the genetic "transitions zone population". Stock mixing proportions in the transition zone population would need to be estimated for these areas, based on a genetic split of commercial and survey data. Subsequently, TACs can be allocated to exiting management areas, but should be informed by genetic estimates of mixing proportions in the different management areas to link estimated harvest rates in geographical areas to the stock assessments/advice for the underlying biological populations. components not accounted for with genetic split data, ii) how to allocate area-specific TACs, and iii) how to deal with social and political considerations.

1. Background and outline of the project

The waters from the North Sea to the Baltic Sea are an economically important area for Danish fisheries. The area serves as a transitional zone from a fully marine environment in the North Sea to a brackish environment in the Baltic Sea. In this zone, many fish species, including Atlantic herring (Bekkevold *et al.*, 2011), cod (Nielsen *et al.*, 2009), and sprat (Limborg *et al.*, 2009), have developed genetically distinct populations, each biologically adapted to their respective environments. Although it has long been known that the individual stocks overlap due to widespread migrations, it has been challenging to account for stock structure and mixtures in stock assessment and fisheries management. This is primarily due to insufficiently detailed biological knowledge and the lack of ready-to-use methods for the necessary analyses. In this project, we used Atlantic cod (*Gadus morhua*) stocks from the North Sea to the Baltic Sea as an example.

In this area, cod occur throughout the ICES Subareas and Subdivisions (SD), from the North Sea (Area 4), Skagerrak (SD 20), Kattegat (SD 21), Belt Sea (SD 22), Sound (SD 23), Arkona Sea (SD 24), and to the eastern Baltic Sea (SDs 25 - 32). In the following we will refer to areas by name and not SD numbers. Cod are currently managed as five stocks by ICES: the North Sea–Skagerrak (Area 4 & SD 20), the Kattegat (SD 21), the western Baltic Sea (SD 22-24), and the eastern Baltic Sea (SDs 25 - 32). All cod stocks in this area have experienced significant declines in population size over the past years and, with few exceptions, a general low recruitment (ICES, 2021).

Analyses of stock mixtures in the inner Danish waters are based on an innovative approach that combines interdisciplinary methods. This approach involves genetically identifying the origin stock of the cod and conducting shape and microchemical analyses of otoliths to identify the marine areas the cod have inhabited (Hemmer-Hansen *et al.*, 2019, 2020; Hüssy *et al.*, 2021b). When combined, these two methods provide answers regarding the stock a cod belongs to and where it has been at different stages of its life, which can be translated into migration rates between areas. Previous projects have focused on migrations and stock mixing between the North Sea and Kattegat (33113-B-16-034) (Hemmer-Hansen *et al.*, 2020) and between the western and eastern Baltic Sea (3744-11-k-0216) (Hüssy *et al.*, 2013). In order to implement mixing patterns throughout the entire transitional area between the North Sea and the Baltic Sea, this project assessed the lacking stock genetics and migrations between Kattegat and the western Baltic Sea, and the question of which biological stock cod in the Sound belong to.

Fish stock assessments traditionally focus on fish in a specific geographical area and therefore do not take into account stock mixtures and migrations. As a result, stock assessments are not biologically accurate and can lead to ineffective management (Reiss *et al.*, 2009; Maggini *et al.*, 2022). When migrations between areas are not taken into account, it becomes difficult to quantify fishing mortality for individual stocks (especially Kattegat cod), and it hinders the understanding of stock productivity. As a result, stock assessment becomes uncertain, and management is not optimal. Experience from the stock mixing case between eastern and western Baltic Sea cod in the Arkona Sea has shown that detailed quantification of stock mixture proportions requires a significant amount of data, while also complicating the implementation of management. Yet, new approaches to population-based stock assessment and management have recently been implemented for cod in Greenland, showing that an integrated approach is feasible (ICES, 2023b).

Therefore, there is a need to develop a balanced management approach that allows for a simple and manageable management model, while still considering the necessary biological complexity and optimizing resources for data collection and analysis. In order to develop such a balanced management approach, in-depth biological knowledge of the distribution of individual stocks in space and time is necessary. Based on the provided knowledge and data, measures can then be developed and optimized, ultimately leading to the most effective management model that ensures the quality of individual stock assessments while optimizing the need for data collection and costly sample analyses.

To find long-term stable solutions to stock mixing related assessment issues, the following objectives were addressed in this project:

- Collection of biological knowledge of the dynamics of stock migrations and how they can be incorporated into a realistic model that describes the spatial and temporal overlap of stocks.
- Investigation of how the observed migrations affect the stock assessment for the involved stocks.
- Cost-benefit evaluation of the types and amounts of data needed to achieve accurate stock assessments and sustainable management in the future.

2. Historical data and sample collection

2.1 Introduction

The objective of this WP was to 1) collate existing information on stock mixing of cod from the Nort Sea to the Baltic Sea from various data sources, and 2) provide contemporary samples to provide updated information on stock mixing and connectivity.

Tagging data*: The aim of this task was to digitize data from historical Danish tagging projects, and to collate all national and international data from historical and recent tagging projects into a single data base. Over the last decades, many countries bordering the North Sea, Skagerrak, Kattegat and Baltic Sea have carried out tagging projects, where fish are caught, tagged with externally visible tags with an individual number and released into the wild. Upon recapture, all available information has been collected on hard copy information sheets (Fig. 1). Archives of these cards have been stored at fisheries research institutes and digitized over the years. Most of the tagging data of the Baltic Sea was collated and digitized during the TABACOD project (Mion *et al.*, 2020, 2021).

Otolith chemistry data: During various earlier projects (CODYSSEY (Righton *et al.*, 2006); DE-CODE (DECODE, 2009), TABACOD (Hüssy *et al.*, 2020a), and the EMFF-funded *Sustainable management of cod* (33113-B-16-034) (Hemmer-Hansen *et al.*, 2020) a database with chemical profiles of otolith has been collated. In FABBIO, this database was expanded with a subset of samples from the new sample selection in order to provide information that allows the reconstruction of each individuals movement patterns.

Collection of new samples*: The primary task was to collect contemporary samples for genetic stock identification and estimation of stock mixing, as well as individual movement patterns of a subset of these fish. Particular focus was on evaluating to what extent it is possible to discriminate genetically between cod stocks from the Kattegat, Sound and western Baltic Sea.

2.2 Materials and Methods

2.2.1 Historical tagging data

During FABBIO, information sheets of tagging data (Fig. 1) from the Inner Danish waters, particularly from the western Baltic Sea and the Sound (ICES areas 22 and 23) were digitized and collated with the TABACOD database. Each new entry was subjected to quality control and entries double checked. Further tagging data from the North Sea was obtained through collaboration with scientists from the Centre for Environment, Fisheries and Agricultural Sciences (CEFAS) in the UK (Righton *et al.*, 2006). This data was also formatted to the TABACOD database format to allow for combined analyses (Lundgreen *et al.*, 2022). The database now consists of 21.530 individual fish data entries (Table 1). Information collected for each fish were typically release location, date and fish size. Recapture information includes location, date and biological data (size, sex, maturity, age). Not all information is available for each fish, but where information was available, this was noted in the database. During the FABBIO project additionally a total of 7.962 information sheets of Danish taggings were digitized (Lundgreen *et al.*, 2023). The database now holds 21.530 individual fish where the release location was known. Release numbers by ICES SD and decade in which the taggings were carried out are shown in Table 1.

Fig. 1. Example of information sheet of a fish tagged on 14-03-1984 and recaptured on the 11-11-1986.

Table 1. Overview of the number of individual fish entries in the compiled cod tagging database shown by ICES Subdivision (SD) and decade. Maps show the location of the ICES SDs.

| ICES SD | Decade | | | | | | | Total |
	1950s	1960s	1970s	1980s	1990s	2000s	2010s	
20/3.a	4							4
21	41	114	481	374				1 010
22	1807	914		1064				3 785
23	208	231	262	96				797
24	15	807	525			68	185	1 600
25	513	2278	660	106	42	166	140	3 905
26	272	809	185				60	1 326
27		492						492
28.2	19	1189	131					1 339
29	76	84		65				225
30	11	1		84	5			101
32			401	2				403
4.a		1	306			12		319
4.b		19	1902	758	9	91		2 779
4.c		439	708	2203	24	41	30	3 445
Total	2 966	7 378	5 561	4 752	80	378	415	21 530
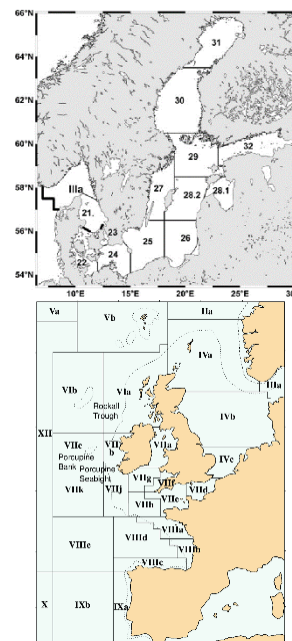
2.2.2 Historic otolith samples

The majority of the otolith samples in the Skagerrak/Kattegat/western Baltic Sea originate from the EMFF-funded *Sustainable management of cod* (Hemmer-Hansen *et al.*, 2020), while the samples from the eastern Baltic Sea originate from the tagging projects TABACOD and CODYSSEY (Righton *et al.*, 2006; Hüssy *et al.*, 2020a). A full overview of the samples available by fish age is shown in Table 2. During the initial analyses in FABBIO, it became evident that the samples from the tagging projects are not complementary to the new samples because they primarily consist of much older individuals and it was not possible to discriminate between area effects and sampling year. They were therefore not used in the analyses.

Table 2. Overview of otolith samples available from various projects by ICES SD and age. Numbers in italic indicate samples that were not used for the analyses in this project.

| Age | ICES SD | | | | | | | | | Total |
	20	21	22	23	24	25	26	4A	4B	
0	10	91	7							106
1	15	20	4			*2*				48
2	23	52	9		*6*	*27*	*1*		*8*	122
3	8	16	5		*11*	*28*	*2*		*5*	217
4	8	52	16		*13*	*69*	*4*		*4*	121
5	1	6	4		*59*	*93*	*17*	*1*	*2*	201
6		7	1		*55*	*75*	*12*	*1*	*1*	160
7		2	2		*9*	*10*				21
8		1	1		*3*	*1*	*1*			7
Total	65	248	49		141	296	37	2	15	1103

2.2.3 Collection of new samples

Originally, we had planned to collect cod for a genetic baseline collection (spawning individuals during the spawning season in January - March) and the fishery (outside the spawning season). Baseline samples were collected as planned from DTU Aqua's standard surveys KASU, Cod survey and Sole survey (Skagerrak, Kattegat, Belt Sea and Sound) in 2020 and 2021. In addition, baseline samples were provided by the Thünen Institute in Rostock (Belt Sea and Arkona Sea). Baseline samples from the Arkona Sea were collected in spring to target the wester Baltic cod population, as the eastern Baltic cod also spawns in that area, but with peak spawning in summer (Hemmer-Hansen et al. 2019). Baseline samples for genome sequencing were further comple-mented with earlier collections to represent North Sea ("Viking" and "Dogger" components) and eastern Baltic Sea (SD25) populations (Table 4). Fishery samples were collected from harbour landings and from the recreational catches of tour operators in 2020 and 2021. To estimate mixing outside spawning periods and for life stages not in spawning condition, we collected genetic data for 1946 fish sampled from KASU, cod and sole surveys in 2020-2022 (Table 3). These samples represent what would be targeted by the fishery and supplements the originally planned fishery samples, which were more difficult to obtain in larger quantities due to general restrictions in cod fisheries during the duration of the project. Thus, survey collected fish secured the important broad geographical and temporal coverage of sampling. A full overview of the new samples col-lected for genetic analyses, and the subset thereof used for otolith chemistry is shown in Table 4, and a map of sampling locations in Fig. 2. A summary of the samples used by age group is shown in Table 5 Note the balanced sampling design between Kattegat and Belt Sea, but lack of small individuals in the Sound.

Table 3. Number of samples used to estimate population mixing with genetic data in Skagerrak (SD 20), Kattegat (SD 21), Belt Sea (SD 22), Sound (AD 23) and Arkona Sea (SD 24), by quarter (Q)

| | ICES SD and Quarter | | | | | | | | | |
| | 20 | | 21 | | 22 | | 23 | | 24 | |
Sampling year	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4
2020	8	106	73	255	63	77	18		161	
2021			95	97	114	67		12	123	40
2022	7		135		207		151		137	

Table 4. Number of samples used for the Genomic analysis of spawning baseline

Region	No of samples
North Sea (Dogger)	18
North Sea (Viking)	20
ICES SD 21	22
ICES SD 22	20
ICES SD 23	20
ICES SD 24 (spring, i.e. western spawners)	19
ICES SD 25	23

Table 5. Number of new samples selected for otolith analysis by age

	ICES SD			
Age	21	22	23	Total
1	88	35	1	124
2	58	31	2	91
3	16	97	56	169
4	5	27	57	89
5	19	17	98	134
6	1	2	5	8
7	0	0	5	5
Total	187	209	224	620
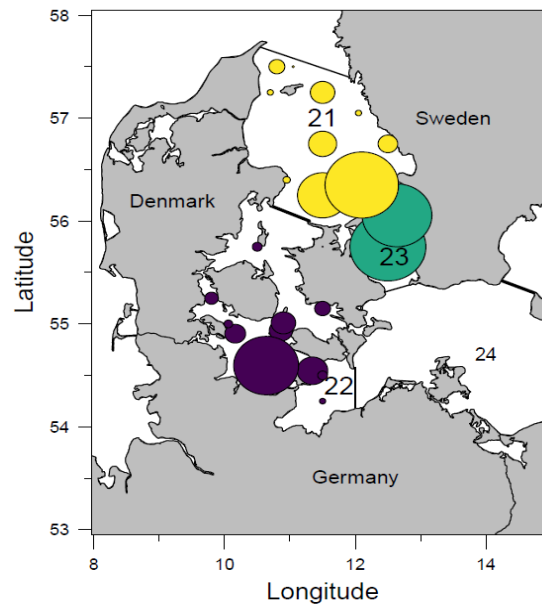
Fig. 2. Map of sample locations of the new samples collected during FABBIO, color coded by ICES Subdivision: Skagerrak (SD 20), Kattegat (SD 21), Belt Sea (SD 22). Symbols are scaled by number of individuals, from smallest dots: < 10 individuals to largest dots: > 100 individuals.

2.3 Conclusions

- A combined database with tagging data spanning the entire area from southern North Sea to northern Baltic Sea and covering 50 years of tagging projects is now available.
- New baseline and fishery samples have been collected in 2020, 2021 and 2022, where a total of 1946 cod were sampled.
- We have compiled samples for spawning baseline, by complementing newly collected samples with existing samples from reference populations in the North Sea and Baltic Sea
- A large number of tissue samples were collected for genetic estimates of mixture proportions in the North Sea-Baltic Sea transition zone.
- Cod otolith chemistry data from all projects, spawning all ICES SDs from the North Sea to the eastern Baltic Sea have been standardized and compiled.

3. Stock identification

3.1 Introduction

The population structure of Atlantic cod in the North Sea - Baltic Sea is characterized as a hybrid zone between North Sea and eastern Baltic Sea parental populations (Nielsen *et al*. 2003; Hemmer-Hansen *et al*. 2019). While the transition zone has been described as a genetic gradient (Nielsen *et al*. 2003) and new genomic technology has improved our understanding of populations structure in the region (Berg *et al*. 2015; Barth *et al*. 2019), pervious work has either suffered from using limited genomic information or from applying a relatively moderate number of samples that were furthermore not always collected at spawning time. Mapping of population structure should focus on the collection of individuals in spawning condition, since the reproductive unit is the basis for generating (genetic) population structure within a species (Waples and Gaggiotti 2006). Thus, our first aim with this work was to use full genome re-sequencing data to examine population structure of spawning fish collected throughout the North Sea - Baltic Sea transition zone, with particular reference to spawning populations outside the transition zone. Subsequently, we extended previous work that has estimated mixing between populations in the transition zone and North Sea/Baltic Sea (Hemmer-Hansen *et al*. 2019, 2020). The transition zone has been described as a "retention zone" for juveniles, suggesting that juveniles recruit primarily to local geographical areas (Nielsen *et al*. 2005), however previous work has suggested some mixing in particular in the edges of the transition zone (Kattegat and Arkona Sea). However, so far, estimates have been limited to these management areas and no study has integrated estimates of mixing for different life stages through the system from the North Sea to the Baltic Sea.

The objectives of WP 2 were to:
- Establish a genetic baseline using full genome sequencing.
- Perform genetic stock identification of fisheries samples.

3.2 Materials and Methods

3.2.1 Baseline

DNA extraction and genomic library preparation
All laboratory work was carried out at a specialized DNA laboratory at DTU Aqua in Silkeborg. DNA extractions were made using Omega Ezna DNA extraction kits. Genomic libraries were prepared using the method described in the article by Therkildsen and Palumbi (2017), except that adaptors were prepared at IDT for Illumina Nextera Dual Indexes. We sequenced all individuals at a NovaSeq6000 at an external provider, aiming for ~3X coverage per individual, i.e. on average at least three reads that cover the whole Atlantic cod DNA sequence, also known as *genome*.

Bioinformatics and filtering: We processed the genomic data using specific bioinformatic software designed for this type of data. First, we examined the quality of the data sequencing using FASTQC (Andrews, 2010). We removed the adaptor sequence that remained in the raw data, and used the mapping software BWA (mem algorithm) (Li, 2013) to map back the reads to the reference genome of the Atlantic cod, using the gadMor2.0 version assembled by Tørresen *et al*. (2017) which we previously had indexed. We marked duplicates and re-aligned the reads. Next,

we estimated genotype likelihoods for each marker sequenced in the genome (Single Nucleotide Polymorphism, SNP) using ANGSD (Korneliussen *et al*., 2014). In order to call the genotype likelihoods, we first measured the depth of the reads for the whole dataset using – *doDepth* 1 in ANGSD, that allowed us to establish the right parameters for filtering. Finally, we called SNPs using a p-value of -*SNP_pval* 10^{-6}, filtering out SNPs that had a lower Minor Allele Frequency than 0.015 and came from a read that had a mapping quality less than 20. We obtained a total of 6885302 SNPs, which we used to analyse the population structure and genomic differentiation of the populations included in this WP.

Population structure: To study the population structure among the individual samples, we performed a Principal Component Analysis using PCAngsd (Meisner and Albrechtsen, 2018), which calculates a covariance matrix that later was visualized using a custom-made R script. We calculated the overall F_{ST} per pair of populations using ANGSD; the F_{ST} measures the differentiation between pairs of populations, and it varies between 0 and 1. When two populations present a F_{ST} of 0, means that the pair of populations are genetically indistinguishable and share genetic flow. A F_{ST} of 1 means that the two populations are genetically completely different. We also performed a MultiDimensional Scaling analysis (Principal Coordinate Analysis - PCoA), using the R-package *ape* (Paradis *et al*., 2004), based on pairwise F_{ST} estimates.

Levels of genetic differentiation and adaptation: We evaluated the differences at the genomic level between all pairs of populations analysed by calculating again the F_{ST} per site in the genome (individual SNPs), using ANGSD (realSFS fst). We visualized the patterns by using Manhattan plots of this pairwise F_{ST} in nonoverlapping windows of 1, 5, 10 and 20K SNPs along the genome.

3.2.2 Population mixing

We used previously developed methodology to identify the most likely population of origin for individuals collected in the North Sea - Baltic Sea transition zone (Hemmer-Hansen *et al*., 2020). Briefly, we used a panel of 187 Single Nucleotide Polymorphism (SNP) genetic markers that have been developed to identify populations in the North Sea – Baltic Sea transition zone, using merged samples from spawning Kattegat, Sound, and Belt Sea as a transition zone reporting group (referred to here as "Kattegat"). In addition, North Sea and eastern Baltic Sea were included as baseline reporting groups in the analyses. This configuration assumes limited genetic difference among spawning individuals from Kattegat, Belt Sea, Sound and spring spawning cod from the Arkona Sea, that were merged into the transition zone ("Kattegat") reporting group. Thus, an important outcome from 3.1 was also to assess the validity of this assumption (see results 3.3.1). The assignment is based on assignment scores calculated as genotype likelihood in a given baseline sample divided by the sum of all likelihoods.

3.3 Results and discussion

3.3.1 Baseline

The principal component analysis based on all baseline individuals (global PCA) showed a clear separation of the populations from the North Sea ("Viking" and "Dogger" components) and the eastern Baltic Sea (SD 25) along axis 1 (Fig. 3). Spawning individuals from the transition zone (i.e., Kattegat, Belt Sea, Sound and Arkona Sea) grouped intermittently on axis 1 without any clear separation between areas. These results match earlier studies reporting a clear genetic break between the North Sea and Baltic Sea (Nielsen *et al.* 2003; Berg *et al.* 2015, Barth *et al.*, 2019).

The PCA results were corroborated by the sample site based PCoA on pairwise FST estimates, which also showed clear divergence of North Sea and eastern Baltic Sea while transition zone areas grouped together without clear geographical signals in the data (Fig. 4).
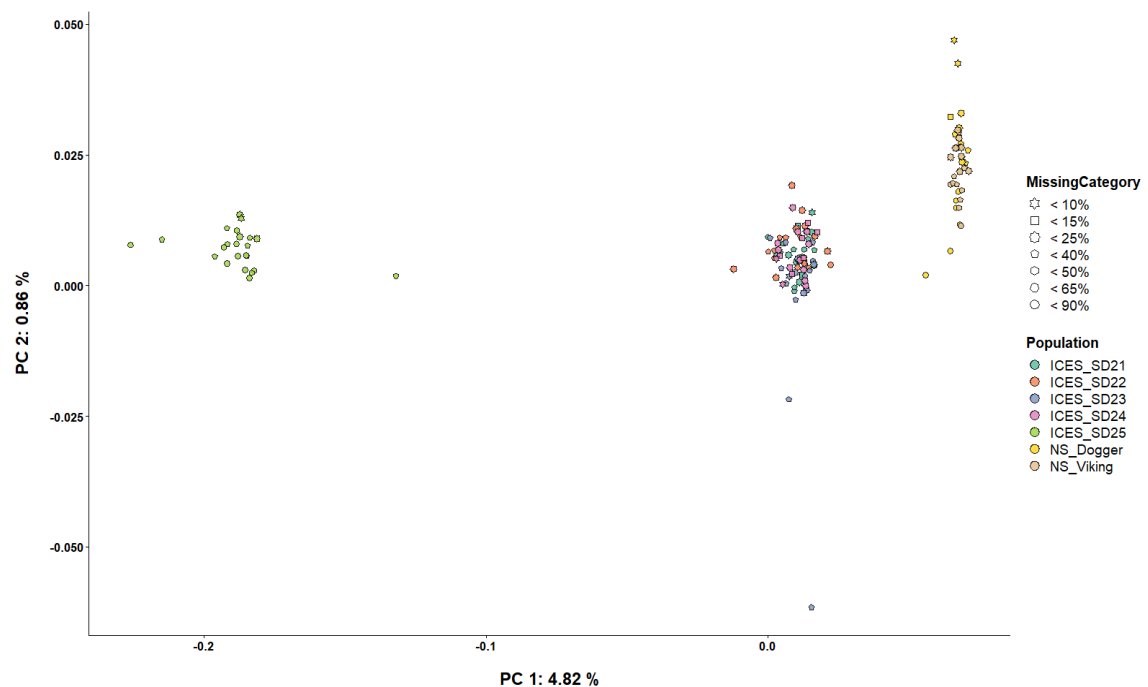
Fig. 3. Principal components analysis (global PCA) of all baseline individuals. Colors represent different capture areas (ICES SDs). The shape indicates the amount of missing data from each individual sample. "Missing category" refers to the proportion of missing data for each individual.

The sliding window analyses of pairwise F_{ST} estimates confirmed low genome wide divergence in all pairwise comparisons involving transition zone samples (Fig. 5). In contrast, the eastern Baltic Sea sampled in SD 25, was highly divergent from other samples across the full genome (Fig. 6). This comparison is included here mainly for comparison to aid interpretation of the overall low levels observed across the genome in all comparisons in the transition zone.
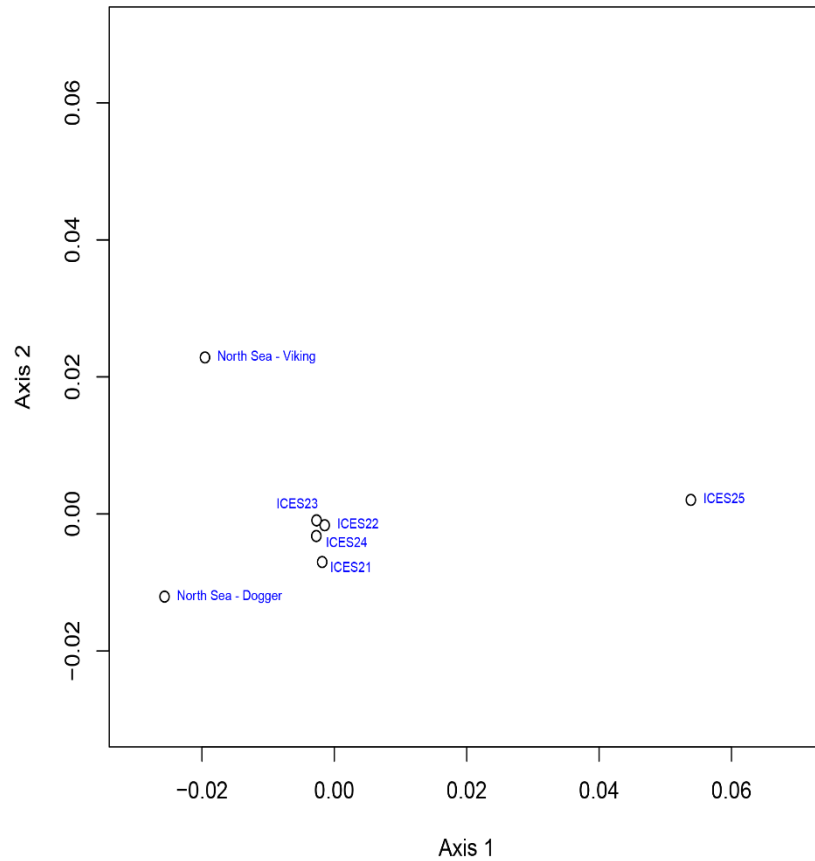
Fig. 4. **Principal Coordinate Analysis (PCoA) of all the baseline populations, with two North Sea populations (Viking and Dogger), Kattegat (ICES21), Belt Sea (ICES22), Sound (ICES23), Arkona Sea (ICES24), and eastern Baltic Sea (ICES25)**
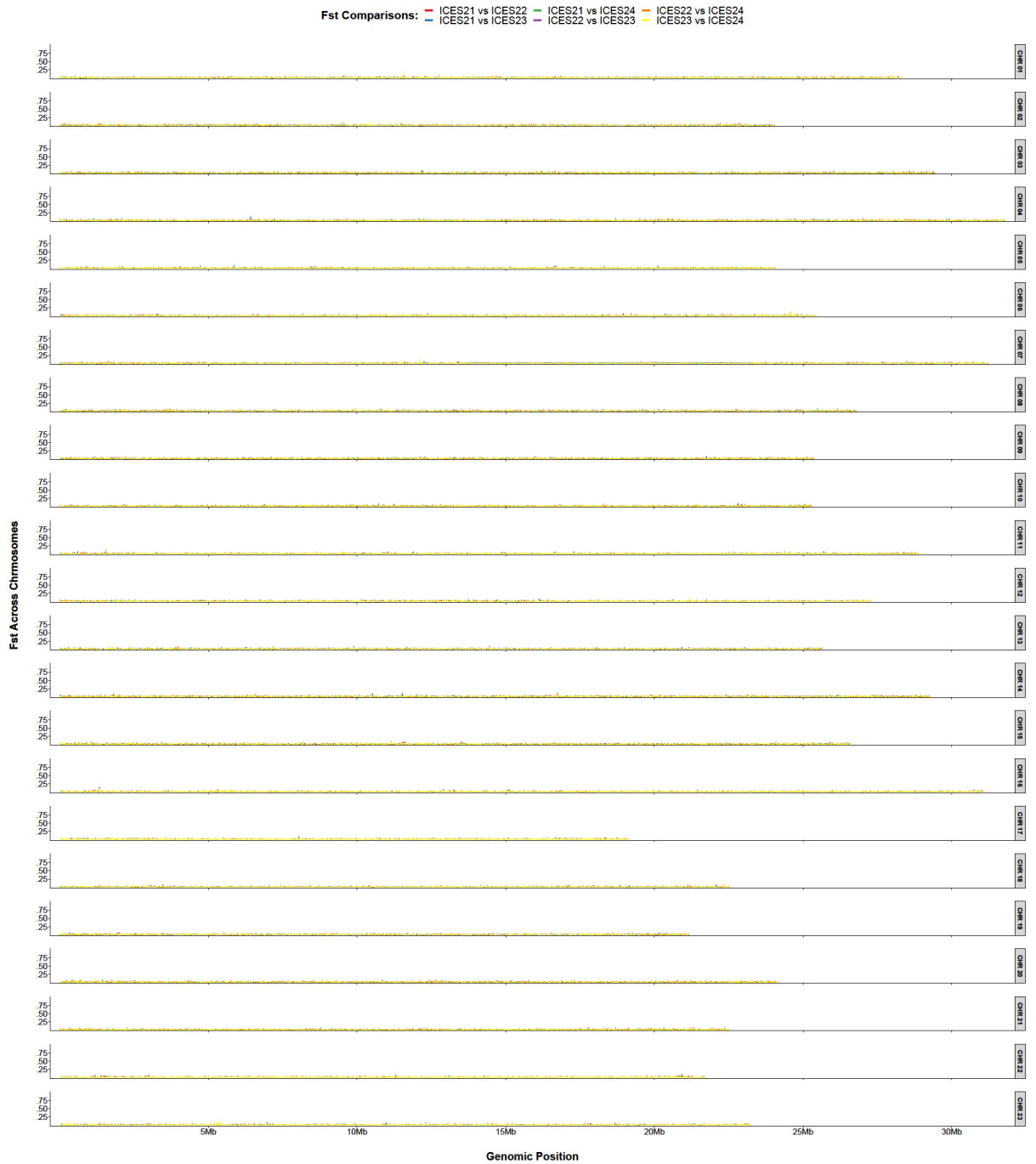
Fig. 5. Sliding window analyses of pairwise F$_{ST}$ between sampling sites in the North Sea – eastern Baltic Sea transition zone. Each panel in the plot corresponds to one chromosome in the Atlantic cod genome of cod caught in Kattegat (ICES21), Belt Sea (ICES22), Sound (ICES23), Arkona Sea (ICES24), and eastern Baltic Sea (ICES25)

Management of mixed cod stocks in the transition zone between the North Sea and the Baltic Sea   19
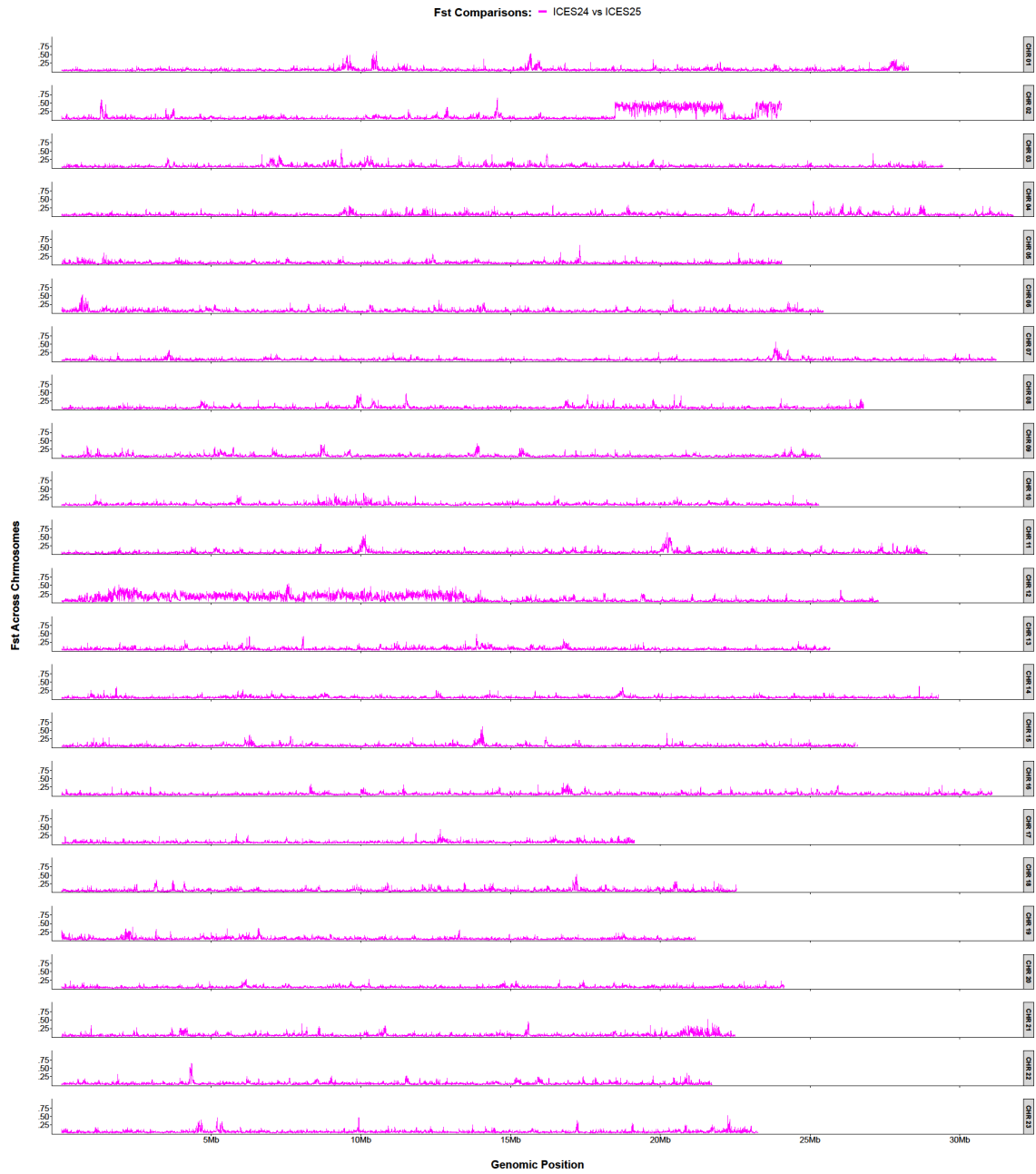
Fig. 6. Sliding window analyses of pairwise F$_{ST}$ between sampling sites in the eastern Baltic Sea (ICES25) and spring spawners in the Arkona Sea (ICES24). Each panel in the plot corresponds to one chromosome in the Atlantic cod genome.
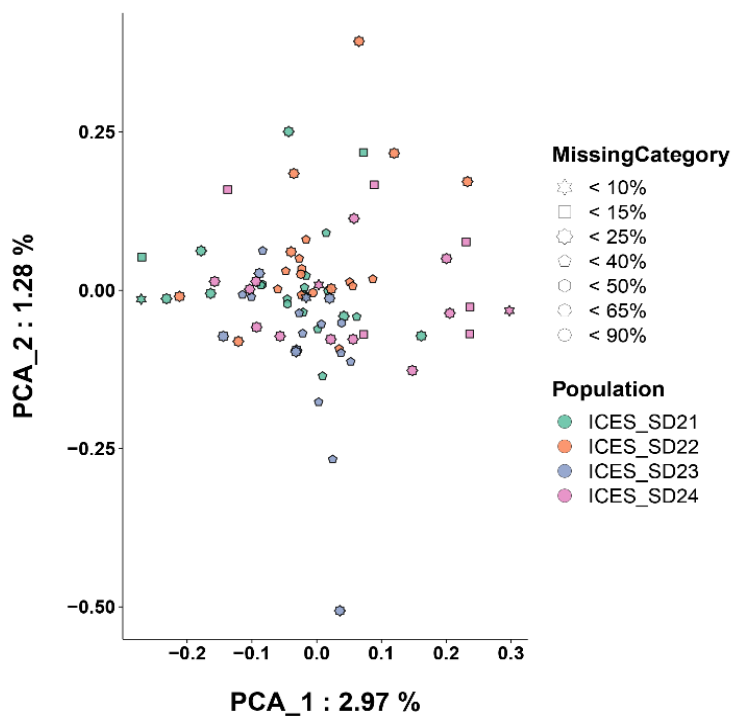
Fig. 7. Principal component analysis of the individuals from the Kattegat to the Arkona Sea (Kattegat (ICES21), Belt Sea (ICES22), Sound (ICES23), Arkona Sea (ICES24)) for all loci excluding four major inversions, i.e. major blocks of non-independent genetic markers, that may distort small scale patterns of structuring. The shape indicates the amount of missing data from each individual sample.

To examine population structure among spawning samples collected in the transition zone, we conducted a PCA only on samples from the Kattegat to the Arkona Sea to exclude signals introduced by the highly divergent samples collected outside the transition zone. This plot showed limited divergence, however there was a tendency to a gradient related to ICES SD along axis 1 (Fig. 7). This result is consistent with those reported by Barth *et al*. (2019) who also identified a gradient pattern in samples collected in Kiel Bight (SD 22) and the Sound (SD 23), which was, however (and as observed in our study), much weaker than any differences observed between these samples and samples from the North Sea and eastern Baltic Sea. This weaker gradient pattern within the transition zone may be related to signals from introgression from parental populations in the North Sea-Baltic Sea hybrid zone (Nielsen *et al*. 2003; Hemmer-Hansen *et al*. 2019).

In conclusion, the genomic data confirmed earlier work suggesting strong genetic divergence between spawning cod from the transition zone and the North Sea and Baltic Sea. In contrast, any difference between spawning cod from the transitions zone (Kattegat, Belt Sea, Sound and Arkona Sea) were much smaller (if at all present), suggesting that – from an evolutionary point – spawning components from the Kattegat are more closely related to other transition zone regions (Belt Sea, Sound and spring spawners in Arkona Sea) than to the North Sea. Further studies with larger sample sizes may refine these patterns in the transition zone, but they are not expected to change the overall relationship between spawning cod from the regions included in the present study.

3.3.2 Population mixing

The original 1946 fish collected from the transition zone for mixture analyses were reduced to 1500 after removing fish without age information and fish with assignment score below 95. Results were plotted by year class and sampling year to investigate mixing dynamics in time and space (Fig. 8).

Fig. 8. Proportion of fish from three different populations (North Sea in red, Kattegat, i.e. transition zone in yellow, and Baltic Sea in blue), shown here by year class and collection year (2020, 2021, 2022). In this first panel, we show the proportions of the Year class <u>2021</u>, older year classes are shown in the subsequent panels. Note that the size of the pie charts reflects sample sizes within each plot but are not comparable across plots due to differences in sample sizes.
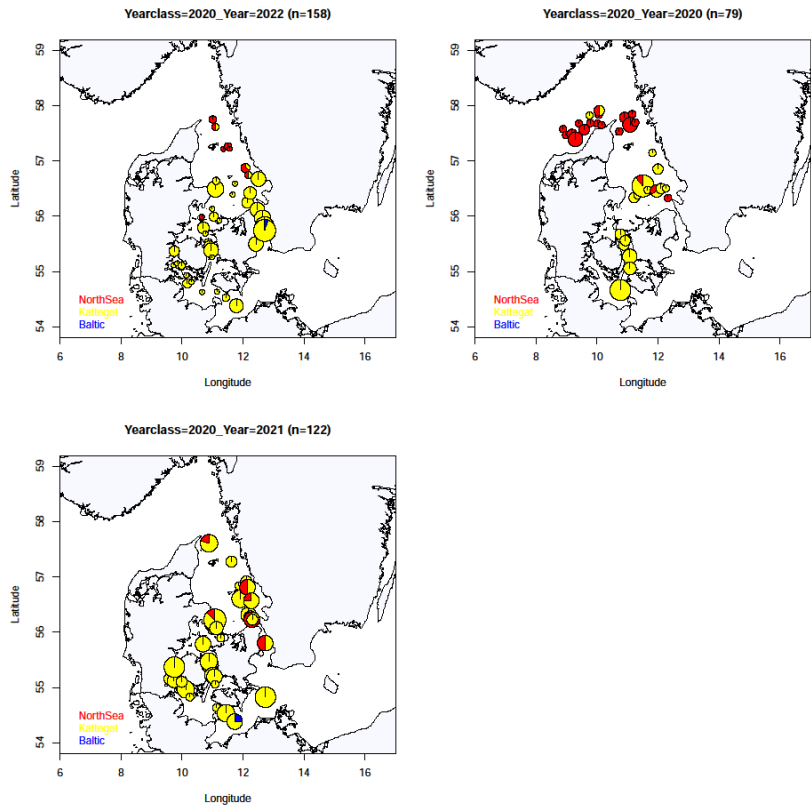
Fig. 8. continued - Year class <u>2020</u> and collection year (2020, 2021, 2022).
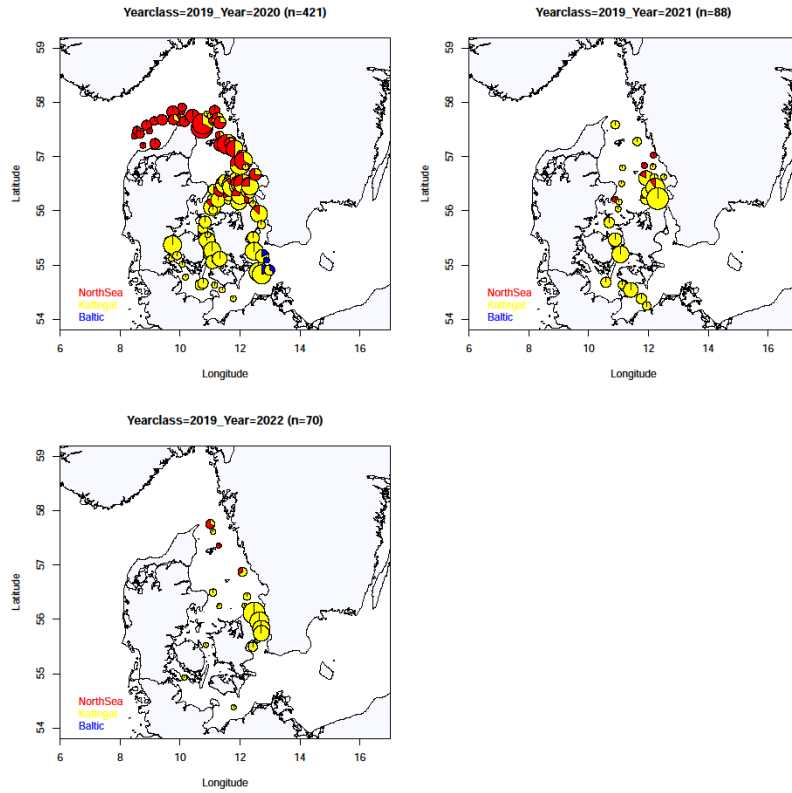
Fig. 8. continued - Year class <u>2019</u> and collection year (2020, 2021, 2022).
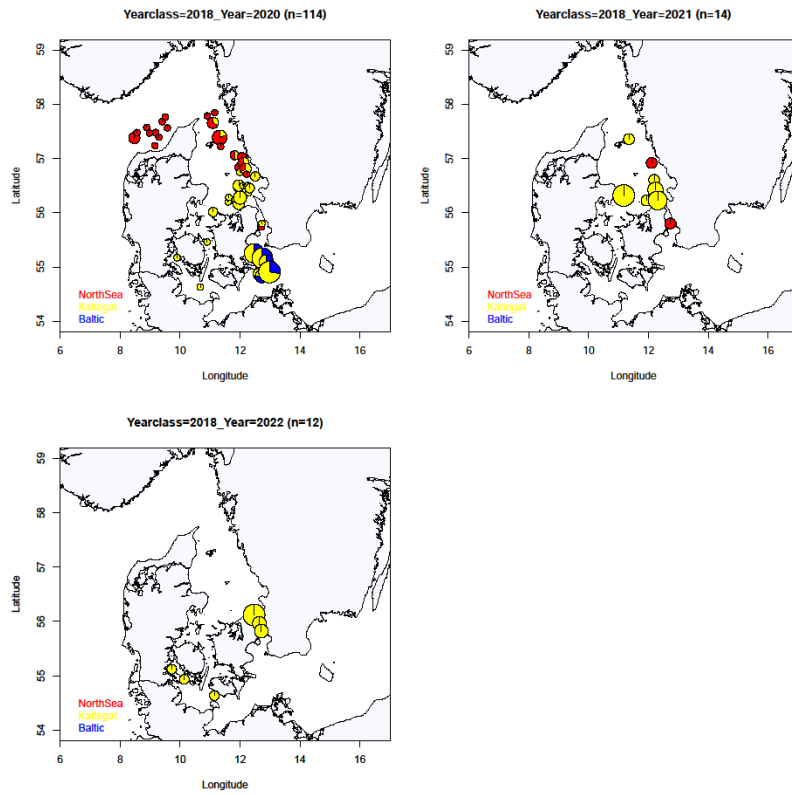
Fig. 8. continued - Year class <u>2018</u> and collection year (2020, 2021, 2022).
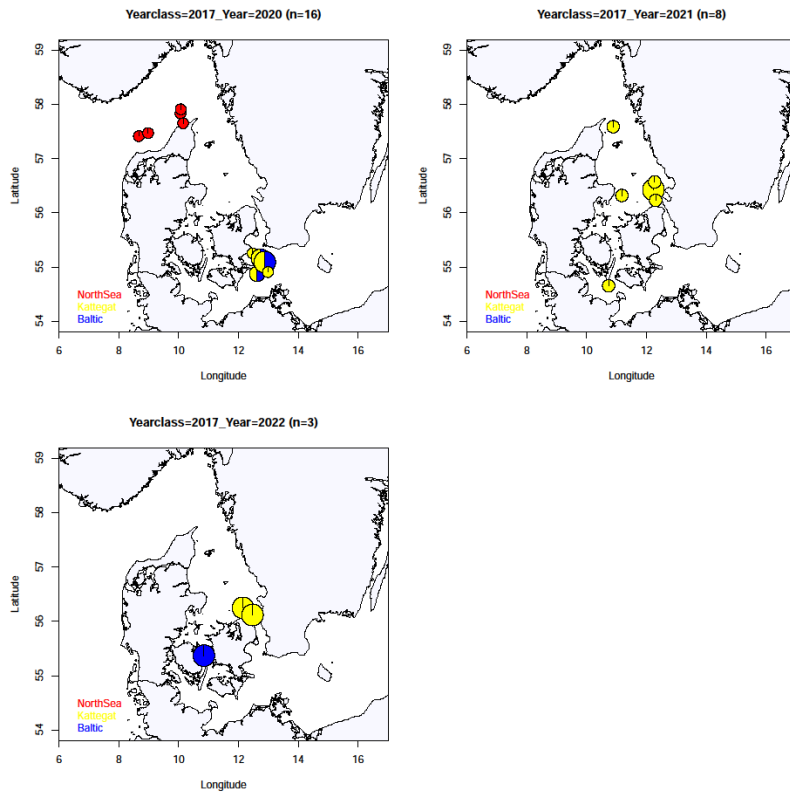
Fig. 8. continued - Year class _2017_ and collection year (2020, 2021, 2022).
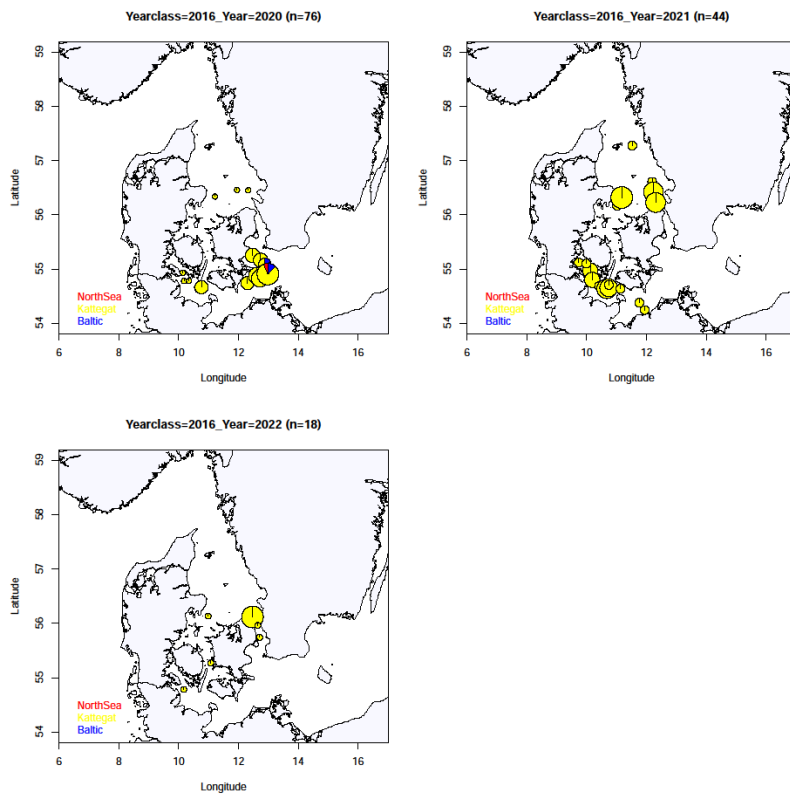
Fig. 8. continued - Year class _2016_ and collection year (2020, 2021, 2022).
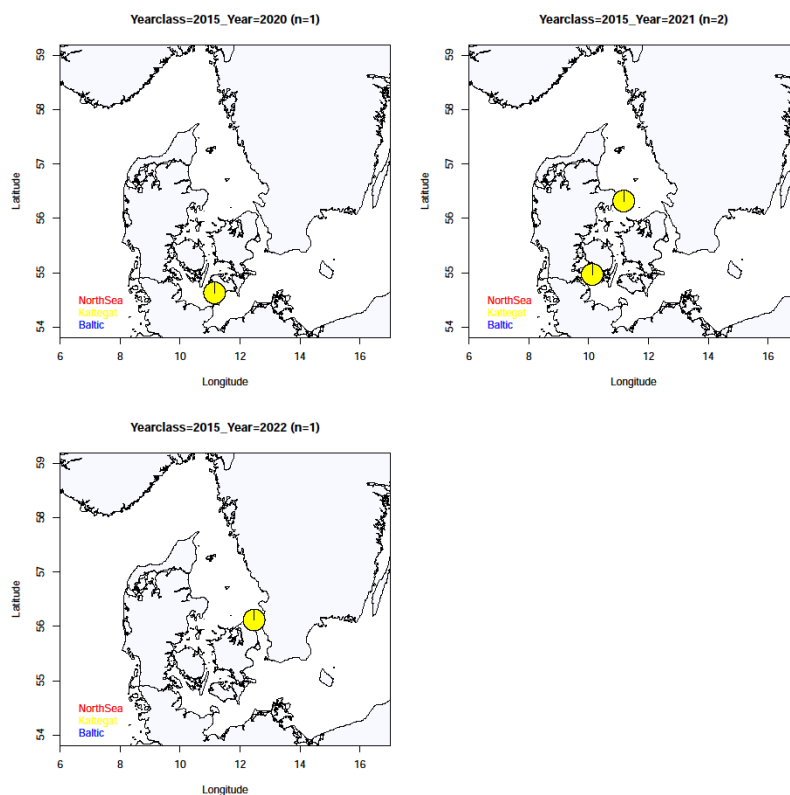
Fig. 8. continued - Year class <u>2015</u> and collection year (2020, 2021, 2022).

The results confirmed earlier findings of substantial mixing in the Kattegat (Hemmer-Hansen *et al*., 2020) and the Arkona Sea (Hemmer-Hansen *et al*. 2019), with North Sea cod entering Kattegat and eastern Baltic Sea cod entering the Arkona Sea. In contrast, we found limited presence of North Sea and eastern Baltic Sea cod in the Belt Sea and the Sound, indicating that the majority of cod in these regions are of local transition zone origin. These results support earlier findings suggesting local retention in the transition zone (Nielsen *et al*. 2005), but they also show that there is a mix of local and non-local fish in the transition zone edges in Kattegat and the Arkona Sea. Although our sample sizes for older individuals are quite limited, we also found support for earlier findings of higher proportions of North Sea cod among younger year classes in the Kattegat, hypothesized to originate from a combination of early immigration of juveniles to the Kattegat followed by return migration to the North Sea for spawning (André *et al*., 2016; Hemmer-Hansen *et al*., 2020; Hüssy *et al*., 2021b).

3.4   Conclusions

- We found limited genome wide divergence between spawning cod from the transition zone, Kattegat, Belt Sea, Sound and Arkona Sea (SD 21, SD 22, SD 23 and SD24 - spring spawners only), suggesting that spawning cod in these areas belong to the same biological population. In contrast, we found strong genetic divergence between cod from

these transition zone areas and cod from the North Sea and the eastern Baltic Sea respectively. To accurately capture the biology of spawning cod in the transition zone, the southern parts of the Kattegat, the core spawning areas of the transition zone population in the Kattegat (Börjeson *et al*. 2013; Hemmer-Hansen *et al*. 2020), should be merged with spawning components in the Belt Sea and the Sound.

- We confirmed previous findings of substantial mixing in the Kattegat and the Arkona Sea, with North Sea fish entering Kattegat as early life stages and leaving again when they reach maturity, with considerable inter-annual fluctuations in the extent of mixing and geographical range of population overlap.

- Spawning of both western and eastern Baltic Sea cod occurs in the Arkona Sea, but spawning of the two populations appears to be temporally and spatially separated (Hemmer-Hansen *et al*. 2019), although our data to map the spawning components are still relatively limited. Consistent with this, we have shown here that the western Baltic component in the Arkona Sea belongs genetically to the other transition zone areas and the current practice of splitting survey and landings data in the Arkona Sea by genetic population and treating Arkona Sea as a mixed stock area is reflecting the biology of cod in this area.

- Mixing with North Sea and eastern Baltic Sea populations in the Belt Sea and the Sound is limited. This also applies to juveniles, suggesting local recruitment in these areas (Nielsen *et al*., 2005). However, we confirmed previous findings of substantial mixing in the Kattegat and Arkona Sea, with North Sea fish entering the Kattegat and eastern Baltic Sea fish in the Arkona Sea. Stock assessment and management would need to account for this mixing in both Kattegat and Arkona Sea.

Future considerations
- We would recommend to extend the sampling of juveniles to increase data availability of juvenile mixing proportions and also to conduct more checks to confirm the limited extent of mixing in the Belt Sea and the Sound reported in this study. In addition, a more detailed mapping of the spawning components in the Arkona Sea would be needed to get a better understanding of temporal and spatial dynamics.

4. Migrations and stock mixing

4.1 Introduction

The Kattegat, Sound and the Belt Sea, are generally considered as a transition zone between the marine North Sea and the brackish Baltic Sea. The topography in this area becomes successively shallower from > 120 m in 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. In addition, there are spatial gradients in nutrient loading with highest concentrations in the western Baltic, decreasing towards the Skagerrak. These environmental gradients provide an ideal setup for studying fish migrations based on chronological analyses of the chemical composition of their otoliths.

The otoliths of fish are composed mainly of calcium carbonate (about 98%) and organic matrix (about 2%), as well as trace amounts of various elements. The formation of otoliths is regulated by physiological processes that result in both daily growth increments and annual growth zones, reflecting seasonal fluctuations in temperature and food availability (Beckman *et al.*, 1988; Weidman and Millner, 2000; Høie and Folkvord, 2006). The trace elements found in otoliths are primarily absorbed from the surrounding water through the gills, providing a record of the environmental conditions experienced by the fish (Watanabe *et al.*, 1997; Campana, 1999; Milton and Chenery, 2001). As the chemical composition of the water is influenced by the local geochemistry of the catchment, otoliths can act as a fingerprint for a specific area (Walther and Limburg, 2012). Otolith chemistry has become an increasingly important tool in fisheries science, allowing researchers to study fish stock dynamics, migration patterns, pollution exposure, and connectivity between habitats, and aiding in the development of effective fisheries management strategies (Campana, 1999; Campana and Thorrold, 2001; Elsdon *et al.*, 2008; Carlson *et al.*, 2017). In the EMFF funded project *Sustainable management of cod* we use of the environmental salinity gradient, which is reflected in the otolith Sr content, and thus provides a reliable tool to analyse north/south movements within the transition zone. However, given that the salinity is similar in the Belt Sea and the Sound, classification of chemical signatures to either area using otolith Sr alone is not suitable. In FABBIO, we therefore expanded the analyses, making use of all measured elements, the area-specific chemical fingerprints.

We supplemented these analyses with estimates of stock mixing derived from historic tagging projects. The resulting stock mixing estimates are not directly comparable, in that there is no temporal overlap between the two data sources (tagging: 1950s – 1980s; otolith chemistry: 2020s). Tagging data furthermore only provide information on release and recapture locations, and thus give an overall picture of the direction of cod movements.

The objectives of WP 3 were to:
1. Establish area-specific otolith chemistry fingerprint baselines.
2. Classify which area individual cod had inhabited throughout their lives using these fingerprints and the life-long chronological records of chemical composition from hatch to death.
3. Estimate stock mixing proportions from the life-long habitat classifications from otolith chemistry and compare these with movement rates estimated from tagging data.

4.2 Materials and Methods

4.2.1 Otolith chemistry

Sample preparation

Otolith preparation procedures have been described in detail in Hüssy *et al*. (2020a, 2021b). In brief, otoliths were embedded in Epoxy resin (Struers ®) and sectioned through the core using an Accutom-100 multicut sectioning machine. 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), using a NWR213 laser system from Elemental Scientific Lasers that was coupled to an ELEMENT 2 mass spectrometer from Thermo-Fisher Scientific. The otoliths were analysed along a transect from the core to the dorsal edge of the otolith (Figure 3). Concentrations of the following elements were analysed: Barium (^{137}Ba),

Fig. 9. Image of cod otolith cross section, where the line indicates the transect in which the chemical composition was analysed with LA-ICP-MS from core to edge. As an example, the strontium profile is shown, including the growth zone into which the LA-ICP-MS data were parsed, where the numbers indicate which age of the fish's life (ageLife) and season with each age (season) the sections correspond to.

calcium(^{44}Ca), copper (^{65}Cu), iron (^{57}Fe), potassium (^{39}K), lithium (^{7}Li), magnesium (^{25}Mg), manganese (^{55}Mn), lead (^{208}Pb), strontium (^{88}Sr), and zink (^{66}Zn). Measurement values > 4 standard deviations < from the mean were treated as outliers and discarded (percentage of data discarded no more than 1-4%). The concenrations of all these elements together are considered as the "chemical fingerprint" of each fish.

The otolith sections were further viewed under reflected light using a Leica MZ12 microscope and DCF290 camera setup. Otolith growth chronologies were obtained for each individual by measuring the widths of successive opaque and translucent growth zones – representative of annual and seasonal growth zones covering the entire life of the fish - along the laser track, from the core to the edge using ImageJ (Rueden *et al*., 2017) (Fig. 9). LA-ICP-MS data were thereafter parsed to the corresponding age zones of the otolith and element concentration values averaged by season and age of life, thereby allowing to assess the data on a temporal scale representing different years in the fish's life. In addition to average values by year of life, element concentrations were also averaged from the first 50 mm representative of the core (E_{core}) and the last 50 mm at the edge of the otolith (E_{edge}) representative of the element signature at the time of capture.

Statistical analyses

Chemical signatures at hatching: The first step of the analyses was to assess whether there are area-specific signals in the chemical fingerprints of the otolith core, which is representative of the signal at hatching and early larval stage. The chemical fingerprints with all elements in the
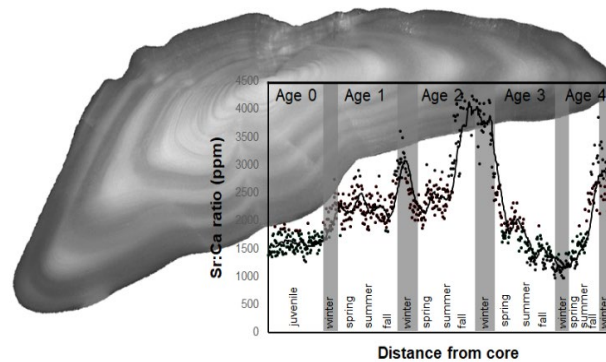
core (E_{core}) were analyzed with Multivariate Analysis of Variance (MANOVA), using the following model, and post-hoc groupwise comparison with the R package "emmeans":

$$\text{Fingerprint} = \text{factor(ICES SD)} + \varepsilon_i, \text{ where } \varepsilon = N(0,\sigma^2)$$

Identification of natal origin: For the identification of number of potential spawning areas the chemical fingerprint with all measured elements in the core only (E_{core}) was used. Number of potential spawning components were assessed with a commonly used cluster analysis approach using the "kmeans" and "factoextract" packages of R (R Core Team, 2020). k-means clustering is an unsupervised method for dividing n observation in a data set into k clusters in which each observation belongs to the cluster with the nearest mean. k-means cluster analysis was performed on scaled (dividing by standard deviation) and centered (subtracting mean) element concentrations, and using the Euclidean distance between observations. The optimal number of k was determined using the *Average Silhouette Width* method, which measures the cluster quality (Batool and Hennig, 2020). The *Silhouette Width* for an observation i is calculated as the proportion of the difference in the average distance to other observations in the cluster to which is was assigned (b_i), and the average distance to observations in the nearest cluster to which it was <u>not</u> assigned (a_i), in relation to the maximal inter-cluster distance ($\max(a_i, b_i)$). The *Average Silhouette Width* ($\overline{S}(k)$) of a dataset with n observations and k clusters is thus:

$$\overline{S}(k) = \frac{1}{n} + \sum_{i=1}^{n} \left(\frac{b_i - a_i}{\max(a_i, b_i)} \right)$$

The larger the values of the Silhouette width, the better the clustering quality, so that the optimal number of k has the largest value of $\overline{S}(k)$.

Chronological element patterns and reconstruction of life-long area occupancy: The first step in this analysis was to assess the extent of area-specific differences in lifetime element concentrations. To that end, Linear mixed-effects (LME) models were fitted to the chronological patterns across the entire lifespan of the fish in order to assess effects of area and fish age using the "lme4" package in R (R Core Team, 2020). Models were fitted using individual fish as random variable to allow variable intercepts, accounting for inter-individual differences. The full model included all fixed effects, including *area* (ICES SD at capture), *season* (spring, summer, fall, winter), and *ageLife* (each year in the fish's life, from age = 0 to age at capture), with E representing elements, subscripts i representing individual fish and j individual chemistry measurements:

$$E_{ij} = \text{factor}(\text{area}_{ij}) + \text{factor}(\text{season}_{ij}) + \text{factor}(\text{ageLife}_{ij}) + (1|\text{ fish}_i) + \varepsilon_i, \text{ where } \varepsilon = N(0,\sigma^2)$$

Interactions between variables could not be included due to data constraints. The most parsimonious model was identified by ranking all possible models by the Akaike information criterion (AIC) using the "dredge" function of the MuMIn package in *R*. The final model was selected as the model with an AIC difference > 2 compared to all other models.

The next step of the analysis was to classify habitat occupancy for each fish and throughout its entire life. To that end, we selected suitable elements from the LME analysis, i.e. elements that differ significantly between areas and occurred at concentrations that can be reliable measured: Ba, Fe, K, Mg, Mn, Sr, and Zn. Pb, Li and Cu were omitted, owing to a high percentage of outliers and negative concentrations relating to the low concentration of these elements in the otoliths. Since a significant *ageLife* effect was evident in all elements, the classical Linear Discriminant Analysis (LDA) approach was modified to an LDA-like model that is conditional on covariates

(*ageLife*), that also allows for cases with missing element data. The effect of *ageLife* was modelled as a spline function, where elements were assumed to be independent allowing for different variances between elements and the same degree of freedom between groups. Conditional LDA functions were established for each ICES SD using the element measurements at the edge (E_{edge}) where we included samples of a wide age range (Table 2, Table 5). Classification success was assessed using jack-knived leave-one-out cross validation. The conditional LDA functions were then used to classify the element signatures of each *ageLife* and *season* interval of each fish to the most likely ICES SD occupied, thus reconstructing life-long area occupancy of each fish.

Stock mixing: From the reconstructed life-long area occupancy profiles of individual fish, area-specific mixing proportions were calculated as total proportion of fish classifications by *ageLife* and *season* for the ICES SD fish were captured in.

4.2.2 Tagging data

General movement patterns: The direction of the general movements from the release point was illustrated using a mean movement vector $\hat{a}$, and a diffusion coefficient $\hat{D}$ around the mean release coordinates following (Nielsen 2004). Movement direction and dispersion were estimated for each ICES area with sufficient releases separately. Days at liberty differ between individuals, therefore the general direction and dispersion parameters are estimated per time unit. In the present case a time unit of 6 months was used. A full description of methods and details of data quality assurance are available in (Lundgreen *et al.*, 2022). These parameters are a qualitative representation of the general movement patterns that are useful for a comparative assessment of the temporal stability of the movement patterns obtained from otolith chemistry analysis.

$$\hat{\alpha} = \left(\frac{\sum \Delta x}{\sum \Delta t}, \frac{\sum \Delta y}{\sum \Delta t}\right)'$$

$$\hat{D} = \frac{1}{4(n-1)}\left(\sum\left(\frac{\Delta x^2}{\Delta t}\right) - \frac{(\sum \Delta x)^2}{\sum \Delta t} + \sum\left(\frac{\Delta y^2}{\Delta t}\right) - \frac{(\sum \Delta y)^2}{\sum \Delta t}\right)$$

Stock mixing: In addition to assessing the general movement patterns, we evaluated whether the data was suitable for estimating movement rates between adjacent areas. There is unfortunately only very sparse tagging data available from the Skagerrak and Sound. However, data of a total of n = 797 releases is available from the Sound. Since one of the objectives of this projects was to assess stock connectivity between the Sound and adjacent areas, we used this data to calculate the probability of a cod tagged in the Sound to remain in the Sound, to move toward the Kattegat, or toward the Arkona Sea. For this analysis release dates were grouped into two time periods: Spawning (January-March) and non-spawning (April-December), and a time step of one week based on observed swimming speeds in resident cod. The full analytical setup, including quality assurance and data selection may be found in (Lundgreen *et al.*, 2023). The likelihood of a certain movement pattern was estimated for each individual fish using a transition matrix, which basically is a three-cell grid where the cod has the options of staying in a grid cell (= area), or moving to an adjacent cell (either north or south). The negative log likelihood for each individual fish was calculated and summed to get the overall most likely transition probabilities for the entire dataset based on all recaptures:

$$L(\theta) = -\sum_i \log (P_\theta^{t_i})_{k,j}$$

4.3   Results and discussion

4.3.1   Natal origin

The objective of this analysis was to address the question whether cod caught in the different ICES SDs originate from the same spawning areas? This question was addressed with a MANOVA analysis of the core signatures to assess whether there are area-specific fingerprints, and subsequently with a *k*-means cluster analysis for identifying the proportional distribution of spawning area signatures over the entire sampling area.

Chemical signatures at hatching: The chemical fingerprints of the core differed significantly between all ICES SDs (MANOVA, df = 20, p = < 0.05). Contributing significantly to the separation between SDs were all elements (Ba, Cu, Fe, K, Li, Mg, Mn, P, Sr, and Zn). This indicates that cod in Skagerrak, Kattegat, Belt Sea and the Sound to some extent originate from different spawning areas and that stock separation to some degree has been maintained throughout the life of the fish (otherwise there would be no differences). All elements were therefore used in the cluster analysis to identify how many potential spawning areas cod in the transition originate from.

Identification of natal spawning components: Examples of the cluster analysis for *k* = 2, 3, 4, and 5 clusters area shown in (Fig. 10). The *Average Silhouette* method identified the optimal number of clusters as *k* = 3. For k=3, a relatively discrete red cluster was separated from blue and green clusters, where the spatial distribution of datapoints appears more like a large homogenous cloud without a clear separation between clusters (at least in the two dimensions shown here). Consequently, the *Average Silhouette Width* is only marginally smaller for *k* = 2 than *k* = 3 (Fig. 10). Thus, this latter separation should be interpreted with caution. However, cod are know to spawn through large areas in the North Sea (Fox *et al.*, 2008), in two geographically separated areas within the Kattegat (Vitale *et al.*, 2008; Børjeson *et al.*, 2013) and Belt Sea (Bleil *et al.*, 2009; Hüssy, 2011) and in the eastern Baltic Sea (Bagge, 1994, Wieland *et al.*, 2000). While North Sea cod are known to occur in the Kattegat (Hemmer-Hansen *et al.*, 2020), eastern Baltic cod are generally not found in the Belt Sea (this report). A likely interpretation of the clusters for k=3 is therefore that the red cluster corresponds to North Sea/Skagerrak core signatures while the blue/green clusters correspond to transition zone core signatures. The proportional distribution of chemical fingerprints from the core per ICES statistical rectangle is shown for the new samples collected during FABBIO, and the existing samples from the *Sustainable management of cod* project separately (Fig. 11) and for the FABBIO samples by year class (Fig. 12). These figures show a spatial gradient in the proportional origin of cod. Notable is, that one of the core fingerprint clusters (green), only occurs in the northern part of the Kattegat. This group consists presumably of genetically distinct North Sea cod drifting into the Kattegat as early life stages (Hemmer-Hansen *et al.*, 2020; Hüssy *et al.*, 2021b). There are conspicuous differences in mixing proportions between projects (Fig. 11). These are most likely attributable to the strength of inflow currents that entrain early life stages from the North Sea, in that the proportion of this cluster varies considerably between year classes, where particularly the year class 2019 is characterized by a large contribution of a cluster (Fig. 12) that are presumably of North Sea origin (Fig. 8). The majority of the remaining cod in Kattegat are classified into the pink cluster and cod in the Belt Sea and Sound originate from a mix of purple and pink clusters, suggesting that the cod in the transition zone originate from somewhere in the transition zone and not from the North Sea. Unfortunately, it is not possible to extrapolate the chemical fingerprint at the edge of the otoliths, representative of the true area-specific fingerprint, to the fingerprint at the core of the otolith due to ontogenetic

changes in otolith growth processes. Our data therefore does not allow us to identify which spawning areas the cod originate from. These analyses only give us information about whether they come from similar or different spawning areas. Historically, spawning areas of cod in this area have been located in the southern Kattegat extending into the northern Sound (Vitale *et al.*, 2008) and in the deeper parts of the Belt Sea (Bleil *et al*, 2009; Hüssy, 2011). It therefore seems likely that the blue/green core clusters identified here represent these spawning areas – but it is not possible to identify which ones. A study on drift patterns of eggs and early larvae released in the known spawning areas within the southern Kattegat, Sound, and Belt Sea has shown that early life stages released in any of these spawning areas has the potential to be distributed throughout the entire areas (Huwer *et al.*, 2016). The microchemistry-based spawning cluster identifications is thus entirely in line with these drift simulations.

Our results thus suggest that cod caught in the transition zone originate from multiple spawning areas within the transition zone - in addition to the North Sea component which dominates in the northern part of the Kattegat. To what extent the observed mixing of populations and/or stock components occurs as drift of early life stages, or active migration of adult individuals, will be addressed in the paragraph on reconstruction of area occupancy.
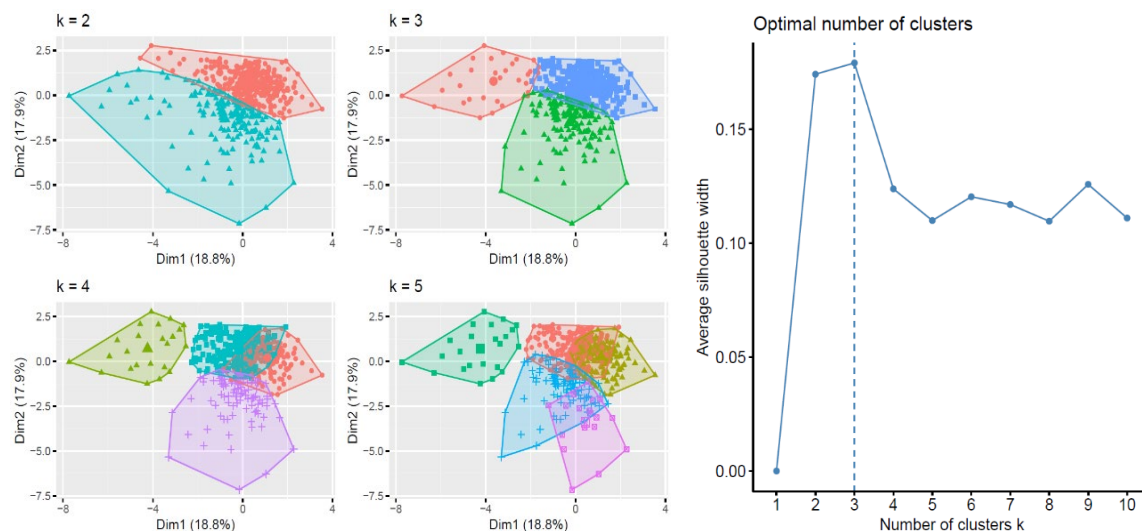
Fig. 10. Biplots of the k-means cluster analysis performed in the core element fingerprints of Atlantic cod, where the four panels show examples with k = 2, 3, 4, and 5 clusters (left panel). Average Silhouette Width values for each k, with identification of the optimal number of clusters (right panel).
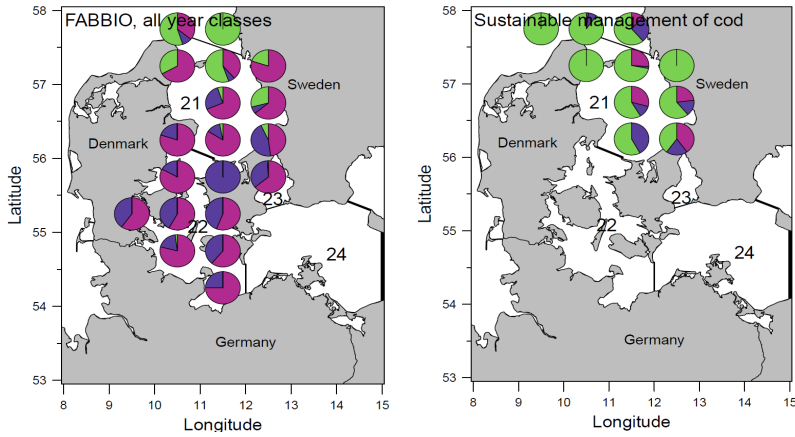
Fig. 11. Proportional composition of the three clusters of chemical fingerprints in the core by ICES statistical rectangle for the new samples collected during FABBIO (2020 and 2021) and the "Sustainable management of cod" (2016) project. Note that sample sizes differ between rectangles, while the pie charts shown here are of equal size. Pies are placed at the center of the rectangles to avoid overlapping, which results in some pies being placed on land.
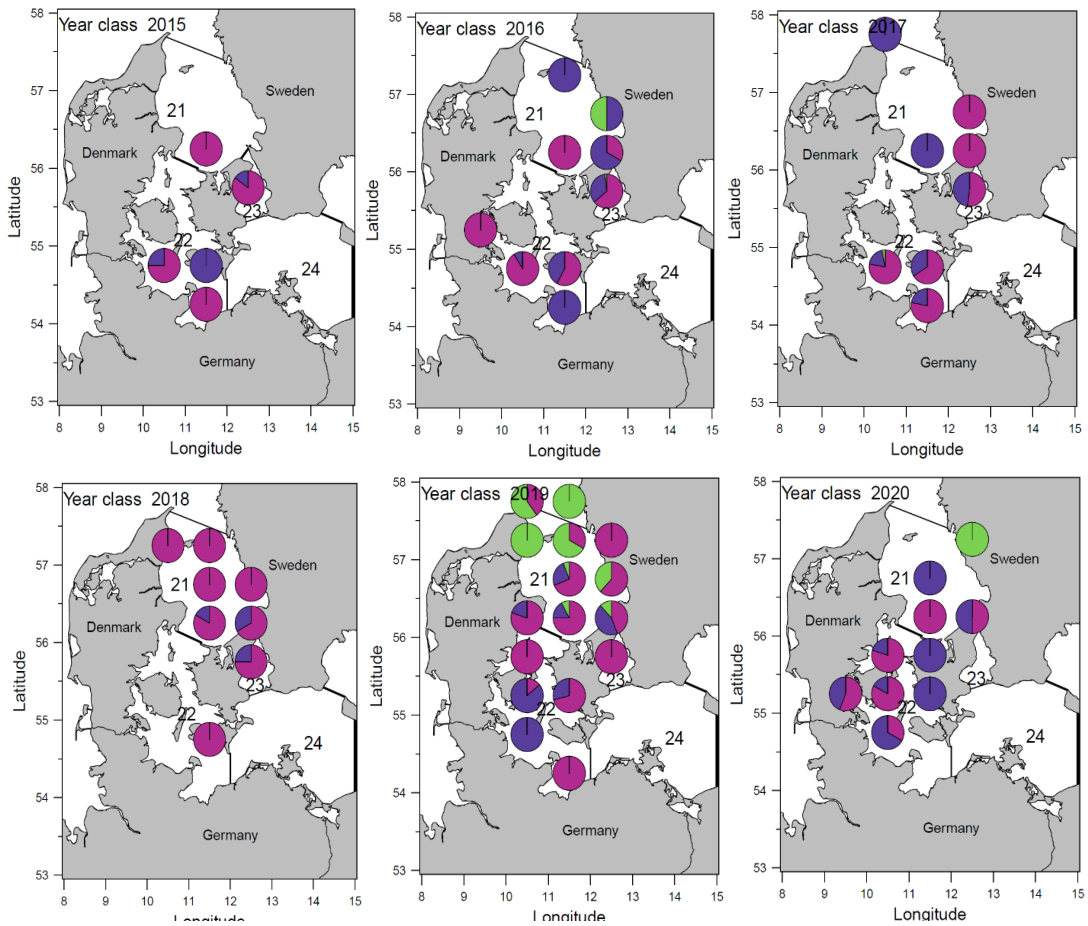
Fig. 12. Proportional composition of the three clusters of chemical fingerprints in the core represented by ICES statistical rectangle for the FABBIO samples represented by year class. Note that sample sizes differ between rectangles, while the pie charts shown here are of equal size. Pies are placed at the center of the rectangles to avoid overlapping, which results in some pies being placed on land.

4.3.2 Reconstruction of life-long area occupancy

The objective of this analysis was to address the question: Have cod remained resident in the ICES SD they were caught in throughout their adult life? We addressed this objective by first assessing lifelong chronological patterns in element concentrations and identify drivers that have a significant influence. We then used the elements exhibiting significant area effects to reconstruct where each individual fish had been throughout its adult life. Finally, we estimated life-long stock mixing proportions for each ICES SD separately.

Lifelong chronological element patterns: The results of these analyses are first presented as mean element concentration profiles by area to visually mean element concentration profiles between areas the fish were captured in. Following this, the results of the LME analysis assessing the statistical significance of area-specific differences as well as season and age in the fish's life are presented.

Fig. 13. Chronological profiles of element concentrations (mean ± SD) averaged across seasons for each age in the life of the fish. Colors represent ICES SDs, with Skagerrak (SD 20) = lime, Kattegat (SD 21) = yellow, Belt Sea (SD 22) = purple, and Sound (SD 23) = green. Note that the elements Fe, K, Li and Pb were not analyzed in the "Sustainable management of cod" project, from which all Skagerrak samples originated.

The mean element concentration profiles by ICES SD (Fig. 13) exhibit some well-known trends with respect to area and fish age. Elements that are known 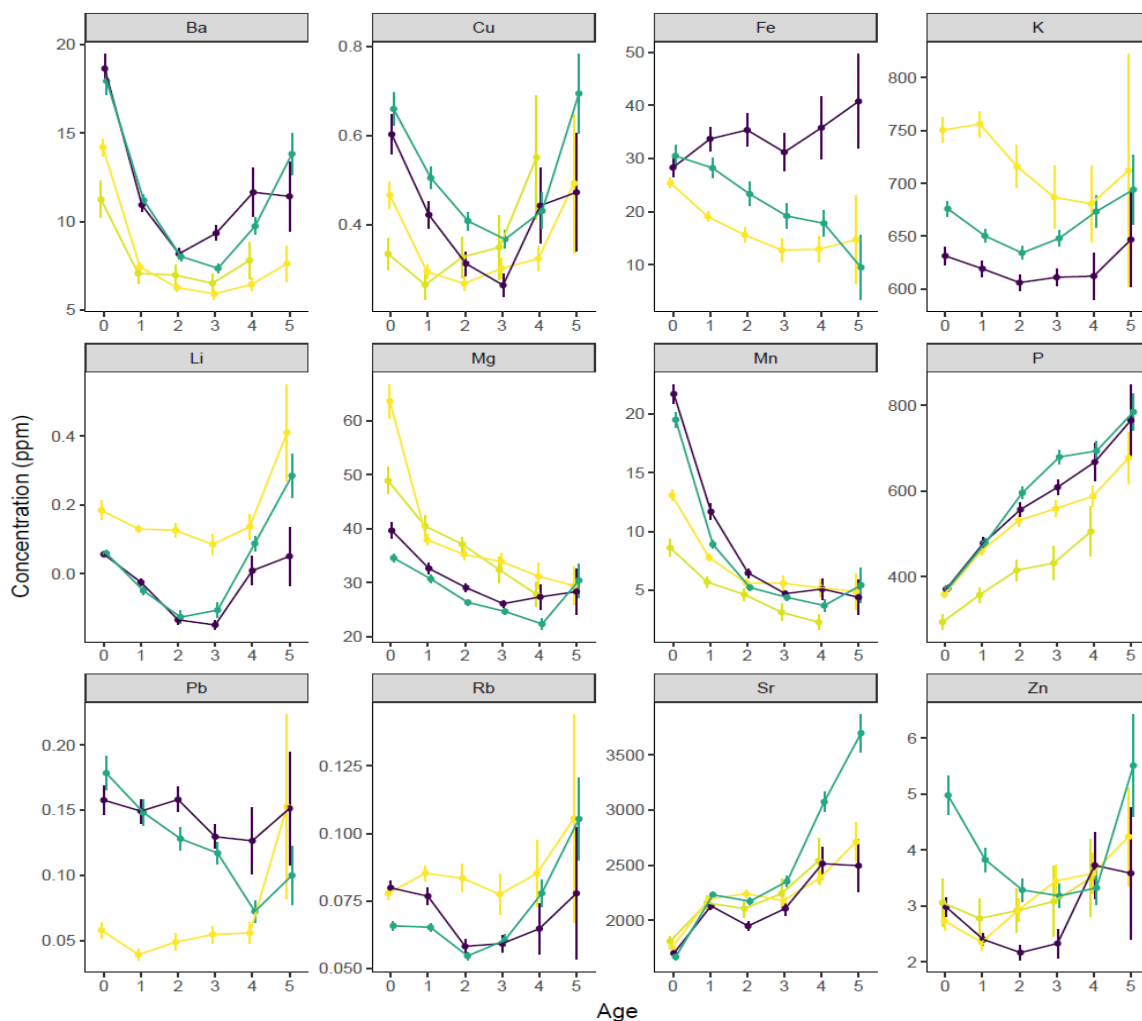to reflect specific environmental conditions, show spatial patterns that are expected from known environmental gradients, and under a scenario with limited stock mixing (Hemmer-Hansen et al., 2020; Hüssy et al., 2020b, 2021b). Note that sample sizes of age 4 and even more so of age 5 fish are limited in the Kattegat and Skagerrak (Table 5), which therefore may influence the visual appearance of age-related patterns. Sr for example, is a proxy for water salinity and is lower in the Belt Sea compared to the other areas, in accordance with the spatial gradient in salinity in that area. Ba on the other hand reflects proximity of the fish to the coast and is higher in cod from the Belt Sea and Sound compared to the Kattegat and Skagerrak. Finally, Mn, which is considered a proxy for the occurrence of hypoxia, shows a gradient from highest concentrations in the Belt Sea/Sound, where regular summer hypoxia in shallow waters occurs in addition to persistent hypoxia in the deepest areas, and lowest in the Skagerrak in the western Baltic. Also in elements, the incorporation of which is known to be exclusively under physiological control (P, Mg, Cu and K) (Hüssy *et al.*, 2020b), differ between areas indicating with cod from Skagerrak similar to those from Kattegat, and similarities between cod from the Belt and Sound as well.

The element-by-element LDA analysis revealed highly significant effects of both area (ICES SD), age (age in the life of the fish) and season (spring, summer, fall winter) in virtually all elements. The predicted effects of these variables on element concentration are shown below for elements that reflect environmental concentrations (Ba, Fe, Li, Mn, Pb, Sr) (Fig. 14) and in elements that are under physiological regulation (Cu, K, Mg, P, Zn) (Fig. 15). The statistics relating to these figures may be found in the Appendix (Table A1).

Effect of age: The concentration of most elements, both those under environmental and physiological regulation, decreases with fish age. This is presumably attributable to the fact that the weight proportion of calcium increases with fish age, and that element concentrations are calculated in relation to Ca. Increasing Sr concentrations with fish age on the other hand are often observed in field samples and are hypothesized to reflect size/age related shifts in habitat (Secor and Rooker, 2000; Jessop *et al.*, 2008; Brown and Severin, 2009). The combination of vertical salinity stratification from the Kattegat to the eastern Baltic Sea and the age/size related depth distribution of cod (Pihl and Ulmestrand, 1993; Oeberst, 2008) thus provide a credible explanation for the observed size effect in Sr (Fig. 14). The increase in P with fish age on the other hand seems to be related to otolith formation dynamics (Heimbrand *et al.*, 2020; Hüssy *et al.*, 2020b) (Fig. 15). This effect of fish age on otolith element concentration occurring across most elements made it necessary to incorporate the effect of fish age in the habitat reconstruction analyses (see below).

Effect of season: Significant differences also occur in relation to season, where summer concentrations are lowest in Sr and highest in Ba, indicating a movement towards shallower near-coastal areas with lower salinity and a higher terrestrial element signal (Elsdon and Gillanders, 2005; Walther and Limburg, 2012) (Fig. 14). All elements under physiological regulation (Cu, K, Mg, Zn) show identical seasonal patterns with highest concentrations during summer and lowest during winter (Fig. 15). P concentrations somewhat surprisingly, exhibit an inverse pattern with highest concentrations during winter. Mg is thought to reflect fish metabolism (Limburg *et al.*, 2018), P has been shown to reflect seasonality in growth (Heimbrand *et al.*, 2020; Hüssy *et al.*,

2021a). The precise mechanisms driving seasonal concentration characteristics are, however, yet unresolved.

Effect of area: When interpreting differences in element concentration between areas, it is important to note that significant differences between areas suggest limited mixing between individuals from the two areas. But no difference between two areas does not necessarily mean that the fish have been mixing freely between these areas. It could also be that the chemical concentrations in the water happen to be the same. Here, we highlight some of the most interesting elements and how to interpret the results:

Strontium (Sr): The concentration of Sr in otoliths has a strong correlation with its concentration in the environment, as multiple studies have found correlations > 0.95 (review of studies in Hüssy et al., 2020b). This makes otolith Sr a useful indicator of environmental salinity (Albertsen *et al.*, 2021; Hüssy *et al.*, 2021b) and it is widely used to track fish movements in estuaries and between freshwater and marine habitats on a global scale (Bath *et al.*, 2000; Elsdon and Gillanders, 2003; Miller, 2011; Sturrock *et al.*, 2012). Sr is a good example of how complex the interpretation of otolith chemistry results can be. We found that otolith Sr did not differ between the Skagerrak, Kattegat and the Sound on one hand but these areas differed significantly from the Belt Sea on the other hand (Fig. 14) (Table A1). Environmental salinity decreases only slowly from the Skagerrak towards the northern Sound, then decreases steeply in the northern Belt Sea and the Sound. Otolith Sr is thus consistent with environmental salinities in the areas fish were caught in.

Barium (Ba): 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). Ba shows a nutrient-like environmental distribution environment that gets depleted 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), which in our transition zone is found not only in nearshore coastal waters, but also south of the southern Kattegat. Consistent with this, we found a significant area effect on otolith Ba, with significantly increasing concentrations from the Skagerrak to the more coastal type environments in the Belt Sea/Sound, with no differences between the latter (Fig. 14) (Table A1). This supports the Sr-based conclusion of limited mixing between the Belt Sea and the Kattegat, but also suggests limited mixing between the Sound and the Kattegat.

Manganese (Mn): Owing to the reduction of manganese oxides from the sediment with decreasing ambient oxygen content, otolith Mn concentrations are known to increase in hypoxic areas. Otolith Mn concentrations have proven useful for tracking hypoxia exposure (Mohan and Walther, 2016; Altenritter *et al.*, 2018; Altenritter and Walther, 2019), including in Baltic cod (Limburg *et al.*, 2011, 2015). Prolonged seasonal hypoxia is known to occur in the Kattegat (Rosenberg *et al.*, 1992, 1996) and even more pronounced in the Baltic Sea (Conley et al., 2011; Carstensen and Conley, 2019; Kõuts et al., 2021). Otolith Mn also reflects these dynamics, increasing significantly from the towards the highest levels in the Belt Sea, but with Sound otolith Mn significantly lower than the Belt Sea (Fig. 14) (Table A1). Otolith Mn thus also suggests that mixing between stocks is relatively limited between all areas.

Copper (Cu), Phosphorus (P), magnesium (Mg) and zinc (Zn): These are elements that are all either essential constituents of the otolith's organic matrix, or co-factors in metabolic processes (Sturrock *et al.*, 2014; Thomas and Swearer, 2019). Their ions also appear to be incorporated into the otolith randomly trapped in the crystal lattice (Miller *et al.*, 2006; Izzo *et al.*, 2016; Thomas *et al.*, 2017). As such, these elements are assumed to be under strong physiological control, but their ambient concentrations exhibit large horizontal, vertical and seasonal variations gradients (Sylva, 1976; Conley *et al.*, 2002; Lebrato *et al.*, 2020; Naumann *et al.*, 2020) (Sylva, 1976; Cox, 1989; Conley *et al.*, 2002; Lebrato *et al.*, 2020; Naumann *et al.*, 2020), which may contribute to area-specific differences. As a consequence, the geographic patterns of these elements suggest a lack of mixing between the Skagerrak/ Kattegat and the Belt Sea/Sound (Cu, Mg), between all areas (K), and between the Skagerrak, Kattegat and the Sound, but not between the Belt Sea and the Sound (Fig. 15) (Table A1).

These element-by-element analysis results are somewhat inconclusive for considering stock separation/mixing, even though each element is in line with accepted hypotheses for what concentrations we should expect in the transition zone (Heimbrand *et al.*, 2020; Hüssy *et al.*, 2020b, 2021b). The overall picture is that there may be a geographic pattern with variable degrees of mixing between areas. To make full use of otolith chemistry we need to combine all elements in an analysis that takes into consideration spatial, seasonal and ontogenetic patterns, an example of which is presented in the next paragraph.

Fig. 14. Predicted effects of ICES SD (Area, with Kattegat (SD 21), Belt Sea (SD 22) and Sound (SD 23)), age (age of the fish throughout its entire life) and season (spring, summer, fall, winter) for elements that reflect ENVIRONMENTAL concentrations (Ba, Fe, Li, Mn, Pb, Sr). Corresponding statistics may be found in Table A1
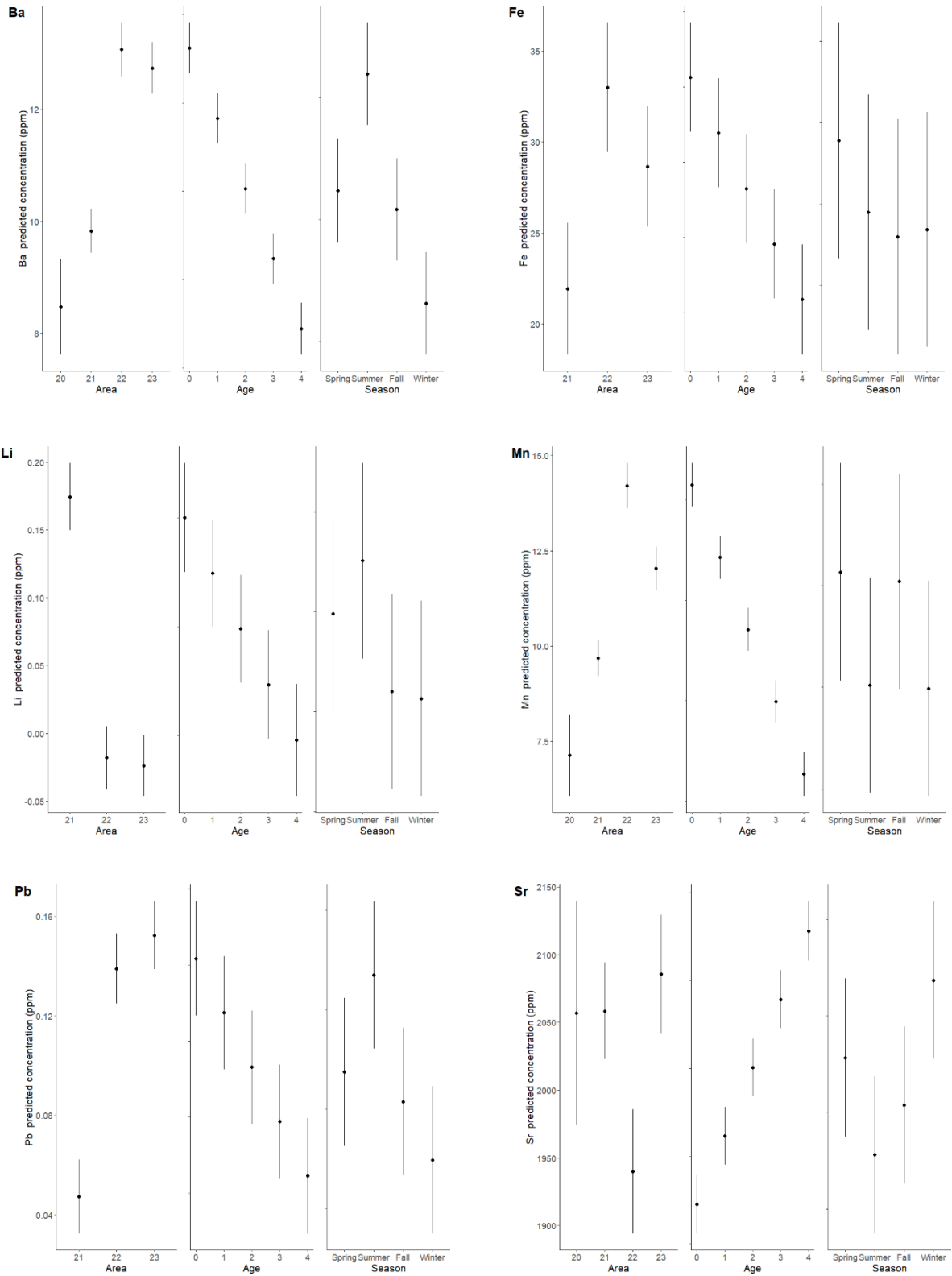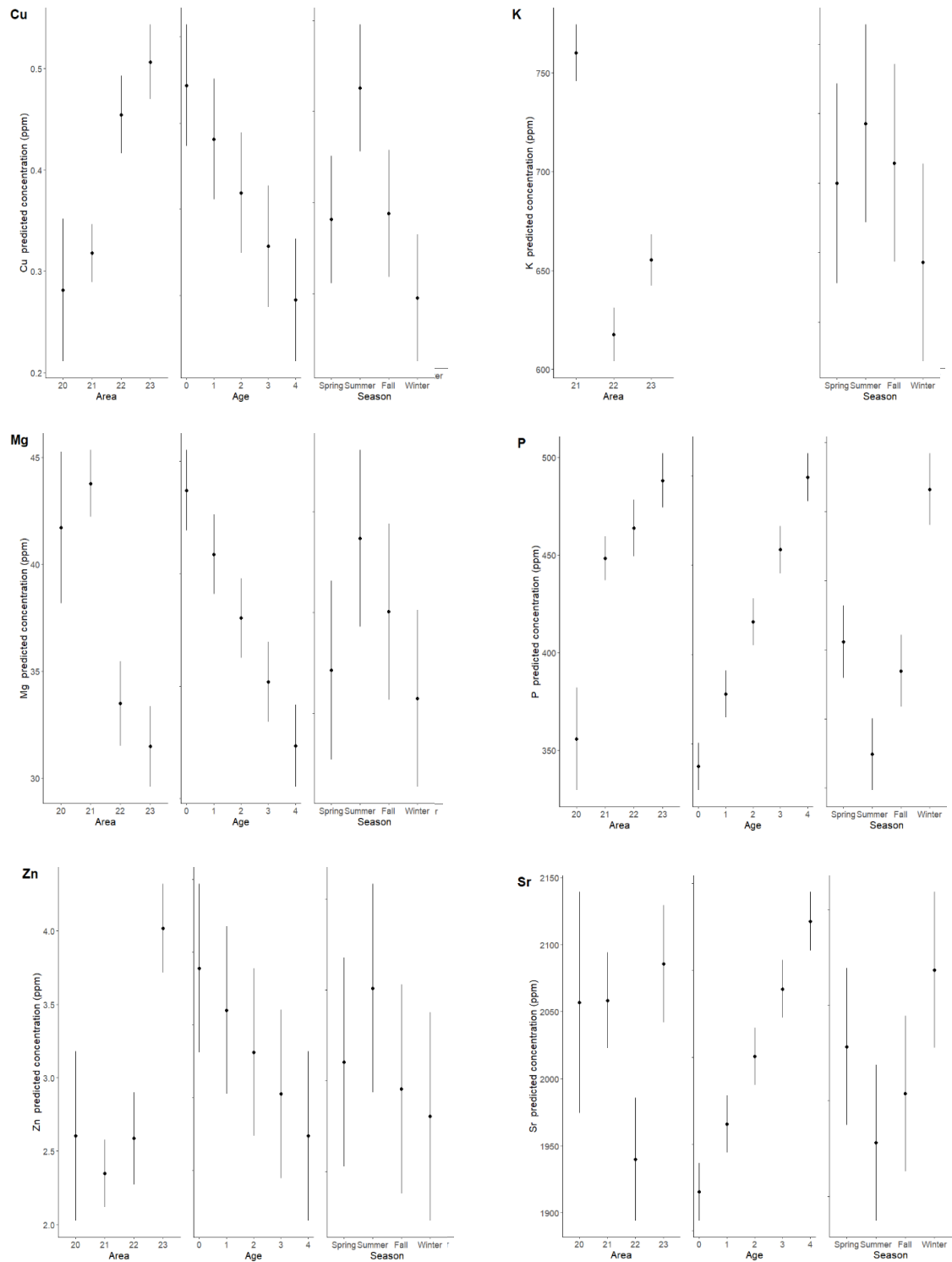
Fig. 15. Predicted effects of ICES SD (Area, with Kattegat (SD 21), Belt Sea (SD 22) and Sound (SD 23)), age (age of the fish throughout its entire life) and season (spring, summer, fall, winter) for elements that are subject to PHYSIOLOGICAL control during incorporation into the otolith (Cu, P, K, Mg, Zn). Corresponding statistics may be found in Table A1

Reconstruction of life-long area occupancy: For the reconstruction of life-long area occupancy we used the otolith edge fingerprint representative of the environmental water composition in the area where the cod was captured., with all relevant elements identified above, in a Linear Discriminant Analysis approach that included a parameter that modelled this age effect (Table A1). The edge fingerprint analysis found significant differences between areas (MANOVA, df = 9, p < 0.05), with an overall conditional LDA classification success of 63% and jack-knived leave-one-out cross validation success for Skagerrak: 75%, Kattegat: 31%, Belt Sea: 68%, Sound: 59%. The habitat reconstructions are shown for each individual as color-coded maps grouped by the ICES SD the fish were caught (Fig. 16). Only fish older than 2 years are shown to decrease the size of the figures. Individuals in these maps are ordered by latitude of their capture position. It is important to highlight that these mixing patterns do NOT address the movement of North Sea cod in and out of the Kattegat. Otolith chemistry depends on environmental element concentrations and is useful for inferring movement patterns. Consequently, cod that have spent their lives in the same area will have the same chemical profiles regardless of which genetic population they belong to. In summary, these maps show the following:

Skagerrak (SD 20): Most notable in this figure is that all individuals captured in the Skagerrak have a mixture of Skagerrak and Kattegat signals at various times in their lives. This may reflect considerable movement of cod between the two areas as tagging studies suggest (Danielssen, 1969; Svedäng *et al.*, 2007; Righton *et al.*, 2010). Or, alternatively, that the chemical signals are somewhat similar and assignment accuracy thereby hampered. Noteworthy however is, that not a single individual had a signal indicating that it had been in Belt Sea/Sound.

Kattegat (SD 21): Here too a mix of fingerprints assigned to the Skagerrak and the Kattegat occur, consistent with considerable movements between the northern part of these areas (Danielssen, 1969; Svedäng *et al.*, 2007; Righton *et al.*, 2010). We found an interesting geographic pattern, where a considerable number of individuals in the southern Kattegat have a Belt Sea signal - and have had so consistently throughout their whole life. Two scenarios can explain these patterns: 1) A number of cod that have been primarily in the Belt Sea and Sound throughout their lives moved into the Kattegat within a sufficiently short time prior to capture to not leave a chemical Kattegat signal, or 2) The capture location of these cod in relation to the water masses dominating and the SD boundary area. The largest change in the environmental gradient occurs north of the boundary between western Baltic and Kattegat management areas. This environmental gradient is the result of mixing of saline Atlantic water and brackish Baltic Sea water, that not only results in the salinity gradient shown in (Fig. A1) but also chemical properties of the different water masses in general. The cod with the Belt Sea signal were captured just north of the ICES SD 21 boundaries, which seems to be dominated by Baltic Sea water (Fig. A1). While the latter seems like the most likely explanation, the movement-based scenario cannot be excluded.
Cod tagged in the southern–central Kattegat and the Sound have been found to be primarily resident, with no indications of a pronounced migration further south than the northernmost Sound (Svedäng *et al.*, 2007), which is consistent with the clean reconstructions without Belt Sea/Sound signals throughout the life of the fish. It is not clear why a small group of individuals captured in northern locations have fingerprints consistent with the Belt Sea.

Belt Sea (SD 22): Cod captured in the Belt Sea have a highly distinct elemental fingerprint and confidence in the area assignment is therefore high. None of these fish have immigrated from other areas as adults.

Sound (SD 23): In the Sound, a clear geographic pattern in early life habitat occupancy is evident. In cod younger than 3 years, most cod captured in the southern Sound originate from the Belt Sea, while the cod captured in the northern Sound have a clean "Sound" fingerprint. Cod older than 3 years on the other side, have on average been more frequently present in the Sound, even though most individuals seem to have undertaken movements into the Belt Sea. These results are consistent with two tagging studies that showed that on averaged 70% of cod tagged in the Sound were recaptured within the Sound, 19% were recaptured just north of the SD 21/23 boundary around Kullen (where the hydrographical conditions are more similar to the western Baltic Sea than the Kattegat (Fig. A1), while only 2% were recaptured further north in the Kattegat (Svedäng *et al.*, 2010). A few individuals have a Kattegat signal in winter/spring, which coincides with the spawning season. In eastern Baltic cod, the spawning season of individual fish lasts for ca 95 days according to data from archival tags (Nielsen *et al.*, 2013). This information is not available for cod from the Sound/Kattegat, but is presumably of a similar duration. Combined with the results from the tagging data (see 4.3.3), it cannot be excluded that some individuals from the Sound move to the Kattegat for spawning, but the otolith chemistry signals do not support a large-scale spawning migration towards the Kattegat. It is quite striking that only few individuals have been identified as having spent short periods of time in the Kattegat, and only three individuals seem to have spent > 1 year in the Kattegat.

Arkona Sea (SD 24): Unfortunately, we do not have adequate samples from the Arkona Sea to address whether these classifications to the Belt Sea are in fact the result of movements into the Arkona Sea, which is directly connected to the Sound.

Fig. 16. Lifetime area occupancy in cod captured within the four ICES SDs (SD indicated above each figure). Cells represent individual fish and each year of its entire lifespan from the first winter to catch. Individuals are ordered according to latitude of their capture position, with individuals captured further north at the top of the panel. The time from hatch to the first winter is not included in this plot, as the area assignment for this age group using the conditional LDA approach is rather imprecise due to the ontogenetic shift in habitat occupation. Colors indicate area occupancy assignments: Skagerrak (SD 20) = lime, Kattegat (SD 21) = yellow, Belt Sea (SD 22) = purple, Sound (SD 23) = green.

4.3.3 Stock mixing

In the following we have estimated measures of stock mixing from otolith chemistry and tagging data. The nature of these data does not allow a direct comparison of derived estimates, but rather as complementary information.

Otolith chemistry-based stock mixing estimates: The mixing proportions of cod captured in the different ICES SD was calculated by age of life and season from the reconstructed individual area occupancy assignments. Again, it is important to remember that these mixing proportions, like the individual movement patterns above, do NOT separate between genetically distinct populations (i.e. between North Sea and Kattegat cod), but exclusively address ecological connectivity where cod that have spent their lives in the same area will have the same chemical profiles regardless of which genetic population they belong to. The resulting mixing proportions are shown as number fish (Fig. 17). It is evident from these results, that mixing occurs between different ICES SDs (i.e. Skagerrak/Kattegat and Belt Sea/Sound), and that the mixing proportions seem fairly stable over time. Cod captured in the Skagerrak had on average spent 60% of their time in Skagerrak and 40% in Kattegat, with some apparently random variation between age classes and seasons. Cod captured in Kattegat had on average spent 36% of their time in Skagerrak, 34% in the Kattegat, and 29% in the Sound/Belt Sea. The latter seems to be attributable to a mismatch between management area boundaries and environmental gradients. Cod captured in the Belt Sea had on average spent 89% of their time in the Belt Sea, 10% (primarily age > 2) in the Sound and only 1% in the Kattegat. Cod captured in the Sound had up to an age of 3 years on average spent 63% of their time in the Belt Sea, 33% in the Sound and 4% in the Kattegat. Older cod spent about equal amounts of time in the Sound and Belt Sea. This suggests that cod in the Sound are indeed a component of the western Baltic cod stock according to the current management units.

Note that stock mixing of North Sea and Kattegat, where North Sea cod are entrained to the Kattegat as early juveniles and leave again upon reaching maturity, is not addressed in the present context (due to lack of adequate data). The proportions of stock mixing over the seasons and fish ages suggest the presence of an ecological separation between the northern Sound/Belt Sea and the southern Kattegat. An earlier study combining genetics and otolith chemistry of cod tagged in the Sound (Svedäng *et al.*, 2010), suggested that cod in the northern Sound undertake spawning migrations into the southern Kattegat. Such a migration is not evident from our data.
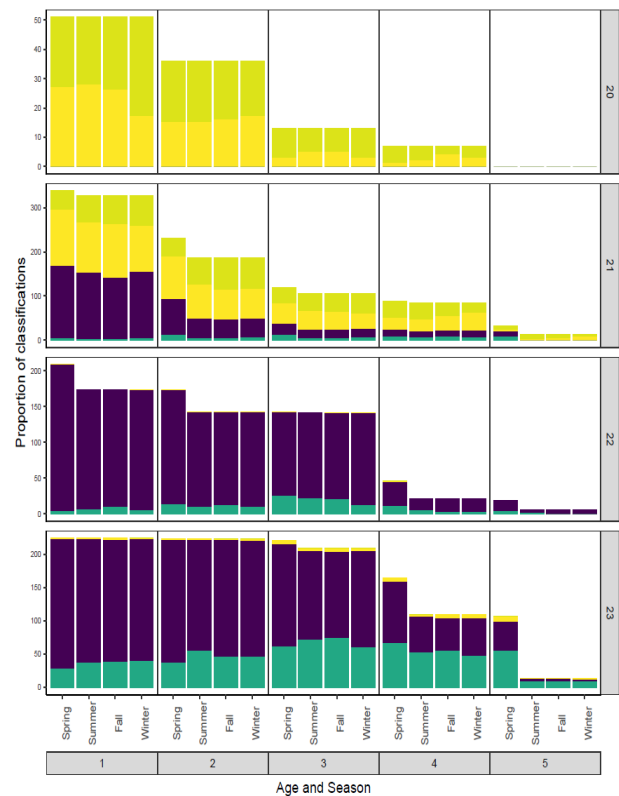

Fig. 17. Number of cod classified to either Skagerrak (SD20; lime), Kattegat (SD 21; yellow), Belt Sea (SD22; purple) or the Sound (SD 23; green) by age and season, for each of the four ICES Subdivisions samples originated from. Colors represent ICES SD the cod were assigned to.

Tagging based stock mixing estimates: The movement patterns from the historical tagging data showed some area-specific trend, with cod in the North Sea and eastern Baltic Sea moving longer distances and dispersing further away from the release positions than cod in the western Baltic, Belt Sea and Sound (Fig. 18), where population seem to consist of both resident coastal and migratory offshore stock components (Lundgreen *et al.*, 2022). Stock mixing between western and eastern Baltic cod has previously been documented using genetics (Hemmer-Hansen *et al.*, 2019), otolith shape (Hüssy *et al.*, 2016a, 2016b), and tagging data (Mion *et al.*, 2022). Mixing proportions of survey samples and landings are estimated annually, and used in stock assessment. Similarly, mixing of North Sea and Kattegat stocks in the Kattegat have been addressed with genetics and otolith chemistry (Hemmer-Hansen *et al.*, 2020; Albertsen *et al.*, 2021; Hüssy *et al.*, 2021b). Here, we therefore focus on the Sound, where abundant tagging data is available, and where the question of stock structuring and mixing has not been adequately addressed.
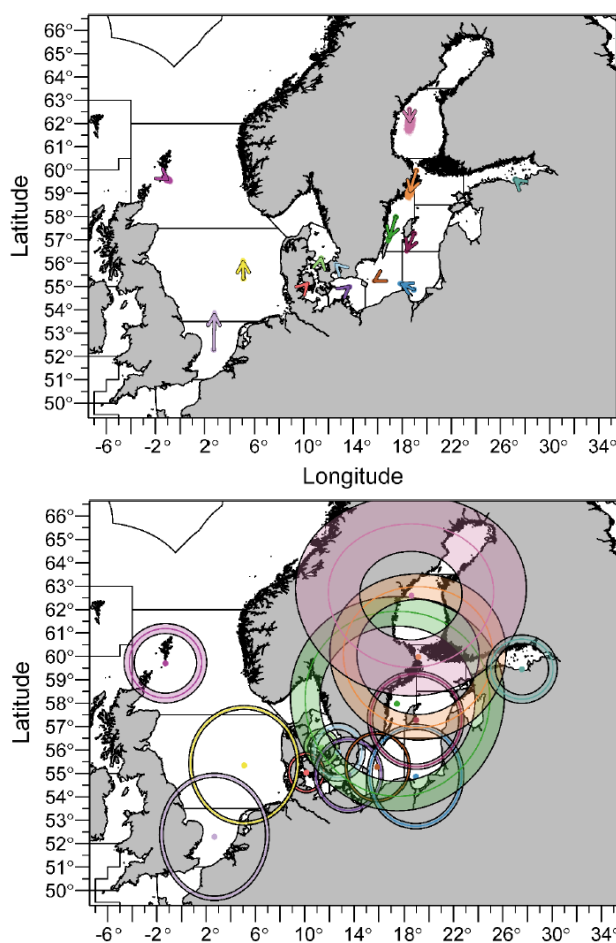

Fig. 18. General patterns of movement direction (left) and dispersion (right) for all tagging years combined and color coded by ICES area the cod were released in. The length of the arrow indicates the strength of the directional movement, while the circles show the mean dispersion and 95% confidence intervals from the mean release coordinates. From Lundgreen et al. (2022).

The recapture positions of the newly digitized individuals tagged in the Sound are shown in Fig. 19a. Note that there are a total of 797 dots, whereby far the majority are superimposed on each other in the Sound. Recaptures outside the Sound thus visually dominate considerably more than the majority of individuals. From these tag/recapture data, the likelihood of moving from the Sound towards the Kattegat within any week during either the spawning season or the feeding season was estimated. These movement probabilities are shown in Table 6 and further details on analytical setup and results are presented in Lundgreen *et al.* (2023). The numbers in Table 6 mean that in any given week during the spawning period (January-February) (Vitale *et al.*, 2005; Lundgreen *et al.*, 2023) there is a probability of 0.71 that cod stay in the Sound, and a probability of 0.29 that they move to Kattegat. The cod that move to Kattegat have then a probability of 0.77 for staying in Kattegat during the next week. So movement of cod between areas is calculated by multiplying the probability of staying/leaving an area with the numbers from the previous week. The ecological connectivity between the Sound and southern Kattegat thus appears to have been considerable, primarily during the spawning season. These results are thus not entirely consistent with Svedäng *et al.*

(2010) who found that 89% of cod tagged in the Sound remained within the Sound/Kullen area and only 2% further north into the southern Kattegat. However, it is important to note here, that the tagging data span the years 1957 and 1987. Since stock dynamics have changed significantly since then (ICES, 2019), it may be that connectivity between the Sound and the Kattegat has changed.

It was unfortunately not possible to estimate movement probabilities from the Sound to the Arkona Sea because there was not sufficient data. Despite area-specific fishing patterns, which inherently influence where and when tagged cod are recaptured, this scarcity of recaptures in the Belt and Arkona Sea suggests limited movements from the Sound towards the south (Lundgreen *et al.*, 2023). However, a few cod tagged in other parts of the western and eastern Baltic Sea were recaptured in the Sound (Fig. 19b), documenting that a certain degree of exchange between areas is presumably occurring throughout the area (Lundgreen *et al.*, 2023).
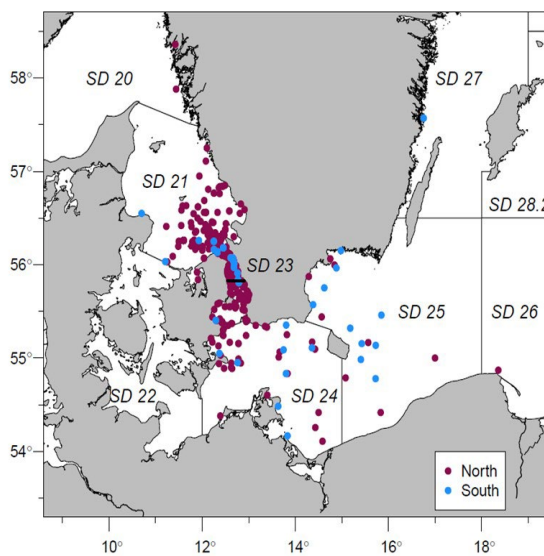
Fig. 19a. Recapture positions of cod tagged and released in the northern Sound (SD 23; red dots) and the southern Sound (SD 23; blue dots). The black horizontal line indicates the geographical divide between the two areas based on topographic features in combination with the salinity gradient prevailing in the Sound. Note that there are a total of n = 797 dots, where by far the majority are superimposed on each other in the Sound. Recaptures outside the Sound thus visually dominate considerably more than the majority of individuals.
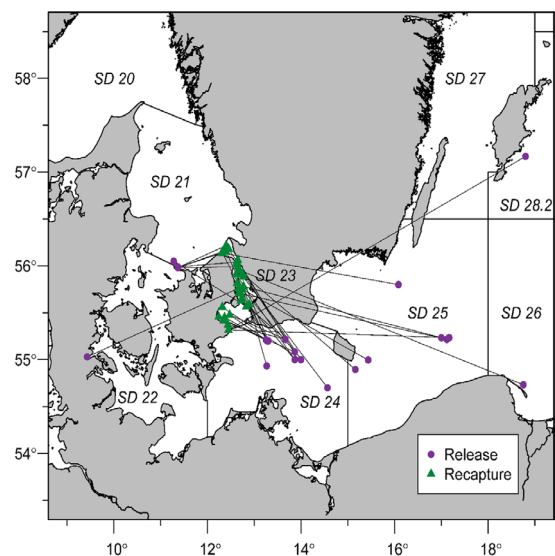
Fig. 19b. Recaptures in the Sound released in other areas. Recaptures originated from Kattegat (Sd 21; n = 11), the Belt Sea (SD 22; n = 1), the Arkona Basin (SD 24; n = 20), Bornholm Sea (SD 25; n = 6), the south-eastern Baltic Sea (SD 26; n = 1), and the Gotland Sea (SD 28; n = 1).

Table 6. Seasonal transition matrices between Kattegat and the Sound during peak spawning (January-February) and feeding season (March-December) showing the most likely area transitions. The table shows the probability of <u>a cod tagged in the Sound</u> moving from the current area (row) to a neighboring area (column) within a random week during either the spawning or feeding season.

Season	Current area	New area	
		Kattegat (SD 21)	Sound (SD 23)
Spawning	Kattegat (SD 21)	0.77	0.23
	Sound (SD 23)	0.29	**0.71**
Feeding	Kattegat (SD 21)	0.82	0.18
	Sound (SD 23)	0.04	**0.96**

4.4 Conclusions

Natal origin
- Spatial differences in the chemical fingerprint in the otolith cores, representing the natal origin, indicate that cod in the transition zone originate from different spawning areas.
- Three spawning area clusters were identified, although clear separation was only evident for two of the clusters. Clusters cannot be assigned to specific spawning areas without baseline samples from cod larvae from the different spawning areas. However, the spatial distribution of the clusters suggests the most likely origin of the clusters as 1) North Sea cluster occurring primarily in the Skagerrak and northern Kattegat, and 2) Two transition zone clusters occurring throughout the southern Kattegat, Belt Sea and the Sound.
- The geographic distribution of spawning area clusters differed considerably between year classes.

Adult movements
- The elements analysed in this study reflect gradients in environmental conditions and physiological processes in line with known mechanisms of otolith biomineralization. Otolith chemistry is therefore suitable for assessing movement patterns of fish in the transition zone.
- Cod in the Skagerrak and northern Kattegat have either a similar chemical fingerprint throughout their lives - or mix extensively throughout their lives. Cod from Skagerrak and Kattegat do generally not move south into the western Baltic Sea. Nevertheless, some cod in the southernmost Kattegat have a Belt Sea/Sound signal throughout their lives, which may be the result of active movement from the Belt Sea into the Kattegat or, alternatively, the fact that the environmental gradient is not entirely aligned with the boundaries between management areas.
- Cod in the Belt Sea are almost exclusively resident in that area throughout their lives.
- Cod in the Sound show some connectivity with the Belt Sea. Most of the cod immigrated into the Sound from the Belt Sea before the age of 3 - and remained largely resident within the Sound thereafter. However, most cod have Belt Sea signals at irregular times in their lives, suggesting some movement in and out of the Sound.

Movement-based Stock mixing
- Estimates of stock mixing between the Sound and the southern Kattegat from historic tagging data and contemporary otolith chemistry are somewhat contradictory. Tagging data indicate

that a considerable proportion of Sound cod move into the southern Kattegat for spawning, while there was no evidence of such a strong connectivity from the otolith chemistry. It is not clear, whether stock mixing across the Sound/Kattegat management area border has changed over time in relation to general changes in stock dynamics.

- Cod captured in the eastern Skagerrak and northern Kattegat are not moving south into the Belt Sea and Sound. Cod captured in the Sound spent about equal amounts of time in the Sound and Belt Sea, but virtually no time in the Kattegat.

- Overall, the combined analysis of natal origin and adult movements suggests that while cod in the transition zone may originate from different spawning areas, they are largely resident within the respective management areas as adults. Our results are indicative of stock structuring in the transition zone with an ecological separation into distinct components in 1) the (eastern) Skagerrak and the Kattegat, 2) the Belt Sea and the Sound. One issue that remains to be resolved is to what extent the occurrence of cod with a life-long Belt Sea signal in the Kattegat is the result of active movement of cod from the Belt Sea to the Kattegat, or whether it is caused by the fact that SD boundaries and strongest change in environmental gradient are not entirely aligned.

Future considerations

- In order to improve the precision of the otolith chemistry-based reconstruction of movement patterns, we suggest in the future to 1. Include samples from the Skagerrak to consolidate the ecological connectivity thereof with the Kattegat, 2. Include samples from the Arkona Sea to identify whether the connectivity between Belt Sea and Sound observed in this study occurs primarily north or south of Zealand, 3. Expand the analysis of movement patterns available so far with particular focus on testing differences between genetically distinct populations capture in the same locations.

- While the otolith chemistry-based stock mixing estimates suggested an ecological stock separation into Skagerrak/Kattegat and Belt Sea/Sound components, the estimates from historic tagging data suggests extensive mixing between the Sound and the southern Kattegat. Owing to the discrepancy in the stock mixing results between these approaches, we recommend that future initiatives should focus on validating the movements of cod in the Sound based on an approach that combines tagging and otolith chemistry.

5. Implications for stock assessment

5.1 Introduction

Fisheries stock assessment utilizes biological information, available knowledge and assumptions to model population dynamics and, in turn, estimate vital parameters and stock size. Often, the models are validated and tested in self-simulations or within a larger management strategy evaluation. However, these often rely on the same, or similar, model structure to generate 'true' simulated data. In this project, a working prototype for a generic simulation tool was built (project task 4.1) to evaluate the robustness of stock assessment models under a variety of biological assumptions that may or may not be similar to the model being tested. The simulation tool is suitable for evaluating fisheries stock assessment models, management strategies, and data collection methods under different biological scenarios.

Stock structuring and -mixing has been identified by the stock assessment working group as the likely cause of area-specific trends in recruitment and spawning biomass trends in the North Sea and adjacent waters (ICES, 2019b). At the Benchmark workshop in 2023 (ICES, 2023a) the decision to split the North See into three different stocks (Northern Shelf stock, Southern stock and Viking stock) was taken. Movement between areas are known to exist, but the extent of the spatial and temporal distribution of the three stocks is not well described. The decision was therefore taken to use this setup as a first case study for a prototype assessment. All available data on genetic stock identification, biological data and migration patterns from tagging was compiled during the ICES workshop on stock identification of cod in the North Sea and adjacent waters (ICES, 2020). The Northern Shelf and Kattegat cod stocks were therefore selected as a first case studies to test a prototype of this tool, using state-of-the-art knowledge about migration patterns and stock mixing from work packages 1-3. The simulation procedure included detail assumptions about migration, spatial distribution, maturity, mortality, and fishing based on the current best available knowledge. The results of the simulations facilitate multiple evaluations of current management practice. First, the simulation procedure can be used to test the robustness of fisheries stock assessment models to different assumptions about the biological system. Second, the simulations can be used to evaluate the effect of different management procedures. Finally, they can be used to evaluate costs and benefits of different sampling - or modelling - procedures to collect biological information or catch data under different scenarios (project task 4.2).

The Northern Shelf complex simulations were used to illustrate three potential analyses facilitated by the simulation tool. The first analysis evaluates the effect of ignoring North Sea cod migration into the Kattegat area on data that goes into the Kattegat assessment. Such an analysis could, in turn, be used to run assessments for each simulation. The second analysis evaluates the effect of closing the Kattegat area at latitudes below 57 degrees North on conservation efforts of the Kattegat cod stock. The final analysis uses one of the simulations for a cost-benefit analysis of genetic versus otolith sampling for splitting mixed catches of Kattegat and North Sea cod stocks on a quarter 1 bottom trawl index for the Kattegat stock.

In summary, the objectives of WP 4 were to:
1. Development of a generic simulation model that can incorporate state-of-the-art knowledge about migration patterns and stock mixing.
2. Evaluation of costs and benefits of incorporating different sampling or modelling procedures to collect biological or catch data under different scenarios.

5.2 Materials and Methods

5.2.1 Simulation framework

During the project, a software package for the R statistical software was built to simulate detailed fish stock dynamics. Based on user input, The tool consisted of a C++ library implementing the simulations and a PDE solver. Further, the tool had an interface for the statistical programming language R, implemented as a package.

Several specifications were considered and tested regarding the number of parameters used to keep track of stock abundance. The final implementation used a cohort-based implementation with abundance per grid cell and maturity. Other implementations also considered abundance per length. However, as a trade-off between biological accuracy and computational complexity, the final implementation was chosen.

Simulation model implementation: The simulation tool was implemented as a continuous time stochastic model, discretized in arbitrary - user specified - time steps. Further, the user supplies a grid for spatial discretization. Functionality is included to construct such grids. Finally, the user must provide a list of stocks and a list of fleets for the simulations.

For each stock in the model, the user must specify migration patterns at an individual fish level in the form of a stochastic differential equation reflecting observed stock mixing proportions (i.e. Hüssy et al., 2016b; Hemmer-Hansen et al., 2019; ICES, 2020; Lundgreen et al., 2022), a weight-length relation, natural mortality hazard rate, maturity hazard rate, and recruitment function. Individual level migrations were scaled to population level through the Fokker-Planck equation and solved on the user specified grid with an implementation of the finite volume method.

For each fishing fleet in the model, a function must be given to determine effort (based on previous effort and total stock biomass), a function for conversion from effort to F, a function to determine availability, and a function to determine retention probabilities.

In each time step, effort per fleet is updated first. Afterwards, stocks - and each cohort within them - are updated sequentially. Within a cohort, recruitment is updated first, followed by survival (fishing and other causes are updated simultaneously), maturity, and movement. Finally, values are saved for outputs.

Application to Northern Shelf and Kattegat cod: The simulation tool was applied to the combined Kattegat-Northern Shelf cod complex. The stock complex consists of four stocks: Kattegat, Viking, Northwestern Dogger, and Southern Dogger. Further, 22 fishing fleets were included in the simulations. The fleets were defined by gear type, mesh size, ICES area, and target species. The stock and fleet assumptions in the simulations are described below. Note that not only the parameters, but also the functional forms of the relationships are flexible and determined by the user of the simulation tool.

Migration patterns: Migration patterns per stock were based on the best available knowledge and tuned from initial simulations. Migrations were specified as individual level stochastic differential equations,

$$dX_t = f(X_t)dt + SdB_t,$$

where X_t are coordinates, $f(X_t)$ is a drift function, S determines the variability, and B_t is a Brownian motion.

For the Kattegat stock, migration drift patterns ($f(X_t)$) included a combination of depth preference, spawning migrations, and home range attraction. The stock was assumed to prefer depths of 40m at age 0 and 80m at older ages. At spawning time, the stock was assumed to be attracted towards the centroids of the statistical rectangles "41G2" and "42G2". Finally, the stock was specified to be attracted to the point (675, 6300) in UTM coordinates. The diffusion (S) was a diagonal matrix with 150 in the diagonal.

For the Northwestern stock, migration drift patterns ($f(X_t)$) included a combination of depth preference, spawning migrations, and home range attraction. The stock was assumed to have a bi-modal preference at depths of 40m and 140m to reflect the inshore and offshore individuals of the stock. At spawning time, the stock was assumed to be attracted towards the centroids of the statistical rectangles "45E3","45E4","46E3","46E4","46E5", and "45E7". Finally, the stock was specified to be attracted to the point (-400, 6600) in UTM coordinates. The diffusion (S) was a diagonal matrix with 520 in the diagonal.

For the Southern stock, migration drift patterns ($f(X_t)$) included a combination of depth preference and spawning migrations. The stock was assumed to have a depth preference of 40m. At spawning time, the stock was assumed to be attracted towards the centroids of the statistical rectangles "36F1", "36F2", "37F1", "37F2", "37F7", "38F5", "38F6", "38F7", "39F5", "39F6", "39F7", "40F5", "40F6", "40F7", "41F5", "41F6", "41F7", "42F4", "42F5", "43F2", "43F3", and "43F3". The diffusion (S) was a diagonal matrix with 340 in the diagonal.

For the Viking stock, migration drift patterns ($f(X_t)$) included a combination of depth preference, spawning migrations, and home range attraction. The stock was assumed to have a depth preference of 60m for immature individuals and 130m for mature individuals. At spawning time, the stock was assumed to be attracted towards the centroids of the statistical rectangles "49F0" and "49F1". Finally, the mature part of the stock was specified to be attracted to the point (100, 6700) in UTM coordinates. The diffusion (S) was a diagonal matrix with 150 in the diagonal for mature individuals and 440 for immature fish. The values were selected to reflect that the stock utilize Kattegat as a nursing area while adults tend to aggregate close to the Norwegian trench.

For all four stocks, drift ($f(X_t)$) was capped to be between -20 and 20.

Initial distribution and spatial grid: A spatial grid was constructed for the Kattegat, North Sea, and west of Scotland areas (ICES areas 3.a.20, 3.a.21, 4.a, 4.b, 4.c, 6.a, and 7.d). Grid cells were constructed to be 30 x 30 km squares. Initial spatial distributions for each stock were found by projecting the migration models for 100 time steps over 10 years using the PDE solver. The spatial distribution of catch from the IBTS Q1 was used to initialize the migration model projection. The spatial grid and initial distributions are illustrated below (Fig. 20).
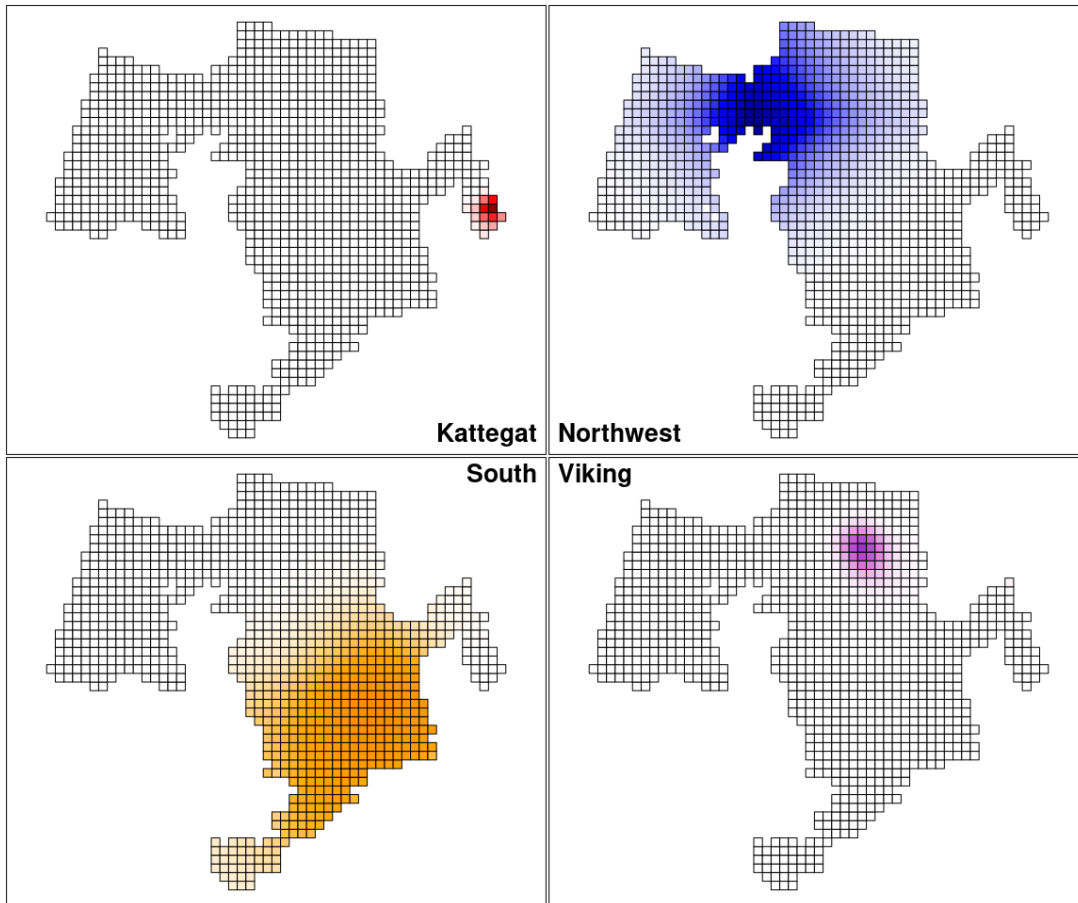
Fig. 20. Spatial grid and initial distribution of the four simulated stocks. Note that intensity colors are not on the same scale, but intended for illustration only.

Length-at-age relation: The relationship between fish age and length was assumed to follow a von Bertalanffy growth curve. Parameters were estimated per stock from IBTS Q1 data split by area (Table 7).

Table 7. Estimated length-at-age relations used in the simulations.

Area	$\log(L_\infty)$	$\log K$	t_0	
Kattegat	4.599539	-1.508548	0.2221364	1.947655
Northwest	4.837883	-1.672297	0.1629521	2.002408
South	5.236399	-2.221675	-0.0392078	2.015357
Viking	4.917175	-1.766522	0.2062893	1.989504

Length-weight relation: The relationship between fish length and weight was assumed to follow the relationship:

$$W = a \cdot L^b$$

Parameters a and b were estimated per stock from IBTS Q1 data split by area (Table 8).

Table 8. Estimated length-weight relations used in the simulations.

Area	a	b
Kattegat	0.0063128	3.111781
Northwest	0.0053612	3.175868
South	0.0064936	3.117642
Viking	0.0062750	3.123217

Natural mortality: Natural mortality was included through a hazard function in a survival analysis framework. The hazard function determines the instantaneous risk of dying. A Weibull hazard function was fitted to the 2019 SMS key run age-wise estimated natural mortality rates,

$$h_M(a) = \lambda_M \cdot \theta_M \cdot a^{\theta_M - 1},$$

where a is the age and λ_M and θ_M are parameters.

To determine the risk of dying from non-fisheries causes, natural mortality was combined with fisheries induced mortality hazards to calculate the cause specific cumulative incidence with competing risks.

Maturity: Similar to natural mortality, maturity was included through a hazard function in a survival analysis-like framework. The hazard function determines the instantaneous 'risk' of moving from immature to mature. That is, similar to the instantaneous risk of dying in a survival context.

A Weibull hazard was fitted to IBTS Q1 maturity data for geographical areas corresponding to the four stocks,

$$h_{Mat}(a) = \lambda_{Mat} \cdot \theta_{Mat} \cdot a^{\theta_{Mat} - 1},$$

where a is the age of the fish, and b and k are parameters to determine the risk. Consequently, the chance of "surviving" maturity (i.e., staying mature) until age a is

$$P_{Mat}(a) = \exp\left(-\int_0^a h_{Mat}(t)dt\right) = \exp(-\lambda_{Mat} \cdot a_{Mat}^{\theta})$$

The estimated parameters are listed in Table 9 below:

Table 9. Estimated maturity parameters used in the simulation.

Area	b	k
Kattegat	0.4009278	3.504833
Northwest	0.3472627	3.196841
South	0.3864690	2.962741
Viking	0.2434789	3.528071

Recruitment: For all four stocks, median recruitment was assumed to follow a Beverton-Holt stock recruitment relationship per grid cell,

$$R(S,t) = 20000 \cdot \frac{S}{S + 200} \cdot \tau(t),$$

where $\tau(t)$ was a function distributing recruitment throughout the year. With monthly time steps, $\tau(t)$ was set to distribute 6% of recruitment to January, 88% to February, and 6% to March. The maximum recruitment of the curve is $2000 \cdot \tau(t)$ while half of the maximum recruitment is reached at an SSB of 200, within a grid cell. In general, it would be preferable to let the parameters depend on the grid size. For a tiny grid cell, an SSB of 200 may be a lot, while for a large grid cell, an SSB

of 200 is not. However, in this simulation, all grid cells have the same size, and the simulations were only run with one grid.

In initial setups, we experimented with only allowing recruitment in the respective spawning areas. However, this leads to unsustainably low recruitment. Therefore, the scenario presented here allows recruitment in any geographical position.

Fishing fleets: A total of 22 fishing fleets were included in the simulations. Spatial landing and effort data were downloaded from the European Commission Joint Research Centre Data Catalogue (Gibin *et al.*, 2022). Effort and landings were collected to fleets by aggregating to ICES area, gear (Gillnet/Trawl), targeting cod (demersal fisheries with more than 10% cod landings) or not, and net shape and size.

For each of the 22 fleets, effort (scaled to days) was calculated per model grid cell and used as effort in the model. Daily effort was used as the fully selected fishing mortality rate in the model for the fleet with the largest LPUE in the data. For remaining fleets, LPUE relative to the largest LPUE was used as the relative availability, scaling fishing mortality between fleets. Finally, selectivity for trawl fleets were included as a logistic curve, while gillnet selectivity was included with a bell curve. Effort per grid cell for each of the fleets is illustrated in Fig. 21.

Fig. 21. Illustration of the effort per fleet and grid cell used in the simulations.

5.2.2 Evaluation of simulations

Effect of migration patterns on stock assessment data: The effect of migration patterns on stock assessment input data was evaluated from the simulated scenario. To evaluate the effect, catch and abundance of the true Kattegat stock was compared to the perceived catch and abundance within the geographical Kattegat area. This difference directly translates into the difference between accurately managing the Kattegat stock and managing the stock through a geographical approximation.

Effect of closing areas to fishing: As an example of using the simulation tool to evaluate management policies, additional simulations were run. In the additional simulations, fishing in Kattegat south of 57 degrees latitude was prohibited. Any effort in the area was distributed evenly between grid cells in the remaining part of Kattegat. Finally, SSB and TSB were compared between the simulations. While the simulations are appropriate to evaluate the conservation effect on Kattegat, any potential economic effects on the fisheries, primarily targeting crustaceans, are not evaluated. However, they could be, by including the target species in the simulations.

5.2.3 Cost-benefit analysis

In the final analysis based on the simulations, a Monte Carlo method was used to evaluate the cost benefit trade-off between using genetic and otolith shape samples for estimating stock composition to split survey indices. In general, genetic samples are more accurate than otolith shape for estimating stock composition. However, the cost per fish is higher. Further, otoliths often need to be collected for age reading, thereby lowering the marginal cost per fish of otolith shape analysis. In contrast, otolith images need more post-processing than genotype data, which makes the (sample size independent) statistical analysis cost of otolith shape higher for otolith shape than genetics.

In the analysis, BITS Q1 survey bottom trawl haul samples were constructed based on a simulated abundance. Likewise, either genetic or otolith shape samples were simulated. For these, true stock abundance was simulated using the 'true' simulated abundance from one of the simulations from the simulation tool. Since the proportion of Kattegat cod was very high in the simulations, the composition was set to 50%, 75%, 85%, and 95% Kattegat cod, respectively. The cost-benefit was assessed for each composition proportion. In turn, observed stock classifications (Kattegat/North Sea) were simulated. For genetic samples, the accuracy was 99.9%, while it was 80% for otolith shape, estimated from published values (Hüssy *et al.*, 2016a; Hemmer-Hansen *et al.*, 2019, 2020). Based on the simulated hauls and stock classifications, a depth stratified CPUE index was calculated using a procedure similar to the BITS survey. In the simulations, the CPUE index was calculated directly for each age (i.e. without going through age-length keys). For simplicity, the procedure did not correct for bias when using uncertain classification for stock splitting. The procedure was replicated 1000 times for different numbers of stock composition samples, and the standard deviation of the mean relative error was calculated.

Finally, the price to obtain a certain standard deviation was calculated and compared between the two sampling procedures. For this analysis, a per fish laboratory cost of €15 is assumed for genetic samples and €5 for otolith shape. Subsequent analysis cost is assumed to be €1000 for genetics and €3000 for otolith shape, which requires more post processing of images and more computational power.

5.3 Results and discussion

5.3.1 Simulation model

In this project, a fully functional prototype for a simulation tool was implemented. The simulation tool was used to evaluate the impact of migrations on stock assessment and how to best mitigate the effects through stock composition sampling efforts.

While the tool is fully functional, improvements can still be made. For example, it would be beneficial for users to add documentation and tutorials to the package. Likewise, the package could benefit from user friendly implementations of functional forms for recruitment, growth, and movement, such that a user only needs to give, e.g., the von Bertalanffy growth parameters for a growth curve or L_{50} and selection range for a selectivity curve. Further improvements could include simulations of genetics, otolith shape, otolith micro-chemistry, tagging data, survey hauls, observer information and biological information within the framework. Finally, it would be useful to implement common functional forms in C++ to speed up computations. The package is expected to be released as open-source software when funding is obtained to improve the user interface and documentation.

5.3.2 Evaluation of simulations

Application to Northern Shelf and Kattegat cod: Specifying a complex simulation scenario is difficult. The simulations are highly dependent on e.g. stock-recruitment, natural mortality, and fishing mortality assumptions - on which there are typically limited data (Fig. 22). Especially at a fine spatial resolution. Further, combining different information about migration patterns and space use preferences can be difficult. In reality, preferences may be fluid depending on the current condition and environment of the fish. However, in a model with multiple preferences, one preference may easily mask the others. In the present example (Fig. 23), the amount of immature Viking fish in Skagerrak appears to be too low. However, the simulations reflect the best achieved specification. Likewise, the stock-recruitment / mortality balance seems to be too pessimistic for the Kattegat stock and too optimistic for the Southern stock. The results below must therefore be interpreted in this light.
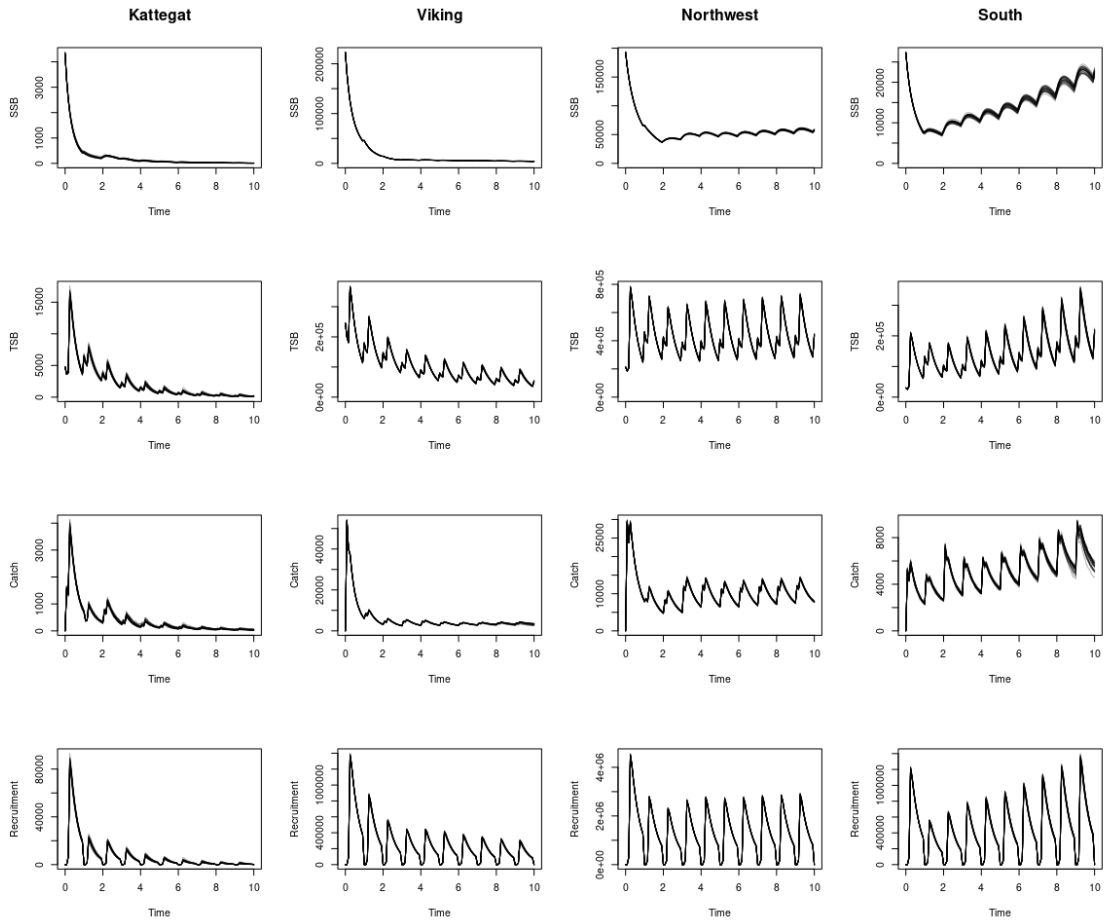
Fig. 22. Simulated trajectories of SSB, TSB, Catch numbers, and number of recruits for the four stocks as a function of year after simulation start (Time).
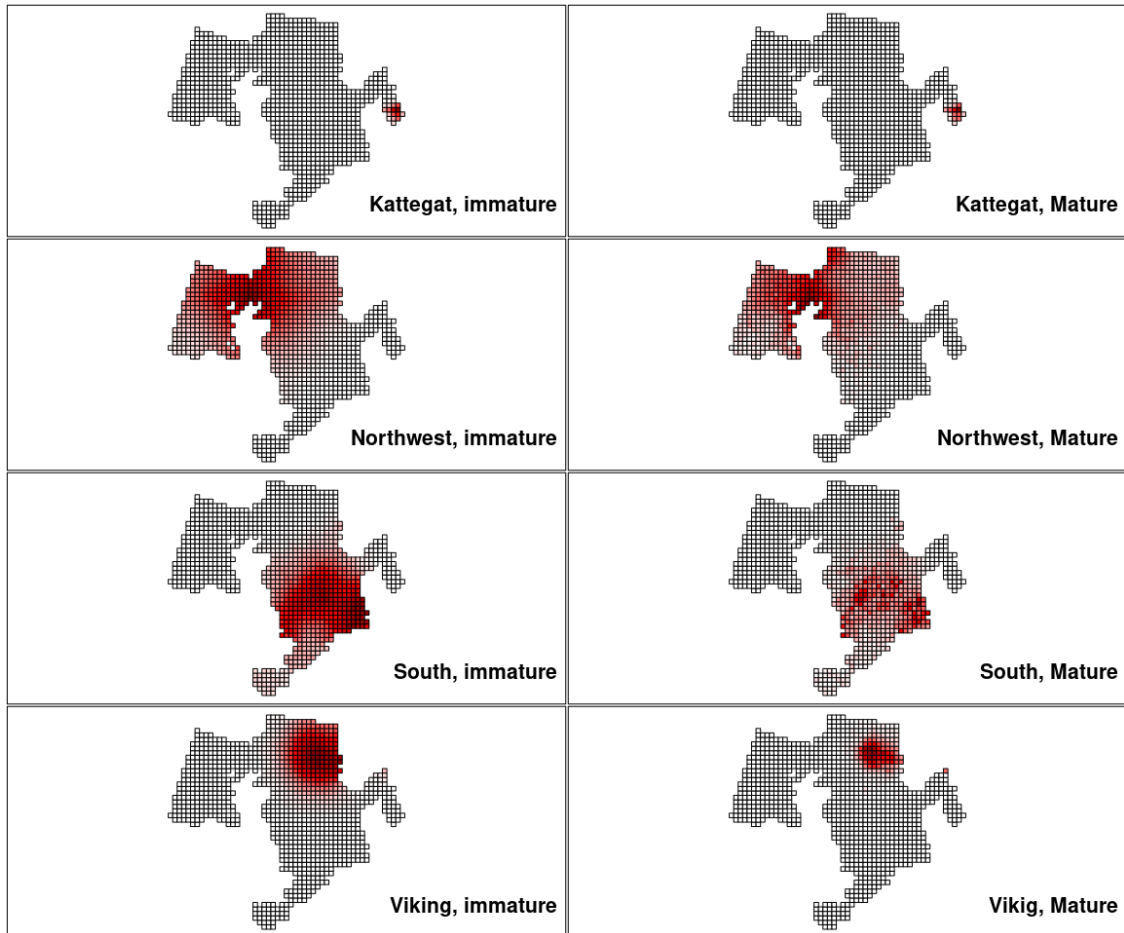
Fig. 23. Illustration of a simulated spatial distribution in February of the third simulated year. Note that intensity colors are not on the same scale, but intended for illustration only.

Effect of closing areas to fishing

To illustrate the utility of the simulation tool, it was used to evaluate the effect of closing parts of Kattegat to fishing. To this end, an additional scenario - closing fisheries in Kattegat - was simulated. While the Kattegat stock is heavily overexploited in the baseline scenario, the stock is steadily recovering in the scenario where most of the Kattegat area is closed (Fig. 24). Naturally, the closure is reflected in the catch of the two fleets in the area (Fig. 25). For both fleets, cod is a bycatch species. Since the target species were not included in the simulations, the economic effect of the closure cannot be evaluated with the simulations conducted here. However, it can be concluded that moving the effort of the two fleets further north, would drastically aid the recovery of the Kattegat stock.
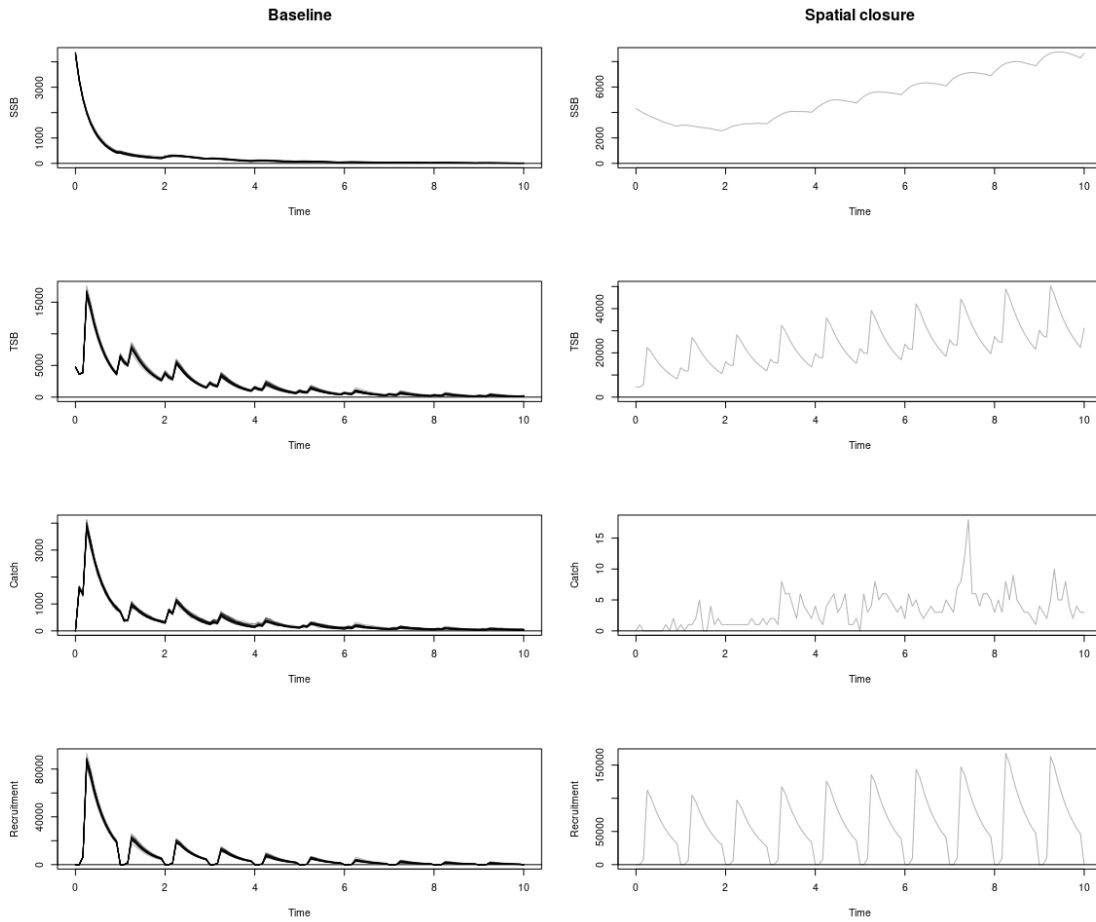
Fig. 24. Simulated trajectories of SSB, TSB, Catch numbers, and number of recruits for genetic Kattegat cod in the two scenarios as a function of year after simulation start (Time).
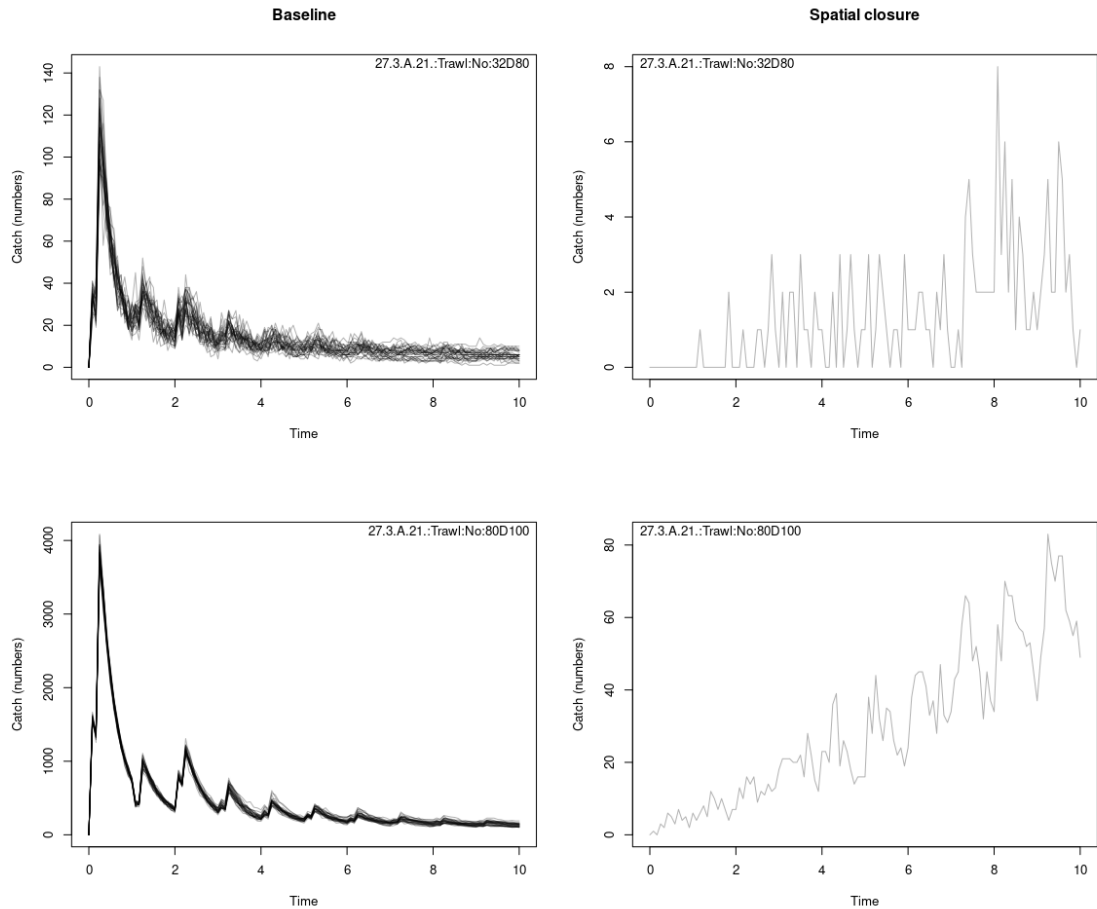
Fig. 25. Catch number trajectories for the fleets affected by the closure as a function of year after simulation start (Time).

5.3.3 Cost-benefit analysis

An important application of the simulation tool is the option to do cost-benefit analyses of sampling efforts in order to identify which sampling strategy and analytical methodology will provide the preferred precision in stock indices. In this project, a cost-benefit analysis of using genetic samples versus otolith shape for stock composition estimation for survey indices was conducted. The results are shown in Fig. 26. Genetic samples have a higher accuracy but are more costly than otolith shape analysis. On the other hand, subsequent analysis of otolith shape is more labor and computationally intensive. In the analysis, this was reflected by a higher initial cost.

We performed a cost-benefit analysis using the Kattegat cod as case study. The analysis is based on the above simulations to determine abundance, where survey index calculations were simulated. The simulations included both trawl hauls and stock composition samples. Based on 1000 replications, the standard deviation of the index was estimated for different sample sizes. Finally, the Fisher information and irreducible error (i.e., the standard deviation remaining with known stock composition) was estimated assuming independence and used to calculate the cost of obtaining a specific standard deviation. The study was replicated assuming 50%, 75%, 85%, and 95% Kattegat cod in the composition, respectively.

Due to the higher precision of stock identification and lower analysis cost, reasonable precision in estimated survey indices is best obtained with genetic samples. However, when aiming for very high precision in estimated survey indices, the marginal cost of adding genetic samples increases

rapidly. Therefore, otolith shape analysis was calculated to be cheaper for very high precision of estimated survey indices. The analysis was sensitive to the variability in the simulations. Lower variability in estimated cost functions could be obtained by increasing the number of replicated survey index calculations. However, this also comes at a high computational cost. Further, the cost-benefit analysis does not account for the cost of collecting samples or the cost of creating and maintaining baselines.
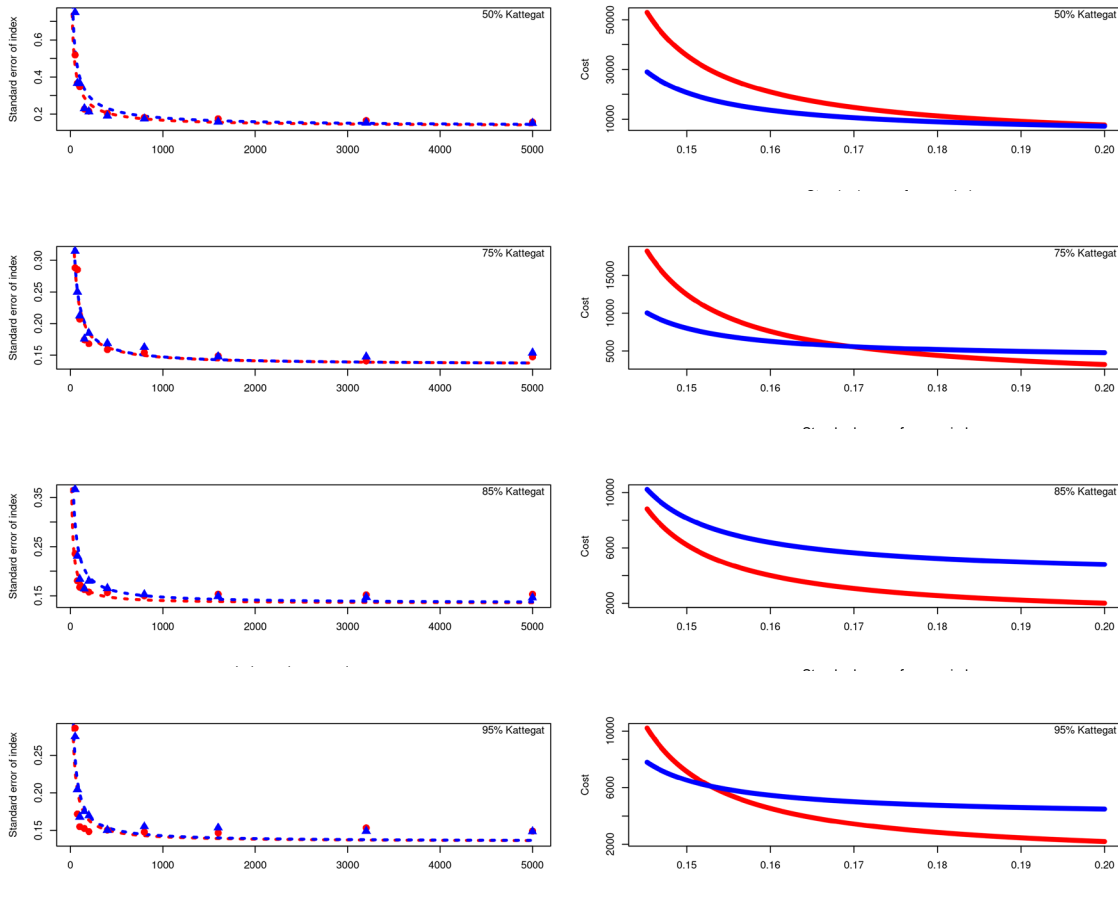

Fig. 26. Estimated standard deviation of survey indices with different stock composition sample sizes (left panels) and estimated cost to obtain a given standard deviation (right panels) for genetics (red) and otolith shape analysis (blue).

5.4 Conclusions

Simulation tool

- A fully functional prototype of a software package for the R statistical software for simulating the impact of stock mixing on stock assessment precision was implemented. The package is expected to be released as open source software when funding is obtained to improve the user interface and documentation.
- The results of this project can be directly used to improve the stock assessment and management of cod populations in Danish waters. Furthermore, the methods and framework developed and refined in this project are generic, and can therefore be used to address similar issues concerning stock mixing of other species, populations, and areas.
- Given the new biological knowledge on stock structure, movement patterns and stock mixing rates between the Kattegat and the western Baltic Sea and Sound, this will be the next focus area for application of this tool.

Evaluation of simulation scenarios

- *Application to Northern Shelf and Kattegat cod*: This study highlighted that specifying a complex simulation scenario including migrations and stock mixing is difficult. The simulations are highly dependent on a number of parameters, including stock-recruitment relationships, natural mortality, and fishing mortality assumptions. Data on these parameters are typically limited, particularly on spatial scales smaller than management units. Simulations therefore exclusively reflect the best achieved specification of model parameters.
- *Effect of migration on assessment data*: Using Kattegat cod stock as a case study, integration of migration patterns showed limited influence on the relative difference between total stock size and the size of the genetic Kattegat stock. However, the current model configuration is presumably underestimating actual numbers of immigrants from the North Sea, and the results should therefore be considered with that in mind.
- *Effect of closing areas to fishing*: Closure of most of the Kattegat, particularly a re-allocation of fishing effort to the northern Kattegat, would result in a steadily recovering cod population. However, the economic effect cannot be estimated as cod is a bycatch species in the fishery in Kattegat, and the target species (*Nephrops*) was not included in the simulation.

Cost-benefit analysis

- At low sample sizes, genetic stock identification provides a more precise and cost-efficient method for estimating stock mixing than otolith shape analysis.
- For high precision of estimated stock mixing proportions and large sample sizes, otolith shape-based stock identification is the cheapest method.
- However, the otolith shape-based approach requires calibration with genetics to assess mis-classifications and the additional cost of genotyping need to be factored into the otolith based approach. As such, the shape-based approach will still also require some volume of genetic analyses to secure high quality, and these additional cost of genotyping were not included in the modelling exercise. Thus, the above cost/benefit balance between the methods should be modified with these considerations.

Future considerations

- The simulation tool is expected to be released as open source software. To that end, there will be a need for technical improvements of the simulation tool itself, streamlining of the user interface to make it accessible to a broader user group, and development of suitable documentation. In the longer perspective, the tool may support management strategy evaluations (MSE), as demonstrated here with the closed areas in Kattegat, and an add-on to stock assessment models.

- The combined genetics/otolith shape-based stock identification approach between the western and eastern Baltic cod relies on established genotype-specific differences in otolith shape. Prior to application of this approach to stock mixing scenarios elsewhere, it needs to be established whether similar differences in otolith shape exist between other populations and/or stock components.

6. General Conclusions

Collectively, the compilation of knowledge gained from historical data and new samples, from genome sequencing and otolith chemistry, indicate that there is considerable genetic and ecological structuring of cod between the North Sea and the Baltic Sea, with three genetically distinct spawning populations: 1. North Sea, 2. eastern Baltic Sea and 3. transition zone (collected from the southern Kattegat, Belt Sea, Sound and spring spawners in Arkona Sea).
The geographic distribution of these populations overlap in the Kattegat and the Arkona Sea. The persevering mixing with eastern Baltic in the Arkona Sea highlights the need for continued monitoring and inclusion of this mixing in stock assessment routines. Accordingly, the spatially and temporally variable mixing dynamics of North Sea cod and local transition zone cod in the Kattegat needs to be considered for an accurate assessment of the stock, similarly to the mixing scenario with the eastern Baltic cod in the Arkona Sea.

While it was not possible to detect genetic differentiation in the transition zone, otolith chemistry revealed considerable ecological stock structuring. This scenario is consistent with considerable exchange of individuals between areas, presumably as a result of drift of early life stages. While cod in the transition zone may originate from different spawning areas, they largely remain within the geographical areas they settle into throughout the rest of their lives. This leads to stock structuring in the transition zone with an ecological separation largely into two distinct components: 1. the (eastern) Skagerrak and most of the Kattegat, 2. the Belt Sea and the Sound (no data from the Arkona Sea). For cod in Belt Sea, the Sound and the spring-spawners in the Arkona Sea, the combined genetic and otolith chemistry results thus indicate that the current management area for western Baltic is in line with the movement-based ecological structuring of the cod. However, there is evidence for ecological connectivity between the southern parts of the Kattegat and the rest of the transition zone, which is in line with genetic results but in conflict with the current management areas, which considers the Kattegat as a separate unit. Consequently, future stock assessment and management should accommodate these patterns.

The simulation studies on impact of stock mixing on stock indices with associated cost-benefit analysis showed that immigration of North Sea cod into the Kattegat has an influence on the relative difference between total stock size and the size of the genetic Kattegat stock. Furthermore, it showed that implementing closed areas in the southern Kattegat would help build up the stock again. With the current low sample sizes in all areas, genetic identification provides the most accurate approach to splitting populations.

Management of fish stocks is based on stock assessment-based advice, but also includes social and political considerations. In this project, we have exclusively dealt with biological knowledge gain and implications this may have on stock assessment, with exclusive focus on dynamics in the transition zone (Kattegat, Belt Sea, Sound and Arkona Sea). Recommendations as to what type of stock assessment and management approach is most suitable for this complex system of genetic and ecological stock structuring is not within the scope of this project. Future discussions on this topic need to include considerations of what type of assessment and management strategy should be adopted in the transition zone. Based on the combined biological knowledge we have obtained now, the following scenarios could be considered:

Area-based assessment and management – current scenario

This is a *status quo* scenario, where current practices are continued with two distinct stocks in the transition zone: Kattegat and the western Baltic Sea (Belt Sea, Sound and Arkona Sea), with separate stock assessments and TACs. As stock management is done by species within a specific area, TACs are allocated by area, irrespective of genetic population. Currently, stock mixing of eastern/wester Baltic cod in the Arkona Sea is monitored using genetic split of landings and survey data, and the resulting mixing proportion is implemented in the stock assessments of the two stocks. By not addressing stock mixing of North Sea/Kattegat populations, the severely declined Kattegat population is at risk of local depletion. This will be particularly pronounced following years of high inflow of North Sea individuals, that artificially boost stock size in the Kattegat. Consequently, future monitoring of stock mixing in the Kattegat, with particular reference to the estimation of proportions of North Sea cod in catches, should be considered to inform spatial management measures considered to protect local Kattegat cod.

Area-based assessment and management – updated scenario

This scenario implies continuing with current practices of separate stock assessments for the current management areas Kattegat and western Baltic Sea (Belt Sea, Sound and Arkona Sea)..
In addition to the stock mixing of eastern/wester Baltic cod in the Arkona Sea (scenario 1), the mixing of North Sea and Kattegat cod in the Kattegat should be addressed in a similar approach. This scenario disregards the fact that cod in the transition zone are genetically the same population, but would on the other hand reflect the ecological stock structuring, and thereby minimize the risk of local depletion of population components. This stock assessment scenario is thus, at least within the transition zone, similar to the approach used in the North Sea sandeel assessment, where limited genetic structuring is found throughout the North Sea, but the assessment is carried out by areas representing ecologically connected banks (ICES, 2022). In terms of management, the current management practices with area-specific TACs for the Kattegat and western Baltic Sea could continue.

Population-based stock assessment and management

A population-based approach to stock assessment would require cod from the Kattegat, Belt Sea, Sound and Arkona Sea to be combined into a single stock representing the genetic "transitions zone population". Given the documented mixing with North Sea cod in the Kattegat, and eastern Baltic cod in the Arkona Sea, stock mixing proportions in the transition zone population would need to be estimated for these areas, based on a genetic split of commercial and survey data similar to the updated Area-based stock assessment scenario. A population-based approach to stock assessment similar to the North Sea/transition zone/Baltic Sea complex has recently been adopted for cod in the Greenland/Iceland region, where cod have been divided into four genetically distinct populations. Survey and commercial data are genetically split, and both stock assessments and advice is given for each genetic population separately (ICES, 2023b). Subsequently, TACs can be allocated to exiting management areas, but should be informed by genetic estimates of mixing proportions in the different management areas to link estimated harvest rates in geographical areas to the stock assessments/advice for the underlying biological populations. In the North Sea-Baltic Sea transition zone, this would mean three separate stock assessments (North Sea, transition zone, eastern Baltic Sea) followed by area based TACs, which could follow current management areas, but which would need to be informed by estimates of mixing proportions estimated for the three biological populations. The main management challenges for this

scenario would therefore be: i) how to address the observed ecological structuring and the resulting area-specific biological parameters (growth, maturity, mortality, size distribution etc.) to avoid overexploitation of specific ecological population components not accounted for with genetic split data, ii) how to allocate area-specific TACs, and iii) how to deal with social and political considerations.

Consequently, the scenarios with updated area-based (2) and population-based (3) assessment and management require that stock mixing in the Kattegat would need to be estimated in addition to the current practice in the Arcona Sea, based on genetic split of commercial and survey data. This would require only a limited additional effort since this is already done in the Arcona Sea as part of regular data collection (ICES, 2019a; 2021) and all routines associated with this approach ae well established. For scenario 1 (status quo), estimates of proportions of North Sea/Kattegat cod in catches would be important for informing the implementation of spatial management measures to protect local Kattegat/transition zone cod.

Acknowledgements

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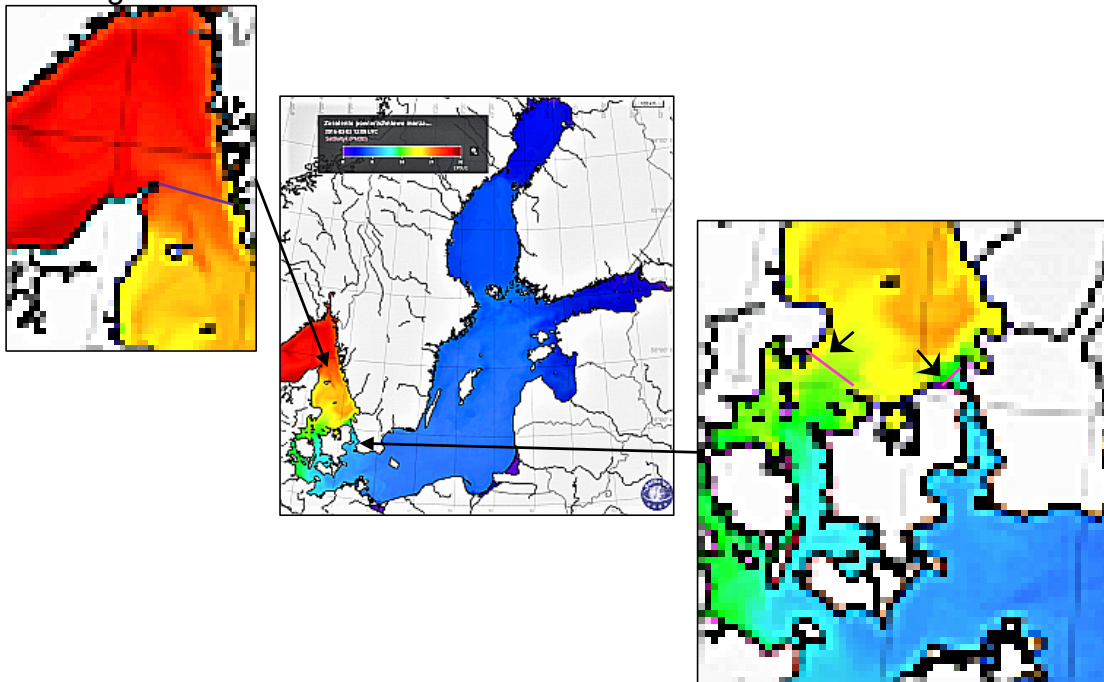# Appendix A. Supplementary Figures and Tables

A.1. Figure

Fig. A1 Map of average bottom salinity available from the MARE Foundation (https://fundacja-mare.pl/en/hydrology-of-the-baltic-sea/). Magenta lines indicate ICES SD borders. Magnified figures highlight: 1) Eastern Skagerrak/northern Kattegat: Salinity rather similar across the management border, and 2) Kattegat/Sound/Belt Sea: Strongest gradient in salinity change occurs north of the management border between the Kattegat and the two adjacent areas. The two black arrows indicate the catch position of individuals in the southern Baltic Sea with a Belt Sea/Sound signal in otolith chemistry.

A.2. Table

Table A1 Summary statistics of the Linear Mixed Effects model examining the variables influencing otolith element concentrations in cod. Marginal r^2 = variance explained only by fixed effects, conditional r^2 = variance explained by the entire model, p = pseudo p-values.

	Fixed effect Conditional r^2	Direction of effect	*F-value*	*p*	Marginal	r^2
Environmental regulation						
Ba	ICES SD 0.312	20 < 21 < 22 = 23	24.2	<0.001	0.214	
	AgeLife	- ve	1812.3	<0.001		
	Season	2 > 1 = 3 > 4	160.2	<0.001		
Fe [1]	ICES SD 0.833	21 < 22 = 23	7.6	<0.001	0.041	
	AgeLife	- ve	994.5	<0.001		
	Season	1 > 2 = 3 = 4	24.9	<0.001		
Li [1]	ICES SD 0.612	21 > 22 = 23	109.2	<0.001	0.158	
	AgeLife	- ve	310.2	<0.001		
	Season	2 > 1 > 3 = 4	26.6	<0.001		
Mn	ICES SD 0.509	20 < 21 < 22 > 23	51.9	<0.001	0.346	
	AgeLife	+ ve	4720.1	<0.001		
	Season	1 = 3 > 2 = 4	25.5	<0.001		
Pb [1]	ICES SD 0.769	21 < 22 = 23	49.4	<0.001	0.121	
	AgeLife	- ve	601.7	<0.001		
	Season	1 < 2 > 3 > 4	144.8	<0.001		
Sr	ICES SD 0.412	20 = 21 = 23 > 22	28.6	<0.001	0.200	
	AgeLife	+ ve	2099.7	<0.001		
	Season	1 > 2 < 3 < 4	61.1	<0.001		
Physiological regulation						
Cu	ICES SD 0.534	20 = 21 < 22 = 23	0.4	<0.001	0.097	
	AgeLife	- ve	713.5	<0.001		
	Season	2 > 1 = 3 > 4	296.1	<0.001		
K [1]	ICES SD 0.631	21>23>22	113.8	<0.001	0.153	
	AgeLife		0.4	ns		
	Season	1 < 2 = 3 > 4	22.7	<0.001		
Mg	ICES SD 0.461	20 = 21 > 22 = 23	43.8	<0. 001	0.123	
	AgeLife	- ve	962.9	<0. 001		
	Season	1 < 2 > 3 > 4	34.2	<0.001		
P	ICES SD 0.563	20 < 21=22 < 23	39.6	<0.001	0.400	
	AgeLife	+ ve	4209.0	<0.001		
	Season	4 > 1 > 3 > 2	828.9	<0.001		
Zn	ICES SD 0.572	20 = 21 = 22 < 23	53.7	<0.001	0.111	
	AgeLife	+ ve	216.4	<0.001		
	Season	1 < 2 > 3 = 4	54.2	<0.001		

[1] Elements which were measured in the FABBIO samples, but not in the Sustainable cod management project.
[2] Season: 1 = Spring, 2 = Summer, 3 = Fall, and 4 = Winter

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