DTU Aqua
National Institute of Aquatic Resources

Improved knowledge for management of flatfish stocks in Danish waters (FORFLAD)

Jakob Hemmer-Hansen, Karin Hüssy, Belén Jiménez Mena, Jon C Svendsen, Maria Krüger-Johnsen, Josianne G. Støttrup, Alexandros Kokkalis, Elliot John Brown, Henrik Mosegaard, Anders Nielsen, Casper W. Berg and Jesper Boje

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Colophon

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DTU Aqua Report no.:	473-2024
Year:	Scientific work finalized March 2023. Report published December 2024
Reference:	Hemmer-Hansen, J., Hüssy, K., Jiménez-Mena, B., Svendsen, J.C., Krüger-Johnsen, M., Støttrup, J.G., Kokkalis, A., Brown, E.J., Mosegaard, H., Nielsen, A., Berg, C.W., Boje, J. (2024). Improved knowledge for management of flatfish stocks in Danish waters (FORFLAD). DTU Aqua. DTU Aqua Rapport no. 473-2024.https://doi.org/10.11581/60e738ef-cc68-424f-9c49-14411651757e
Cover photo:	Flatfish. Photo: Mikael van Deurs
Published by:	National Institute of Aquatic Resources, Technical University of Denmark, Henrik Dams Allé, 2800 Kgs. Lyngby, Denmark
Download:	www.aqua.dtu.dk/publikationer
ISSN:	1395-8216
ISBN:	978-87-7481-416-0

Preface

This report is based on the project "Improved basis for the management of flatfish stocks in Danish waters (FORFLAD). The project is financed by the European Maritime and Fisheries Fund and the Danish Fisheries Agency with grant no 33113-B-19-149. The project period was from June 2019 to March 2023. The project leader was Jakob Hemmer-Hansen.

In Danish the project title is "Forbedret grundlag for forvaltning af fladfiskebestande i danske farvande (FORFLAD)".

Silkeborg, December 2024

Jakob Hemmer-Hansen

Senior Researcher

European Union
European Maritime and Fisheries Fund

HAV & FISK

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Project summary

This report summarizes the outcome of the project “Improved knowledge for management of flatfish stocks in Danish waters”, funded by the European Maritime and Fisheries Fund and the Danish Fisheries Agency (“Fiskeri, natur og miljø - Marin biodiversitet”). The project focused on improving the biological data to support advice and sustainable management of three flatfish species in Denmark: turbot (*Scophthalmus maximus*), common sole (*Solea solea*) and European plaice (*Pleuronectes platessa*). These species are important to Danish fisheries, and they currently suffer from a lack of information on a number of questions of importance for proper stock assessment and management. While there were differences between knowledge gaps of species, there were also common themes, such as the identification of biological populations, their geographical distributions and migration between areas which are currently challenging a sustainable management, in particular in the Skagerrak/Kattegat, which was a focus area in all species in the current study.

In turbot, we reviewed how available evidence for population structure matches current stock assessment and management units for the species. Importantly, Kattegat and Skagerrak are currently merged into one management unit. However, merging Kattegat and Skagerrak did not seem to match available biological evidence, suggesting the presence of two different biological populations within the management unit, with interaction between North Sea/Atlantic and Baltic Sea populations mainly occurring in the Kattegat. This is based on substantial data, including genetic evidence and long-term data series. However, more information on the distribution of these populations in and outside spawning periods will be needed to refine current stock assessment and management practices to better match underlying biological variation (e.g. geographical distribution of populations across life stages). In addition, we addressed issues related to data quality for the stock assessment and confirmed the validity of catch data series and survey design in relation to the distribution of fishing efforts.

In sole, we used genetic data in combination with otolith microchemistry to identify populations and their migrations in the North Sea-Baltic Sea transition zone, with a particular focus on Kattegat/Skagerrak. In this area, previous work had indicated evidence for population mixing not well aligned with current assessment units, where Skagerrak and Kattegat are merged with the rest of the Baltic Sea transition zone. We found evidence for the presence of two biological populations in our data, with a strong degree of mixing in particular in the Skagerrak, with indications of the presence of spawning individuals from both populations. In addition, we found stronger mixing outside spawning season and also found mixing in other areas (e.g. northern part of the North Sea). Migration data and larval origin supported a hypothesis of extended distribution into the Skagerrak of a Baltic Sea population, and hence recruits to the western Baltic Sea may have been spawned as far north as the Skagerrak, where our current study indicates that both Baltic Sea and North Sea/Atlantic populations may reproduce. However, data also indicated that common sole, once recruited to a specific geographical area within the distribution of the two populations, are fairly stationary and do not migrate to a notable extent. Collectively, the results have provided an improved understanding of biological variability and population dynamics in the management areas, supporting a change in management procedures towards a population based assessment rather than the present area based assessment. However, while such population based stock assessment and management have been implemented in other species, further work, in particular related to more fine scale and quantitative estimation of population presence and migration will be needed to implement this change for sole.

In plaice, we focused on issues related to an apparent mis-match between stock assessment and quotas and the perception of the stock status by fishermen. In particular, in the Skagerrak where fishermen since 2017 are far from utilizing the quota that is based on the advice. In the project, we found distribution shifts possibly resulting in the availability of fewer plaice in the Skagerrak, matching the perception from the fishery. While the underlying reasons for these changes are unknown at present, we discuss potential sources of this variability, in particular in relation to potential local depletion and environmental changes that may have driven distribution changes in the Skagerrak.

Collectively, the project has improved our knowledge on stock dynamics and biological parameters to support the quality of stock assessment and management in all three species significantly. While there are still open questions, the results have also shown interesting common patterns across species, such as the identification of specific geographical areas with high level of interaction between biological populations in several species. As such, and in addition to improving single species biological advice, our project could also have broader impacts, for example in relation to future ecosystem based approaches to management, where an integrated understanding across species will be needed to secure an implementation on a sustainable basis. In addition, climate change will also challenge current management procedures and the balance of quota allocation between countries through changes in distributions and migrations of exploited populations. As such, an improved understanding and quantification of these changes will provide crucial decision support for future management.

Project background

Sustainable management of aquatic resources requires a thorough understanding of the underlying biology in the managed species. In this project, we focused on filling existing knowledge gaps in three flatfish species of relevance to the Danish commercial and recreational fishery: turbot (*Scophthalmus maximus*), common sole (*Solea solea*) and European plaice (*Pleuronectes platessa*). For these species, a range of questions regarding for example the identification of populations and their mixing and migration, and the quality assurance of biological data supporting stock assessments were in common across species. Furthermore, all species are exploited by Danish fishermen in Skagerrak/Kattegat, where previous work had indicated potential challenges for stock assessment related to either data quality or biological effects from e.g. mixing of populations. Thus, both the overall questions and the geographical focus areas were in common for the three species, and the project hence also aimed to collate information across the species in order to make more general inferences. There were also difference between the three species, for example related to how the project could support advice and management. For turbot, the project results were directly integrated into an ICES benchmark process, while sole and plaice insights potentially will improve the advisory process through improved biological understanding and hence improving the quality of advice. Consequently, results from all species will directly impact the advisory processes and thus improve sustainability of resource exploitation in the marine environment.

The current report is structured in three main sections covering the three species individually, each providing more specific background to the topics and questions addressed for the species. These species-specific sections are then followed by two common sections where we discuss management implications and draw general conclusions based on the collated information across the project.

1. Support for turbot benchmark

1.1 Background

Turbot (*Scophthalmus maximus*) is a species of flatfish belonging to the Scophthalmidae family. Turbot is a relatively large, demersal flatfish species distributed across marine and brackish environments in the Mediterranean See, Baltic Sea and other parts of the Northeast Atlantic Ocean. Turbot is a valuable fish species, targeted by commercial fishing fleets and recreational fishing.

This work package covers turbot management in the marine areas Kattegat and Skagerrak, known as ICES Division 27.3.a (Figure 1.1).

Figure 1.1. Boundaries of ICES Division 27.3.a. ICES subdivisions and codes: Skagerrak (Subdivision 27.3.a.20), Kattegat (Subdivision 27.3.a.21), Belt Sea (Division 27.3.c), Sound (Division 27.3.b), Baltic Sea (Division 27.3.d), Northern North Sea (Division 27.4.a), Central North Sea (Division 27.4.b), Southern North Sea (Division 27.4.c).

In ICES Division 27.3.a., Danish turbot landings represent the largest share compared to other countries (Table 1.1). Specifically, Danish turbot landings exceed 75% of the total landings followed by the Netherlands (14%) and Sweden (6%).

Table 1.1. Turbot landings in ICES Area 3.a. (tur.27.3a). Total landings (tonnes) and average percent of landings per country in the years 2015 - 2018. From ICES (2020).

Country	Total (2015-2018)	%
DK	543	75.87
NL	98.89	13.82
SE	43.72	6.11
NO	28.79	4.02
DE	0.893	0.12
GB	0.372	0.05

The life cycle of turbot includes both pelagic and demersal phases, often covering coastal and more offshore areas. Turbot eggs and larvae disperse pelagically, prior to the juveniles settling on seabed for a demersal lifestyle (Figure 1.2).

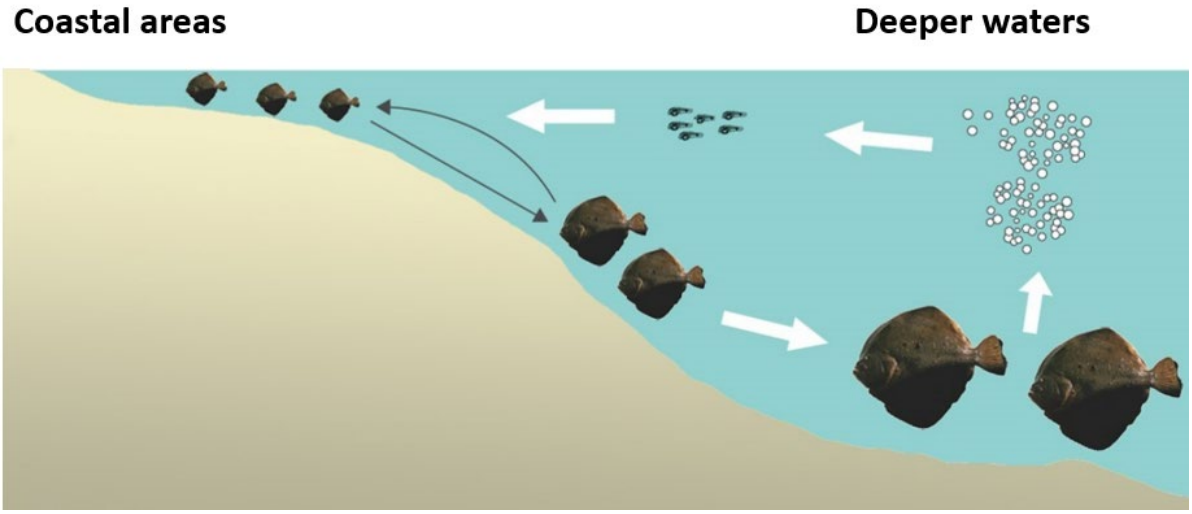


Figure 1.2. Schematic illustration of turbot life cycle. Details of the life cycle likely vary between locations. Figure produced by Mette K. Schiønning and Jon C. Svendsen.

Turbot in the North Sea and the Baltic Sea have been subjected to scrutiny, whereas limited information is available about turbot in ICES Division 27.3.a. Turbot male and female growth curves diverge markedly from about age three and onwards, females growing larger than males in both the North Sea and the Baltic Sea (Jones 1974; Stankus 2003). Females may approach 100 cm in body length, but fish larger than 75 cm (> 12 kg) are very rare in ICES Division 27.3.a. The maximum length of male turbot approaches 50 cm. In the North Sea, evidence suggests that 50% of turbot females have reached maturity when they are about 46 cm in body length, and they are all sexually mature at a body length of approximately 55 cm (Jones, 1974). In contrast, Stankus (2003) found that all turbot females are sexually mature when they are about 28 cm in body length in the Baltic Sea. Here, females often carry about two million eggs kg^{-1} (Stankus 2003), whereas females in the North Sea carry about one million eggs kg^{-1} (Jones 1974). In terms of the von Bertalanffy growth equation, turbot parameters differ between the North Sea and the Baltic Sea, including female L_∞, which approaches 64.8 cm and 53.5 cm in the North Sea and Baltic Sea, respectively. Corresponding parameters for ICES Division 27.3.a have not been determined, but the parameters for the area could vary between the parameters representing the North Sea and Baltic Sea. During the first years of life, females grow up to 8-10 cm per year. Turbot females continue to grow about 1–2 cm per year at ages exceeding ten years.

In ICES Division 27.3.a, turbot is mainly caught as bycatch in trawl, trammel net and gillnet fisheries, although due to its high economic value, targeted fisheries may occur in specific areas and seasons.

Turbot stock definition

Turbot occurs widely in the eastern North Atlantic, ranging from the Mediterranean Sea to Iceland and Lofoten in Norway. Specifically, turbot lives in the North Sea, Skagerrak, Kattegat and large parts of the Baltic Sea, including ICES Division 27.3.a. Several studies have revealed genetic structures and migration patterns of turbot. Genetic studies on turbot by Vandamme *et al.* (2014) identified an Atlantic group, a Baltic Sea group, a group on the Irish Shelf, and an additional genetic break in the North Sea, subdividing turbot in southern and northern Atlantic areas. In the Baltic Sea and Kattegat, Florin and Höglund (2007) reported low genetic differentiation and no evidence of isolation by distance. Analyzing adult turbot, Baden *et al.* (2022) documented limited spawning migration, suggesting a relatively sedentary lifestyle. Nielsen *et al.* (2004) reported genetic differentiation going from the low salinity Baltic Sea to the high salinity North Sea. According to the authors, the data were explained best by two divergent populations, connected by a hybrid zone. Le Moan (2019) identified clear genetic differences between individual turbot from the Baltic Sea and the North Sea. Sampling included the Western Baltic Sea and Kattegat, but not Skagerrak. The individual fish sampled in Kattegat were typically genetically intermediate compared to turbot sampled in the Baltic Sea and the North Sea. However, several individual turbot from the Kattegat matched genetically turbot sampled in the Baltic Sea or the North Sea. Thus, individual turbot, with genetic compositions reflecting Baltic Sea, North Sea or their hybrids, occur in Kattegat (Figure 1.3), suggesting that the Kattegat is an area of interaction between North Sea/Atlantic and Baltic Sea turbot populations.

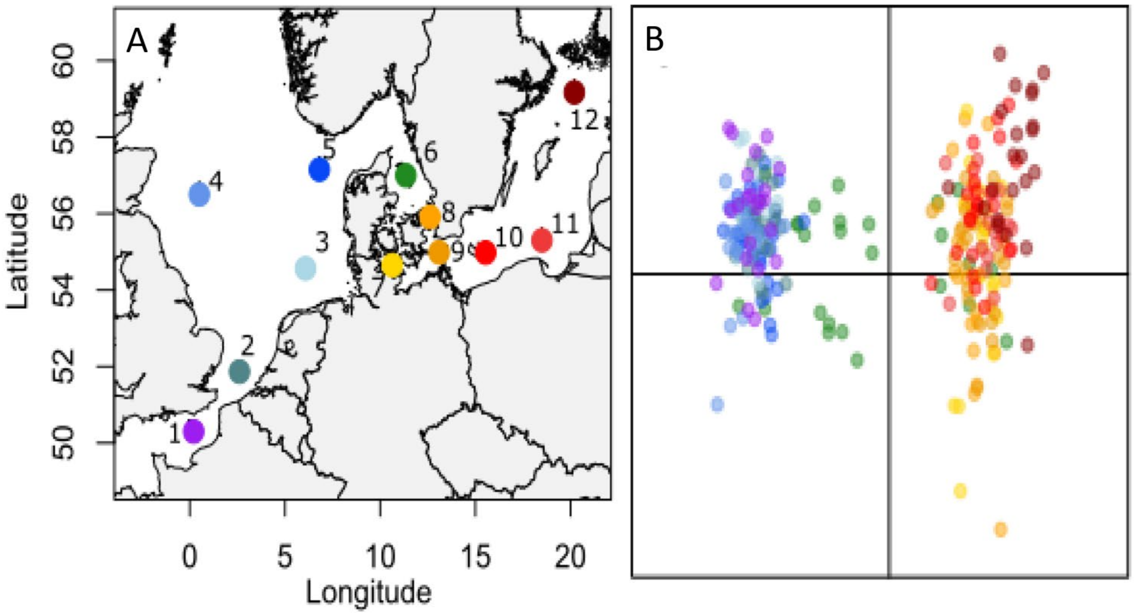


Figure 1.3. Genetic sampling ranging from the North Sea (1-5) to the Baltic Sea areas (6-12) in (A). Genetic profiles of individual turbot (B), with individual color codes corresponding to the sampling locations (A). Turbot sampled in the Kattegat (A; green; point 6) are usually intermediate between the North Sea and the Baltic Sea, however, individual turbot with a genetic profile reflecting either North Sea or Baltic Sea turbot are also occurring in the Kattegat. Figure modified from Le Moan (2019).

While genetic differences between turbot from the North Sea, Kattegat and the Baltic Sea have been subjected to detailed studies, there is less knowledge about genetic differentiation related to the Skagerrak. However, Nielsen *et al.* (2004) reported genetic differentiation between the Skagerrak and the North Sea.

The main purpose of this work package was to contribute to ICES benchmark processes for turbot in the Skagerrak and Kattegat (ICES Division 27.3.a) covering issues related to the identification of populations and their mixing. In addition, three issues related to landings and the correspondence between landing and survey data were explored during the process. Specifically, the three issues were:

a) Provide a closer description of the spatial distribution of landings (ICES rectangle) in relation to the coverage of the survey data

b) The spiked Dutch landings from 1976–1980 are questionable. Clarify if they appear to be missing from North Sea landings, or if the Dutch landings remain questionable

c) Clarify if sampled water depth in the IBTS surveys and the BITS surveys align with the water depths at which turbot are landed.

1.2 Materials and methods

Stock ID and mixing

In total, five different survey series were included in the present analysis to investigate the spatial distribution of turbot. The results were subsequently compared to available genetic data. Three of these surveys are available via the DATRAS database, specifically the beam trawl survey (BTS), the North Sea International Bottom Trawl Survey (NS-IBTS), and the Baltic International Trawl Survey (BITS). The remaining two surveys (TN and TOR) are Danish national surveys covering ICES Division 27.3.a.

Issue a)

The analyses related to issue a) targeted the Danish fisheries, because these fisheries provide the majority of the landings in ICES Division 27.3.a. compared to other countries involved in the turbot fishery. The purpose was to examine if there is a spatial overlap between commercial turbot landings and survey data (IBTS and BITS). Landings data included VMS data from the Danish commercial turbot fishery for vessels above 15 meters for the years 2005-2011 and vessels above 12 meters from 2012 and onwards. For 2005-2011, VMS coverage was about 55%, whereas it increased to approximately 80% after 2011. For each year, landings were summed for Skagerrak and Kattegat, i.e., ICES Division 27.3.a. Locations of the two international bottom trawl surveys IBTS and BITS in Skagerrak and Kattegat were aggregated and superimposed in a common plot.

Issue b)

During the years 1976–1979, the Netherlands reported landings between 87–389 tonnes in ICES Division 27.3.a., considered a dramatic increase compared to reported landings in other years (Figure 1.4).

The unusual turbot landings could potentially originate from the North Sea, and the landings might be missing from the landings reported from the North Sea over the same years (1976–1979). To examine the issue, landings from the North Sea (Area 27.4) were plotted and compared across years.

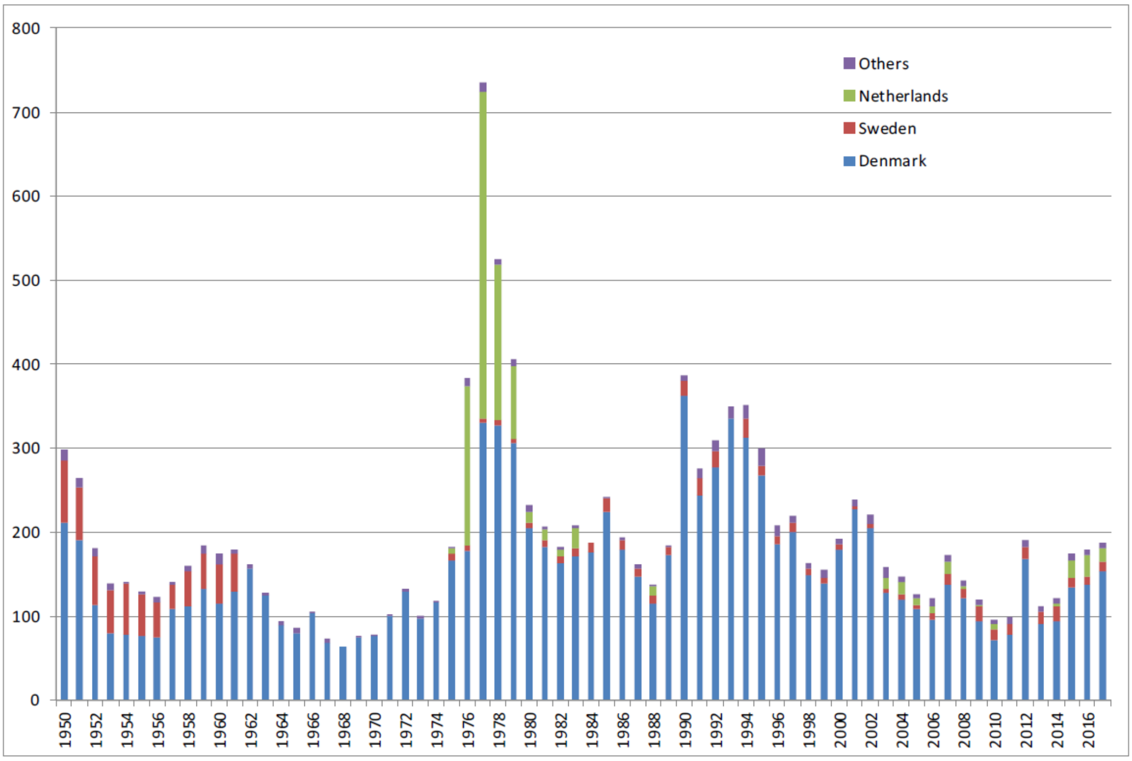


Figure 1.4. Country specific landings from Skagerrak and Kattegat (ICES Division 27.3.a.) between 1950 and 2017. From ICES (2020).

Issue c)

It was unknown if the sampled water depths in the IBTS survey and the BITS survey aligned with the water depths from which turbot is landed. To address the issue, a comparison was made involving the water depths at which IBTS and BITS are conducted in Skagerrak and Kattegat and the water depths of the Danish commercial fishery in the same waters. The comparison targeted the Danish turbot fisheries, because Danish landings constitute the majority of the turbot landings in ICES Division 27.3.a. The analysis covered the years 2005-2016. Water depths reflecting the IBTS surveys and the BITS surveys were narrowed down to coordinates in Skagerrak and Kattegat. Using VMS data, turbot landings of the Danish commercial fishery and water depths of ICES c-squares (0.05 degree) were derived. The data included turbot landings from vessels >15 meters until year 2011 and > 12 meters after year 2011. Data were plotted with water depths on the x-axis and surveys and landings on the y-axis. This was conducted to reveal if survey water depths and landing water depths overlap.

1.3 Results and Discussion

Stock ID and mixing

Broadly speaking, the genetic evidence is consistent with turbot distributions reported by bottom trawl surveys in the North Sea, Skagerrak, Kattegat, and the Baltic Sea (Figures 1.5-1.6) and with recorded landings (Figure 1.7).

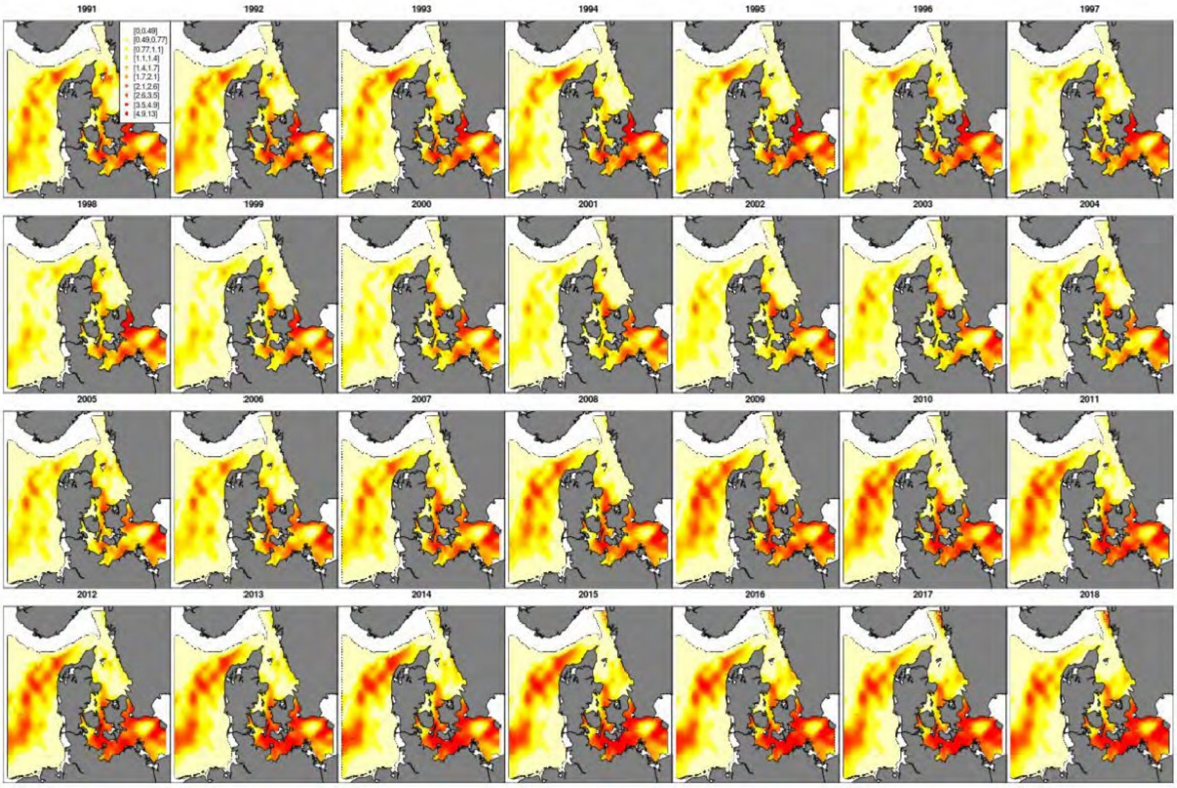


Figure 1.5. Combined survey data for turbot in quarter four covering the North Sea, Skagerrak, Kattegat and the Baltic Sea. The survey data included the beam trawl survey (BTS), the North Sea International Bottom Trawl Survey (NS-IBTS), the Baltic International Trawl Survey (BITS) and two additional surveys (TN and TOR). From ICES (2020).

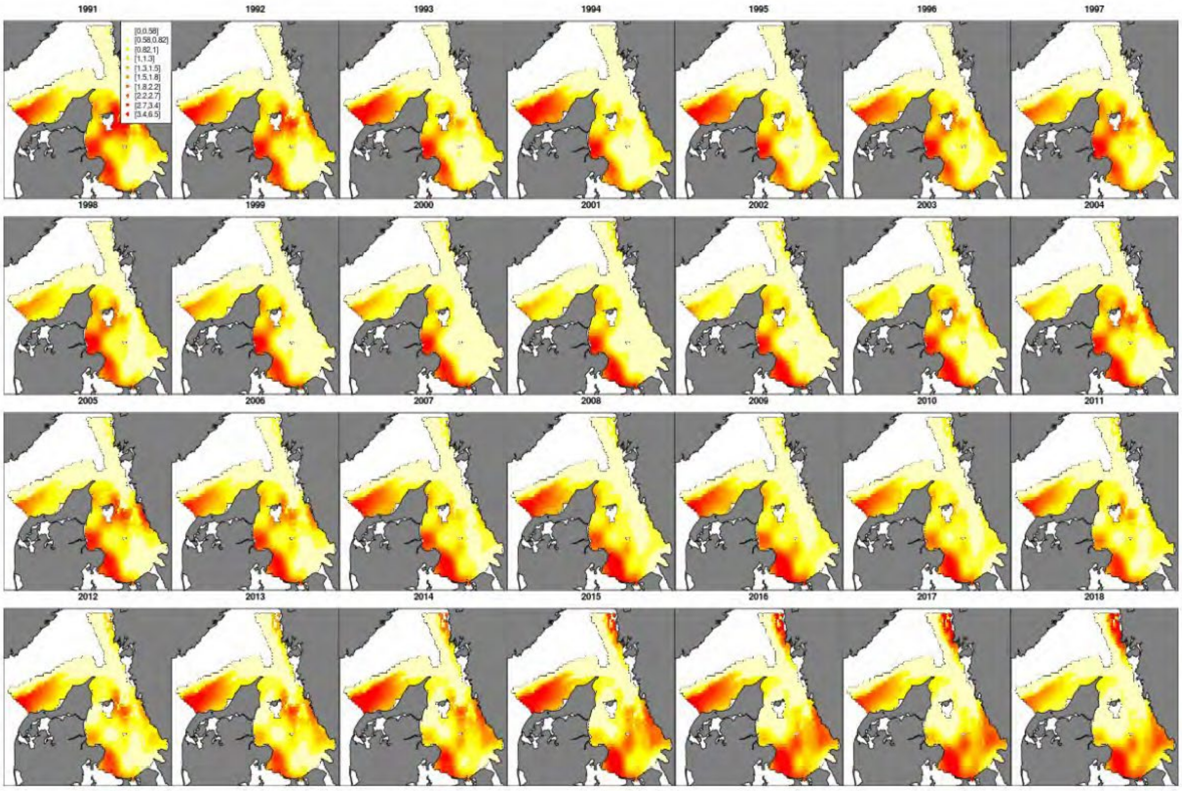


Figure 1.6. Combined survey data for turbot in quarter four covering ICES Dicision 27.3.a. Highest turbot abundances occur near the boundaries to the neighbouring areas, specifically in southwestern Skagerrak and southern Kattegat. From ICES (2020).

Summarising the genetic evidence, and combining with survey data and landings data for ICES Division 27.3.a., this study indicated that turbot in Skagerrak are connected to North Sea turbot, while turbot in Kattegat are more, although not exclusively, connected to Baltic Sea turbot.

Issue a)

The analyses covered 2005-2018 with results presented as kg year^{-1}. Landings included the beam trawl, demersal trawl, gillnet, and Danish Seine/anchor dragging fishery. Recreational landings remain unknown. Landings predominantly originated from the southwestern part of Skagerrak. The landings are adjacent to the border of the Central North Sea, including division 27.4.a and b, and are relatively consistent between years. In Kattegat, landings are less aggregated with relatively high landings in the southern parts of Kattegat, southeast of Anholt and east of Ebeltoft in Denmark (Figure 1.7). Data on the landings are largely overlapping survey data. Specifically, the spatial distribution of IBTS and BITS in Skagerrak and Kattegat during 2005-2018 includes areas with both high and low levels of turbot landings (Figures 1.7 and 1.8). A comparison between the landings and survey data shows significant geographical overlap between the landings and surveys.

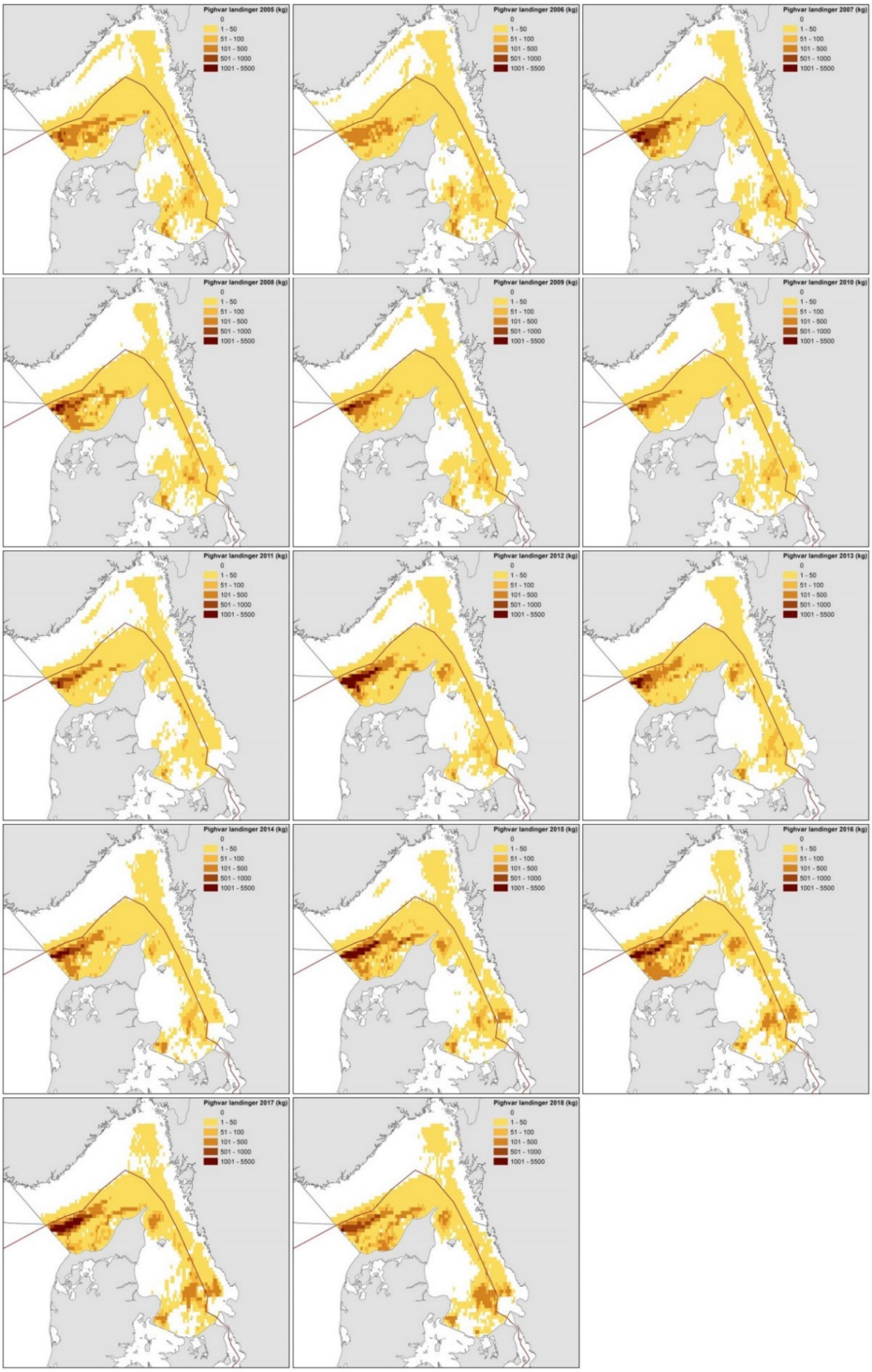


Figure 1.7. Locations of Danish commercial turbot landings in Denmark from 2005-2018. The Danish landings constitute the majority of the turbot landings in ICES Division 27.3.a. and are predominantly aggregated in southwestern Skagerrak and southern Kattegat. From ICES (2020).

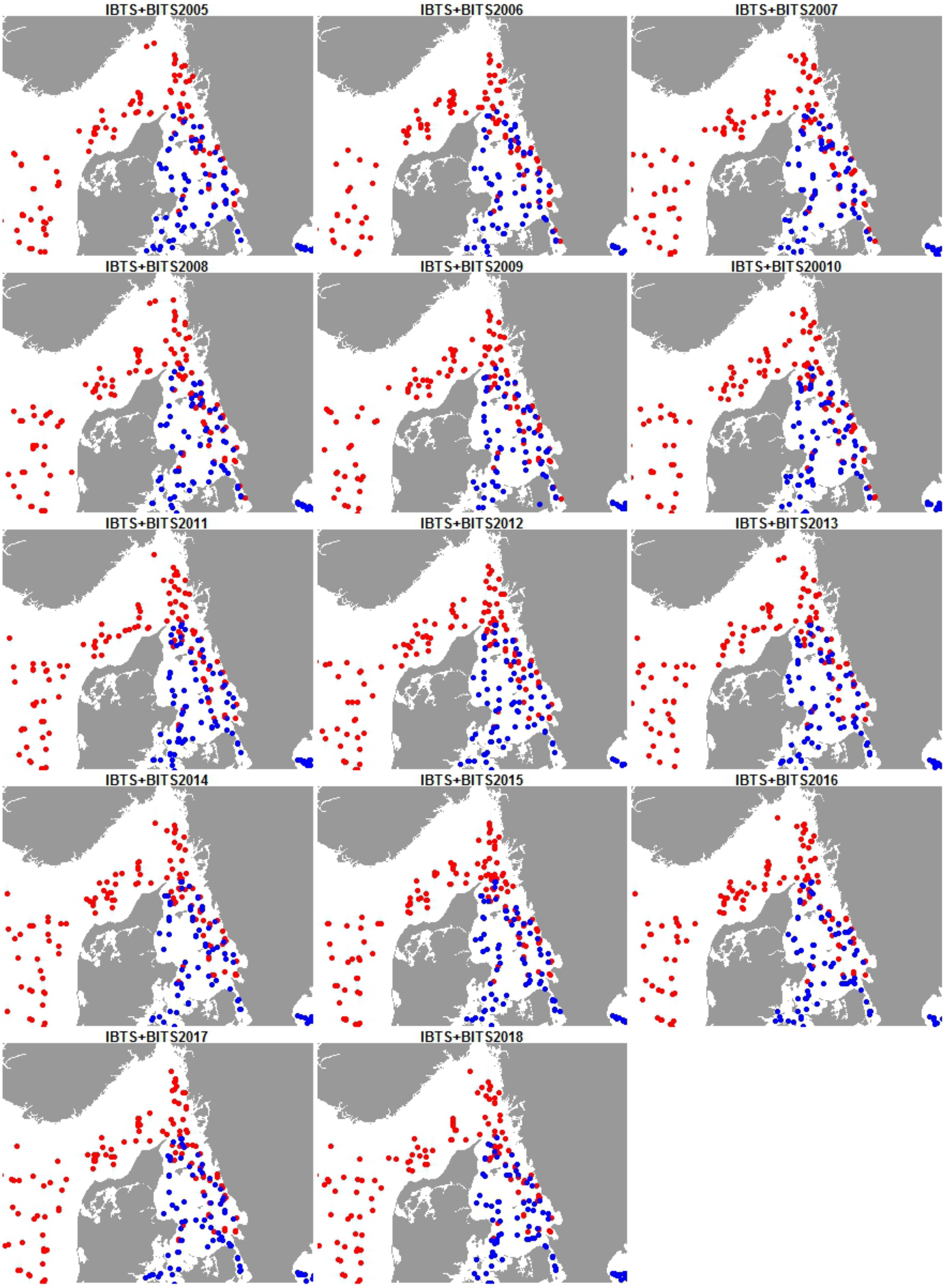


Figure 1.8. Locations of sampling stations of the two international bottom trawl surveys IBTS (red circles) and BITS (blue circles) in Skagerrak and Kattegat (i.e., ICES Division 27.3.a.) between 2005—2018. The IBTS and BITS survey data overlap the areas of turbot landings. From ICES (2020).

Issue b)

Although the Dutch landings approached 389 tonnes in ICES Division 27.3.a. (in 1976-1979), the Dutch landings in the North Sea in the same period were about 10-fold larger (3000-4000 tons; Figure 1.9). This means that it is not possible to detect if the large Dutch landings observed in ICES Division 27.3.a. across the years 1976-1979 were missing from the North Sea landings over the same years.

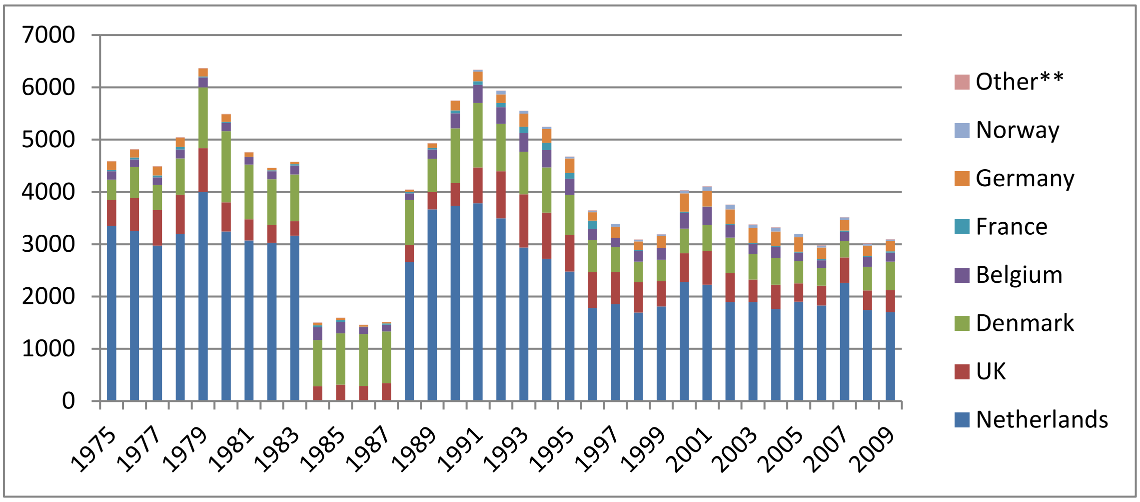


Figure 1.9. Country specific landings from the North Sea (Division 27.4) between 1975 and 2009.

During the benchmark meetings, relevant stakeholders reported that the period (1976-1979) corresponded with the introduction of ITQs in the North Sea, and consequently a reduction in the fishing opportunities for Dutch vessels in the North Sea. As a result, some of these vessels temporarily moved their efforts into ICES Division 27.3.a. This information suggests that the elevated Dutch landings during 1976-1979 represent valid data. From ICES (2020).

Issue c)

The analysis revealed overlap between the water depths covered by the two surveys (IBTS and BITS) and the water depths from which turbot are landed in the Danish commercial fishery (Figure 1.10). This suggests that the surveys provide information relevant for the fisheries management.

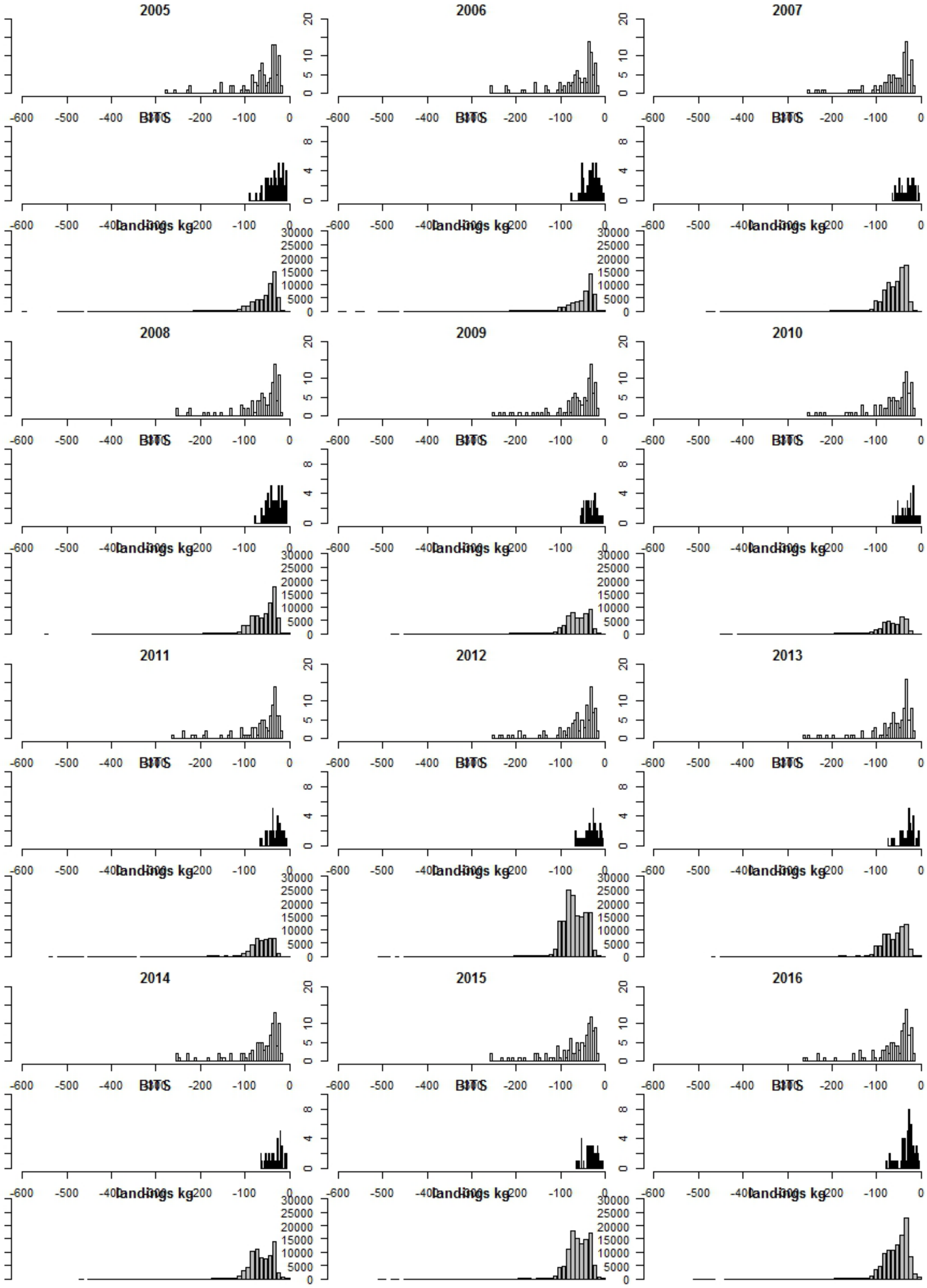


Figure 1.10. A comparison of water depths associated with surveys and landings of turbot. The graph shows the water depth distribution in IBTS surveys (1st, 4th, 7th, 10th row), BITS surveys (2nd, 5th, 8th, 11th row) and turbot landings in the Danish fishery. For the landings data, the label for the x-axis has been omitted, but it goes from -600 – 0 meters (3rd, 6th, 9th, 12th row). The graphs reveal overlapping water depths associated with surveys and turbot landings. From ICES (2020).

1.4 Conclusions

Stock ID and mixing

Collectively, the available data indicate a separation of turbot populations in the North Sea and Baltic Sea through ICES Division 27.3.a., especially through Kattegat. In this area, data suggest a partial hybrid zone and relatively low presence of turbot. This turbot distribution seems to deviate from the current stock separation (i.e., ICES Division 27.3.a). On this basis, it was recommended to consider stock identity in ICES Division 27.3.a. and possibly merge the Skagerrak part of the stock with the North Sea stock and the Kattegat part of the stock with the Baltic Sea stock.

Issue a)

Analyses of turbot landings and survey data from the Kattegat and Skagerrak areas revealed overlapping data. Generally speaking, survey efforts cover areas with significant turbot landings as well as areas with fewer landings of turbot.

Issue b)

It was not possible to detect if the large Dutch landings observed in ICES Division 27.3.a. across the years 1976-1979 were missing from the North Sea landings over the same years. Stakeholders reported that during the period (1976-1979) some vessels temporarily moved their efforts into ICES Division 27.3.a. This information suggests that the elevated Dutch landings during 1976-1979 represent valid data.

Issue c)

The analysis revealed overlap between the water depths covered by the surveys and the water depths from which turbot are landed in the Danish commercial fishery, suggesting that the surveys provide information relevant for the turbot fisheries management.

In conclusion, the present study provided information to support the ICES benchmark covering turbot fisheries in ICES Division 27.3.a. The benchmark was completed, and details are available in the resulting report (ICES 2020).

2. Stock identification and migration in common sole

2.1 Background

Common sole (*Solea solea*) in Subdivisions 20-24 represents the Skagerrak (ICES Subdivision 20), Kattegat (ICES Subdivision 21), the Belts (ICES Subdivisions 22 and 23) and the western Baltic (ICES Subdivision 24) and is therefore part of the transition area between the saline North Sea and the less saline Baltic Sea. Sole seems to be more abundant in the Kattegat and Belts than in Skagerrak. Distribution of sole beyond the Belts into the Baltic Sea is limited by salinity which decreases further eastward. Sole is therefore found only in low abundances in the western Baltic (ICES Subdivision 24) and the Øresund (ICES Subdivision 23) (Støttrup *et al.* 2019). However, within the past decade the fishery for sole indicates that the size of the population inhabiting the Belts and western Baltic (SD 22) has been increasing.

Spawning areas for sole in inner Danish waters are believed to be located in the Kattegat and Skagerrak but their specific locations are not well known. Spawning is occurring in the pelagic and peaks in May-June (Støttrup *et al.* 2019). Previous work has indicated small although statistically significant genetic differences between sole in the North Sea and the North Sea-Baltic Sea transition zone (Cuveliers *et al.* 2012; Vandamme *et al.* 2021), suggesting the presence of unique genetic populations in the area. However, one earlier study also found conflicting results for samples collected in the Skagerrak-Kattegat (Cuveliers *et al.* 2012). While Skagerrak grouped with the Baltic Sea genetic cluster, one sample from the Kattegat was grouping with North Sea while another sample was grouping with the Baltic Sea genetic cluster (Cuveliers *et al.* 2012). These samples were collected in November (i.e. not in spawning season) and may indicate complex patterns of migration and population mixing in the area. Consequently, interactions and exchanges between sole within SDs 20-24 and the neighbouring North Sea may occur but have previously been poorly documented. There seems to be a continuum in abundance from the Baltic to the North Sea without any natural density boundaries between any of the seas/divisions. Former stock boundaries in the east (i.e., limit at border between Kattegat and the Belts/Øresund (SDs 22-23) were biologically based on the abundance scarcity beyond the Kattegat. The boundary to the west (i.e. between Skagerrak and the North Sea) is likely porous to some extent due to potential mix/migration of adults and/or drift of sole eggs and larvae. However, neither the direction nor magnitudes of exchanges have previously been thoroughly described.

In a previous EMFF project (“Improvement of the biological advice for Common Sole in Danish waters” (J. nr. 33113-B-16-021)) a range of questions regarding the biology and distribution of common sole in Danish waters, in particular in Kattegat and Skagerrak, were resolved. The results indicated that there are genetic difference between spawning sole in the two areas, but growth and survey data also suggested some exchange between the areas (Boje *et al.* 2019). Consequently, the basis for changes in stock assessment and management practices were not present at the time, and more information about the existence and distribution of different populations as well as their interactions were needed to move forward. The overall purpose of this work package in the current project was therefore to improve our understanding of genetic differences between sole in the North Sea, Skagerrak, Kattegat and western Baltic (with a specific focus on Skagerrak) and to map migrations and mixing of populations in these areas. The specific objectives of this work package were to a) identify populations and their mixing through the use of genetic analyses, and b) to map migration patterns through the use of microchemistry signals from otoliths.

2.2 Materials and methods

Genetics

Sample overview

A total of 226 individuals were collected in 2021 at spawning time from different locations in the North Sea, Skagerrak, Kattegat and Baltic Sea (we will refer to these samples as Collection 2022). This dataset was combined with 136 individuals that had been collected between 2016 and 2018 in similar locations but at different times of the year (we will refer to these samples as 2019 collection), to increase the number of samples and therefore the power of the analysis carried out (Table 2.1). In addition, the comparisons of samples collected at two different time points allows for exploration of important temporal dimensions in the data, since temporal stability would be important in relation to more permanent changes in management practice. The individuals collected in 2016 and 2018 consisted of a mix of fish collected in and outside of main spawning season. Thus, the two data set also allowed for a comparison of patterns across different seasons.

Table 2.1. Number of sole used for the genomic analysis after the extensive genomic filtering described in the Methods.

Region	No. of individuals - 2019 collection	No. of individuals - 2022 collection
IVb South	23	
IVb North	17	
IVb Central		47
ICES SD 20	29	87
ICES SD 21	30	29
ICES SD 22	15	63
ICES SD 23	20	
ICES SD 24	2	

DNA extraction and genomic library preparation

All laboratory work was carried out at a specialized DNA laboratory at DTU Aqua premises in Silkeborg. DNA was extracted using DNeasy Blood Tissue kit (Qiagen). The approach chosen for the genomic work was to analyse single nucleotide polymorphism (SNP) genetic markers through the double-digestion Restriction-site Associated DNA (ddRAD) approach; this genomic approach cuts the DNA in different sites where we find a specific DNA nucleotide sequence (a restriction site). To cut the genome into pieces, we used two restriction enzymes, Msp1 and Pst1. In total for the 2016-2022 dataset, we built five independent libraries following the protocol by (Poland & Rife, 2012). After size-selection to a size between 300-400 base pairs (bp) and 12-cycles of PCR amplification, we checked the quality of the libraries using a Bioanalyzer 2100 with the High Sensitivity Kit. Finally, the genomic libraries were sent to an external company for sequencing. The two libraries sequenced in 2019 from the individuals collected between 2016-2018 were sequenced at a HiSeq4000 Illumina sequencer using 100bp paired-end reads. Due to the unavailability in 2022 of the DNA sequencer used in 2019, the three libraries with the individuals collected in 2021 were sequenced at a HiSeqX10 Illumina sequencer using slightly longer reads (150bp paired-end reads).

Bioinformatics and filtering

We processed the genomic data using state-of-the art bioinformatic software. The raw genomic data from the sequencing of the five libraries was received in FASTQ format. We demultiplexed the genomic data using *process_radtags* from STACKS v.2.60 (Catchen *et al.* 2013), that allows to separate the data from individual fish samples. We also removed the adaptor traces from the raw genomic data. After quality control using FASTQC (Andrews 2010), we removed reads that had a mean base quality lower than 30 and collapsed pair-end reads when possible. In order to set up the reads into the right «order» in the genome (i.e. mapping), we made use of a reference genome, which is an established representation of the DNA of a given species. At the time of analyses, there was no available reference genome for the common sole (*Sole solea*). Consequently, we used the reference genome of a closely related species, *Solea senegalensis* (Guerrero-Cózar *et al.* 2021). We mapped the reads back to the *S. senegalensis* assembly using BWA-mem algorithm (Li, 2013), implemented in PALEOMIX v.1.2.13.2 (Schubert *et al.* 2014). We re-aligned the mapped reads to correct for some biases in the mapping, and used the re-aligned BAM file to call single nucleotide polymorphisms (SNPs) using the "cstacks" module from STACKS. We obtained the final SNP file using the "populations" module from STACKS.

We extensively filtered the data for quality control using VCFtools v.1.16 (Danecek *et al.* 2011). We included only biallelic SNPs, excluded indels and selected loci with a minor allele frequeny higher than 0.05 and a minor allele count of minimum 2. We allowed a maximum missing data for individuals of 80% and a maximum missing data for loci of 3%, which are quite stringent filtering conditions. We allowed for a maximum mean depth of 110 and excluded loci that were not under an overall Hardy-Weinberg Equilibrium (HWE), using a pvalue threshold of 0.05. Finally, we checked for relatedness, excluding any potential clones using the algorithm behind the relatedness2 option in VCFtools. We generated two datasets: **dataset 1** consisting of loci that passed all these filtering steps, independently of their physical position in the genome, and **dataset 2**, where we further filtered dataset 1 to select one SNP *per RAD tag*, to be able to have SNPs that are sufficiently separated in the genome to be considered *unlinked*, which is needed for specific genetic analysis, e.g. population structure.

Population structure

In order to study the population structure of the samples analysed, we carried out a Principal Component Analysis (PCA) using the R-package *adegenet* (Jombart 2008; Jombart & Ahmed 2011). We also estimated the overall degree of differentiation between pairs of populations using the F_{ST} measure; for this, we used the R-package *StAMPP* (Pembleton *et al.* 2013). The measure of F_{ST} ranges from 0 to 1, where 0 means that the pair of populations cannot be genetically distinguished and share genetic information among them, whereas a value of 1 means that the two populations are completely different. Using the F_{ST} values, we did a Principal Coordinate Analysis, which is a type of Multi Dimensional Scaling analysis, using the R-package *ape* (Paradis *et al.* 2004). We used dataset 2 for this set of analysis.

Admixture

We calculated admixture proportions using the DAPC implemented in the R-package *adegenet* (Jombart *et al.* 2010) for different numbers of putative clusters. We chose the best cluster scenario by using the function find.clusters() implemented in *adegenet.* After conversion of the format file using PGDSpider, we also calculated hybridization proportions using STRUCTURE (Pritchard *et al.* 2000). We used dataset 2 for this set of analysis.

Levels of genetic differentiation and adaptation

We studied the levels of genetic differentiation between the samples obtained in ICES Area IVb and ICES area 22, where we could observe most differentiation. We used the 2022 collection as it had the largest number of samples, which is essential to eliminate any potential bias due to sampling bias, and the highest number of individuals collected in spawning season. For this scenario, first, we calculated the levels of differentiation (F_{ST}) across the genome using VCFtools. This is a similar analysis as we did when calculating the mean F_{ST} per populations, but this time we calculated per site in the DNA to examine potential signals of selection. Secondly, we performed a more specific selection scan, where we looked for potential selective areas; for this we conducted an outlier analysis using

BAYESCAN (Foll & Gaggiotti 2008). We checked which genes were located near those genomic areas highlighted as outliers in this test with a local alignment search tool, BLASTn (Camacho *et al.* 2009). We used dataset 1 for this set of analysis.

Microchemistry

Sample overview

Samples for microchemistry analyses were collected from ICES SD 4B (statistical rectangle 41F7), SD 20 (44F9, 44G0) and SD 22 (38G0) during May and June 2020 and 2021 (Figure 2.1). A total of 158 individuals in the age range 2 – 19 years were sampled (Table 2.2). Length and weight were available for all individuals, while information on sex was only partly available for SD 4B. Size at age of the samples resolved by SD and sex (where possible) are shown in Figure 2.2. The available data did unfortunately not allow for an assessment of the impact of sex in the following analyses. It appears, however, that differences in size at age differ more between sex in the same area, than between sex in different areas. Furthermore, otolith samples from the Kattegat (SD 21) were not available for chemical analysis.

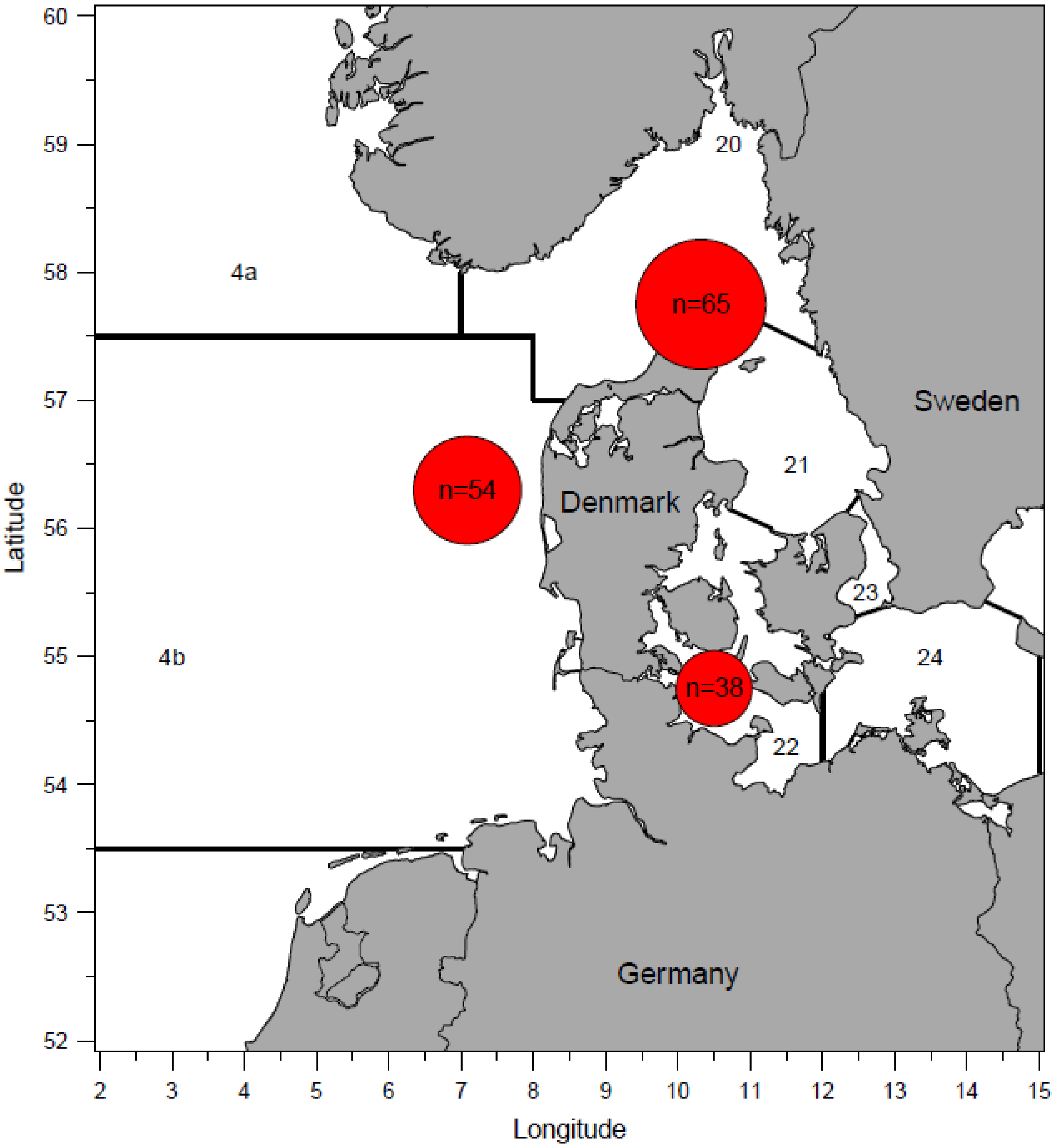


Figure 2.1. Map of the sampling locations and number of common sole sampled per ICES SD for microchemistry analyses. Numbers indicate ICES SDs, horizontal and vertical lines the SD boundaries.

Table 2.2. **Number of common sole per ICES SD and age group for microchemistry analyses.**

	ICES SD			
Age	20	22	4B	Total
2	-	-	2	2
3	2	1	6	9
4	2	11	12	26
5	1	4	8	13
6	6	8	3	17
7	14	10	3	27
8	8	3	5	16
9	2	1	4	7
10	3	-	5	8
11	9	-	2	11
12	5	-	1	6
13	2	-	1	3
14	2	-	1	3
15	1	-	-	2
16	4	-	1	5
18	2	-	-	1
19	2	-	-	2
Total	65	38	54	158

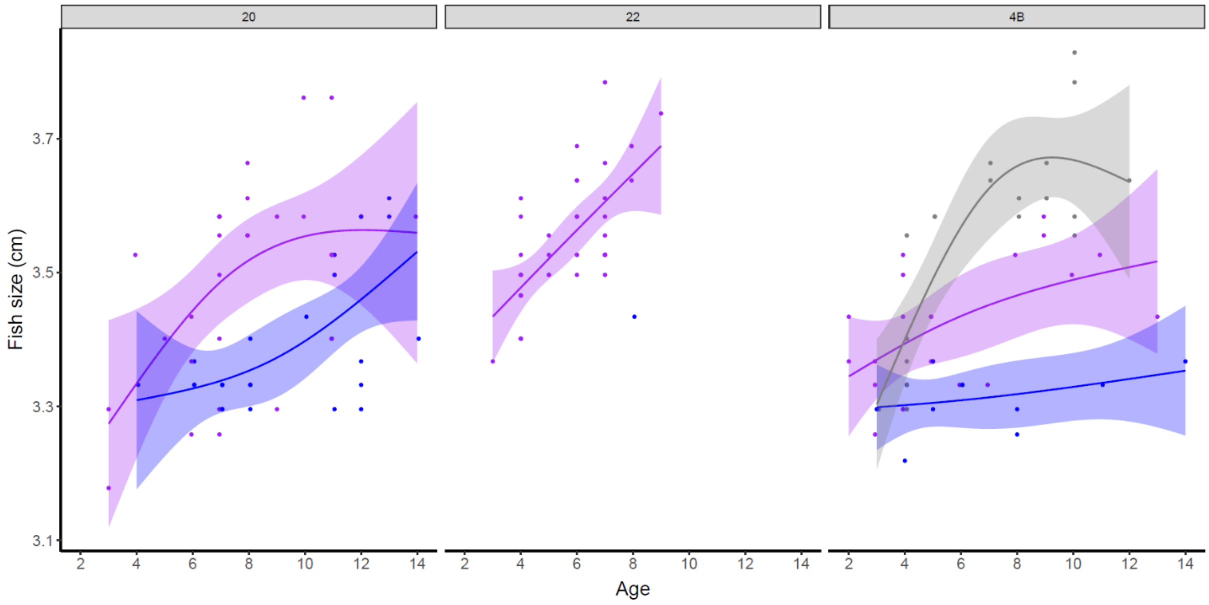


Figure 2.2. Size at age of sole by ICES SD and sex, where females = purple, males = blue, and unsexed individuals = grey). Graphs are loess smoothed mean values with span = 0.2 and standard error band. Note that most fish in SD 4B were not sexed.

Chemical analysis of otoliths

Otolith preparation procedures have been described in detail in Hüssy *et al.* (2020a, 2021a). 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 nucleus to the dorsal edge (Figure 2.3). Concentrations of the following elements were analysed: Barium (^{137}Ba), calcium(^{44}Ca), copper (^{65}Cu), iron (^{57}Fe), potassium (^{39}K), litium (^{7}Li), magnesium (^{25}Mg), manganese (^{55}Mn), lead (^{208}Pb), strontium (^{88}Sr), and zink (^{66}Zn). Measurement values below and above 4 standard deviations from the mean were treated as outliers and discarded (percentage of data discarded no more than 1-3%). 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 growth zones covering the entire life of the fish - along the laser track, from the nucleus to the edge using ImageJ (Rueden *et al.* 2017) (Figure 2.3). LA-ICP-MS data were thereafter parsed to the corresponding age zones of the otolith and element concentration values averaged by 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 µm representative of the nucleus (E_{nucleus}) and the last 50 µm at the edge of the otolith (E_{edge}) representative of the element signature at the time of capture.

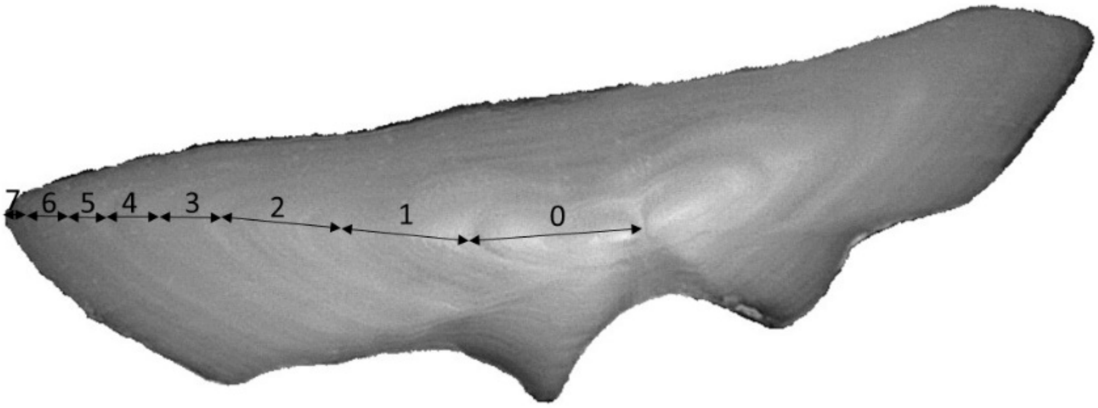


Figure 2.3. Image of common sole otolith cross section, where the sequence of arrows indicates the transect in which the chemical composition was analysed with LA-ICP-MS. Each arrow also indicates an annual growth zone into which the LA-ICP-MS data were parsed, and the numbers indicate the corresponding fish ages.

Statistical analyses

Chemical signatures at hatching: The chemical fingerprints with all elements in the nucleus (E_{nucleus}) were analyzed with Multivariate Analysis of Variance (MANOVA), using the following model, and post-hoc groupwise comparison with the R package “emmeans”:

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

Subsequently, this analysis was repeated, but using genetic clusters instead of ICES SDs. In the genetic analysis, fish from SD 4B clustered together and were separate from the other areas. Fish caught in SD 20 grouped in two clusters, one similar to SD 4B, the other different to SD 4B. Out of 64 soles caught in SD 20, 50 were genetic SD 20 individuals, and 14 genetic 4B individuals. The MANOVA therefore used the following genetic cluster groups: SD 4B$_{\text{genetic 4B}}$, SD 22$_{\text{genetic 22}}$, SD 20$_{\text{genetic 20}}$ and SD 20$_{\text{genetic 4B}}$:

$\text{Fingerprint} = \text{factor}(\text{geneticCluster}) + \varepsilon_{\text{i}}$, where □□= N(0,□□)

<u>Identification of natal origin</u>: Also for the identification of number of potential spawning components the chemical fingerprint with all measured elements in the nucleus only (E_{nucleus}) was used. The number of potential spawning components were assessed with a commonly used cluster analysis approach using the “kmeans” and “factoextract” packages of (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 centred (subtracting mean) element concentrations, and using the Euclidean distance between observations and cluster means. The optimal number of *k* was determined using the average Silhouette method.

<u>Lifelong chronological element patterns</u>: 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), *yearClass* (year the fish was born), 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:

$\text{E}_{\text{ij}} = \text{factor}(\text{area}_{\text{ij}}) + \text{factor}(yearClass_{ij}) + \text{ageLife}_{\text{ij}} + (1|\ \text{fish}_{\text{i}}) + \varepsilon_{\text{i}}$, where □□= N(0,□□)

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.

<u>Reconstruction of life-long area affiliation</u>: From the LME analysis, elements that differed significantly between areas and were not subject to a year class effect were selected: Ba, Cu, Li, Mn, Sr, Zn. 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, 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}). These functions were then used to classify the element signatures of each *ageLife* interval of each fish to the most likely ICES SD occupied, thus reconstructing life-long area affiliation of each fish.

2.3 Results and Discussion

Genetics

Population structure

After filtering, dataset 1 consisted of 4028 SNPs and dataset 2 of 3274 SNPs, for 362 individuals from both collections (2019 and 2022). A first inspection of the genetic differentiation of the individuals used for this study revealed a slight difference between the collections of 2019 and 2022 when merging data sets together; this difference appears to be due to a technical bias originated to the difference in read length or sequencing technology. Therefore, we decided to analyse the two sets of individuals separately which allowed us to check for consistency in the results, i.e. whether the same patterns were observed in both collections.

The genetic map, represented in the Principal Component Analysis, revealed that the fish sampled in the North Sea (ICES area IV) were genetically different from other areas included in this study. SD 21-24 were very similar genetically, forming a single cluster (Figure 2.4). Fish collected in area ICES SD 20 seemed to share the cluster with both the fish in areas IV and the other ICES areas in Kattegat and the Baltic Sea. The collection in 2019 had two sampling locations in the North Sea (one located in the south and the other in the north); we observed that the northern location shared more genetic characteristics with the fish in SD 20 (i.e. the presence of more fish with a genetic profile that matched the profiles of fish in the North Sea-Baltic Sea transition zone, SD 20- SD24), than the southern location showing what appears to be a gradient in mixing from the North Sea and into the Baltic Sea.

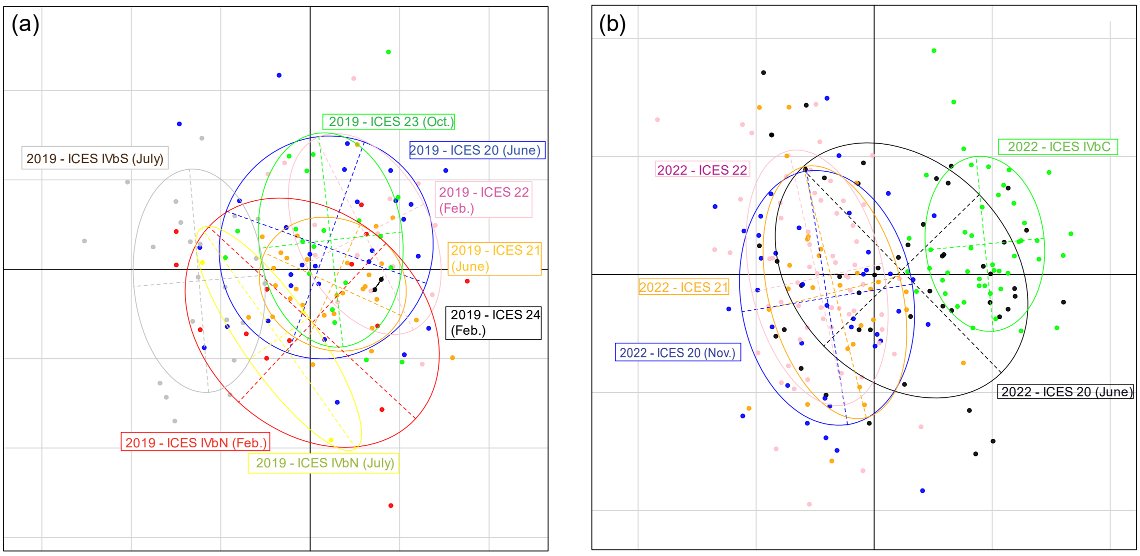
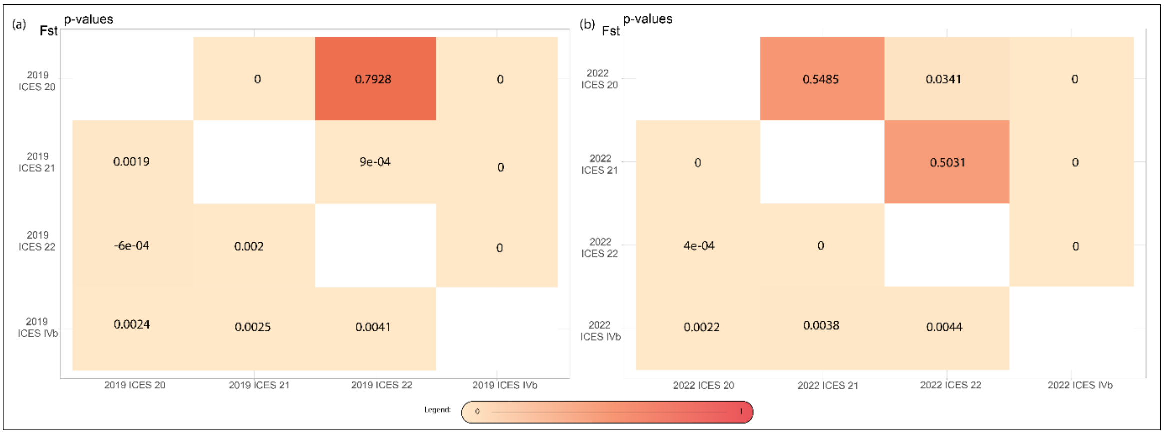


Figure 2.4. Genetic map (Principal Component Analysis, PCA) of the individuals analysed as part of this study, for 2019 (a) and 2022 (b) collections. Each point represents an individual fish, and each colour in each individual PCA represents each population sample (colours differ between a) and b)). In the legend names we indicated both the area where the samples were collected (corresponding to Table 1) as well as the time of year of sample collection.

This genetic connectivity gradient observed in the PCAs was also corroborated in the F_{ST} analysis (Table 2.3). Both collections showed the same pattern of genetic differentiation. Individuals in area IVb had an F_{ST} that increased as we moved towards ICES SD 20 (0.0022), SD 21 (0.0038) and SD 22 (0.0044) for collection 2022, illustrating this gradient (Table 2.3b). The same pattern was observed for the collection in 2019 (Table 2.3a). All these comparisons reflected statistically significant differences between sampling locations (Table 2.3). In contrast, F_{ST} between the samples from ICES SDs 20-22 were very close to 0 and in many cases not statistically significant, suggesting a similar genetic profile in these locations.

Table 2.3. F_{ST} values (lower diagonal) and corresponding p-values (upper diagonal) represented in a heatmap, for 2019 (a) and 2022 (b) collections.



(a)

Fst \ p-values	2019 ICES 20	2019 ICES 21	2019 ICES 22	2019 ICES IVb
2019 ICES 20		0	0.7928	0
2019 ICES 21	0.0019		9e-04	0
2019 ICES 22	-6e-04	0.002		0
2019 ICES IVb	0.0024	0.0025	0.0041	

(b)

Fst \ p-values	2022 ICES 20	2022 ICES 21	2022 ICES 22	2022 ICES IVb
2022 ICES 20		0.5485	0.0341	0
2022 ICES 21	0		0.5031	0
2022 ICES 22	4e-04	0		0
2022 ICES IVb	0.0022	0.0038	0.0044	

Admixture analysis

The admixture analysis revealed two different putative populations (K) as the most plausible scenario (results not shown), so we performed the rest of the analysis assuming K=2. Both clusters 1 (pink) and 2 (yellow) were found in all areas studied; however, their proportions within areas varied (Figure 2.5). Cluster 1 (pink) was found predominantly in ICES IVb, whereas cluster 2 (yellow) was more frequent in the Baltic Sea (Figure 2.5a and 2.5b). In all areas we found individuals that had mixed genomic proportions from the two clusters; however, ICES area IVb, SD 22 and SD 23 seemed to have more individuals that were homogeneous for one cluster and less admixed. By contrast, ICES SD 20 and the Northern part of area IVb seemed to have a larger proportion of samples with mixed proportions from both clusters. These results are consistent with a genetic model of isolation by distance (IBD; i.e. a gradual genetic change with increasing geographic distance), which has also been reported in other species in the North Sea – Baltic Sea transition zone, e.g. European plaice, *Pleuronectes platessa* (Le Moan *et al.* 2021). The results also indicated the presence of both genetic clusters in the Skagerrak at spawning time, suggesting the presence of two spawning populations in the area. In addition, the differences between spawning time (June) and non-spawning time indicate dynamic patterns of mixing in the areas.

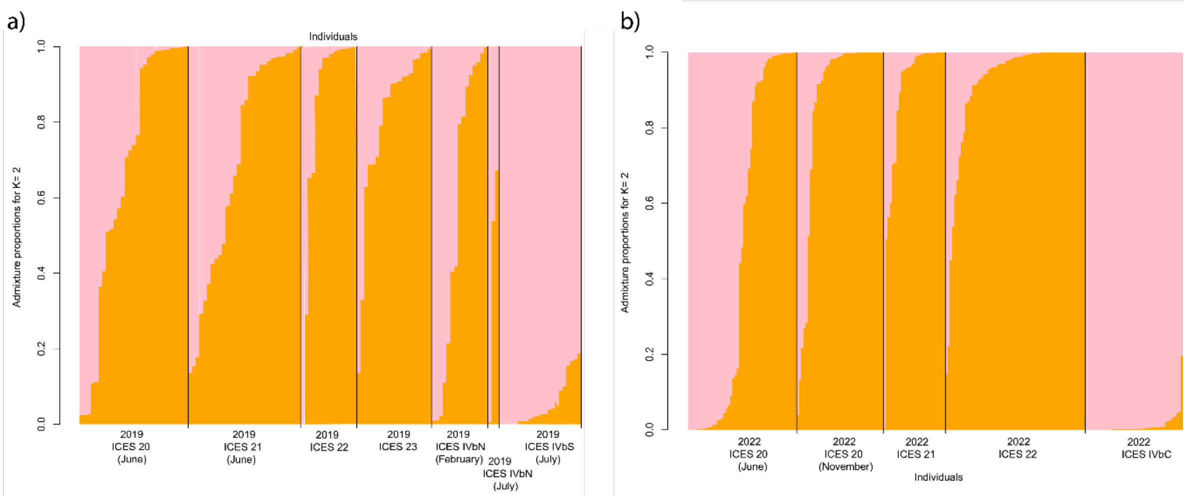


Figure 2.5. Admixture plot for the 2019 collection (a) and the 2022 collection (b).

Levels of genetic differentiation and adaptation

The outlier analysis for the comparison “IVb vs ICES SD 22” highlighted three outlier loci (Figure 2.6). The genomic sequences where these three outlier SNPs were found did not map to any gene from the NCBI data base, therefore it was not possible to identify any biological function associated with these genomic regions. The pairwise F_{ST} did not show any specific areas under strong selection (Figure 2.7); the three outliers corresponding to the outlier scan mapped to different areas of the genome on chromosomes 18, 19 and 22. Although the adaptation analysis did not result in the identification of any known genes, we cannot exclude the possibility that these genomic regions are related to important physiological processes that allow the sole to adapt to their specific habitats in area IVb or SD 22 (and the gradient occurring between the areas). For instance, the European plaice was found to have specific regions of its genome (known as Structural Variants) with different gene versions along the transition zone in the North Sea towards the Baltic Sea, which seemed to be related to salinity adaptation (Le Moan *et al.* 2021), and similar signatures have been found in a range of other species in the transition zone (Johannesson *et al.* 2019).

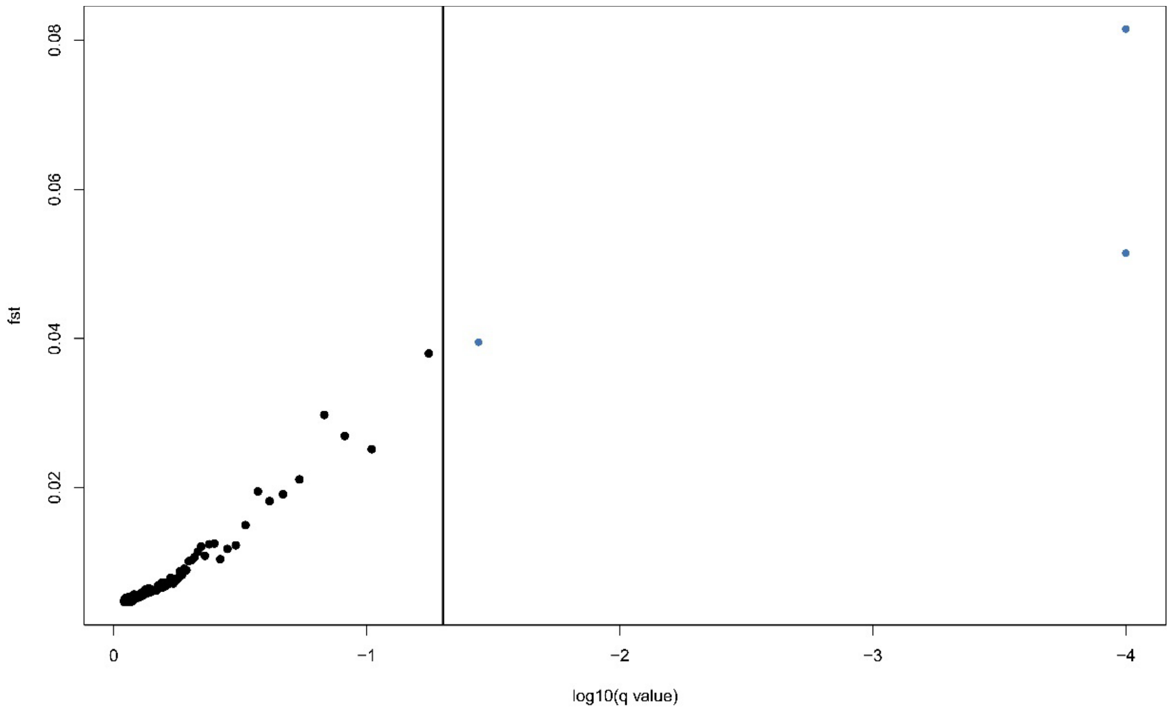


Figure 2.6. Outlier analysis (Bayescan analysis) for the scenario “ICES area IVb vs ICES SD 22”, from the 2022 collection. The plot shows DNA markers that display particularly strong genetic differences between the populations. The markers that appear to the right of the vertical line highlighted in blue correspond to significantly diverging loci, which could be showing a signal of local adaptation.

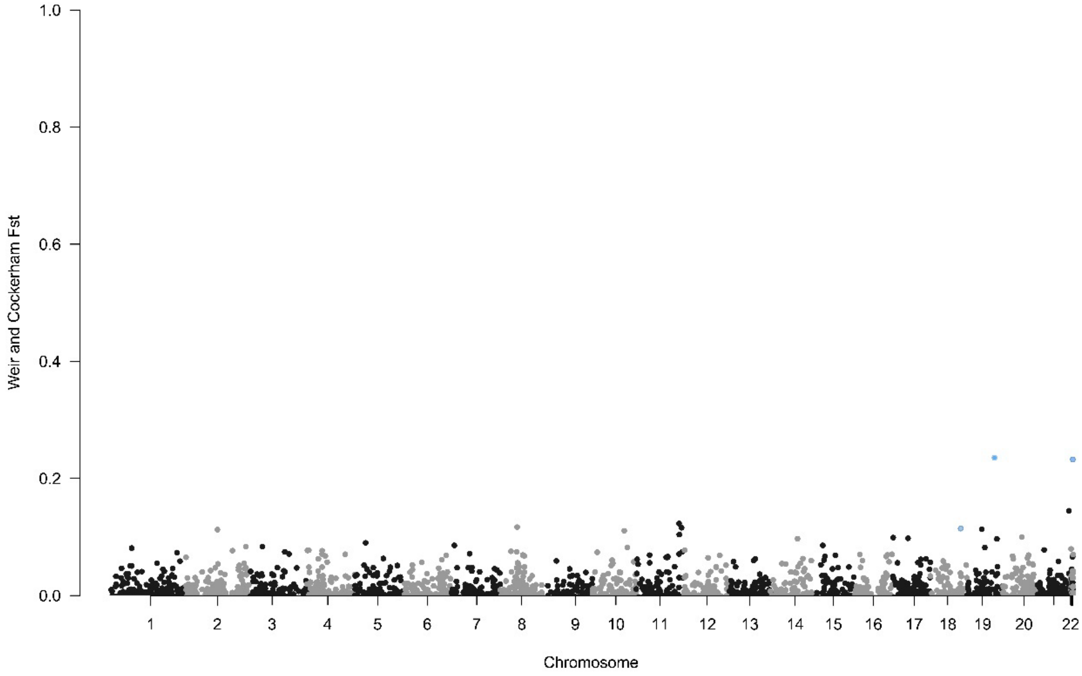


Figure 2.7. Pairwise F_{ST} across the genome for the scenario "ICES area IVb vs ICES SD 22", from the 2022 collection. Loci in blue are the same loci highlighted as outliers in the outlier analysis (Figure 2.6). The different chromosomes in the sole genome are indicated with alternating grey and black dots (one dot for each genetic marker analysed in the present study).

Microchemistry

Identification of natal origin

The objective of this analysis was to address the question: Do sole originate from the same spawning areas?

Chemical signatures at hatching: The chemical fingerprints of the nucleus were similar between SD 4B and SD 20 (MANOVA, df = 142, p = 0.13). Since there were no differences in chemical fingerprints between SD 20 and 4B, the splitting of the samples into genetic clusters did not result in any statistical differences in core fingerprints of SD $20_{\text{genetic 20}}$ and SD $20_{\text{genetic 4B}}$ sole (MANOVA, df = 141, p = 0.99), between SD $20_{\text{genetic 4B}}$ and SD $4B_{\text{genetic 4B}}$ sole (MANOVA, df = 141, p = 0.74), or between the genetically distinct SD $20_{\text{genetic 20}}$ and SD $4B_{\text{genetic 4B}}$ sole (MANOVA, df = 141, p = 0.23), suggesting that both (genetic) populations may be spawning in the Skagerrak, thereby obtaining a similar chemical core fingerprint. Individuals from SD 4B and 20, on the other hand, both differed from SD 22 (MANOVA, df = 142, $p < 0.05$), indicating ecological separation between these areas. Contributing significantly to the separation between SDs were the elements Ba, Cu, Li, Mn, Pb, Sr, and Zn. Given the lack of samples from the Kattegat, it is unfortunately not possible to assess to what extent the "western Baltic" signal extends northward.

Identification of natal origin: While the MANOVA analysis provides information on whether there are differences in chemical fingerprints between areas, it does not provide any information on potential mixing proportions of putative spawning areas. To that end, the cluster analysis provides an unsupervised estimate of how many potential spawning areas samples may originate from. Examples of the cluster analysis for *k* = 2, 3, 4, and 5 clusters area shown in Figure 2.8. The average Silhouette method identified the optimal number of clusters as *k* = 2, which shows a complete separation of the two clusters. The proportional distribution of chemical fingerprints from the nucleus per ICES SD the samples originate from using *k* = 2 clusters is shown in Figure 2.9.

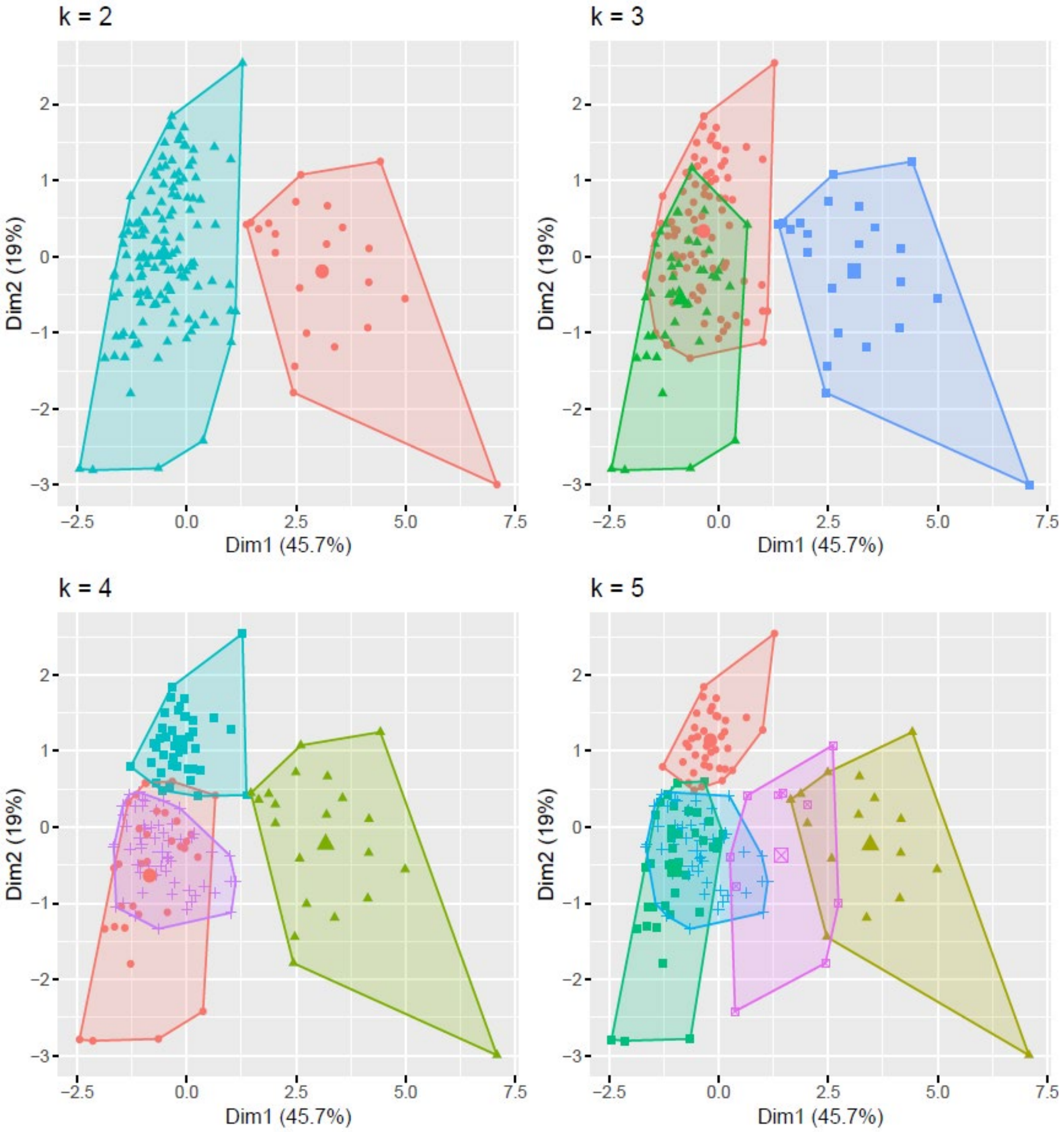


Figure 2.8. Biplots of the k-means cluster analysis performed in the nucleus element fingerprints of sole, where the four panels show examples with k = 2, 3, 4, and 5 clusters.

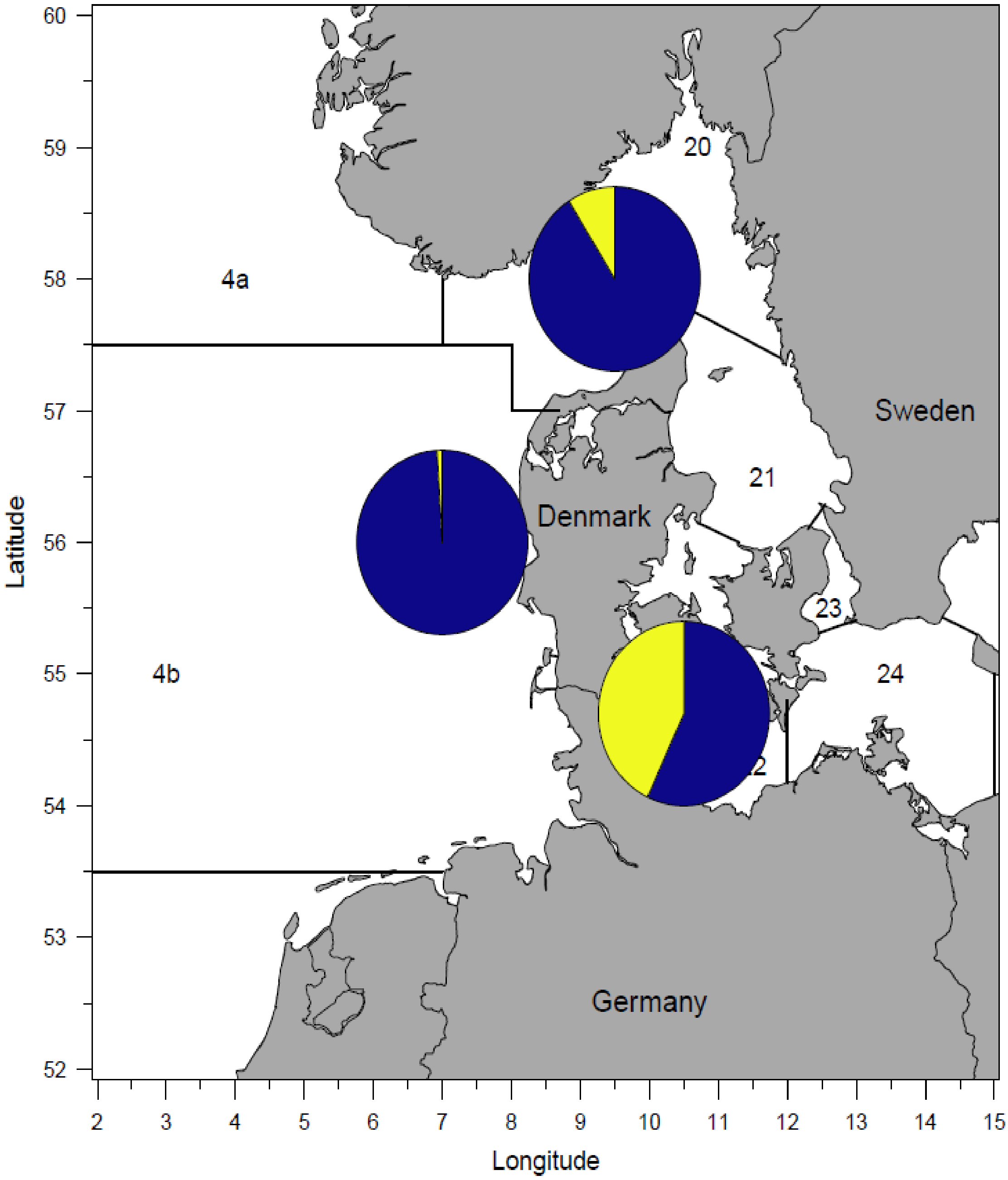


Figure 2.9. Proportional composition of the two clusters of chemical fingerprints in the nucleus by capture area.

This analysis revealed that the sole captured in the North Sea (SD 4B) and Skagerrak (SD 20) seem to have the same natal origin (99% and 91% of individuals belonging to cluster 1), which was to be expected from the MANOVA analysis that did not detect any significant difference between areas. Individuals from that (cluster 1 - blue) spawning origin also make up 57% of the sole captured in the western Baltic (SD 22). The component that is represented by the remaining 43% of sole in the western Baltic (cluster 2 - yellow) also stretches north with a minor contribution (9%) to the population in the Skagerrak. The natal origin fingerprints do not overlap, and therefore suggest that sole in the study area come from two distinct spawning areas/populations. Sole spawning in the North Sea/Skagerrak would therefore seem to have been spawned there themselves, while sole spawning in the western Baltic Sea may have been spawned in either the Skagerrak or the western Baltic Sea themselves.

Overall, the present results indicate that sole originate from at least two chemically distinct spawning areas: One in the North Sea/Skagerrak area and one presumably in the western Baltic. Consequently, these results seem to support the scenario with two distinct populations from the genetic analyses.

The results area in line with Brown *et al.* (2019) who found significant differences in chemical fingerprints of juvenile sole caught in shallow nursery areas in the northern and southern Kattegat and the Belt Sea. While they found some overlap in signatures in the Kattegat, the clearest separation occurred between the southern Kattegat and the Belt Sea, with only 12% of Belt Sea juvenile sole having a southern Kattegat element signature. Together, these results suggest that common sole in the study area consist of two distinct spawning components, one originating from the North Sea/Skagerrak, the other from the Baltic Sea and Kattegat, but extending also into the Skagerrak, where the two populations spawn. Without chemical data from the Kattegat it is not possible to identify the exact location of the spawning areas in the inner Danish waters.

The greatest weakness with this and the following analyses is the fact that there may be spawning areas that are missing in our sampling design. This is particularly evident for the missing sample coverage in SD 21, but also applies to the geographical resolution of our samples, as most samples originate from few ICES rectangles only.

Reconstruction of adult movements

The objective of this analysis was to address the question: Do sole mix during 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.

Lifelong chronological element patterns

The element-by-element analysis revealed that elements, the incorporation of which is known to be exclusively under physiological control (P, K), exclusively depends on the age of the fish. Notable exceptions are Cu, Mg, and Zn, where an additional area effect was also evident (Table 2.4) (Hüssy *et al.* 2020b). In elements known to be incorporated as a function of their concentration in the surrounding water (Ba, Sr, Li, Mn, Pb and Sr) area-specific differences in element concentrations were found. The elements that are known to reflect specific environmental conditions, behave just as expected – and in accordance with element concentrations found in cod (Hemmer-Hansen *et al.* 2020; Hüssy *et al.* 2020b, 2021b). Sr, for example, is a proxy for water salinity and is significantly higher in sole from the North Sea and Skagerrak compared to the western Baltic Sea, in accordance with the spatial gradient in salinity in that area (Figure 2.10). Ba, on the other hand, reflects proximity of the fish to the coast and is much higher in sole from the Skagerrak and western Baltic than the North Sea (Figure 2.10). Finally, Mn, which is considered a proxy for the occurrence of hypoxia, is much higher in the western Baltic where regular summer hypoxia in shallow waters occurs in addition to persistent hypoxia in the deepest areas (Figure 2.10). The fact that we found significant effects of area (ICES SD) in virtually all elements indicates that stock mixing of sole in the transition area between the North Sea and the western Baltic Sea is relatively limited.

Figure 2.10. Lifelong mean element concentration profiles for all elements analysed in common sole captured in the three ICES SD. Of particular interest for stock separation are the elements Sr (proxy for salinity), Ba (proxy for coastal affiliation) and Mn (proxy for hypoxia exposure). Colours indicate the habitat area assignments: North Sea = yellow, Skagerrak = green, western Baltic = blue.

Table 2.4. Summary statistics of the Linear Mixed Effects model examining the variables influencing otolith element concentrations in sole. Marginal r^2 = variance explained only by fixed effects, conditional r^2 = variance explained by the entire model.

	Fixed effect	Direction of effect	*F-value*	*p*	Marginal r^2	Conditional r^2
Ba*	Area	4B < 20 = 22	28.5	<0.001	0.162	0.323
	AgeLife	- ve	151.3	<0.001		
	Year Class			ns		
Cu*	Area	4B < 20, 20 > 22	10.7	<0.001	0.113	0.412
	AgeLife	+ ve	131.7	<0.001		
	Year Class			ns		
Fe	Area			ns	0.005	0.72
	AgeLife	+ ve	30.5	<0.001		
	Year Class			ns		
K	Area			ns	0.071	0.288
	AgeLife	+ ve	128.4	<0.001		
	Year Class			ns		
Li*	Area	4B = 20 > 22	23.0	<0.001	0.116	0.506
	AgeLife	- ve	33.5	<0.001		
	Year Class			ns		
Mg*	Area	4B < 20 < 22	12.9	<0.001	0.108	0.146
	AgeLife	- ve	25.9	<0.001		
	Year Class	increasing since 2005	2.3	<0.01		
Mn*	Area	4B > 20, 20 < 22	25.4	<0.001	0.213	0.440
	AgeLife	- ve	126.7	<0.001		
	Year Class			ns		
P	Area			ns	0.336	0.433
	AgeLife	+ ve	670.3	<0.001		
	Year Class			ns		
Pb	Area	4B = 20 < 22	25.0	<0.001	0.161	0.257
	AgeLife	+ ve	156.2	<0.001		
	Year Class	increasing since 2002	1.7	< 0.05		
Sr*	Area	4B = 20 > 22	7.5	<0.001	0.405	0.656
	AgeLife	+ ve	993.3	<0.001		
	Year Class			ns		
Zn*	Area	4B = 20 < 22	17.1	<0.001	0.126	0.562
	AgeLife	+ ve	138.1	<0.001		
	Year Class			ns		

* Elements used for the reconstruction of lifetime area occupation (conditional Linear Discriminant Analysis)

These analyses were repeated for individuals caught in SD 20, comparing the two groups of genetic clusters (SD $20_{\text{genetic 4B}}$ and SD $20_{\text{genetic 20}}$). No statistical differences were found between the two groups in any element (Figure 2.11). This result may be explained by two different scenarios: 1) sole captured in SD 20 have spent their entire life in the same area, or 2) sole captured in SD 20 have inhabited areas with a similar chemical water composition. Given the significant differences in chemical signatures between sole captured in SD 4B and SD 20, it seems most likely that the two genetic groups have lived most of their lives in the same habitat.

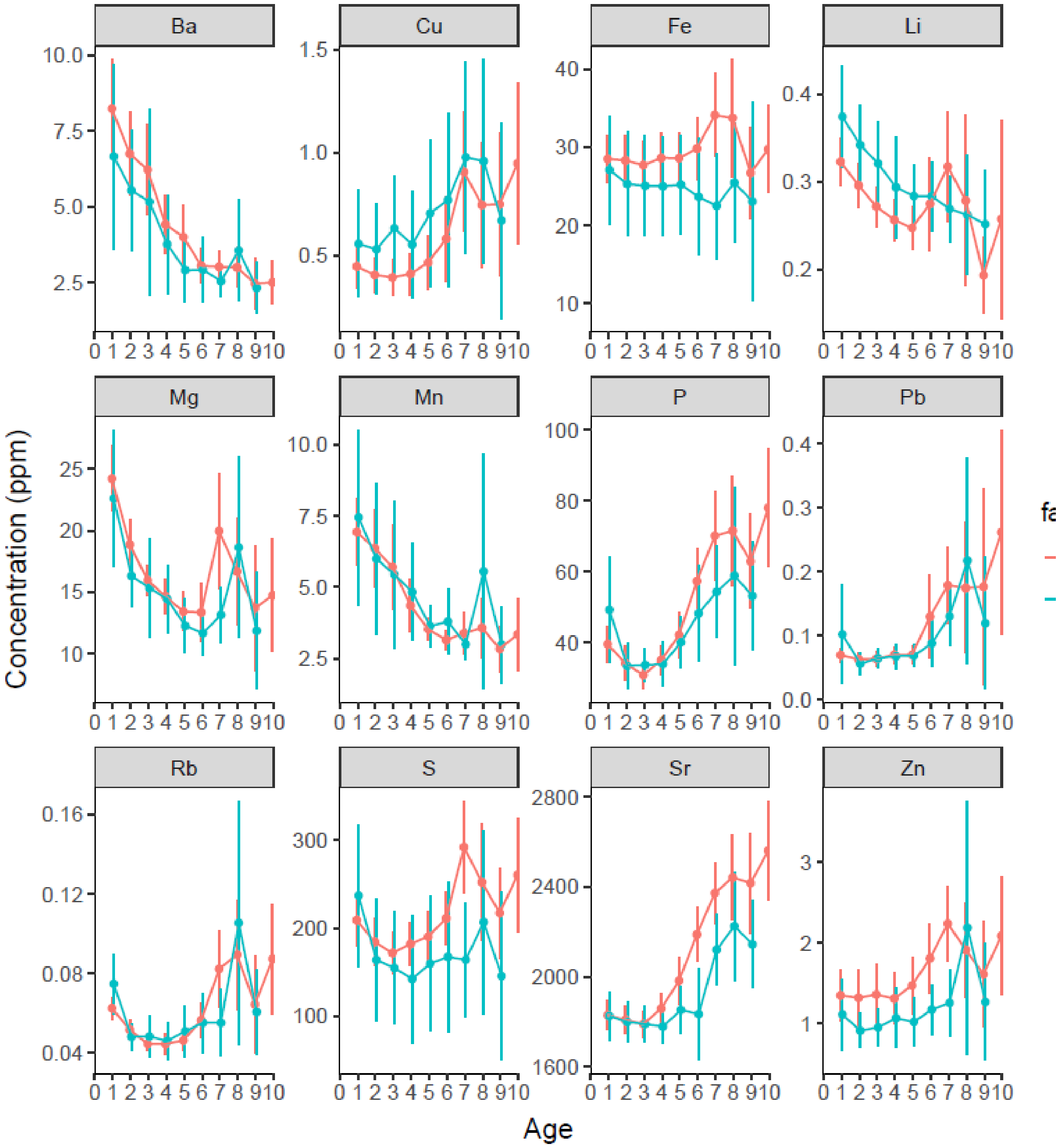


Figure 2.11. Lifelong mean element concentration profiles for all elements analysed in common sole captured in ICES SD 20, comparing the two groups of individuals clustering with the genetically identified clusters, i.e. sole clustering with the genetic SD 4B individuals (SD 20$_{\text{genetic 4B}}$ – green) and individuals clustering with the genetic SD 20 individuals (SD 20$_{\text{genetic 20}}$ - red). There were no statistically significant differences between the two groups in any of the elements analysed, indicating that 1) sole captured in SD 20 have spent their entire life in the same area, or 2) sole captured in SD 20 have inhabited areas with a similar chemical water composition.

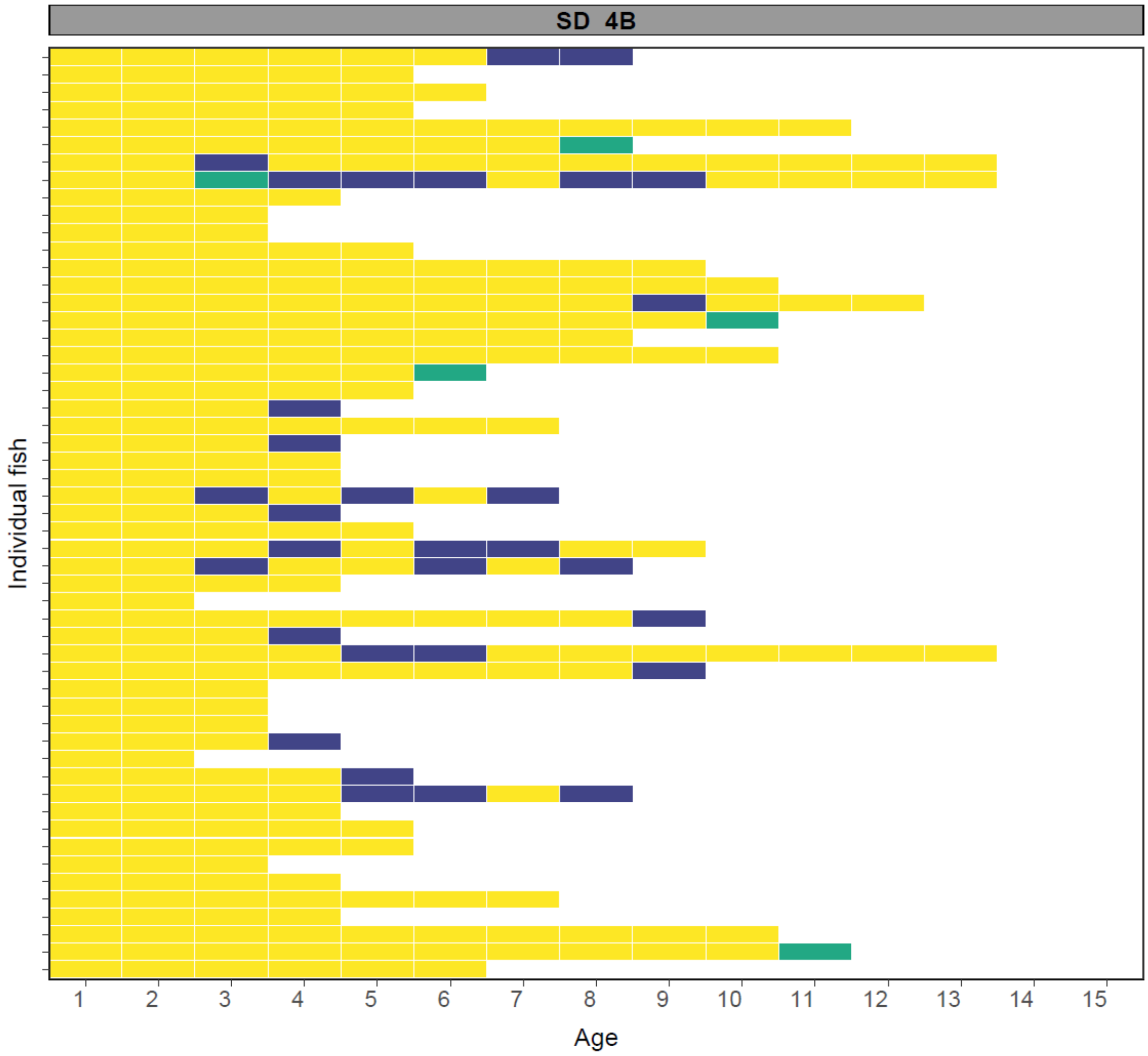
Reconstruction of area of occupation

For the reconstruction of which SDs each individual had occupied throughout its life, we used the elements with significant differences in concentration between areas, identified with the LME approach in the previous section: Ba, Cu, Li, Mg, Mn, Sr and Zn (Table 2.4). The habitat reconstructions are shown for each individual as colour-coded maps grouped by the ICES SD where the fish were caught (Figure 2.12).

SD 4B: Most notable in this figure is that the majority of individuals captured in the North Sea have a clean (yellow) “SD 4B signal”. Only a few individuals seem to have moved between areas in asynchronous patterns over their lives.

SD 20: Common sole in Skagerrak have a rather heterogenous chemical fingerprint that leads to assignments to all areas. There seems to be some consistency in that most individuals have been assigned predominantly to the North Sea (yellow) and to some degree also the western Baltic Sea (blue), while the (green) Skagerrak signal from the otolith edge (representative of the environmental conditions at capture) occurs much less frequently. When comparing the individuals from this SD with the individuals captured in the North Sea and western Baltic Sea, it seems evident that the Skagerrak is a mixing area, where individuals with a (yellow) North Sea signal co-occur with (blue) individuals from the western Baltic Sea (or Kattegat, which we cannot assess due to the lack of samples in that area), and that the mixing appears to be without a consistent pattern related to age.

SD 22: The cluster analysis examined the fingerprints in the nucleus and showed that 57% of individuals had a North Sea/Skagerrak signal (Figure 2.9). At first glance, it looks like this pattern continues into ages 1 and 2, after which most individuals have a purely western Baltic chemical fingerprint (Figure 2.12). Since the chemical fingerprint of an area is laid down without much time lag, this would seem to suggest that individuals keep moving into the western Baltic from the North Sea/Skagerrak for the first three years of their lives. However, we believe that this classification pattern of age 1 and 2 individuals from SD 22 is attributable to methodological challenges. Particularly the fact that there are clear ontogenetic patterns in otolith element concentration, i.e. a strong decrease in concentration of Ba, Mg, Mn, Rb and S (Figure 2.10) with age. The area-classification approach was therefore modified to account for this effect of fish age. An accurate area-classification thus requires that all age classes are represented with adequate sample sizes. For this study, sample sizes of fish younger than 4 years are limited (only two fish aged two years, none younger – Table 2.2).


SD 4B
Individual fish
Age
1
2
3
4
5
6
7
8
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11
12
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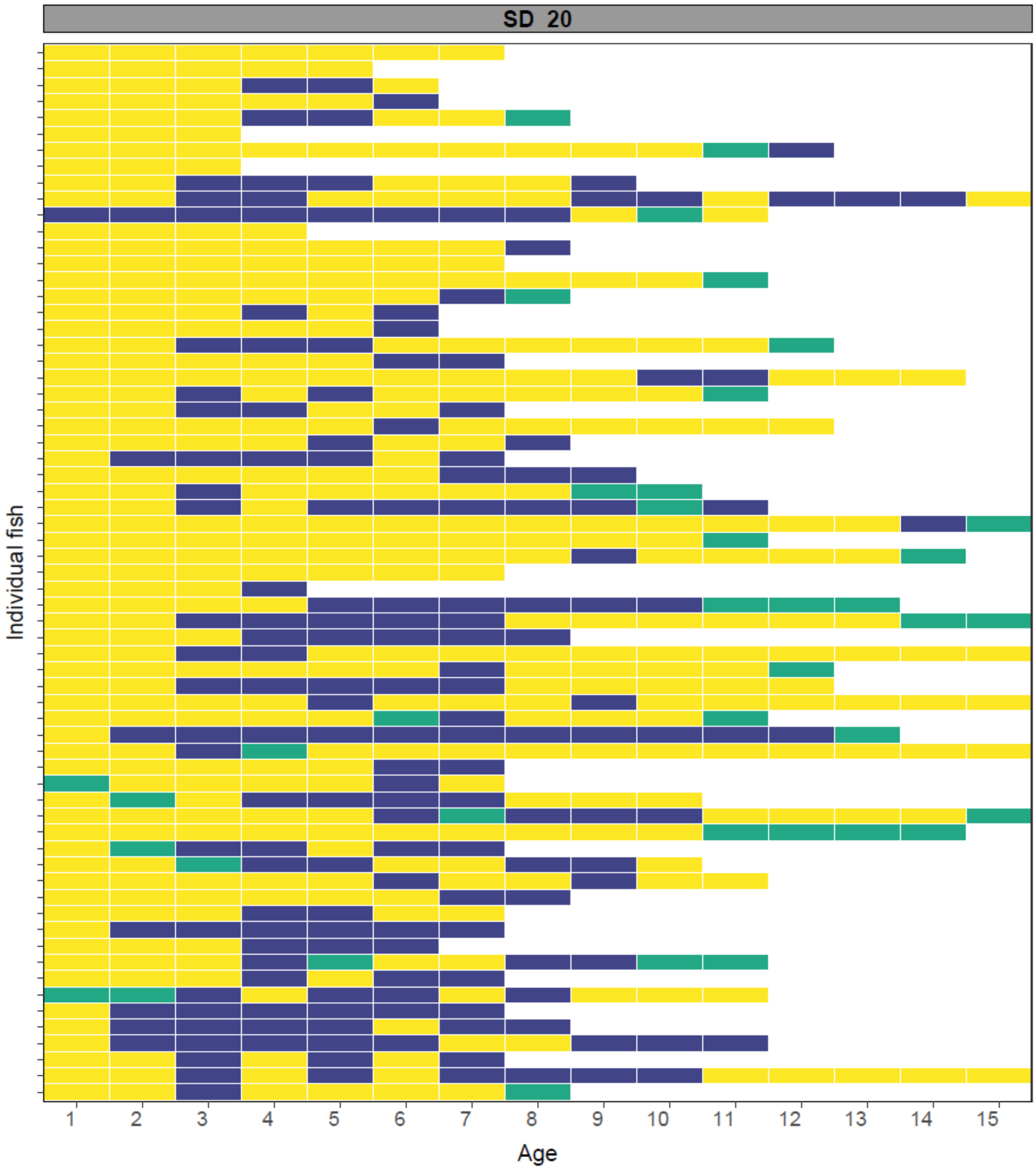
14
15


SD 20
Individual fish
Age
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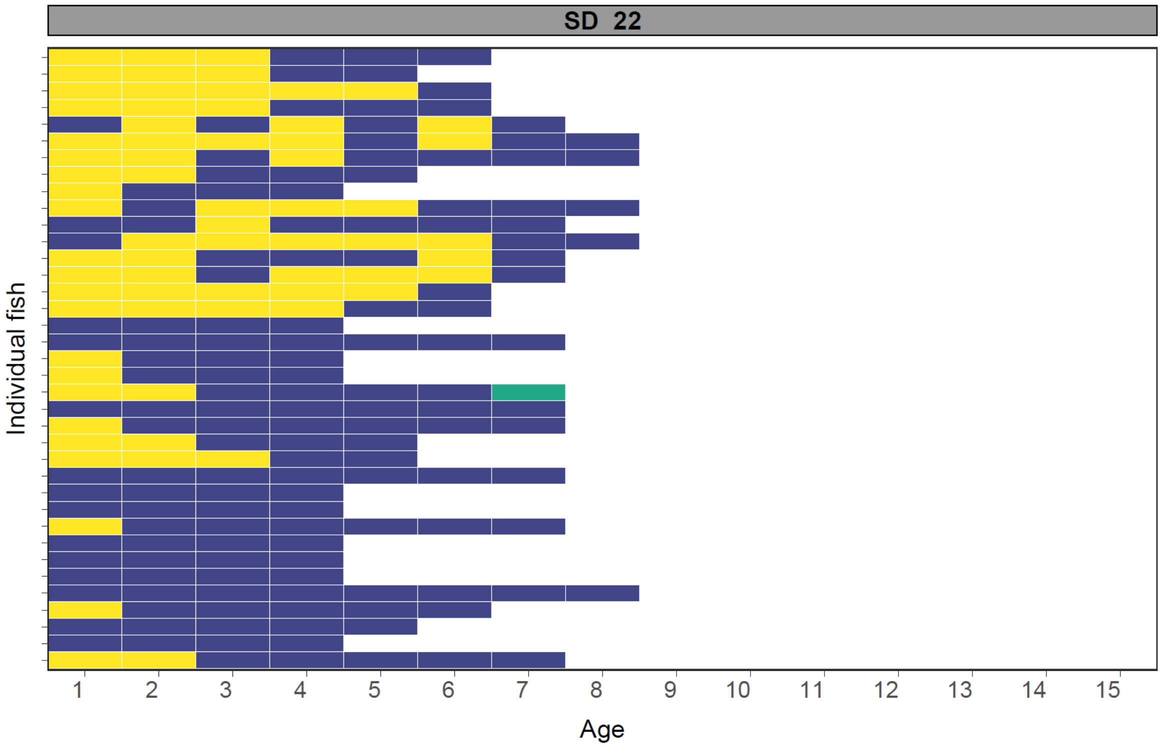


Figure 2.12. Lifetime area use in common sole captured within the three ICES SDs in this study (SD indicated above each figure). Cells represent individual fish and each year of its entire lifespan from the first winter to catch. Individuals are ordered according to latitude of their capture position, 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. Colours indicate the habitat area assignments: North Sea = yellow, Skagerrak = green, western Baltic = blue.

2.4 Conclusions

The genetic data showed clear evidence for the presence of two reproductive populations in the areas under study: one North Sea population and one western Baltic Sea population. These differences were most pronounced at spawning time, stressing the importance of sampling individuals in spawning conditions when mapping population structure. Skagerrak seems to consist of a mix of the two populations, also at spawning time, suggesting that this is a central area where both populations spawn, meet and interact. However, the maintenance of the relatively clear genetic differences between these groups requires some separation (in space and/or time) of spawning or that hybrids have reduced reproductive success. The temporal data suggest that there may be temporal variation in where and when the two populations spawn in the Skagerrak. However, it should also be noted that while we targeted spawning season for sampling in order to increase the likelihood of obtaining spawning fish, a proportion of the fish were actually characterized as “recovering from spawning”. Consequently, future work could concentrate on a more detailed geographic mapping of spawning areas used by the two populations. The genetic data also suggest significant mixing of populations in non-spawning season, in particular evident in Kattegat and Skagerrak, which appear as very dynamic areas of interaction between the two populations.

The chemical fingerprint of the nucleus suggests that there are two distinct spawning areas of common sole in the area from the North Sea to the western Baltic Sea: One spawning area which virtually all individuals in the North Sea and Skagerrak and 55% in the western Baltic Sea originate from. Additionally there is a further spawning area that primarily contributes to recruitment in the western Baltic

Sea. The chemical signatures in otolith cores in the western Baltic Sea suggest that some fish recruiting to the western Baltic Sea were spawned in environments resembling the North Sea/Skagerrak. As the fish collected in SD22 show a relatively clean Baltic Sea signature (i.e. with limited evidence for the presence of North Sea fish this far into the Baltic), these fish genetically assigned to the Baltic Sea may belong to a reproductive population that expands from the western Baltic Sea to the Skagerrak. Thus, the combination of insights from genetics and microchemistry suggests a system with a spatial distribution of a Baltic Sea population extending as far north as the Skagerrak which may supply recruits to the western Baltic Sea. Missing samples for chemistry analyses from the Kattegat unfortunately precludes more detailed spatial investigations into these dynamics at present.

The chronological records of single element concentrations from hatch to capture show a generic ontogenetic pattern with fish age. Additionally, virtually all elements known to reflect concentrations in the environment show a significant area effect. This indicates that common sole, once recruited to a specific geographical area within the distribution of the two populations, are fairly stationary and do not migrate to a notable extent. Reconstruction of area association using the combined area-specific fingerprint supports the conclusion that common sole in Danish waters consist of a North Sea component and a western Baltic Sea (or Kattegat) component, and that these components mix to some extent.

3. The state and development of plaice stocks

3.1 Background

The European plaice (*Pleuronectes platessa*) is an important fisheries species in Denmark. While it is not the most highly valued fish, its abundance and accessibility to a variety of fleets make it an important species for different fisheries operating at a variety of scales.

This attribute of accessibility has been called in to question by Danish fishers of the Skagerrak coast, where reports of difficulties in catching quotas have filtered in to DTU Aqua researchers via fishery organisations (pers. comms., Josianne Støttrup). These same reports identify the mid 2000s as the point in time when plaice started to be less available. The International Council for the Exploration of the Seas, the organisation responsible for providing fisheries advice to the EU, has been consistently advising relatively high catches for the plaice stock in the North Sea and Skagerrak area (ple.27.4-3a.20), while the European Commission has been setting the Total Allowable Catch (TAC) lower than the single species advised catch since the management plan switched from stage I to stage II, whereby quotas are set in a multi-species context, accounting for the abundance of other stocks in what is a mixed fishery. In spite of these high advised catches and lower corresponding TACs, catches in recent years have been consistently below TACs.

Since 2016, the Skagerrak plaice has been included in the North Sea stock for the purposes of both stock assessment and management. This was based on sound biological reasons such as genetic stock structure and life-history connectivity. However, given the North Sea population covers a large area, there is a risk that evaluations of the stock are not able to detect over-exploitation of local populations, for example in the Skagerrak, either due to shifting species distributions or unequal fishing pressure distribution.

It is the hypothesis of this study that plaice distributions have shifted such that densities are lower in coastal areas, including the Skagerrak, reducing their availability to more coastal fleets. In order to investigate this hypothesis we undertake two main lines of enquiry, the first is to document the distribution of plaice over time, looking specifically for a “breakpoint” where distributions shifted significantly. The second is to investigate the distribution of effort and efficiency of plaice capture in fisheries for which plaice is an important species.

In support of these correlative studies, looking to explain changes backwards in time, we also sought to investigate the feasibility of juvenile habitat surveys along the Danish west coast, using the Skagerrak as a pilot study. Future studies of the drivers of juvenile growth and survival may contribute to better predictions of stock recruitment. In order to maintain a productive fishery, bottle-necks to production must be managed across the exploited species whole life-history. In plaice, much like many other flatfish species, juvenile habitat availability and quantity can limit the survival from settling larvae to recruits. The processes that restrict the survival of individuals through the juvenile phase are many and to understand how human activities potentially exacerbate the limits imposed in juvenile habitats, we must first know where they exist in space and how the natural components of the environment effect the growth and survival of juveniles to recruitment.

This work package concentrated of four major tasks:

a) Mapping of Adult and Juvenile Distributions

The overall aim of this task was to first map the distributions of plaice over time and to quantify if any major changes in this distribution have been observed. Specifically we set out to model the abundance of plaice by age classes, over time utilising fisheries independent survey data. Furthermore we aimed to detect if any significant change in distribution over time occurred and if so, in which year did this occur.

b) Fishers' Interpretations

The aim of this task was to present our findings and interpretations of both the fish distributions and the fishery distributions to fishers in order to validate that our data and approaches do not appear biased and that our results seem sensible to those with real world experience of the stock and the fishery.

c) Mapping of Fisheries Activities

This task set out to corroborate the perspectives of the fishers and their representatives that plaice were not as available to the fishery in the Skagerrak as they once were. To enable this investigation we first aimed to identify those metiers that are relevant for plaice in the mixed demersal fishery of the North Sea and Skagerrak. Secondly, we aimed to produce maps of these relevant fishing activities over time to compare where effort and catch efficiency have changed

d) Coastal Juvenile Habitat – A Pilot Study

In this task we aimed to utilise the juvenile sampling procedures developed for fjords and coastal waters of the inner Danish waters to investigate the presence, abundance and distribution of juvenile plaice along the more exposed Skagerrak coast.

3.2 Materials and methods

a) Mapping of Adult and Juvenile Distributions

Data

The North Sea International Bottom Trawl Survey data were utilised for the plaice abundances. Haul level depth and abundances, by age (measured as length distributions with age-length-keys applied), from the quarter-one survey were attributed to the mid-point (latitude and longitude) of the haul and the year in which they took place.

Spatial Distribution

To address the first aim, spatially explicit Generalised Additive Models (GAMs; Wood 2016) were employed to correlate changes in abundance with changes in spatial and depth distribution while accounting for annual variation in the overall population size and different sampling effort. The package `mgcv` (Wood *et al.* 2017) in R (R Core Team 2020) was used to fit the model which was specified as:

$$Abundance_{age} \approx Year + S(lon, lat) + S(Depth) + Offset(\log(Haul\ Length))$$

Where each age is modelled independently, *Year* is a factor to account for annual variation in the observed abundances from changes in population size. *S(lon, lat)* is a Duchlon spline of the two horizontal positions, longitude and latitude with 50 knots. *S(Depth)* is a thin plate regression spline of haul depth. The model includes an offset, *Offset(log(Haul Length)), that* accounts for the variation in haul duration, by creating a 1:1 relationship with abundance and the reported duration of the haul on the scale of the response without the log link.

The resultant model is used to predict abundances across space (within North Sea and Skagerrak) for each year of the study (interpolation, not extrapolation).

Changes in Spatial Distribution

To address the second aim of detecting potential changes in spatial distribution, and when in time any change occurred, a binary variable d was added to the dataset used in the spatial distribution model above, indicating whether a year was included in a "before" or "after" period. The value assigned to each year was modified so that a sequence of datasets were available to fit the model to. The first dataset in this sequence had only the first year (1993) assigned to “before”, and the rest to “after”, while the middle dataset in the sequence had the years 1993-2006 assigned to “before” and the years 2007-2020 assigned to “after”. The spatial model was then modified to incorporate this “dummy” variable of period:

$$Abundance_{age} \approx Year + d + S(lon, lat * d) + S(Depth * d) + Offset(\log(Haul\,Length))$$

Such that the dummy variable in the independent position accounts for the differences in mean absolute abundance at a point “before” or “after” some hypothesised break point. By including the dummy variable as an interaction in the spatial smoother and the depth smoother we allow the horizontal and vertical distributions of plaice to be estimated independently for each of the periods in these “before” and “after” periods.

The sequence of datasets with the changing year in which the dummy variable transitioned from “before” to “after” were used to fit the model for each age, independently. Within each age, the Akaike Information Criterion (AIC; Akaike 1998) was used to select the most parsimonious model, i.e. the dataset with the before/after sequence that produced the best fitting model.

To ensure that the most parsimonious fit was not biased to select for a date in the middle of the time-series (with even data on each side), we undertook a sensitivity analysis, where we removed either the beginning or the end of the time series and re-ran the above procedure to find the most parsimonious model.

b) Fishers’ Interpretations

Originally, group interviews were planned, where fisheries representatives and researchers would jointly host fishers from the Skagerrak. Unfortunately due to project delays, the lead competence for this task retired before any group interviews could be undertaken.

To replace the group interviews, a workshop was organized together with representatives from the fishing industry in which the project’s methods, results and conclusions were presented and discussed. The fisheries representatives were free to ask questions and probe the evidence and justification for our conclusions, and the workshop was used to get important insights from professional and recreational fishermen to supplement the work presented in this report.

c) Mapping of Fisheries Activities

To address the first aim of identifying the appropriate metiers for tracking the plaice fishery, yearly average landing values by DCF métiers were found for the period 2005-2019. Métiers where the average landings value of plaice exceeded 30% of the total landings value of that métier, or métiers where the average value of landings of plaice exceeded 100.000 DKK during the period were selected as being significant for the plaice fishery.

To map the distributions of these relevant metiers over time we utilised Danish VMS logbook and VMS data. Fishing trips by Danish vessels from the North Sea and Skagerrak were selected from logbooks, coupled with VMS data for the period 2005-2019, and filtered by speed to only keep positions where fishing activity are assumed. During the years 2005-2011 VMS was mandatory on fishing vessels larger than 15 meters, from 2012 onwards it has been mandatory on fishing vessels larger than 12 meters.

The métiers have been split into active and passive gears because they have different effort measurements. For active gears, the fishing effort is measured as vessel power multiplied by fishing time (kilowatt hours, kWh), while for passive gears the fishing gear effort (soaking time) is not known from the available data, so the vessel fishing effort as fishing hours is used as effort measurement.

The CPUE per vessel and date was calculated as:

- Active gears:
    - Plaice CPUE = KG PLE/kW*Fishinghours
    - Total CPUE = KG TOT/kW*Fishinghours
- Passive gears:
    - Plaice CPUE = KG PLE/Fishinghours
    - Total CPUE = KG TOT/Fishinghours

The average daily CPUE per vessel and date was merged to the VMS positions averaged in a 5 km^2 grid and mapped by year. The plaice landings per vessel and day are distributed on the VMS positions with fishing activity summed.

d) Coastal Juvenile Habitat – A Pilot Study

Sampling Design

Five locations along the coast between Hantsholm and Hirtshals were selected with a combination of positions within the middle of a bay or at one end of a bay (Figure 3.10). At each location five depth strata were planned for sampling: 0-1m, 1-2m, 2-3m, 3-4m, 4-6m.

Sampling Procedure

On July the 25th, 27th and August the 13th of 2020, the vessel *Havørreden* (eight metres, 110 hp) was used to tow a juvenile beam trawl (two metres wide, five millimetre stretched mesh, single tickler chain in front of the ground rope, at approximately one knot, for three minutes and twenty seconds) at each depth strata, at each location. The planned 0-1m stratum was only accessible at one location and the 1-2m stratum was not accessible at all locations, due to the surf break. For this same reason it was not tenable to land the vessel and undertake the sampling of the shallowest stratum by hand, as was normal procedure. Due to few fish being caught in the shallower depths, stations were added at ~6m and ~8m depth.

GPS positions were recorded at the deployment and retrieval sites of the trawl, together with date and times. While the trawl was deployed and fishing, environmental parameters were recorded from just above the sea floor, using a Multi 3620 IDS hand held meter with FDO® 925 and TetraCon® 925 probes (WTW). Environmental conditions recorded were: temperature (°C), salinity (practical salinity scale), oxygen concentration ($mg{\cdot}L^{-1}$) and oxygen saturation.

Flatfish under 18cm were killed using and overdose of benzocaine dissolved in seawater ($250mg.L^{-1}$), larger flatfish were measured for total length and released while non-target species were counted and released. The retained fish were individually labelled and bagged, stored on ice in the vessel and frozen down at the end of the day until processing in the laboratory.

Fish Identification

The identification of juvenile flatfish can be difficult, especially distinguishing between European plaice and flounder, or between turbot and brill. Therefore, morphometric analyses were undertaken to properly identify young of the year fish to species level. Traits such as mouth shape, body shape, colouration and eye-placement were used to identify species to the family level, then a combination of dorsal, anal and caudal fin-ray counts were used to distinguish between con-familials. Differentiation was based on counts from Haynes (2011) and corroborated by Munk & Nielsen (2005).

3.3 Results and Discussion

a) Mapping of Adult and Juvenile Distributions

Spatial Distribution

The plaice is distributed in space differently depending on age. Young plaice (age 1) is mostly gathered close to the coast along the south-western Danish coast, the German bight and the Southern bight. Ages 2-4 are concentrated in deeper parts further away of the coast of the same areas, but also in Skagerrak and in the area off Scotland and Shetland islands. Plaice of six years and older are mainly observed in Scottish waters (Figure 3.1).

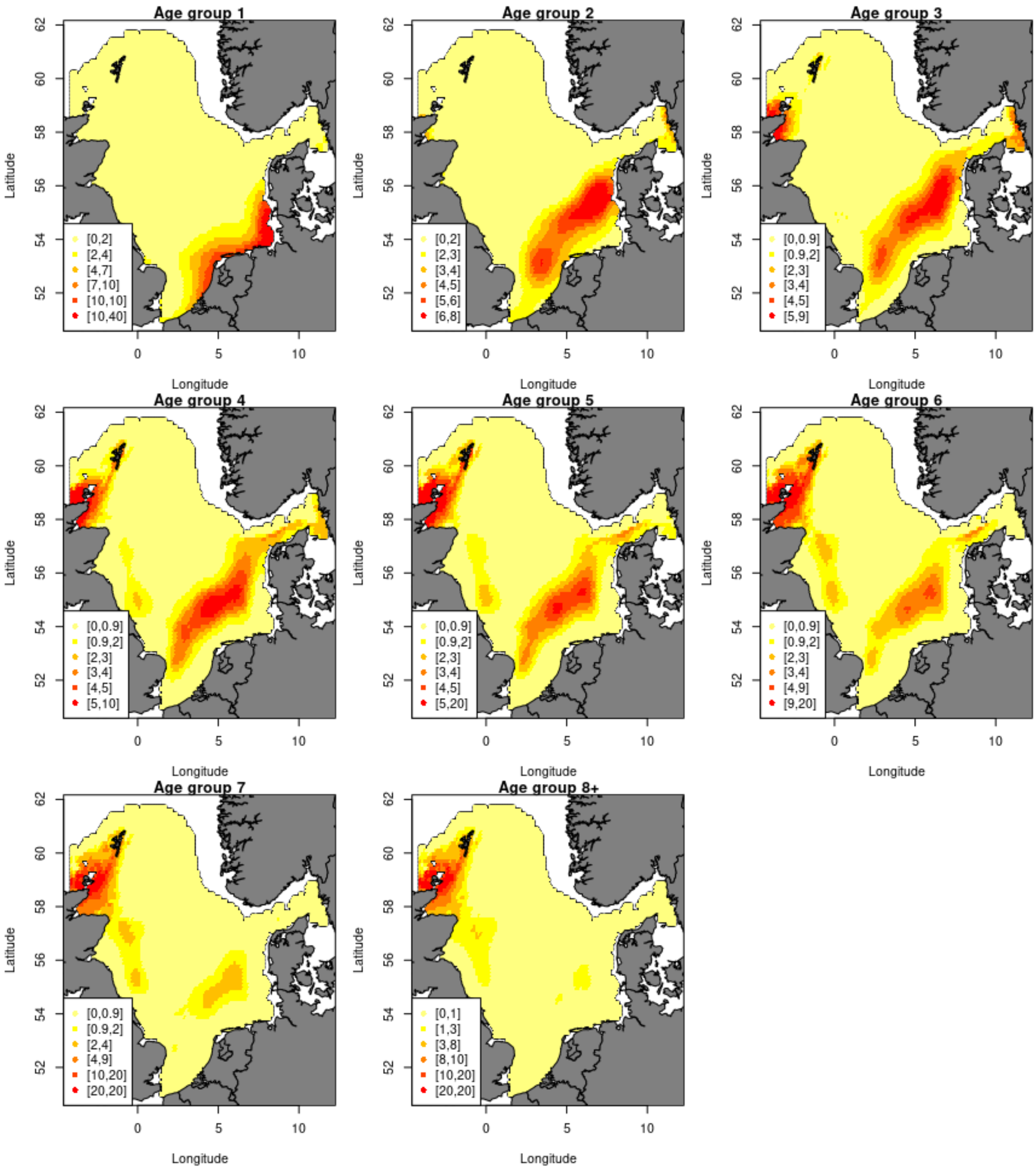


Figure 3.1. Plaice distribution by age, combined for all years. The colour indicates concentration of plaice, increasing from yellow to red.

Changes in Spatial Distribution

The models with different distributional change assumption were ranked according to AIC. For each age, distribution changes seem to have occurred in different years, with younger ages having the change earlier. Young age one plaice appear to have shifted in distributions around 2000. Ages 2-6 around 2006-2007 and older ages in mid 2010s (Figure 3.2). Models that have AIC values close to the best model, i.e., AIC difference lower than six compared to the best model, are shown in Figure 3.2 as smaller full circles as they are also plausible alternatives.

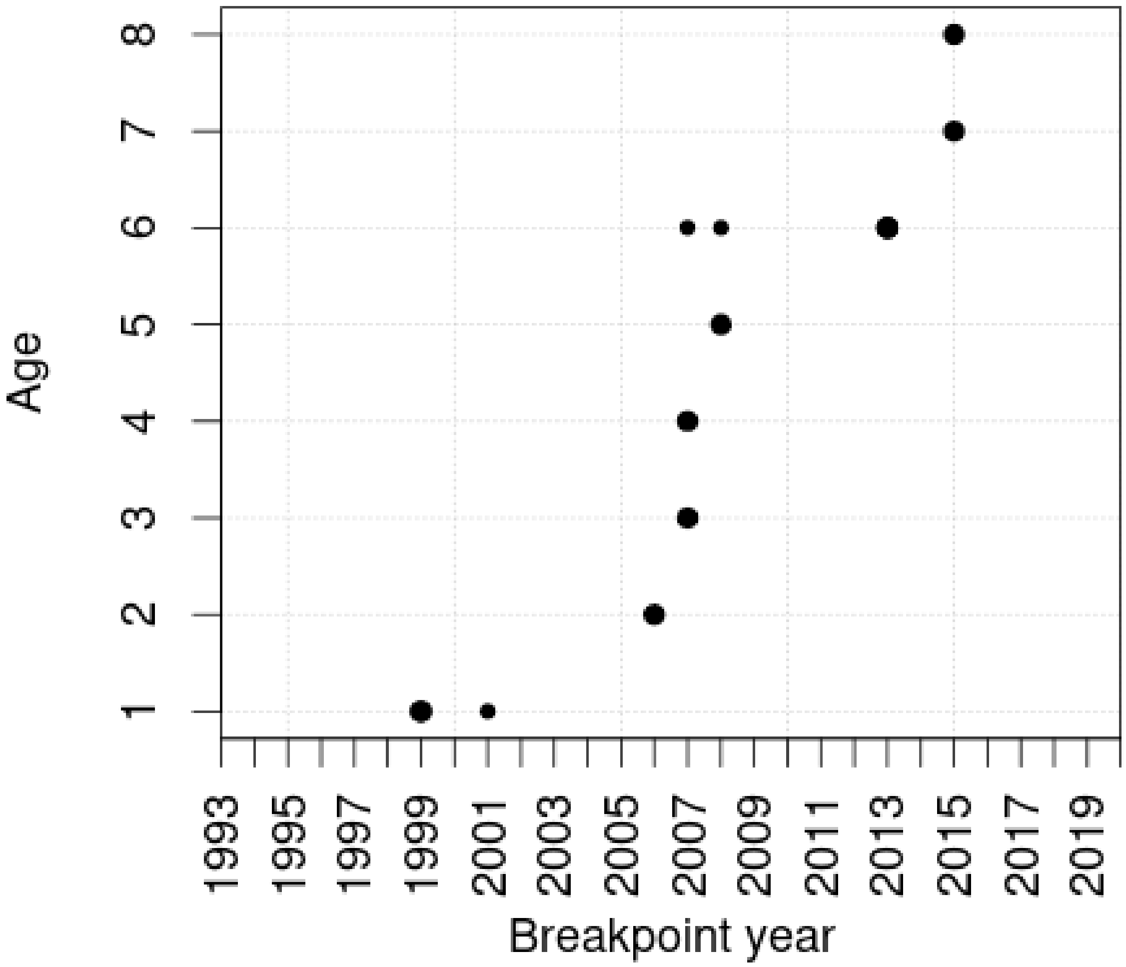


Figure 3.2. The year of most probable distribution changes by age. The best model is shown with larger full circles and alternative similarly plausible models with smaller circles.

The distributions before and after 2007 are presented in Figure 3.3 for ages 1-4 and in Figure 3.4 for ages 5-8. Young plaice of age one is more spread and expanded to more offshore areas comparing their distribution before and after 2007. Plaice distribution for ages 2-5 has moved significantly further away from the Danish coast. Plaice of ages 3-6 are no longer found in the Skagerrak in the abundances that they were before 2007.

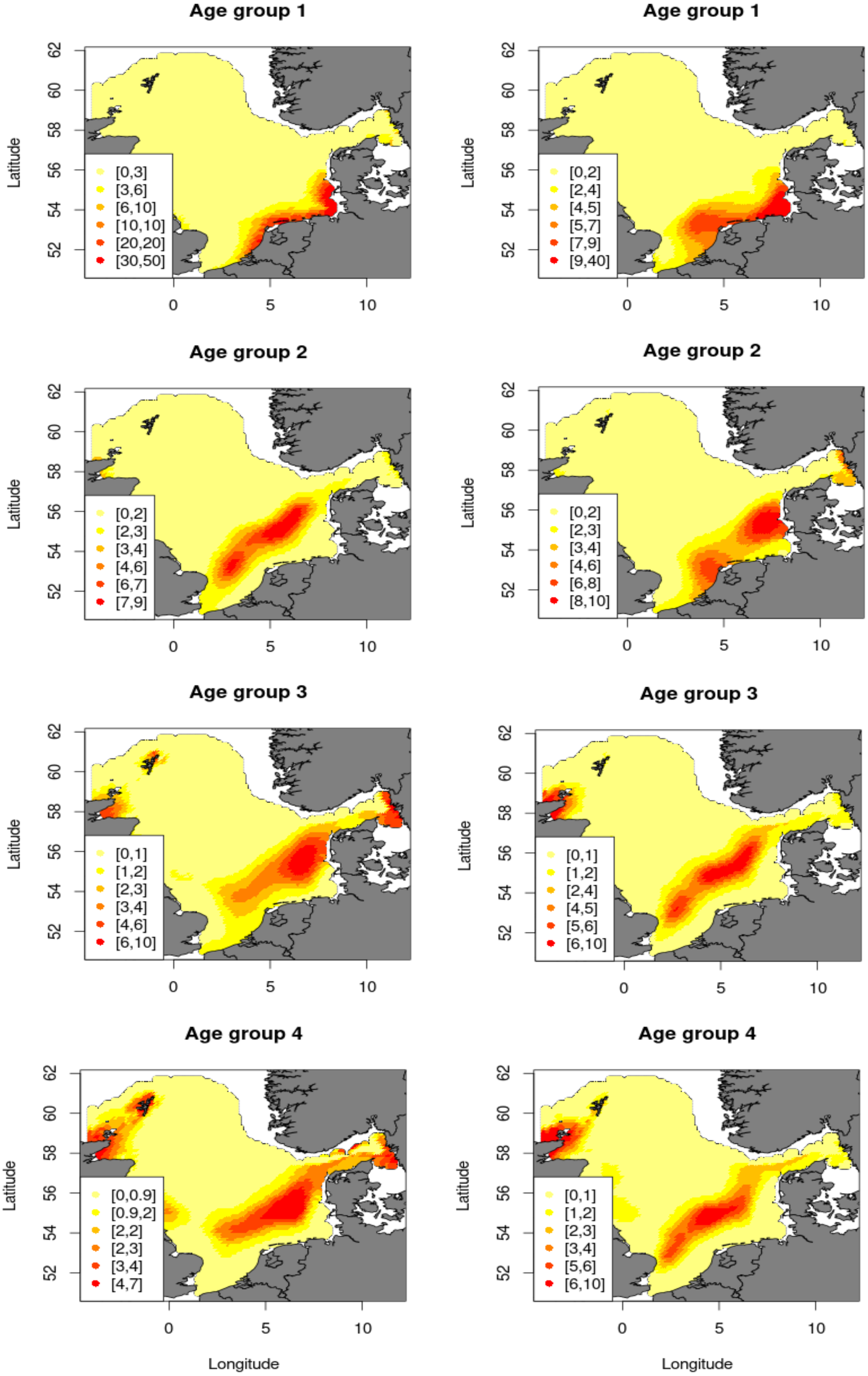


Figure 3.3. Distribution of plaice before 2007 (left) and after (right) for ages 1-4 (from top to bottom).

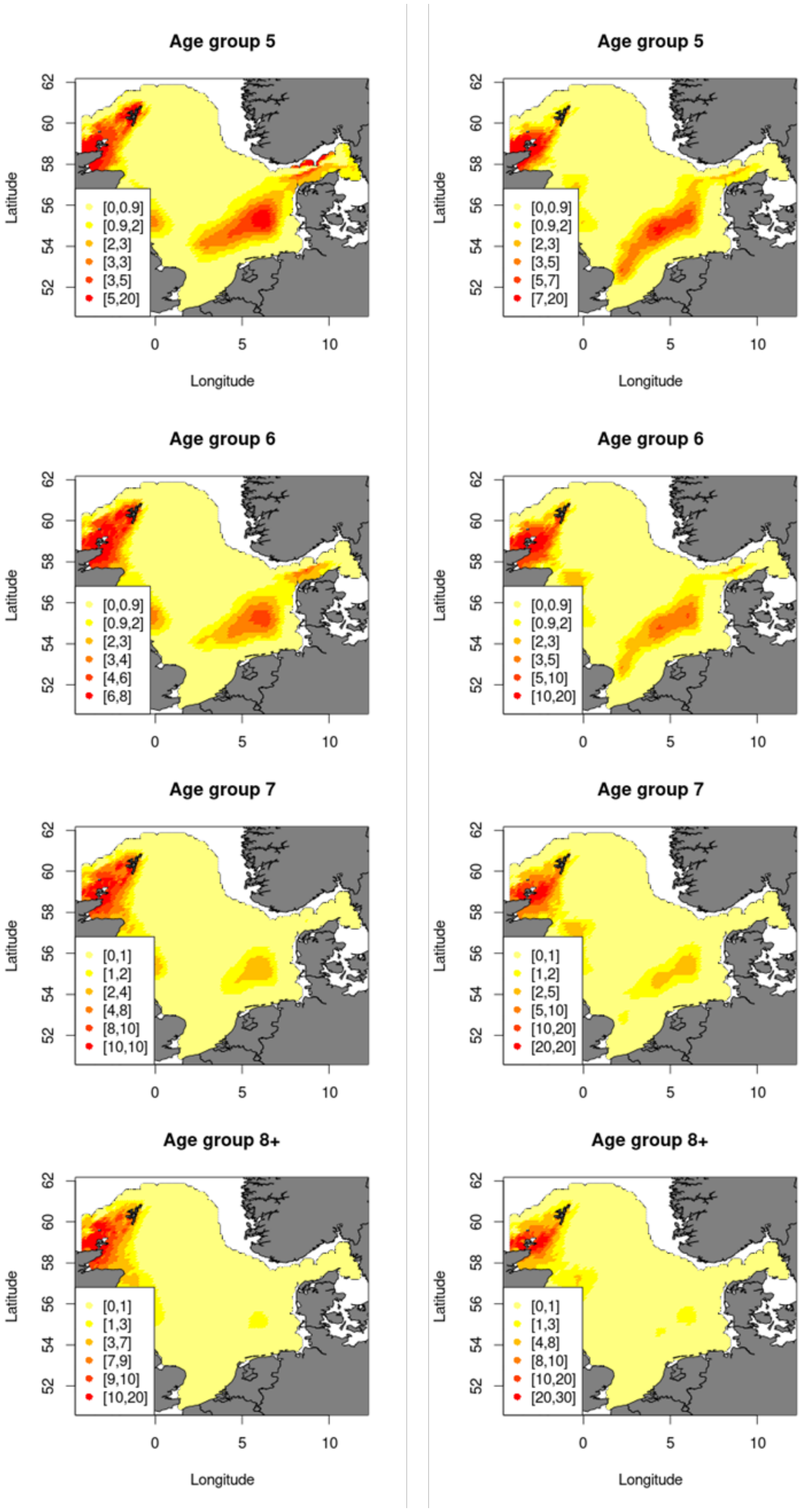


Figure 3.4. Distribution of plaice before 2007 (left) and after (right) for ages 5-8 (from top to bottom).

Finally, we present the changes in depth distribution for the two periods, before and after 2007 (Figure 3.5). The partial depth effect from the GAMs indicates that plaice is now in deeper water than before 2007.

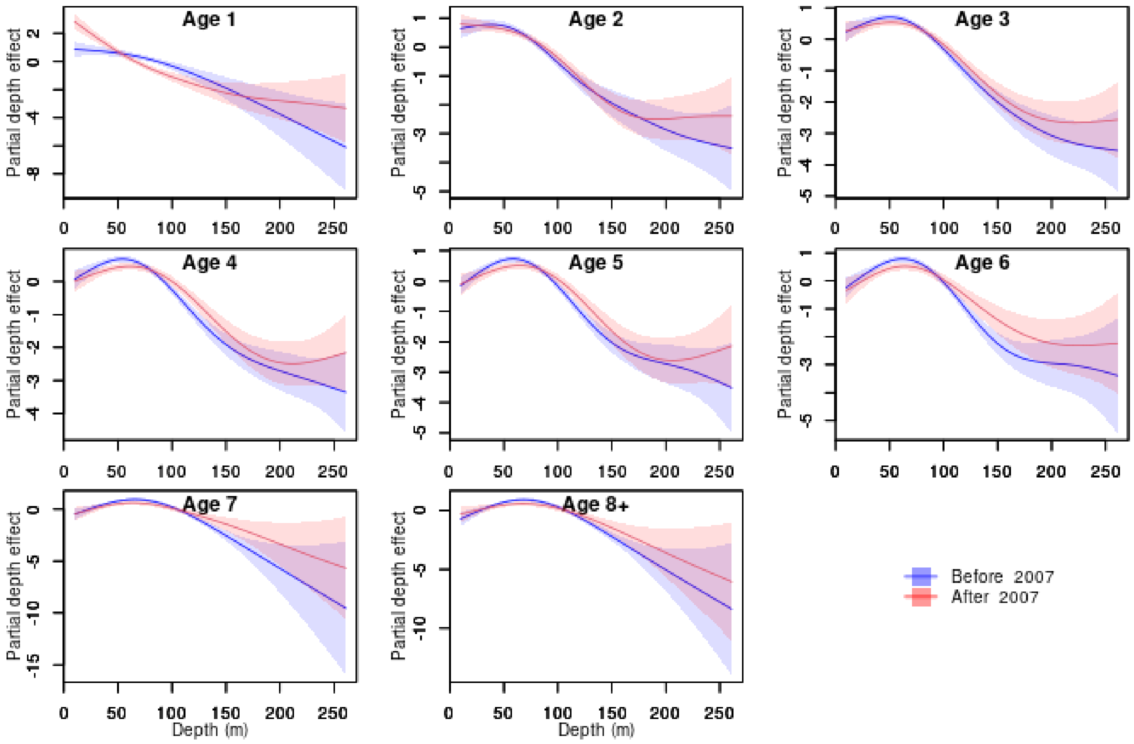


Figure 3.5. Partial depth effect from the GAMs before 2007 (blue) and after (red lines). The shaded areas show 95% confidence intervals.

Discussion

Here we investigated the spatial distribution of plaice in the North Sea and Skagerrak. It is apparent that the distribution of plaice has shifted over the years to deeper waters further away from the West coast of Denmark and with much reduced presence in Skagerrak. The aim of the modelling presented here was to map the distribution of plaice in different ages, but not to identify drivers that led to the observed distribution or to the distribution changes. Further work is needed to investigate such drivers. Potential candidates are environmental conditions (e.g., temperature), predators or prey, and fishing pressures.

b) Fishers' Interpretations

At early stages of the work, we had multiple, more informal, interactions with individual fishermen, where we discussed the apparent mis-match between stock assessments, advice and the perceptions of the stock status by fishermen. These helped formulate the more specific objectives of the work.

A more formal workshop was arranged towards the end of the project to provide the opportunity for both individual fishermen and their organizations to provide feedback on project results, and to discuss ways forward (Table 3.1). This provided a unique opportunity to "pressure test" some of the conclusions from the project. Six representatives from four organisations attended the workshop and provided direct feedback. In addition, the material presented at the meeting was circulated to all registered participants, one of which was not able to attend on the day of the workshop, to allow for broader impact and contributions from a broader group.

Table 3.1. Registered participants for FORFLAD workshop at DTU.

Organisation	Representatives	Representative Position
Danmarks Fiskeriforening Producent Organisation	1	Biologist
Dansk Amatørfiskerforening	1	Chairman
Danmarks Sportsfiskerforbund	1	Biologist
Foreningen for Skånsomt Kystfiskeri Producent Organisation	1	Biologist
Gershøj Fritidsfiskerforening	3	Chairman and members

While the finer resolution of individual fisher's responses were not collected, the representatives of the fisheries organisations (who are/were also fishers themselves) provided valuable feedback to the project. The workshop was characterized by a constructive dialogue and also allowed the project to disseminate project results more broadly.

c) Mapping of Fisheries Activities

The mixed nature of the fishery that includes plaice is evident in the proportions of species landed by metier, from only those metiers we determined to be of significance for plaice (Figure 3.6).

Five to six of the selected metiers were responsible for large portions of the total plaice landings value (Figure 3.7). There were many metiers with low absolute values landings values, but were retained as they received greater than 30% of value from plaice landings, indicating that it is a relatively important species for their operation (Figure 3.8).

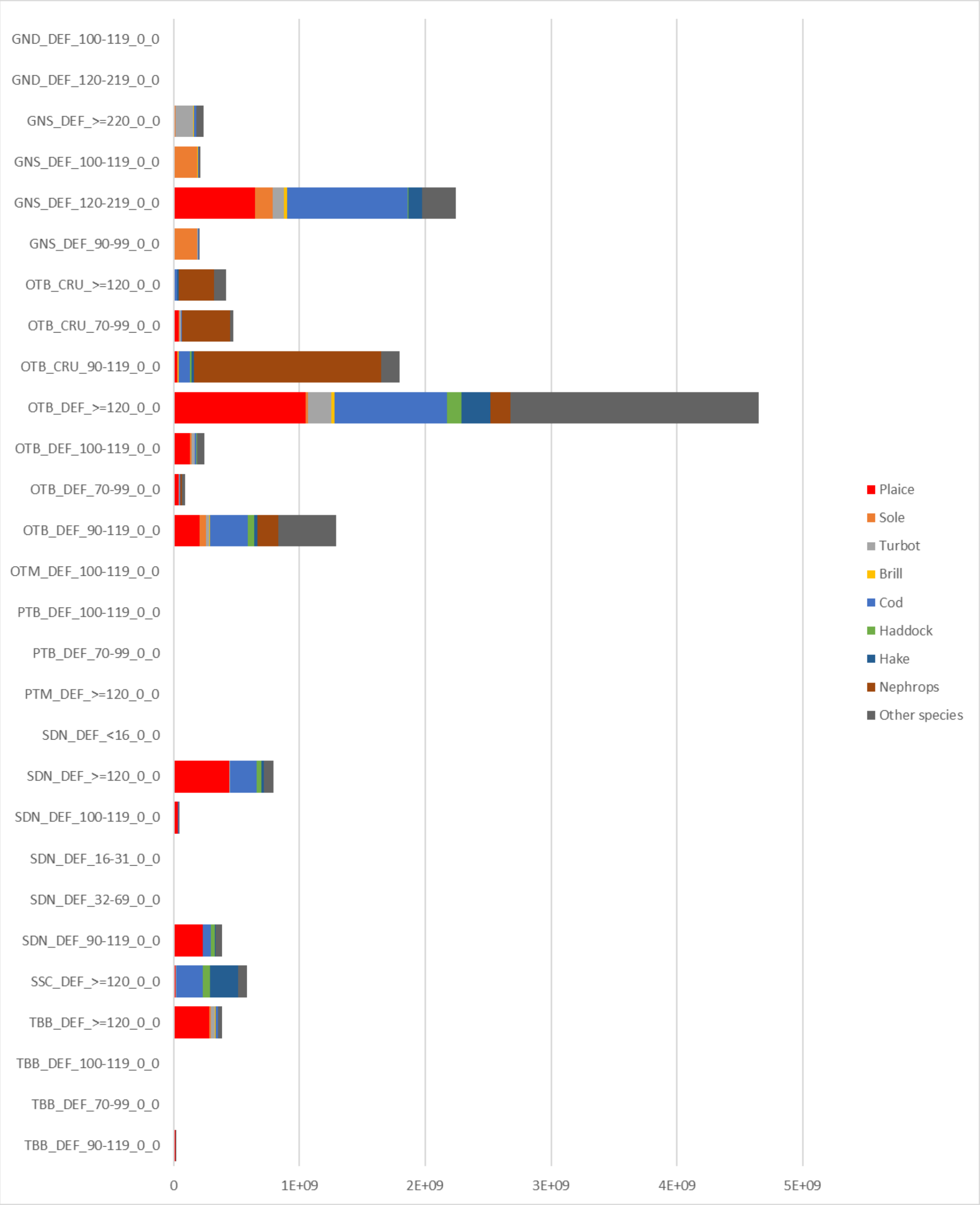


Figure 3.6. Landings values (DKK) by selected metier, coloured by species for Danish vessels of the Northern North Sea/Skagerrak in the period 2005-2019. Metier were included based on absolute value of plaice landings and proportions of landings value from plaice.

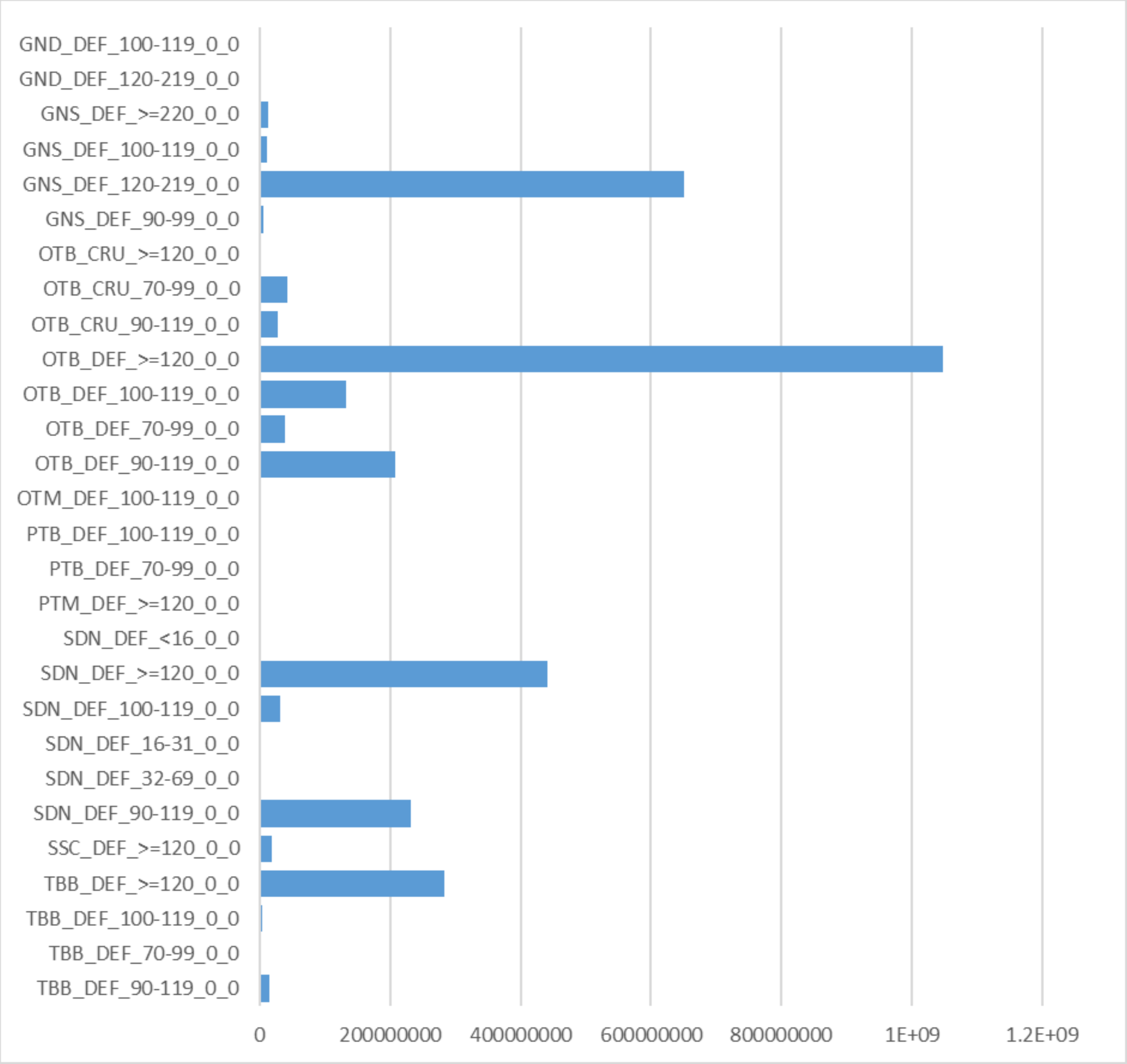


Figure 3.7. Landings value (DKK) of plaice from Danish vessels of the Northern North Sea and Skagerrak by select metiers, most relevant to the fishery for plaice.

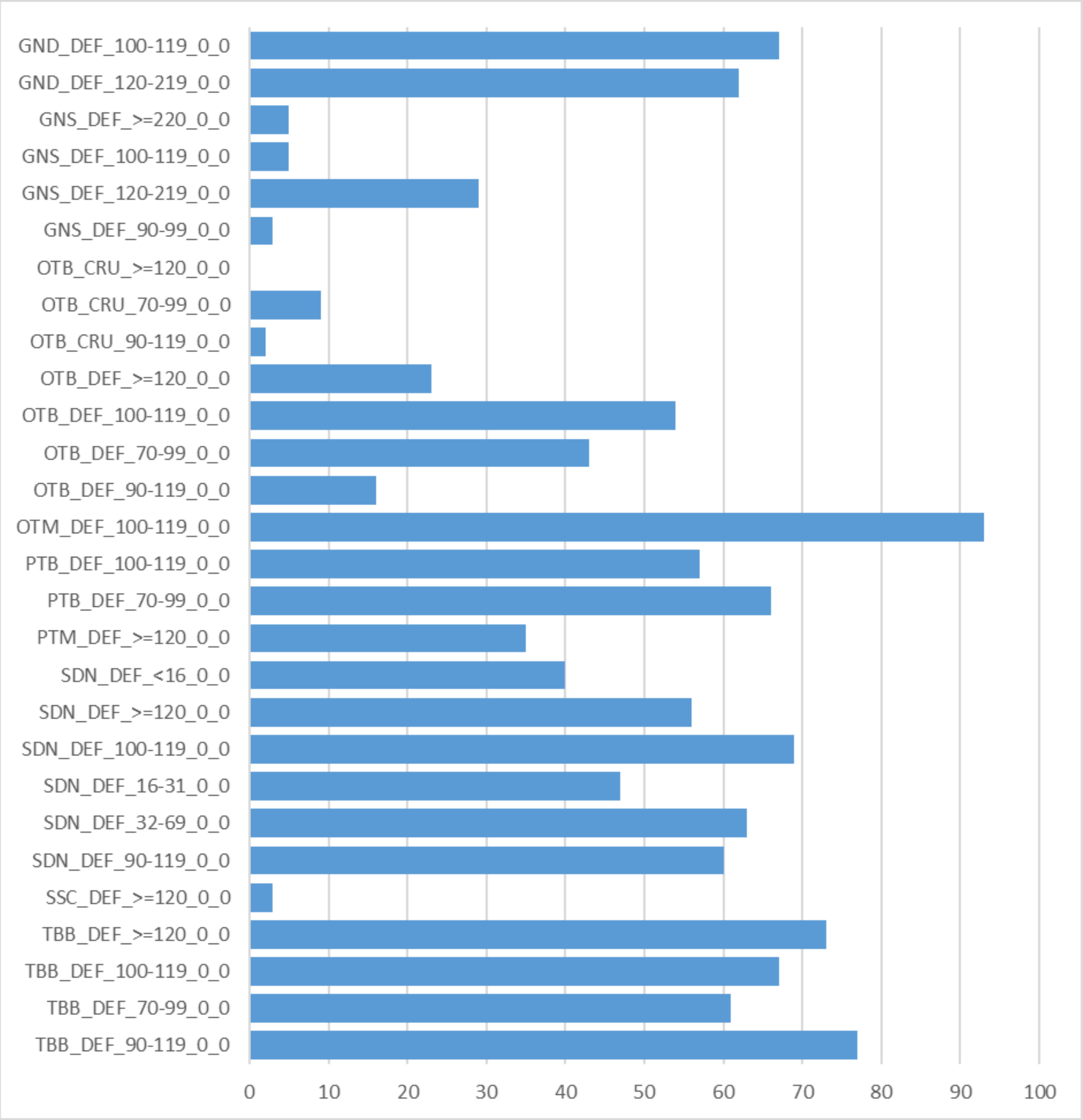


Figure 3.8. Percentage of landings value from plaice, by selected metiers, from Danish fishing vessels operating in the North Sea and Skagerrak over the period 2005-2019.

Series of annual maps were produced (see "Appendix (plaice)"), showing the landings and CPUE for plaice and all species in both active and passive gears. While inter-annual variation can be observed in the distribution of effort and CPUE, there is not a monotonic (unidirectional) trend in where the fishery either operates or catches plaice more efficiently (Figure 3.9). In the whole time series it appears more plaice were being caught in offshore waters through the middle of the period in question, while at the end of the period, the distribution of CPUE was back to what was experienced at the beginning.

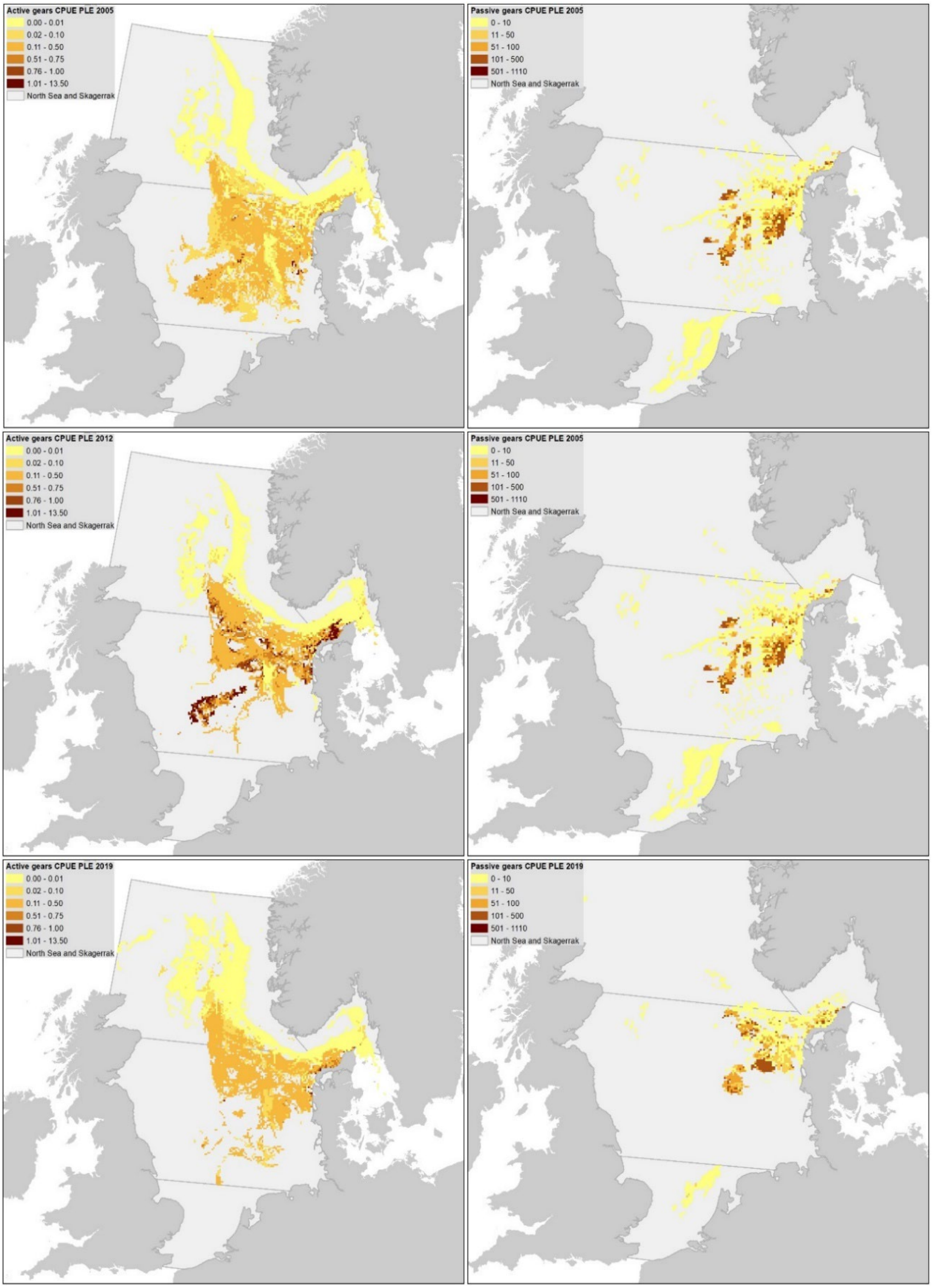


Figure 3.9. Catch Per Unit Effort for selected active gears (left column) and passive gears (right column), from the beginning of data availability (top row = 2005), the middle of the data availability period (middle row = 2012), and the end of the study period (bottom row = 2019).

Discussion

While we were able to isolate those metiers which contribute most to the plaice fishery, we were not able to see the trends in catchability that fishers have described. This is most likely due to the fact that the time-series of data from VMS only allows us to investigate back to 2005, which is about the point in time that fishers have identified as the turning point in the availability of plaice for the fishery

d) Coastal Juvenile Habitat – A Pilot Study

Thirty-two sites were investigating at the five locations (Figure 3.10). The majority of hauls were made in the 2-4m range, while some hauls were extended out to approximately eight metres to investigate the presence of juveniles in the deeper waters of the open bays (Figure 3.11).

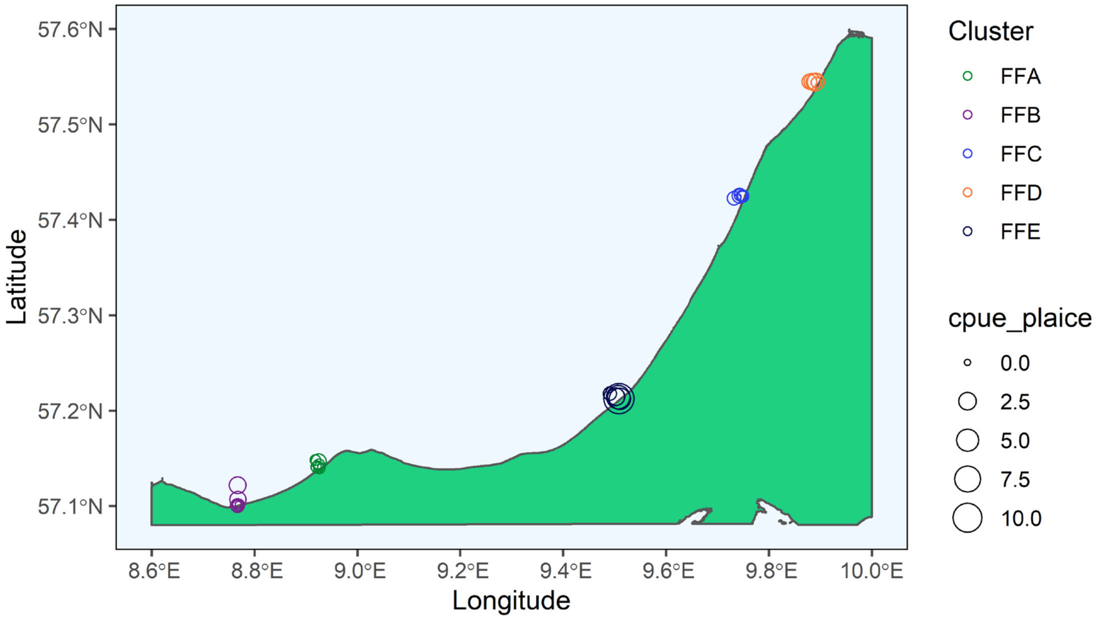


Figure 3.10. Map of study sites for the pilot study of a juvenile fish survey on the open Skagerrak coast. Colours represent the different locations along the coast, while the size of the circle represents the number of individual plaice caught at each sampling station.

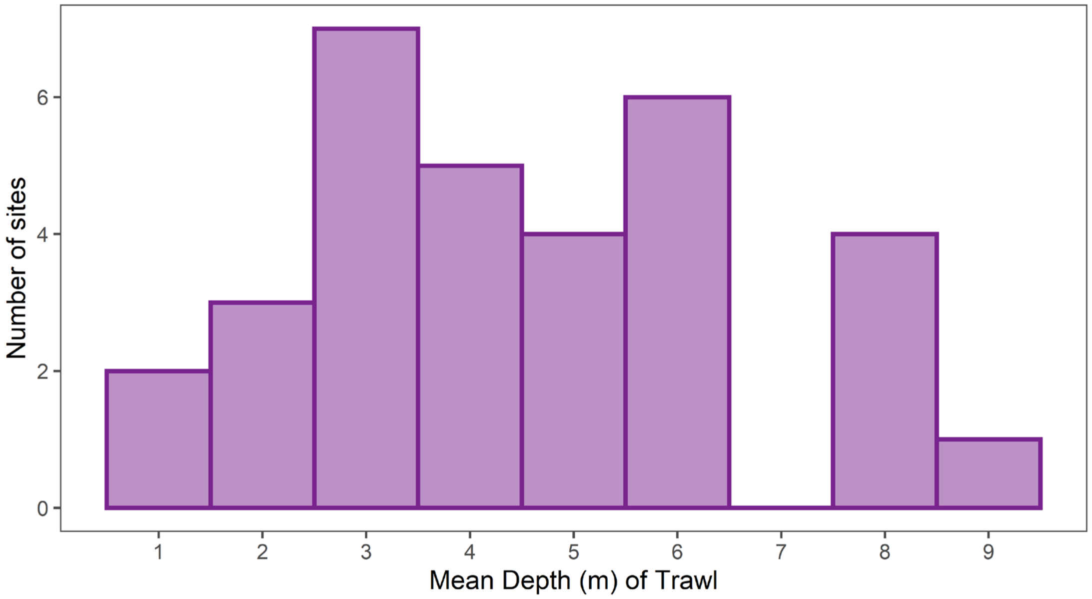


Figure 3.11. Frequency of depths of sampling stations, where the mean is the mean over the duration of the haul and these are binned into one metre categories.

Of all juvenile fish caught in the survey, juvenile plaice made-up the vast majority by number (Figure 3.12). The open coast along the study area had higher rates of occurrence of plaice, compared to sites investigated in the west coast fjords in another study (Figure 3.13). However, the highest densities observed along the Skagerrak never reached the densities seen at some sites in the fjords.

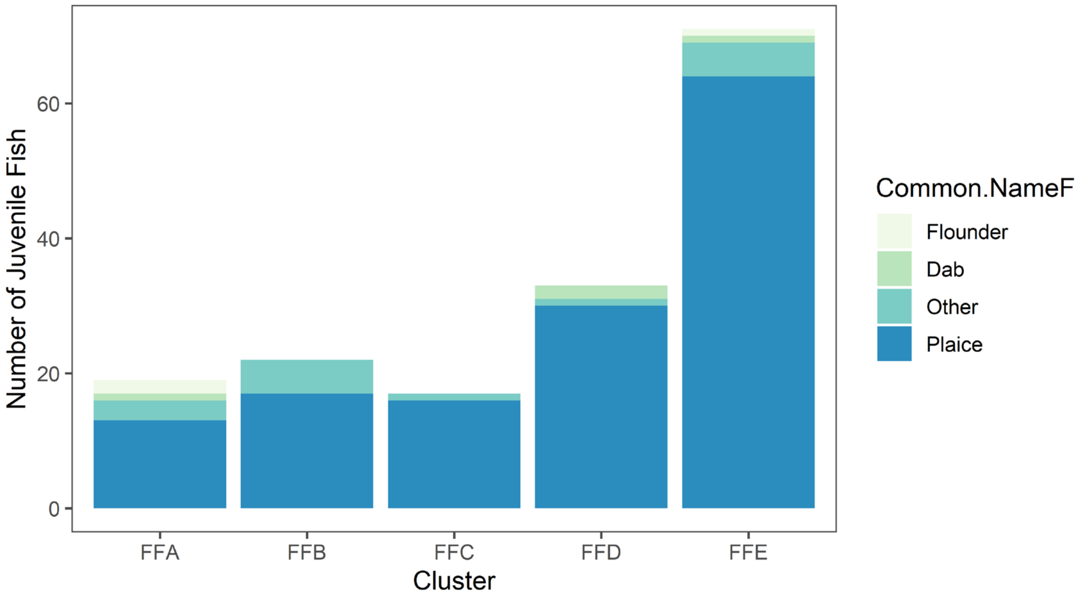


Figure 3.12. Numbers of juvenile fish caught per location (summed across all sampled depths). Colours represent different species (where "Other" is mostly juvenile roundfish that were not identified to species level). The cluster codes correspond to those on the map in Figure 3.10.

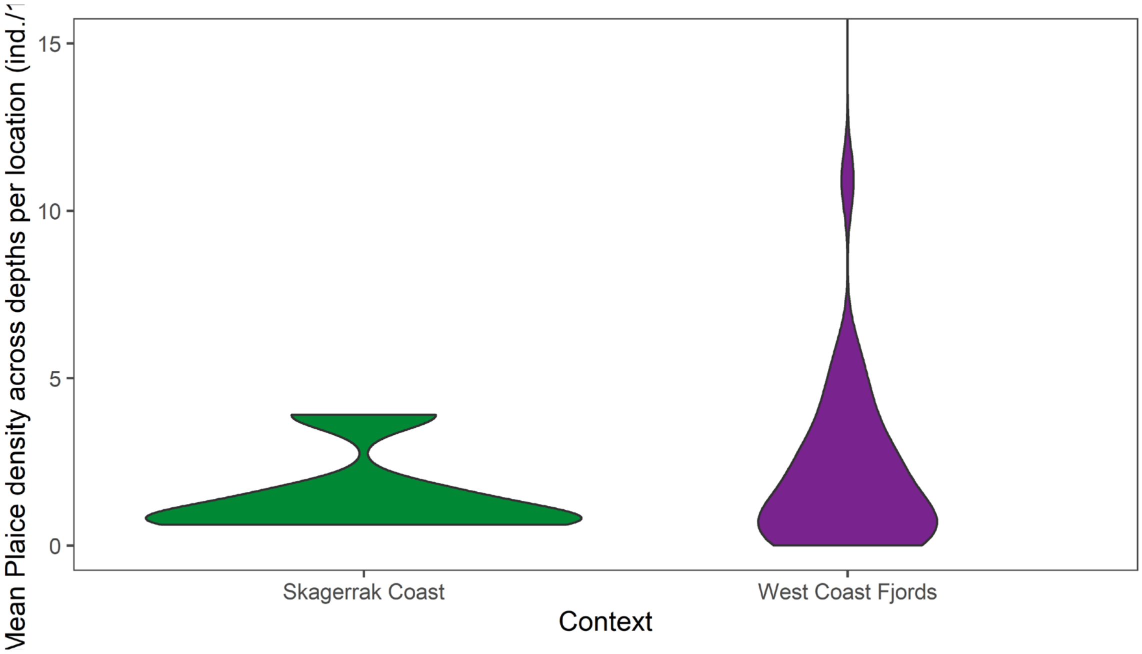


Figure 3.13. Density (individuals per 100 m2) of plaice juveniles caught along the open Skagerrak coast (this study) and within the neighbouring west coast fjords (External Study). Widths of "violin" figures represent the number of observations at the given density.

Greater depths were sampled than normally investigated in studies of juvenile habitat. The densities of plaice appear relatively uniform across the depths investigated. There were too few data in the shallow depths to try and model changes in juvenile plaice density over depth (Figure 3.14).

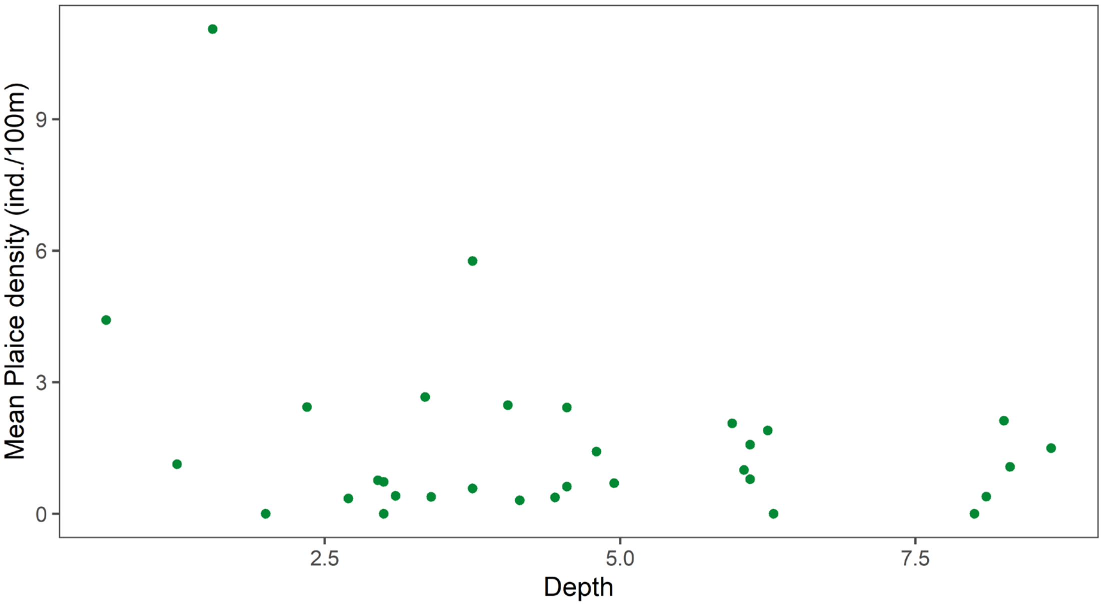


Figure 3.14. Densities of Juvenile plaice sampled over various depths along the Skagerrak coast.

Discussion

This pilot study suggests that plaice are the dominant species in the coastal juvenile habitats of the Skagerrak coast and that they can be found over a larger range of depths than has been indicated in earlier work.

The densities of plaice observed in this pilot study on open coast habitats are similar to those found in the west-coast fjords (based on data from another project). Juvenile plaice were more routinely found on the open coast (fewer zero catches), which is expected given the more uniform nature of the habitat compared to the diversity found in in the brackish fjords. Interestingly, some of the locations in the fjords produced much higher densities than were observed on the open coast. This is probably due to the aggregation effects of juvenile plaice at the entrance to the fjords, where the combination of suitable salinity and higher food availability can support higher densities, however, this is conjecture based on preliminary work in other studies.

In order to produce recruits to support a commercial fishery, suitable juvenile habitats that support the survival, growth and eventual connectivity to the adult population are required. While the high densities observed in the fjord entrances seem like logical and efficient places to protect to ensure the production of recruits to the fishery, their relatively small area means that the absolute production of recruits is probably relatively low. These smaller areas of extraordinary densities are defined as “nurseries” (Beck *et al.* 2001). Conversely, large areas of medium or even slightly below average densities of juveniles may contribute large proportions of the recruits that support fisheries (Dahlgren *et al.* 2006).

To better understand the contributions of these large, open-coast and smaller enclosed brackish juvenile habitats, further research is needed. To properly quantify the quality of these habitats and their value for local fisheries, we must understand survival, growth and connectivity of juveniles in these habitats. In more detail, how different habitats effect the survival of settling larvae through to recruitment, the growth rates of juveniles over their time in the juvenile habitats, and the proportion of the fish caught in various fisheries that come from these different habitats.

Currently, DTU Aqua has samples to support investigations into the value of the various west coast fjords as juvenile fish habitat, via analyses of pre-collected otoliths. However further sampling is required on the open west coast habitats to quantify these areas’ importance for producing juvenile plaice. This pilot study has proven that while existing methods are feasible for most of the coast, supplementary methods need development to better sample the shallow surf zone of the west coast beaches.

3.4 Conclusions

To address our main hypotheses: we have detected that there was a significant shift in spatial distribution of plaice, in the key ages exploited by the fishery, in the period 2006-2007. This corresponds to lower abundances both in the coastal North Sea (which corresponds with the loss of plaice from the plaice box) and lower abundances in the Skagerrak.

The data on fisheries effort and distributions only started in 2005, and we are therefore unable to determine if there was a corresponding spatial shift in effort, or if fisheries effort remained constant in space over this period of shifting fish distributions. One reason that fisheries might not respond to a shift in fish distributions may be that vessels were limited to their normal range of operations, which prevented them fishing in areas further afield, where plaice were now more abundant. If this case is true, then the movement of plaice could mean lower catches or having to fish at the extremes of their normal areas of operations in order to catch plaice efficiently. A second reason that fisheries might not respond to a shift in plaice distribution, specifically, is because plaice is a relatively low value species caught in a range of mixed fisheries. These fishers are unlikely to change fishing behaviour in an attempt to catch plaice at the expense of catching higher value species.

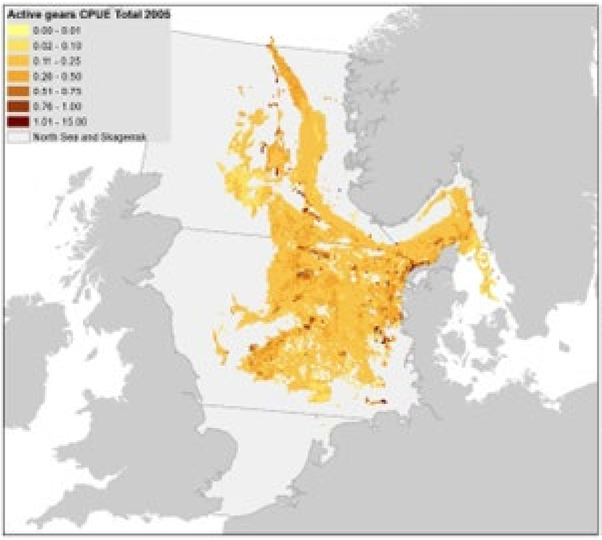
Therefore, it is our evaluation that a shift in plaice distributions around the year 2006 led to a lower level of coincidence between plaice and established fisheries. This lack of overlap between fish and fishers, helps to explain the incongruous observations that the population size is large and in some cases increasing, while fishers are not able to catch their allowed quotas.

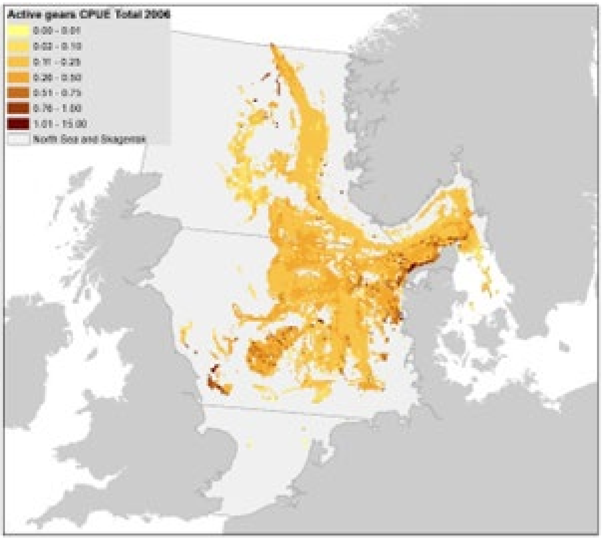
While we are able to document this shift in space, we have not yet been able to attribute a cause to this shift. There is much literature on the movement of temperature limited species to deeper water to avoid higher temperatures in shallow areas, however our results do not detect a significant change in depth corresponding with the shift in distribution. There may be other environmental factors that are driving this shift in distribution and future work should integrate environmental covariates from either observations on surveys or newly available hydro-bio-geochemical models.

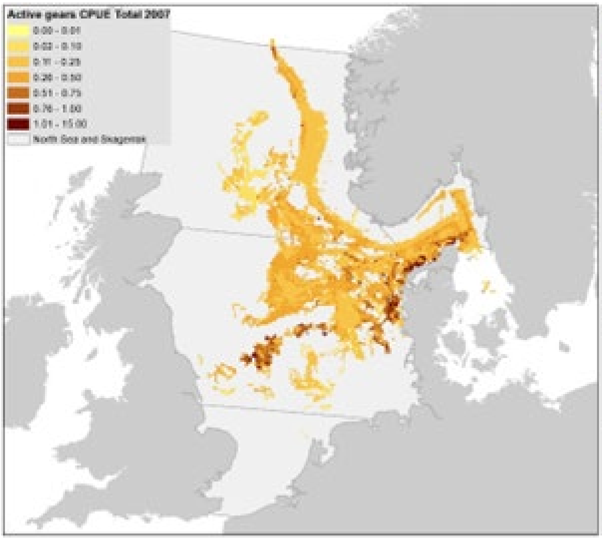
Furthermore, fisheries themselves play a role in depleting local populations, and therefore the effect of fishing effort in space should be accounted for in future work to identify drivers of this plaice distribution shift. The Skagerrak is an area of known population mixing, with an overlap of a North Sea component and a presumably smaller local Skagerrak/Kattegat component (Ulrich *et al.* 2017). As such, a risk of local depletion was highlighted as a potential effect when merging the Skagerrak with the larger North Sea area for stock assessment and management (Hemmer-Hansen *et al.* 2015; Ulrich *et al.* 2017). Because the largest changes in distribution appear to be in shallower, more coastal regions, the extractions made by small scale fisheries may be important for explaining local reductions in abundance. To properly quantify fishing pressure from smaller vessels in the small-scale fisheries, better methods are required. The methods used in this study rely on speed alone, which provide some insight, while models incorporating speed, turning angle and other variables available from AIS may be more accurate.

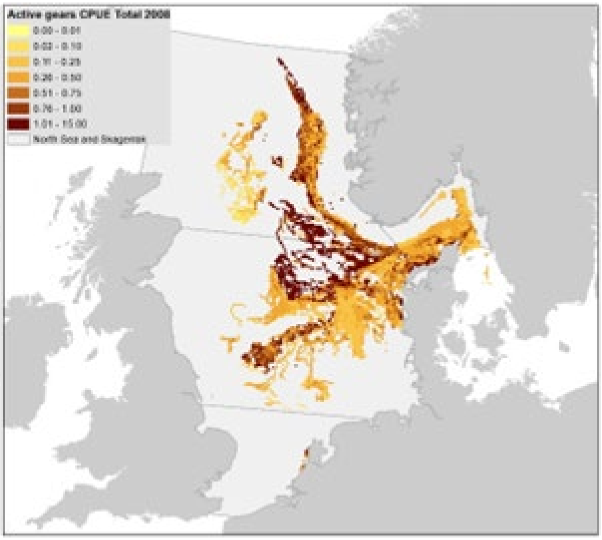
Because plaice relies on shallow, coastal juvenile habitat to produce recruits, the documented distribution shift could have implications for life-history stage connectivity and the importance of various areas or habitats for sustaining the population. Therefore, future work on the productivity of various juvenile habitats (namely larval supply, growth, survival) and their contributions to the fishery should be prioritised to inform both spatial management and better models of recruitment for stock assessment.

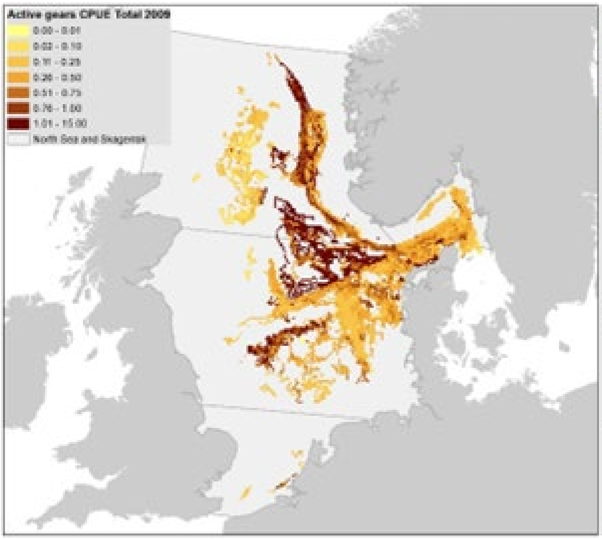
3.5 Appendix (plaice)

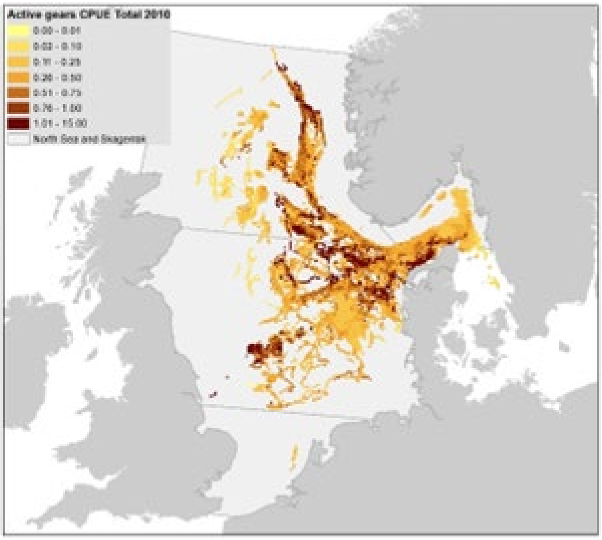


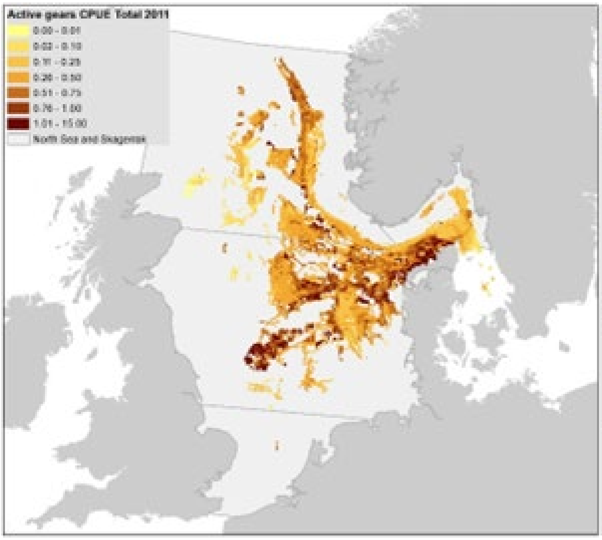








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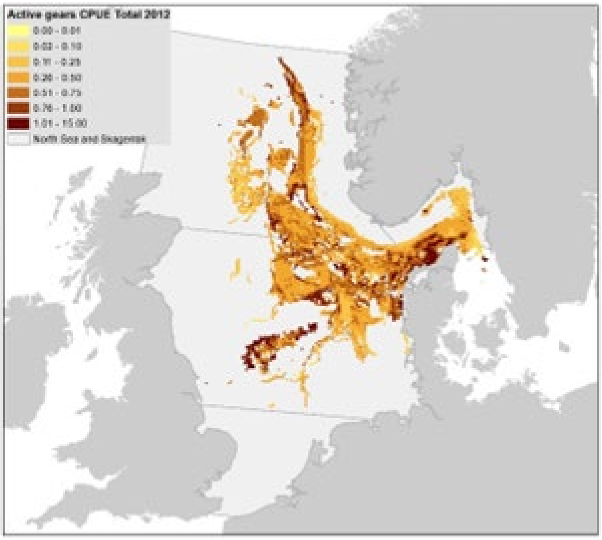
North Sea and Skagerrak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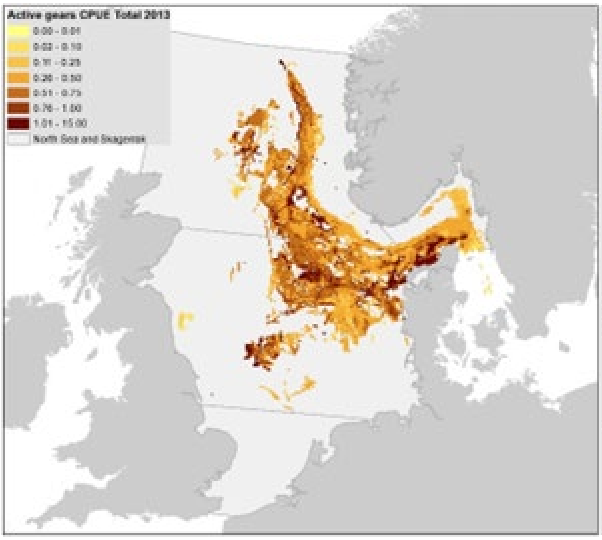
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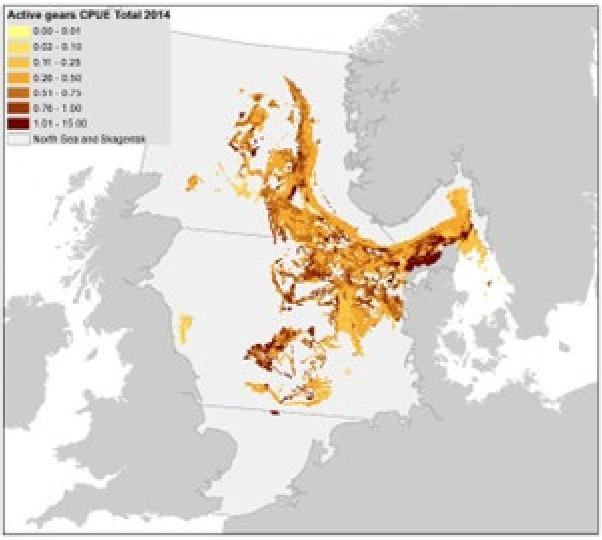
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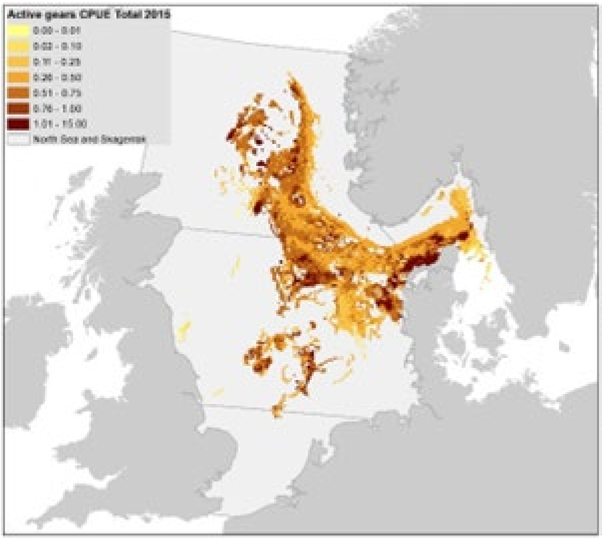
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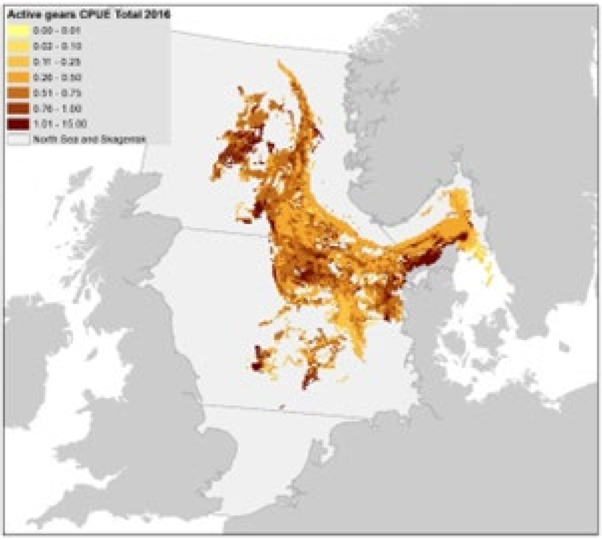
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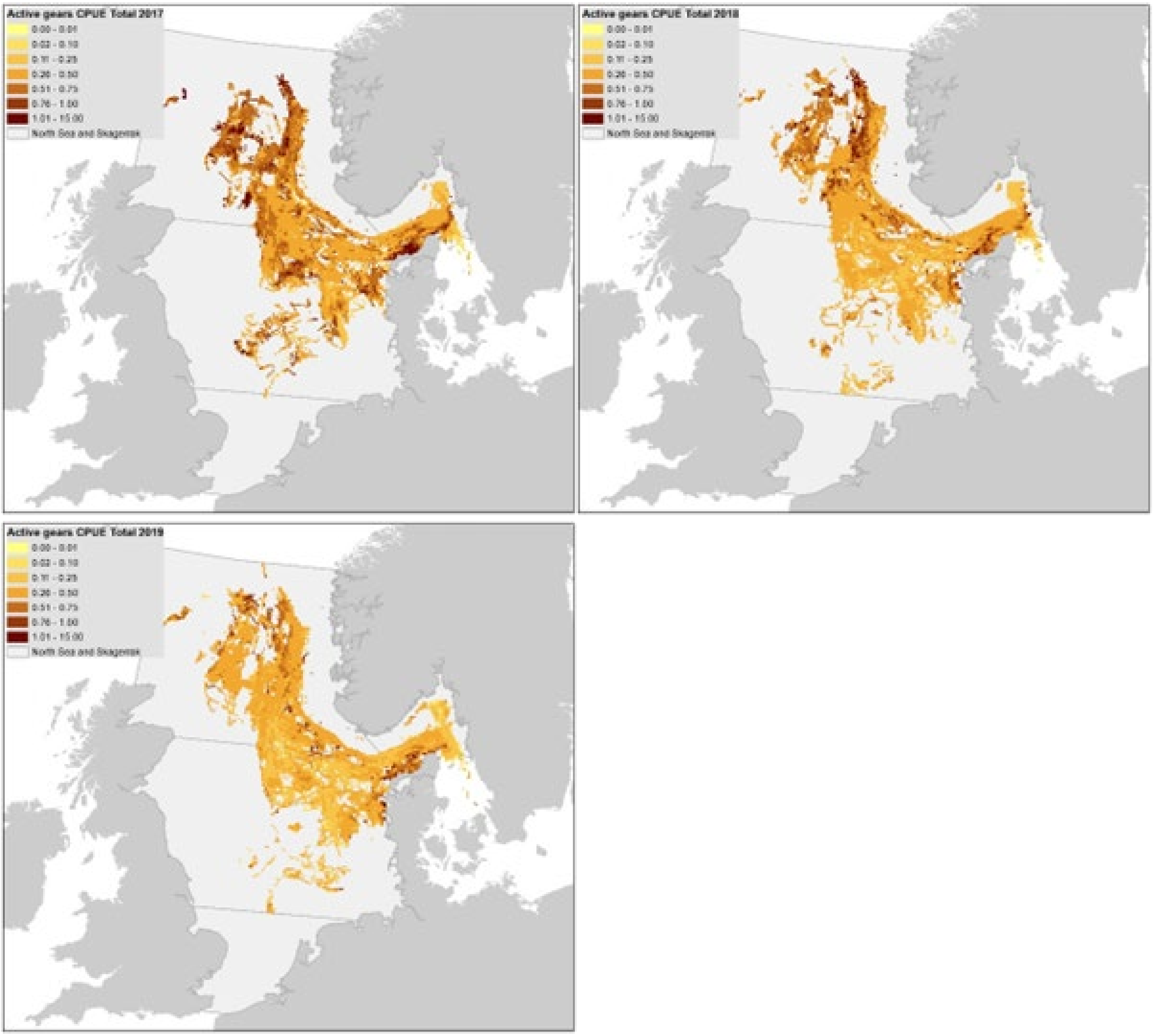
North Sea and Skagerrak

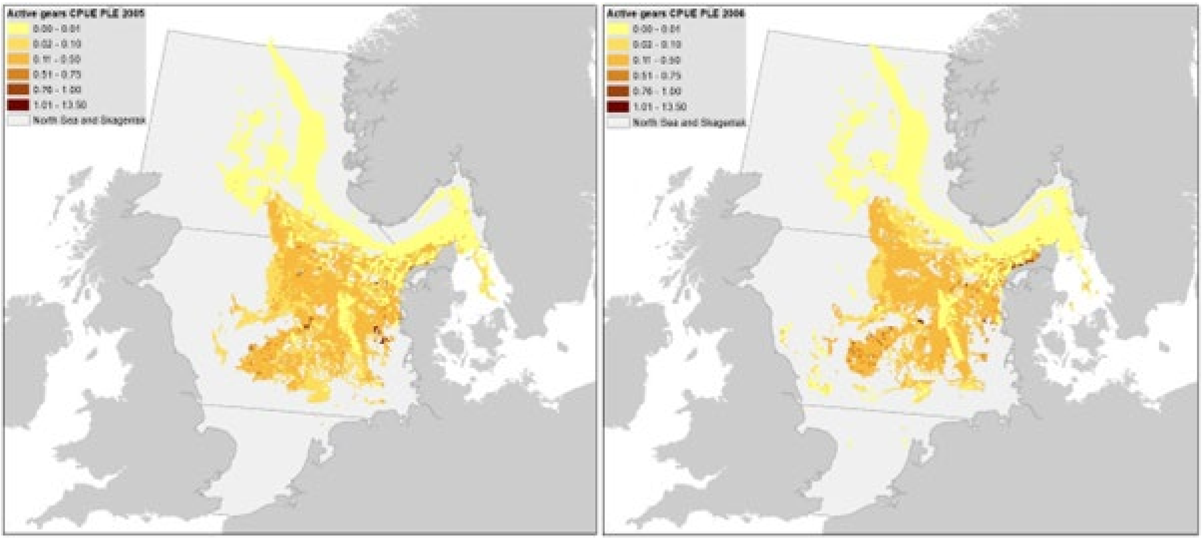


Active gears CPUE Total 2017

Active gears CPUE Total 2018

Active gears CPUE Total 2019

Figure A1. Maps of plaice catch per unit effort (CPUE, kg/hr) from selected métiers with active gears 2005-2019 (from top to bottom and left to right).



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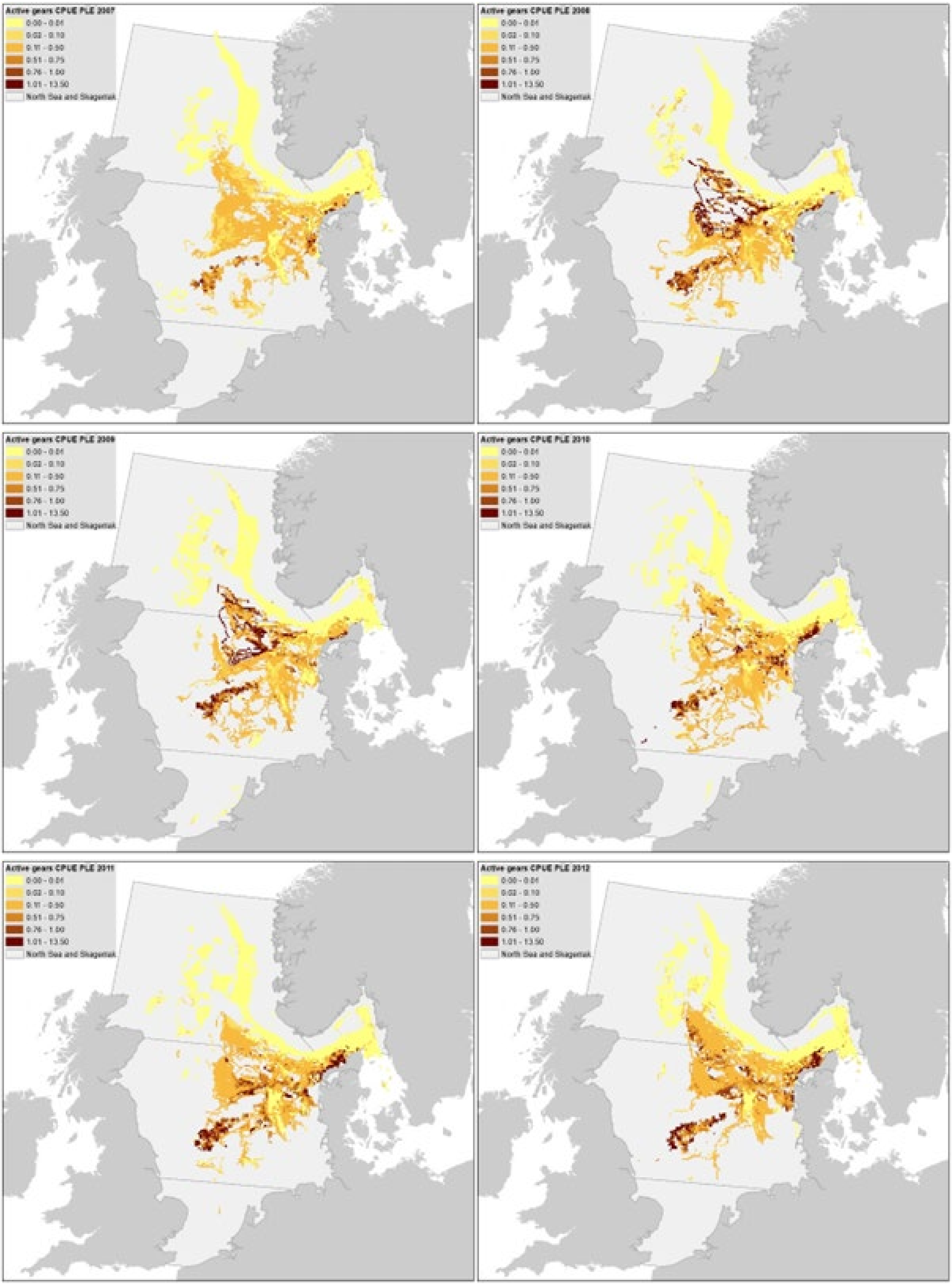
Active gears CPUE PLE 2006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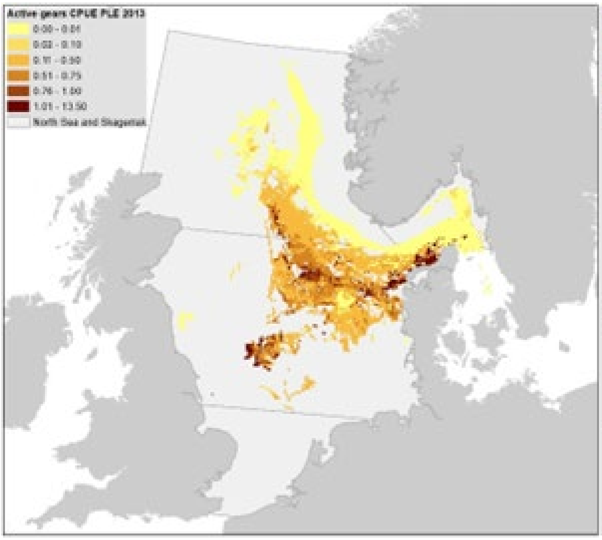
North Sea and Skagerrak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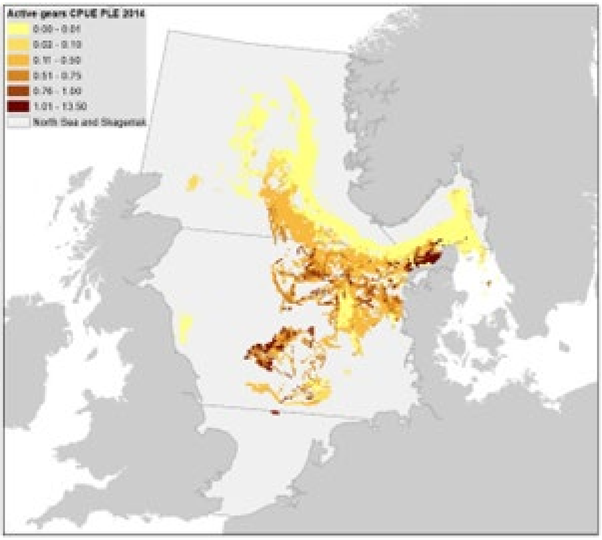
North Sea and Skagerrak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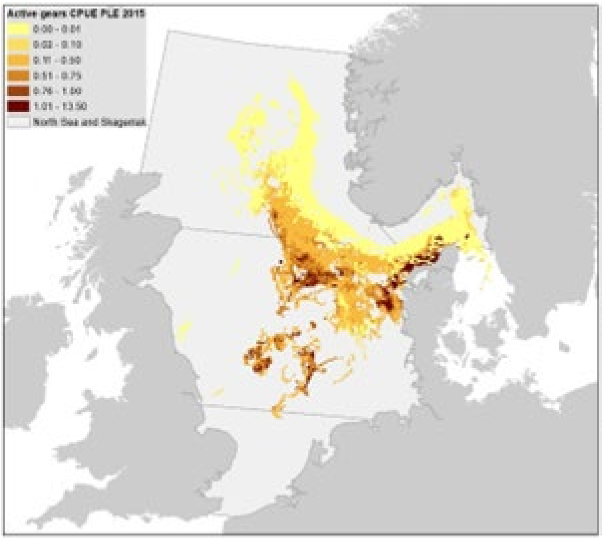
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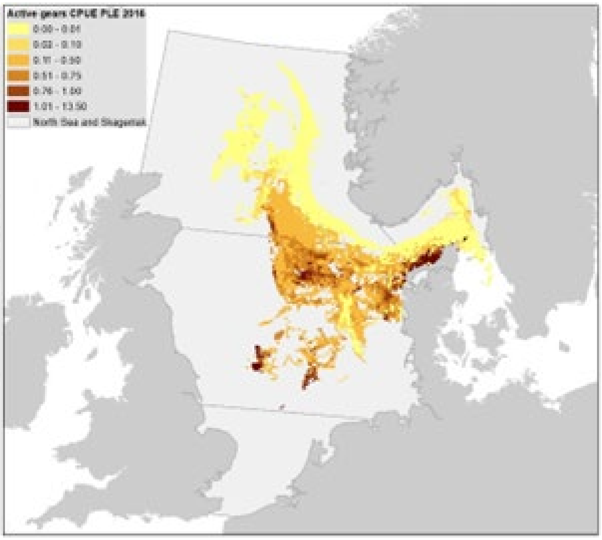
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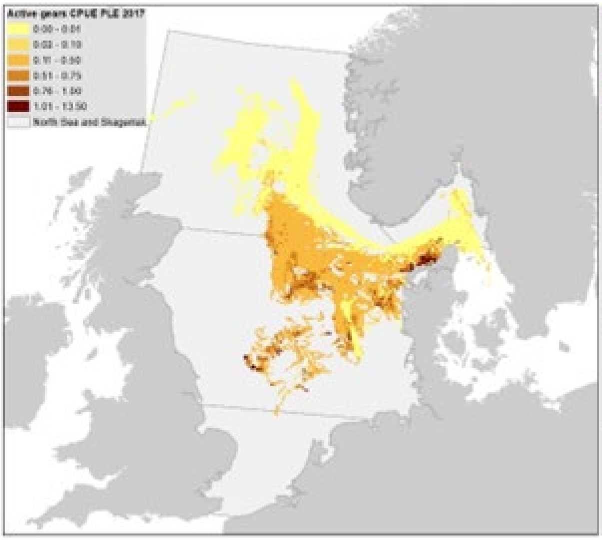
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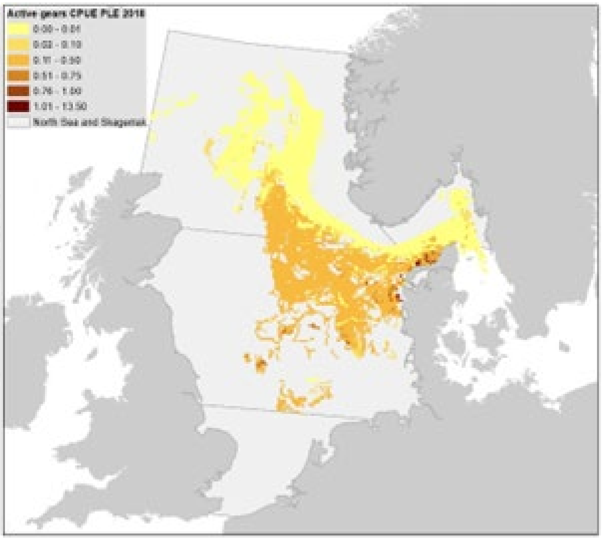
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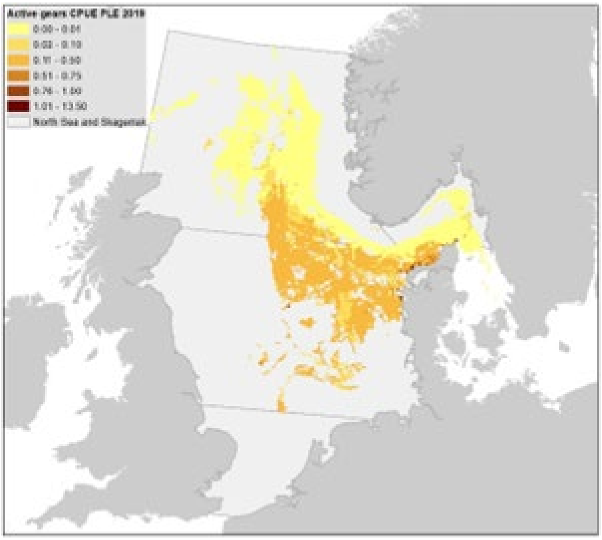

Figure A2. Maps of plaice catch per unit effort (CPUE, kg/kW/hr) from selected métiers with passive gears 2005-2019 (from top to bottom and left to right).

4. Management of flatfish stocks, synthesis of results - Implications to stock assessment and management

4.1 Turbot

This project addressed management issues relevant for benchmark approaches and synthesized information about the distribution of turbot populations, with a particular focus on the Skagerrak and Kattegat. One important insight from this work is an apparent mis-match between current stock assessment and management units and the geographical distribution of turbot populations. In particular, the Kattegat seems to be a dynamic area where two populations meet, hybridize and interact, and which is currently not considered for stock assessment and management as Kattegat is merged with the Skagerrak in terms of management. It is recommended that future work include more detailed genetic analyses of the distribution of these turbot populations, both in and outside of spawning season. Further size at age distributions with genetic assignments should be analysed for a further quantification of population components and indications of different productivity in line with work done on Kattegat cod (Hemmer-Hansen *et al.* 2020). Moreover, studies on the dispersal of turbot eggs and larvae are recommended in conjunction with studies on the migration of adult turbot, especially for identifying spawning areas (Baden *et al.* 2022) and the dispersal of juveniles from the areas. This knowledge will be important for future benchmark processes concerning this species with a focus on aligning biological information with stock assessment and management units.

4.2 Sole

This project provided important information to substantiate any suggested perception of changes in biological stock distribution and subsequently in defined management areas. With the obvious connectivity between Skagerrak and the North Sea and the apparent mix of North Sea fish into Kattegat and the Belts based on the genetic studies and the otolith microchemistry, the former view of a unique stock in Skagerrak, Kattegat, the Belts and western Baltic need revision. Both stock assessment, catch advice and fisheries management must consider this mix in order to ensure a sustainable utilization of the sole stocks.

Assessment and catch advice considerations

If area assessment is the preferred option, mix or migration between areas need to be quantified in order to be implemented in the assessment model. Presently there is not sufficient data to quantify the mix. Assumptions could be made on such a quantification, however, the mix from North Sea to the Skagerrak, Kattegat, Belts and Western Baltic stock and vice versa have more levels to consider, e.g., life stage and season, which our study did not include in sufficient detail. Some qualitative and pragmatic considerations could be considered in the assessment and advice process, such as taking account of stock status and reproductive success between the two neighbouring stocks to avoid unbalanced harvesting of one or the other stock. This is especially the case for the smallest stock in SD 20-24.

Alternatively, an assessment based on biological reproductive populations rather than geographic areas could be an option. This requires genetic split for all management areas into known spawning populations. The assessment and management will then consider biological units over more areas that spatially mix but is assessed and advised separately, as recently implemented for cod stocks in Greenland (ICES 2023). This demands a continuous sampling for split of catches and is for sole not considered to be realistic to implement at present, given the relatively small and patchy catches in SD 20-24.

Management considerations

The management implications of an existent mix of the SD20-24 population with the North Sea sole population depends on how the stock assessment and catch advice addresses this issue. Within the near future (~5 years) the present stock perception for the assessment and the assessment method will likely not change due to lack of quantitative information to justify assessment input changes. A partial advice and management of the SD20-24 stock with SD20 (Skagerrak) being linked to the North Sea stock status could be an intermediate scenario until connectivity in the areas are fully quantified.

If an approach of assessment by reproductive population is followed, it will put more demand on management cooperation between nations since genetically defined populations/stocks will inevitably be wider in distribution crossing more national jurisdictions than a stock assigned by area.

More accurate determination of spawning sites and more quantitative information on mixing rates in catches from different areas, based on model studies of likely drift of egg and larvae and further genetic work, need to arrive before the present assessment and catch advice can improve considerably in quality to reflect the dynamics as inferred from this study. Consequently, we recommend further work to improve data availability to support future changes in management practice for the species.

4.3 Plaice

For European plaice, our main findings align with the perception from local fishermen and show that the distribution of plaice has shifted geographically, so that abundance in the Skagerrak is now at overall lower levels. In contrast, abundance seems currently high in the North Sea and Baltic Sea/transition zone. Thus, the area of interaction between the two main populations coincides with the area with observed changes in abundance. Since the reasons for these changes are unknown at present, further work is needed to investigate potential drivers for observed changes in distribution. Potential candidates are environmental conditions (e.g., temperature), predators or prey, and fishing pressures in combination with dynamics of potential local populations. For example, time series analyses of genetic data in combination with chemical signatures in otoliths could help to address questions related to potential local depletion in Skagerrak by investigating changes over time in composition of the population components present in the Skagerrak. Also, future work should focus on improving our understanding of connectivity between juvenile nursery areas and adult spawning areas, which is important for sustainable management at population scale.

5. Conclusions and outlook

As highlighted above, the project has significantly advanced our understanding of the underlying biology and ecology of managed flatfish species in Danish waters. Although there are still outstanding questions, the results from the project can improve sustainable utilization and management of all species.

One common theme across the three species included in this project is the similar signals of dynamic interaction between biological populations from the North Sea and Baltic Sea in the Kattegat/Skagerrak region. While this is interesting in terms of improving our understanding of natural biodiversity, it could also have implications to resource management. Ecosystem based approaches to the management of marine resources will benefit from this kind of integrated knowledge across species, for example through the identification of biodiversity hotspots or specific geographical areas where multiple species show similar patterns of population structure and connectivity, which can guide spatial management priorities on short and longer time scales. The current project also shows that recent technological developments have enabled novel and integrated approaches to the study of complex patterns of population structure, connectivity and adaptation in natural populations, and future management approaches can benefit from integrated multiple approach studies, as applied in the current project.

With climate change the species distributions and their underlying population structure are also changing, often leading to a continuing mismatch between experienced abundances within management units and allocated quota shares between countries. Managers will soon have to deal with these issues at an overall level for all fisheries. More exact information about population migration, structure and dynamics of all fisheries species will here be a crucial decision support tool for managers.

6. Acknowledgements

We thank Danmarks Fiskeriforening PO (DFPO), Dansk Amatørfiskerforening and Danmarks Sportsfiskerforening for valuable input and discussions during project. We are grateful to the fisheries control at Nymindegab for help with sample collection for genetic analysis.

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