#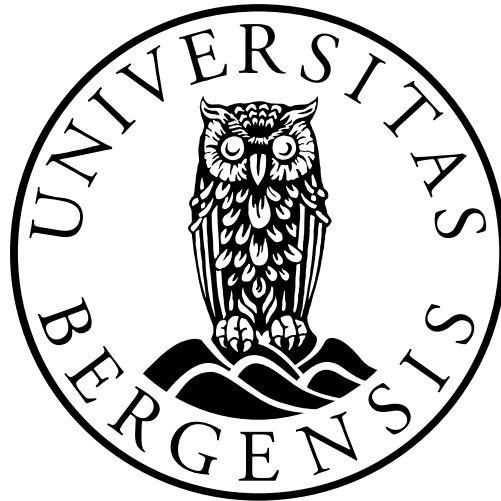 Spatial and Temporal Variation in Atlantic herring (*Clupea harengus*) Otolith Shape During the Summer Feeding Migration in the North Sea

Tomine Grov Lekven

Acknowledgements

Table of contents

Abstract

Otolith shape has been successfully utilized in stock identification for a number of fish species. Nevertheless, there is uncertainty regarding potential temporal instability in otolith shape. Age, growth and year-class effects pose the threat of falsely detecting stock-structure within stocks. The North Sea autumn spawners (NSAS) and western Baltic spring spawners (WBSS) are two stocks of Atlantic herring (*Clupea harengus*) co-occurring at various degrees throughout the North Sea during their summer feeding migration. In this thesis, herring otoliths collected in the North Sea in June-July were analyzed by means of shape analysis. Otolith shape and size were described by digitally extracted wavelets and linear measurements, respectively. Both spatial and temporal analyses were conducted to examine spatial variation and to clarify the possibility of between-cohort stability in otolith shape. Otolith shape was found to differ spatially throughout the North Sea as a possible result of different degrees of stock-overlap. The results also suggested a highly stable otolith shape in the eastern part of the North Sea, indicating no year-class effect within stocks, in addition to a relatively stable overlap of stocks for three consecutive cohorts. Providing management with evidence of temporal stability in otolith shape for commercial fish species could further strengthen the use of otolith shape analysis as an easy and cost-effective method for stock assessment. Further studies should thus allocate individuals to their stock of origin to remove genetic effects on otolith shape, and to answer the question of temporal stability in otolith shape for a longer period of time.

1. Introduction

A central topic in fisheries science and management is sustainability, i.e., implementing management that is beneficial for the production of populations of exploited species in the long run (Reiss et al. 2009). To sustainably manage a species in the long run, one needs knowledge of its population structure (Kutkuhn 1981, Begg and Waldman 1999). In terms of management, species are managed as stocks. The term 'stock' has been debated and given several definitions (Booke 1981, Pawson and Jennings 1996). However, for the purpose of management, stocks are described as separate units which can be exploited independently of one another (Cadrin et al. 2013). Put in simple terms, a stock can be, but is not limited to, a group of one or more populations of a species which is partly isolated and therefore self-sustaining (Hilborn and Walters 1992, Cadrin et al. 2013). However, one can have areas of stock overlap where several stocks are exploited together in the same fishery (Gilbey et al. 2017). In such cases it is of great importance to recognize the temporal and spatial overlap between stocks due to less productive parts facing the threat of being overfished if not accounted for (Begg et al. 1999). This is where the methods of stock identification play a key role.

Stock identification involves identifying and discriminating a species into its associated components (Cadrin et al. 2013). Several methods have been developed by fishery scientists and biologists to achieve the identification of stocks. These methods involve; mark-recapture, catch-data, life history characteristics, parasites as biological tags, morphometrics, meristics and genetics (Begg and Waldman 1999).

Variation in phenotypic traits such as morphometric (relative size of body parts) or meristic (e.g. number of vertebrae or finrays) characters have long been used for stock discrimination (Swain and Foote 1999). Both morphometric and meristic characters are highly controlled by genetics, but their phenotypic expression is also commonly influenced by environment in early development (Beacham 1990, Wainwright et al. 1991). Applications of these traits are valuable as they can reveal stock structure that should be accounted for when developing appropriate management plans. Turan (2004) conducted a study on horse mackerel *Trachurus mediterraneus* using relative body measurements and finrays where no prior knowledge of stock structure between fishing areas existed, and the fish thus were managed as one stock. The study found heterogeneity in the studied traits between groups of fish, indicating stock structure, and Turan (2004) suggested that if these differences persisted over time, they had to be taken

into account and could be reason for reevaluating management. Another structure often used in morphometric studies of fish are otoliths. Otoliths are paired calcified structures in the inner ear of teleost fish (Figure 1). There are three pairs of otoliths; the saggitae, lapilli and asterisci, where saggitae, being the largest, has proven most useful in morphometric analysis (Campana and Casselman 1993). Morphometric analysis of otoliths describe the shape of the otolith by morphometric characteristics such as length, width, area and perimeter, but also by more complex methods where image analysis software traces the otolith outlines and generates shape coefficients (Libungan and Pálsson 2015). The software development has revolutionized morphometric research, making otolith shape analysis both an inexpensive and effective tool useful for studying stock structure (Cadrin and Friedland 1999, Libungan and Pálsson 2015, Afanasyev et al. 2017).
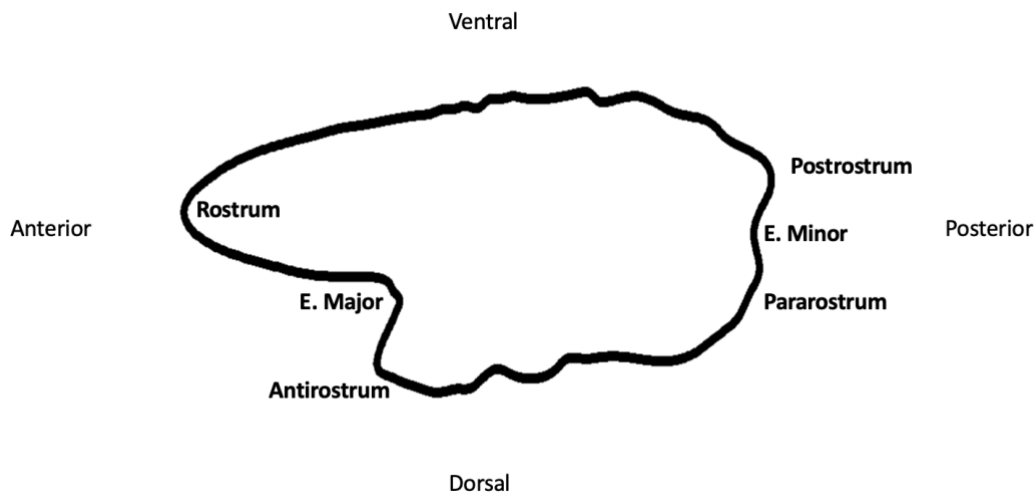
Figure 1: The orientation of a saggitae fish otolith and its different parts. E. Major = Excisura Major, E. Minor = Excisura Minor (Mitsui et al. 2020).

Otoliths are a valuable and widely used tool in stock discrimination because they continue to grow throughout the life of the fish, and once the material is deposited, it will not be reabsorbed (Campana 1999). In addition, otolith characteristics such as size and shape are species specific and highly determined by genetics (Berg et al. 2018), but are also affected by growth and environment (L'Abée-Lund 1988). Thus, otoliths function as recorders of the life history of fish and the use of these structures in morphometric analyses enables differentiation of species, but also differentiation of stocks of the same species in cases where environmental and genetic differences exist between partially separated stocks (Campana and Casselman 1993). However, people have questioned the application of otolith shape analysis as a tool for stock

discrimination due to the possibility of mistaking within-stock variation for stock structure (Begg and Brown 2000). Such variation might occur within a stock due to age effects and year-class variability arising from temporal differences in food availability, temperature and growth (Castonguay et al. 1991, Campana and Casselman 1993, Denechaud et al. 2020, Jónsson et al. 2021). Another factor believed to contribute to within-stock variation in otolith shape is sex, however several studies have been conducted and shown that the effect of this factor is not of any significance (Castonguay et al. 1991, Cardinale et al. 2011, Libungan et al. 2015a). Still, based on possible temporal instabilities, Begg and Brown (2000) suggested that otolith shape characteristics should be recalculated each year.

Despite potential temporal instabilities in terms of discriminating stocks and populations, otolith shape analysis has been successfully utilized for a number of marine species such as Atlantic herring *Clupea harengus* (Turan 2000, Eggers et al. 2014, Libungan et al. 2015a), forkbeard *Phycis phycis* (Vieira et al. 2014), king mackerel *Scomberomorus cavalla* (DeVries et al. 2002) and cod *Gadus morhua* (Campana and Casselman 1993). This method can thus be a promising tool for evaluating and monitoring levels of mixing of co-occurring stocks (Pérez et al. 2013, Hüssy et al. 2016) where sampled otoliths can provide time-series estimates of proportion of mixing (ICES 2015b). In addition, studies conducted on the temporal stability in otolith shape have also been successful, challenging the suggestion of an annual recalculation of shape characteristics. A study conducted on Northeast Arctic cod otoliths by Denechaud et al. (2020) reported temporal stability in otolith shape for a period of over 80 years, showing that the morphology of the otolith stays highly similar within one stock despite environmental fluctuations. The work by Libungan et al. (2015b) was the first to report temporal stability in otolith shape for local Norwegian herring. It is not clear, however, that this conclusion applies to what is referred to as oceanic herring. Typical for local populations is that they are small and somewhat stationary in contrast to the oceanic herring which are highly migratory (Lie et al. 1978, Silva et al. 2013) with larvae that drift at long distances to nursery grounds with oceanic currents (ICES-FIshMap, Vikebø et al. 2010). The spatial scale of the larval drift in addition to the long migrations of adult oceanic herring results in different growth patterns and exposure to climatic fluctuations at larger spatial scales compared to the more stationary local herring (Ottersen et al. 2010, Silva et al. 2013). Thus, further studies regarding the temporal stability in otolith shape of oceanic herring is warranted.

Atlantic herring *Clupea harengus*

Atlantic herring is a pelagic, schooling clupeid fish distributed in the North Atlantic Ocean. Its distribution has a broad range within both the Northeast and Northwest Atlantic (Iles and Sinclair 1982, Whitehead 1985). Herring often undergo long feeding and spawning migrations which are seasonally driven (ICES-FIshMap). The population structure of herring is complex due to spatial and temporal differences in migration routes and spawning grounds (Whitehead 1985, Hay et al. 2001, Geffen 2009). Herring is separated into stocks based on these differences (Smith and Jamieson 1986). Spawning occurs once a year where benthic eggs are deposited on gravel or sandy substrate on the seabed (Hay et al. 2001, ICES 2017). Herring is of high commercial importance and have been heavily exploited (Whitehead 1985, ICES 2018), and there have been cases where depletion of herring populations have occurred (Hay et al. 2001). Due to a lack of proper management, the exploitation of herring stocks in the Northeast Atlantic resulted in a collapse in the late 1960s and early 1970s followed by a closing of the North Sea herring fishery in 1977 (Jakobsson 1985). In addition to being a commercially important species, herring plays a key role in the trophic web as a planktonic predator, and prey to fish, marine mammals and sea birds (ICES 2018), emphasizing the importance of sustainable management.

Biological characters of herring often differ between stocks due to the spatial and temporal differences resulting in stocks being partially isolated and exposed to different environments (Whitehead 1985). The North Sea Autumn Spawners (NSAS) and Western Baltic Spring Spawners (WBSS) are two such stocks. NSAS consists of several spawning components, i.e. groups of herring that spawn at different locations along the eastern English coast and in the English channel in the autumn and winter (ICES 2018). The spawning components display some phenotypic variation (Turan 2000), however, little genetic differences exist between the spawning components (Mariani et al. 2005). The NSAS larvae drift from the spawning locations towards nursery grounds in the southeastern part of the North Sea and into ICES Division IIIa in Skagerrak/Kattegat (Figure 2) where they mix with individuals of the WBSS stock (Rosenberg and Palmén 1981, Ulrich et al. 2012). The WBSS stock is more complex containing several populations of different genetic origin (Bekkevold et al. 2005, ICES 2019). The main spawning aggregation of WBSS occurs off the Rügen island (Figure 2) spawning during March-May in the western Baltic Sea (Bekkevold et al. 2005). Juveniles migrate to Kattegat and the eastern part of Skagerrak (Clausen et al. 2015). Adults from the two stocks, NSAS and WBSS,

are temporally and spatially separated during parts of the year but mix during their summer feeding migration (ICES 2019). During summer, mature individuals of NSAS are distributed in the central and northern parts of the North Sea, and mature individuals from WBSS migrate out to Kattegat and Skagerrak and the eastern part of Division IVa (Rosenberg and Palmén 1981, Bekkevold et al. 2011, ICES 2020). Here they are caught together in fisheries targeting herring in the North Sea in what is called the 'Transfer Area' (Figure 2), making them a mixed fishery (ICES 2020). WBSS herring caught in the 'Transfer Area' are transferred to the Baltic assessment, hence its name (ICES 2018). To assess the proportion of the two stocks in the catches, mean vertebrae counts (VS) have been used as the two stocks display differences in this meristic trait (Rosenberg and Palmén 1981).
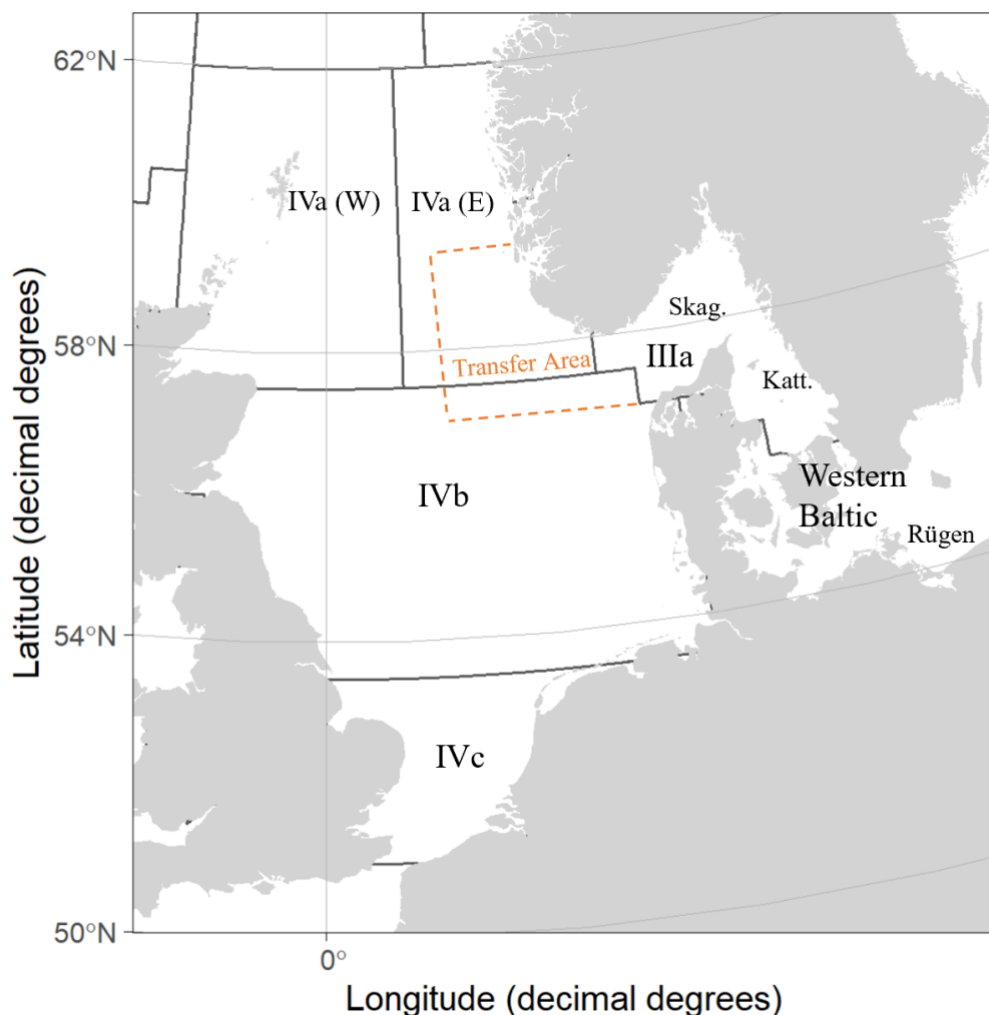

Figure 2: The North Sea ICES Divisions IVa (west and east), IVb and IVc and Division IIIa (Skag. = Skagerrak, Katt. = Kattegat).

Both NSAS and WBSS herring mature at 2-3 years of age, and normally reach an age of 8-9 years (Whitehead 1985, ICES 2020). Due to NSAS spawning in the autumn and usually passing their first winter as larvae, they do not attain their first winter ring (wr) until over a year after being born (Rosenberg and Palmén 1981). Thus, they attain their first wr at the same time as the spring spawning WBSS stock. When the age of herring is determined, their number of wr are counted which results in NSAS being able to grow up to several months more than WBSS before our ageing method register them as age 1 herring. When referring to age, one is referring to number of wr. Therefore, same-age individuals from the two stocks do not belong to the same year-class.

Aims of this study

This study aims to clarify the possibility of temporal stability in otolith shape in an area of stock overlap. The study will provide insight into the temporal variation in otolith shape as well as a temporal trend in mixing of two stocks. This again could be valuable for mixed fisheries such as NSAS and WBSS, where monitoring catches and ensuring the productivity of less productive parts is of great interest and importance.

For this study, herring otoliths sampled in June-July in 2014-2021 from the eastern part of the North Sea, and in June-July in 2016-2018 from the western part of the North Sea, were analyzed by means of shape analysis (1) to test the hypothesis that otolith shape will vary spatially depending on the degree of mixing of the two stocks NSAS and WBSS, and (2) to investigate the temporal trend in otolith shape by evaluating between cohort differences. The individuals in the samples used in this study were not allocated to their stock of origin and were therefore treated as three different units divided by sampling location; the northwest, northeast and southeast in the North Sea.

2. Materials & Methods

2.1 Sampling

Herring otoliths have routinely been sampled by the Institute of Marine Research (IMR) in the North Sea, forming a time series of herring otoliths to be used in this study (Figure 3). The sampling has taken place in June-July during an annual Herring Acoustic survey (HERAS) from 2014 to 2021. Samplings were conducted using standard pelagic and bottom trawls, see HERAS protocol for details (ICES 2015a). The HERAS survey is coordinated by the International Council for the Exploration of the Sea (ICES) and Working Group of International Pelagic Surveys (WGIPS) and is part of an international sampling effort. Among others, the sampling effort involves Scotland which also contributed with herring otolith samples from 2016-2018, to be used in this study (Figure 3, Table 1).
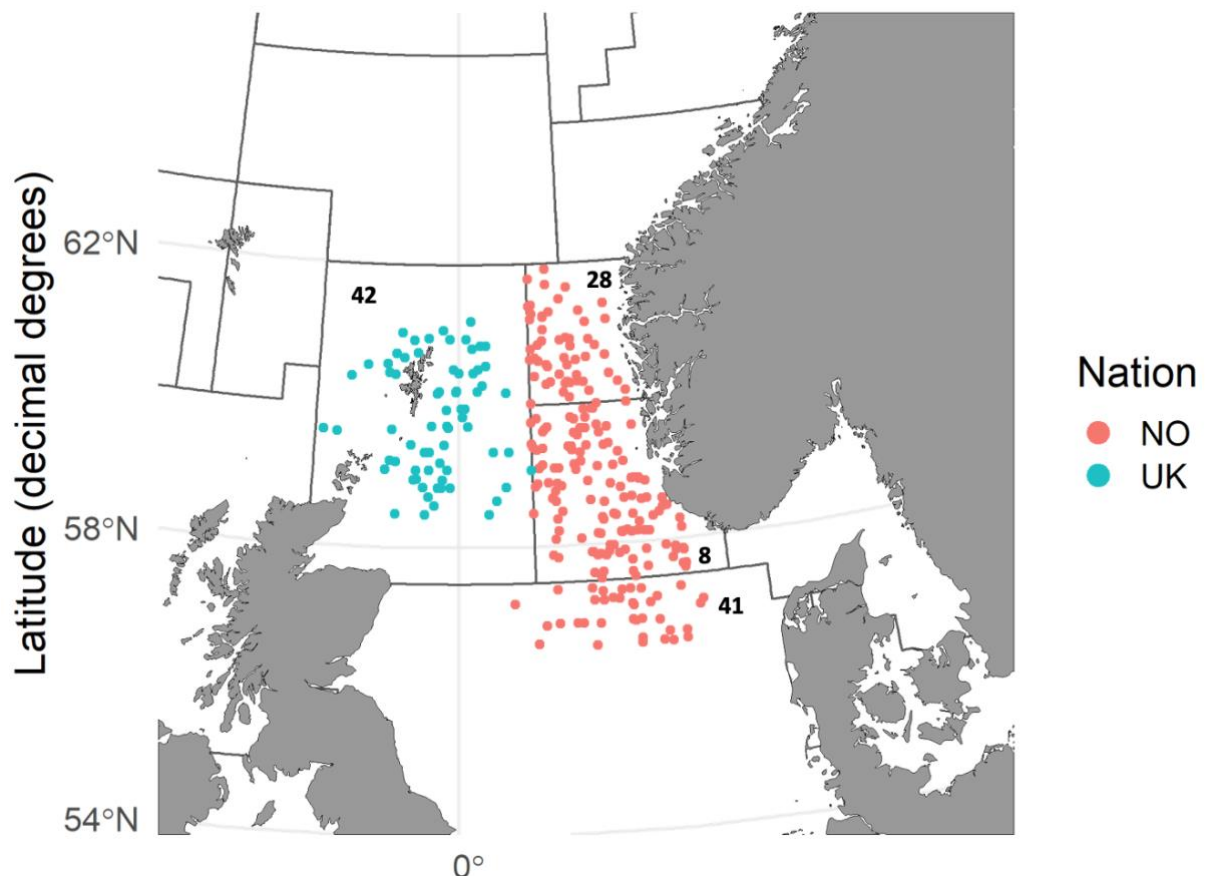

Figure 3: Map of study area with trawl stations from Norwegian (NO) and Scottish (Folkvord et al.) vessels. Norwegian samples are from 2014 to 2021, and Scottish samples are from 2016 to 2018. The map is divided into the Norwegian fishing regions; area 42 = Northwest, area 28 = Northeast, area 8 and 41 = Southeast.

For both the Scottish and Norwegian samples, length measurements were taken to the nearest 0.5 cm below. Age was determined by extracting otoliths (saggitae) and counting winter rings (wr) and, for the Norwegian samples only, the number of vertebrae were counted. Otoliths in the Scottish samples were extracted following a length stratified sampling scheme where more otoliths were collected per length group for larger individuals ($\geq$ 28 cm). This was done to optimize the age at length data as the presence of several age groups are expected within larger length classes. An estimate of the true age distribution for the Scottish samples were then constructed by calculating the proportions of age groups within each length class. See HERAS protocol for more details on biological data collection (ICES 2015a).

Table 1: Total number of individuals per age, sampled in June/July each year (2014 to 2021) in the North Sea (Figure 3), where otoliths have been extracted, photographed and shape coefficients have been generated. Numbers in brackets [] are the proportion of the total number that are Scottish samples sampled from 2016 to 2018.

	Year							
Age [wr]	2014	2015	2016	2017	2018	2019	2020	2021
0	2	0	0	0	0	0	0	0
1	319	529	36 [9]	116 [7]	44 [42]	255	300	249
2	139	513	799 [474]	176 [31]	240 [163]	97	146	149
3	93	166	918 [592]	593 [170]	596 [447]	107	57	105
4	163	83	337 [236]	475 [100]	1042 [811]	96	101	68
5	108	102	326 [269]	114 [26]	720 [398]	144	39	55
6	65	31	391 [312]	59 [9]	271 [173]	94	113	35
7	43	67	209 [179]	56 [6]	231 [155]	40	97	59
8	23	50	127 [93]	29 [8]	150 [102]	23	29	51
9	21	10	58 [44]	42 [2]	88 [39]	37	19	20
10	21	6	22 [12]	26	45 [14]	11	8	8
11	21	6	9 [6]	5	17 [9]	13	8	3
12	9	3	9 [5]	3	4	4	5	4
13	1	0	1 [1]	4	0	1	1	1
14	0	0	1 [1]	1	0	0	1	0
Total	1028	1566	3243 [2233]	1699 [359]	3448 [2353]	922	924	807

2.2 Otolith images

Digital images of each individual otolith were taken in .tif format using a Nikon SMZ745T stereo microscope together with the software NIS-Elements D 5.30.02 (Build 1545). Otoliths that still had residue of tissue attached were cleaned with water and photographed when dry.

The right otoliths were preferred for the images, but in cases where the right otolith was missing or broken, the left one was used. The otoliths were placed under the stereo microscope on a clean black background with concave side facing up (sulcus facing down). Right otoliths were orientated with their rostrum pointing to the left, and the left otoliths with rostrum pointing to the right. The microscope was adjusted to get the best possible contrast between background and otolith. The white balance of the images was adjusted using the auto white balance function in NIS-Elements.

A calibration image of a calibration stick in good focus was taken for size reference. This image had to be taken at the same microscope magnification as the otoliths. A new calibration image was taken for each magnification used.

Otolith images were stored in unique folders named by the cruise and survey the otoliths were sampled from. The individual otolith images were named to make it easy to identify them; with station number and fish ID (Norwegian data), or cruise and haul number and fish ID (Scottish data), e.g.: 24007_01 or 0817S_159_01, respectively.

2.3 Handling of otolith images prior to outline extraction

All images were converted from .tif format to .jpg format using the IrfanView 4.58 ® image editing software. The same software was used to convert the images to a smaller resolution to speed up the process of the analysis. When changing the resolution, the images were checked to make sure that their quality was still good. These changes were also done to the calibration stick images. The images of the left otoliths were flipped horizontally to give them the same orientation as the right otolith images. The calibration stick images were used to convert 1 mm to pixels. Resolution of the images ranged from 121 to 597 pixels per mm.

The unique folders containing the otolith images were then placed in folders and a datafile was made as described in the procedure by Libungan and Pálsson (2015).

2.4 Outline extraction

The otolith outline extraction was done in R (R Core Team 2021) using the ShapeR package by following the procedure from Libungan and Pálsson (2015). When extracting the outlines using the ShapeR package, a threshold argument is set by the user to distinguish white (otolith) from black (background). Each otolith outline is automatically saved as a .png file in a new folder called 'Original_with_outline' (Figure 4a). Each extracted outline was visually checked to see if the outline fit the otolith image. In cases where the outlines did not fit the image (Figure 4b), the threshold argument was changed, or the image was edited using the software GIMP (The GIMP Development Team 2019).
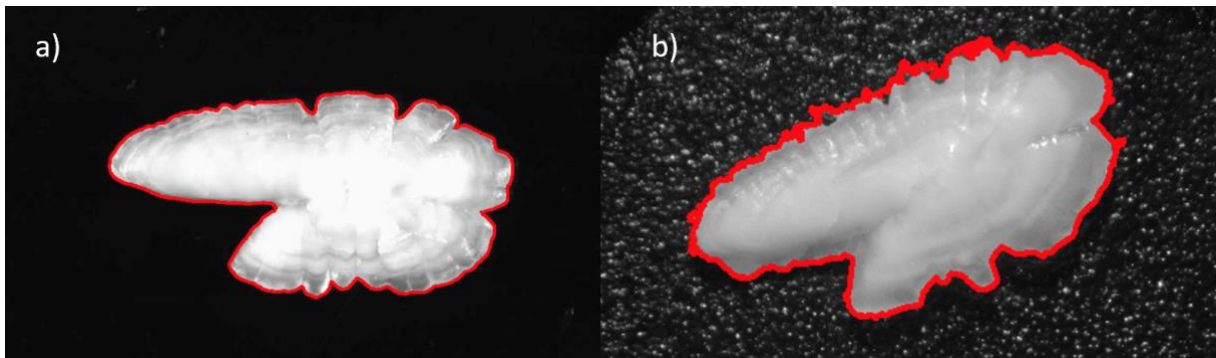
Figure 4: Example of extracted outline from an otolith that a) does and b) does not fit the otolith image.

After the outline extraction, the shape coefficients (Wavelet and Fourier) and otolith indices (otolith length, width, area and perimeter) were estimated and collected as described in the procedure by Libungan and Pálsson (2015).

2.5 Statistical analysis

All statistical analyses were conducted using the software R (R Core Team 2021), with the statistical packages vegan and car (Fox and Weisberg 2019, Oksanen et al. 2019) with a type 1 error probability of 0.05. Maps were made using the package ggOceanMaps (Vihtakari 2021). Data exploration was done following the procedure by Zuur et al. (2010). Boxplots were made by plotting otolith indices (otolith length, width, area and perimeter) by age, and Cleveland dotplots were made by plotting the otolith perimeter by age to look for potential outliers/measurement errors. Outliers that seemed to be due to measurement errors were removed. These parameters seemed either too small or too large to be true. The removal of

outliers was only conducted for age groups 1 to 6, as these were the age groups used in further analyses. This resulted in the removal of 35 individuals out of a total of 11728 (Appendix Figure A1).

NSAS juveniles are known to stay in the southeastern part of the North Sea in their nursery area up until about 2 years of age (Clausen et al. 2015) and a higher proportion of age 1 and 2 herring are thus expected to aggregate in this area. The otolith morphology of juvenile fish has a higher variation in shape than matured fish, and the shape of the otolith evens out as the fish matures (Hüssy 2008). Due to this, herring of age 1 and 2 were excluded from analyses relating to otolith shape differences between locations, and temporal stability of otolith shape. Only age groups 3 to 6 were used for these analyses as they had the highest sample sizes.

Due to the possibility of mixing of NSAS and WBSS herring in the samples, the data was separated into the northwestern (NW), northeastern (NE) and southeastern (SE) part of the North Sea (Figure 3, Table 2). The separation into locations was based on the assumption that NW would mainly comprise individuals of NSAS herring, and that NE and SE contained a mix of NSAS and WBSS, with SE containing larger proportions of WBSS. The Transfer Area was included in the SE unit (Figure 2).

Table 2: Number of individuals of age 1 to 6 per sampling year per location (the northwestern (NW), northeastern (NE) and southeastern (SE) parts of the North Sea (Figure 3)), where otoliths have been extracted and photographed. Removed outliers are not included (Appendix Figure A1).

Year	Age [wr]					
	1	**2**	**3**	**4**	**5**	**6**
	NW					
2016	9	474	592	236	269	312
2017	7	31	170	100	26	9
2018	14	153	442	809	397	173
	NE					
2014	0	3	21	58	63	32
2015						
2016	0	31	162	49	29	47
2017	0	12	187	193	48	23
2018	0	6	13	75	90	32
2019	2	7	26	23	95	65
2020	0	1	0	38	11	66
2021	1	6	26	23	17	13
	SE					
2014	319	136	72	105	45	33
2015	529	513	166	83	102	31
2016	27	294	164	52	28	32
2017	109	133	236	182	40	27
2018	2	71	136	156	232	66
2019	253	90	81	73	49	29
2020	299	145	57	63	28	47
2021	243	135	73	43	27	20

2.5.1 Univariate and multivariate analyses

Otoliths were investigated using otolith indices and wavelet coefficients in univariate and multivariate analyses, respectively. Indices were used as a descriptor for size and wavelets as the descriptor for otolith shape. Out of the four otolith indices (length, width, area and perimeter), only otolith length was chosen for analyses as all four indices are correlated (Appendix Figure A2). Otolith length was checked for homogeneity of variance by Levene´s test, and for normal distributions by means of histograms. Otolith length was investigated by

ANOVA followed by Tukey HSD to see where significant differences appeared between analyzed groups. The extracted wavelet coefficients were used to plot mean otolith shapes and in multivariate analyses to determine shape differences between groups. This was done by performing a Canonical Analysis of Principal coordinates (CAP) followed by an ANOVA-like permutation test (1000 permutations) and by visualizing the canonical scores on the first two discriminating axes; CAP1 and CAP2. Further, post-hoc tests were conducted using CAP and the ANOVA-like permutation test (1000 permutations) to investigate which groups contributed to significant differences in shape. Multiple comparison was corrected for in the multivariate analysis with the Bonferroni method.

2.5.2 Age effects

As age has previously been proven to influence otolith shape (Castonguay et al. 1991), this was investigated using ages 1 to 6. Individuals of age 1 and 2 were only included in this analysis to visualize the change in shape from juvenile to mature fish. For the analysis, the SE samples were used due to this location being representative for all six age groups. To match Scottish and Norwegian sampling years, only herring sampled in 2016-2018 were used in the analysis. The assumption was made that the chosen samples would be representative for how shape changes with age for all three locations (Table 2).

2.5.3 Northwest, northeast and southeast in the North Sea

All analyses conducted on the three locations were done using samples from year 2016 to 2018 to match the Scottish and Norwegian sampling years (Table 2).

2.5.3.1 Length and age distributions

Total length and age distributions per location were plotted to visualize possible differences in the parameters between locations. A two-sample Kolmogorov-Smirnov test was conducted on the length distributions to investigate if the distributions were significantly different between the three locations. The same method was used for the length distributions of single age groups of age 3 to 6.

2.5.3.2 Comparing otolith shape between locations

Otolith shape was investigated between locations using herring of age 3 and 4 as these were the most abundant age classes across the three locations.

For the univariate analysis, an otolith length – fish length ratio was calculated by dividing the otolith length (mm) of each individual by its respective body length (mm). This was done to consider the possible differences in fish size vs otolith size relations between the locations. Typically otolith length and fish length have been shown to strongly correlate, but can differ under different environmental conditions (Hare and Cowen 1995). As including the NW and NE samples in the analysis violated the assumption of homogeneity of variance, the otolith length – fish length ratio was log transformed to uphold this assumption. The log transformed ratio was investigated for the two independent variables, location and age using a two-way ANOVA.

The multivariate analysis was conducted by combining age group 3 and 4 by random subsampling to make a balanced dataset where each age group represented the same number of samples within each location. The balanced dataset was made due to the results from the age effects analysis (section 2.5.2) showing significant differences in shape between the two groups.

2.5.4 Temporal stability in otolith shape

2.5.4.1 Comparing otolith shape between cohorts

The results of the analyses conducted on length distributions and otolith characteristics between locations (section 2.5.3) indicated large similarities for NE and SE. Thus, temporal stability in otolith shape was investigated by combining the two locations.

Otolith shape was compared between cohorts containing age groups 3 to 6. To balance out the age effect on otolith shape, a balanced dataset was made by random subsampling so that each cohort contained the same number of individuals within each age group (Table 3).

Table 3: Number of individuals of age 3 to 6 per cohort sampled in the eastern part of the North Sea (Norwegian samples, Figure 3) from year 2014 to 2021, where otoliths have been extracted and photographed and coefficients have been generated. Number of samples per age per cohort (N balanced) used in the balanced dataset is also shown.

COHORT	AGE [WR]				N BALANCED
	3	4	5	6	
2008				65	
2009			108	31	
2010		163	102	79	
2011	93	83	57	50	50
2012	166	101	88	98	88
2013	326	375	322	94	94
2014	423	231	144	113	113
2015	149	96	39	33	33
2016	107	101	44		
2017	57	66			
2018	99				

As otolith length is a shape parameter prone to environmental influence, the otolith length – fish length ratio was used in the univariate analysis to remove the possible differences in growth between cohorts caused by exposure to different environments, to be able to see if otolith growth is stable over time. Including cohort 2013 in the analysis violated the assumption of homogeneity of variance, and log-transforming the data did not correct for this. However, the analysis was conducted with this in mind. Violation of homogeneity of variance increases the probability of falsely rejecting the null-hypothesis. Further, the cohorts were analyzed in the multivariate analysis to investigate the stability in otolith shape over time.

2.5.4.2 Mean proportion of WBSS herring

To further examine how the overlap of NSAS and WBSS might influence the temporal variation in otolith shape, the vertebrae counts (VS) collected by Norway were used to calculate the proportion of WBSS with the equation:

$$\%WBSS = \frac{56.5 - Sample\_VS}{56.5 - 55.8}$$

Sample_VS is the sample mean, and the assumed population mean VS for NSAS is 56.5, and 55.8 for WBSS (ICES 2020). Values less than 0 assume a proportion of WBSS equal 0%, and values above 1 assume a proportion of WBSS equal 100%. Mean VS were calculated per sampling station, cohort and age, and the mean was then again calculated per cohort and age. This to avoid sampling bias between stations. The proportion of WBSS was then calculated per cohort and age group using the equation above.

3. Results

3.1 Age effects

The average otolith shape for age 1 individuals were more circular compared to the older herring, deviating at areas such as the rostrum, ventral edge and postrostrum. Average shape for age 2 herring showed similarities to the older individuals but displayed some deviation at the same areas as age 1 (Figure 5).
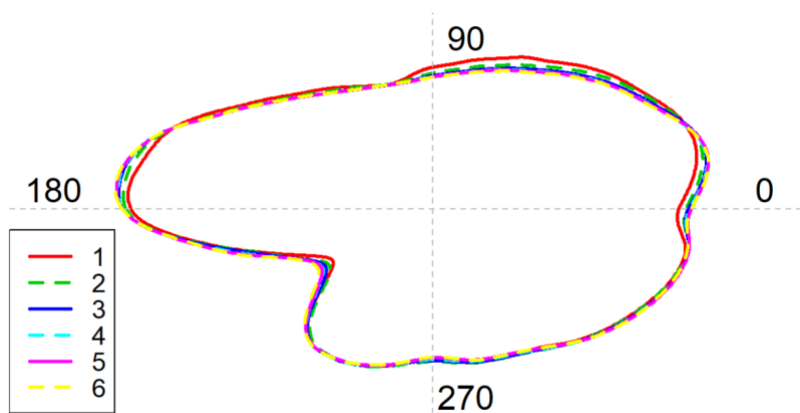

Figure 5: Mean otolith shape for age 1 to 6 wr sampled in the Southeastern part of the North Sea in year 2016 to 2018.

The Tukey HSD test showed significant differences in otolith length between all tested age groups ($p < 0.05$, Figure 6a). Post-hoc tests revealed significant differences in otolith shape between all age groups (CAP, $p < 0.05$, Appendix Table A1) except between age 5 and 6. By visual inspection of the mean CAP values for the age groups (Figure 6b), age group 1 and 2 showed the largest difference compared to the other ages on the first discriminating axis

(CAP1), and the older age groups (age 3 to 6) were more similarly distributed on CAP1, indicating less variation in shape as the fish matures.
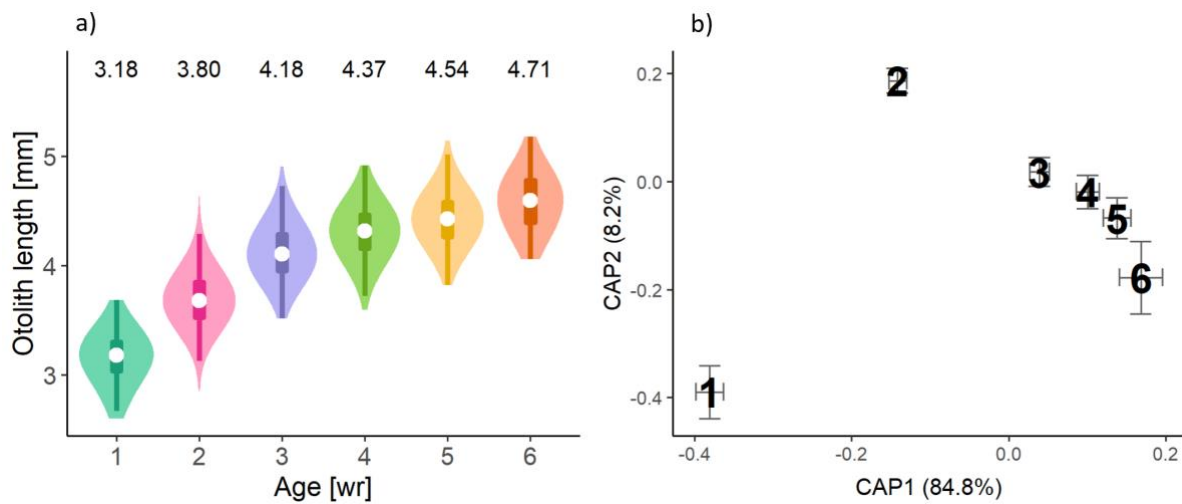

Figure 6: Samples from the southeastern part of the North Sea from year 2016 to 2018. a) Otolith length for individuals of age 1 to 6. The violin plot shows the distribution of the data, and the white dots and bars show the medians and quartiles while the whiskers show the extremes within 1.5 times the interquartile range. Values on top are mean otolith length per age. All age groups are significantly different in otolith length (p < 0.05). b) Canonical scores on the first two discriminating axes, CAP1 and CAP2 (and their explained variation), for individuals of age 1 to 6. Black numbers in the plot represent mean canonical value ± 1SE for each age group. All age groups significantly different in shape (p < 0.05) except for age 5 and 6.

3.2 Northwest, northeast and southeast in the North Sea

3.2.1 Length and age distributions

The length distributions for the three locations differed significantly (Table 4, p < 0.001). Southeast (SE) seemed to show a broader length distribution compared to the other two locations where northeast (NE) had the narrowest distribution (Figure 7). The age distribution for the three locations also differed, reflecting the differences in length, where the highest proportion of age 1 and 2 herring were sampled in SE.
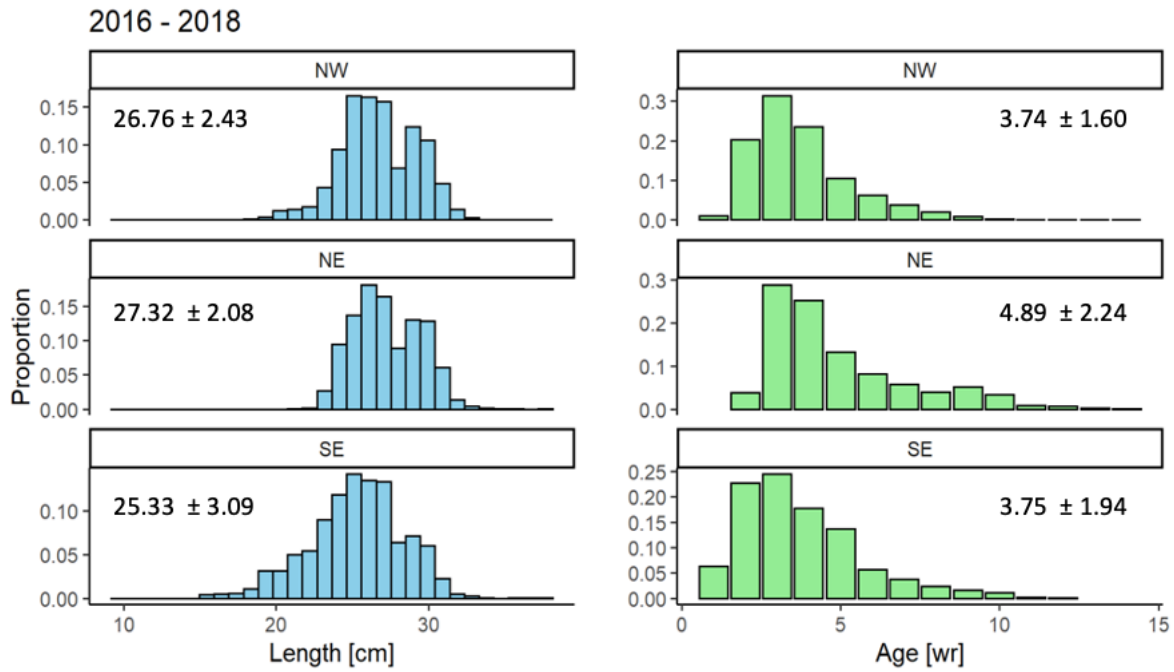
Figure 7: Length and age distribution of individuals sampled in the northwest (NW), northeast (NE) and southeast (SE) in the North Sea in year 2016 to 2018. Numbers in the plots represents mean ± SD.

Table 4: Sample size (N) per location (NW = northwest, NE = northeast and SE = southeast in the North Sea) and results from the two-sample Kolmogorov-Smirnov (KS) tests conducted on the total length distributions for the three locations. The table shows the KS statistic (D) value and p-value (P). P < 0.05 indicates significant differences.

LOCATIONS	N	KS TEST	D	P
NW	25824	NW – NE	0.144	< 0.001
NE	1255	NW – SE	0.209	< 0.001
SE	2195	NE – SE	0.285	< 0.001

Length distributions for the single age groups 3 to 6 showed a general trend of smaller herring in NE and SE, especially seen for age 5 and 6 (Figure 8). Significant differences in length distributions within each age group were found when comparing northeast (NW) to the two other locations (Table 5, p < 0.001). When comparing length distributions within the age groups between NE and SE, no significant differences were found for age 4 to 6 (Table 5, p > 0.05).
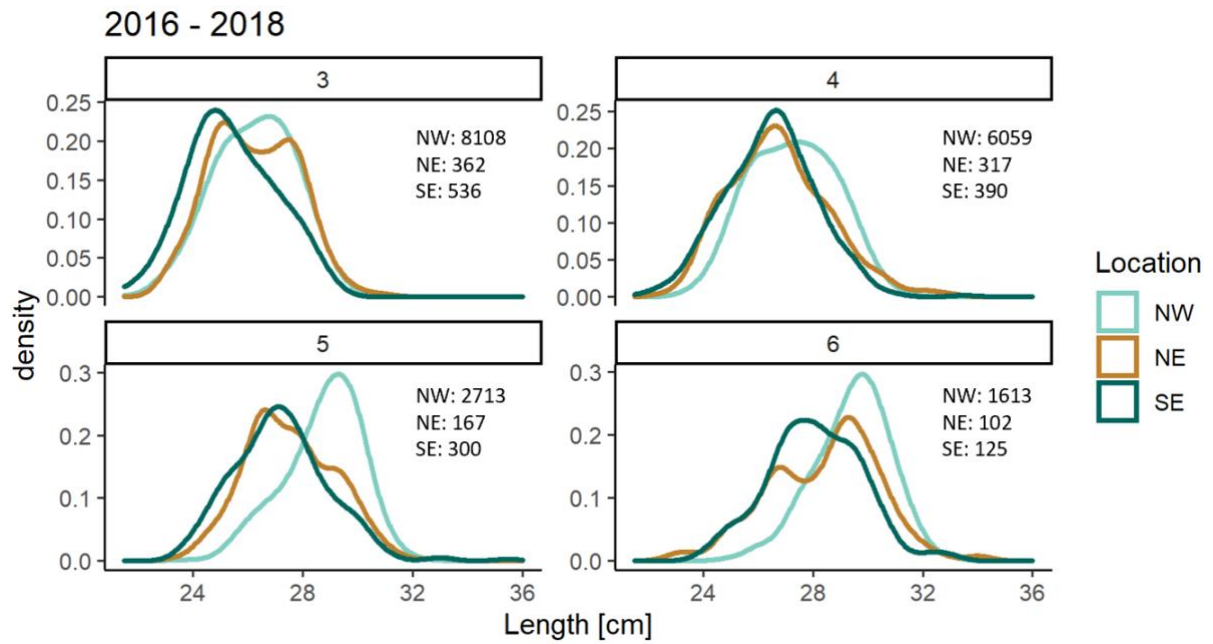
Figure 8: Length distribution for age 3 to 6 wr per location (NW = northwest, NE = northeast and SE = southeast) sampled in year 2016 to 2018. Numbers in the top panels represents age (wr), and numbers in plots represents sample size per location per age.

Table 5: Results from two-sample Kolmogorov-Smirnov tests conducted on length distributions for age groups 3 to 6 wr between locations (NW = northwest, NE = northeast and SE = southeast). The table shows the KS statistic (D) value and p-value (P). P < 0.05 indicates significant differences.

AGE [WR]	D	P
	NW – NE	
3	0.146	< 0.001
4	0.160	< 0.001
5	0.422	< 0.001
6	0.340	< 0.001
	NW – SE	
3	0.227	< 0.001
4	0.211	< 0.001
5	0.509	< 0.001
6	0.462	< 0.001
	NE – SE	
3	0.182	< 0.001
4	0.0836	> 0.05
5	0.0938	> 0.05
6	0.176	> 0.05

3.2.2 Otolith length – fish length ratio

The two-way ANOVA revealed a significant effect of location ($p < 0.001$) on the otolith length – fish length ratio, and no significant effect of age ($p > 0.05$). The Tukey HSD test showed significant differences in ratio when comparing individuals from NW to both NE and SE (Figure 9, $p < 0.05$). No significant differences were found between individuals from NE and SE (Figure 9, $p > 0.05$). There seemed to be a trend of a slightly higher ratio for NE and SE compared to NW (Figure 9).
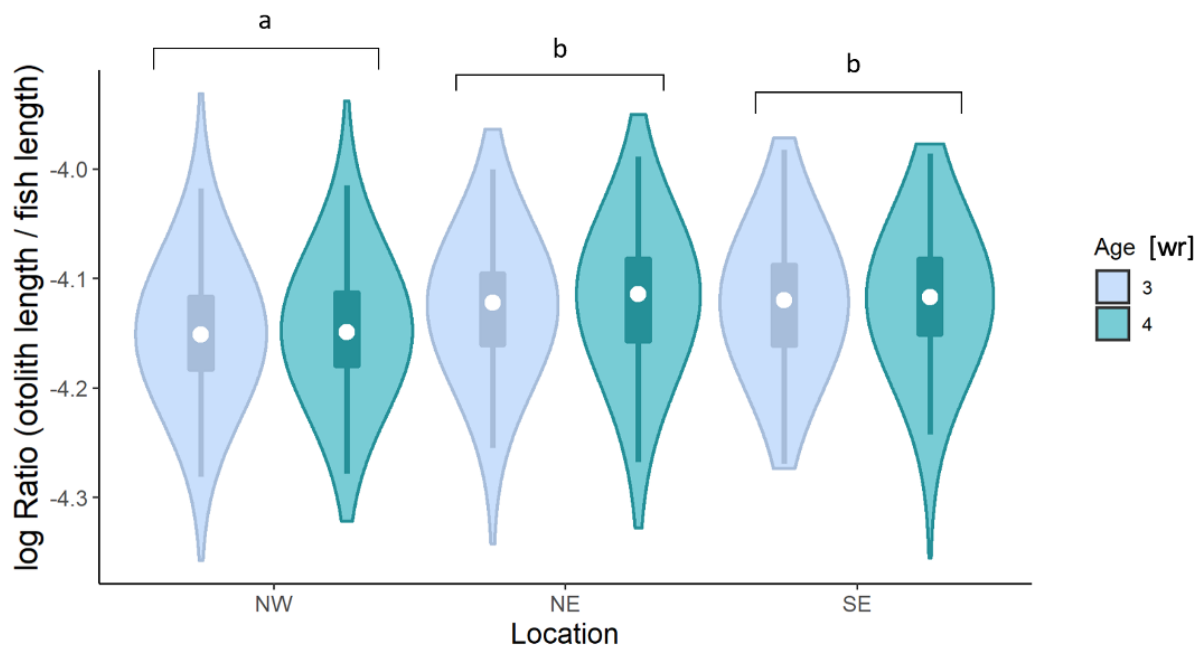
Figure 9: log Otolith length – fish length ratio for individuals of age 3 and age 4 wr per location (NW = northwest, NE = northeast and SE = southeast in the North Sea) sampled in year 2016 to 2018. The violins show the distribution of the data, and the white dots and bars show the medians and quartiles while the whiskers show the extremes within 1.5 times the interquartile range. Different letters represent significant differences ($p < 0.05$).

It is worth mentioning that even though significant differences were detected, the differences in mean log ratios and standard deviations between locations were minor (Figure 9, Table 6) with a maximum difference in mean log-ratio (between NW and SE) being $< 1\%$.

Table 6: Mean log otolith length – fish length ratio, standard deviation (SD) and sample size (N) per location for individuals of age 3 and age 4 wr sampled in year 2016 to 2018 per location (NW = northwest, NE = northeast and SE = southeast in the North Sea).

Location	Age 3			Age 4		
	Mean log-ratio	SD	N	Mean log-ratio	SD	N
NW	-4.15	0.0514	1204	-4.15	0.0532	1145
NE	-4.13	0.0521	362	-4.12	0.0586	317
SE	-4.12	0.0519	536	-4.12	0.0550	390

3.2.3 Main shape features and Multivariate analysis

Comparison of mean otolith shape of age 3 and 4 herring by combining all three locations (Figure 10a) revealed no clear differences between the two age groups. The comparison of mean otolith shape between locations by combining age 3 and 4 also revealed no clear differences (Figure 10b).
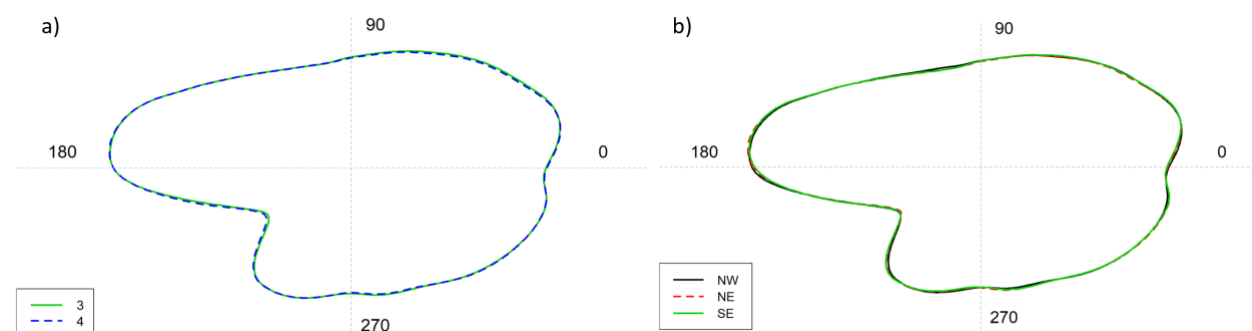

Figure 10: a) Mean otolith shape of individuals of age 3 wr and individuals of age 4 wr sampled in year 2016 to 2018 with all three locations combined. b) Mean otolith shape for individuals of age 3 and 4 combined per location (NW = northwest, NE = Northeast and SE = southeast in the North Sea) sampled in year 2016 to 2018.

Despite the mean shape plots indicating identical shape for age 3 and 4 across locations, post-hoc tests revealed significant differences in otolith shape between all three locations (CAP, $p < 0.05$, Table 7). However, when visually inspecting the individual CAP scores, there were large proportions of overlap between locations (Figure 11). Mean CAP values for NE and SE were also overlapping at CAP1, and the distribution of individual CAP scores along the CAP1 axis (explaining 89.1% of the variation) showed a trend of larger proportions of individuals from these two locations being distributed to the right, while individuals from NW were distributed

to the left in comparison. NE and SE showed different distributions along the CAP2 axis, where a small but higher proportion of individuals from SE were distributed further up on the axis, and individuals from NE further down. The overlap of CAP scores indicated large similarities in otolith shape for a high proportion of individuals from all three locations.
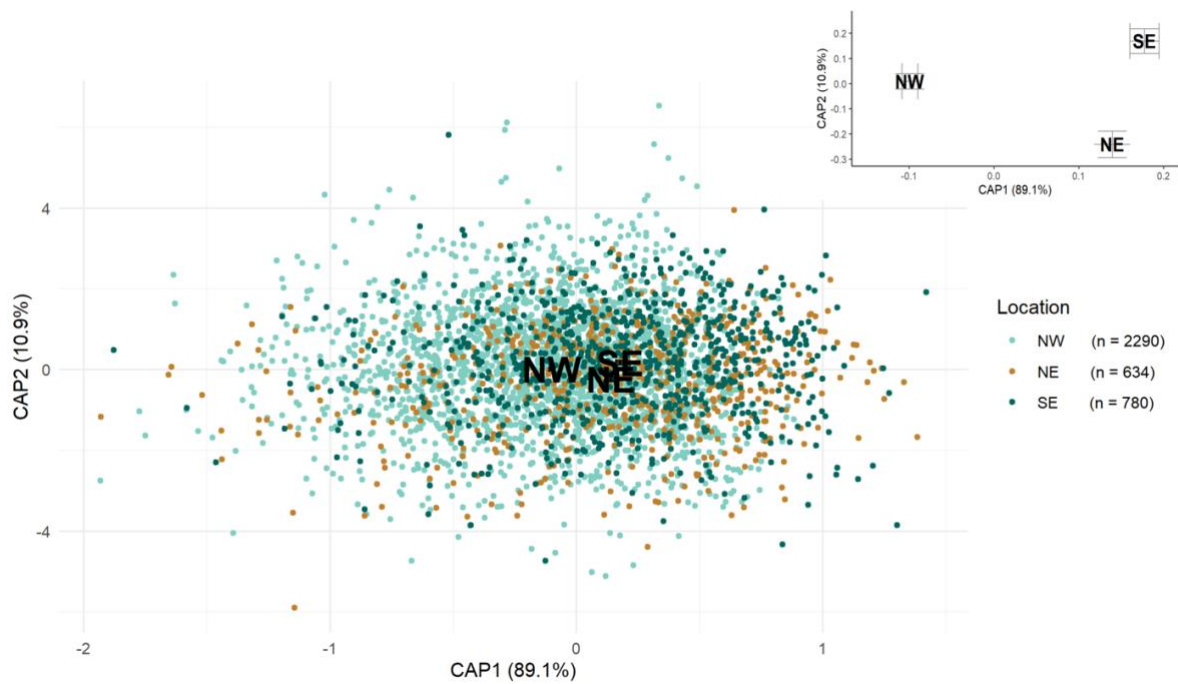
Figure 11: Canonical scores on the first two discriminating axes, CAP1 and CAP2 (and their explained variation), for individuals of age 3 and 4 wr combined per location (NW = northwest, NE = northeast and SE = southeast in the North Sea) sampled in year 2016 to 2018. Black letters in the plot represent mean canonical value for each location, and the top right plot represent the mean canonical values ± 1SE without the canonical scores for a clearer picture of their spread along the axes. n represents number of individuals per location.

Table 7: Output from Canonical analysis of principal coordinates followed by an ANOVA-like permutation test (1000 permutations) conducted on otolith shape grouped by location (NW = northwest, NE = northeast and SE = southeast in the North Sea). The table shows overall analysis and post-hoc tests, degrees of freedom (Df), variance, F-value (F) and p-value (P), where $P < 0.05$ indicates a significant effect.

Location	Df	Variance	F	P
NW – NE – SE	2	0.00563	11.006	0.001
Post-hoc				
NW – NE	1	0.00343	10.553	0.001
NW – SE	1	0.00472	15.312	0.001
NE – SE	1	0.00171	2.562	0.017

3.3 Temporal stability in otolith shape

Large similarities in length distributions (Figure 8, Table 5) and no significant differences in otolith length – fish length ratio (Figure 9) for NE and SE herring, in addition to visual inspection of the distribution of CAP scores (Figure 11) lead to the decision of combining the two locations for further analyses.

3.3.1 Otolith length – fish length ratio

The otolith length – fish length ratio for the five cohorts 2011 to 2015 indicated a trend of somewhat stable otolith growth (Figure 12). However, cohort 2014 was significantly different from cohorts 2011, 2012 and 2013 ($p < 0.01$). Despite the significant differences, the mean otolith length – fish length ratios showed minor differences between cohorts (Table 8).
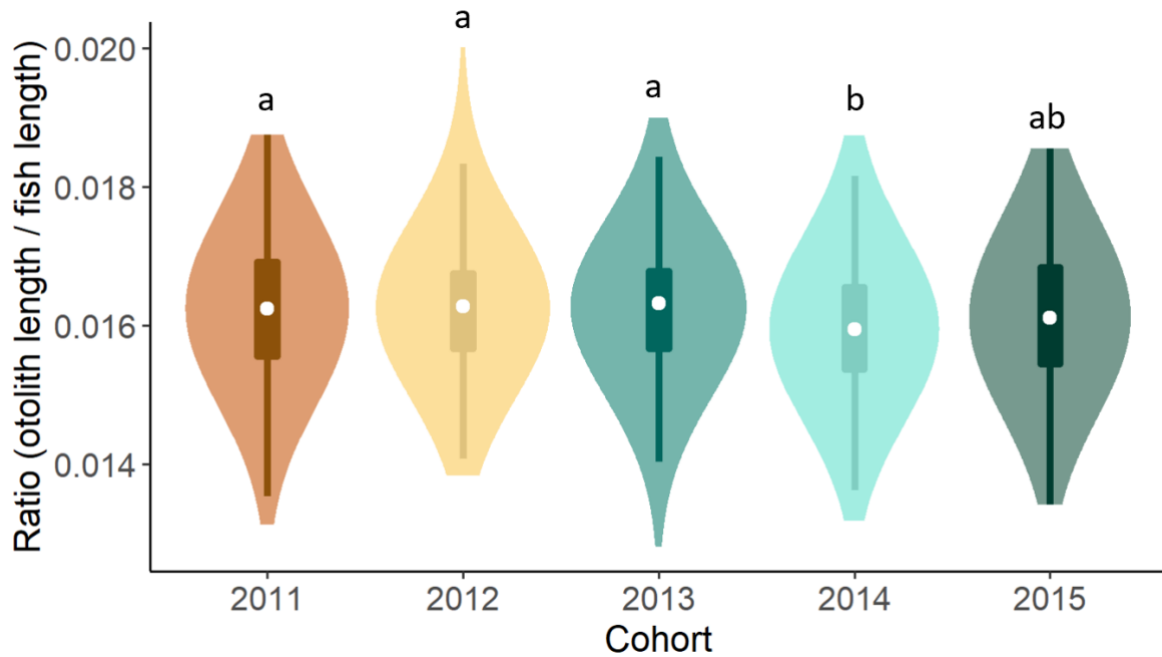
Figure 12: Otolith length – fish length ratio for individuals of age 3 to 6 wr combined per cohort sampled in the eastern part of the North Sea (area 28, 8 and 41, Figure 3). The violins show the distribution of the data, and the white dots and bars show the medians and quartiles while the whiskers show the extremes within 1.5 times the interquartile range. Different letters represent significant differences (p < 0.05).

Table 8: Mean otolith length – fish length ratio (Mean ratio), standard deviation (SD) and sample size (N) per cohort for individuals of age 3 to 6 wr combined, sampled in the eastern part of the North Sea (area 28, 8 and 41, Figure 3).

Cohort	Mean ratio	SD	N
2011	0.0162	0.000970	200
2012	0.0163	0.000891	352
2013	0.0162	0.000866	376
2014	0.0160	0.000900	452
2015	0.0161	0.000980	132

3.3.2 Main shape features and Multivariate analysis

Comparison of mean otolith shape for the five cohorts using ages 3 to 6 combined showed a consistent overlap indicating an overall stable otolith shape between cohorts (Figure 13). When separating the cohorts into single age groups, deviations in outlines became slightly clearer, especially in age group 6 when comparing cohort 2015 to the others, where it deviated along the rostrum to the antirostrum, and at the postrostrum (Figure 14). Cohort 2014 also showed

some deviation along the post- and pararostrum when compared to the other cohorts (Figure 13 and 14).
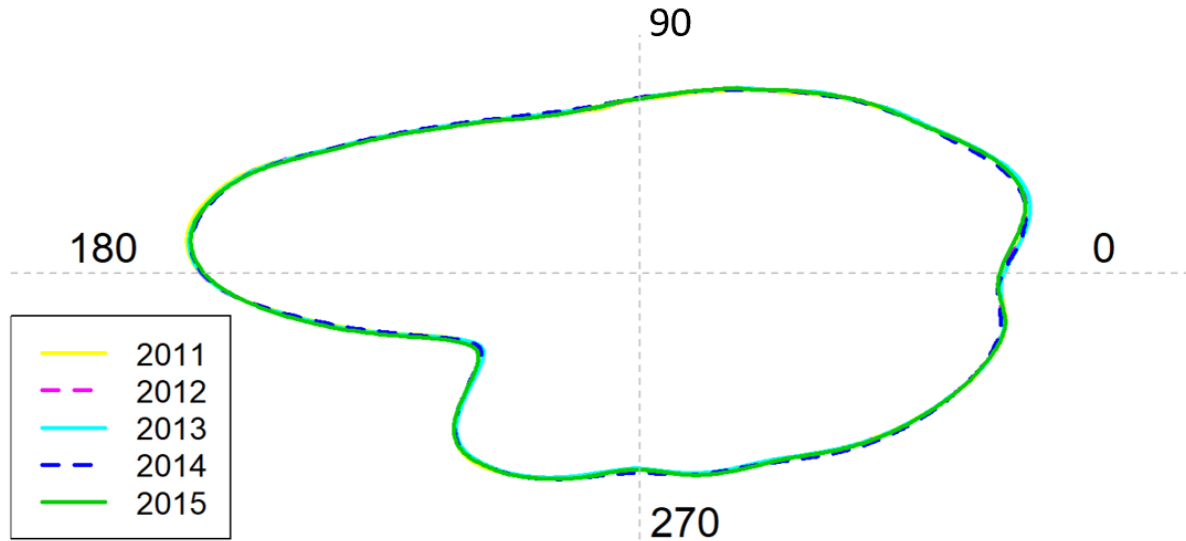
Figure 13: Mean otolith shape per cohort for age groups 3 to 6 wr combined, sampled in the eastern part of the North Sea (area 28, 8 and 41, Figure 3).

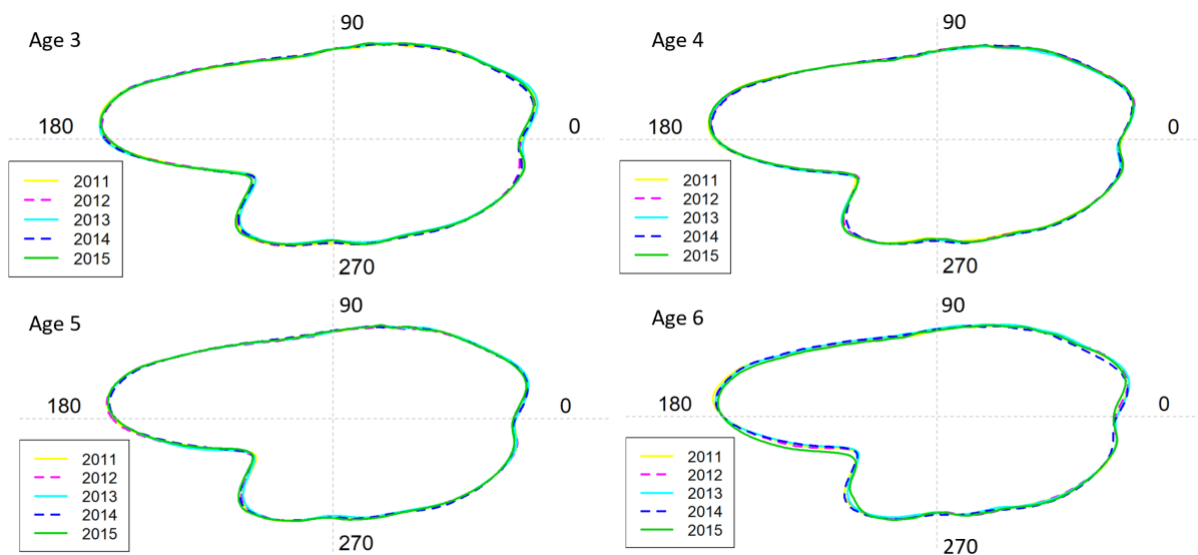*Figure 14: Mean otolith shape for cohorts 2011 to 2015 per age group (age 3 to 6 wr), sampled in the eastern part of the North Sea (area 28, 8 and 41, Figure 3).*

Post-hoc tests showed that otolith shape differed significantly when comparing cohort 2014 to all other cohorts, and when comparing cohort 2013 to 2015 (CAP, p < 0.05, Table 9). No significant differences were found between the remaining cohorts (CAP, p > 0.05, Table 9).

When inspecting the mean CAP values for the cohorts, the significant result for cohort 2014 was reflected in its distribution along the CAP1 axis (explaining 62.4% of the variation) where it was distributed towards the left compared to the other mean CAP values (Figure 15 first panel). Cohort 2011, 2012 and 2013 showed clustering of mean CAP values and their individual CAP scores showed large similarities in distribution along both CAP axes (Figure 15). Despite the significant shape differences between some cohorts, the individual CAP scores showed overlap between all cohorts and no clear clustering were indicated (Figure 15). This indicated large similarities in otolith shape for a high proportion of individuals across the five cohorts. Statistical output shown in Appendix Table A2.
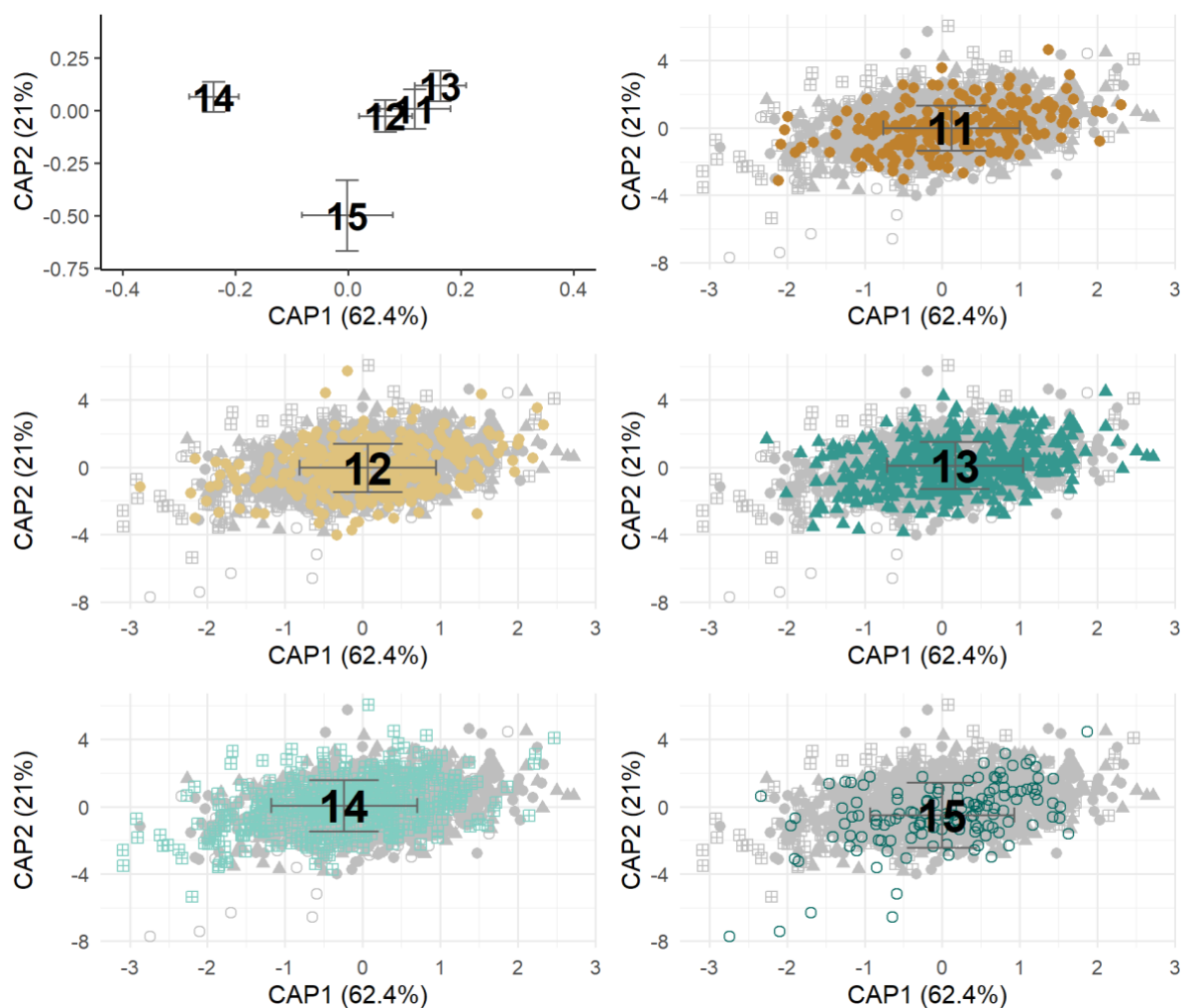
Figure 15: The first plot (upper left) represents mean canonical values ± 1SE for all five cohorts. The other five plots represent canonical scores on the first two discriminating axes, CAP1 and CAP2 (and their explained variation), for individuals of age 3 to 6 wr within cohorts 2011 to 2015 sampled in the eastern part of the North Sea (area 28, 8 and 41, Figure 3). Black numbers in the plot represent mean canonical value ± 1SD for each cohort (11 = 2011, 12 = 2012, 13 = 2013, 14 = 2014 and 15 = 2015).

Cohort	Homogenous groups
2011	ab
2012	ab
2013	a
2014	c
2015	b

The calculated proportions varied both between cohorts, and between the age groups within cohorts (Figure 16). Cohorts 2014 and 2015 displayed the lowest and highest proportions of WBSS, respectively.

Figure 16: Calculated proportion of Western Baltic Spring Spawning herring (%WBSS) per age per cohort in the eastern North Sea samples (area 28, 8 and 41, Figure 3). Numbers above the lines shows mean proportion WBSS per cohort. Lines added for graphical purposes.
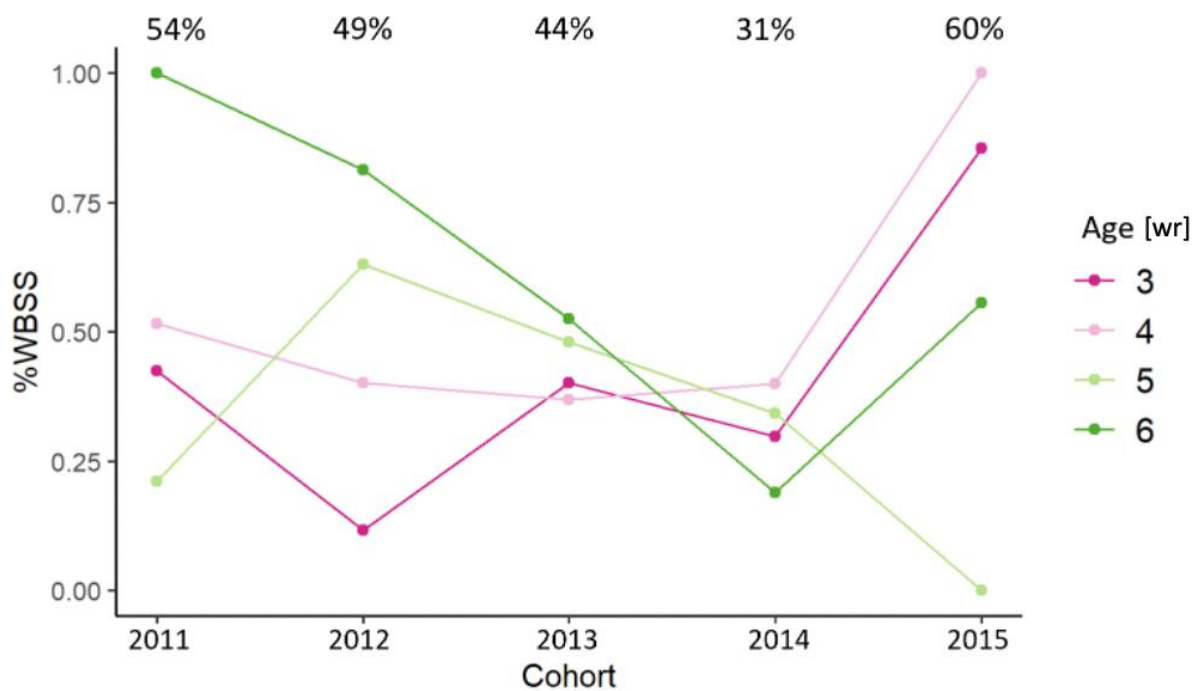
4. Discussion

The aim of this thesis was to clarify the possibility of temporal stability in otolith shape in an area of stock overlap in the North Sea by investigating the temporal variation in shape, and the temporal and spatial trend in mixing between stocks by means of shape analysis. It was hypothesized that otolith shape would vary spatially depending on the degree of mixing between the two main stocks in the area, NSAS and WBSS. The results showed significant shape differences between locations supporting the hypothesis of spatial variation as a result of various degrees of overlap between the two stocks. As for the question regarding temporal stability, herring from the eastern part of the North Sea demonstrated stability in otolith shape for three consecutive years.

Age has been shown to contribute to within-stock variation in otolith shape, and it has thus been suggested that this effect should be carefully assessed before drawing conclusions regarding stock structure (Castonguay et al. 1991). The analysis conducted in this thesis to demonstrate the effect of age on otolith shape in age 1 to 6 Atlantic herring indicated a decrease in variation with age. Less variation was observed from age 3+ winter rings (wr) which is when the majority of individuals (>90% for NSAS and >70% for WBSS) are assumed to have reached maturity (ICES 2020). The decrease in variation with age has also been observed for local Norwegian herring populations (Libungan et al. 2015b). However, it is important to note that the samples used in this thesis were sampled in an area of stock-overlap, thus possible differences in the degree of overlap within the age groups might also have contributed to some of the variation observed.

4.1 Spatial variation in otolith shape

Mean length-at-age for catches of age 3+ herring is generally smaller in the eastern part of the North Sea Division IVa compared to the western (ICES 2020). WBSS herring have shown a slightly smaller length-at-age than NSAS (Clausen et al. 2015), and their overlap during summer feeding in the eastern North Sea might explain the smaller length distributions for catches in this area. As was also observed in this thesis by the slightly smaller length distributions for age 3 to 6 herring sampled in the east, compared to those sampled in the west. The occurrence of WBSS herring during summer have previously been traced into the North Sea and further up north along the west coast of Norway (Berg et al. 2017, Berg et al. 2019).

The similar results for the north- and southeastern herring otolith length – fish length ratio (hereon referred to as relative otolith size) and shape found in this thesis reflect a possible similar latitudinal distribution of the two stocks in the eastern North Sea during their summer feeding. In the Transfer Area (Figure 2) the overlap is taken into consideration and the two stocks are split into proportions for assessment. Because WBSS herring seem to migrate and mix with NSAS further north than the Transfer Area, this should be taken into account in the assessment of the WBSS stock as it would be exposed to fishing mortality outside the transfer area as well. ICES (2020) have suggested discussing the geographical boundary for the Transfer Area.

Somatic growth and otolith growth are phenotypic expressions arising from different factors such as temperature, growth and food availability (Folkvord et al. 2000, Folkvord et al. 2004, Hüssy 2008). Several studies have reported a strong correlation between otolith size and fish size (Yilmaz et al. 2014, Aneesh Kumar et al. 2017, Souza et al. 2019), suggesting different growth rates to be an important factor for variation in otolith characteristics. It is, however, important to mention that studies have found that the otolith size – fish size relationship changes during the life of the fish due to the otolith continuing to grow even though fish growth decrease or come to a halt (Hare and Cowen 1995), resulting in a larger relative otolith size (Reznick et al. 1989, Pryce and Scott 1993). The relationship is thus not necessarily proportional. A study conducted on the size and shape of red snapper *Etelis carbunculus* otoliths revealed regional similarities between individuals with similar growth, and regional differences between individuals of different growth (Smith 1992). As was observed in this thesis where larger differences in length distributions and otolith parameters were observed between the western and eastern samples, than among the eastern samples. Feeding level have shown to have significant impact on the formation of the otolith shape during early stages and into the juvenile phase (Hüssy 2008), and it is believed that the shape of the otolith is determined during these stages and that layers are added to this shape as the fish grow (Libungan et al. 2015a). The otolith shape would then be a result of both genetics and the environment experienced at spawning and nursery grounds (Couillard et al. 2022), and differences in otolith size and shape would then be expected to occur at spatial scales with differing environments. The work conducted by Begg and Brown (2000) on otolith shape of haddock *Melanogrammus aeglefinus* of western and eastern Georges Bank found differences between the two areas, and suggested both differences in environment and genetics to be contributing factors to the observed stock structure. The samples from the eastern part of the North Sea assumingly constitute a mixture

of mainly NSAS and WBSS herring and otolith shape as described by wavelets from this area would then, to some extent, result in an intermediate of the otolith shapes for the two stocks. The western samples were assumed to constitute primarily NSAS herring, and, if this assumption holds true, the otolith shape described by wavelets from this area would then be more representative to the NSAS stock. This could be the reason for the larger difference observed in otolith parameters between west and east compared to among eastern samples.

4.2 Year-class variation

The results demonstrated temporal stability in otolith shape for three consecutive years, i.e., no significant year-class effects were observed between cohorts 2011, 2012 and 2013. In the cases where significant differences in otolith parameters did occur between cohorts, this was somewhat reflected by relative otolith size and the distribution of individual CAP scores. As was especially the case for cohort 2014. Cohort 2014 deviated to a greater extent in mean canonical value from the rest of the cohorts, and displayed similarities in relative otolith size with herring sampled northwest in the North Sea which were assumed to comprise mainly individuals of the NSAS stock. The calculated mean proportion of WBSS herring for cohort 2014 was also lower compared to the rest of the analyzed cohorts. These results suggest a larger proportion of NSAS in cohort 2014, compared to the other cohorts. In 2014 and 2015 a larger than average age 0-wr and 1-wr abundance of NSAS were recorded, respectively (ICES 2020, 2021b), agreeing with the results of a presence of a strong year-class of NSAS herring (i.e. cohort 2014). In addition, cohort 2015 has been recorded as lower than average for NSAS (ICES 2021b) which seems to be reflected in the larger proportion calculated for WBSS herring in the cohort. Because individuals of NSAS do not attain their first wr until a year after they are born their true age is wr+1. Consequently, individuals of NSAS do not belong to the same year-class as WBSS with the same number of wr. For example, cohort 2014 and 2015 in the present study are referred to as 2013 and 2014 in assessment, respectively (ICES 2021a).

Year-class strength of Atlantic herring is to a large degree determined during the larval and juvenile phases (Nash and Dickey-Collas 2005, ICES 2020). Even though not fully understood yet (Brosset et al. 2019), larval survival and strong and weak year-classes of herring have been strongly linked to food availability which again is closely linked to sea surface temperatures and wind patterns affecting coastal currents (Corten 2013, Lusseau et al. 2014, Skagseth et al. 2015). These are some of the same factors suggested to influence otolith growth and shape

either directly or through somatic growth (Hüssy 2008, Denechaud et al. 2020). Differences in year-class strength could then be indicative of annual fluctuations in environment, and thus year-class variation in otolith shape. Denechaud et al. (2020) demonstrated a significant year-class effect in a study conducted on the long-term stability in cod otoliths. However, the study showed that the contribution of this effect to the overall variation was minor and their results suggested a highly stabile otolith shape between cohorts within a stock over time (Denechaud et al. 2020). Another study conducted on spring spawning components of Atlantic herring in the Northwest Atlantic found no year-class variation in otolith shape for several of the components over a time period of three years (Couillard et al. 2022). The shape differences observed between cohorts in this study might be a result of the different year-class strengths affecting the proportions of the two stocks, NSAS and WBSS, in the samples. The effect of various degrees of stock-overlap on the observed otolith shapes is especially indicated by the non-significant differences in shape between non-consecutive cohorts (i.e., no difference in shape were observed between cohorts 2011 and 2015, and between cohorts 2012 and 2015). However, the possibility of a significant year-class effect on the otolith shape itself cannot be ruled out without removing the genetic effect (allocating individuals to their stock of origin).

The majority of individuals used for investigating both the spatial variation and the temporal stability overlapped at a great extent in otolith shape indicating highly similar otolith shapes between large proportions of individuals. However, small proportions of individuals deviated from the majority at various extents, suggesting the presence of herring from other genetic and/or environmental origin. The Norwegian spring spawning herring (NSSH) have been shown to migrate south of 62°N during their spawning season, where they mix with the two stocks NSAS and WBSS in the eastern part of the North Sea and Skagerrak (Eggers et al. 2014, ICES 2020). Genetic assignment of individuals caught during the Norwegian HERAS survey in 2020 also identified the presence of NSSH in the northeastern part of the North Sea for ages 4+ wr (ICES 2021a). Herring catches in the eastern part of the North Sea and Division IIIa are split and assessed as part of either NSAS or WBSS. However, despite being treated as one stock in these areas, spring spawning herring display local stock diversity (ICES 2019) and population structure of local populations along the south-west coast of Norway has been revealed by otolith shape analysis (Libungan et al. 2015b). If individuals from the NSSH stock and/or local herring are present in my samples, this might have contributed to the small proportions of individuals deviating in shape.

4.3 Implications for management

Identifying stocks, especially in mixed-stock fisheries, is crucial for management purposes to enable correct assessment and sustainable utilization of our marine resources. Currently the splitting methods for mixed catches of NSAS and WBSS in the Transfer Area and Division IIIa are mean vertebrae counts (VS) (Norwegian catches) and otolith microstructure (OM) analysis (Danish catches) (ICES 2019). The use of mean VS is based on the assumption that the mixed catches only comprise two stocks, and is thus sensitive to influence of individuals from a different origin than the stocks taken into account (Mosegaard and Madsen 1996). In contrast to the otolith shape analysis that utilizes computer software to extract shape contours, the mean VS and OM methods are based on visual inspection, and thus require high precision and experienced technicians. Studies have been successful in both validating and utilizing otolith shape as a tool for stock identification (Campana and Casselman 1993, Turan 2000, DeVries et al. 2002, Eggers et al. 2014, Vieira et al. 2014, Libungan et al. 2015a). In addition, otoliths are routinely sampled, as these structures are used for ageing of fish, making them easily available for further research such as in shape analysis. Previous studies have been conducted by collecting fish during spawning season where mixing is assumed to be minimal and using these shape characteristics as a baseline to monitor and estimate stock composition in mixed fisheries (DeVries et al. 2002, Hüssy et al. 2016, Berg et al. 2019). However, there are some challenges and uncertainty regarding the baseline method due to the potential temporal variation in otolith shape that, if significant, would require an annual recalculation of the baseline (Begg and Brown 2000). Providing management with evidence of temporal stability in otolith shape for different fish species would then strengthen this method in regard to using the same baselines over several years, which again would support the implementation of this method as a valuable and highly efficient tool for monitoring and allocating mixed catches for assessment.

4.4 Limitations

Applying otolith shape analysis using individuals of unknown origin sampled at a location and time of stock-overlap, as done in this thesis, leaves uncertainty about the contribution of factors to the observed variation. These factors are as mentioned mainly believed to be genetics and environment (Cardinale et al. 2011, Berg et al. 2018). Thus, the analysis regarding spatial variation do not depict an exact otolith shape for a true managed stock, as the shapes observed, especially in the eastern North Sea, are intermediate shapes of a mixture of individuals from

different genetic origin. In addition, the analysis on temporal stability in otolith shape cannot answer as to what extent the stock-overlap or the potential year-class variation contribute to the variation in otolith shape between the analyzed cohorts.

Using mean VS to calculate the proportion of WBSS herring in the samples might provide an indication of the extent of the overlap. However, the sensitivity to the influence of individuals from other stocks/populations in the samples leads to some uncertainty. Mean VS for NSSH is higher than for NSAS and WBSS (Haraldsvik 1968). In addition, the winter spawning component of NSAS have previously been shown to display a slightly higher mean VS than the autumn spawning components (Hulme 1995), which would affect the calculated proportion of WBSS herring (Mosegaard and Madsen 1996). Nevertheless, Berg et al. (2017) reported that the mean VS in both the North Sea and western Baltic have been relatively stable throughout the years.

4.5 Recommendations for future studies

To better clarify the possibility for temporal stability in otolith shape, future studies should allocate individuals to their stock of origin prior to analysis when using mixed samples as in this study. Identifying the origin of individuals would help remove some of the variation in shape caused by the overlap and provide clarity of within-stock variation in shape. Allocating individuals to their respective stock could be accomplished by combining shape analysis with different methods such as DNA analysis (Ruzzante et al. 2006, Gilbey et al. 2017, Dahle et al. 2018) or OM analysis (Clausen et al. 2007) to identify origin and/or spawning time of individuals. This would however require more comprehensive research. Another approach would be sampling during spawning at spawning locations, as done by Hüssy et al. (2016) when creating a baseline for Baltic Sea cod. However, to answer the question of temporal stability, this would require several years of sampling.

5. Conclusion

This study demonstrated spatial variation in otolith shape throughout the North Sea during the summer feeding migration of Atlantic herring as a result of possible differences in overlap between herring stocks. Temporal stability in otolith shape for three consecutive years was demonstrated, indicating no within-stock year-class variation in otolith shape as well as a somewhat stable degree of mixing between stocks in the eastern North Sea for the analyzed cohorts. It is often taken for granted that there is temporal instability in otolith shape which thus require annual recalculation of shape characteristics. However, few studies have investigated this assumption, and further studies regarding temporal stability in otolith shape for other commercially important fish species should be conducted.

Otolith shape analysis using time-series of otoliths sampled in areas of stock overlap seem like an easy and cost-effective method to apply when monitoring the temporal trend in mixing and the possibility for temporal stability in otolith shape within a stock. However, to determine the contribution of mixing and other influential factors on otolith shape this method requires a combination with an established baseline or other methods for allocating individuals to their stock of origin. The type of analysis used in this thesis is useful in the way that it might provide indications of a somewhat stable degree of stock-overlap between cohorts, and a stable otolith shape within stocks (temporal stability). Alternatively, if the mixing of stocks is fluctuating to a large extent, one can to some extent gain insight into historical fluctuations, when using time series of collected otoliths.

6. References

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Appendix
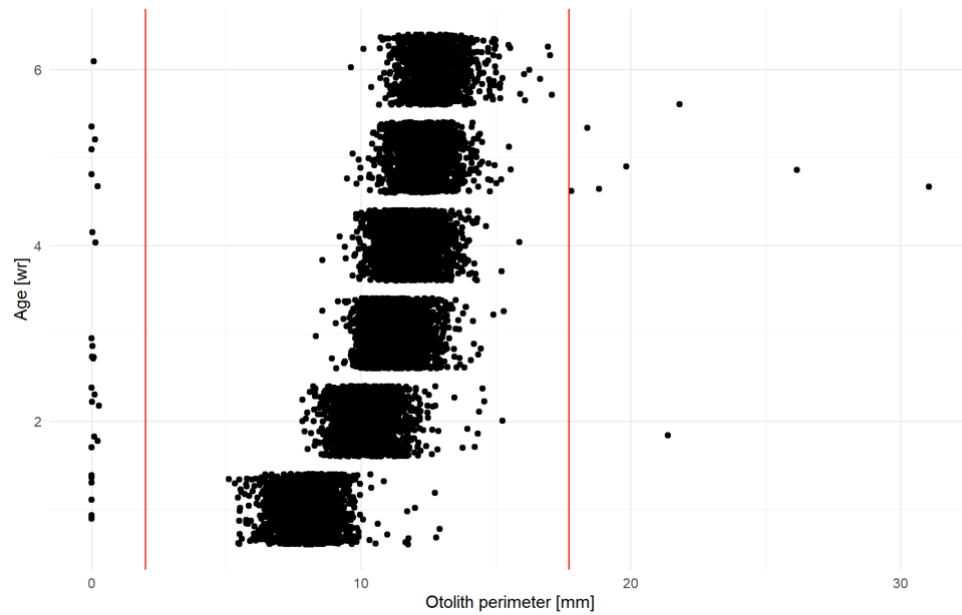
Removal of outliers:

Figure A 1: Cleveland dotplot of otolith perimeter for age 1 to 6. Red lines indicate ´cut-off´ lines for the removal of outliers which seem either too large or too small to be true.
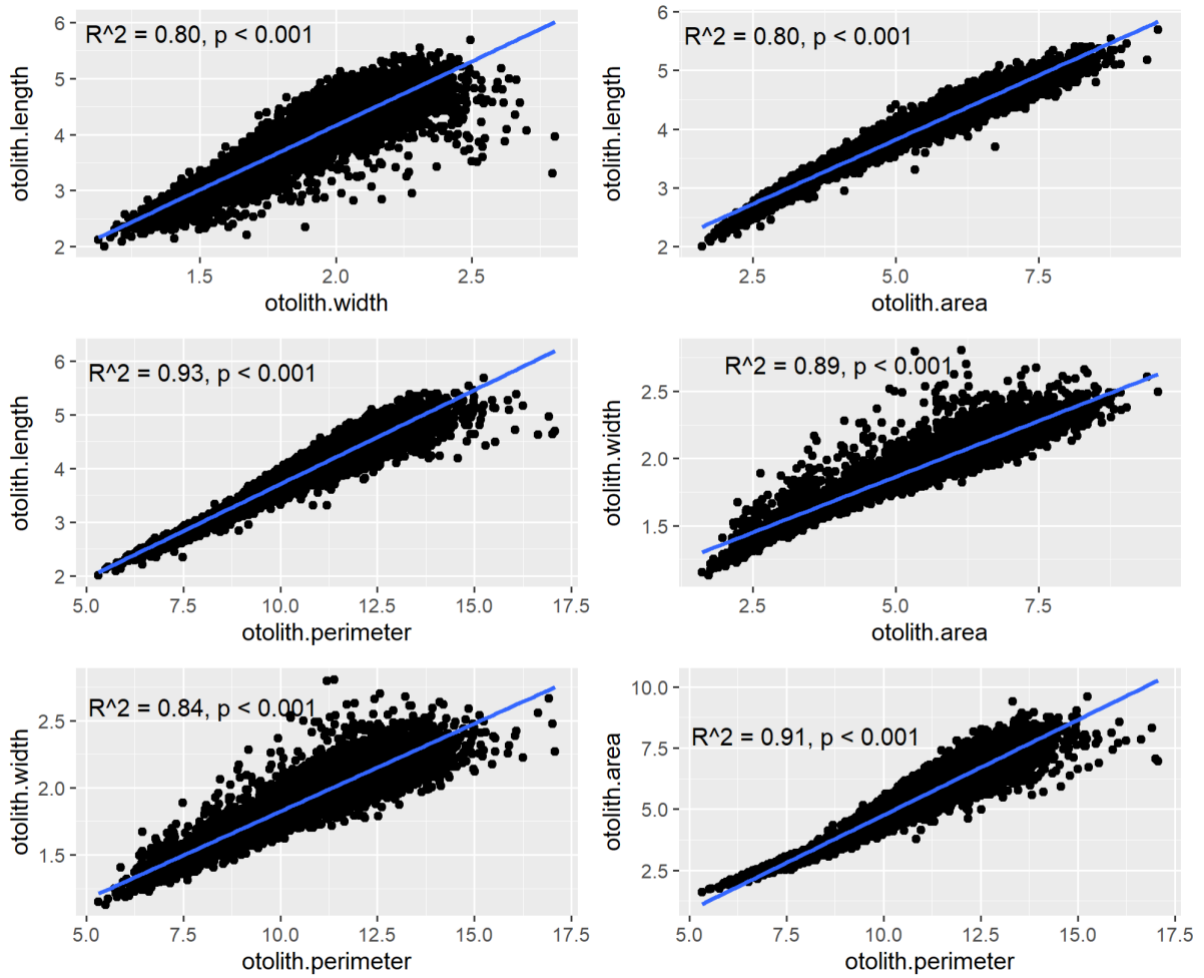
Correlation of otolith indices:

Figure A 2: Correlation of the four otolith indices, otolith length, width, area and perimeter for individuals of age 1 to 6, showing significant positive correlation between all four indices. $p < 0.05$ indicates a significant effect, and R^2 close to 1 indicates a high correlation.

Age effects:

Table A 1: Output from Canonical analysis of principal coordinates followed by an ANOVA-like permutation test (1000 permutations) conducted on otolith shape for southeastern samples (SE) grouped by age (for age groups 1 to 6). The table shows overall analysis and post-hoc tests, degrees of freedom (Df), variance, F-value (F) and p-value (P), where P < 0.05 indicates a significant effect.

Age	Df	Var	F	P
1 to 6	5	0.041	18.02	0.001
		Post hoc		
1-2	1	0.024	20.60	0.001
1-3	1	0.050	37.79	0.001
1-4	1	0.075	44.76	0.001
1-5	1	0.097	42.47	0.001
1-6	1	0.12	34.18	0.001
2-3	1	0.016	19.99	0.001
2-4	1	0.026	27.29	0.001
2-5	1	0.033	29.32	0.001
2-6	1	0.028	20.97	0.001
3-4	1	0.0028	2.79	0.009
3-5	1	0.0074	6.23	0.001
3-6	1	0.0078	5.30	0.001
4-5	1	0.0036	2.49	0.008
4-6	1	0.0037	1.94	0.047
5-6	1	0.0030	1.16	0.273

Temporal stability:

Table A 2: Output from Canonical analysis of principal coordinates followed by an ANOVA-like permutation test (1000 permutations) conducted on otolith shape of samples from the eastern part of the North Sea (area 28, 8 and 41, Figure 3), grouped by cohorts. The table shows overall analysis and post-hoc tests, degrees of freedom (Df), variance, F-value (F) and p-value (P), where P < 0.05 indicates a significant effect.

cohorts	Df	Var	F	P
2011 to 2015	4	0.0066	2.34	0.001
Post-hoc:				
2011-2012	1	0.0019	1.03	0.377
2011-2013	1	0.0023	1.31	0.213
2011-2014	1	0.0053	3.27	0.007
2011-2015	1	0.0055	1.60	0.104
2012-2013	1	0.0011	0.77	0.595
2012-2014	1	0.0039	2.94	0.007
2012-2015	1	0.0028	1.21	0.262
2013-2014	1	0.0064	5.08	0.002
2013-2015	1	0.0046	2.19	0.03
2014-2015	1	0.0045	2.30	0.03