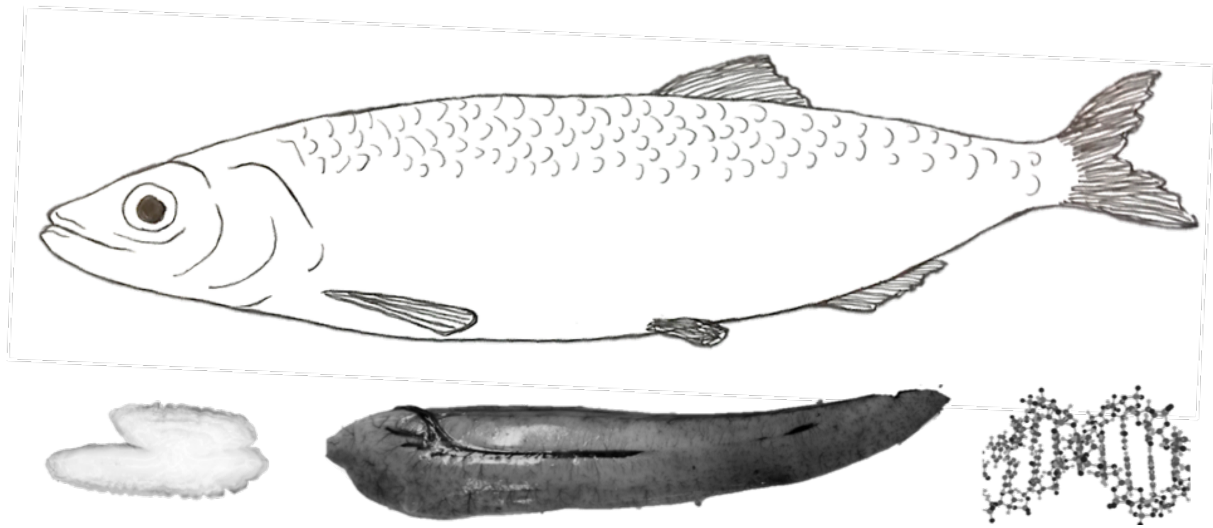
Master of science in Marine biology

Spawning season fidelity in spring and autumn spawning Atlantic herring, *Clupea harengus*

- A comparative study of genomic and phenotypic separation methods



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Bergen, June 2019
Hedda Dugstad Østgaard

So long, and thanks for all the fish.
><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>-><>

Abstract

Atlantic herring have a complex population structure and show a wide range of life history strategies, including spawning times and locations. Time of spawning is a key characteristic for population separation. Spawning timing fidelity is often assumed in herring, that is they spawn in the same season as they hatched. However, low rates of spawning season switching (straying), is observed in a number of stocks suggesting interbreeding between populations. Spring and autumn are the most common spawning seasons among Northeast Atlantic herring populations. Seasonally spawning herring are traditionally separated on their gonadal maturity stage at sampling, but otolith microstructure has also been used to separate herring form mixed catches. Genetic discreteness was recently documented for spring and autumn spawning herring.

The present thesis investigates population structure and dynamics between spring and autumn spawning herring. Phenotypic maturity status was related to two genomic SNPs markers. In addition, hatching season was determined through visual reading of otolith microstructures, corroborating the already known spawning season pattern. Herring were sampled during spring and autumn at the same locations in western Norway from autumn 2016-2018. Spawning herring (ripe and running) were found in both sampling seasons but spring spawning herring dominated in all samples. Herring were successfully separated to spawning type spring or autumn by the three separation methods. Correspondence was generally high, but some spawning season switching was also observed. In autumn samples, straying was documented among the autumn spawning herring (45.0%), while the spring sampled spring spawning herring showed high fidelity (95.2%). These results suggest an increased effect of straying on the less abundant autumn spawning herring. When comparing separation methods two by two the correspondence was highest between hatching season and genetic spawning season. It is suggested that adaption to spawn in spring or autumn causes selective pressures which keeps isolation between seasonal spawning populations. When comparing all separation methods, the correspondence was highest for the SNPs test. SNPs markers have great potential for separating spring and autumn spawning herring from mixed catches and should be implemented as a separation method in assessment of mixed herring stocks.

Keywords: *Atlantic herring, population structure, otolith microstructure, maturity stages, genetic markers, phenotypic plasticity.*

CONTENTS

ACKNOWLEDGEMENTS **2**

ABSTRACT **3**

1 INTRODUCTION **6**

1.1 The Atlantic herring 6

1.2 Population structure and dynamics 6

1.3 Separating herring populations 8

1.3.1 Phenotypic separation methods *9*

1.3.2 Genotypic separation methods *10*

1.3.3 Combining separation methods *11*

1.4 Aim of the study 12

2 MATERIALS AND METHODS **13**

2.1 Study area and field sampling methods 13

2.2 Wet lab procedures and ageing 14

2.3 Separation to spawning type spring or autumn 15

2.3.1 Maturity stages and separation to spawning season *16*

2.3.2 SNPs markers and separation to genetic spawning season *16*

2.3.3 Otolith microstructure analysis and separation to hatching season *18*

2.4 Data material 21

2.4.1 Statistical analysis and software *22*

3 RESULTS **23**

3.1 Separation to spawning type 23

3.1.1 Maturity stages and maturity spawning season *23*

3.1.2 SNPs and genetic spawning season *24*

3.1.3 Otolith microstructure and hatching season *25*

3.2 Comparing spawning season separation methods 26

3.2.1 Maturity spawning season and genetic spawning season *26*

3.2.2 Otolith hatching season and maturity spawning season *27*

3.2.3 Otolith hatching season and genetic spawning season *28*

3.3 Spawning season dynamics of subsample 29

3.4 Length and age data 30

3.5 Population abundance estimates 30

4 DISCUSSION **32**

4.1 Discussion of results 32

4.1.1 Spawning types; correspondence and switching *32*

4.1.2 *The effect of relative abundances* ... 36
4.1.3 *Evidence of a third spawning group?* ... 37
4.1.4 *Population affiliation* ... 38
4.2 DISCUSSION OF METHODOLOGY ... 39
4.2.1 *Maturity staging and spawning season* ... 39
4.2.2 *SNPs and genetic spawning season* ... 40
4.2.3 *Otolith microstructure and hatching season* ... 41
4.2.4 *Sampling equipment* ... 43
4.3 MANAGEMENT IMPLICATIONS ... 43
4.4 FUTURE STUDIES ... 44
5 REFERENCES ... 46
6 APPENDIX ... 52

TERMINOLOGY

Spawning type	Spring or autumn. In this study herring were separated to spring or autumn by three separation methods. Spawning type refers to the combined result of these methods. A “spring type herring” hatched in spring, spawned in spring and was genetically assigned to spring spawning season.
Maturity spawning season	Spawning season of herring inferred from gonad development.
Otolith hatching season	Hatching season of herring inferred from otolith microstructures.
Genetic spawning season	Genetic spawning season of herring inferred from two genomic SNPs markers.
Fidelity	Hatch and spawn in the same season.
Straying	Hatch in one season, spawn in the other.
Correspondence and mixing	Used when comparing genetic spawning season with otolith hatching season or maturity spawning season.
NSS	Norwegian spring spawning herring.

1 INTRODUCTION

1.1 The Atlantic herring

The Atlantic herring *(Clupea harengus, L. 1758)* is a commercially and ecologically important fish species with a long and fundamental history in fisheries science. It is a schooling pelagic fish with a complex population structure. Atlantic herring has a wide distribution area, reaching from Northwestern Atlantic to Eastern Atlantic and adjacent seas, and thrive in a variety of environmental conditions. Global catches in 2015 were 1 500 000 tonnes (FAO, 2017), and more than 500 000 tonnes was landed in Norway in 2018 (SSB, 2018). Many herring populations are known to the North east Atlantic which can be separated by their life history characteristics. Some are highly migratory, while others are more stationary. The migratory behaviour of herring populations is known to be relatively stable between years, but changes in migration patterns occur. The largest migratory stock in the North Atlantic is the Norwegian Spring Spawning herring (NSS), which spawns along the west coast of Norway. Examples of resident populations along the Norwegian coast are the Lindåspollene herring (LP) (Johannessen et al., 2014), Landvik herring (Eggers et al., 2014) and the Balsfjord herring (Jørstad et al., 1994). Little is known about the level of interbreeding between these stocks. Herring populations can be separated on their spawning area and/or season but are sometimes found mixing on feeding and overwintering grounds where they are also caught in mixed catches. Separation of such mixed populations can be challenging. For sustainable fisheries, and conservation of herring population diversity, knowledge on the structure and dynamics between herring populations must be implemented to fisheries assessment and management models. It is therefore necessary to find good methods for separation of herring populations.

1.2 Population structure and dynamics

A population can be defined as *"A group of individuals of the same species living in close enough proximity that any member of the group can potentially mate with any other member"* (Waples and Gaggiotti, 2006). This evolutionary definition of a population emphasizes the importance of timing and location of spawning when studying the structure of fish populations. Atlantic herring populations spawn once a year at specific locations and during discrete time periods (Iles and Sinclair, 1982). This works to isolate populations and decrease interbreeding between groups (McQuinn, 1997a). However, there is a number of life history traits in herring

that might cause both temporal and spatial discreteness or connectivity (through interbreeding) between populations. Population -density and -dynamics is primarily controlled by birth and death rates within the population, as well as immigration and emigration between populations (Campbell et al., 2015). In the present thesis the dynamics *between* herring populations will be further investigated.

Spawning season fidelity (hatching and spawning in the same season) is often assumed in herring populations. Herring stocks are known to have specific spawning periods which is also an important trait for classification and separation of different stocks (Sinclair and Tremblay 1984). Mean spawning time differ substantially between stocks, and occurs throughout the year (Sinclair and Tremblay, 1984). Also, location of spawning in herring is documented to be relatively fixed. Tagging experiments on herring from the North west Atlantic documented 90% return to spawning grounds (Wheeler and Winters, 1984). At the same time, natal homing (hatching and spawning on the same grounds) has not yet been clearly been documented for herring (Stephenson et al., 2009). The reproductive strategies controlling spawning time and location are not fully understood, and different theories exist which will also have implications on the level of interbreeding between populations. Iles and Sinclair (1982) suggested that both time and location of spawning is predetermined at larval retention areas. They observed that herring larvae are found in limited geographical areas with specific oceanographic characteristics which herring will return to for spawning, thereby keeping discreteness between populations (Iles and Sinclair 1982). Spawning season fidelity is investigated in herring populations, and high rates of spawning season fidelity is documented in a number of studies (Husebø et al., 2005, Brophy et al., 2006, Clausen et al., 2007). Although there is evidence of discreteness in timing and location of spawning between herring populations, mixing during different life history stages suggests that populations are not completely isolated (Stephenson et al., 2009). Mixing of herring populations occur primarily on feeding or overwintering grounds by juveniles and adults (Stephenson et al., 2009). Connectivity between herring populations has been documented as different rates of straying (hatching in one season, spawn in the other) between populations (McQuinn 1997b, Brophy et al., 2006). McQuinn (1997a) suggested that timing and location of spawning is learnt from adults co-existing with first year spawning herring during prerecruitment life phases. Herring matures at ages between 3 and 5 years, recruits to an adult population, and start an annual lifecycle with migrations between spawning, feeding and wintering grounds (Corten, 2002). After maturation herring tend to follow the same migratory routes and come back to the same spawning area. The hypotheses of social transmission of spawning behaviours from adults to the recruiting year classes is named the adopted-migrant model (McQuinn 1997a) and it contrasts to the dicsreete populaiton concept which suggested that spwning timing and locaiton is predetermined at

nursery areas (Iles and sincalir 1982). Straying in herring might be explained by the adopted-migrant model, and if straying leads to interbreeding there is connectivity between seasonally spawning herring populations. At the same time, population structure and dynamics will be influenced by a number of other factors (e.g. population densities, life histories, hydrographic and environmental barriers). Straying might occur on the individual level with low rates of spawning season switching as documented by Brophy et al. (2006), or it might be that a substantial proportion of a year class spawns in the opposite season of hatching. This was documented by MqQuinn (1997b) who explained straying through variability in juvenile growth rates. Due to variability in growth conditions (primarily temperature, feeding and density dependence) herring of the same cohort can grow at different rates, affecting the time of first maturation (McQuinn 1997b). Length, rather than age, is suggested to be the main factor affecting which season the herring will adopt to for spawning, and after first maturation herring will continue to spawn in the same season (McQuinn, 1997b). The rates of fidelity within and straying between populations will determine how much genetic contact there is between them, and number of genetic techniques have been used to find structure between herring populations (Dahle and Eriksen, 1990; André et al., 2010; Barrio et al., 2016). (Lamichhaney et al., 2017) found loci associated with spawning time with strong genetic differentiation between spring and autumn spawning herring. However, the level of interbreeding between populations is still a question to research, and the genetic basis of timing of reproduction is not fully understood. In the following I present herring population separation techniques, including the ones used in this thesis.

1.3 Separating herring populations

Separation of populations requires some measurement of variation between populations. Both genetic differences and environmental influences cause variability among individuals of a species (Beebee and Rowe, 2004). This has led to a distinction between the genotypes (all genes/alleles) and phenotypes (morphological, physiological, biochemical and behavioural characteristics) of an organism (Beebee and Rowe, 2004). Marine fish separation methods are often used to separate stocks as a management unit, but the same separation methods can be applied to populations. In management and assessment of fish stocks the structure of populations may in some cases be overlooked as stocks may represent an aggregation of biological subpopulations (Hay et.al., 2001).

1.3.1 Phenotypic separation methods

Atlantic herring populations and/or stocks have been separated through a number of phenotypic traits (variants of a phenotypic character). Measurements based on morphometric and meristic characters are common (Begg and Waldman, 1999). Morphometric analyses compare the shape of morphological features, e.g. otolith shape, while meristic measurements are countable structures such as number of vertebrae. Meristic characters are set early in ontogeny and will therefore reflect the experienced environment during early life phases (Begg and Waldman, 1999). Also, life history characteristics such as growth rates (length at age) or reproductive characteristics (e.g. fecundity-at-age, spawning time) can separate populations (Begg and Waldman, 1999). For example, western Baltic spring-spawning herring and central Baltic herring are mixing in the western Baltic. These stocks were separated for management purposes through length-at-age data (Grohsler et al., 2013). Frequency distributions of measured morphometric and meristic characters can overlap between populations, preventing separation at the individual level (McQuinn 1989). However, combining methods can increase the discriminatory power of such analyses.

Fish otoliths are highly useful for management and fisheries research purposes, as they contain information about age and growth, both on annual and daily levels (Folkvord et al., 2000). Otoliths are hard, calcified structures located in the inner ear cavity of teleost fish (Mendoza, 2006). The shape of the otolith is species specific. Most bony fish have 3 otoliths on each side; sagittae, lapilli and asteriscus. For management purpose the sagittae is most often used, as it is the largest (Campana, 2004). Otolith growth is not resorbed (Mendoza, 2006). This is convenient because the growth pattern observed in the otolith reflects the environment in which the fish experienced (e.g. water temperature or food resources). Most of the otolith is composed of calcium carbonate ($CaCO_3$) which is deposited in the otolith throughout the fish's life (Mendoza, 2006). Trace elements derived from the surrounding environment are also deposited (Mendoza, 2006). Otoliths are therefore useful when studying life histories of bony fishes.

At the larval stage daily growth increments are deposited in the otolith, forming a microstructure that can give information on daily age and growth patterns (Campana, 2004). Polishing of the otolith sagittae (hereafter called otolith) reveals this microstructure, found as a cross section from the core (=center) of the otolith. One daily increment (formed over 24 hours) appears as one dark and one lighter increment when viewed in a light microscope (Kalish et al., 1995, p. 726). In herring larvae, the daily increment deposition rate is found to correlate with both growth and size (Folkvord et al., 2000). Increment width therefore reflect the growth rate of the larvae,

where fast- growing larvae have larger increments than slow- growing larvae (Moksness and Wespestad, 1989). Otolith growth is affected by the surrounding environment, for example temperature (Folkvord et al., 2004). This allows for identification of larvae originating from different prey-, and temperature-, regimes, enabling the separation of herring hatching in different seasons and at different locations. Spring and autumn hatched herring have been separated through otolith microstructure growth patterns in a number of studies (Clausen et al., 2007; Husebø et al., 2005; Moksness and Fossum, 1991), and otolith microstructures are currently used to separate western Baltic spring spawners (WBSS) and North Sea autumn spawning herring (NSAS) for management purposes (ICES, 2018).

Methods that separate herring populations through hatching season assume spawning time fidelity in herring, but herring can also be separated directly through examination of gonads. Spring- and autumn- spawning herring stocks mixing in the Gulf of St. Lawrence were separated through gonad stages (McQuinn, 1989). For management purpose, the gonadal development of herring is visually divided into maturity stages, allowing for quantitative studies of gonad development (Mjanger et al., 2017). Seasonal spawning herring populations have been separated through otolith microstructure in a number of studies. When compared with maturity stages the results are inconclusive; indicating both fidelity to spawning season and straying. Spawning season fidelity was documented through otolith microstructure in autumn spawners mixing with NSS (Husebø et al., 2005), suggesting that herring spawn in discrete seasons. Autumn- and winter- spawning herring in the Irish and Celtic seas were also successfully separated on maturity stages and otolith microstructure (Brophy et al., 2006). However, low estimates of switching was observed, which may indicate straying between populations (Brophy et al., 2006). McQuinn (1997b) used otolith microstructure and maturity stages to separate mixed herring populations in the northern Gulf of St. Lawrence. Straying was observed, and it was concluded that juveniles may recruit to a population with a different spawning season (McQuinn, 1997b).

1.3.2 Genotypic separation methods

Genetic differentiation, the accumulation of differences in allelic frequencies between completely or partly isolated populations, is required for genetic population separation. This can be caused by either natural selection or genetic drift. The first studies on population structure between seasonal spawning Atlantic herring did not succeed in finding such structure (Dahle and Eriksen, 1990). However, André et al. (2010) found that loci influenced by selection can serve as powerful markers for detecting population structure in herring. Previous genetic

analysis, using neutral markers, did not find any genetic structure because neutral markers can only capture differentiation resulting from genetic drift, which is generally small in large populations (Kerr et al., 2018). Genetic differentiation found in large populations, such as Atlantic herring, is typically caused by selection resulting from local adaption (Barrio et al., 2016; Lamichhaney et al., 2017).

Using whole-genome resequencing data of Atlantic herring populations from both sides of the Atlantic Ocean Lamichhaney et al. (2017) found that there is a genetic basis for spawning timing in herring. Loci associated with spawning time showed strong genetic differentiation between spring- and autumn- spawning herring and were even linked to genes of significant role in reproductive biology (Lamichhaney et al., 2017). Kerr *et al.* (2018) also found temporal stability of spawning timing in Northwest Atlantic herring using 64 highly differentiated single nucleotide polymorphisms (SNPs) found by Lamichhaney et al (2017). The combined results give evidence for both temporally and spatially consistency of local adaption to asynchronous reproduction in herring, emphasizing the applicability of SNP subsets for characterization of spawning season in Atlantic herring. It also demonstrates that spatial and temporal isolation between seasonal spawning herring is sufficient to keep some genetic population structure between spring and autumn spawning herring.

1.3.3 Combining separation methods

Phenotypic plasticity is the ability of an individual’s genotype to respond to environmental influences, generating different phenotypes across environments (Beebee and Rowe, 2004). Many herring populations have been identified through phenotypic traits. However, few studies documented the genetic basis of such traits. Florian et al. (2018) found a genetic basis for otolith shape, suggesting that genes can have an effect on phenotypic structures in herring. Using six polymorphic loci Balsfjord herring was found to be genetically more similar to Pacific herring than Atlanto-scandian herring found in the same area (Jørstad et al., 1994). The local Balsfjord herring was also separated from other local populations through meristic and morphological characters, i.e. number of vertebrae, and the spawning behaviour of Balsfjord herring is different from the Atlanto-scandian one (Jørstad et al., 1994). More studies comparing genetic structure and phenotypic traits in herring should be performed to increase the knowledge on the genetic structure of populations already separated by phenotypic traits.

1.4 Aim of the study

The aim of this thesis is to further increase the knowledge on herring population structure and dynamics through comparing phenotypic and genetic separation methods of spring- and autumn- spawning herring. Herring were sampled at the same locations in both spring and autumn spawning seasons to be classified into spawning type spring or autumn by three separation methods; Phenotypic maturity status was related to two genomic SNPs markers. In addition, hatching season was determined through visual reading of otolith microstructures, corroborating the already known spawning season pattern. Based on previous studies, it is hypothesized that genetic markers will separate herring to spawning season responding to what is found from maturity stages. Spawning time fidelity is also expected, implying that the three separation methods will assign herring to the same spawning season. The combined data gives essential information for understanding the underlying structure of seasonal spawning Atlantic herring populations. The results are also highly relevant to management because we link genotype (adaptive genes for spawning season) with phenotypes that are currently used in management for separation of seasonal spawning groups (maturity stages and otolith microstructure).

The three main objectives were thus to:

- Relate spawning season of SNPs markers to maturity spawning season.
- Relate maturity spawning season to otolith hatching season
- Relate genetic- and maturity- spawning season to otolith hatching season.

2 MATERIALS AND METHODS

2.1 Study area and field sampling methods

Herring were sampled at three locations in western Norway, Hordaland county (Figure 1; Appendix a). Sampling was conducted during spring and autumn spawning seasons between autumn 2016 and autumn 2018. All sampling locations have small rivers running out in the fjord near the sampling stations. Rishålo have higher inflow of freshwater, followed by Kobbevågen and Garnvika. Salinity and temperature was measured at each sampling (Table 1). Hydrography and topography on sampling sites vary; Rishålo is a protected locality, but with good water flow. Occasionally there is great supply of freshwater from the nearby river. Bottom consists of rock, sand and mud. Garnvika is exposed to wind from north and northwest, also with good water exchange. Kobbevågen is protected by a shallow inlet with an island in the middle, and the bottom consists mainly of mud. There is a threshold here with a max depth of 4 meters. Water exchange occurs with changing tides and wind from north.

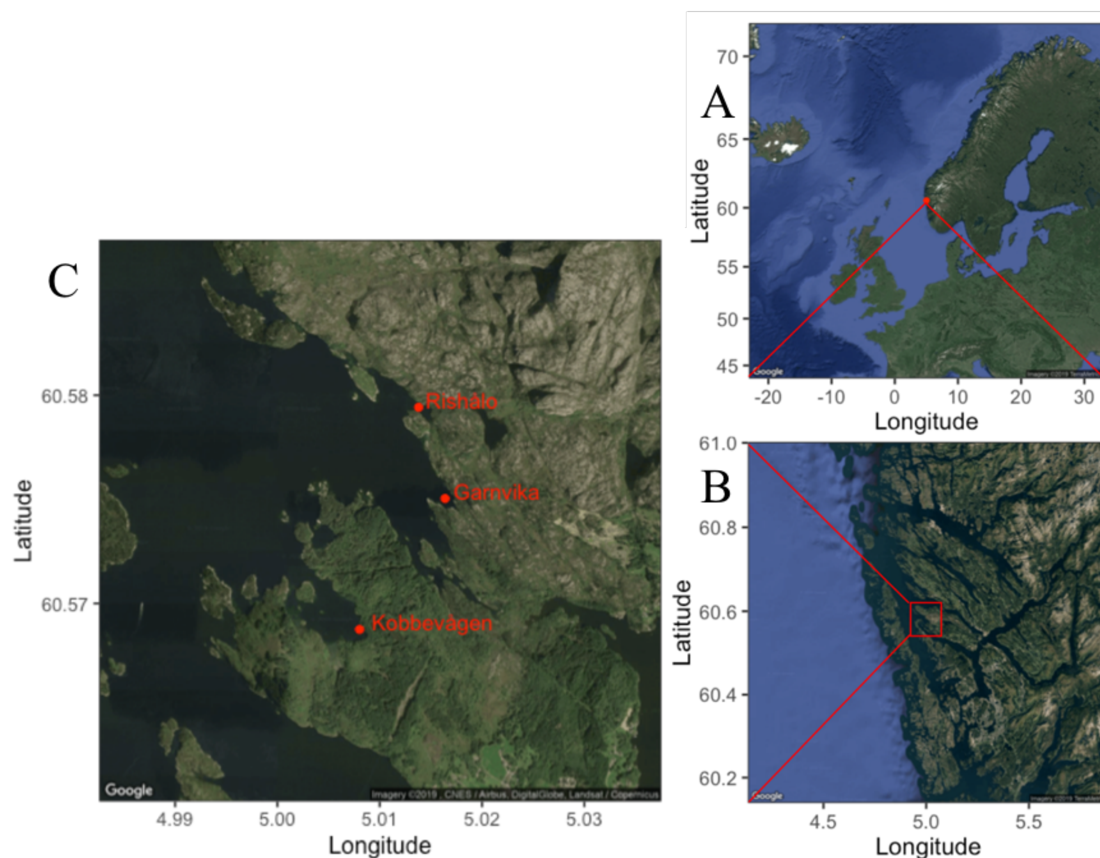

Figure 1 *Location of sampling sites, western Norway (A), Location in Hordaland county (B), Sampling sites; Rishålo, Garnvika and Kobbevågen (C).*

Herring were sampled using 5-meter-wide gillnets, with 29, 31 and 34 mm mesh size (Appendix b). Depth were gillnets were placed varied; 6-8 meters in Rishålo, 6-25 meters from start to end in Garnvika and 6-10 meters in Kobbevågen. The nets were set out in the evening, to be collected the next morning. The small mesh size of gillnets did little harm to the herring, so they could be sampled carefully without losing many scales and injure the fish. Floats were connected to the upper end of nets, and lead weights at net ends oriented them vertically in the water column. The sampled herring were kept cold in Styrofoam boxes filled with ice and brought to the lab for analysis. Other species were caught in the gillnets but were not further considered in this study.

Table 1 *Mean temperature (°C) and salinity measures from sampling sites; Rishålo, Garnvika and Kobbevågen. One measurement was taken at each location. SD = Standard deviations. *No measurements from Garnvika due to bad sampling conditions. **Salinity measurements not taken due to equipment malfunction.*

Sampling date	Mean temperature ± SD	Mean salinity ± SD
09/09/2016	15.8 ± 0.2	17.4 ± 0.3
29/09/2016**	14.6 ± 0.1	-
17/10/2016	10.8 ± 0.4	24.1 ± 0.7
31/10/2016	11.1 ± 0.1	29.0 ± 0.2
29/03/2017	6.4 ± 0.2	28.0 ± 0.4
04/05/2017	9.3 ± 0.7	22.1 ± 1.1
04/09/2017	14.3 ± 0.3	19.6 ± 0.7
20/09/2017	13.9 ± 0.2	26.0 ± 0.6
02/10/2017*	13.7 ± 0.1	27.1 ± 0.7
30/10/2017*	10.0 ± 2.0	28.0 ± 2.6
08/03/2018	2.7 ± 0.1	29.5 ± 0.4
24/04/2018*	8.0 ± 01	20.9 ± 1.2
18/09/2018	13.0 ± 0.1	17.0 ± 0.8
Mean autumn	**13.1 ± 1.9**	**23.3 ± 4.7**
Mean spring	**6.5 ± 2.7**	**25.5 ± 3.9**
Mean total	**11.1 ± 3.7**	**24.0 ± 4.5**

2.2 Wet lab procedures and ageing

Somatic measurements were conducted on sampled fish. Individuals were measured from snout to the end of the tail (total length in cm, 1 decimal), and weighted (grams, 1 decimal). Gonads were extracted for sex determination, weighted (grams, 2 decimals), and visually separated to maturity stage (Figure 2B). For further description of maturity staging see section

2.3.1. Tail fin clips and otoliths were saved for genetic analysis and otolith microstructure analysis, respectively (Figure 2A,C). Tail fin clips were stored in Eppendorf tubes filled with 150 ml 100% ethanol. The two otoliths were removed from the herring brain cavity using tweezers (Figure 2A). Visible tissue was removed from the otoliths before placing them into marked trays. In addition, scales were removed but not further considered in this study.

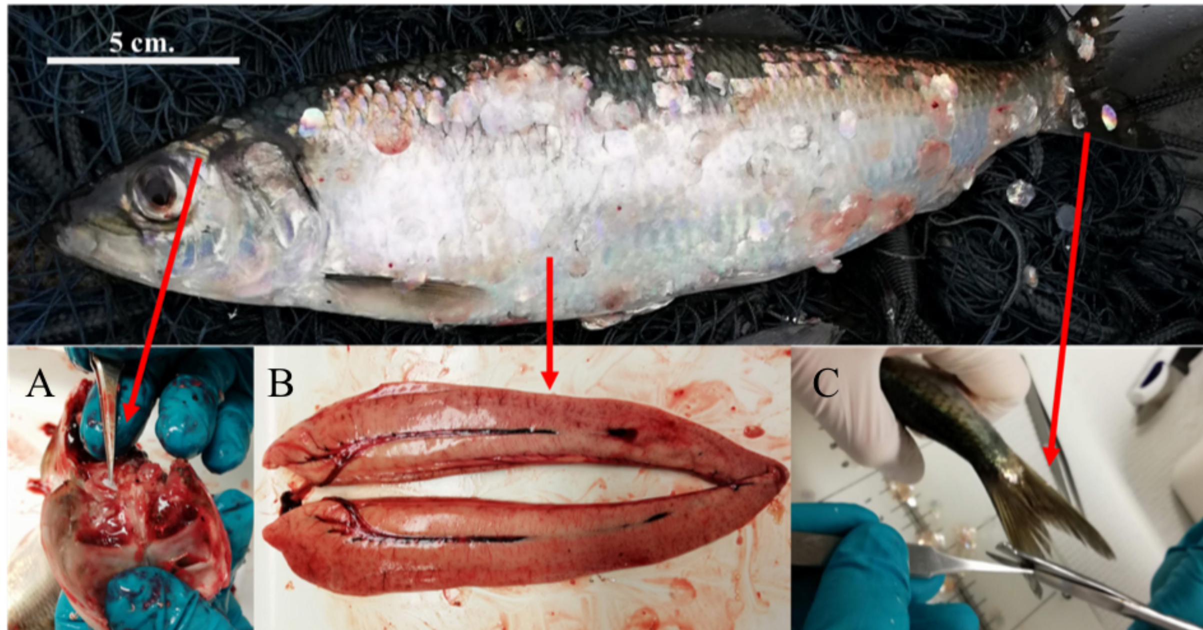

Figure 2 *Biological material extracted in wet lab for separation to spawning type spring or autumn. Otolith (A), gonads (B) and tail fin clip (C) from an individual herring. Material was used to determine hatching season (otolith microstructure), spawning season (gonadal maturity stages) and genetic spawning season (Allelic discrimination of SNPs markers) respectively.*

Some herring were aged to check for patterns in age composition (N=58). Herring were picked out randomly from sampling dates, but it was attempted to get both spring and autumn type herring from both sampling seasons (based on genetic- and maturity- spawning season). One intact otolith was used to age the herring. A moist pencil was used to move the otolith from storage tray to smaller black trays filled with a thin layer of Milli-Q water. Otoliths were then read under a stereomicroscope by an experienced age reader.

2.3 Separation to spawning type spring or autumn

Three methods were used to separate herring to spawning type. *Maturity spawning season* was found through visual inspection of gonad stages. Herring gonads were visually separated to maturity stages 2-9 following a macroscopic staging key used by the Marine Institute of Norway (Mjanger et al., 2017). *Hatching season* was found through otolith microstructure

analysis. Otolith microstructure was revealed through grinding of otoliths and photos of microstructures were used to visually separate herring to hatching season. Separation of spring- and autumn- hatched herring followed guidelines for visual discrimination of otolith microstructure according to Clausen et al. (2007). *Genetic spawning season* was found through genomic analysis of two diagnostic SNPs markers. A Taqman assay was used for allelic discrimination of the two SNPs through qPCR. SNPs were found on independent loci with consistent allele frequency differences by Lamichhaney et al. (2017). Methods and procedures are further described in the following.

2.3.1 Maturity stages and separation to spawning season

Maturity stages were determined visually following the scale: Immature = 1–2, Maturing = 3–4, Ripe = 5, Spawning/Running = 6, Spent = 7, Recovering = 8 and Abnormal = 9. Descriptions of each stadium are given in the standard quality system of the marine institute of Norway (Mjanger et al., 2017). For separation to spawning season it was assumed that herring assigned maturity stages 5-8 would spawn in the sampling season, while maturity stages 3-4 would spawn in the opposite season. Stages 2 (Maturing) and 9 (Abnormal) were not assigned a spawning season and were therefore not included when comparing spawning type separation methods. Among the maturity staged herring were 557 herring classified to stages 3-8, which will be used when combining spawning season variables.

2.3.2 SNPs markers and separation to genetic spawning season

DNA from tail fin clips were amplified though polymerase chain reaction (PCR). The HotSHOT* genomic DNA preparation technique was followed, according to manufacturer's instructions (Camper Lab, 2000 based on Truett et al., 2000). In short, tail fin clips (about 1x1 mm) were cut out and added to marked 0.65 mL wells. Alkaline lysis reagent (75 µL) was added before PCR amplification in thermocycler. Neutralization buffer (75 µL) was then added to wells and plates were kept cool in a fridge. TaqMan genetic analysis was performed on two diagnostic SNPs using two genetic kits; KIT481 and KIT1420. Reporter sequences for these kits are found in Table 2.

Table 2 *Genetic kits with assay names and reporter sequences. Kits were used to separate spring and autumn spawning herring.*

KIT	Assay name	Reporter 1 Sequence	Reporter 2 Sequence
481	scaffold481_2824	AGCATAGCGTAGCTGTT	CATAGCGCAGCTGTT
1420	scaffold1420_137	ATTTGTTTGTTAAGAAGGAC	TGTTTGTTCAGAAGGAC

Allelic separation of SNPs was preformed using Assays-on-Demand™ SNP Genotyping Product (P/N 4331183, Applied Biosystems, California US). The SNP Genotyping Assay follows the TaqMan® SNP Genotyping Assays Protocol (P/N: 4332856, Applied Biosystems, California, US). The genetic analysis was preformed according to the manufacturer's instructions. In short, a reaction mix consisting of Taqman Universal PCR Master Mix (12.5 µL) and 20x assay mix (1.25 µL; assay and ddH2O in 1:1 ratio) was added to wells on a DNA reaction plate (96 wells). Amplified genomic DNA (11.25 µL) was added to wells. All plates were marked so allelic result from single wells could be traced back to individual herring. The DNA reaction plate was placed in a quantitative polymerase chain reaction (=qPCR) machine (CFX96™ Real-Time System, Bio-Rad, California US), and run for 40 cycles with specific thermal cycler conditions (Appendix c). CFX Manager™ software (Bio-Rad, California US) was used for allelic discrimination into spawning type spring or autumn (Figure 3). The procedure was performed twice, once for each of the two SNPs markers with each genetic Kit.

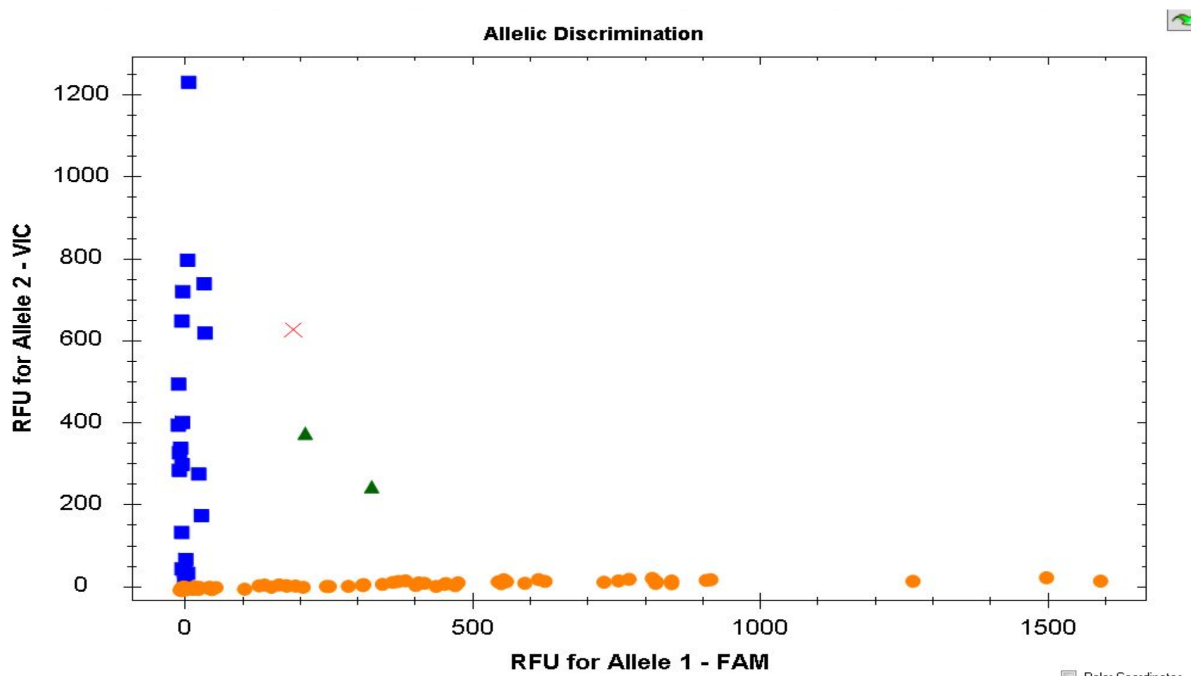

Figure 3 Allelic discrimination output in the CFX Manager™ program for one well plate with Atlantic herring DNA samples run in qPCR. Orange dots are spring spawners (Allele 1), blue squares are autumn spawners (Allele 2). Green triangles were classified as heterozygous, while the red x could not be classified by the program. RFU values are the relative fluorescent units for the two alleles.

The genetic analysis was conducted on randomly selected herring (N=446, 77.3% of total sample). To get enough genetic autumn spawning herring most of autumn sampled herring were analysed (98.5% of autumn sample). Fewer spring sampled herring were analysed due to dominance by maturity spring spawners (58.90% of spring sample). In some cases, the CFX

Manager^{TM} program could not classify herring to genetic spawning season (red cross in Figure 5, 3.4% of total analysed). These fish were excluded from further analysis, leading to a total of 431 herring with genetic spawning season. The overall result for the two SNPs markers can be found in Appendix d. Allelic discrimination led to 6 possible outcomes (Table 3). For data interpretation these categories were simplified. Herring classified as Heterozygous on one SNPs genomic test were included as spring or autumn spawners respectively, while Spring:Autumn and Heterozygote:Heterozygote herring were pooled into a mixed category (Table 3).

Table 3 *Possible spawning season categories from two SNPs genomic markers, with spawning season simplification used for analysis.*

Spawning season from two SNPs markers	Spawning season used for analysis
Spring:Spring Heterozygote:Spring	Genetic spring spawner
Autumn:Autumn Heterozygote:Autumn	Genetic autumn spawner
Spring:Autumn Heterozygote:Heterozygote	Mix

2.3.3 Otolith microstructure analysis and separation to hatching season

At each sampling date 15-25 otoliths were randomly selected depending on the number of herring otoliths available for microstructure analysis. However, as autumn types were few compared to spring types, a stratified sample was selected to get enough data for the comparison of seasonal spawning types. This included some selectivity to otoliths from genetic- and maturity- autumn spawning herring, resulting in a sample that is random with respect to hatching season. A higher proportion of autumn sampled herring were analysed than spring sampled herring (58.6% of autumn samples and 27.51% of spring samples), N_{total}=161.

Otoliths were polished to document the otolith microstructure at the otolith nucleus and a transect towards the edge. Otoliths were first mounted on microscope slides using thermoplastic resin (Crystalbond 509, melt point 77°C), (Figure 4A). The right otolith from each herring was used when it was not broken. Slides were marked with sampling date and individual fish number for tracking of all individuals. A hotplate SH2 from Stuart Scientific (Figure 4B) was used to heat the microscope slides enough to melt the Crystalbond when it

was applied to slides. Otoliths were mounted with the sulcus acusticus facing up, and slides were left on a worktable to cool down to room temperature. Otoliths were then grinded on a Saphir 330E sanding machine (Figure 4C), using first a coarse P1200, then fine P2500 emery paper. The sanding machine was set to 325 rpm, and the microscope slide was held over the emery paper with the weight of two fingers for smooth grinding of otoliths. The sanding machine turns the emery paper so the level of grinding could be managed through regulating rotation speed and time of grinding. Grinding was performed to optimize the visual resolution at a focal plane through the otolith nucleus, and the fine emery paper was used to polish the otolith to get a smooth surface and clear photos. Slides were inspected under a Leica DMBL light microscope with objective lens 20x (Figure 4D; Figure 5). The objective lens had a long distance between focus and lens to facilitate viewing of the otolith microstructure through the microscope slide. This gave the opportunity to view the microstructure from both sides when taking photos of the microstructure. Otoliths were grinded until the sulcus acusticus was not visible, as the otolith nucleus is located here. Slides were then heated up again on the hotplate and the otolith was turned for grinding from the dorsal side. The approximation to the nucleus was monitored through regular visualization under the microscope. A Nikon camera (camera: DS-Fi2, control unit: DS-U3) was attached to the microscope making the microstructure visible on a computer screen in the program Nis Elements D (Figure 4D). When approaching the nucleus photos were taken with 20x magnification in the same program. Further grinding was preformed, and multiple pictures were taken to secure a photo of well visualized daily increments around the nucleus (Figure 5). Otoliths were turned a third time though re-heating on hotplate when necessary. Good documentation of each individual otolith during the grinding process secured visualization of the microstructure before an undesired over-grinding took place.

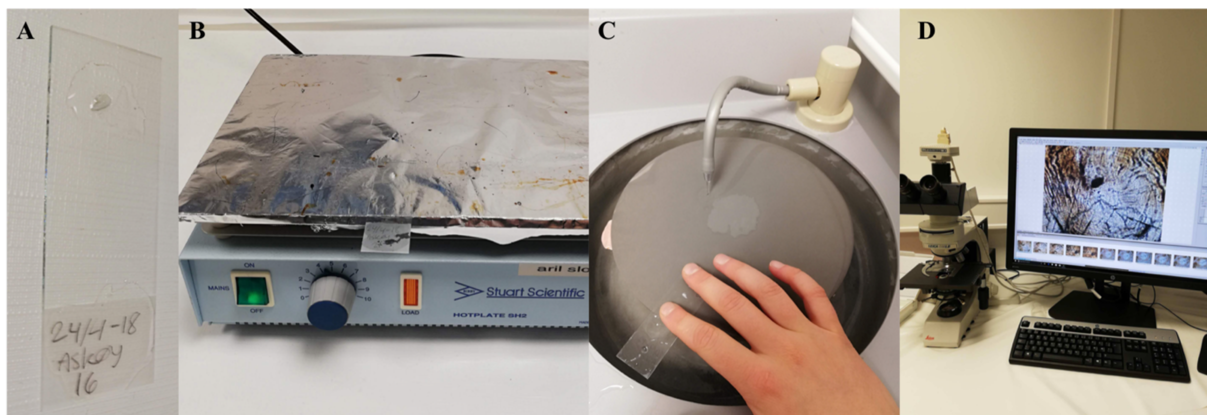

Figure 4 *Process of otolith grinding for microstructure analysis. Otolith mounted on microscope slide (A), hotplate (B), sanding machine (C), microscope with camera and computer used to visualize and photograph the microstructure (D).*

Photos of the season-specific daily increment pattern for the larval period were used to visually determine hatching season. One photo from each individual was sorted out to be categorized, through visual inspection, into hatching season spring or autumn (Figure 5). Photos with a clear microstructure, meaning good visibility of the daily increments, were selected. To avoid bias the photos were randomly coded so the reader could not know the sampling date. Methods and guidelines for determining the hatch season followed the same procedure as Clausen et al., (2007). Spring hatched herring have wide increments that increase in width from the centre of the otolith and increment widths can also be highly variable. Autumn hatched herring have narrower increments with close to constant widths (Clausen et al., 2007). Readers focused on increments found between 20 μm and 100 μm from the nucleus. Two readers looked through coded photos individually, giving them one of three categories; "spring", "autumn" or "undetermined". Agreement between readers was 89.6% (Appendix e). Reader classifications were compared. Upon disagreement photos were discussed and re-evaluated (N=16). Upon agreement the spawning season was assigned to either spring or autumn accordingly. In some herring the second otolith was grinded to get better photos of microstructures (N=11). The final sample included 242 herring. Hatching season was not detectable in some otoliths (5.8%, N=14). This led to final determination of hatching season for 228 herring which will be used when presenting results.

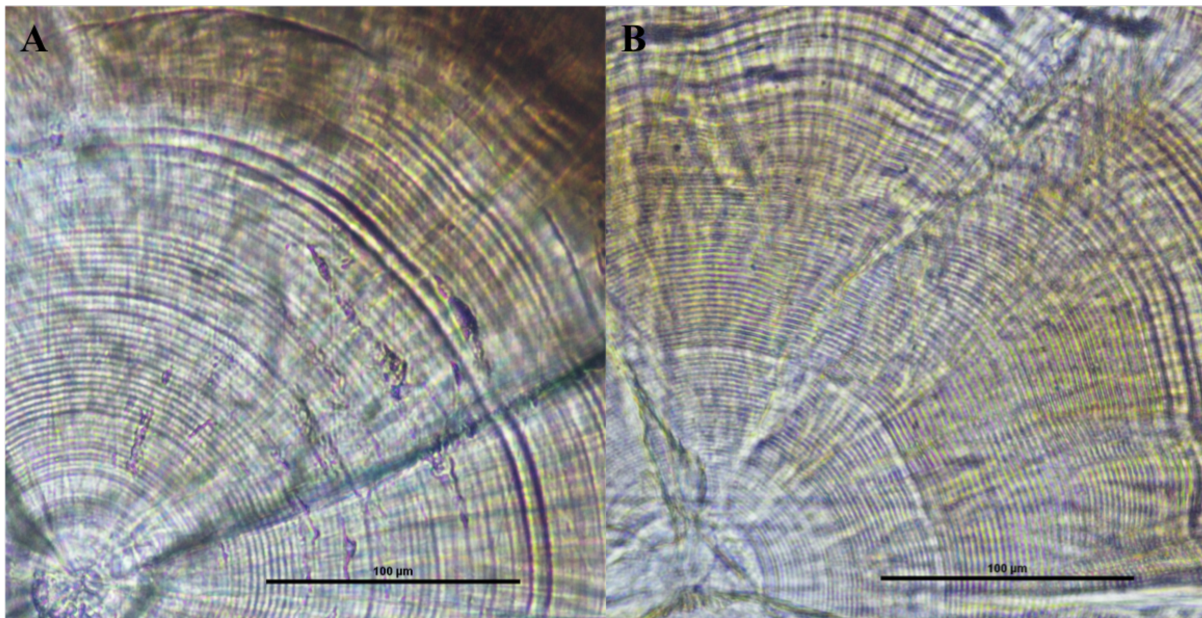

Figure 5 *Photographs of otolith microstructure from spring hatched herring (A), and autumn hatched herring (B). Black line is 100 μm.*

2.4 Data material

A total of 577 herring sampled over 13 dates are included in this study, 9 autumn samples and 4 spring samples (Table 4). Data from the three sampling stations were pooled as the question of the thesis is not to compare results between sites. Total catches were counted when N <100. When N >100 numbers are estimated from total weight, expecting a mean fish weight of 300g. Among the sampled herring were 563 maturity staged, 242 analysed for otolith microstructure and 431 analysed for genetic spawning season (Table 4). A subset (N=210) was analysed for all spawning season variables (Table 4).

Table 4 *Available data per sampling date, total numbers. In the analysed total sample the length, weight, GSI and Sex was found for all individuals, with a few exceptions. Subset is herring with maturity spawning season, genetic spawning season and otolith hatching season data available. Herring that could not be discriminated genetically are not included in the table.*

Date	Total catch	Total sample	Maturity staged	Otolith microstructure	Genetic test	Subset
09/09/2016	11	11	10	11	11	10
29/09/2016	13	13	12	5	13	5
17/10/2016	17	17	17	14	17	14
31/10/2016	12	12	12	10	12	10
29/03/2017	100	61	51	24	35	16
04/05/2017	110	72	72	24	67	22
04/09/2017	20	20	19	17	20	11
20/09/2017	11	11	11	10	11	10
02/10/2017	38	38	37	25	35	21
30/10/2017	50	50	50	11	44	9
08/03/2018	500	100	100	20	39	18
24/04/2018	120	76	76	17	31	15
18/09/2018	164	96	96	54	96	49
Total Autumn	**336**	**268**	**264**	**157**	**259**	**139**
Total Spring	**830**	**309**	**299**	**85**	**172**	**71**
Grand Total	**1166**	**577**	**563**	**242**	**431**	**210**

2.4.1 Statistical analysis and software

Statistical analysis was preformed, and figures were made using RStudio, Version 1.1.463 and R software, version 3.5.1. Various R packages were used for making figures and tables (Appendix f). A parametric pearson's Chi-squared test was preformed to demonstrate difference in maturity stage composition between sampling seasons.

Frequency tables were made to relate spawning type results. Tables included herring analysed on the two spawning season variables in question (maturity spawning season * genetic spawning season, maturity spawning season * otolith hatching season or genetic spawning season * otolith hatching season). All tables were sorted to sampling season for biological interpretation. Rates of correspondence and switching between spawning types were calculated vertically and horizontally as:

$$\frac{N\ herring\ with\ correspondence\ or\ switching}{\sum herring\ analysed\ to\ spring\ or\ autumn\ type\ on\ that\ spawning\ season\ variable} * 100$$

Alluvial plots were made to compare all three spawning season variables, and to show the result of the genetic analyses.

Independent two sample t-test were calculated to compare means of total length measures of herring sorted to sampling season and spawning season variable ($\alpha \leq 0.05$).

Rough population estimates and catch per unit effort (CPUE) was calculated for each sampling season with all spawning season variables included. A frequency table of the subsample (N=210) was first made and fractions of spring- and autumn- hatching herring were calculated from this frequency table. For the first estimate these fractions were multiplied with the number of herring analysed for genetic- and phenotypic- spawning season that were not yet included in the frequency table (N=422). This estimate was then multiplied with the fraction of herring in the total catch of that sampling season that were not yet included in the estimate. CPUE was then found dividing this estimate on the number of nets used to catch herring in that sampling season. The resulting CPUE is a measure of relative abundances of spawning types in spring and autumn sampling seasons.

3 RESULTS

3.1 Separation to spawning type

3.1.1 Maturity stages and maturity spawning season

Overall, herring sampled in autumn were in an earlier stage of maturation than herring sampled in spring (Figure 6; Table 4). The composition between sampling seasons clearly differed (Figure 6; X-squared = 422.79, df = 5, p-value < 2.2e-16). In spring the composition changed from mostly spawning and spent herring (stages 6 and 7) in March towards spent and resting herring (stages 7 and 8) in late April/beginning May (Figure 6). In autumn the composition did not change as much throughout the season (Figure 6). Stage 3 dominated in autumn samples (46.2%), while stage 6 dominated in spring samples (59.9%) (Table 5).

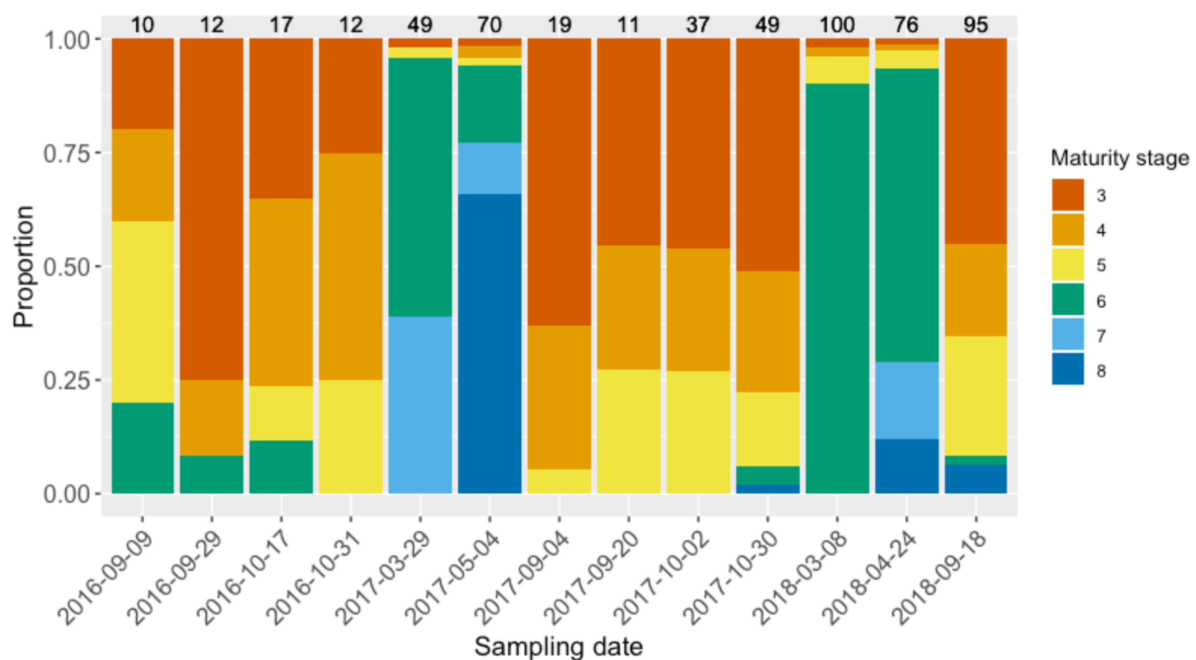

***Figure 6**. Maturity stage composition (stages 3-8) at sampling dates. Total numbers are included above each sampling date column (N_{total}=557).*

Assignments to spawning seasons resulted in 475 spring spawners and 82 autumn spawners, irrespective of sampling season. Spring spawners dominated in both sampling seasons (97% in spring, 73% in autumn; Table 5). Among the spring spawners were 60.0% sampled in spring, while 87.8 % of autumn spawners were sampled in autumn. Ripe and running herring (stages

5 and 6) were present in both sampling seasons, indicating that sampling was conducted on or near spawning grounds for both spring- and autumn- spawning herring. However, the relative frequency of spawning herring was higher in spring than in autumn (Spring; 63.5%, Autumn; 24.6%). Spawning on sampling grounds has been documented in spring through freediving (28.04.2018, Berg pers.comm.), confirming that sampling was conducted on spawning grounds. The presence of ripe and running herring in autumn samples also suggest that herring spawn in the area in autumn. However, since spring and autumn spawning herring mix in autumn samples it might be that autumn type herring are spawning in the area, rather than on the exact same physical grounds as was observed for the spring spawning herring.

Table 5 *Number of herring per maturity stage and sampling season, with assignment to maturity spawning season. Numbers in ( ) are proportions of spring and autumn spawners in the given sampling season.*

Maturity stage	Autumn Sampling	Maturity spawn season	Spring sampling	Maturity spawn season	Total
2	0		1		1
3	122	Spring	5	Autumn	127
4	68	Spring	5	Autumn	73
5	56	Autumn	11	Spring	67
6	9	Autumn	179	Spring	188
7	0	Autumn	40	Spring	40
8	7	Autumn	55	Spring	62
9	2		3		5
Total (3-4)	**190 (0.73)**	**Spring**	**10 (0.03)**	**Autumn**	**200**
Total (5-8)	**72 (0.27)**	**Autumn**	**285 (0.97)**	**Spring**	**357**
Total (2 and 9)	2	x	4	x	7
Grand Total	**264**		**299**		**563**

3.1.2 SNPs and genetic spawning season

Spring spawning herring also dominated using genetic the genetic method (Table 6). Among the herring sampled in autumn were 18.5% autumn spawning while 89.5% of spring sampled herring spawned in spring (Table 6). Only 3.9% of all analysed herring were in the “mix” group. Genetic spring spawning herring dominated the genetic spawning season composition at all sampling dates, but no samples consisted of only genetic spring spawners (Appendix g).

Table 6 *Number of herring assigned to genetic spawning season (spring, autumn or mix) from two SNPs markers, sorted to sampling season. Numbers in ( ) are portions of genetic spring, autumn and mix herring in the given sampling season.*

Sampling season	**Genetic spawning season**			
	Autumn	**Spring**	**Mix**	**Total**
Autumn	48 (0.19)	200 (0.77)	11 (0.04)	259
Spring	12 (0.07)	154 (0.90)	6 (0.03)	172
Total	60	354	17	431

Most herring were assigned the same spawning season on the two SNPs tests also before simplification of the genetic result (86.3% of total). Among these were mostly spring spawners (83.9%) followed by autumn spawners (14.0%) and Heterozygous (2.2%). The remaining herring were assigned different spawning seasons on the two SNPs tests (13.7%). Among these were mostly Heterozygous:Spring herring (71.2%). A visual presentation of the genetic spawning season assigned from the two SNPs markers in each sampling season is found in appendix h.

3.1.3 Otolith microstructure and hatching season

Spring hatched herring dominated in both sampling seasons (Table 7). The proportion of otolith autumn hatchers might be higher than what is found by the other separation methods because otoliths were selectively selected for microstructure analysis (stratified sample).

Table 7 *Number of herring assigned to hatching season from visual inspection of otolith microstructure, sorted to sampling season. Numbers in ( ) are portions of spring or autumn hatched herring in the given sampling season, in the stratified sample.*

Sampling season	**Otolith hatch season**		
	Autumn	**Spring**	**Total**
Autumn	45 (0.31)	102 (0.69)	147
Spring	13 (0.16)	68 (0.84)	81
Total	58	170	228

3.2 Comparing spawning season separation methods

3.2.1 Maturity spawning season and genetic spawning season

Genetic and maturity spawning seasons were compared through estimating rates of correspondence and switching. Correspondence was generally high except when there were few individuals available for calculation (N<11). In autumn the correspondence to spawn in autumn was low (55.9%) with considerable switching (33.8%), and some in the mixed group (10.3%) (Table 8). This means that a substantial part of maturity autumn spawners were genetically assigned to spring spawning season, or they were genetically not spring or autumn spawners (Mix). However, most of the genetic autumn spawning herring were also maturity autumn spawning (84.4%). Spring samples were dominated by spring spawning herring with high correspondence between spawning season variables. However, few autumn type herring explain the high correspondence.

Table 8 *Estimated rates of spawning season match (correspondence) and switching. Sorted to sampling season (N_{total}=422).*

		Genetic autumn	Genetic spring	Genetic mix	Switch from spawn %	Match to spawn %	Mix %	Sum
Autumn sampling N=253	**Spawn autumn**	38	23	7	33.8	55.9	10.3	68
	Spawn spring	7	174	4	3.8	94.1	2.2	185
	Switch from genetic %	15.6	11.7	*				
	Match to genetic %	84.4	88.3	*				
	Sum	45	197	11				
Spring sampling N=169	**Spawn autumn**	2	5	0	71.4	28.6	0.0	7
	Spawn spring	9	147	6	5.6	90.7	3.7	162
	Switch from genetic %	81.8	3.3	*				
	Match to genetic %	18.2	96.7	*				
	Sum	11	152	6				

Ripe and running herring (maturity stages 5 and 6) showed similar rates of relationship with genetic spawning season, although with a small increase in match between the two markers. Autumn sampled autumn spawning herring (stages 5 and 6) showed 59.7% fidelity, 32.3%

switching, and 8.1% mix while spring sampled maturity spring spawners (stages 5 and 6) showed 92.0% correspondence, 2.7% switching and 3.6% mix (Appendix i).

3.2.2 Otolith hatching season and maturity spawning season

Fidelity rates were generally high, except for small sample sizes (N<12). In autumn samples substantial straying was found for maturity autumn spawners (46.9%), meaning almost half of autumn spawning herring hatched in spring (Table 9). Spring hatched herring sampled in autumn also showed some straying with 3 out of 10 herring spawning in autumn. In spring fidelity was high for spring spawners. Most autumn hatched herring were sampled in a spawning state (83.3% switching, N=12).

Table 9 *Otolith hatching season and maturity spawning season with calculated fidelity and stray (crossover) rates. Sorted to sampling season (N_{total}=222).*

		Maturity autumn	Maturity spring	Stray from hatch %	Fidelity to hatch %	Sum
Autumn sampling	**Hatch autumn**	34	8	19.0	81.0	42
	Hatch spring	30	70	30.0	70.0	100
	Stray from spawn %	46.9	10.3			
N=142	**Fidelity to spawn %**	53.1	89.7			
	Sum	64	78			
Spring sampling	**Hatch autumn**	2	10	83.3	16.7	12
	Hatch spring	6	62	8.8	91.2	68
	Stray from spawn %	75.0	13.9			
N=80	**Fidelity to spawn %**	25.0	86.1			
	Sum	8	72			

Compared only with ripe and running herring (maturity stages 5 and 6) the fidelity to hatching season increased from 86.1% to 95.2% for spring sampled spring spawners, while fidelity to hatch season for autumn sampled autumn spawners (stages 5 and 6) remained at low levels (from 53.1% to 55.0%) (Appendix j).

3.2.3 Otolith hatching season and genetic spawning season

Correspondence between hatching season and genetic spawning season was high, also in small samples (N≤11). Autumn sampled genetic autumn spawners showed some switching (17.1%), meaning they hatched in spring (Table 10). Correspondence to hatching season was 75% for autumn hatched herring sampled in autumn (Table 10). The remaining autumn hatched herring were genetically spring or mix, meaning that a considerate part of autumn hatched herring were genetically not autumn spawners (24.4%). In spring overall correspondence was above 80% for autumn type herring (N=11), and above 90% for spring spawning herring (Table 10).

Table 10 *Otolith hatching season and genetic spawning season with calculated match (correspondence) and switching rates. sorted to sampling season (N_{total}=216).*

		Genetic autumn	Genetic spring	Genetic mix	Switch from hatch %	Match to hatch %	Mix %	Sum
Autumn sampling	**Hatch autumn**	34	5	6	11.1	75.6	13.3	45
	Hatch spring	7	87	5	7.1	87.9	5.1	99
N=144	**Switch from genetic %**	17.1	5.4	*				
	Match to genetic %	82.9	94.6	*				
	Sum	41	92	11				
Spring sampling	**Hatch autumn**	9	1	1	9.1	81.8	9.1	11
	Hatch spring	2	55	4	3.3	90.2	6.6	61
N=72	**Switch from genetic %**	18.2	1.8	*				
	Match to genetic %	81.8	98.2	*				
	Sum	11	56	5				

3.3 Spawning season dynamics of subsample

Full correspondence to either spring or autumn type was found for 55.7% of herring analysed for all spawning season variables (N_{total}=210). In autumn the autumn hatched herring were mostly genetic- and maturity- autumn spawning (59.5%), while full correspondence for maturity autumn type herring was only 40.3% (Figure 7). A substantial part of maturity autumn spawning herring were found to hatch in spring, and most of these were also genetically spring spawning (Figure 7). Most genetic autumn spawning herring hatched and reproduced in autumn (73%). For autumn sampled spring spawners the correspondence was highest for the genetic spring spawners (71.0%), followed by maturity spawning season (63.6%) and otolith hatching season (50.5%). Very few autumn spawners were present in spring samples, but otolith- and genetic-analysis revealed more autumn spawners than the maturity spawning season method (Figure 7). Correspondence for spring types sampled in spring was highest for genetic spring spawners (89.6%), followed by spring hatched herring (70.5%), and maturity spring spawners (66.2%).

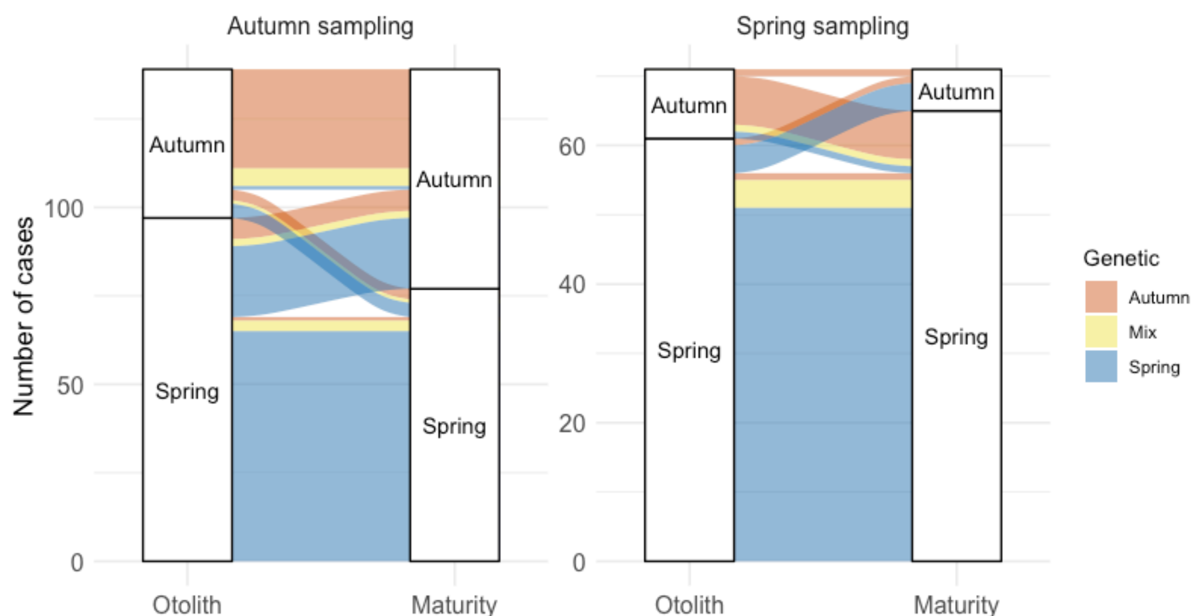

Figure 7 *Coherence of spawning season variables; Otolith hatch season (Otolith) and maturity spawning season (Maturity) assignments with genetic spawning season assignment in colour (Genetic), sorted to sampling season (N_{total}=210).*

3.4 Length and age data

Spring type herring were significantly longer than autumn type herring, independent of spawning season variable (Table 11). Also, spring sampled herring were significantly longer than autumn sampled herring (Table 11). Length distributions were highly overlapping (Appendix k). Among the aged herring (N=58) there was found no clear trend in age distribution (Appendix l). Herring between 3 and 12 years were among the herring and the overall mean age was 5.78 (± 1.92).

Table 11 *Mean total length (cm) for herring sorted to spawning season variable. N = number of herring within the given season and method. SD = Standard deviation.*

		N	Mean total length (cm)	SD	T-test; p-value
Sampling season	**Spring**	309	33.37	2.13	< 0.001
	Autumn	268	31.60	2.34	
Genetic spawning season	**Spring**	354	32.48	2.52	< 0.001
	Autumn	60	31.24	1.97	
Otolith hatching season	**Spring**	170	32.45	2.57	0.005
	Autumn	58	31.41	1.82	
Maturity spawning season	**Spring**	475	32.62	2.40	0.049
	Autumn	82	32.05	2.26	

3.5 Population abundance estimates

Catch per unit effort (CPUE) was highest in spring (herring caught per gillnet was 8 in autumn against 55 in spring), and spring type herring clearly dominated in both sampling seasons (75.0% in autumn and 85.5% in spring; Table 12). The remaining herring were spread out among spawning season variables. In autumn samples 25.0% of herring spawned in autumn, but half of these hatched in spring and were maturity spring spawning.

Table 12 *Frequency table of spawning season variables sorted to sampling season, with population and CPUE estimates. Numbers in ( ) are relative portions of spring and autumn types of herring analysed for at all spawning season variables. N pheno.geno. is the number of herring analysed for both maturity spawning season and genetic spawning season, but not for otolith hatch season. Sample estimate 1 accounts for N pheno.geno. Sample estimate 2 adds sampling season data of herring in the total catch. CPUE estimates divides the 2nd sample estimate on the number of gillnets used for sampling in that season. *'Spring' and 'Autumn' categories below populaiton estimates and CPUE is the hatching season.*

Sampling season	Maturity spawning season	Genetic spawning season	N pheno. geno.	Otolith hatching season		Sample estimate 1 (Fraction hatch x N pheno.geno.)		Sample estimate 2 Total catch (N=1166)		CPUE estimate, N total catch / N of gillnets	
				Spring	Autumn	*Spring	*Autumn	*Spring	*Autumn	*Spring	*Autumn
Autumn	**Autumn**	**Autumn**	38	6 (0.18)	28 (0.82)	7	31	9	42	0	1
Autumn	**Spring**	**Autumn**	7	1 (0.25)	3 (0.75)	2	5	2	7	0	0
Autumn	**Autumn**	**Spring**	23	20 (0.95)	1 (0.05)	22	1	29	1	1	0
Autumn	**Spring**	**Spring**	174	65 (0.94)	4 (0.06)	164	10	218	13	6	0
Autumn	**Autumn**	**Mix**	7	2 (0.29)	5 (0.71)	2	5	3	7	0	0
Autumn	**Spring**	**Mix**	4	3 (0.75)	1 (0.25)	3	1	4	1	0	0
Spring	**Autumn**	**Autumn**	2	1 (0.50)	1 (0.50)	1	1	5	5	0	0
Spring	**Spring**	**Autumn**	9	1 (0.13)	7 (0.88)	1	8	6	39	0	3
Spring	**Autumn**	**Spring**	5	4 (1.00)	0 (0.00)	5	0	25	0	2	0
Spring	**Spring**	**Spring**	147	51 (0.98)	1 (0.02)	144	3	708	14	47	1
Spring	**Autumn**	**Mix**	0	0	0	0	0	0	0	0	0
Spring	**Spring**	**Mix**	6	4 (0.80)	1 (0.20)	5	1	24	6	2	0

4 DISCUSSION

4.1 Discussion of results

4.1.1 Spawning types; correspondence and switching

In this study herring were successfully separated to spawning type spring or autumn through three methods. The correspondence between methods was generally high, but some switching was also observed. Due to few autumn type herring in spring samples the dynamic between seasonal spawning types was most prominent in autumn samples. Autumn samples were also dominated by spring types, but the presence of autumn type herring increased. According to rough CPUE estimates autumn type herring constituted 12.5% of the overall autumn caught herring. However, due to increased sampling effort in autumn, there was enough autumn herring to analyse the dynamics between spring and autumn type herring. A trend was observed in the data; Most of the switching was found between spawning season and hatching season (straying) or spawning season and genetic spawning season. Hatching season and genetic spawning season corresponded better. Trends were similar when comparing all three spawning season variables. The SNPs test corresponded best with the other two separation methods. However, the genetic "mix" herring was not assigned a spawning season. In the following I present possible explanations to the observed trends in correspondence and switching between spawning season variables and compare them with the results of previous studies.

It has been demonstrated that spring- and autumn- spawning herring populations can be separated genetically. Lamichhaney et al. (2017) predicted actual spawning season based on maturity stages at the time of sampling, and Kerr et al. (2018) grouped herring in the right genetic spawning season according to their maturity stage. Kerr et al. (2018) analysed spring sampled herring on 64 SNPs and found that all non-spawning herring were genetically autumn spawning, and all but one ripe herring was genetically assigned to the spring spawning component. Fewer herring were analysed than for the present theses (N_{total}=97, Kerr et al., 2018). Even though the results of this study demonstrate correspondence between maturity stages and genetic spawning season, the rates of correspondence are lower than what was found in previous studies. Correspondence between maturity spawning season and genetic spawning season was generally high for spring spawning herring in both seasons (90.7% for spring, and 94.1% for autumn), but a considerable part of autumn spawning herring from

autumn samples were genetically spring spawning (33.8%). Although few in numbers, most of spring sampled genetic autumn spawners also spawned in spring (N=11, 81.8%). Discrepancies between the present study and previous studies might be explained by biological differences between the populations analysed or it might be a result of methodological differences. Biologically the results suggest less discreteness between spring- and autumn- spawning herring. Methodologically there is a possibility that autumn caught ripe herring might spawn in early spring, meaning some maturity autumn spawning herring are assigned to the wrong spawning season (see discussion of methodology; 4.2.1). The low number of SNPs might also decrease the discriminatory power of the genetic separation method by providing less genetic information (see; 4.2.2). Switching was highest for herring spawning in the sampling season, but with genetics for the opposite season, suggesting that the genetic basis for spawning timing is less rigid than what was found by (Lamichhaney et al., 2017).

A general conclusion from studies on spawning time fidelity is that most herring spawn in the same season as they hatched themselves, implying discreteness between populations. However, some studies also found low rates straying between seasonally spawning populations. Husebø et al. (2005) studied autumn- winter-, and spring spawning herring caught in mixed catches on feeding and wintering areas in the North-east Norwegian sea and the Barents Sea. Otolith micro-increments were measured and a discriminant analyses was applied to separate spring and autumn hatched herring. Based on overall results it was concluded that the mixing populations are discrete units (Husebø et al., 2005), implying spawning season fidelity. Due to different methodology it is hard to compare the results with findings of the present theses. Clausen et al. (2007) validated otolith microstructure as a stock identification method through assuming spawning time fidelity in herring sampled in the North Sea and western Baltic. Ripe herring were sampled during three main spawning seasons; spring, autumn and winter, and otolith microstructures were visually assigned to hatching season according to the same criteria as those used for the present thesis (Clausen et al., 2007). Fidelity to spawning season was generally high; 97% for spring spawning herring, 92% for autumn spawning herring and 68% for winter spawning herring (Table 3 in Clausen et al., 2007). Compared to findings in the present thesis fidelity rates were high for ripe and running spring spawned herring (95.2%), but fidelity in autumn sampled autumn spawning herring was only 55.0%. The remaining autumn spawning herring hatched in spring, suggesting straying of spring herring to the autumn spawning herring. Clausen et al. (2007) also calculated back-tracked hatch dates which corresponded better to the visual separation method than separation assuming fidelity, suggesting low rates of spawning season straying (Tables 3 and 4, Clausen et al. 2007). Brophy et al. (2006) separated autumn- and winter- spawned herring

from spawning and feeding grounds in the Irish and Celtic seas. Switching rates below 10% were documented (Brophy et al. 2006). In accordance with previous studies the present thesis present high overall spawning season fidelity, especially in spring spawning herring (89.7% in autumn and 86.1 % in spring). High rates of spawning season fidelity suggest that season of spawning is predetermined by season of hatching, and possibly also location of hatching (Brophy et al., 2006). Iles and Sinclair (1982) argued that larval retention areas keep herring populations segregated, implying that spawning timing and location is either predetermined or determined in early life phases of herring. Such mechanisms will work to isolate herring populations. However, the switching documented in this thesis suggest straying between spring- and autumn- spawning populations. As many as 30% of spring hatched herring from autumn samples were maturity autumn spawning, and the same herring accounted for 46.9% of autumn sampled maturity autumn spawning herring, thereby constituting a significant part of overall autumn spawning herring. According to McQuinn (1997b) spawning behaviours are not predetermined as suggested by Iles and Sinclair (1982) but learnt by recruiting year classes from adults mixing with juveniles. Little is known about the first life phases of herring collected in this study. Also, too few herring are sampled and aged to investigate rates of fidelity and straying of single cohorts. However, the documented switching between spawning- and hatching- season suggests that straying has occurred in some of the herring sampled for this thesis. Primarily spring hatched herring strayed to spawn in autumn.

In the present thesis correspondence between otolith hatching season and genetic spawning season was higher than when the same spawning season variables were compared with maturity spawning season. Correspondence was high in both sampling seasons and hatching season seemed to match the genetic spawning season also in small sample sizes (overall correspondence > 75%). This stood in contrast to the low correspondence observed for maturity autumn spawning herring sampled in spring (25% fidelity, N=8; 28.6% correspondence to genetic, N=7). To my knowledge, this is the first study that compares hatch season with SNPs markers for genetic spawning season in herring. It is also the first study to document an increased correspondence between hatching season and genetic spawning season compared to maturity spawning season. A possible explanation to such dynamic is that natural selection will keep structure between populations because herring with genes for one season only will have higher chances to survive and reproduce (higher relative fitness) compared to the offspring with mixed genes. In such a scenario a spring hatched strayer with typical spring genes might successfully reproduce with an autumn hatched herring with typical autumn genes, but the offspring with mixed genes will struggle to survive and reproduce successfully (lower relative fitness). Such mechanism should work to structure timing of reproduction in herring populations because offspring with higher fitness become more

dominant (specialization to timing of spawning). Discreteness between seasonal spawning populations is kept, even if some herring stray between spawning seasons. The genetic differentiation between seasonal spawning herring can be linked to seasonal adaption because of low genetic drift in large populations (Lamichhaney et al., 2017), and Kerr et al. (2018) found temporal stability in a number of genomic regions linked to season of spawning. In other words; genetic factors controlling time of spawning are linked to adaptive traits that confront environmental conditions met in either spring or autumn. Seasonal light and/or temperature regimes could possibly play crucial role here. Isolation by time causes adaption because of variation in selection between the reproductive seasons (Hendry and Day, 2005). Also, adaption to variation in the seasonal local environment can be observed as variation in phenotypic traits between seasonal spawning herring. Such temporal variation in phenotypic traits are then caused by genetic differences (adaption), rather than phenotypic plasticity which causes variation in phenotypes due to variation in the external environment. It is suggested that genetic structure of spring- and autumn- spawning herring is linked to adaption to seasonal spawning times and that selective pressures keeps structure between seasonal spawning populations. Straying may occur but adaptive traits linked to spawning season cause reduced survival of offspring from mixed seasonal spawning herring.

A strong genetic component to spawning season in herring is suggested but straying might occur due to external cues met at certain life stages. In other words: straying is a result of the environment experienced by those herring which counteract the genetic adaptation to spawning season. Winters and Wheeler (1996) documented that maturation in herring is affected by physiological factors (condition and size of fish) and environmental factors. It was found that spring spawners in Newfoundland coastal waters adjusted their spawning times to the winter sea temperatures to match the environmental conditions met during spawning (Winters and Wheeler, 1996). Variation in timing of spawning in herring has also been linked to feeding conditions prior to spawning (Rajasilta, 2011). This suggests that time of spawning is not fixed, but rather a dynamic process influenced by the external environment. Although it is suggested that the relative fitness of the offspring of seasonally mixed herring is reduced, there is a possibility of survival to reproduction. The genetic heterogeneity (heterozygosity and rare occurrences of Spring:Autumn herring) observed in this study might possibly be the result of such event. It is suggested that the external environment met by herring in some life phases can cause straying of herring which in some cases leads to successful breeding of offspring with seasonally mixed genetics. The effect of strayers is still to be investigated, but it might explain why genetic differentiation between populations is low in herring (Lamichhaney et al., 2017).

4.1.2 The effect of relative abundances

In this study CPUE estimates were calculated to get a rough idea about the relative abundance of herring types within and between spring and autumn sampling seasons. Spring type herring clearly dominated in both seasons. The rates of switching between genetic spawning season, otolith hatching season, and maturity spawning season documented in the present thesis might be specific for the populations studied and remain constant over time, or it might be an adaptive characteristic that changes with encountered conditions (Harma et al., 2012). Also, the rates of correspondence and switching might be an effect of relative abundances. If straying occurs at equal and steady rates between spring- and autumn- spawning herring the straying will naturally have a larger effect on the smallest population. In the present study 45.0% of the less abundant autumn spawning herring (ripe and running) hatched in spring, suggesting that genetic mixing will be large in autumn spawning herring. It can be speculated that a successful autumn spawning event will cause higher mortality of autumn hatched herring compared to spring hatched herring because of lower fitness in seasonally genetically mixed herring. Over time this could possibly lead to a collapse of the autumn spawning component. At the same time, interannual variability in environment and recruitment in each population have unknown effects on straying rates and dynamics between the seasonally spawning components.

Variability in spawning type composition and abundance within sampling seasons and between years was not considered in this thesis. However, recruitment in herring populations is variable. Both abundance of a population and number of strayers within a population is therefore expected to change over time. For example, variability in stock size is documented to affect the recruitment pattern of components of NSS herring (Holst and Slotte, 1998). Holst and Slotte (1998) suggested the that the relative strength of individual year classes in two NSS components might involve factors such as spawning stock size, geographic spawning distribution, oceanographic conditions, growth, natural mortality and fishing mortality. In all, there is a number of factors which might affect the year to year recruitment to a population. Toresen and Østvedt (2000) documented a positive correlation between recruitment and average temperatures of inflowing Atlantic water masses, emphasizing the effect of temperature on long-term trends in abundance of spawning stocks or populations. McQuinn (1997b) suggested that a strong year class could lead to increased straying because there would be more juveniles that could possibly stray to the other season. The number of strayers would depend on growth of juveniles (McQuinn 1997b). Also, as documented in this thesis, the straying might have an effect on the fitness of the following generation. The effects of temporal varation in environment and recruitment on herring popualiton dynamics, including levels of straying and/or fidelity, remains to be established.

4.1.3 Evidence of a third spawning group?

In this study it was assumed that two spawning types (spring or autumn) would be detectable when separating herring through genetic and phenotypic techniques. However, otolith microstructure suggests that a third group of winter hatched herring were present in samples (N=21). Otolith microstructure was found similar to that of the winter-spawning Downs herring found in mixed catches in the North Sea (Clausen et al., 2007). In the present study hatching season was related to spring- and autumn- spawning types only, so winter hatched herring were separated to spring (38.1%) or autumn (61.9%) hatching seasons. The presence of winter hatched herring in samples is however possible. These herring might have recruited to a population spawning in another season than the parental population, or winter spawning herring might exist in the area.

The two SNPs used in this study showed allelic differentiation between spring- and autumn-spawning herring, allowing for separation at the individual level. This in consistent with the findings of Lamichhaney et al. (2017). However, the two SNPs also revealed some heterogeneity between tests and heterozygosity was mostly found on only one out of the two SNPs tests. For comparison between methods these herring were added to spring- and autumn- genetic spawning season respectively. Interestingly, in 2013 Lamichhaney et al. (2017) found considerable heterogeneity at some loci in herring sampled from the same sampling location as was used in the present thesis (see AB2 station in Lamichhaney et al. 2017). The variability found in these herring contrasted to the homogeneity observed in the AB1 sample of Norwegian spring spawning herring in February 1980 (Lamichhaney et al., 2017). The heterogeneity observed in this study and by Lamichhaney et al. (2017) could possibly be explained by mixing between populations in the area, and the possibility of a third spawning group cannot be excluded.

4.1.4 Population affiliation

The herring sampled for this thesis might be a part of a larger migratory stock and/or local herring adapted to local conditions. Most of the dominant spring spawning herring were caught in a running state in spring (stadium 6) and were maturing in autumn (stadium 3). In other words; spring spawning herring stay throughout the year, suggesting that there is a local spring spawning population in the area. At the same time, the abundance of spring spawners increased in spring with 88.7% according to CPUE estimates. This suggests that most of the spring spawning herring migrate to other areas for feeding and overwintering. Herring with long migrations are known to have high growth and be of larger size at maturity than other populations (Husebø *et al.*, 2005). Mean length of spring and autumn type herring caught for this thesis was similar, and length distributions were overlapping, but spring type herring were still significantly longer than autumn type herring (Table 11, Appendix k). Length distributions might suggest that the autumn spawning herring migrate less than the spring type, but further investigations are recommended. Differences in length might also be due to different cohort structure, but the aged herring did not show any trends in age structure between sampling seasons (Appendix l). Ageing more herring would give more information here. The highly migratory NSS herring stock is known to spawn along the Norwegian coast in spring (February to March) (Holst and Slotte 1998), and the spring spawning herring caught in spring might possibly be NSS herring. Co-occurrence of dominant NSS herring and less dominant autumn spawning herring was reported by Husebø et.al. (2005). The discreteness documented between the seasonally spawning components suggest that these should be managed separately (Husebø et al., 2005). The autumn spawning herring caught for this thesis were highly influenced by straying spring hatched herring, questioning the discreteness of autumn spawners. However, the correspondence between genetic spawning season and otolith hatching season suggests that there is indeed an autumn spawning component in the area which can be genetically separated from the spring spawners.

4.2 Discussion of methodology

4.2.1 Maturity staging and spawning season

Sexual maturity staging of herring gonads is the standard method to quantify gonad development (Mjanger et al., 2017). In this study herring samples were maturity staged the day of sampling, allowing for analysis of fresh specimens. Still, there are some limitations. Human errors can happen due to lack of experience with maturity stage estimation, and variability in gonad appearance can make interpretation difficult. In this study maturity stages were subjectively determined by lab personnel which might in some cases interpret the same gonad as different stages. Assigning herring to the wrong maturity stadium will have implications when separating herring to maturity spawning season. It was assumed that stages 5-8 (ripe to resting) spawn in the sampling season while stages 3-4 (maturing) spawn in the other season. This means that stages 4, 5, 8 and 3 are most likely to be prone to misclassification, leading to wrong assumptions about spawning season. Displacement of individual herring within these maturity stages cannot be excluded for the present study, and errors associated with the maturity spawning season method might have had an effect on estimated correspondence rates between spawning season variables. Herring were also sampled throughout spring and autumn spawning seasons, but variability in gonad development within sampling seasons was not considered when assigning herring to maturity spawning season. For example, stage 4 (maturing) herring in an early autumn (or spring) sample might spawn in the given season but will be classified to spawn in the other season.

Seasonal spawning groups of herring can possibly be hard to separate through maturity stages due to synchronized development in parts of the year. Herring gather most of their energy for growth and reproduction during feeding in summer months, and synchronized feeding might result in synchronized development of gonads. Damme et al. (2009) found that autumn- and winter- spawning herring in the North Sea start their maturation at similar times (April or May). The maturation cycle (oocyte development) was similar for the two components, but winter spawning herring continued to develop their oocytes after autumn spawners had spawned. This suggests that seasonal spawning herring groups might be hard to distinguish in early developmental phases due to similar gonad appearance. If the same results can be transferred to the present study the maturity stadium of late autumn sampled herring might indicate autumn spawning (stadium 5, ripe), while it is actually spring spawning herring preparing to spawn in early spring, resulting in wrong seasonal classification. To account for some of the

biases associated with misclassification of maturity spawning season correspondence rates were also calculated for ripe and running herring only (Stages 5 and 6).

Bias in the maturity staging process could possibly be reduced with different methodologies. Documentation through photography would allow for comparison between readers and between samples, securing consistency. Also, photos of uncertain stages could be checked with an experienced gonad reader. Histology is also used to study and enumerate gonad development. Although more time consuming, the method provides higher credibility (McQuinn, 1989). Discriminant analyses on gonadosomatic indexes (GSI) together with maturity stages also demonstrated higher identification accuracy than separating herring groups solely on maturity stages (McQuinn, 1989). However, maturity staging herring gives an idea of the overall composition within and between sampling seasons, and it is therefore considered adequate for the present study.

4.2.2 SNPs and genetic spawning season

Two SNPs were used to genetically separate spring- and autumn- spawning herring through a Taqman assay. In contrast to maturity staging and otolith microstructure analyses the genetic separation of spring- and autumn- spawners is not dependent on reader interpretation, providing high reliability. The choice of the two SNPs was based on findings from previous studies (Barrio et al., 2016; Lamichhaney et al., 2017). These studies were the first to document that autumn- and spring-spawning herring are genetically distinct. The results of Lamichhaney et al. (2017) were based on seasonal spawning herring populations from both sides of the Atlantic Ocean. Genetic differentiation between spring- and autumn- spawning herring was found high in some loci compared to low genetic differentiation in other parts of the genome. This was explained by the large effective population sizes in herring that result in low genetic drift and increase the chances that genetic differentiation is caused by natural selection. Genetic differentiation between seasonal spawning herring populations can therefore be linked to ecological adaption to timing of spawning. Lamichhaney et al. (2017) found significant differentiation between spring- and autumn- spawning herring populations on some loci including genes that play a significant role in reproductive biology. Among these were the thyroid-stimulating hormone receptor (TSHR) known to play a key role in photoperiodic regulation of reproduction in birds and mammals (Hanon et al., 2008). The SNPs used in this study are found upstream of the TSHR locus. Based on these findings the SNPs used in this study should be highly applicable for separation of spring- and autumn- spawning herring.

It can be questioned whether more than two SNPs should be applied to secure correct separation. Adaptive traits are expected to be controlled by several genes (Pritchard and Di Rienzo, 2010), and loci associated with timing of spawning in herring are many Barrio et al., 2016). Lamichhaney et al. (2017) found 60 000 SNPs with significant differentiation between spring- and autumn- spawning herring, suggesting that a range of SNPs can be used for separation. Kerr et al. (2018) used thinning methods to determine which SNPs could be used for individual assignment to spawning season. They found that 6 SNPs was sufficient for 100% separation of spring- and autumn- spawning herring. These results suggest that the two SNPs used in the present study might not be sufficient for 100% discrimination. Some heterogeneity was found between the two SNPs tests which might be explained by the choice and number of SNPs used to separate spring- and autumn- spawners.

4.2.3 Otolith microstructure and hatching season

In this study herring were successfully separated to spring- and autumn- hatching season based on the assumption that daily increment growth of otoliths will be progressively wider in spring than in autumn (Clausen et al., 2007). Otolith microstructures were revealed by grinding of otoliths, documented through photography, and visually separated to hatching season by two readers. Such qualitative assessment of otolith structures requires experience and training of otolith microstructure readers. In this study the documentation of otolith microstructures helped to diminish reader bias because otoliths could be re-read when readers were in doubt. Documentation also made it possible to compare otolith microstructures from different fish, and between readers, assuring consistency in interpretation of microstructures. However, the readers were not always certain of hatching season assignments, and 5.8% of photographed otoliths could not be assigned to a hatching season. To reduce possible errors associated with human interpretation of the otolith microstructure the increment width of otolith microstructures can be measured allowing for quantitative separation to hatching season (Husebø et al., 2005). Reader bias will be diminished through this method and increment widths can be compared with other studies using the same method. However, this was not achievable within the time frame of present study.

Unclear microstructure patterns might be explained by the environment in which the herring experienced after hatching, by the genotype of the herring or by human errors associated with grinding and preparation of otoliths. In this study many otoliths revealed a clear microstructure only in parts of the growth zones at the determined reading spectrum (20-100 µm from the nucleus). The translucency also varied, where some otoliths appeared too translucent to see

much of the microstructure pattern. Variability in the opacity and translucency of otoliths can be explained by mechanisms controlling the otolith biomineralization process. Temperature and metabolism (food availability) are the main factors affecting such otolith structure (Fablet et al., 2011). Although otolith microstructure can be explained by variations in these factors it will be hard to separate herring to hatching season when microstructures deviate from the predetermined assumptions about seasonal otolith microstructure patterns. Variability in otolith microstructure growth is a natural effect of variation in the environment met by herring larvae. Herring from different year classes are therefore expected to show some variation in otolith microstructure growth. However, Husebø et al. (2005) did not find any significant influence of otolith microstructure growth between year classes. Otolith microstructure growth has been studied in herring larvae experiencing different prey levels. Fox et al. (2003) found irregular crystal formation and incremental structure in slow- growing larvae. Also, Geffen (1982) found that increment deposition rate can be below one per day due to low feeding conditions. If these findings can be transferred to herring caught in this study, slow growth could possibly explain the difficulty of interpreting the otolith microstructure found in some of the analysed herring. Little is known about the genetic effect on otolith microstructure patterns, but as suggested by Clausen et al. (2007), variability in otolith microstructures may be an effect of straying. Naturally overlapping spawning seasons could also be a source of variability in otolith microstructure patterns.

The technique used to reveal otolith microstructure might also explain why some herring could not be separated to hatching season. Grinding of herring otoliths is a delicate process, and microstructure photos are not always taken on the exact plane though the core. Grinding of the second otolith might then give better photos. In this study the second otolith was grinded for 11 herring. Some of these could then be separated to hatching season but others were still hard to interpret. The visible microstructure might also be limited by the resolution of the light microscope and narrow increments might not always be detected (Campana, 2004). Even though some difficulties were met when separating herring though otolith microstructures, the method have successfully separated mixing herring populations to spawning season in a number of studies (Moksness and Fossum, 1991; Husebø et al., 2005), and validation studies showed high discriminatory power (Clausen et al., 2007). The method is also used so separate western Baltic spring spawners (WBSS) and North Sea autumn spawning herring (NSAS) for management purposes (ICES, 2018). Based on previous research otolith microstructure is therefore considered an adequate method to separate herring to hatching season.

4.2.4 Sampling equipment

The mesh size of gillnets used in this study (29, 31 and 34 mm) caused size selectiveness (in width) of caught herring. The mean size of the natural population might be lower than what is observed among sampled individuals. If spring spawning herring are significantly wider than autumn spawners the relative amounts of each spawning type will not represent that of a natural population. Spring- and autumn- sampled herring, and spawning types (sorted to spawning season variable), were found to be significantly different in length, suggesting that there might also be significant differences in width of the fish. However, population estimates presented in this thesis are very rough, and other sources of variability (e.g. between year variability) will most probably be more important than the size of caught herring. The relative abundance of each spawning type was also not the main focus when discussing correspondence between spawning season variables. The samples are therefore considered suitable for this study.

4.3 Management implications

Identification of populations and/or stocks in necessary, especially when they are mixing, for sustainable management. Within-species diversity is an important element of an ecosystem approach to management (Stephenson et al., 2009). Each population should also be monitored separately to preserve life-cycle diversity that may give resilience to climate- and fisheries- induced changes (Harma et al., 2012). Also, while management and assessment tools are mainly based on phenotypic traits, the management aims (such as the sustainability of spawning stock biomass and conservation of genetic diversity) is linked to genetic structure (Reiss et al., 2009). Including genetic analysis among fisheries management tools is therefore necessary in order to meet management aims. In this study the discriminatory power of SNPs was documented to be high when compared with otolith hatching season and maturity spawning season. This suggests that the genomic SNPs markers used in this study have great potential in separating mixed spring and autumn spawning herring populations. Genetic separation of spring and autumn spawning herring can also be performed on herring regardless of developmental stage. For example, can mixing juveniles be separated genetically for use in recruitment estimates. Two cod stock components found mixing in the Lofoten area, one local and one migratory, are currently separated for management purpose through the use of diagnostic SNPs (Dahle et al., 2018). Genetic separation methods of stocks and/or populations through SNPs can be conducted rapidly and with simple equipment. Also, genetic methods do not require highly trained personnel (e.g. an experienced otolith- or gonad-

reader). Implementing genetic population separation methods for mixed catches of herring is therefore recommended. New genetic techniques might also be able to detect genomic regions unique to specific herring populations, providing population specific genetic markers highly useful to management. Standardized genetic methods for separating seasonal spawning herring will then be a requirement, making the choice of SNPs subsets an important part of the discussion on SNPs genetic separation methods. Future genetic studies should aim to develop population specific markers which are connected to genomic regions that show temporal stability (Kerr et al., 2018). Through whole genome sequencing the GENSINC project could possibly reveal genetic structure which can be used for stock identification and separation in herring, contributing more sustainable exploitation of the species. However, a holistic approach to management should include all available stock identification information, implying that other methods based on phenotypic characters should not suffer from the application of genetic methods (Begg and Waldman, 1999).

4.4 Future studies

Age composition is relevant for studies on population structure and dynamics as it allows for studies of single cohorts and year class dynamics. Combining genetic studies with cohort structure could be a powerful tool to detect population dynamics between seasonal spawning groups as it allows for detection of generational changes. The rates of fidelity and straying observed in this study might be a fixed population trait or it might change according to the conditions encountered (Harma et al., 2012). Future studies should aim to assess temporal variability in rates of fidelity and straying and integrate the abundances of single cohorts in genetic and phenotypic analyses of population structure and dynamics between seasonally spawning herring populations. Population affiliation of herring sampled for the present study should also be further investigated. Measuring otolith microstructure growth would allow for comparison of growth of other already identified populations. Length at age and somatic weight at length could also be used to compare herring in the present study area with other herring populations. Population affiliation based on such phenotypic characters are useful because results can be compared with studies that already exist. Also, genetic analyses and the development of population specific genetic markers could be used to compare the genetic structure of herring in the sampling area with other herring populations (Barrio et al, 2016). A third winter spawning group was suggested to exist in the sampling area which might also be detected by the mentioned methods. The *location* of spawning should be close to sampling grounds for herring sampled in a spawning state (Maturity stadium 5 and 6). However, the

autumn spawning event is not yet documented in the area. Diving or video recording could be used to document autumn spawning grounds. Also, hatching season was found for the herring sampled for this thesis but we do not know the location of hatching. Thorrold et al. (2001) studied spawning site fidelity (natal homing) through isotope analysis of otoliths on weakfish. Spatial structure of Atlantic herring populations should be considered when discussing population dynamics of seasonal hatching and spawning herring, and isotope analysis of otoliths could possibly be used to study natal homing in herring.

A major question which emerged from this thesis is the faith of offspring with both spring and autumn genes. Based on the findings of this thesis it is suggested that adaption to spawn in spring or autumn causes selective pressures which keeps isolation between seasonal spawning populations. Herring with seasonally mixed genes will then have lower relative fitness than herring with genes for one spawning season only. However, the biological significance of the genetic structure between seasonal spawning herring populations is still not fully understood. An experimental population consisting of hybrids between spring and autumn spawning herring can be used to study the effect of a typical spring or autumn environmental regime on herring with seasonally mixed genes. Survival and variation in phenotypic traits (e.g. growth) can then be compared between herring with genes for one season and herring with seasonally mixed genes. Such experiment is planned within the GENSINC project and it will probably improve the understanding of the genetic basis of timing of reproduction in herring.

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6 APPENDIX

Appendix a: *Sampling sites with coordinates*

Sampling station	Coordinate in decimals
Rishålo	N60º34´45.9´´, E5º00´45.7´´
Kobbevågen	N60º34´7.5´´, E5º00´28.9´´
Garnvika	N60º34´30.2´´, E5º00´59.0´´

Appendix b: *Gillnets used for sampling herring.*

Type of net	Mesh size (mm)	Width (m)
Herring net	29	5.8
Mackerel net	32	5.5
Mackerel net	34	5.5

Appendix c *Temperature cycles for qPCR.*

Temperature (°C)	Time	Cycles
95	10 min.	
92	15 s.	x 40
60	1 min.	

Appendix d *Number of herring assigned to a genetic spawning season through genetic analyses with two SNPs; KIT1420 and KIT481.*

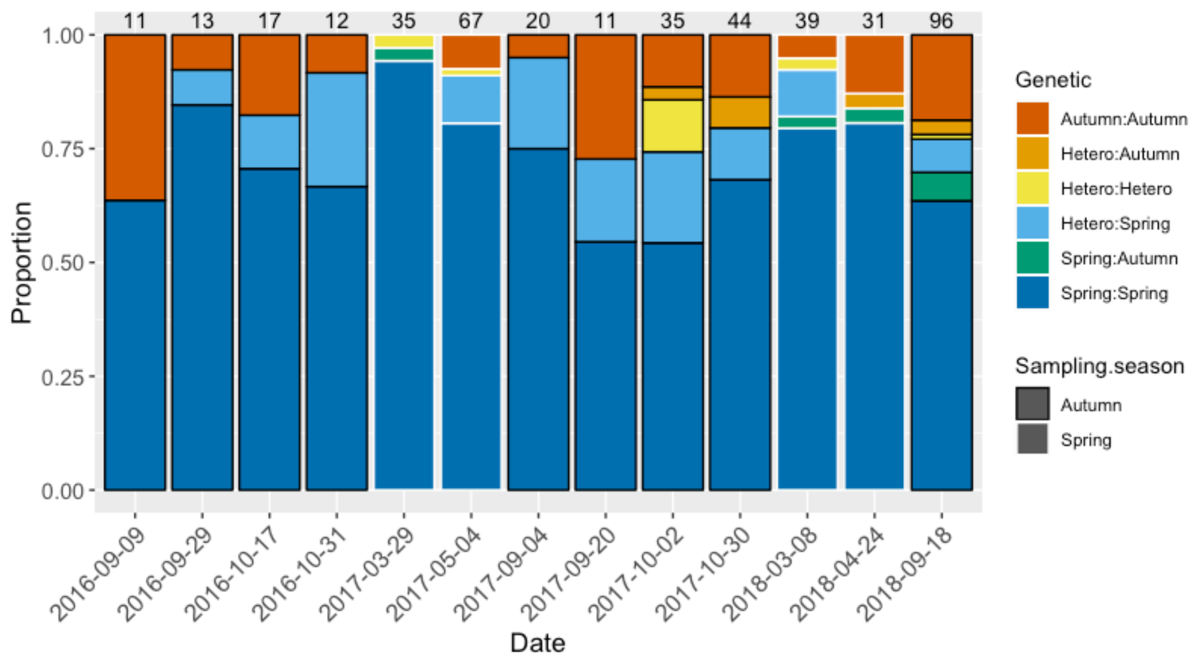
KIT1420	**KIT481**				
	Autumn	**Spring**	**Heterozygote**	**Undetermined**	**Total**
Autumn	52	2	4	1	**59**
Spring	7	312	27	1	**347**
Heterozygote	4	15	8	3	**30**
Undetermined	3	3	1	3	**10**
Total	**66**	**332**	**40**	**8**	**446**

Appendix e *Number of herring assigned a hatching season, determined by visual inspection of otolith microstructure. Performed by two readers (reader 1 and 2), before comparison.*

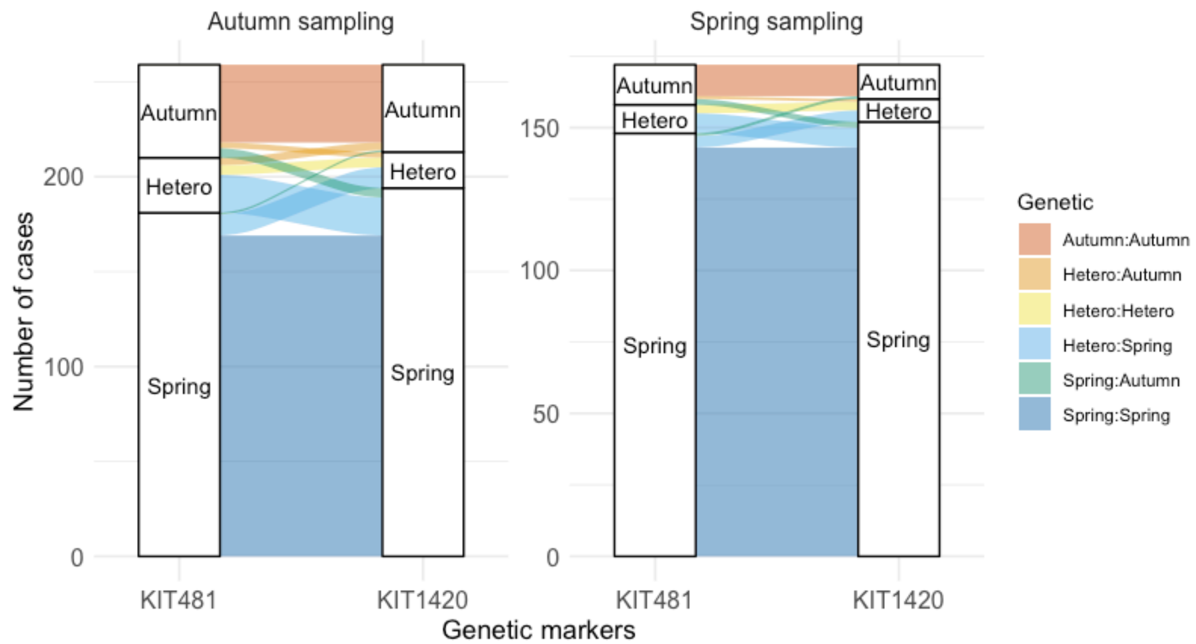
Reader 1	**Reader 2**			
	Autumn	**Spring**	**Undetermined**	**Total**
Autumn	41	5	1	47
Spring	11	164	2	177
Undetermined	0	6	11	17
Total	52	175	14	241

Appendix f: *R packages used for statistical analysis.*

R package	Developer and reference
library(readxl)	Hadley Wickham and Jennifer Bryan (2018). readxl: Read Excel Files. R package version 1.1.0. https://CRAN.R-project.org/package=readxl
library(tidyverse)	Hadley Wickham (2017). tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse
library(ggalluvial)	Jason Cory Brunson (2018). ggalluvial: Alluvial Diagrams in 'ggplot2'. R package version 0.9.1. https://CRAN.R-project.org/package=ggalluvial
library(ggmap)	D. Kahle and H. Wickham. ggmap: Spatial Visualization with ggplot2. The R Journal, 5(1), 144-161. URL http://journal.r-project.org/archive/2013-1/kahle-wickham.pdf



Appendix g *Genetic assignment to spawning season by two SNP's; KIT481 and KIT1420, sorted to sampling date. Numbers above axis are total numbers of herring analysed on both SNPs at that sampling date.*



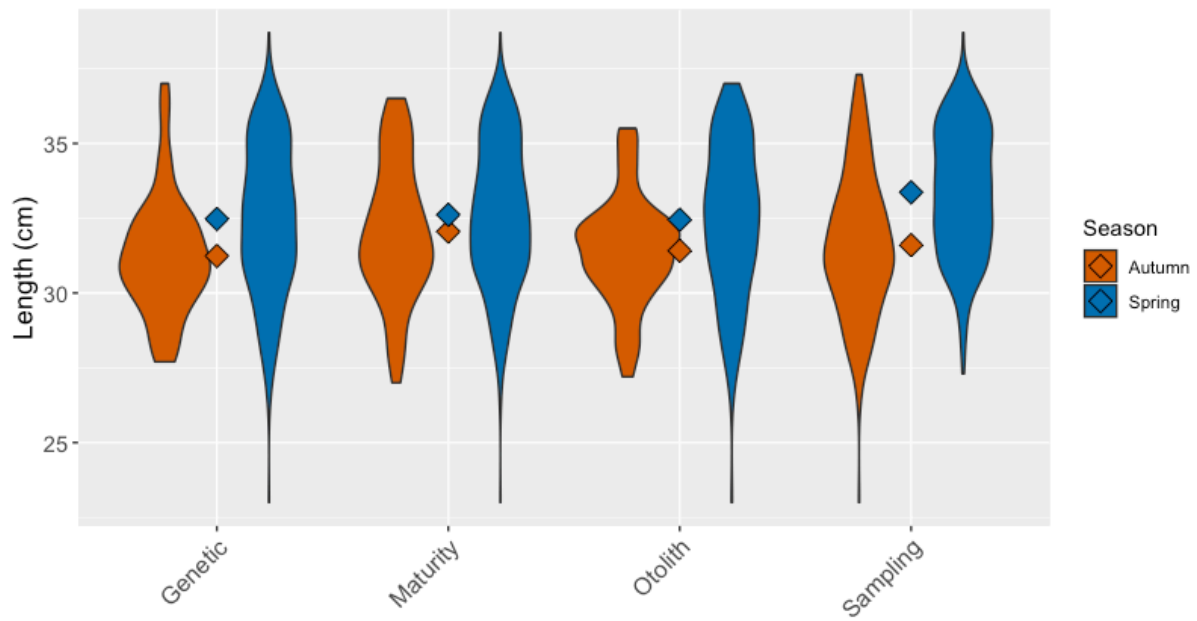
Appendix h *Alluvial plot of two SNPs; KIT481 and KIT1420. "Genetic" are categories assigned to herring when combining the two kits before genetic simplification. Heterozygote herring is simplified to "Hetero". Genetically undetermined herring are not included in the figure (N_{total}=431).*

Appendix i *Genetic spawning season for ripe and running herring (maturity stages 5 and 6) with correspondence and switch (crossover) rates. Sorted to sampling season (N_{total}=137).*

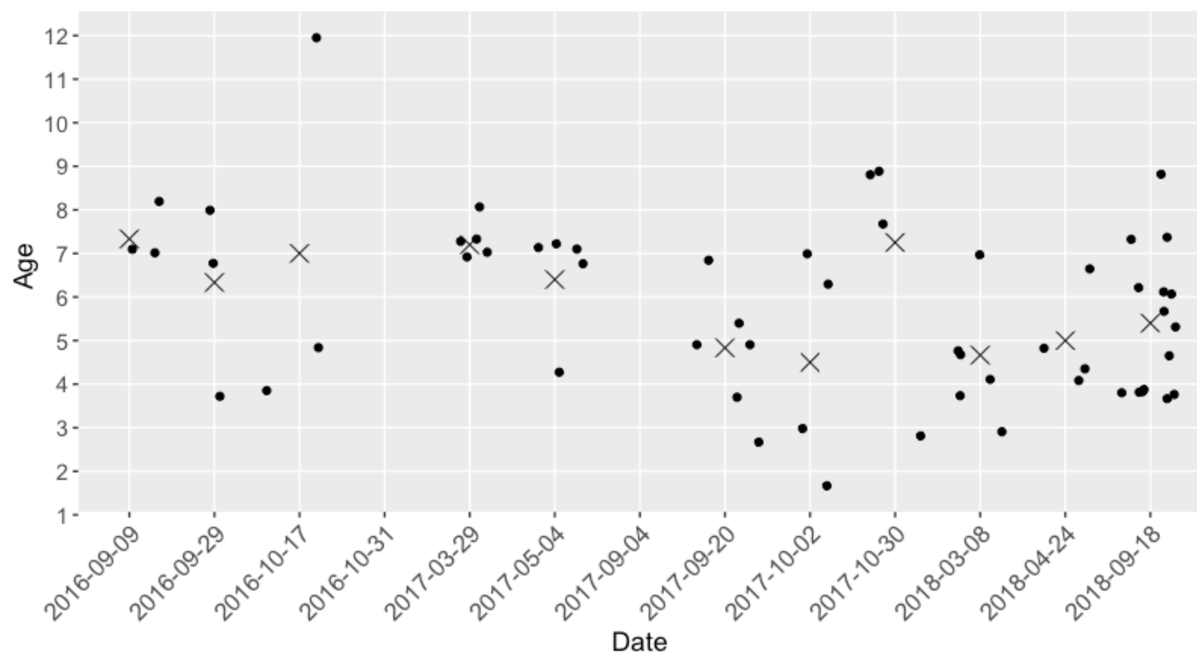
Sampling season	Genetic autumn	Genetic Spring	Genetic mix	% Switch from spawn	% Match to pawn	Mix %	Sum
Autumn	37	20	5	32.3	59.7	8.1	62
Spring	2	69	4	2.7	92.0	3.6	75

Appendix j *Hatching season for ripe and running herring (maturity stages 5 and 6) with fidelity and straying (crossover) rates. Sorted to sampling season (N_{total}=137).*

Sampling season	Hatch autumn	Hatch spring	Stray from spawn %	Fidelity to spawn %	Sum
Autumn	33	27	45.0	55.0	60
Spring	2	40	4.8	95.2	42



Appendix k *Violin plot of length distributions of spring- and autumn-type herring split by spawning season variable; genetic spawning season (Genetic), Maturity spawning season (Maturity), Otolith hatching season (Otolith) and sampling season (Sampling). Points are means per method and sampling season.*



Appendix l *Age distribution, sorted to sampling date (N=58). Crosses are means per sampling date.*