Population structure and dynamics of Atlantic herring

A case study of herring inhabiting marginal habitats in Landvikvannet and its vicinity

Florian Berg

Thesis for the Degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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”When you study herring,

there are no wrong answers.”

A. J. Geffen at ‘Linking herring’ Symposium, 2009

Scientific environment

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Abstract

Knowledge about the structure and dynamics of marine fish populations is essential for their conservation and management to maintain biodiversity and population complexity. Atlantic herring (*Clupea harengus*) is one of the ecologically and commercially most important fish species in the northeastern Atlantic and well-known for its complex population structure. However, the actual population structure and dynamics are still debated and partly resolved. To expand the knowledge, the purposes of this thesis were (1) to identify herring populations and their population structure in a case study area based on phenotypic and biological characteristics, and (2) to examine whether these distinct characteristics can be used for population discrimination on a broader scale.

Within the case study area, three distinct herring populations could be identified based on behavioral and phenotypic differences such as vertebral counts, length-at-age, otolith shape, and otolith microstructure. The spatial and temporal overlap and potential interbreeding between these three populations suggest that they form a metapopulation. The existence of a metapopulation would have a significant influence on the current management approach. Further, mixing of several populations could have been demonstrated on a broader scale, but an individual assignment being essential for a sustainable management was not feasible. Finally, hybrids of two herring populations have been reared until maturity in a common garden experiment showing that phenotypic characteristics were controlled by genetic factors to a larger extent than by salinity.

The results of this thesis provide novel insight into the population structure and dynamics of herring, the factors influencing phenotypic traits, and potential implications for management purposes. Furthermore, the findings contribute new knowledge about several population identification methods strengthening their application to resolve the complex population structure of Atlantic herring. This thesis highlights the importance of recognizing herring dynamics and understanding the mixing of populations as a challenge for management of herring.

Keywords: metapopulation, population structure, phenotypic plasticity

List of publications

Paper I

Eggers, F., Slotte, A., Libungan, L. A., Johannessen, A., Kvamme, C., Moland, E., Olsen, E. M., and Nash, R. D. M. 2014. Seasonal dynamics of Atlantic herring (*Clupea harengus* L.) populations spawning in the vicinity of marginal habitats. PLoS ONE **9**(11): e111985. doi: 10.1371/journal.pone.0111985.

Paper II

Eggers, F., Olsen, E. M., Moland, E., and Slotte, A. 2015. Individual habitat transitions of Atlantic herring *Clupea harengus* in a human-modified coastal system. Mar. Ecol. Prog. Ser. **520**: 245-256. doi: 10.3354/meps11103.

Paper III

Berg, F., Husebø, Å., Godiksen, J. A., Slotte, A., and Folkvord, A. 2017. Spawning time of Atlantic herring (*Clupea harengus*) populations within a restricted area reflects their otolith growth at the larval stage. Fish. Res. **194**: 68-75. doi: 10.1016/j.fishres.2017.05.009.

Paper IV

Berg, F., Slotte, A., Johannessen, A., Kvamme, C., Clausen, L. A. W., and Nash, R. D. M. 2017. Comparative biology and population mixing among local, coastal and offshore Atlantic herring (*Clupea harengus*) in the North Sea, Skagerrak, Kattegat and western Baltic. PLoS ONE **12**(10): e187374. doi: 10.1371/journal.pone.0187374.

Paper V

Berg, F., Almeland, O. W., Skadal, J., Slotte, A., Andersson, L., andFolkvord, A. 2017. Genetic factors have a major effect on growth, number of vertebrae and otolith shape in Atlantic herring (*Clupea harengus*). PLoS ONE. **under revision**.

List of abbreviations

CSS	Coastal Skagerrak spring spawners
NSAS	North Sea autumn spawners
NSS	Norwegian spring spawners
SNP	Single nucleotide polymorphism
VS	Mean vertebral counts
WBSS	Western Baltic spring spawners

CONTENTS

1 INTRODUCTION

1.1 Theoretical background and definition of a population

The biological species concept of Mayr (1942) is a centerpiece and fundamental when studying biology, especially ecology. “Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups (Mayr, 1942)” is a familiar species definition for most modern biologists. This concept had an important influence on the systematics in particular, and the evolutionary biology in general (de Queiroz, 2005). However, as Mayr already stated, an additional concept, the population, is necessary. Populations are essential and central for ecologists who need to know how the abundance of species changes over time and space. Ecological dynamics of populations over space and time have wide implications for conservation and management of the biodiversity (Camus and Lima, 2002). Despite the importance of populations, there is no single and consistent definition that could be applied directly to species in the wild. Given such a definition, which should be objective and quantitative, independent researchers could apply it and, for example, determine how many populations exist in a particular area and how the relationship between them is characterized. Furthermore, a single definition would achieve the same results of a common problem when applied independently. “A group of organisms of the same species occupying a particular space at a particular time (Krebs, 1994)” is a commonly used definition of a population. Berryman (2002) expanded this definition allowing for dispersal and/or migration of individuals within the particular space, but the dynamics of a population are mostly determined by internal birth and death processes. It is also important to distinguish if the population definition is used under the ecological paradigm (demographic cohesion) or the evolutionary paradigm (reproductive cohesion; Waples and Gaggiotti, 2006). Throughout, the population definition of Berryman (2002) will be used in the evolutionary context as a reproductive group of individuals of the same species (see Fact box 1).

Fact box 1: Definitions

Population: Group of individuals occurring at the same time in a defined geographical area, which is of sufficient size to permit dispersal and/or migration of individuals, and representing a reproductive group where all individuals can potentially interbreed with any other member. Death and birth processes largely determine the dynamics of a population.

Metapopulation: Spatially complex population consisting of several locally breeding subpopulations ($n \geq 2$) in a defined geographical area where the subpopulations are linked (e.g., by migration) and gene flow between subpopulations exist.

Subpopulation: A population within a spatially complex population.

Local population: A population occupying and spawning in a local, regional area.

Population dynamics = Birth (B) - Death (D) + Immigration (I) - Emigration (E) (only immigration and emigration will be considered in this thesis).

Stock: Parts of a population (or several populations) with similar life history parameters occupying a defined geographical area and being subject to a distinct fishery.

1.2 Population concepts

Although a population is now defined as a reproductive group, gene flow between different populations cannot be unambiguously excluded. All populations, both terrestrial and marine, are to some extent spatially structured (Goodwin and Fahrig, 1998) because an ecological barrier surrounds each population (Andrewartha and Birch, 1984). The spatial structure is an essential feature of the population dynamics (Dunning et al., 1992). Population dynamics are mainly driven by (1) birth and death processes, and (2) emigration and immigration (Fact box 1). This thesis will solely focus on the influence of immigration and emigration of individuals on the population dynamics. However, depending on the simplicity or complexity of aggregation levels of populations within their spatial structure, several expanding population concepts have been described (Table 1; Ciannelli et al., 2013).

Within the sympatric discrete population concept (Iles and Sinclair, 1982) the definition of populations as reproductive group can be applied. Under the sympatric discrete population concept, several populations can aggregate in the same space during at least one phase of their lifetime. However, the populations of these aggregations are mostly reproductively and genetically isolated populations. Also, the discreteness and reproductive isolation of populations is accomplished by natal homing, allowing for temporal persistence and ensuring life-cycle closure, and the reduced viability of none returning individuals (Iles and Sinclair, 1982).

Table 1: Population concepts based on their genetic and demographic characteristics, modified after Ciannelli et al. (2013). B = Births, D = Deaths, E = Emigration, I = Immigration.

Concept	**Definition**	**Demography**	**Genetic**
Sympatric discrete populations	Reproductively and genetically isolated populations which might occupy overlapping habitats, at least during one phase of their lifetime	B + D >> E + I	Structured
Spatially complex populations	Locally breeding subpopulations which might be genetically connected via dispersal	B + D $\geq$ E + I	Homogenous and weakly structured
Panmictic population	Interbreeding individuals that are heterogeneously distributed over space	B + D << E + I	Homogenous

In contrast, within aggregations of individuals, which are comprised as a panmictic population, all individuals can heterogeneously distribute over space. In addition, each individual can interbreed with any other member of the panmictic population. Dynamics within a panmictic population are regulated by emigration and immigration of individuals between different places.

Apart from these either loose or well-structured aggregations of populations, spatially

complex aggregations exist. Within the spatially complex population concept, several locally breeding subpopulations which might be genetically connected via dispersal are combined as one overall population, for example as a metapopulation (Levins, 1968) or a sink-source population (Pulliam, 1988). The dynamics of the local subpopulations within a spatially complex population are mainly driven by emigration and immigration, whereas the dynamics of the entire spatially complex population is driven by birth and death processes. At the larger spatial scale, the spatially complex population constitutes an independent biological unit (Camus and Lima, 2002).

1.3 Population structure in marine and terrestrial ecosystems

A common prerequisite of all three population concepts is the spatial structure. Therefore, it is essential to know the area occupied by the populations. When sampling spatially complex populations, the sampling area determines whether the whole population is collected rather than a local population or spatially segregated part of the population. Differences in the physical environments between marine and terrestrial ecosystems affect both ecological and evolutionary processes leading to varying spatial population structures and dynamics (Carr et al., 2003). The marine ecosystem has relatively few physical barriers, and areas are characterized by variations in abiotic and biotic factors (Turner, 1989). Many marine species have pelagic larvae, freely drifting in the water column, and possibly resulting in the population structure and dynamics being determined over a vast area. Without physical barriers, the gene flow of marine species usually tends to be higher than for most terrestrial species (Utter and Ryman, 1993; Waples, 1998). High levels of gene flow within a species increase genetic diversity. Consequently, genetic differentiation is typically not be detectable (Wright, 1965). Still, genetic differentiation has been found between populations in many marine species (Hauser and Carvalho, 2008; Ovenden et al., 2015) suggesting that marine populations do not always behave as panmictic populations. Further, marine populations have, in general, a high genetic diversity reflecting fundamental differences, compared to terrestrial populations, affecting the spatial structure of populations (Carr et al., 2003). The response time of terrestrial

ecosystems to physical changes leading to differences in spatial population structure is more substantial (over centuries) compared to marine ecosystems (over decades; Steele, 1991). Considering these principal differences between terrestrial and marine ecosystems, defining the area of occupancy of marine populations is even more challenging and might change over shorter time periods than in terrestrial species.

Spatial scaling is important when studying population structure (Wiens, 1989) because the term "local" can be used in different ways. In most marine and terrestrial studies, also within this thesis, "local" refers to a regional or geographical area encompassing a particular population (Fact box 1). If individuals are selected based on a regional scale, then the term "local" is biologically meaningless. An alternative practice is to use the "local" scale to describe an ecological unit (e.g., a "local" population) within functional boundaries (e.g., a metapopulation), referred to as subpopulations in this thesis (Fact box 1). In this case, individuals in a subpopulation respond to a particular environment and subpopulations have a high probability of extinction or recolonization because they are strongly influenced by emigration and immigration from other subpopulations (Hanski, 1998). However, in some cases, regional "local" populations can at the same time represent subpopulations within a metapopulation. The maintenance and diversity of local populations or subpopulations, in general, is an important target in conservation management (Smedbol and Stephenson, 2001; Baguette and Schtickzelle, 2003). Overexploitation, especially of marine fish, can lead to a destabilization of local population dynamics (Kerr et al., 2017).

1.4 Population discrimination and identification methods

Within marine fish species, the research field of population discrimination based on morphology, behavior, life history and genetic differentiation is continuously developing (Cadrin et al., 2014). The main characteristic defining the distinctness of populations is the independence of a population as a reproductive group. Therefore, rapidly developing genetic analyses should be the key elements to discriminate between populations. Where traditional genetic methods have previously failed to detect genetic differentia-

tion between populations, recent whole-genome resequencing might help in resolving the population structure of several species (Fuentes-Pardo and Ruzzante, 2017). In the last 50 years, genetic variation between populations has been examined by only using a handful of molecular markers (Allendorf, 2017). However, if genetic methods fail to discriminate between populations, other methods are required (Nielsen et al., 2004; Svedäng et al., 2010; Imsland et al., 2014). A variety of methods have been applied to distinguish populations based on phenotypic characteristics: (1) Meristic characters, like number of vertebrae or fin rays, which are fixed during the early development of fish and remain stable throughout life (Tåning, 1952; Swain et al., 2001; Reimchen and Cox, 2015), (2) morphometric differences (Cadrin, 2000; Turan, 2004), (3) otolith characteristics such as otolith microstructure (Barnett-Johnson et al., 2007; Brophy and King, 2007; Sponaugle, 2010), otolith shape (Begg and Brown, 2000; Bacha et al., 2014; Mahe et al., 2016) and otolith chemistry (Chang and Geffen, 2013; Tanner et al., 2016). All of these phenotypic traits expressed by fish reflect environmental conditions experienced during particular periods of their life history. The number of meristic characters, for example, is explicitly influenced by the environmental conditions during the early life history (from incubation until metamorphosis). On the other hand, otoliths are lifetime recorders and even changes experienced in the last days or month can be traced. This ability to display different phenotypes in response to environmental factors is known as phenotypic plasticity (Via et al., 1995). Therefore, differences in phenotypic characteristics can be considered as population-specific traits, in the absence of genetic differentiation, because their variation suggests that individuals of a population lived under specific environmental conditions. Further, migration studies of fish populations can support the assumptions of experiencing different environmental conditions and provide evidence of different spawning or feeding areas based on parasites (MacKenzie, 2002) or tagging/telemetry (Pine et al., 2003; Block et al., 2005).

1.5 Life history of Atlantic herring

Atlantic herring (*Clupea harengus*) is one of the most important fish species, both ecologically and commercially, in the northeastern Atlantic. The population structure of Atlantic herring has been a research topic since the end of the 19th century (Heincke, 1898; Hjort, 1914). However, more than a century later, it is still debated how herring populations are spatially and temporally structured (Mariani et al., 2005; Reiss et al., 2009; Martinez Barrio et al., 2016). Within this single species, evidence exists for all three population concepts from spatial discrete populations (Iles and Sinclair, 1982), though metapopulations (McQuinn, 1997a), to a panmictic population (Smith and Jamieson, 1986; Ciannelli et al., 2013). The biological and ecological diversity of Atlantic herring is large. Herring can, for example, inhabit ecosystems ranging from nearly freshwater to fully marine conditions or from small local lakes or fjords to the oceanic waters. Further, spawning periods of herring range from early spring to late autumn or even winter. This variety is, in theory, a good prerequisite when studying the population structure of herring, because genetic differentiation might arise based on isolation-by-distance (Wright, 1965) or environmental differences leading to phenotypic variation. Phenotypic differences of Atlantic herring have been well studied (see e.g., Rosenberg and Palmén, 1982; Brophy and Danilowicz, 2002; Libungan et al., 2015a) and recent studies also suggested genetic differentiation between populations (Lamichhaney et al., 2012; Pampoulie et al., 2015; Martinez Barrio et al., 2016). However, herring populations are often highly migratory and undertake long-distance migrations from a few 100 km to more than 1000 km (Slotte, 1999) between feeding, overwintering, and spawning areas. During these migrations, mixing (e.g., spatial and temporal overlap) of several populations is known in particular areas, such as the transition zone between the North Sea and the Skagerrak (Clausen et al., 2015), but the connectivity (see Cowen et al. (2007) for definition) between populations remains unclear. The management of Atlantic herring stocks in the northeastern Atlantic is an additional challenge. Instead of ”population”, fisheries managers use the term ”stock” which is defined as parts of a herring population (or several populations) with similar life history parameters occupying a defined geographical area

and being subject to a distinct fishery (Fact box 1; ICES, 2012). Three of the main herring stocks managed in the northeastern Atlantic are: Norwegian spring spawners (NSS), North Sea autumn spawners (NSAS) and western Baltic spring spawners (WBSS). All three stocks comprise of more than one population, but the spatial and temporal population structure within and/or between these stocks is not resolved as yet.

Further studies are necessary to investigate the population structure and dynamics of Atlantic herring. The Norwegian coastline with its numerous fjords and semi-enclosed coastal marine ecosystem offers a vast variety of study areas where the population structure of herring can be investigated. The relatively small area of fjords and semi-enclosed coastal marine ecosystem are well suited for undertaking studies on herring dynamics in well-defined natural ecosystems. In addition, small local areas might represent the entire area occupied by one or several populations. This is, to a large extent, possible in the case study area of the present thesis. To study the population structure and dynamics of Atlantic herring, the marginal habitats of Landvikvannet (Fact box 2) and its vicinity were selected as a case study. Three distinct herring populations occur in the case study area during the spawning season. Their overlap in space and time, as well as possible connectivity, were investigated and results from this case study might be applied on a broader scale being of relevance for the population structure of herring in the northeastern Atlantic.

Fact box 2: Landvikvannet and adjacent fjords

Landvikvannet (Figure 1) is a 1.85 km² brackish lake located on the Norwegian Skagerrak coast. This inland lake was artificially connected to the marine environment of the adjacent fjord (Strandfjorden) via a canal in 1877. The canal, called Reddal canal, is approximately 3 km long and quite narrow, 1-4 m deep. The canal was constructed to drain water from Landvikvannet and thereby increase the surrounding agricultural areas, as well as was to get logs down to the shipbuilding locations on the adjacent fjord. Further, the canal transformed Landvikvannet into a brackish water environment and lowered the water level in the lake by 3 m. A small 25 m deep basin is located at the entrance of the lake. Otherwise, the bottom depth is between 7-10 m. The shoreline is mostly rocky and steep or covered by reeds. There is an inflow of saltwater over the tidal cycle, whereas freshwater empties into the lake from streams, resulting in a stratified water column with a transition depth at 4 m. Typically, in May the upper layer has low salinity (<20), high temperature (>10 °C) and oxygen content above 5 ml/l. In contrast, the lower layer has high salinity, low and constant temperature (~8 °C) and no oxygen but toxic hydrogensulphide. Due to these environmental conditions, Landvikvannet resembles a miniature Baltic Sea system. Landvikvannet was colonized by a local sea trout (*Salmo trutta*) population shortly after the canal was opened. Nowadays, the dominant species is common rudd (*Scardinius erythrophthalmus*) which is an invasive species and was introduced in 2013 probably by fishermen using it as live-bait. Typical marine species caught in Landvikvannet are Atlantic herring (*Clupea harengus*) and European sprat (*Sprattus sprattus*).

In contrast, Strandfjorden is sheltered from the outer coast and has fully marine conditions. The outer part of the fjord is narrow and shallow (1-7 m), whereas the inner part is relatively deep (10-13 m). Between those two parts, fish must cross a shallow sill of only 1 m.

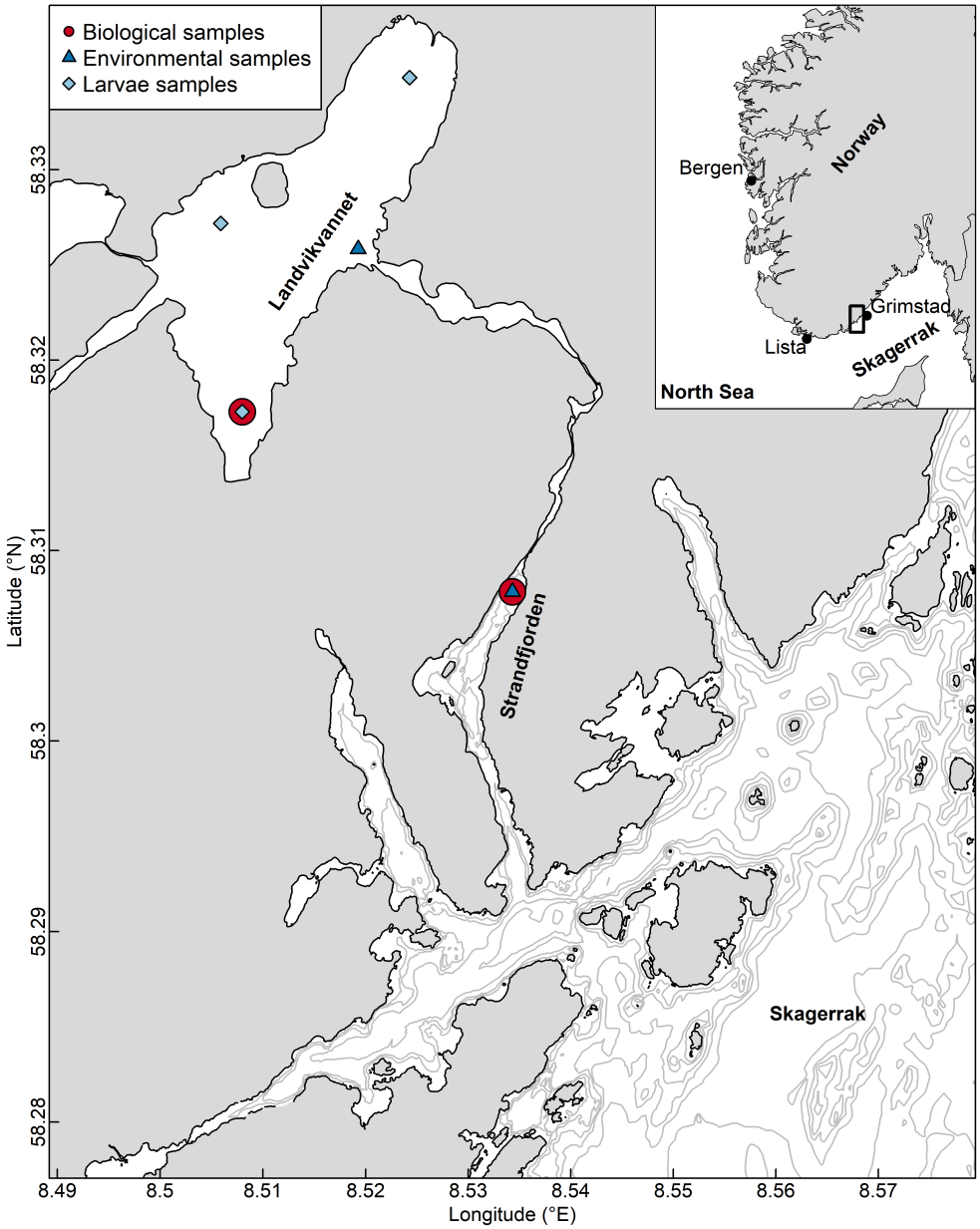

Figure 1: Map of the case study area, including Landvikvannet and adjacent fjords, indicating the sampling locations of biological (circles), environmental (triangles) and larvae data (diamonds).

2 OBJECTIVES

Given the need to improve our understandings of herring population structure and dynamics, the two primary objectives of this thesis were (1) to evaluate phenotypic and biological characteristics distinguishing the three herring populations in the case study area and their population structure, and (2) to validate whether the distinct characteristics can be used for population discrimination on a broader scale.

To achieve the first objective (**Paper I-III**) biological data collected in the case study area, mostly during the spawning season in 2012, were analyzed. Varying methods, previously demonstrating significant variation between other herring populations, were applied to explore phenotypic and behavioral differentiation between the three herring populations in Landvikvannet and its vicinity. Further, these results were investigated to clarify the population structure and possible connectivity. Analyzed phenotypic characteristics were the number of vertebrae (**Paper I**), growth (**Paper I**), otolith shape (**Paper I**), and otolith microstructure (**Paper III**). Behavioral traits like spawning time (**Paper I**) and migration patterns (**Paper II**) were also investigated.

Historical data from 1970-2015 (**Paper IV**), as well as data from common garden experiments (**Paper V**) were used to accomplish the second objective. Chiefly, the number of vertebrae (**Paper IV-V**), growth (**Paper IV**) and otolith shape (**Paper V**) data were analyzed to validate if the differences found between herring populations are population-specific traits (influenced by genetics) or the results of varying environmental conditions.

3 SUMMARY OF PAPERS

3.1 Discrimination characteristics of populations

Paper I

Seasonal dynamics of Atlantic herring (*Clupea harengus* L.) populations spawning in the vicinity of marginal habitats

F. Eggers, A. Slotte, L. A. Libungan, A. Johannessen, C. Kvamme, E. Moland, E. M. Olsen, and R. D. M. Nash

The putative herring populations, (1) Norwegian spring spawners, (2) coastal Skagerrak spring spawners, and (3) Landvik herring, were identified in Landvikvannet and adjacent fjords by differences in vertebral counts, otolith shape, and growth. These populations mix over the spawning season (February-June) in the case study area. Norwegian spring spawners and coastal Skagerrak spring spawners occurred mainly in the adjacent fjords and had a peak spawning in March-April. Landvik herring spawned later in the season, May-June, inside Landvikvannet. The occurrence and spawning of Landvik herring inside the lake could be explained by local adaptations to the environmental conditions and seasonal changes of this marginal habitat. Despite differences in peak spawning and utilization of different habitats between the three putative populations, there was an apparent temporal and spatial overlap of spawning herring suggesting potential interbreeding being in accordance with the metapopulation concept.

Paper II

Individual habitat transitions of Atlantic herring *Clupea harengus* in a human-modified coastal system

F. Eggers, E. M. Olsen, E. Moland, and A. Slotte

The migration pattern of herring is usually examined at population- or school-level, while less is known about individual movement characteristics and habitat transitions. The behavior of Atlantic herring was monitored in Landvikvannet and adjacent fjords with the use of acoustic tags and moored receivers over two subsequent spawning seasons. Approximately 10% of tagged herring entered Landvikvannet where they resided for up to five weeks. All herring left the monitored fjord area into the open ocean by early August. This habitat transition occurred in three main pulses, which were assumed to be formed by the three putative populations mixing in the case study area. Before leaving the monitoring system, herring migrated between different habitats (coast, fjord, lake). Most migration happened during night-time regardless of tidal cycle, and it is suggested that spawning is the primary driver for entering Landvikvannet and its vicinity. Later detections at a separate receiver system indicate that some herring might overwinter in coastal areas. Further, some herring returned to their original tagging location in the subsequent spawning season. There was no clear evidence for either natal or repeated homing to this specific area. This study reveals new aspects of the migration behavior of the three populations occurring in Landvikvannet and adjacent fjords and suggests that capacity for individual behaviors in schooling fish might be underestimated.

Paper III

Spawning time of Atlantic herring (*Clupea harengus*) populations within a restricted area reflects their otolith growth at the larval stage

F. Berg, Å. Husebø, J. A. Godiksen,it was A. Slotte, and A. Folkvord

Larval growth from the three putative populations was estimated by microstructure analysis of otoliths of four year classes of adult herring sampled over a full spawning season in Landvikvannet and adjacent fjords during the years 2012-2015. Landvik herring had significantly higher mean widths of daily increments compared with the two other populations. Based on spawning times of these populations, the differences were highly consistent with expected temperature-dependent larval growth. Also, daily otolith growth tended to decrease with increasing vertebral counts within the populations. This implies that timing of spawning is population-specific with a tendency of adult herring to spawn at the same time and under the same conditions as they hatched themselves. These results signify the importance of otolith growth history and number of vertebrae for studies on population discrimination and population structure in herring, even within the same spawning season.

3.2 Validation of population characteristics

Paper IV

Comparative biology and population mixing among local, coastal and offshore Atlantic herring (*Clupea harengus*) in the North Sea, Skagerrak, Kattegat and western Baltic

F. Berg, A. Slotte, A. Johannessen, C. Kvamme, L. A. W. Clausen, and R. D. M. Nash

Biological and environmental data from 1970-2015 were analyzed to study the complex population structure of Atlantic herring from 13 defined areas in the northeast Atlantic. Herring from the 13 areas varied in phenotypic characteristics such as mean vertebral counts, growth and maturity ogives. Temporal, as well as intra-annual, dynamics of mean vertebral counts were demonstrated, but the dynamics were not affected by environmental factors. The dynamics can be explained by variation in presence/absence of herring populations in specific areas. Based on temporal and spatial variation in phenotypic characteristics, Norwegian spring spawners, western Baltic spring spawners and North Sea autumn spawners, the three of the main stocks in the northeast Atlantic, were identified, as well as several local populations along the coast. Direct mixing of local populations with the main stocks could not be demonstrated. However, local populations are included in the management of the three stocks, without knowing the extent of mixing. Our results clearly validated the use of mean vertebral counts as a population-specific characteristic and further highlighted the importance of recognizing and understanding herring dynamics and mixing of populations as this is a challenge for the management of herring.

Paper V

Genetic factors have a major effect on growth, number of vertebrae and otolith shape in herring (*Clupea harengus*)

F. Berg, O. W. Almeland, J. Skadal, A. Slotte, L. Andersson, and A. Folkvord

To study the influence of genetic factors and salinity on phenotypic characteristics of Atlantic herring, ripe spring spawning herring were collected in fully marine (salinity 35, Atlantic Ocean) and brackish water (salinity 6, Baltic Sea) conditions. One Atlantic herring female was crossed with one Atlantic and one Baltic male generating an F1-generation consisting of Atlantic purebreds and Atlantic/Baltic hybrids which were incubated and later co-reared at two different salinities, 16 and 35, for three years until their first maturation. Mean vertebral counts were higher for purebreds than hybrids, consistent with higher counts in Atlantic parental herring, but there was no effect of salinity. Otolith shape analysis demonstrated significant differences between purebreds and hybrids, as well as between the salinities. Hybrids had a lower otolith aspect ratio than purebreds, being consistent with the aspect ratio of the parental groups. The variation in otolith shape between herring was analyzed by a Canonical Analysis of Principal Coordinates. Differences between purebreds and hybrids were clearly identified on the first discriminating axis and the minor effect of salinity on the second axis. These results demonstrate that otolith shape and vertebral counts have a strong genetic component and are therefore useful for studies on population dynamics and connectivity.

4 SYNTHESIS AND GENERAL DISCUSSION

This thesis confirms the notion of three Atlantic herring populations in Landvikvannet and adjacent fjords as results of several population identification methods (**Paper I-III**). The three identified populations are local Landvik herring (Fact box 3), coastal Skagerrak spring spawners (CSS), and Norwegian spring spawners (NSS). Depending on the identification methods and the interpretation of their results, the structure of these three populations can be described by two out of the three introduced population concepts. Applying analyses on one of the major divergent characteristics between the three populations, mean vertebral counts (VS), on a broader scale, identified several herring populations in the northeast Atlantic and revealed evident mixing and dynamics between them (**Paper IV**). The uncertainty whether these distinct biological characteristics are population-specific traits (influenced by genetics) or based on the environmental conditions is partly resolved for vertebral counts and otolith shape in this thesis (**Paper V**).

4.1 Population structure of herring in Landvikvannet and adjacent fjords

Within this thesis, a variety of methods has been used to identify different herring populations in Landvikvannet and adjacent fjords. Prior to the actual discrimination analyses in **Paper I & III**, herring were pre-selected based on criteria potentially identifying different populations. Firstly, individual NSS were identified subjectively based on otolith growth characteristics (**Paper I**) and treated as a single population. Secondly, herring were grouped based on differences in geographical sampling origin because individual identification of CSS and Landvik herring was not possible. Therefore, individuals sampled inside Landvikvannet were all classified as Landvik herring, whereas those sampled in the adjacent fjords were classified as CSS. This pre-selection is supported by the following results of all identification methods, used within this thesis, demonstrating clear differences between these three populations.

The major divergent characteristic between the three populations is the number of vertebrae (**Paper I**). Early spawning NSS experiencing coldest temperatures after hatching

Fact box 3: The life history and biology of Landvik herring

Landvik herring belong to a local population that can be found during the spawning season from March-June in Landvikvannet. Landvik herring are characterized, for example, by low mean vertebral counts (55.7), smaller length-at-age, or higher larval otolith growth compared to other herring in adjacent areas. When the ambient water temperature in Landvikvannet is approximately 9°C, Landvik herring start to migrate through the canal into the lake. Most Landvik herring are in spawning condition with a relative high gonadosomatic index (GSI). Individual Landvik herring resided more than five weeks in the lake before all of them leave the system in July-August. Immature Landvik herring (2-year-olds or younger) have never been found inside the lake. The juvenile nursery and adult feeding grounds of Landvik herring remain unclear, except that they are not inside the lake. Also, the actual ecological role of Landvik herring in the lake is ambiguous. Even though most herring are in spawning condition, no direct evidence of spawning exists. During several diving surveys along the coastline, no spawned eggs were found. However, newly hatched larvae (younger <24h) occur inside Landvikvannet at the beginning of July (Figure 2). Further, preliminary genetic results indicate that Landvik herring are similar to other herring originating from brackish water conditions (pers. comm. Leif Andersson).

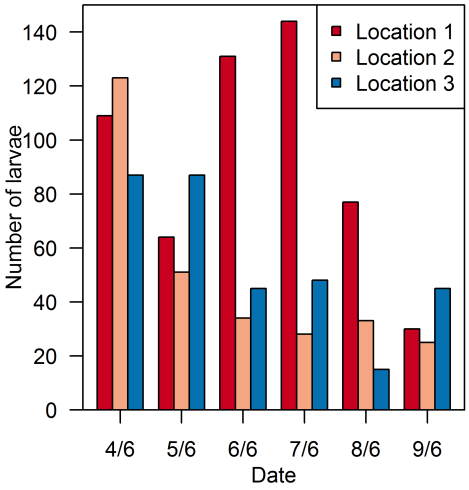

Figure 2: Total number of herring larvae sampled on six subsequent days at three different locations within Landvikvannet during the spawning season 2015 (see Figure 1). Numbers of larvae were standardized to a sampling time of 15 minutes, towing speed and gear were identical during sampling.

had the highest VS (>57.5), followed by CSS (~56.6) spawning and hatching approximately a month later. Lowest VS (~55.7) were found in Landvik herring spawning last

of the three populations. A similar relationship exists between spawning time and larval otolith growth of these populations (**Paper III**). The later herring spawn in the spring, the higher their larval otolith growth. In contrast to these two life-history traits, which are defined and fixed during the early development of fish, the otolith shape is changing throughout the entire lifetime. Still, clear differentiation between the otolith shape of the three populations could be found reflecting differences in their life history, e.g., the timing of feeding and spawning migrations or the generally different locations of feeding or overwintering areas. Such behavioral differences were observed between the three populations based on acoustic telemetry (**Paper II**). Without any pre-selection, herring were tagged with acoustic transmitters, and their migration behavior within Landvikvannet and adjacent fjords were recorded. Based on the residence time within the case study area, three types of behavior were identified and could be linked to the populations of NSS, CSS, and Landvik herring. Now the remaining question is how the three populations in this case study are structured.

There are several indications that NSS, CSS and Landvik herring in spawning condition mix and to some extent interbreed, which is a prerequisite for the existence of a metapopulation (McQuinn, 1997a). First of all, NSS were caught at the same location and time with running gonads (close to spawning) together with either CSS or Landvik herring (**Paper I**), and individuals of all three populations were tagged with acoustic transmitters at the same location and time (**Paper II**). Secondly, the seasonal dynamics of biological characteristics (**Paper I**) for CSS and Landvik herring indicate that these two populations are, to some degree, mixing both in Landvikvannet and adjacent fjords. Thirdly, the larval otolith growth of individual CSS and Landvik herring of the same year class are overlapping (**Paper III**), indicating that those herring are grown up under similar environmental conditions. Fourthly, if these three populations represent discrete populations, an individual assignment based on the otolith shape should be feasible (**Paper V**); however, this is not documented in my thesis. Further, it seems that VS of wild populations is not directly linked to temperature or salinity (**Paper IV**) in contrast to what has been observed in laboratory studies (Tåning, 1952; Pavlov and Shadrin, 1998) and that VS is

clearly affected by genetic factors (**Paper V**). Likewise, the heredity of vertebrae is well known for different marine species with a complex population structure (Christiansen et al., 1988; Løken and Pedersen, 1996). Thus, the historical increase in VS over the last 30 years in Landvikvannet (Eggers, 2013) might be a result of interbreeding CSS and Landvik herring. Therefore, the existence of a metapopulation comprising three subpopulations (NSS, CSS and Landvik herring) is most likely in Landvikvannet and adjacent fjords (Figure 3).

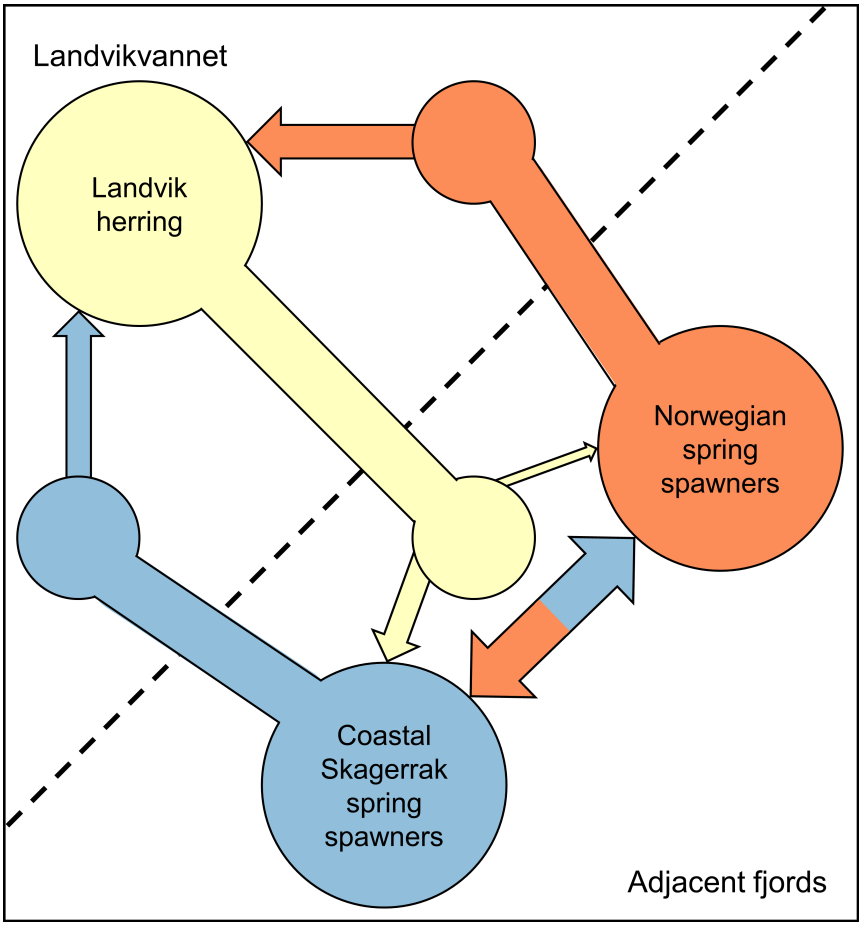

Figure 3: A schematic model of subpopulation dynamics which are comprised of metapopulation in the study area including Landvikvannet and adjacent fjords. The size of the circles indicates the where the majority of herring from each subpopulation occurs. Potential connectivity between the subpopulations are demonstrated by arrows; the size represents the prospective amount of gene flow between subpopulations.

Even though a metapopulation would automatically reject the discrete population concept, the results of this thesis could potentially also be interpreted that NSS, CSS and Landvik herring are three sympatric discrete populations (Iles and Sinclair, 1982). Differences in peak spawning times (**Paper I**) might lead to different phenotypic characteristics and distinct populations (McPherson et al., 2001). Different behavior and migration patterns of the three populations (**Paper II**) support different spawning times because herring leave their spawning grounds shortly after spawning (Stephenson, 1999). Consequently, these three populations would have grown up under differential environmental conditions resulting in phenotypic differences (**Paper I & III**). Also, genetic differentiation is demonstrated, at least, for NSS and Landvik herring (Pampoulie et al., 2015). Spawning time and location of herring are affected by genetic factors (Martinez Barrio et al., 2016) supporting that NSS, CSS and Landvik herring might represent discrete populations. Even though no direct evidence for natal homing being essential for the discrete population concept (Iles and Sinclair, 1982) exist in this thesis **Paper II**, there are some indications that fidelity of adult individuals to time and conditions of spawning resemble their own situation at the larval stage (**Paper III**). Further, the tendency of homing herring is supported by other studies (Brophy and Danilowicz, 2002; Husebø et al., 2005).

In contrast, a panmictic population is maintained by the heterogeneity of environmental conditions and by the connectivity and behavioral interactions between individuals (Cianelli et al., 2013). The apparent biological differences between the three populations are either determined by varying environments (**Paper III**) or genetic differentiation (**Paper V**). Further, behavioral differences between the populations exist (**Paper II**). Consequently, a single panmictic population model can be excluded.

4.2 Consequences of a metapopulation for Norwegian spring spawners

In case of a metapopulation, the study area might need to be redefined to cover the “true” metapopulation (Berryman, 2002), since CSS can be found along the entire Norwegian Skagerrak coast and their spawning grounds are not explicitly the adjacent fjords of

Landvikvannet (**Paper II & IV**). Studies on the otolith shape of herring sampled at different locations along the Norwegian Skagerrak coast also indicated that these herring could be grouped (Libungan et al., 2015b). CSS have a relatively small distribution area, in contrast to NSS. Usually, NSS are distributed in the Norwegian Sea during their feeding migrations, overwintering in the fjords and the coastal regions of northern Norway and spawn along the Norwegian west coast (Dragesund et al., 1997; Varpe et al., 2005; Huse et al., 2010). Depending on the fluctuations in population size, NSS also change their distribution area and migration routes (Dragesund et al., 1997).

However, within the thesis, NSS were recognized along the Skagerrak coast of Norway (east of Lista, Figure 1) in 2012 for the first time (**Paper I**). Historical data sampled along the Skagerrak coast of Norway also indicate that NSS might have occurred earlier in that area (**Paper IV**). So far, it was assumed that NSS spawn only along the west coast of Norway from Lofoten (69° N) in the north to Lista (57.5° N) in the south of Norway (Slotte, 1999; Røttingen and Slotte, 2001). Further, no clear evidence for the migration of NSS to the east of Lista and into the Skagerrak area has been published. In spite of the length-dependent spawning migration of NSS (Devold, 1963; Slotte, 1999; Slotte and Fiksen, 2000), NSS in Landvikvannet and adjacent fjords are most likely first time spawners (**Paper I**). Thus, they have probably not conducted the general migration triangle (Harden Jones, 1968) implying overwintering and nursery areas in northern Norway. The majority of NSS in the case study area belongs to the 2009-10 year classes, produced by a high abundance of old and large NSS spawning along the southern west coast of Norway (Slotte et al., 2009; Directorate of Fisheries, 2013). Within a metapopulation, the recruitment of a strong year class may disperse over several local populations (McQuinn, 1997a). Instead of drifting northwards to the common nursery areas (Fossum and Moksness, 1993; Holst et al., 2004), these year classes might have allocated Lysefjorden as their primary nursery area (**Paper IV**). Nonetheless, NSS caught in Landvikvannet and adjacent fjords are more migratory than the other two populations (**Paper I**; Silva et al., 2013), and they might join other NSS on their common migration routes after spawning.

Besides the metapopulation in the case study area, NSS are comprised of another metapopulation occurring in Lindåspollene at the Norwegian west coast (Johannessen et al., 2009, 2014). In Balsfjord in northern Norway, NSS and another local spring spawning population are mixing with very limited gene flow (Jørstad and Pedersen, 1986). However, even limited gene flow is sufficient to form a metapopulation. Along the west coast of Norway, several local populations have been identified, and potential interbreeding with NSS cannot be excluded (**Paper IV**; Runnstrøm, 1941; Aasen, 1952, 1953). Consequently, the metapopulation structure including NSS is far more complex than observed in the case study area, and further studies are necessary to investigate the interbreeding and connectivity of NSS with other local populations, as well as the connectivity between NSS spawning along the west and east coast of Norway.

4.3 Benefits and relevance of a metapopulation structure

A better understanding of population dynamic processes of spatially structured species can be achieved through the metapopulation concept (Bailey et al., 1999; Smedbol and Wroblewski, 2002). Failures of correct population identifications and the following loss of population diversity can lead to a delayed recovery of collapsed populations (Stephenson, 1999; Smedbol and Stephenson, 2001). Discrete populations can potentially be depleted by local exploitation, and the consequent loss of genetic diversity will not lead to recolonization from other populations (Heath et al., 2008). The subpopulation dynamics of metapopulations, on the other hand, are strongly dependent on local demographic processes and influenced by replenishment between subpopulations (Hanski, 1998; Kritzer and Sale, 2004). For the maintenance of genetic diversity within a metapopulation, a closure for the fishery of local areas inhabiting subpopulations might be effective (Wright et al., 2006). The application of fishery closures is advocated to prevent the disruption of spawning activity and the loss of biodiversity regarding extinct subpopulations (Hu and Wroblewski, 2009; Zemeckis et al., 2014). Connectivity between subpopulations is expected to support recolonization by expanding the spawning migration of other subpopulations (Stephenson, 1999). Further, metapopulation dyna-

mics improve the likelihood of recolonization within shorter timescales, whereas discrete population dynamics imply long-term replenishment after depletion (Rose et al., 2010). This would explain the rapid colonization of Landvikvannet and establishment of a new subpopulation in less than 150 years.

4.4 Advantages of Landvikvannet and its vicinity

From an ecological perspective, Landvikvannet and its vicinity is a highly interesting case study area. The unique composition of two completely different environmental habitats allows herring populations to develop local adaptations to a given environment (Conover, 1998; Hutchings et al., 2007; Jensen et al., 2008). In contrast to other local areas inhabiting herring populations, Landvikvannet is a relatively young habitat. This demonstrates how efficient and fast evolution (<150 years) can provoke the establishment of new well-adapted populations (Neb, 1970; Nævdal, 1972).

Case studies aim to transfer and apply their small-scale results on a broader aspect. A suitable area for the application of used identification methods and their results within this case study could be the transition zone between the North Sea and Baltic Sea, where several herring populations mix during their annual migrations (**Paper IV**; Ruzzante et al., 2006; Clausen et al., 2015). These populations are comprised and managed as three main stocks (see Fact box 1) and the assignment of individual herring to a given stock is essential for the assessment and management of fish stocks (ICES, 2016). This application can be justified by two aspects, (1) the environmental conditions and (2) the similarities of biological characteristics between the populations identified in the case study area and the stocks managed in the transition zone.

The environmental conditions of Landvikvannet and adjacent fjords provide an application to large-scale habitats such as the transition zone between the North Sea and Baltic Sea. The brackish water system Landvikvannet with an anoxic layer resembles a miniature Baltic Sea system, whereas the adjacent fjords resemble marine environments like the neighboring North Sea. It can be expected that herring growing up in habitats with

similar environmental conditions develop comparable biological characteristics as a consequence of phenotypic plasticity (Via et al., 1995). The plasticity of herring (Geffen, 2009) increases the potential for local adaptations to a specific habitat or environment (Kawecki and Ebert, 2004; Ghalambor et al., 2007) favoring the colonization of these habitats such as Landvikvannet.

Besides the environmental similarities, all three populations identified within the case study area have counterparts in the transition zone and can be compared with one of the three stocks managed and occurring in this area. Most apparent is the linkage of NSS identified in the case study area and the corresponding stock of NSS mostly distributed along the west coast of Norway and in the Norwegian Sea. Except for the spawning time, the biological characteristics defining CSS are quite similar to those of the stock called North Sea autumn spawners (NSAS; **Paper IV**). The environmental conditions, regarding temperature and salinity, during spawning of CSS and NSAS are comparable, resulting in similar phenotypic characters between CSS and NSAS. Year class twinning could be an explanation for different spawning times (McQuinn, 1997b), but not for differences in biological characteristics. Currently, CSS are, however, included in the management of another stock, the western Baltic spring spawners (WBSS). The majority of WBSS belong to a population spawning around the island of Rügen in the western Baltic, conducting annual feeding migrations in the Skagerrak (Biester, 1979; Aro, 1989) whose biological characteristics are almost identical with those of Landvik herring (**Paper I & IV**). Both populations, Landvik and Rügen, spawn in late spring (warm temperatures) in low saline waters which might explain the low number of vertebrae and similar growth patterns. This similarity between the populations and stocks can help to establish reliable methods to identify and assign individual herring to their actual stock/population.

On the other hand, all herring leave the case study area after spawning, and the consequences are uncertain. The populations start their migration, probably to their feeding grounds, at different times (**Paper II**). Thus, the questions asked are where the herring migrate to, if they mix again or not and if so, with whom. During this time, these

herring populations are potentially exposed to exploitation by a mixed fishery, and the smaller subpopulations need to be protected from over-exploitation to maintain diversity (Schindler et al., 2010). Migration routes and patterns are learned by first-time spawners as they join repeat spawners (McQuinn, 1997a; Huse et al., 2002). This learning of behavioral traits is essential in maintaining population-specific characteristics (Petitgas et al., 2010). If the three populations conduct individual migrations, this would increase the population integrity and reinforce their biological differences, whereas mixing provides a higher potential for connectivity (Stephenson et al., 2009). High abundances of inexperienced fish, however, might change migration patterns and specific overwintering areas or even spawning grounds (Huse et al., 2010), which might explain that first-time spawning NSS occurred in Landvikvannet and adjacent fjords. Similar to the westward orientated feeding migration based on the predictive mechanism of NSS (Fernö et al., 1998), a southward orientated spawning migration could have led first-time spawning NSS from their nursery areas in southern Norway into the case study area. Based on their reproductive investment it can also be assumed that NSS have longer migration routes than CSS or Landvik herring (**Paper I**).

4.5 Validation of population characteristics

4.5.1 Factors influencing phenotypic characteristics

So far, the population discriminations within this thesis are solely based on differences in phenotypic traits. Still, the mechanisms driving differences in phenotypes and the relative importance of genetic and environmental factors on the determination of those phenotypic discrepancies are generally unclear (Swain and Foote, 1999; Mitchell-Olds et al., 2007; Barrett and Hoekstra, 2011). Many studies have ascribed changes in phenotypes to environmental changes without questioning whether they are a result of phenotypic plasticity or inherited from previous generations (Merilä and Hendry, 2014). Further, salinity conditions not only influence phenotypes but are also a clear genetically structuring factor in population integrity (Nielsen et al., 2004; Bekkevold et al., 2007;

Martinez Barrio et al., 2016). To answer the question of whether the environment, genetic differentiation or a combination of both is the driving factor for varying phenotypic traits, common garden experiments, as conducted in **Paper V**, are necessary (Swain and Foote, 1999). One disadvantage of common garden experiments is that the number of investigated factors is limited. However, common garden experiments can play an essential role in resolving population structures (Hutchings et al., 2007) because they are ideally suited to dissect the relative importance of environmental and genetic factors causing phenotypic differences (de Villemereuil et al., 2016).

Although all phenotypic traits investigated within this thesis are influenced to a certain extent by environmental factors (Table 2), the common garden experiment demonstrates a clear influence of genetic factors on the development of some phenotypic traits (**Paper V**). However, it remains unclear to what extent the genetic or other environmental factors influence the phenotypic traits and if one might dominate the other. Despite the high impact on phenotypic characteristics of the well-studied environmental factor temperature, in some cases it has no effect (Hüssy, 2008) or the genotype still has a higher impact (Løken and Pedersen, 1996; Hutchings et al., 2007; Jensen et al., 2008). Unfortunately, the current experimental design of the common garden experiment in **Paper V** did not allow an investigation of the temperature impact. Even though the effect of salinity on phenotypic traits, investigated in **Paper V** (otolith shape and VS), was minor compared to the genetic effect, those differences are relevant for population structure analysis. In the absence of genetic differences between subpopulations, otolith shapes are used to discriminate them (DeVries et al., 2002). These results indicate that fish on different spawning grounds having divergent phenotypic characters are not randomly mixing even if they are genetically indistinguishable due to low levels of gene flow between them. Further, individuals of a population occupying different environments during their life history (e.g., various feeding grounds or nursery areas) might be identified based on their phenotypes. For NSS, phenotypic traits, especially the otolith shape, might be useful to distinguish between individuals utilizing the fjords along the coast as nursery areas and those drifted as larvae into the Barents Sea (Holst and Slotte, 1998;

Skagseth et al., 2015). Knowing the proportion of herring having different nursery areas could provide new insight into the population recruitment of herring. Similar analyses on otolith shape can be used to discriminate between Norwegian coastal cod and their counterpart the Northeast Arctic cod (Stransky et al., 2008). Despite the huge variety of phenotypes, they are not as informative as direct genetic data because a lack of phenotypic differences does not prove a lack of genetic differentiation. On the other hand, it is, however, challenging to detect genetic factors affecting phenotypic traits when no differences in phenotypes exist (Barrett and Hoekstra, 2011).

Table 2: Overview of factors determining the development of phenotypic traits investigated within this thesis.

Phenotypic trait	**Determining factors**	**References**
Number of vertebrae	Temperature	Reimchen and Cox (2015)
	Salinity	Tåning (1952)
	Genetics	**Paper V**
Growth	Temperature	Morrongiello and Thresher (2015)
	Salinity	Bœuf and Payan (2001)
	Food	Werner and Blaxter (1980)
	Genetics	**Paper V**
Otolith microstructure	Temperature	Folkvord et al. (2004); **Paper III**
	Photoperiod	Mugiya (1987)
	Prey density	Johannessen et al. (2000)
Otolith shape	Temperature	Begg et al. (2001); Cardinale et al. (2004)
	Genetics	Söllner et al. (2003); **Paper V**

4.5.2 Using phenotypic differences on a broader scale

An enormous benefit of common garden experiments is the exact knowledge of individual origins. In contrast, the origin of wild samples can only be assumed, and it is ambiguous if all individuals belong to the same population. As demonstrated in **Paper**

IV and other studies (Swain and Foote, 1999), it is possible to identify wild populations based on phenotypic characteristics. Further, their dynamics regarding emigration and immigration between populations could be demonstrated. Geographical local and stationary populations often display high phenotypic variation compared to oceanic and migratory populations. As long as an individual population assignment of herring is not realizable, despite the general rapid development of genetic analysis (Fuentes-Pardo and Ruzzante, 2017), phenotypic identification methods are still a suitable and accurate alternative. Nevertheless, a clear separation of populations when mixing occurs is necessary for fish management and assessment to allow more sustainable exploitation of the populations (Schindler et al., 2010).

In particular otolith shape analyses have a great and promising potential to provide an assignment of individuals based on the clear genetic component affecting the otolith shape (**Paper V**). Previous studies have successfully separated mixed samples into individual populations (Brophy et al., 2015; Hüssy et al., 2016). These studies mostly assigned individuals only into two distinct populations. Other phenotypic traits can also enable separation into two components, for example, when individual herring are identified as autumn or spring spawners based on their otolith microstructure (Clausen et al., 2007). This method is currently used to discriminate between the stocks of NSAS and WBSS, but the specific populations comprised of these stocks are neglected. As soon as the populations have the same spawning period, even though the timing is differentiated, an individual assignment is ambitious, is not impossible (**Paper III**). Even the number of vertebrae can be used as a sole method to calculate the proportions of two different populations from a mixed sample (Gröger and Gröhsler, 2001), but an individual assignment is impossible. Furthermore, this method fails when more than two populations are involved. Hence, a combination of several phenotypic traits might be needed for an individual assignment on the population level. In the case study area, for instance, NSS can be individually identified and separated from other populations based on their otolith appearance (**Paper I**). Further, the methods described by Gröger and Gröhsler (2001) could help to estimate the extent of mixing between CSS and Landvik herring during

the spawning season resulting in the seasonal dynamics (**Paper I**). Known identification methods and new technological opportunities due to increasing computing power allow for analyses of large data sets, for example, generated when transforming the otolith shape into wavelet coefficients. For fisheries managers, an unambiguous identification and separation into stock would already be sufficient. However, the criteria defining a stock might become invalid when population assignments are feasible.

4.6 Management implication

Most herring populations are migratory and aggregations on feeding or overwintering grounds likely consist of mixtures of individuals from several populations. Therefore, the definition of a stock limited to a geographical area, and mostly considering discrete populations (Fact box 1), is not a straight-forward assumption. Further, each of the three main stocks mentioned in this thesis (Norwegian spring spawners (NSS), North Sea autumn spawners (NSAS) and western Baltic spring spawners (WBSS)) are comprised of more than one population and several spawning grounds. The mixing and potential connectivity between populations within a stock, or even between stocks, especially within a metapopulation, is the most challenging part of the assessment and management of marine fisheries which aim to maintain biodiversity by protecting local and stationary populations from overexploitation (Stephenson, 1999; Bierman et al., 2010). Developing precision of identification methods leads to an increasing knowledge of the genetic and phenotypic diversity of many populations in the distribution areas of NSS, NSAS and WBSS (Ruzzante et al., 2006; Bekkevold et al., 2011; Pampoulie et al., 2015). Although local populations are in theory included in the management of the three stocks, in general, they are neglected by fisheries managers since the extent of mixing remains unclear. Further, fisheries managers should aim to maintain the biocomplexity of herring by sustaining the diverse life-history traits and geographical spawning grounds of local populations (Hilborn et al., 2003).

In cases of a metapopulation, the fisheries managers should incorporate all subpopulations and not only the largest one. In general, persistence and stability of dynamics within

a metapopulation are affected by connectivity through straying and entrainment (Secor et al., 2009). To provide a successful management of a metapopulation, extensive assessment of population size and demography, connectivity, genetics and estimation of mortality is required (Jones, 2006). Disproportional fishing effort and overexploitation of local populations when aggregating with other populations must be prevented. Furthermore, lumping of subpopulations comprised of a metapopulation for management purposes will have long-term effects underestimating the risk of a collapse and overestimating the probability of recovery (Kell et al., 2009). The possible management implications following such considerations will be further discussed based on the findings in this thesis.

Besides the impacts of a metapopulation structure on the current concept of a stock, most considerations should emphasize the occurrence of NSS migrating east of Lista (**Paper I**). The abundance of NSS occurring outside of their traditional management area and their harvesting rates in other areas are unknown and have so far been neglected by fisheries managers. Based on historical data, it can be assumed that the proportion of NSS along the east coast has increased in the last 15 years (**Paper IV**). Such a discrepancy between the spatial mismatch of management (stock) and biological (population) units can bias stock assessment and impede sustainable fisheries (Kerr et al., 2017). Unrecognized NSS in Skagerrak catches might even affect the assessment of WBSS to a lesser or greater extent.

In theory, geographical locations inhabited by subpopulations will always be recolonized by straying from other subpopulations after extinction or overfishing (Hanski, 1998). That is, a recovery of local populations can be expected, but the strategies for spatial management of a metapopulation do not necessarily have intuitive outcomes (Heath et al., 2008). However, preserving the diversity and adaptions of local populations can be necessary for a metapopulation regarding maintaining its potential to sustain variable environmental conditions (Hilborn et al., 2003). For a successful maintenance of a metapopulation, fisheries managers should incorporate strategies to stabilize dynamics and migration between subpopulations (Secor et al., 2009). On the other hand, it is not ne-

cessarily that local populations would be overfished and become extinct. In cases when the dominating subpopulation is close to collapsing or already collapsed, other local and stationary subpopulations can act as a buffer and thus contribute to the recovery by new recruitment and straying of individuals. Therefore, the findings of this thesis (**Paper I & IV**) and other studies (e.g., Johannessen et al., 2009) demonstrating that NSS form a metapopulation with local and stationary populations along the Norwegian coast might explain the recovery of NSS after the collapse in the 1970s. More effort should thus be made to preserve the biocomplexity of metapopulations, for example, by closing fjords for the commercial fishery. Further, the natural stability and resilience of subpopulations should be maintained by responsive management in connection with continuous monitoring through applications of population identification methods (Kerr et al., 2017). Nevertheless, false assumptions of connectivity between discrete populations could lead to overexploitation, whereas assuming discreteness when actually connectivity within a metapopulation occurs would have no consequences on the population structure (Stephenson, 1999).

5 FUTURE PERSPECTIVES

More research effort is needed to understand the spatial and temporal distribution of herring thoroughly even though this thesis provides novel insight into the population structure and dynamics of Atlantic herring, as well as potential population identification methods. In particular, the connectivity between populations needs to be investigated to develop sound fisheries management. The attempt to identify and separate populations in this thesis was based on phenotypic traits and behavioral differences and not by using genetic markers. Basically, it can be expected that genetic methods should resolve the population structure of herring at the beginning of the 21st century but despite the rapid development, from microsatellite DNA analyses (Mariani et al., 2005; Bekkevold et al., 2007) over single nucleotide polymorphism (SNP) analyses (Lamichhaney et al., 2012; Limborg et al., 2012) to whole-genome sequencing (Martinez Barrio et al., 2016; Lami-

chhaney et al., 2017), identification at individual levels is not yet possible. Altogether, further refinements of genetic analyses are necessary, and in combination with phenotypic traits, we might be able to identify and separate individual fish from a mixed herring fishery in the near future .

Still, there is a lack of knowledge on how genetic and/or environmental factors influence the development of phenotypic characteristics. A first approach with a common garden experiment was conducted in **Paper V**, but more experiments are necessary to investigate the influence of other factors, like temperature and food availability. To apply the results of common garden experiments on the population levels, more parental crosses should be considered. In addition to its effect on the phenotypic response, genetic analyses can also be conducted to examine the expression of specific genes in varying environments. With the experimental design applied in **Paper V**, the mutation rate of Atlantic herring could be estimated (Feng et al., 2017). In an ongoing experiment under common garden conditions, we rear herring in two different light regimes (spring and autumn) to investigate the impact of light on the maturation development. Since the spawning time of herring is affected by genetic factors (Lamichhaney et al., 2017), results from the current experiment might shed light on how and which other factors affect switching spawning times in herring. This might influence the management of the Norwegian spring spawning stock, which is also comprised of a smaller population of Norwegian autumn spawners. However, further common garden experiments are definitely needed to understand phenotypic plasticity, as well as to develop new tools and methods for identifying and assigning individuals to a population.

Although the mean vertebral counts (VS) are an essential character defining a herring populations, VS are not optimal for individual assignments. A changing VS could either indicate a temporal and spatial aggregation of two populations or previous interbreeding of two populations resulting in an intermediate VS (**Paper V**). In contrast, individual assignments based on otolith shape yielded in relatively high classification success (**Paper V**; Brophy et al., 2015; Libungan et al., 2015a). It seems that the otolith shape is both population-specific and to a large extent affected by genetic factors. This combination

might allow separating individual herring from mixed catches into at least corresponding stocks. An assignment down to the population level would be even better to understand the influence of smaller populations in the stock and to estimate their abundance compared to those most abundant one. An advantage for individual assignments based on otolith shape is the development of learning machines in the last recent years (Kotsiantis, 2007; Van Bocxlaer and Schultheiß, 2010). If fisheries managers and scientists can agree on a standard protocol, an extensive reference database could be established on samples from spawning grounds. This reference database can further be used to train the learning machine which later automatically assigns new otoliths to one of the populations or stocks included in the database. Preliminary results indicate that this method might be feasible to identify and assign individual herring when the background information is sufficient. In the initial phase of establishing such a database, complementary information, such as VS, might be necessary.

Besides the more general aspects of future population structure studies, the investigations in Landvikvannet and adjacent fjords should continue to resolve several unanswered questions. First of all, detailed genetic analyses on the three populations in the case study area should be conducted. Preliminary genetic analysis demonstrated no significant differences between the three populations. However, it is questionable how reliable these results are, since only a small number of SNPs were analyzed. Pampoulie et al. (2015) indicated that Landvik herring and other local populations in Norway are genetically different from NSS. However, it is not necessary the case that NSS caught in the study area are genetically indistinguishable from NSS found in other regions. Further, detailed analyses might provide new insights of the origin of the Landvik herring. First implementations of Landvik herring in a phylogenetic tree demonstrated that they are more closely related to other North Sea, Skagerrak and Kattegat populations than populations in the western or central Baltic Sea (pers. comm. Leif Andersson). Also, there are indications that Landvik herring are hatched in intermediate salinity conditions (pers. comm. Leif Andersson) based on genetic analyses of the fish hatching enzyme which can be linked to ambient salinity conditions during incubation and hatching (Martinez Barrio et al.,

2016). This is contrary to the fact that, despite tremendous effort, no spawned eggs could be found inside Landvikvannet. The hypothesis that newly hatched larvae are initially hatched in Strandfjorden and transported by tidal currents into Landvikvannet shortly after hatching needs to be evaluated. Therefore, crossing experiments were conducted under common garden conditions, and eggs were fertilized at salinities of either 16 or 35. After hatching, half of the offspring were transferred in the opposite salinity. This will simulate two different scenarios, where (1) larvae incubated and reared in the same salinity (either 16 or 35) should have different gene expressions per se, and (2) the gene expression of larvae incubated and reared in opposite salinities should be linked to those of the 1st scenario. Further, larvae of the 2nd scenario will indicate whether the salinity during the incubation and hatching solely determines the gene expression or changes in salinity conditions after hatching influence the gene expression. Genetic analyses investigating the hatching enzyme of these larvae might reject or support the hypothesis that larvae originally hatch in adjacent fjords drifted into Landvikvannet. If the results will support the hypothesis of drifted larvae, this would also explain why the preliminary genetic analyses could not find any genetic differences between the three populations.

Besides genetic analyses, other results need further investigations to understand the life history of herring populations in the case study area. The decreasing otolith growth from 3 to 4-year-olds in Landvik herring and to a minor extent also in coastal Skagerrak spawners (CSS) should be of particular interest (**Paper III**). Currently, almost nothing is known about the early life history of Landvik herring until their return as first-time spawners at the age of 3 years. In general, higher larval growth should be beneficial for fish as this decreases the natural mortality rate and results in earlier maturity (Stearns and Koella, 1986). However, a main question asked is why 3-year-old herring with high otolith growth disappear and do not return the next spawning season. Such a potential trade-off between early maturity and spawning once vs. maturity at higher age but repeated spawning should be further investigated. This investigation might also help to track Landvik herring after they leave the case study area. In summary, several aspects need to be investigated considering and potentially resolving the population structure

and dynamics of herring in Landvikvannet and its vicinity.

6 CONCLUSION

How are herring populations structured and which phenotypic characteristics can be used to discriminate and assign individuals to a population? With regards to the case study area, the answer, in short, is: Herring populations in Landvikvannet and its vicinity are structured as a metapopulation with gene flow between the individual subpopulations. However, an individual assignment of herring to a population is not yet feasible with any of the investigated phenotypic characteristics. Further, the present thesis contributes to the understanding of the population dynamics and structure of herring in the northeast Atlantic and their implications on the management of the herring stocks assessed in this region. Besides these general results, this thesis includes unique applications of methods and provides novel results that need to be highlighted. To my best knowledge, this thesis is the first (1) demonstrating individual habitat transitions of Atlantic herring by the use of acoustic telemetry, (2) showing consistent differences in daily otolith growth in continuous year classes and over several years between herring populations overlapping in spawning season, and (3) rearing hybrids of two herring populations under common garden conditions until maturity. In particular, the results of the common garden experiment, revealing that some phenotypic traits are primarily determined by genetic factors, provide novel information that can be further used to distinguish genetically differentiated populations and to study their dynamics and connectivity on a broader scale. However, the population structure of Atlantic herring is not fully resolved, and definitely, more research is needed to find methods that can assign individual herring, either phenotypically or genetically, to a population to ensure a sustainable management.

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8 PAPER

Paper I

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PLOS ONE

Seasonal Dynamics of Atlantic Herring (*Clupea harengus* L.) Populations Spawning in the Vicinity of Marginal Habitats

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Abstract

Gillnet sampling and analyses of otolith shape, vertebral count and growth indicated the presence of three putative Atlantic herring (*Clupea harengus* L.) populations mixing together over the spawning season February–June inside and outside an inland brackish water lake (Landvikvannet) in southern Norway. Peak spawning of oceanic Norwegian spring spawners and coastal Skagerrak spring spawners occurred in March–April with small proportions of spawners entering the lake. In comparison, spawning of Landvik herring peaked in May–June with high proportions found inside the lake, which could be explained by local adaptations to the environmental conditions and seasonal changes of this marginal habitat. The 1.85 km^2 lake was characterized by oxygen depletion occurring between 2.5 and 5 m depth between March and June. This was followed by changes in salinity from 1–7‰ in the 0–1 m surface layer to levels of 20–25‰ deeper than 10 m. In comparison, outside the 3 km long narrow channel connecting the lake with the neighboring fjord, no anoxic conditions were found. Here salinity in the surface layer increased over the season from 10 to 25‰, whereas deeper than 5 m it was stable at around 35‰. Temperature at 0–5 m depth increased significantly over the season in both habitats, from 7 to 14°C outside and 5 to 17°C inside the lake. Despite differences in peak spawning and utilization of the lake habitat between the three putative populations, there was an apparent temporal and spatial overlap in spawning stages suggesting potential interbreeding in accordance with the metapopulation concept.

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Introduction

Typically, fish species may be split into populations based on their degree of reproductive isolation from each other in space and/or time, which could be reflected in genetic or phenotypic differences driven by diverging environmental conditions [1–3]. Under such circumstances exploitation on one population should have little effect on the population dynamics of a neighboring population, and therefore it is also common to assess and manage such populations separately [4,5]. On the other hand, there are also examples where populations are recognized to be separate with diverging spawning season and/or spawning area, but due to mixing in other seasons a separate management of the populations may be difficult [6,7]. The need to identify the different populations, especially where exploitation occurs on mixtures of populations is important for successful management [8,9]. Fisheries biologists therefore often use the term stock instead of population in their fisheries advice; i.e. sometimes a population is harvested and therefore managed as one stock and at other times several separate populations are harvested and managed as one stock. In Begg et al. [10] the concept of a fish stock was simply defined as characteristics of semi-discrete groups of fish with some definable attributes, which are of interest to fishery managers. The definition of ICES [11] for a stock as a part of a fish population usually with a particular migration pattern, specific spawning grounds, and subject to a distinct fishery, will be used hereby. In theory, all individual fish in an area, being part of the same reproductive process, are comprised as a stock. When referring to fisheries management, the term "stock" is used, otherwise the term "population" is preferred.

Atlantic herring (*Clupea harengus* L.) is characterized by highly complex population structure and migration patterns [12]. It is an iteroparous clupeid, becoming sexually mature at two or three years of age, and a total spawner that aggregates at spawning, laying benthic eggs on shells, gravel, coarse sand and small stones at depths down to 250 m [13]. The larvae hatch after 2–4 weeks depending on temperature [14,15]. They drift with currents until metamorphosis [16–18], with vertical migration increasing throughout ontogeny [19,20] and affecting the dispersal trajectories of larvae. The different herring populations are generally

classified according to their spawning grounds, which, due to the specific spawning substratum requirements, are fixed geographically and used at a predictable time of the year. Due to physical and geographical barriers, such as prevailing currents and general location of nursery areas, there is often little mixing of larvae, thus tending to isolate the different populations. However, there are occasions where larvae and juveniles may co-occur. Under these circumstances identification of individuals or groups of individuals is undertaken using otolith or meristic characters [1,21–24] as well as genetic markers [25–28]. In the 1950–60s experimental studies [29–31] demonstrated that myotome counts in herring were influenced by both temperature (negatively) and salinity (positively) experienced during the incubation period. The consequence is that mean vertebral count of adult herring is an indicator of spawning ground and spawning times and in some cases also population.

In Norwegian waters some herring populations occupy marginal habitats along the coastline and deep inside fjords, most of which are thought to be stationary with adaptations to local conditions. Hence, they are often phenotypically and, in some occasions, genotypically different from the nearby oceanic population. Examples of such local herring populations are Trondheimsfjord herring [32,33], Borge Poll herring [34], Lusterfjord herring [35], Lindåspollene herring [36], Balsfjord herring [37], Lake Rossfjord herring [38] and the summer/autumn spawners in northern Norway [39]. Despite the discovery of these local populations, the overall research effort targeting marginal areas along the Norwegian coast has been rather low, and it is therefore expected that a number of additional local populations may exist.

Migratory coastal or oceanic populations may occasionally enter the marginal habitats along the Norwegian coast and mix with local herring. This is in accordance with the metapopulation concept, where two or more distinguished subpopulations have variable but moderate interbreeding and significant gene flow [40]. Temporal and spatial overlap during spawning may allow genetic exchange between subpopulations, which is a prerequisite for the existence of metapopulations. An example of such an overlap was demonstrated by Johannessen et al. [41],[42] in the local Lindåspollene herring, where significant changes in life history traits over a 50 year period were linked to genetic exchange with the oceanic population according to the metapopulation concept.

An important mixing area for herring is the northeastern North Sea and Skagerrak, where three different stocks may occur, Norwegian Spring Spawners (NSS), North Sea Autumn Spawners (NSAS) and Western Baltic Spring Spawners (WBSS). Some of these stocks comprise different herring populations, such as coastal Skagerrak spring spawners or more local herring populations, which are not directly subjected to a distinct fishery. The different populations (stocks) can be distinguished by spawning site, spawning season, meristic characters such as the number of vertebrae (VS) and otolith characteristics [23,41].

Of particular interest in the Skagerrak area is a brackish water environment inside Landvikvannet, an inland lake in southern Norway connected to the open sea through an artificial channel. The Institute of Marine Research (IMR) has been sampling herring in Landvikvannet on regular basis since 1984, mainly in May. Data from these investigations demonstrate that herring inside the lake are normally ripe or with running gonads, with a low mean vertebral number (<56.0), slow growth and high fecundity [43,44]. This has led to the hypothesis that the lake is visited on an annual basis by a herring population with specific adaptations to spawning in these brackish water environments. However, in the coastal areas outside the lake, ripe and spawning herring with higher growth and mean vertebral numbers (56.0–57.5) have occurred in samples over the period February–June [43]. This indicates that there may be a mixture of several populations in the area with some temporal and spatial overlap in spawning, which could be linked to spatial seasonal differences in environmental conditions. Such metapopulation dynamics may be revealed by a more detailed seasonal sampling outside the May period normally focused on in IMR's investigations in Landvikvannet. Hence, the principal objective of the present study was to explore the overlap in time, space and maturation stages of phenotypically different herring appearing in Landvikvannet and neighboring fjord areas and their dependence on seasonal changes in environmental conditions.

Material and Methods

Study area

Landvikvannet is a 1.85 km^2 lake located on the Norwegian Skagerrak coast (Figure 1). In 1877 a 3 km long channel (Reddal channel, Figure 1) was constructed, connecting the lake to the open sea. This narrow 1–4 m deep channel transformed Landvikvannet into a brackish system and in addition lowered the water level in the lake by 3 m. At the entrance of the lake there is a small 25 m deep basin. Further into the lake the bottom depth decreases rapidly to 7–10 m. Most of the shoreline is covered by reeds; otherwise the shore is rocky and steep. There is inflow of saltwater over the tidal cycle, whereas freshwater empties into the lake from streams, resulting in a halocline. Oxygen is depleted in the lower layers whereas the surface layer is oxygen rich. In Landvikvannet, herring have been caught by floating gillnets together with trout (*Salmo trutta*) and other freshwater fish since shortly after the channel was opened.

The Reddal channel drains into Strandfjorden (Figure 1), where conditions are estuarine. The outer Strandfjorden is narrow and shallow (1–7 m), whereas the inner part is deeper (10–13 m). Most herring samples were collected in the inner part, close to the mouth of the Reddal channel. The shore is rocky and steep with sparse macroalgae in the upper few meters. At depths >5–6 m the bottom consists of sand and mud. The outermost fjord (Bufjorden, Figure 1) is small with direct connection to Skagerrak. Strandfjorden is connected to the open ocean via Bufjorden (Figure 1). The entrance of Bufjorden is characterized by a 54 m deep basin. The physical environment is similar to Strandfjorden, only less influenced by fresh water runoff. Access to Bufjorden is from the south or east.

Environmental data

To explore whether potential differences in habitat utilization and timing of peak spawning among herring populations were dependent on seasonal changes in environmental conditions, sampling of environmental data was undertaken between March and June 2012 both inside and outside the lake habitat. Note, that no stations could be sampled in February due to ice cover. Water samples were collected at the site where gillnets were moored in the inner part of Strandfjorden and at the entrance of Landvikvannet in the first basin (Figure 1). We measured temperature and salinity at depth with a CTD (STD/CTD – model SD204, SAIV Ltd. Environmental sensors and Systems, Bergen, Norway), while oxygen and hydrogensulfide concentrations were analyzed in the laboratory at the Institute of Marine Research (IMR). In the lake, water samples were collected each 0.5 meter down to the depth of oxygen depletion (hypoxic depth), which was found using the Winkler test [45], thereafter water samples were taken at 5 m

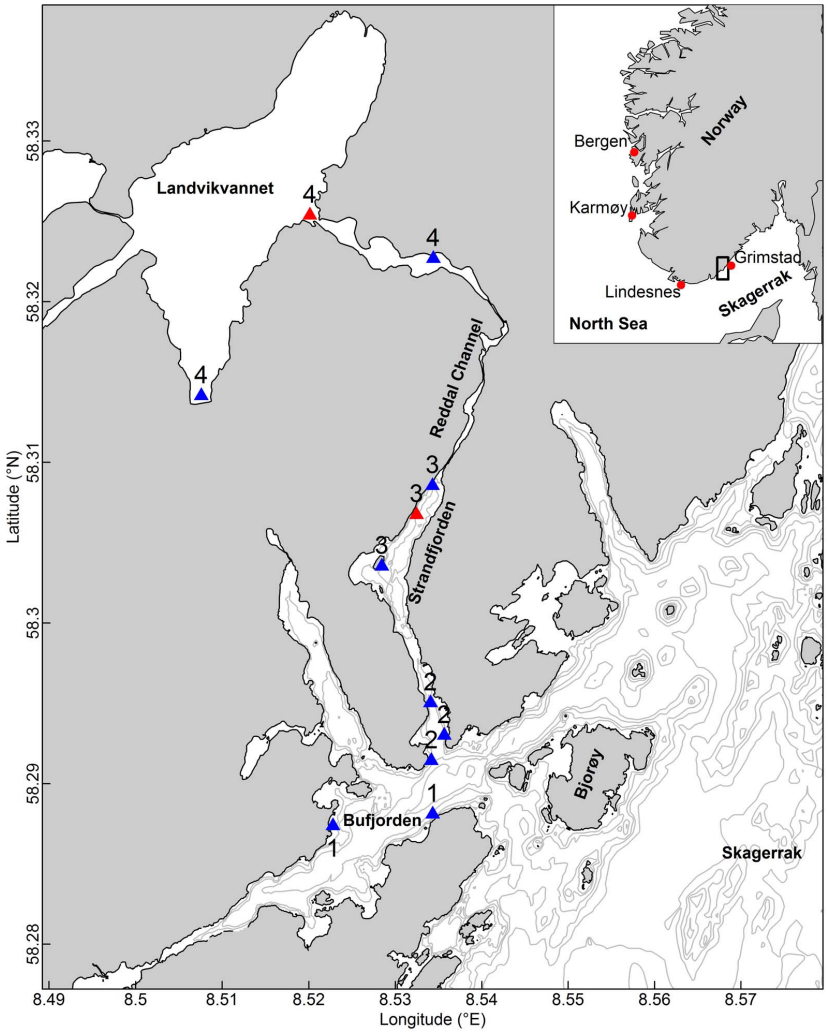

Figure 1. Map of the study area. The map shows CTD-stations (red) and gillnet stations (blue) in 1 = Bufjorden, 2 = Outer part of Strandfjorden, 3 = Inner part of Strandfjorden, 4 = Landvikvannet.
doi:10.1371/journal.pone.0111985.g001

depth intervals. The choice of position for sampling environmental data inside the lake is based on the depth contours of the area. The lake itself is rather shallow, and the bottom depth at most gillnet stations is 2–4 m. However, at the entrance the lake is at its deepest (25 m), which is why this position has been used since investigations started in the area in the 1980s. The environmental conditions at this site between 0 and 10 m have been examined thoroughly over a number of years and are comparable to conditions elsewhere in the lake and as such can be used to characterize the whole lake. These data are therefore representative of all gill net sampling sites.

Biological data

To explore the potential overlap in time, space and maturation stages of phenotypically different herring appearing inside and outside the lake habitat, herring were sampled with gillnet over the full spawning season in 2012 (February–June) concurrently in both habitats (Figure 1, Table 1). In February, due to ice cover both in the lake and inner fjord habitats of Strandfjorden, samples were only taken further out in Bufjorden. The floating gillnets with a mesh size of 26 mm and 29 mm, a depth of 8 m and a length of approximately 10 m were used randomly in all areas. Soak time was 24 hours. This experiment was approved by the Norwegian committee for the use of animals in scientific experiments (FDU). Special permission to fish with floating gillnet inside

Landvikvannet and in the connected fjord system in 2012 was given by the County Governor of Aust-Agder, Department of Climate and Environment, Ragnvald Blakstadsv. 1, Postbox 788 Stoa, 4809 Arendal, Norway. The permission was given to the Institute of Marine Research under the prerequisite that details on the catch were reported when the investigations were finished. The report was delivered to the authorities according to the plan. Our study did not involve endangered or protected species.

Biological samples were analyzed according to IMR standard protocols [46]. The maximum sample size was 100 herring. Biological parameters included in the present study were total length (nearest 0.5 cm below), weight (nearest gram below), sex, stage of maturity, age (otolith readings) and vertebral count (VS). Maturity stages were determined by visual inspection of gonads according to the following scale: immature = 1–2, maturing = 3–4, ripe = 5, spawning/running = 6, spent = 7 and recovering = 8 [46].

Image and shape analyses

Individuals of NSS herring were identified from otoliths, based on a sharper distinction between winter and summer rings compared to local spring spawners (Figure 2). This distinction was also independently tested using image and shape analyses of the otoliths. The rest of the individuals were divided into two populations based on sampling location: local Landvikvannet herring (LV) sampled inside Landvikvannet and coastal Skagerrak spring spawners (CSS) sampled outside Landvikvannet (Table 2). We expected that LV herring would mainly consist of individuals with similar biological characteristics as normally found in May, whereas the CSS herring would mainly consist of spring spawners with characteristics normally found along the Skagerrak coast during February–June. However, some mixture of the two populations would be expected, and this would be evident from results of the biological analyses. To investigate changes in the mixture of NSS, CSS and LV herring in the two habitats, selected biological characters (otolith shape, vertebral count, growth and maturation stage) were analyzed over the full season. The numbers analyzed by month and population are given in Table 2.

Otolith shape was analyzed using the programming language R [47]. Outlines of otoliths were collected from digital images using the package pixmap [48], and applying the conte function [49] to record a matrix of X and Y coordinates (Figure 2a). Mean shape of otoliths differed among the populations, where the modifications in the shape of otoliths mainly were found at the excisura major and antirostrum areas (Figure 2b).

To remove size-induced bias, otolith sizes were standardized to equal area by dividing the coordinates of each otolith with the square root of the otolith area. Equally spaced radiis were drawn from the otolith centroid to the otolith outline, using the regular radius function [49]. Independent Wavelet shape coefficients were obtained by conducting a Discrete Wavelet transform on the

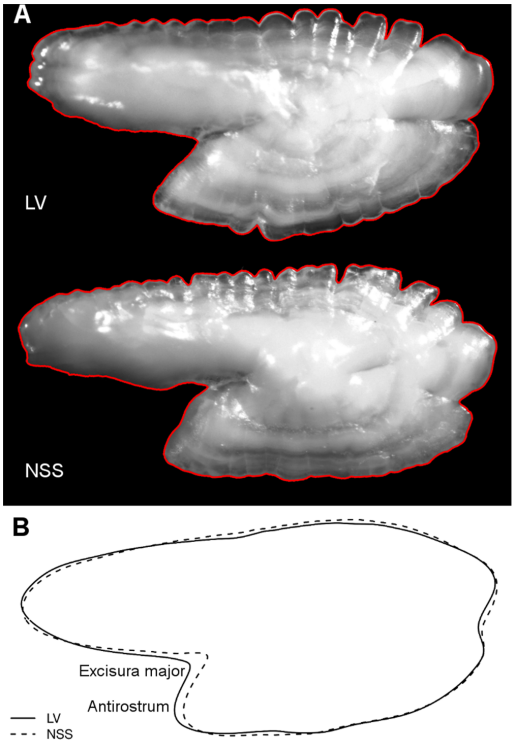

Figure 2. Example of otolith characteristics from two herring populations. A) Example of otoliths used for the shape analysis from Landvikvannet herring (LV) and Norwegian spring-spawning herring (NSS), both at the age of 3 years. Individuals of NSS herring were subjectively identified based on a sharper distinction between winter (dark areas) and summer rings (white areas). Red outline marks the shape of the otolith which was used to compare among populations. B) shows the mean shape of otoliths for the two populations, where the excisura major and antirostrum areas are the most variable areas.
doi:10.1371/journal.pone.0111985.g002

Table 1. Total number of herring caught in the local area for 2012, in brackets number of gillnets; ice = no sampling possible because the area was covered by ice.

Date	Landvikvannet	Inner Strandfjorden	Outer Strandfjorden	Bufjorden
15/2	Ice cover	Ice cover	28 (1)	11 (1)
6/3	4 (3)	129 (1)	119 (1)	
20/3	47 (3)	542 (1)		
26/3	115 (3)	486 (1)		100 (1)
11/4	290 (2)	663 (1)		
14/5	177 (1)	69 (1)		
21/6	82 (1)	66 (1)		
Total	**715**	**1955**	**147**	**111**

doi:10.1371/journal.pone.0111985.t001

Table 2. Total number of herring analyzed in 2012 by month for the three putative herring populations, Norwegian spring spawners (NSS), Coastal Skagerrak spring spawners (CSS) and Landvik herring (LV), in brackets number of NSS inside Landvikvannet.

Month	NSS	CSS	LV
2	7 (0)	32	0
3	108 (38)	440	113
4	32 (14)	68	86
5	8 (5)	61	95
6	0 (0)	66	77
Total	**155 (57)**	**667**	**371**

doi:10.1371/journal.pone.0111985.t002

equally spaced radiuses using the wavethresh package [50]. To determine the number of Wavelet coefficients needed for the analysis, the deviation of the reconstructed Wavelet otolith outline from the original outline was evaluated. To correct for fish length, an ANCOVA was performed on the wavelet coefficients taking fish length as a covariate. Coefficients which could not be adjusted by linear relationships on fish length, due to interaction between the origin and length were excluded from the analysis [51–53]. To adjust the Wavelet coefficients for allometric growth, a normalization technique based on regression was applied to scale the Wavelet coefficients [54].

Data analyses

The number of gillnets varied between Landvikvannet and the neighboring fjord area. Therefore, to estimate the proportions of the LV, CSS and NSS herring, the total catches landed were standardized by catch per unit effort (CPUE), i.e. catch per gillnet.

All statistical analyses were conducted in R (version 3.0.1; [47]). A significance level of $\alpha = 0.05$ was used for all statistical tests. For the plots, mean and standard error (1 SE) are shown. Some samples had very few or no data, and samples with $N<5$ were excluded.

Analysis of Covariance (ANCOVA) was used to test for sex differences in the biological characters (length, age, VS and stage of maturity). Differences in VS among different herring populations were assessed using Analysis of Variance (ANOVA), and a Kruskal-Wallis test for length and age variables as these were not normally distributed. For pairwise comparisons of VS a paired T-test was used, and the Mann-Whitney test for length and age comparisons.

Length-at-age data, used as a proxy for growth of individual herring, were fitted to the von Bertalanffy growth model (VBGM) [55]:

$$L_t = L_\infty(1 - e^{-K(t-t_0)})$$

where L_t is the average length at age t, L_∞ is the asymptotic maximum length, K is the von Bertalanffy growth rate coefficient, i.e. the rate at which length approaches the maximum length asymptote and t_0 is the intercept on the time axis. Growth was compared between the different groups using ANOVA.

Variation in otolith shape, as reflected by the scaled Wavelet coefficients, was analyzed with Canonical Analysis of Principal coordinates (CAP) [56] using the capscale function in the vegan package in R [57]. Using multivariate data to represent otolith shape, an ANOVA like permutation test (vegan package) was used to assess the significance of constraints using 5000 permutations. Variation in otolith shape was analyzed with CAP, while length and VS were compared with ANOVA with respect to herring group: NSS, LV and CSS, the month in which they were caught over the sampling period (Feb–June) and age in years (3–12) using the following models: shape~herring population*month*age, length~herring population*month*age and VS~herring population*month*age. Non-significant interaction terms ($p>0.05$) were excluded from the models. *P*-values for all posteriori comparisons were corrected with the Bonferroni correction [58]. Possible trends of length and VS within herring populations were tested for significance using linear regression, while the stage of maturity was tested with the Spearman's rank correlation coefficient. For the comparisons of environmental data at time of spawning with the VS of herring, measurements from 3 m were used for Landvikvannet due to the depth of oxygen depletion in combination with previous (2010) acoustic observations of school depth [43]. In Strandfjorden, measurements from 5 m were used, based on acoustic observations of herring school depth during tagging experiments and the gillnet sampling [43].

Results

Environmental conditions

The environmental conditions differed considerably between Landvikvannet and the neighboring fjord, and changed over the spawning season in both locations (Figure 3). Anoxic conditions were found in Landvikvannet at increasing depths from 2.5 m in March to 5 m in June. Salinity ILV at 0–1 m increased over the season from 1‰ in March to 7‰ in June, but was stable around 20–25‰ deeper than 10 m. In comparison, there were no anoxic conditions in Strandfjorden, the salinity at 0–1 m increased from 10‰ in March to 25‰ in June and was stable at 35‰ deeper than 5 m. The temperature at 0–5 m depth increased from March to June from 5 to 17°C in Landvikvannet, and from 7 to 14°C in Strandfjorden.

Population structure

A total of 1260 herring were analyzed during the 2012 spawning season. Total length ranged from 22.0–34.5 cm (mean: 28.3 cm) and age from 2–12 years (mean: 4.2 years). None of the biological characters varied between sexes ($p>0.05$). Hence, all further analyzes were carried out with sexes combined.

Mean length, age and vertebral count (VS) differed significantly among the three herring populations ($p<0.001$, Figure 4). For age and length, pairwise comparisons were also significant ($p<0.001$), with the exception of CSS versus LV for age ($p>0.05$). The vertebral count differed significantly ($p<0.001$) for all pairwise comparisons. The main tendency was a significant increase in

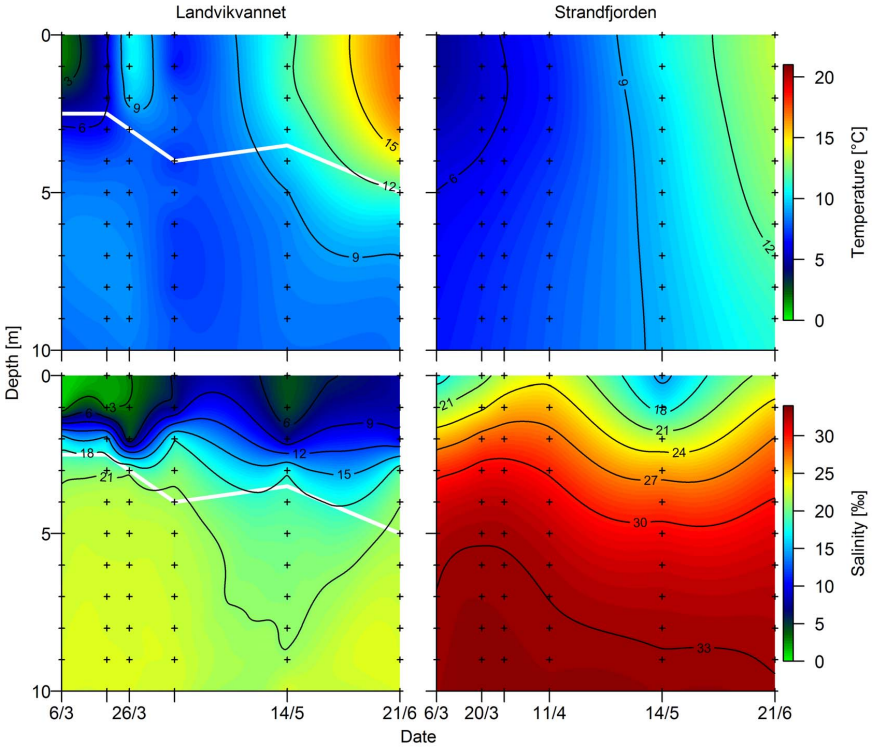

Figure 3. Seasonal change in temperature and salinity by depth. Temperature (upper) and salinity (lower) in Landvikvannet and in Strandfjorden over the study period from March to June. White line indicates the depth of oxygen depletion.
doi:10.1371/journal.pone.0111985.g003

mean body length and VS when moving from LV to CSS to NSS, whereas men age decreased. The most common age was 3 years for NSS, CSS and LV herring. The 4 year olds were also abundant in CSS and LV herring, but hardly present among NSS herring.

Length-at-age data indicated the highest growth for NSS herring, and lowest for LV herring ($p<0.01$) (Figure 5). The von Bertalanffy growth model supported these growth differences (Table 3). Consequently, there were three categories: 'high growth rate' (NSS herring), 'moderate growth rate' (CSS herring) and 'low growth rate' (LV herring).

Between February and June there was a change in the abundance of the different populations (Figure 6). During February–April CPUE was highest for CSS and NSS herring with a low proportion of LV herring (<20%). Also the proportion of NSS herring entering Landvikvannet was insignificant (<10%). The proportion of spawning and spent herring during this period was highest in NSS herring and a little lower for CSS herring, but still indicating peak spawning of two different populations in the fjord habitat during this period. Among the LV herring analyzed in March–April an even lower proportion were in spawning and spent stages than for CSS herring, indicating a later spawning peak for LV herring. This was further demonstrated in the May–June sampling showing a spatial shift in CPUE towards higher abundance of LV than CSS and NSS herring.

Otolith shape differed among the three herring populations ($p<0.001$, Table 4, Figure 7) and also varied though the spawning season ($p<0.001$, Figure 8A). Vertebral count and length differed between the populations ($p<0.001$) and between months ($p<0.001$, Figure 8B, C). Age was a significant factor for all characters ($p<0.001$) and therefore incorporated in the model for all comparisons. Posteriori comparisons showed that LV and CSS differed in otolith shape, VS and length ($p<0.04$, Figure 8, Table 4). NSS and LV ($p<0.001$) as well as NSS and CSS ($p<0.02$) also differed, while no differences were detected for NSS caught inside or outside the lake ($p>0.05$). There was a signifiant ($p<0.001$) negative trend in the mean Canonical scores (CAN1) derrived from the CAP analysis of otolith shape, vertebral count and length for LV and CSS herring at standardized ages over the spawning season, but not for NSS (Figure 8). This indicates that LV herring, characterized by slow growth and low vertebral count, were arriving and mixing with CSS herring.

Maturation and spawning time

Herring in spawning condition were present and overlapped in time for LV, CSS and NSS herring, however, maturation and timing of spawning was delayed in LV compared to NSS and CSS herring (Figure 6). This indicates an adaptation to the environmental conditions and seasonal change in Landvikvannet. Since differences in vertebral count are linked to environmental conditions, the temperature and salinity at depth and time of

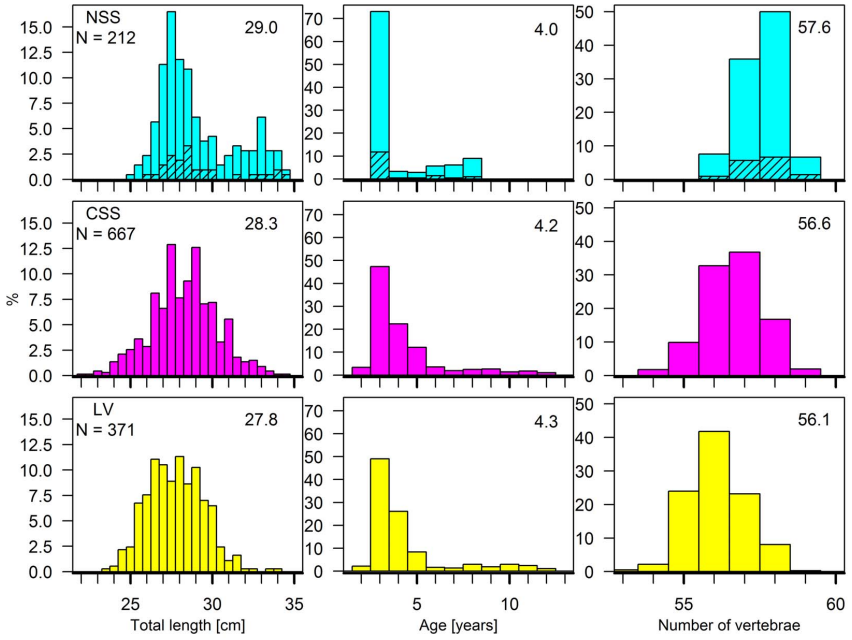

Figure 4. Distribution of length, age and vertebral counts of different herring populations. Comparison between Norwegian spring spawning (NSS), Coastal Skagerrak spring spawning (CSS) and Landvik (LV) herring. Shaded areas are NSS herring inside Landvikvannet. The mean values are included.
doi:10.1371/journal.pone.0111985.g004

spawning affects the vertebral count. The salinity at expected spawning depth in Landvikvannet was distinctly lower (10–15‰) than in the adjacent fjord (>30‰), which could explain the low vertebral count observed in Landvikvannet. The vertebral count was not significantly related to change in salinity over season within habitats; there was negligible change at assumed spawning depth. However, there were significant changes in temperature over season in both habitats, coinciding with a significant decrease in vertebral count at spawning time for both CSS and LV herring ($p<0.05$).

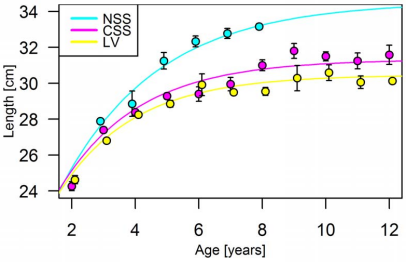

Figure 5. Growth curves of different herring populations. Length-at-age for Norwegian spring spawning (NSS, N = 212), Coastal Skagerrak spring spawning (CSS, N = 667) and Landvik (LV, N = 371) herring in samples pooled over the 2012 spawning season. Means and standard error (1 SE) are given, lines show van Bertalanffy growth models fitted to data.
doi:10.1371/journal.pone.0111985.g005

Discussion

This study reveals strong seasonal dynamics involving three populations of a pelagic migratory fish, the Atlantic herring, in the vicinity of a marginal inland brackish water lake habitat (Landvikvannet) on the Norwegian Skagerrak coast. Gillnet sampling was standardized, implying that the observed differences between herring populations and over season dynamics were not affected by the selectivity normally experienced with gillnet sampling [59]. Three putative herring populations were identified; Norwegian spring spawners (NSS), Landvik herring (LV) and Coastal Skagerrak spring spawners (CSS). Individual NSS herring were identified subjectively based on otolith growth characteristics, and statistically based on otolith shape and mean vertebral count (57.5). NSS herring also had higher growth than the other populations, which is typical for this stock [13,43]. Identification of individual CSS and Landvik herring was not possible. Individuals sampled inside the lake were all classified as LV herring, whereas those sampled outside the channel connecting the lake to the sea were assigned as CSS herring. However, there was a significant decrease in vertebral count over the sampling season in both LV and CSS herring, from levels known as typical for CSS herring (56.5–56.9) in March–April to levels typical for Landvik herring (<56.0) in May–June, again based on historic data [43]. This trend in vertebral count was followed by a decrease in size and change in otolith shape, and a marked change in the relative proportions of the two populations.

The observed seasonal dynamics in biological characters clearly indicate that the assignment of individual fish into CSS and LV herring simply based on sampling location was uncertain, and that

Table 3. Von Bertalanffy growth parameters (L_∞, k, and t_0) of herring populations Norwegian spring spawners (NSS), Coastal Skagerrak spring spawners (CSS) and Landvik herring (LV).

	L_∞	K	t_0
NSS	34.51	0.33	−1.98
CSS	31.31	0.41	−1.98
LV	30.33	0.43	−1.98

doi:10.1371/journal.pone.0111985.t003

the two populations were mixing both inside and outside the lake habitat together with NSS herring showing a different peak occurrence. Early in the season in February–April the biological characteristics indicated that NSS and CSS herring predominated, with only small numbers entering the lake. There was a clear temporal and spatial overlap in spawning individuals from these two populations, although proportions spawning in CSS were comparatively lower than in NSS herring. In May–June there was a significant change with the appearance of a new spawning wave of LV herring, with the highest proportion found inside the lake. Still, the immigration of this population was evident throughout both habitats, where many of the herring found in the fjord would be expected to enter the lake. The data on otolith shape, vertebral count and growth in May tended to differ from the observations in June in both locations, which indicated a spatial and temporal overlap in May between minor proportions of NSS and CSS herring completing their spawning season at the same time as the LV herring was peaking.

All three putative populations were caught at the same location, in the same gillnets, at the same time with running gonads, suggesting that the populations together form a metapopulation [40]. However, there is doubt as to whether interbreeding between distinct populations is occurring despite their proximity in spawning condition. Since breeding was not observed directly,

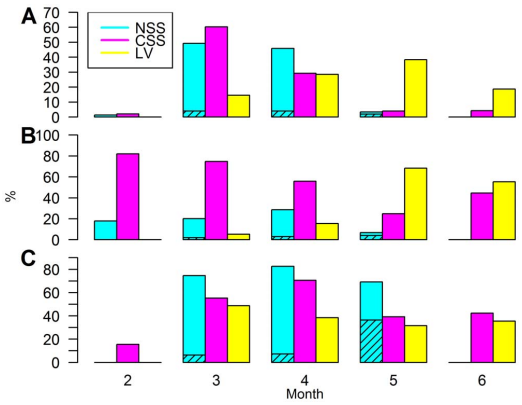

Figure 6. Seasonal change in proportion of different herring populations. Proportion (%), standardized to one gillnet per sample and area, by month of Norwegian spring spawning (NSS), Coastal Skagerrak spring spawning (CSS) and Landvik (LV) herring relative to a) total number analyzed over entire study period (see Table 1 for N), b) total number at month and c) spawning and spent herring (stage of maturity> = 6) relative to total number at month (see Table 2 for N). Shaded areas are NSS herring inside Landvikvannet.
doi:10.1371/journal.pone.0111985.g006

one cannot exclude the possibility that the populations separate for spawning events. Such a full separation seems unlikely for NSS and CSS herring because of the high temporal and spatial overlap; whereas it seems more likely for LV herring considering the limited temporal and spatial overlap with the other populations.

The idea that LV herring is reproductively isolated from other populations may be supported by the low vertebral count and concept of natal homing. Differences in vertebral count stem from the incubation phase and thus reflect the origin of the fish at spawning [60]. In general, there is a positive correlation with salinity [31] and negative with temperature [21,29,61] experienced prior to hatching. Hence, the warmer and less saline ambient environment for herring occurring inside Landvikvannet in May–June compared with that experienced by CSS in March–April in the fjord habitat, could result in the observed differences in vertebral count. The low vertebral count of LV herring and the late timing of spawning is an indication of spawning and adaptations to the environmental conditions of the lake habitat. However, this also implies that natal homing [62,63] of Landvik herring occurs on an annual basis. The vertebral number for LV herring in May has been remarkably stable (55.5–55.8) since 1984 [43], supporting natal homing. The principle of natal homing is central to the discrete population concept [12]. Moreover, recent genetic studies support the occurrence of natal homing of herring in the North and Baltic Seas [6,64]. Likewise, Brophy et al. [65] suggested that spawning season and location of Atlantic herring could be predetermined and not learnt from repeated spawning [66]. Support for natal homing and adaptations of Landvik herring to environmental conditions of its marginal habitat also originates from a recent genetic study using 20 microsatellite markers, where Landvikvannet differed from other local herring in Lindåspollene, Lusterfjord and Trondheimsfjord as well as from other herring populations surrounding the Norwegian Sea [67]. Unpublished results on the microsatellite locus Cpa112, which is non-neutral to salinity variability with allele frequencies varying from 45% in the Baltic to 2–4% in the North Sea [27], have shown that Landvik herring is obvious with a frequency of 15% (Carl André, pers. Comm., Department of Biology and Environmental Sciences - Tjärnö, University of Gothenburg, Strömstad, Sweden).

It seems clear from this study that we can refute the hypothesis of a resident local population inside the lake; LV herring definitely migrates into the lake habitat from coastal areas. In this sense the Landvik herring differs from other local herring populations, such as the Trondheimsfjord or Lindås herring, which can be observed throughout the year in their local areas [32,33,36,41]. This may simply be because of the unsuitability of this location as a nursery area for juveniles and feeding grounds for adults. Both CSS and LV herring may still represent more stationary coastal populations not undertaking large scale oceanic migrations. The observed relatively low investment costs in reproduction (low GSI) of NSS compared with that of LV herring supports the assumption that

Table 4. Comparing otolith shape, vertebral count (VS) and length among herring populations Norwegian spring spawners (NSS), Coastal Skagerrak spring spawners (CSS) and Landvik herring (LV).

		Otolith shape				Vertebral count				Fish length			
Comparison	N	df	Var	F	P	df	Mean Sq	F	P	df	Mean Sq	F	P
Overall	NSS vs LV vs CSS 897	2	3.28	5.36	<0.001	2	109.95	136.44	<0.001	2	129.80	102.58	<0.001
	Month	1	1.20	3.91	<0.001	1	71.49	88.71	<0.001	1	690.00	545.44	<0.001
	Age	10	4.49	1.47	0.001	10	3.87	4.80	<0.001	10	178.20	140.90	<0.001
	Residuals	883	270.41			867	0.81			867	1.30		
Posteriori	LV vs CSS 745	1	0.69	2.22	0.04	1	32.10	36.69	<0.001	1	13.10	10.08	0.006
	NSS vs LV 500	1	1.45	4.76	<0.001	1	219.80	276.99	<0.001	1	250.45	196.30	<0.001
	NSS vs CSS 549	1	0.84	2.72	0.02	1	115.53	149.39	<0.001	1	178.20	114.88	<0.001
	NSS-LV vs NSS-OLV 152	1	0.20	0.65	>0.05	1	0.23	0.47	>0.05	1	1.85	1.65	>0.05

NSS herring were also compared between sampling locations, inside (NSS-ILV) and outside (NSS-OLV) Landvikvannet. ANOVA like permutation tests were used to assess the difference in otolith shape and ANOVA for the vertebral count and fish length comparisons. For otolith shape: df: degrees of freedom, Var: Variance among populations, F: pseudo F-value, P: proportion of permutations which gave as large or larger F-value than the observed one. For the vertebral count and fish length: df: degrees of freedom, Mean Sq: Mean Square, F: F-value, P: P-value. P-values for posteriori comparisons have been corrected with a Bonferroni correction. P<0.05 indicates a significant effect.

doi:10.1371/journal.pone.0111985.t004

Figure 7. Otolith shape compared for different herring populations. Canonical scores for Norwegian spring spawning (NSS, N = 152), Coastal Skagerrak spring spawning (CSS, N = 397) and Landvik (LV, N = 348) herring are shown on discriminating axes 1 and 2. Black letters represent the mean canonical value for each group with standard error of the mean (1 SE).

doi:10.1371/journal.pone.0111985.g007

NSS is more migratory [44]. The fact that growth of CSS was higher than in LV herring, further suggest that these two populations may not overlap much during the nursery period or at adult feeding grounds. In fact, there is probably little or no spatial overlap for most of the year, with overlap only occurring during the spawning season.

The movements of herring between the fjord and Landvikvannet habitats have also been studied with acoustic telemetry [43,68]. The telemetry study showed that some fish moved in and out of the lake habitat, whereas others stayed inside the lake for more than two weeks. Those fish that arrived and only stayed for a short period of time were interpreted as being NSS or CSS, whereas the ones remaining in the area for extended periods of time were thought to be local LV herring. It is likely that some NSS and CSS herring have short visits to the lake as exploratory migrations searching for good habitats cued by the current from the Reddal channel, but migrate out again to spawn in areas which are more characteristic of their normal spawning habitat. Conversely, fish that stay for two weeks inside the lake before leaving is a reasonably good indication of an established adaptation to the lake and to potential spawning within the lake.

The appearance of NSS herring in the habitats within Landvikvannet and adjacent fjords probably does not represent natal homing. The predominance of 3-year-olds among the NSS stock as well as the high stability of growth and meristic characters over the season, suggest independent selection of spawning grounds, as supported by Slotte and Fiksen [69]. In NSS herring specifically, the use of spawning grounds other than their natal ground is common. NSS herring have a tendency to change their spawning ground as they grow older with larger fish tending to migrate further, in this case southward, and thus potentially increase their life time fitness [69–71]. Such straying from natal spawning grounds results in considerable gene flow [72,73]. The predominance of 3-year-old NSS mixing with CSS and Landvik herring in 2012 may be explained by the relatively unusual spawning migrations of NSS herring in 2009–2010. During these two years a significant proportion of the adult NSS migrated from wintering grounds in the northern Norwegian Sea to areas south of 60°N, resulting in the largest fishery in the fjords (e.g. Boknafjorden) east of the traditional spawning grounds off Karmøy since the 1950s [74]. Based on vertebral count and growth data, it was apparent that the fishery was targeting NSS

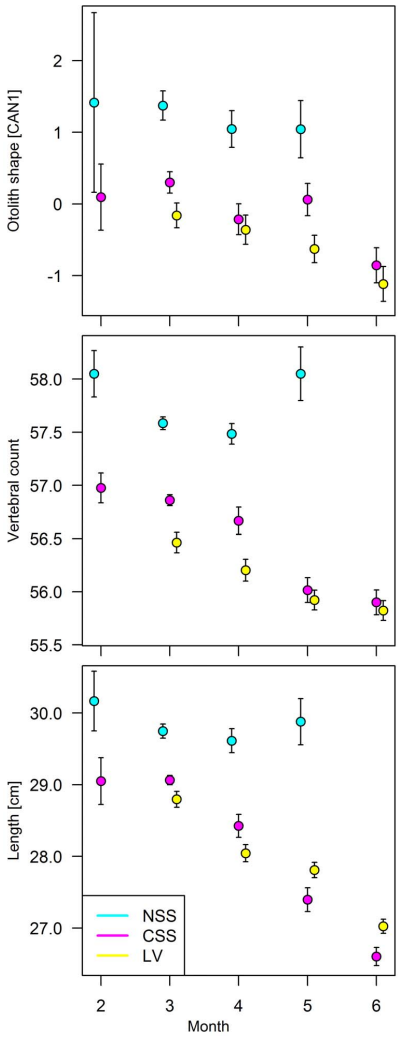

Figure 8. Seasonal changes of otolith shape, vertebral counts and length for different herring populations. For standardized ages. Comparison between Norwegian spring spawning (NSS), Coastal Skagerrak spring spawning (CSS) and Landvik (LV) herring (see Table 2 for N). Values given are means and standard errors (1 SE).
doi:10.1371/journal.pone.0111985.g008

herring [75] and the abundance was high as evaluated by catch levels (Table 5). One hypothesis is that the 3 year old NSS mixing with CSS and Landvik herring in 2012 was a result of this significant spawning at the southern grounds in 2009. Generally, if first time spawners of NSS do not meet older conspecifics and learn to follow their migration towards the spawning grounds then the location of the spawning ground is a chance event [70,71,76,77]. In addition, NSS herring tend to migrate upstream to spawn [69]. Therefore it is not unlikely that NSS from Boknafjorden or further south may have spawned close to their nursery areas or even migrated further south-eastwards against the

Table 5. Commercial catches of herring off Karmøy 2005–2012.

	Year of catch								
Month	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
1					0.1			
2	21.2				172.0	3302.9	609.1	897.3
3	24.5	32.6	16.5		19052.0	14877.0	6528.4	6283.2
4	129.2	0.7	1.0	4.8	2301.2	1000.3	52.0	13.4
8	1.0							
9					0.9			
10			0.1					
11		72.8				0.5		
12	0.2							
Total	**176.1**	**106.1**	**17.6**	**4.8**	**21526.2**	**19180.7**	**7189.5**	**7193.9**

Live weight (tons) calculated from landed weight to live weight equivalent for Norwegian spring spawning herring in the Norwegian statistical area 08 (SW coastal Norway) by month and year as registered in the Directorate of Fisheries database.
doi:10.1371/journal.pone.0111985.t005

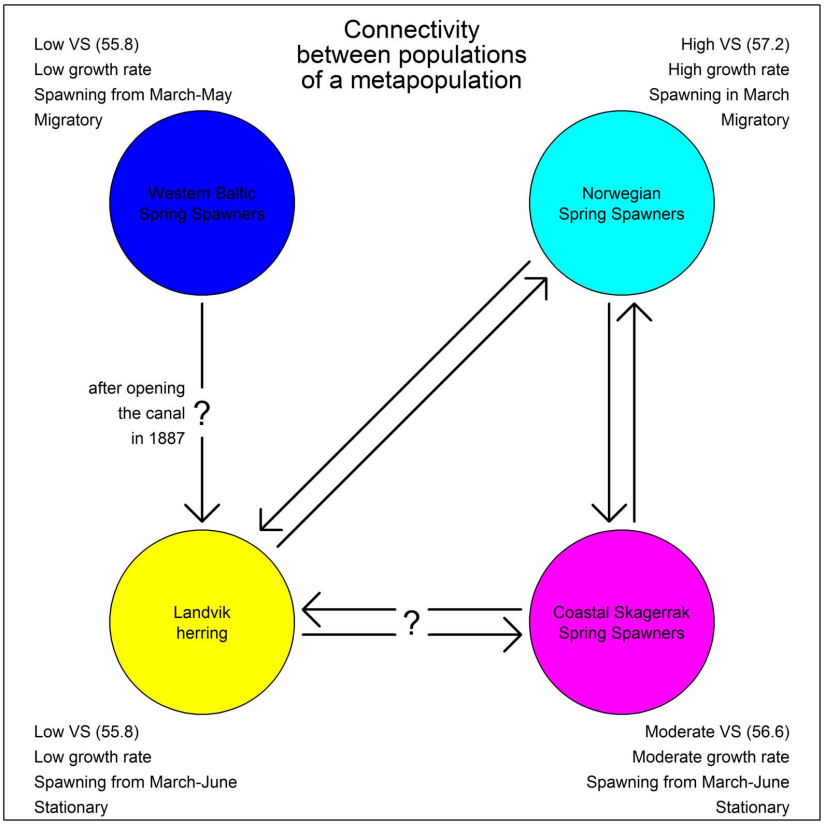

Figure 9. A schematic model of potential metapopulation dynamics in the study area. Potential connectivity between populations of a metapopulation in the study area of Landvikvannet and the connected fjords as hypothesized based on the results of the present study. The biological characteristics (VS = vertebral counts) of the different populations are given.
doi:10.1371/journal.pone.0111985.g009

coastal current to spawn. In addition, school composition tends to involve size-matching among individuals [78], in this case younger, smaller NSS. Three year old NSS (mostly first-time spawners), may have adopted the behavior of the joint local populations with whom they mix during the nursery period as postulated in the adopted-migrant hypothesis [40,79].

From an evolutionary perspective, the Landvikvannet habitat has only been available for marine species for a relatively short period of time. This raises the question of the origin of the herring first colonizing the lake after the opening of the Reddal channel (Figure 9). One possibility is that CSS herring entered the lake sometime after the opening of the channel and successfully spawned there. Due to lower salinity and higher temperature in the lake the offspring developed significantly divergent characters over the years. A strong natal homing effect of herring would lead to the development of a new local population inside Landvikvannet. Hendry and Kinnison [80] concluded that a time span less than 100 years can be sufficient for significant microevolution to develop in response to local agents of selection. Also, Neb [81] demonstrates that such a time interval and differences in salinity are sufficient for herring to diverge in meristic characters. This explanation assumes reproductive isolation during spawning between the original CSS herring and the "new" Landvik herring. A second possibility is that the origin of Landvik herring could be Western Baltic Spring Spawners (WBSS) herring. First time, or even repeated, spawners could have established a new spawning ground in Landvikvannet. The reason for not conducting an annual migration to the original spawning grounds off the island Rügen may be a trade-off between survival of progeny and physiological migration constraints, as shown for NSS by Slotte [70]. WBSS close to their feeding grounds in the Skagerrak could have "discovered" Landvikvannet, cued by similar environmental conditions as those of their original spawning grounds. The continued link to Landvikvannet may have been a result of a fidelity to this site rather than for joining conspecifics in a migration back in to the Baltic region. Huse et al. [76] demonstrate that a high ratio of first-time spawners could lead to the establishment of new wintering grounds. In the case of Landvik herring, it may have led to a new spawning ground.

In conclusion, the present study provides evidence for a distinct small local population of herring associated with Landvikvannet, partly mixing with NSS and CSS herring. This population of LV herring resides, during part of the year in brackish water with many morphometric characteristics indicative of spawning in

warm and low salinity environments. Whilst ripe and spent fish have been found in the area, there is no direct evidence of spawning in the lake. If spawning does occur there are no data to indicate likely survival rates or even the residence time of offspring in the lake. There has been one attempt to find eggs with a diver for 1 hour at one of the many bays in the lake, without success. Also, limited plankton net sampling in selected parts of the lake have failed to capture any larvae. The only evidence of potential spawning in the lake, is from two eels with stomachs full of fertilized herring eggs. There is also no clear evidence of the origin of this population, however, they could have arisen from either WBSS or other local CSS. The presence of mixtures of these and other stocks and populations in the Skagerrak area have been shown previously [6,82]. Recent genetic studies using microsatellite DNA [83] have demonstrated differences between Landvik herring and many other stocks, in addition, unpublished results on one microsatellite locus (Carl André, pers. Comm., Department of Biology and Environmental Sciences - Tjärnö, University of Gothenburg, Strömstad, Sweden) suggesting that Landvikvannet herring has not recently immigrated from the Baltic.

The results of the present study may also have some implications for the official ICES stock assessment of herring in the North Sea and Skagerrak area. The present work demonstrates that there can be a fairly complex population structure in the areas with more than one 'stock' which can be mixed. Whilst this may not be a significant problem for the assessment of NSAS or WBSS due to the relatively small abundances of CSS and LV herring, there is a possibility that these smaller populations could be very vulnerable to overfishing [9]. This is probably not unique for coastal areas as there are a number of relatively small populations bordering the North Sea and Skagerrak area [84].

From management point of view, probably the most striking result of the present study is the conclusive evidence of NSS herring as far southeast as in the Skagerrak. This is the first time that individuals from this historically large herring stock have been studied in the Skagerrak area. By definition this stock is not exploited south of 62°N, with exception of the spawning period when they previously have been found as far south as to Lindesnes (Figure 1). This signifies that migration dynamics and population connectivity among herring in the Northeastern Atlantic may be more dynamic than previously assumed, and this must be taken into account in the future development and implementation of new management strategies.

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Author Contributions

Conceived and designed the experiments: FE AS LAL. Performed the experiments: FE AS LAL. Analyzed the data: FE AS LAL. Contributed reagents/materials/analysis tools: FE AS LAL AJ EMO EM. Wrote the paper: FE AS LAL RDMN. Reviewing the manuscript: FE AS LAL AJ EMO EM CK RDMN.

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Paper II

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Individual habitat transitions of Atlantic herring *Clupea harengus* in a human-modified coastal system

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ABSTRACT: Pelagic marine fish often display highly dynamic migration patterns. However, such movement behaviour is usually studied at the population or school level, while less is known about individual movement characteristics and habitat transitions. During March 2012 to June 2013, we used acoustic tags and moored receivers to monitor the behaviour of Atlantic herring *Clupea harengus* L. (N = 47) throughout a range of habitats on the Skagerrak coast in southern Norway. Five of the tagged herring entered a former lake transformed into an artificial estuary by a human-made canal linking the former lake to the open ocean. Herring resided in this system for up to 36 d. All tagged herring left the fjord where they were tagged by early August 2012. This habitat transition was detected by the receivers as 3 main pulses of tagged individuals, which were assumed to be formed by putative populations mixing in the area. Most transitions occurred during nighttime regardless of tidal cycle, and it is suggested that spawning is the primary driver for entering the fjord and artificial estuary. Later detections at a separate receiver system 17 km to the northeast suggest that some herring may overwinter in coastal areas. In the spring of 2013, 3 of the tagged herring returned to their original fjord tagging location. Our study reveals new aspects of herring migration dynamics linked to anthropogenic modifications of connectivity, and suggests that capacity for individual behaviours in schooling fish may be underestimated.

KEY WORDS: Schooling fish · Acoustic telemetry · Movement behaviour · Migration · Skagerrak

INTRODUCTION

Human activities shape the environments of wild populations around the world, and habitat loss represents a major threat to biodiversity (Fahrig 2003, Cushman 2006, Wiens 2009). In other cases, human-induced habitat changes may also present animals with new opportunities for expanding their range of movements, for instance when new canals connect aquatic ecosystems that have previously been isolated from each other (Silva et al. 2013, Eggers et al. 2014). Such human-induced alterations of connectivity may influence population dynamics as well as interspecific competition and predator–prey relationships. Understanding behavioural responses to human-induced alterations of connectivity in aquatic systems is therefore potentially important from a management and conservation perspective.

The Atlantic herring *Clupea harengus* L. is widely distributed in the North Atlantic and adjacent seas. It has a complex population structure (Iles & Sinclair 1982, Sinclair & Iles 1988) and some populations, such as the Norwegian spring spawning herring, have supported important fisheries for centuries

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(Toresen & Østvedt 2000). This clupeid is an iteroparous total spawner, it matures at 2 or 3 yr of age, aggregates at high densities at spawning time and deposits its sticky eggs on shells, gravel, coarse sand and small stones at depths down to 250 m (Runnstrøm 1941). Herring are thus susceptible to anthropogenic activities affecting the sea bed, e.g. gravel extraction and eutrophication causing oxygen depletion. Herring may also colonize artificial habitats and utilise these for spawning, e.g. the Kiel Canal (Paulsen et al. 2014). Larvae hatch after 2 to 4 wk, depending on temperature (Meyer 1878, Soleim 1942). Early stage larvae drift with the currents until metamorphosis (Russell 1976, Dragesund et al. 1980, Corten 1986), with vertical migration increasing throughout ontogeny, likely affecting the dispersal trajectories of larvae (Woodhead & Woodhead 1955, Blaxter & Parrish 1965).

Mature herring typically conduct annual migrations between wintering, feeding and spawning areas (Varpe et al. 2005). The timing and extent of these migrations are influenced by abiotic environmental factors such as temperature and salinity, as well as biotic factors such as prey distribution (Olsen et al. 2007, Broms et al. 2012). Also, learning and genetic factors may play a role (Fernö et al. 1998). Herring show fidelity, especially to overwintering (Dragesund et al. 1997) and feeding areas (Fernö et al. 1998), while the spawning area depends more on the individual state of the spawners (Slotte & Fiksen 2000). Also, the level of fidelity may change when schools are populated with newly recruited (more naïve) fish (Huse et al. 2010).

Monitoring the movements of individual fish in their natal marine habitat can be challenging, but is enabled by technological developments within the field of acoustic telemetry (Hightower et al. 2001, Pine et al. 2003). Networks of deployed acoustic receivers may be used to continuously log and store data from acoustic transmitter tags implanted in marine animals. This method of acoustic monitoring has been used successfully for species such as blacktip sharks *Carcharhinus limbatus* (Heupel & Simpfendorfer 2002), Atlantic cod *Gadus morhua* (Olsen & Moland 2011), pigeye sharks *C. amboinensis* (Knip et al. 2011) as well as Atlantic herring (Langård et al. 2012).

Individual tracking of fish enables the investigation of different behaviours linked to biological and environmental factors. The monitoring of real-time movements of individual fish results in a determination of the exact abiotic environment. It has been shown that several fish species behave differently according to the influence of environmental factors, like oceanic tides (Lacroix et al. 2004, 2005), season or diel phases (Tolimieri et al. 2009), salinity, temperature and turbidity as characteristics of estuarine tides as well as the tidal phase (Childs et al. 2008). Likewise, biological factors such as size can influence the migration behaviour of fish (Lee et al. 2011). However, none of the above-mentioned studies evaluated the behaviour of typically schooling species such as Atlantic herring.

In schooling fish, the collective output of behavioural decisions forms the results of schooling dynamics, and individuals have to balance stimuli from their neighbouring conspecifics as well as from their environment (Pitcher & Parrish 1993, Parrish & Edelstein-Keshet 1999). To date, the complex nature of schooling has typically been studied by either recording multiple schools over large areas (Nøttestad et al. 1996, Gerlotto et al. 1999) or monitoring single schools over a limited time period (Axelsen et al. 2000).

We investigated individual habitat transitions of Atlantic herring by means of acoustic telemetry. We focused on a coastal system wherein a former lake (Landvikvannet) has been connected to the ocean to form an artificial estuary. Over time, the human-made canal between the former lake and the ocean has changed the freshwater environment into a brackish system, and the connection to a fully marine system allows movement of marine species into this brackish environment, thus making a new habitat available. The very different environment inside and outside Landvikvannet, as well as the tidal effects on currents in the human-made canal, make the area interesting for studying potential environmental drivers of herring behaviour. It is also an interesting area with regard to potential population differences in behaviour and internal drivers, such as maturation status of individual fish. Here, 3 putative herring populations were observed to co-occur at maturing, spawning and spent stages from March to June, where 1 population, 'Landvik herring', showed high fidelity to the artificial estuary (Eggers et al. 2014). In addition to 'Landvik herring', coastal Skagerrak spring spawning herring (CSS) exist in neighbouring fjords, without conducting large annual migrations like those of Norwegian spring spawning herring (NSS), which are also found here. NSS occur in this area mostly in March before starting their annual migration (Eggers et al. 2014). Using individual acoustic tagging and monitoring, herring habitat transitions in this complex artificial estuary-canal-fjord-ocean system were quantified and their pur-

pose evaluated. Our main study goals were (1) to quantify the among-individual synchrony or heterogeneity in movements; (2) to evaluate local environmental conditions as well as the internal status of individual herring as drivers resulting in habitat transitions; and (3) to identify potential behavioural differences supporting biological evidence of putative herring populations in the area.

MATERIALS AND METHODS

Study system

This study was conducted on the Norwegian Skagerrak coast, near the town of Grimstad (Fig. 1). The study area included the brackish former lake Landvikvannet (1.85 km^2, hereafter Landvikvannet) and the neighbouring fjords Strandfjorden and Bufjorden. Landvikvannet is connected to Strandfjorden by a 3 km long and, currently, 1 to 4 m deep canal. In 1877, the depth of the canal was artificially increased to drain water from Landvikvannet and thereby increase the surrounding agricultural areas. This construction allowed salt water and marine organisms to enter Landvikvannet from Strandfjorden, thus transforming the lake into an artificial estuary. Typically, Landvikvannet now has a highly stratified water column with a transition depth at 4 m. In May the upper layer has low salinity (<20 PSU) and higher temperature (>8°C), and is oxygen rich (>1 ml l^{-1}). In contrast, the lower layer has a constant temperature (7 to 8°C) and high salinity (>20 PSU), and no oxygen but toxic hydrogensulphide (for details, see

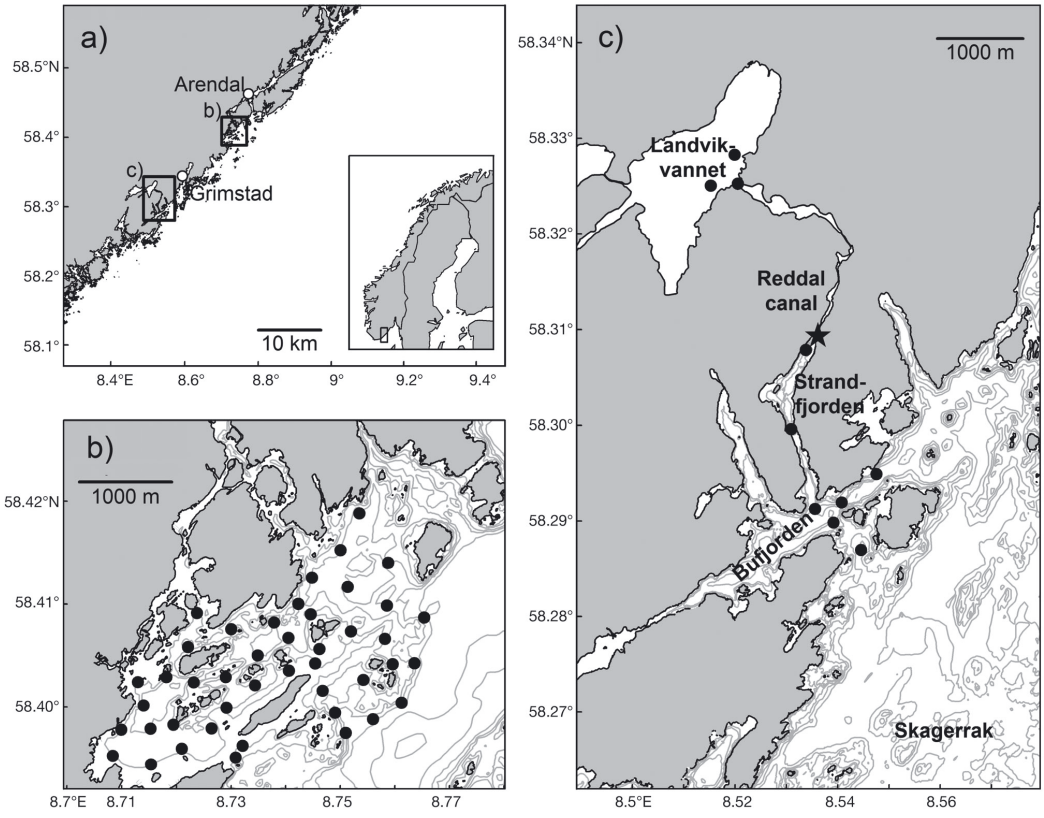

Fig. 1. (a) Study area along the Norwegian Skagerrak coast. (b) Location of the acoustic receiver array in Sømskilen near the town of Arendal. (c) Lake Landvikvannet and the connected fjords Strandfjorden and Bufjorden, showing the point of capture, acoustic tagging and release of Atlantic herring *Clupea harengus* (★), and positions of deployed acoustic receivers (●)

Eggers et al. 2014). Strandfjorden is about 2 km long and sheltered from the outer coast. The inner part has a relatively deep basin (10 to 13 m depth) while the outer part is narrower, and has a shallow sill of only 1 m which fish must cross when moving between inner and outer areas (Fig. 1). Both Strandfjorden and Bufjorden have fully marine conditions (salinity >30, oxygen >0.8 ml l^{-1}) and mainly rocky shorelines with sand and mud in deeper areas. Compared to Strandfjorden, Bufjorden is wider (5 km^2) and deeper (54 m). The fjord has 2 main outlets to the Skagerrak, with sill depths of 30 m (south) and 14 m (east).

Data collection

We captured wild Atlantic herring with hook and line in the inner Strandfjorden during March to June 2012 (Fig. 1). Tagging was conducted in the field. For this purpose, herring were kept in a tank (80 × 50 × 40 cm) in which 10 l of water were exchanged approximately every 10 min. Transmitters were surgically implanted in the abdominal cavity. A small incision was made posterior to the pelvic fins through which the transmitter was inserted. A tissue adhesive (Histoacryl®) was used to close the wound. Total length was measured to the nearest cm, and a few scales were removed for age determination. Tagged herring were left to recover in a separate tank for 30 min and thereafter released close to the site where they had been caught. In total, 11 herring were tagged with 7 mm acoustic transmitters (7.3 × 18 mm, weight in seawater 1.2 g, Thelma Biotel), while 50 herring were tagged with 9 mm acoustic transmitters (9 × 23 mm, weight in seawater 2.5 g, Thelma Biotel). Transmitters were programmed to transmit an identity code every 80 to 180 s, with random intervals to reduce code collision (i.e. 2 or more tags transmitting to the same receiver at the same time). Estimated battery life was 290 and 918 d for the 7 and 9 mm tags, respectively. Langård et al. (2012) used this technique for the first time on herring and concluded that it was suitable for behavioural investigations. Therefore no further analyses or experiments for the survival rate of hooked but untagged herring were conducted to show the effect of surgery on behaviour. The herring sampling and tagging procedure (this study) was reviewed and approved by the Norwegian Animal Research Authority (FDU).

In total, we deployed 10 acoustic receivers (VR2W-69kHz Acoustic Monitoring Receiver, Vemco Division, Amirix Systems) in the Landvik system in March 2012 to record transmitter signals. Receivers were deployed at 3 m depth and kept in position by a trawl float at 2.5 m depth and a 40 kg concrete anchor. Receivers were placed in Landvikvannet, Strandfjorden and Bufjorden (Fig. 1). This allowed us to quantify movements of tagged herring among these habitats, as well as out of the study area and into coastal waters. Stored data were downloaded from the acoustic receivers every 3 to 6 mo until June 2013, when the study ended and the receivers were removed.

In addition to the receiver array described above, we also included data from another receiver array near the town of Arendal, 17 km northeast of our study area, consisting of 44 receivers (Wiig et al. 2013). The reason for this was that some of the tagged herring eventually moved to the Arendal system (see 'Results'). Protocols for deployment of receivers and downloading of data were similar for both telemetry systems.

Data analyses

Presence and movement of fish within the study area were determined from detections at multiple receivers over time. In cases where detections eventually ceased at the edge of the study area (outermost receivers), this was defined as movement out of the study area. For the tag sizes used in our study, detection ranges of receivers in this coastal habitat are typically no less than 200 to 400 m (Olsen & Moland 2011). We were therefore able to detect movements of tagged herring from Strandfjorden to Landvikvannet or Bufjorden.

Probability of entering Landvikvannet

We used generalized linear models (McCullagh & Nelder 1989) to quantify how herring moved among the different habitats in our study system. First, we used logistic regression to estimate the probability of tagged herring entering Landvikvannet from the tagging location in inner Strandfjorden. Herring age (A) and total length (L) were included as continuous explanatory variables in the model; a working hypothesis was that exploratory movement would depend on these individual life-history characteristics:

$$\text{logit}\,(e) = \beta_0 + \beta_1 L + \beta_2 A \quad (1)$$

where β_1 and β_2 describe the estimated effect of length and age, respectively, on the probability (e) of entering Landvikvannet.

Diel and tidal patterns in the habitat transition

Second, we analysed the movement of herring from the inner Strandfjorden to Bufjorden, closer to the open ocean. Since all fish eventually left Strandfjorden (except those that died), this analysis focused on understanding diel patterns in the habitat transition. Specifically, we estimated the probability of moving between Strandfjorden and Bufjorden during daytime (d) versus the night as a function of fish length (L), age (A) and direction (D). D was modelled as a factor with 2 levels (moving outwards to or inwards from Bufjorden). Because some fish moved back and forth between these 2 habitats several times, we included herring individual (i) as a random effect. Daytime was defined as the time interval between 06:00 and 18:00 h, while night was defined as the time interval between 18:00 and 06:00 h. Hence, the probability of moving during daytime was modelled as a dichotomous response variable in a logistic regression model:

$$\text{logit}\,(d) = \beta_0 + \beta_1 L + \beta_2 A + \beta_3 D \quad (2)$$

We did not account for any seasonal changes in daylight hours during the study period, since all habitat transitions out of Strandfjorden took place within 18 d (see 'Results'). A similar approach was conducted to analyse the influence of currents generated by tides. We used the same logistic regression model, but instead of d we used the tides (T). The tides were defined as high and low tides at the start of the migration. While low tides were the time between the highest and lowest water level (decreasing water level), high tides were during increasing water level. The water level and different tides are recorded by Kartverket og Meteorologisk Institutt (Norwegian Hydrographic Service, www.sehavniva.no). This model (without the direction variable) was also used for the departure time of herring.

Migration speed for transitions

Third, we analysed the speed of movement between inner Strandfjorden and Bufjorden. We used a linear mixed effects model including i as a random effect, accounting for repeated observations of individual fish movement. The model included fixed effects of L and A on migration speed (s, in hours). We also explored whether movement speed depended on the direction (D) of movement:

$$s = \beta_0 + \beta_1 L + \beta_2 A + \beta_3 D \quad (3)$$

The significance of β_1, β_2 or β_3 would indicate an effect of length, age or direction on the migration speed of herring.

Duration within the monitoring array

Fourth, we modelled the duration (T) of herring presence within the monitoring array, where they were tagged, south of Grimstad. A linear model was used including total length (L), age (A) and date of the tagging experiment (E) as predictor variables:

$$T = \beta_0 + \beta_1 L + \beta_2 A + \beta_3 E \quad (4)$$

The dependence of these life-history characteristics on duration would be indicated by significant β_1 or β_2 values.

Departure time of herring

Lastly, a K-means clustering (Hartigan & Wong 1979) was conducted to analyse similarities of herring according to their time of departure against total length or age. Day of the year was used as a variable for the departure time. Based on the observed results, we used $K = 3$ clusters for this analysis. The centres of the 3 clusters (C) were compared for each variable — day of the year (Y), total length (L) and age (A) — with a linear model:

$$C = \beta_0 + \beta_1(Y, L \text{ or } A) \quad (5)$$

A significant β_1 term would demonstrate that the 3 clustered groups differed depending on the tested variable.

RESULTS

In total, we tagged 61 herring in the inner Strandfjorden during March to June 2012. The age of the tagged herring ranged from 2 to 15 yr, and the total length ranged from 19 to 34 cm (Table 1). Out of these, a total of 14 herring apparently died within the study area shortly after tagging (inferred from cessation of signals or movement during the first 3 d). These fish were censored from further analyses; thus, 47 individuals were used in our analyses, constituting the tagged population. As we had no reason to infer differences in behaviour of herring tagged with the 2 types of acoustic tags used, all

Table 1. Summary details of acoustically tagged Atlantic herring *Clupea harengus* from Strandfjorden, coastal Skagerrak, showing the tagging dates in 2012, the sample sizes (N), herring total lengths (TL mean and range) and herring ages (mean and range) estimated from scale readings

Tagging date	N	TL (cm)	Age (yr)
28–29 March	43	28.8 (24–34)	4.9 (2–15)
15–16 May	13	25.0 (20–30)	3.4 (2–7)
20–21 June	5	21.8 (19–23)	2.8 (2–3)

analyses were carried out with acoustic tag types merged.

Fidelity of herring to Landvikvannet

A total of 10.6% (N = 5) of the tagged population made the transition from Strandfjorden to Landvikvannet through the human-altered canal. These upstream movements took place during May to July 2012, and were not temporally synchronized (Fig. 2). Time spent swimming up the canal ranged from 10.0 to 27.3 h (mean = 22.3 h), while the duration of the Landvikvannet stay ranged from 2 to 36 d (mean = 17.0 d). When returning to the fjord, time spent swimming down the canal ranged from 12.7 to 107.5 h (mean = 41.9 h). There was no significant effect of total length and age on the probability of entering Landvikvannet ($\beta_1 = -0.18$, SE = 0.21, p = 0.41; $\beta_2 = 0.13$, SE = 0.2, p = 0.52).

Diel and tidal patterns in the habitat transition

Besides movements between Strandfjorden and Landvikvannet, we observed movements between Strandfjorden and Bufjorden. All transitions were made within 18 d after tagging, except for two which took place more than 1 mo after tagging. Most movements were made during the night in both directions (77.3% into Strandfjorden, 97.6% out of Strandfjorden). The probability of moving during the day was not influenced by fish length or age ($\beta_1 = -0.17$, SE = 0.24, p = 0.94; $\beta_2 = -0.09$, SE = 0.22, p = 0.69), but the effect of direction was marginally significant ($\beta_3 = -2.48$, SE = 1.24, p = 0.05). The movement out of Strandfjorden was less likely to happen during the day. Even though all transitions occurred within a limited time interval, none of the herring migrated at the same hour. Herring tended to migrate during low tides, for both directions (In: 61.4%; Out: 63.4%), but the habitat transitions of tagged herring were not significantly influenced by currents generated by tides. Fish length, age and migration direction had no influence on the probability of migration during low or high tide ($\beta_1 = 0.02$, SE = 0.15, p = 0.90; $\beta_2 = -0.04$, SE = 0.12, p = 0.74; $\beta_3 = -0.09$, SE = 0.46, p = 0.85). Even though 63.6% left the system during low tides, the probability of departure during high or low tide was not significantly influenced by fish size or age ($\beta_1 = 0.03$, SE = 0.16, p = 0.84; $\beta_2 = -0.08$, SE = 0.17, p = 0.61).

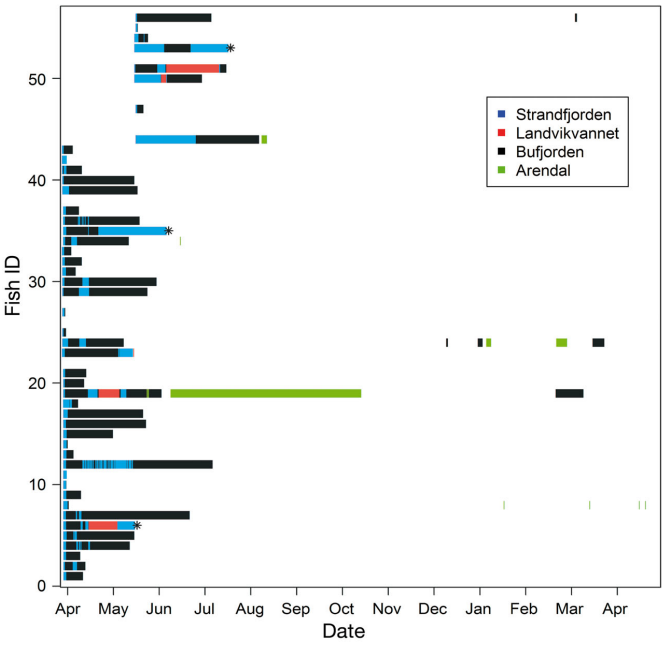

Fig. 2. Acoustic telemetry observations on the duration of presence or absence of individual Atlantic herring *Clupea harengus* (listed by their fish ID number, N = 47) in the sheltered Strandfjorden, through the human-made Reddal canal and the connected Landvikvannet, the more exposed Bufjorden and the Arendal receiver system 17 km farther north-east. See also Fig. 1. Asterisks (*) denote day of presumed expiry for herring that died within the monitoring area. The period shown spans April 2012 to April 2013

Migration speed for transitions

Movement from Bufjorden to Strandfjorden (mean duration: 2.3 h; range: 0.2 to 8.3 h) was significantly longer in duration than the reciprocal one (mean duration: 1.3 h; range: 0.3 to 6.7 h; $\beta_3 = -0.95$, SE = 0.38, p = 0.01). The duration of transitions between the 2 fjords was also significantly influenced by the age of herring ($\beta_2 = 0.25$, SE = 0.1, p = 0.03), whereby younger herring were faster than older ones, but this was not corroborated by total length ($\beta_1 = -0.23$, SE = 0.23, p = 0.06).

Behavioural differences within herring populations

Herring spent between 1 and 99 d after tagging within the Landvik system, with a mean duration of 27.6 d. There was no effect of length, age or date of the tagging experiment on the staying time within the system ($\beta_1 = -0.14$, SE = 3.04, p = 0.96; $\beta_2 = 1.73$, SE = 3.75, p = 0.65; $\beta_3 = 8.61$, SE = 16.46, p = 0.61).

Clustering of individuals according to time of departure

The clustering analyses with $K = 3$ clusters clearly grouped the individual herring according to their time of departure from the study system (Fig. 3). Both analyses showed high accordance comparing the within-clusters sum of squares by cluster, with 93.8% for total length and 94.2% for age. The groups differed significantly in their time of departure within the year ($\beta_1 = -0.02$, SE < 0.01, p < 0.01), but not in their total length ($\beta_1 = 0.05$, SE = 0.04, p = 0.19) or age ($\beta_1 = 0.01$, SE = 0.51, p = 0.87). The departure time of the different clusters was compared to the moon phase, but no significant correlation was found. The range of each cluster covered both, new and full moon, at least for some individuals. The first group showed a tendency to depart at full moon, whereas the second group tended to depart at new moon.

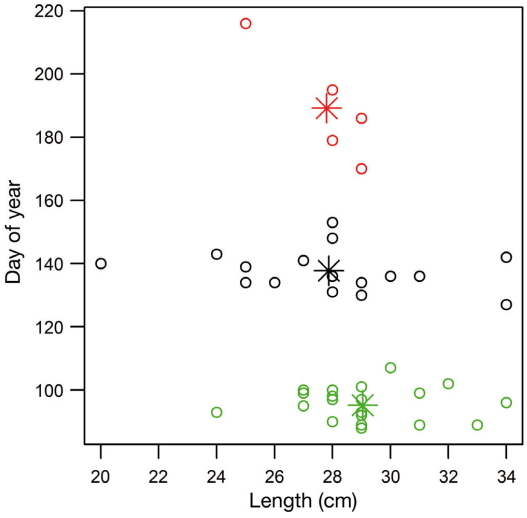

Fig. 3. Clustering analyses of the time of seaward departure by acoustically tagged Atlantic herring *Clupea harengus* (N = 47) from the Landvik system vs. body size (total length, cm) between 29 March and 30 August 2012. The 3 groups were in part assigned to disparate population components: Landvik herring (red symbols), coastal Skagerrak spring spawning herring (green symbols) and Norwegian spring spawning herring (black symbols). Asterisks denote mean departure date for each clustered group

Migratory or stationary population

After leaving the Landvik monitoring array, 3 of the tagged herring (Fig. 2) were detected in the Arendal system and showed different behavioural patterns. One individual even moved back and forth between the 2 monitoring arrays and stayed for a longer time in the Arendal system. The other 2 individuals were only detected by the outermost receivers. Herring needed between 2.1 and 33.5 d to move between the 2 monitoring arrays with a mean travel time of 12.1 d. Excluding the travel time of 33.5 d of 1 herring, the mean travel time was 5 d, which is more precise because the travel duration for the other 3 migrations was at maximum 6.5 d. According to the straight line distance of 17 km between the 2 areas, herring exhibited an average swimming speed of 138 m h^{-1}. These estimates refer to net movement, but actual velocities of individual herring could have been greater as their movement was likely conducted in a back and forth or zigzag manner.

Homing of herring

After overwintering outside the study areas, 3 herring returned to the Landvik system (ID nos. 19, 24, 56) and 1 returned to the Arendal system (Fig. 2, Table 2; ID no. 8). Two herring (ID nos. 19 and 24), which had both been detected in the Landvik system in spring 2013, were also detected in the Arendal sys-

Table 2. Summary details of acoustically tagged Atlantic herring *Clupea harengus* detected during spring 2013. Fish ID as in Fig. 2, herring total lengths (TL) and ages were estimated from scale readings, and cluster affiliation is based on the departure time. The system with which they were affiliated in 2013 is also shown

ID	TL (cm)	Age (yr)	Cluster	System
19	28	4	2	Landvik
24	34	12	2	Arendal + Landvik
56	28	Unknown	2	Landvik
8	28	4	1	Arendal

tem previously. Those herring stayed for a longer time in both systems and were detected by receivers inside the system, but not in Strandfjorden. The other 2 herring individuals were only detected by the outermost receivers for a short time period, indicating that those herring only passed by the different systems, but did not enter them. The ages of these individuals were estimated to be 4, 4 and 12 yr (for 1 herring, age could not be determined from scales) and the lengths were 28, 28, 34 and 28 cm. Except for herring ID no. 8, which left the system directly within 3 d, all herring were grouped in the second cluster (see Fig. 3) for the first departure time from the Landvik system. The 2 herring which had been detected inside the system after returning already showed migratory behaviours during the 2012 season. Both migrated between Bufjorden and Strandfjorden, while ID no. 19 migrated farther into Landvikvannet. Even though ID no. 56 did not migrate between the different habitats, the data indicate a migration into the inner Bufjorden, which is a sheltered and closed fjord, before it finally left the monitoring system.

DISCUSSION

Using acoustic tagging and monitoring, the present study demonstrates how individual herring differ in their movements throughout a complex coastal system, including habitat transitions from an artificial estuary to a fjord through a human-made canal, and farther out into more exposed coastal habitat. In light of our main findings, we discuss to what extent (1) migration of herring could be based more on individual decisions rather than decisions made at the school level; (2) whether the observed movements in our study area could be motivated by environmental conditions and spawning behaviour; and (3) whether the 3 co-occurring putative populations can be separated based on behavioural patterns. Further, we discuss how these individual-based observations can help to improve our understanding of the migration dynamics of pelagic schooling fishes in coastal habitats modified by humans.

Individual movements versus school dynamics

Herring are known for maintaining large schools and conducting synchronized annual migrations. However, our observations indicate that herring entered Landvikvannet more at the individual level, instead of the school level. In fact, we observed herring swimming upstream the canal in schools of less than 10 individuals. Likewise, the recorded movements between Bufjorden and Strandfjorden were not synchronized among individuals. Similar results were observed by Langård et al. (2015), who demonstrated an increase in individual day-to-day variability of spawning herring activity in both horizontal and vertical dimensions, indicating a shift from strong school coherence to high individual variability. Hoare et al. (2004) studied context-dependent grouping size choice in a shoaling fish experimentally, and suggested that fish may individually adjust grouping behaviour without requiring extensive information on the position and movement of all possible shoalmates. This suggests that a highly dynamic environment, such as the artificial estuary-fjord-coast continuum studied herein, may confer variability in spatial decisions. An alternative explanation for the individual habitat transitions could be an effect of disruption of the school structure. After capture, tagging and release, individual herring might have lost contact with their original shoalmates and could not associate with existing herring schools. To avoid such a disruption effect in future studies, tagged and untagged herring could be held together in a larger container from which they are all released simultaneously.

None of the investigated factors, either biological (size or age) or environmental (tides or tidal cycle), explained the observed individual variation in movement patterns. However, our results clearly demonstrated a diel effect on the migration of Atlantic herring. Herring tend to migrate during the night, regardless of tide and current direction. In other species, e.g. Atlantic salmon *Salmo salar*, the tides play an important role for the different migration patterns (Lacroix et al. 2004, 2005). The prevailing direction of the coastal current, known as the Norwegian coastal current, in the general study area was westward along the coast in the Skagerrak (Sætre 2007). The

fact that all herring left the Landvik system through the 2 possible southward or eastward corridors without any clear pattern does not support a strong influence of the Norwegian coastal current on the behaviour of tagged herring.

Motivation for habitat transitions

Habitat transitions in this complex artificial estuary-canal-fjord-ocean system conferred a high predation risk for herring, especially when crossing shallow waters in the canal as well as over the sill between Strandfjorden and the open ocean (see 'Materials and methods'). Consequently, a high motivation must exist for undertaking these potentially hazardous movements. When viewing this trade-off in context with the observed movement patterns, for instance the transitions from Bufjorden to Strandfjorden, spawning can be assumed to be the most likely motivator. With a significantly longer transition (timewise) into Strandfjorden, herring would increase their predation risk even more. Also, older herring spent more time in the shallow part than their younger conspecifics. Older herring are more experienced, repeated spawners, balancing personal information based on past experiences with social information based on the behaviour of other individuals (see e.g. Miller et al. 2013). This trade-off, involving higher predation risk, can be explained by higher probability of successful recruitment due to spawning taking place in better conditions (Candolin 1998).

The only factor influencing the transitions between both areas was the diel cycle, where most migrations occurred during the night, also supporting spawning movements as motivation. However, those diel activity patterns may need to be controlled by several fixed-location control tags to avoid the influence of factors in the absence of animal behaviour (Payne et al. 2010). Typically spawning herring aggregate in schools during daytime in pelagic waters to avoid predation (Nøttestad et al. 1996, Axelsen et al. 2000), and also perform diel vertical migration where shallow habitats are only visited during dark hours (Woodhead & Woodhead 1955, Blaxter & Parrish 1965). In shallow waters, however, as found in our study area, spawning herring may stay in touch with the bottom at all hours and without dispersing closer to the surface during darkness (Slotte 1998). Also, small schools may split from the large aggregation for spawning and migrate to their spawning grounds (Johannessen et al. 1995, Skaret et al. 2003).

Besides Strandfjorden, potential spawning could occur in Landvikvannet as well as in the Reddal canal. Both areas hold suitable habitats for herring to spawn. Herring may colonize artificial habitats for spawning, as seen in the Kiel Canal (Weber 1971, Paulsen et al. 2014) or the Østerbøvatn (Aasen 1953). While the environmental conditions in the Kiel Canal are similar to the Baltic Sea and herring found inside the canal did not form an individual population, the herring in Østerbøvatn are classified as a single population, distinct from the neighbouring population in the full marine habitat. Also, the varying residence time of tagged herring in Landvikvannet of 2 to 36 d suggests that the lake may be suitable for long-term residence for herring populations adapted to the environmental conditions of this marginal habitat (Eggers et al. 2014), whereas populations not adapted may choose to leave quickly. Besides the artificial estuaries Landvikvannet and Østerbøvatn, herring have colonized habitats with similar environmental conditions and can be phenotypically distinct from other populations (Neb 1970, Hognestad 1994).

Population separation based on individual behaviour

Eggers et al. (2014) studied the population structure of herring both inside and outside the Landvikvannet habitat over the full spawning season in 2012, from February to June, by means of monthly gill net sampling and biological analyses. They found that 3 different herring populations seem to co-occur in the study area during spawning: Norwegian spring spawning (NSS), coastal Skagerrak spring spawners (CSS) and a third population termed 'Landvik herring'. This third population is a putative local population presumably spawning inside Landvikvannet, mainly recognised on the basis of consistent low mean vertebral counts in samples collected during 1984 to 2012 (Eggers 2013). The other 2 populations, NSS and CSS, have been suggested to visit the artificial estuary in minor proportions as an explorative behaviour (Eggers et al. 2014). Hence, tagged fish staying inside Landvikvannet for only 2 d would likely belong to the CSS or NSS population, whereas the ones staying for up to 36 d may belong to the locally adapted Landvik herring. The co-occurrence of tree putative populations was also evident when viewing departure dates. The clustering analyses of time of herring departure (see Fig. 3) corroborated this, at least in part, by showing 3 different groups leaving at disparate times of the year. In Eggers et al.

(2014), data on temporal and spatial changes in catch per unit effort combined with observed changes in biological parameters such as stage of maturation, vertebral count, otolith shape and length at age, indicated different peak occurrence and spawning of the 3 herring populations. They found that NSS and CSS herring arrived early in the season in February and March, and with NSS herring finishing spawning at an earlier time than the CSS herring. The Landvik population arrived and entered the artificial estuary in May, having a later spawning peak. The results from the present study support the conclusion from Eggers et al. (2014), where the first (and largest) group of tagged fish leaving early (end of March) were probably NSS herring. Tagged CSS herring were the next to leave in June, followed by individuals of the local Landvik herring which stayed until as late as August.

The potential spawning events demonstrated through migratory behaviour inside the monitoring system, in conjunction with the affinity of CSS, and especially of Landvik herring to this local area, may lead to expectations of a high returning rate of individuals in the tagged herring population. However, in this study only 3 tagged herring returned to the Landvik system and 2 to the Arendal system farther east. One of those fish returned to both systems. This may be an indication of returning herring, but as long as the herring stayed in Bufjorden and did not enter Strandfjorden while the monitoring system was in place (until June 2013), we cannot draw conclusions about homing to a local spawning area.

Furthermore, CSS herring may not be tightly linked to specific spawning locations along the coast, but may vary their preferred spawning grounds according to changes in environmental conditions. The winter 2012 to 2013 was particularly cold, with sea ice remaining until May, which could explain that only few fish (N = 3) returned to the specific Landvik system. Due to late ice cover, potentially returning herring may have spawned at different locations along the coast with more favourable environmental conditions.

Conclusion

Observations gathered by acoustic telemetry demonstrated individual transition of herring between habitats with different environmental conditions. These transitions were neither linked to biological characters such as size or age nor to environmental factors such as tidal cycle. Transitions between fully marine localities were also observed mostly during the night. For habitat transitions between marine locations, spawning is suggested as the primary driver. However, in the local Landvik system, evidence suggests that 3 putative herring groups exist (NSS, CSS and Landvik herring), which differ in their migratory behaviour as well as in their affinity to coastal waters. There was no clear evidence for either natal or repeated homing to this specific area; however, CSS and Landvik herring showed a tendency to stay near the coast. The present study reveals new aspects of herring migration dynamics by demonstrating spatial decisions made by individual herring at intermediate temporal (months) and spatial scales (km). Our work suggests that the capacity for individual behaviours in schooling fish may be underestimated.

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Spawning time of Atlantic herring (*Clupea harengus*) populations within a restricted area reflects their otolith growth at the larval stage

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ABSTRACT

Larval growth from three putative populations was estimated by microstructure analysis of otoliths of four year classes of adult herring sampled over a wide spawning season (February-June) in and around an inland brackish water lake (Landvikvannet) in southern Norway during the years 2012–2015. Mean width of daily increments at distances between 20 and 170 µm from the otolith core were significantly higher in Landvik herring (peak spawning in May) compared with the two other populations, Coastal Skagerrak spring spawners (peak spawning in March-April) and Norwegian spring spawning herring (peak spawning in February-March). These population differences were observed for all studied year classes and years and highly consistent with expected temperature dependent larval growth based on timing of successive spawning events. The observed patterns imply that timing of spawning was population specific with a tendency of adult herring to spawn at the same time and under the same conditions as they hatched themselves. This was also supported by vertebral counts, which are negatively correlated with temperatures during the embryonic stage. Firstly, Landvik herring which experienced higher ambient temperature during the embryonic stage were characterised by significantly lower counts than herring from the two other populations. Secondly, daily otolith growth also tended to decrease with increasing vertebral counts within the populations. The present study signifies the importance of otolith growth history for population discrimination in herring, even within the same spawning season, and further supports the use of vertebral counts in the continuous discussion on herring population structure, assessment and management.

1. Introduction

Population structure of Atlantic herring (*Clupea harengus*) is known to be highly complex (Iles and Sinclair, 1982) and it has been frequently studied in recent years (André et al., 2011; Lamichhaney et al., 2012; Johannessen et al., 2014). Genetic studies have revealed low levels of genetic differentiation among populations that have distinct temporal and spatial spawning locations, but mix during feeding migrations (Ruzzante et al., 2006; Gaggiotti et al., 2009; Bekkevold et al., 2015). However, clear genetic differentiations could be demonstrated among Baltic herring (Corander et al., 2013) as well as geographically isolated populations in Norwegian fjords (Pampoulie et al., 2015). Also, there is a plasticity and a high level of adaptability of herring in terms of contrasting behaviour, morphology and life history (McQuinn, 1997; Geffen, 2009). Hence, biological characteristics, like otolith microstructure or shape, can be used as population markers where genetic markers have not detected any differentiations (Mosegaard and Madsen, 1996; Clausen et al., 2007; Libungan et al., 2015b).

Otolith analysis is a powerful tool when analysing population structures of fish because it allows accurate estimates of age and growth of individuals at both the daily and yearly level (Campana and Thorrold, 2001). In marine species with high gene flow such as Atlantic cod (Cardinale et al., 2004), haddock (Begg and Brown, 2000), blue whiting (Mahe et al., 2016), European anchovy (Bacha et al., 2014) and Atlantic herring (Libungan et al., 2015a), otoliths have been used to detect population structures. Phenotypic information as well as experienced environmental changes can be extracted from otoliths (Campana, 1999). The otolith growth can be influenced by several factors such as temperature (Folkvord et al., 2004), prey density (Johannessen et al., 2000) and photoperiod (Mugiya, 1987). Consequently, differences in adult spawning patterns might be exhibited in the otolith microstructure of their larvae (Fitzhugh et al., 1997).

In Atlantic herring, otolith microstructure has been used to identify spring, autumn and winter spawners (Mosegaard and Madsen, 1996; Clausen et al., 2007). Otolith analysis is also used to separate mixing herring in the Skagerrak region for management and assessment purposes (ICES, 2016). Several studies on population dynamics of herring have used otolith microstructure analysis, but most studies

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have used otoliths of herring with distinct spawning seasons (Brophy and Danilowicz, 2002; Husebø et al., 2005; Brophy et al., 2006).

Within a restricted area in and around an inland brackish water lake (Landvikvannet) in southern Norway, three putative Atlantic herring populations (Norwegian spring spawners = NSS, Coastal Skagerrak spring spawners = CSS, and local Landvik herring = LV) have previously been described to mix during the spawning season based on analyses of vertebral counts, otolith shape and somatic growth (Eggers et al., 2014) as well as behavioural differences (Eggers et al., 2015). However, the actual timing and location of spawning events seems to be population specific; LV herring has peak spawning in May inside the lake, whereas the two other populations tend to spawn outside the lake in February-April (Eggers et al., 2014; Eggers et al., 2015).

In the present study, it is hypothesized that these populations have grown up under different environmental conditions resulting in phenotypic differences, and that they have adapted to the spawning time and location tightly linked to the season and conditions experienced when they hatched themselves. This hypothesis was tested by applying otolith microstructure analysis to a series of year classes of these populations traced over several spawning seasons and to link the daily otolith growth to presumed ambient temperatures experienced by successive larval cohorts. Our hypothesis would allow for population discrimination which, in general, is extremely important not only from ecological point of view but also because of frequent difficulties in stock management. The ecological impact, in terms of losing biodiversity due to sub-optimal exploitation and consequently overfishing of populations, would be immense when population discrimination fails (Begg et al., 1999). Further, existence of population-related differences in otolith microstructure on a small-scale basis will be investigated.

2. Material and methods

2.1. Study area

The study area consists of Landvikvannet and the adjacent fjord (Strandfjorden) along the Norwegian Skagerrak coast (Fig. 1). Strandfjorden has fully marine conditions and is sheltered from the outer coast. The inner part, where samples were collected, is relatively deep (10–13 m) compared to the outer part which is narrow and shallow (1–7 m). Landvikvannet (1.85 km^2) is connected to Strandfjorden and further the open ocean by a 3 km long and narrow 1–4 m deep canal constructed in 1877. This construction allowed marine organisms to enter Landvikvannet from Strandfjorden. Landvikvannet has average depth of 10 m and a maximum depth of 25 m. The saltwater inflow from Strandfjorden and the freshwater from streams result in a highly stratified water column with a transition depth at 4 m. Typically, in May the upper layer has low salinity (< 20), high temperature (> 10 °C) and oxygen content above 5 ml/l. In contrast, the lower layer has high salinity, low and constant temperature (8 °C) and no oxygen (for details, see Eggers et al., 2014).

2.2. Biological data

Adult herring were sampled with gillnets during the spawning season (February-June) between 2012 and 2015 in Landvikvannet and Strandfjorden (Table 1). The maximum sample size was 100 herring per location and sampling date. According to previous results (Eggers et al., 2014) herring were separated into three different populations: Norwegian spring spawners (NSS, n = 139) were separated by subjective otolith shape based on a sharper distinction between winter and summer rings compared to local spring spawners. NSS were found in both Landvikvannet and Strandfjorden. Coastal Skagerrak spring spawners (CSS, outside the lake, n = 333), and Landvik herring (LV, inside the lake, n = 359) were separated by sampling location only (Fig. 1). In total, all 831 available otoliths of adult herring were extracted and analysed. For this study herring of four consecutive year classes, 2009–2012, were chosen (Table 2). Metric (e.g. length, weight) and meristic (number of vertebrae) characters, were measured for each individual herring. Otoliths were extracted for age reading and further analysis of daily otolith growth.

2.3. Environmental data

Ambient temperature is the main factor affecting larval growth (Folkvord et al., 2004; Fey, 2006; Oeberst et al., 2009), and in the present study ambient temperature both during the larval stage and at successive spawning events of the same year class were estimated. Environmental data were measured on the same day as the adult herring were sampled, both in Strandfjorden and in Landvikvannet (Fig. 1, Table 1). Data from the sampling date were averaged for all depths below 2 m in Strandfjorden and between 2 and 5 m in Landvikvannet. The depth was limited to 5 m in Landvikvannet due to anoxic conditions below this depth (Eggers et al., 2014). These values were used as proxies for the spawning temperature. Therefore, only spawning herring (maturity stage = 6, Mjanger et al., 2012) were used for the analyses including ambient water temperature at spawning (Table 1). The majority of non-spawning herring were close to spawning (stage 5). Therefore, the overall temperatures during spawning might be slightly higher, but should not influence the analysis. In addition, continuous temperature measurements from the IMR Flødevigen marine stations (approximately 20 km northwards) were used to calculate mean temperatures for the period when the measured daily otolith increments were generated. Temperatures were measured each day in 1 m and 19 m depths. In general, the seasonal temperature trends were the same in Strandfjorden, Landvikvannet and Flødevigen (Fig. S1). Hence, the data from Flødevigen was used as proxies for the estimation of temperatures during the larval stages.

2.4. Otolith analysis

Otoliths were fixed to glass slides with thermoplastic glue with the sulcus side up and ground with sandpaper (600 and 1200 grid) until the sulcus disappeared. The slides were reheated and the otoliths turned over carefully. Further grinding and polishing was conducted until the nuclei (core) were visible. To avoid over-polishing, the otoliths were repeatedly checked under a Leica DMLB light microscope (Leica Microsystems, Wetzlar, Germany; 40 x magnification) and digital images were taken with a Nikon DS-Fi2 digital camera. From the calibrated digital images (2560 × 1920 pixels) the daily increments were detected and measured using the Caliper function in Image Pro-Plus® version 7.0 (Media Cybernetics, USA). Each otolith annotation was individually verified after the automatic software detection and missing or additional increments were manually added or removed, respectively. Daily increments were registered from the core up to a distance of 170 µm from the core. Only increments with a minimum distance of 20 µm from the core were used for the analyses, because earlier developed increments are not necessarily daily or easily discernible (Geffen, 1982; Campana et al., 1987; Fox et al., 2003).

2.5. Statistical analysis

All statistical analyses and plotting were conducted in the R software (R Core Team, 2016). For all tests, we used the 95% level as the level of significance.

For statistical analyses, we used linear mixed-effects models to indicate the influence of different characteristics on the daily otolith growth of larval herring. The modelling followed a backward selection approach incorporating all fixed and random effects. First the optimal structure of the random effects was tested using likelihood ratio test based on the models fitted by restricted maximum likelihood estimations (REML) (Zuur et al., 2009). Also based on REML fits, the fixed effects structure was optimized using marginal *F*-statistics (Pinheiro and

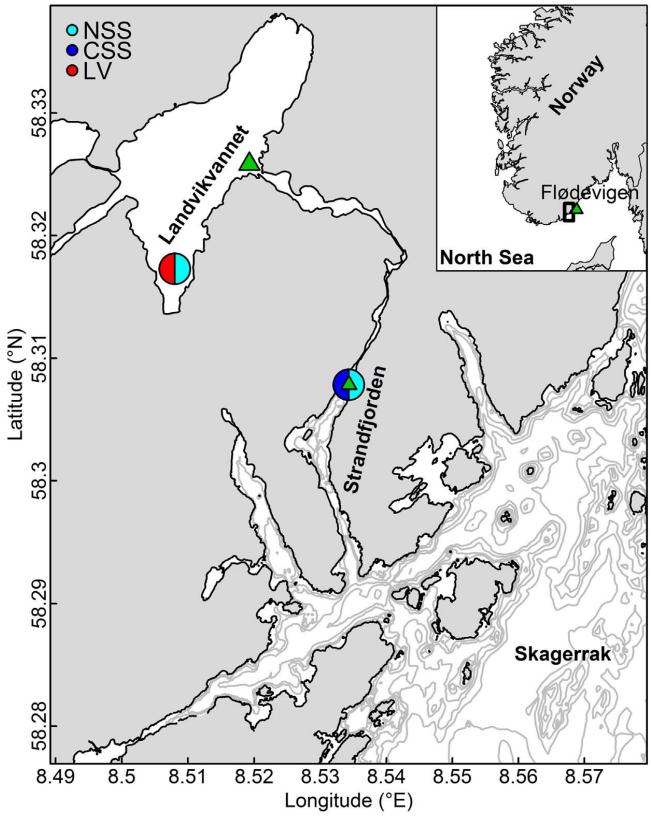

Fig. 1. Map of the study area, including Landvikvannet and adjacent fjords, indicating the sampling locations of three different herring populations (circles) and environmental data (triangles); NSS = Norwegian spring spawners, CSS = coastal Skagerrak spring spawners, LV = Landvik herring.

Bates, 2000). For all models, both the random effect a and the residual ε are assumed to be normally distributed with mean of zero and variance σ^2_{pop}. This structure allows for different residual variances depending on the herring populations. This model structure was also used to test if there were differences between sexes, NSS found at the two locations, and samples within a year. All mixed-effects models were fitted using the 'lme' function within the 'nlme' R-package (Pinheiro and Bates, 2000).

Daily otolith growth showed an approximate linear trend up to a distance of 80 µm from the core. To test the significance of this linear trend of daily otolith growth as a function of the three herring populations and the distance from the core (dis_{ij}) a model was fitted

Table 1
Total number of analysed otoliths per sampling date by herring populations; NSS = Norwegian spring spawners, CSS = coastal Skagerrak spring spawners, LV = Landvik herring. NSS were separated based on the sampling location in SF = Strandfjorden or LA = Landvikvannet. Numbers in brackets indicate number of spawning herring. Temperature were measured in both Strandfjorden and Landvikvannet.

Sampling date	NSS in SF	NSS in LA	CSS	LV	Temperature (° C) in SF	Temperature (° C) in LA
20/03/2012	0	3 (2)	0	9 (5)	6.32	7.50
26/03/2012	14 (11)	12 (4)	25 (18)	19 (8)	6.84	7.95
11/04/2012	10 (6)	8 (2)	19 (11)	20 (5)	7.33	7.65
14/05/2012	6 (4)	5 (3)	23 (7)	29 (7)	9.19	9.74
21/06/2012	0	0	0	21 (4)	12.68	14.10
06/05/2013	12 (10)	4 (0)	40 (19)	41 (14)	7.08	7.54
13/05/2014	14 (3)	1 (0)	22 (8)	37 (7)	9.60	10.12
26/02/2015	0	0	9 (1)	0	6.17	8.24
12/03/2015	3 (2)	0	11 (8)	0	6.27	7.82
15/04/2015	12 (4)	0	62 (14)	0	6.90	8.06
28/04/2015	21 (18)	2 (0)	58 (31)	45 (13)	7.59	9.15
06/05/2015	9 (8)	2 (1)	64 (35)	75 (47)	8.56	9.94
19/05/2015	No sample	1 (0)	No sample	63 (36)	9.46	11.12
Total	101 (66)	**38 (12)**	**333 (152)**	**359 (146)**		

Table 2
Total number of analysed otoliths from different year classes and herring populations (NSS = Norwegian spring spawners, CSS = coastal Skagerrak spring spawners, LV = Landvik herring) and their mean biological characteristics (VS = vertebrae sum). NSS were separated based on the sampling location. Numbers in brackets indicate number of spawning herring.

Herring population	2009	2010	2011	2012	VS	Length (cm)	Age (year)
NSS in Strandfjorden	61 (38)	12 (7)	18 (15)	10 (6)	57.5	29.3	4.0
NSS in Landvikvannet	33 (11)	4 (0)	0 (0)	1 (1)	57.6	28.6	3.4
CSS	129 (60)	87 (35)	69 (37)	48 (20)	56.3	28.2	4.0
LV	161 (56)	95 (38)	77 (35)	26 (17)	55.9	27.7	3.9
Total	384 (165)	**198 (80)**	**164 (87)**	**85 (44)**			

of the form:

$$Width_{ij} = \alpha + \beta_1 \times dis_{ij} + \beta_2 \times pop_i + \beta_3 \times dis_{ij} \times pop_i + a_i + \varepsilon_{ij}$$

$Width_{ij}$ is the width between two increments starting with observation $j = 1$ for first increment after $dis > 20$ of the individual otolith i. The term a_i is the random intercept for the individual otolith i. Due to the linear increase only increments between 20 and 80 µm were included for all further models. The next linear mixed-effects model used to demonstrate differences among the successive year classes in terms of daily otolith growth was of the following form:

$$Width_{ij} = \alpha + \beta_1 \times Yclass_i + \beta_2 \times pop_i + \beta_3 \times dis_{ij} + \beta_4 \times Yclass_i \times dis_{ij} + \beta_5 \times dis_{ij} \times pop_i + a_i + \varepsilon_{ij}$$

$Yclass_i$ and pop_i are categorical variables representing the four year classes and three herring populations, respectively. The differences per year class were incorporated in the random effects structure of further models. Following, a random slope (a_k) for the year classes was included, in addition to the random intercept (a_i) for the individual otolith.

For the further analyses, NSS were excluded due to relatively low numbers and since these were hard to distinguish from CSS and their multiple spawning locations could potentially compromise the interpretation of the data for the two other populations which had different spawning locations. The linear model indicating differences in daily otolith growth including the number of vertebrae (vs_{ik}), one of the main defining characteristics, and the actual age of herring at capture (age_{ik}) had the form:

$$Width_{ijk} = \alpha + \beta_1 \times vs_{ik} + \beta_2 \times pop_{ik} + \beta_3 \times age_{ik} + \beta_4 \times dis_{ijk} + \beta_5 \times pop_{ik} \times dis_{ijk} + \beta_6 \times age_{ik} \times pop_{ik} + \beta_7 \times dis_{ijk} \times age_{ik} + \beta_8 \times dis_{ijk} \times vs_{ik} + a_i + a_k + \varepsilon_{ijk}$$

Temperatures during the larval period corresponding to the formation of the measured daily increments were estimated based on daily temperature measurements in Fløedevigen. Measured temperatures were directly used as a proxy for Strandfjorden. Since temperature samples on the same day indicated that Landvikvannet is on average 1.15 °C warmer than Strandfjorden, this was added for the estimations in Landvikvannet (Table 1). According to Eggers et al. (2014) spawning periods of CSS and LV herring are March-April (Julian $day_{start} = 60 - day_{end} = 120$) and May (Julian day $day_{start} = 121–day_{end} = 151$), respectively. For the estimation, it is assumed that all herring spawn synchronized at the same day in the middle of the spawning period. Consequently, we assume Julian day 90 as the day of spawning (day_{spawn}) in Strandfjorden and Julian day 136 as day_{spawn} in Landvikvannet (Fig. S1). According to Blaxter and Hempel (1961) hatching occurs in average 130 day degrees after spawning. These assumptions allowed for the estimation of the day of hatching (day_{hatch}) for each individual year class of CSS and LV herring:

$$day_{hatch} = day_{spawn} + 130 \div temp_{spawn}$$

$temp_{spawn}$ is the average temperature during the spawning period:

$$temp_{spawn} = \sum_{x=day_{start}}^{day_{end}} \left(\frac{temp_{1\,x} + temp_{19\,x}}{2} \right) \div (day_{end} - day_{start})$$

where $temp_1$ and $temp_{19}$ are the measured temperatures in Fløedevigen at depth 1 m and 19 m, respectively. The temperature at hatching was estimated as:

$$temp_{hatch} = \left(\frac{temp_{1\,day_{hatch}} + temp_{19\,day_{hatch}}}{2} \right)$$

A linear relation among the temperature at hatching ($temp_{hatch}$) and the age of larvae with an otolith radius of 20 µm ($age_{20\,\mu m}$) is assumed. Based on larvae having an average sagitta size of 20 µm at 25 days post hatching = dph at 8 °C (Folkvord et al., 2000) and at 12 dph at 12 °C (Folkvord et al., 2004), the age of larvae with an otolith radius of 20 µm was linearly interpolated as:

$$age_{20\mu m} = 51 - 3.25 \times temp_{hatch}$$

Adding the estimated age and the day of hatching indicated the day ($day_{20\,\mu m}$) when the first measured increments was generated:

$$day_{20\mu m} = day_{hatch} + age_{20\mu m}$$

This day was used as starting point to estimate the average temperature ($temp\_L$) larvae had experienced during the time when they generated the measured daily increments used in this study for the next 30 days:

$$temp\_L = \sum_{x=day_{20\mu m}}^{day_{20\mu m}+30} \left(\frac{temp_{1\,x} + temp_{19\,x}}{2} \right) \div 30$$

Finally, this temperature was used in the next linear model to explain differences in daily otolith growth among the year classes within a population and the general difference among the populations:

$$Width_{ij} = \alpha + \beta_1 \times temp\_L_i + \beta_2 \times pop_i + \beta_3 \times dis_{ij} + \beta_4 \times dis_{ij} \times pop_i + \beta_5 \times temp\_L_i \times pop_i + a_i + \varepsilon_{ij}$$

For the last linear mixed-effects model, only otoliths of spawning herring were included and linked to the ambient water temperature ($temp\_S_{ijk}$) when herring were sampled. This selection was necessary to ensure imminence spawning at the locations, because herring could theoretically migrate between Strandfjorden and Landvikvannet without spawning when they were in stage 5. The model should test whether herring with highest otolith growth spawn at highest temperatures:

$$Width_{ijk} = \alpha + \beta_1 \times tempS_{ik} + \beta_2 \times pop_{ik} + \beta_3 \times dis_{ijk} + a_i + a_k + \varepsilon_{ijk}$$

3. Results

The total body length of herring analysed overall years and year classes ranged from 23.0 to 34.0 cm with a mean value of 28.1 cm. Among the three populations NSS were largest and had the highest mean vertebrae sum = VS, followed by CSS with intermediate length and VS and LV herring being smallest with lowest VS (Table 2). The mean age of selected herring did not differ among the three popula-

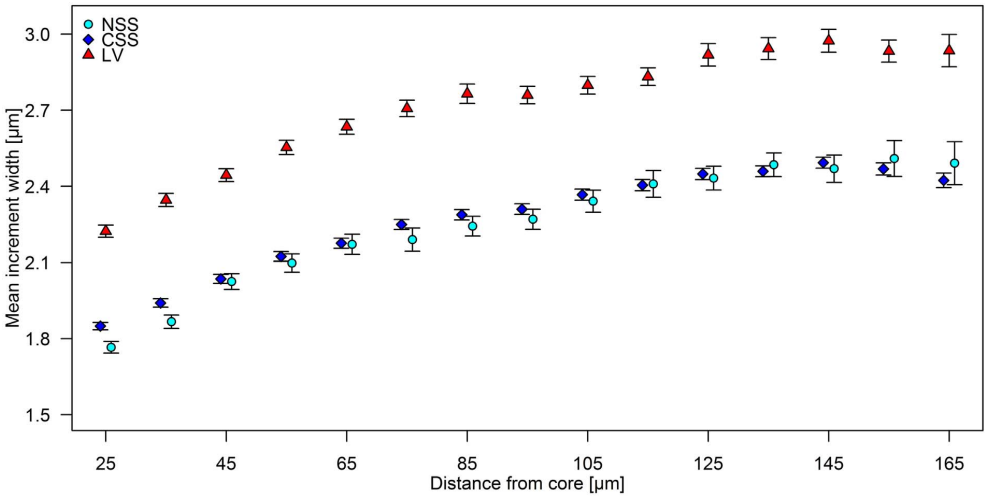

Fig. 2. Mean daily otolith growth of different herring populations as a function of the distance from the core of an otolith: NSS = Norwegian spring spawners, CSS = coastal Skagerrak spring spawners, LV = Landvik herring. Mean values and 1 SE are given.

tions, but NSS inside Landvikvannet were slightly younger, whereas daily otolith growth of NSS herring caught in Landvikvannet did not differ significantly from those in Strandfjorden (ANCOVA: $F = 2.34$, $df = 139$, $p > 0.05$). Neither in 2012 nor in 2015 did daily otolith growth change within the spawning season ($ANOVA_{2012}$: $F = 3.13$, $df = 219$, $p > 0.05$; $ANOVA_{2015}$: $F = 1.90$, $df = 433$, $p > 0.05$). There were no differences in daily otolith growth (mean width of increments) between sexes (ANCOVA: $F = 1.71$, $df = 813$, $p > 0.05$). Therefore, all further analyses were conducted with sexes combined, NSS as a single population, and including all samples per season.

Differences in daily otolith growth among all three populations were obvious by comparing the mean width of increments per distance from the core of an otolith (Fig. 2). The highest daily otolith growth at larval stage could be demonstrated in LV herring, followed by CSS and NSS herring. Otolith growth followed an approximate linear increase up to a distance of 80 µm from the core (ANOVA: $F = 7.67$, $df = 22510$, $p < 0.01$), but levelled out for larger distances and even showed a slight decrease at greatest distances from the core for CSS herring. Daily otolith growth differed for all three herring populations as well as within each population for the four continuous year classes (ANCOVA: $F = 6.8$, $df = 825$, $p < 0.01$) (Fig. 3).

Daily otolith growth was also lower for CSS and LV herring with higher number of vertebrae for CSS and LV herring (ANOVA: $F = 38.06$, $df = 676$, $p < 0.01$), but this factorial differences were more prominent in LV herring (Fig. 4A). For both populations, a higher daily otolith growth was observed for herring with lower age (ANOVA: $F = 5.03$, $df = 676$, $p < 0.05$) (Fig. 4B). This effect was not significant, when excluding 3-year-old herring (ANOVA: $F = 0.65$, $df = 398$, $p > 0.05$).

Higher back-calculated larval stage temperatures for the individual year classes resulted in higher daily otolith growth, also within populations (ANOVA: $F = 3.89$, $df = 688$, $p < 0.05$) (Fig. 5). Furthermore, the general differences in daily otolith growth between CSS and LV herring could be linked to the back-calculated larval stage temperature. Adult herring with highest otolith growth experienced during the larval stage also tended to spawn also at warmer ambient water temperature during successive spawning events (ANOVA: $F = 5.74$, $df = 295$, $p < 0.05$).

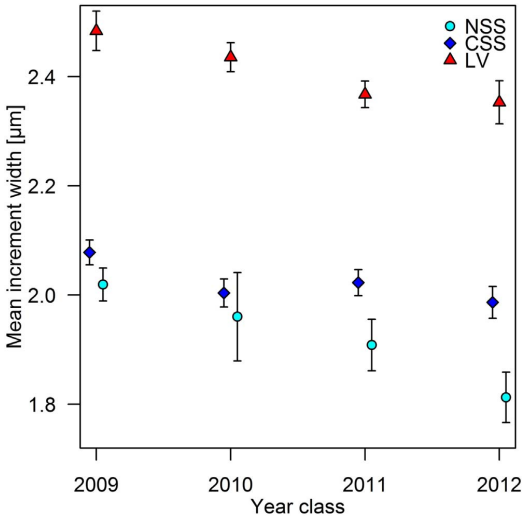

Fig. 3. Average observed daily otolith growth (mean increment width) from 2012 to 2015 of four continuous year classes of three populations; NSS = Norwegian spring spawners, CSS = coastal Skagerrak spring spawners, LV = Landvik herring. Mean values and 1 SE are given.

4. Discussion

Larval growth from three putative herring population estimated by microstructure analysis of otoliths of adult herring showed significant variation among these populations mix during the spawning season in Landvikvannet and adjacent waters. Growth was generally higher in Landvik herring (LV) compared with the two other populations, coastal Skagerrak spring spawners (CSS) and Norwegian spring spawners (NSS). These population differences were consistent for all studied year classes in all years.

NSS were observed in the study area the first time in 2012 (Eggers et al., 2014). They most likely were originating from spawning grounds

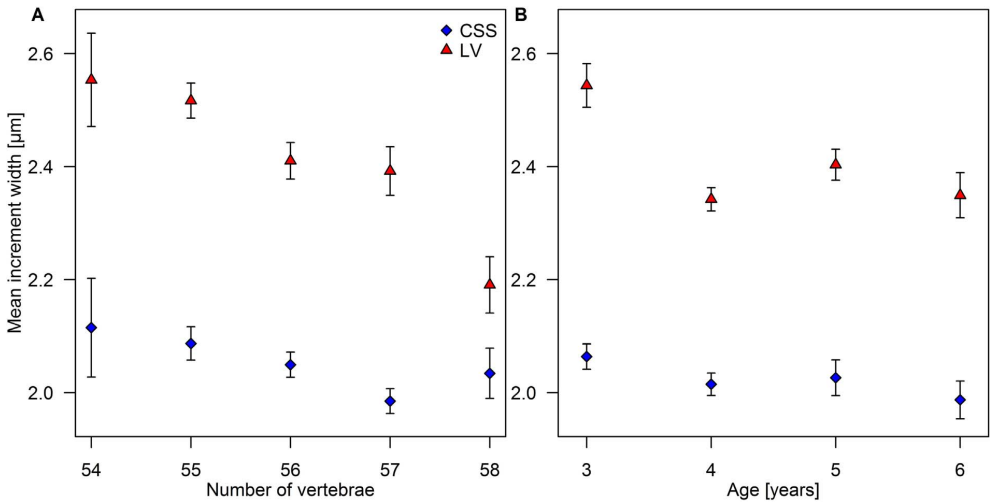

Fig. 4. Mean daily otolith growth versus (A) number of vertebrae and (B) age of herring of two populations: CSS = coastal Skagerrak spring spawners, LV = Landvik herring. Mean values and 1 SE are given.

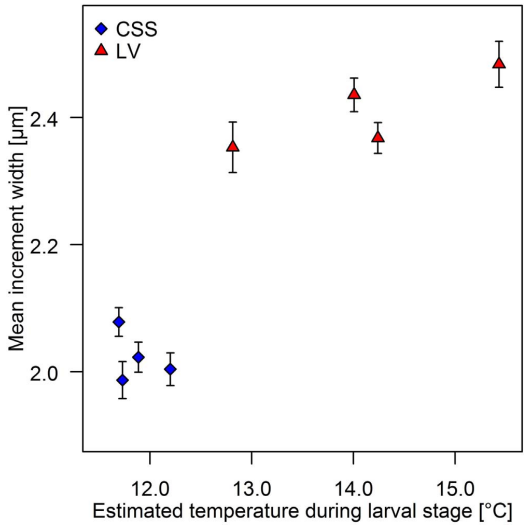

Fig. 5. Average daily otolith growth versus estimated temperatures experienced during the larval stage of different year classes and populations: CSS = coastal Skagerrak spring spawners, LV = Landvik herring. Mean values and 1 SE are given.

at the Norwegian west coast, just exploring new areas as they have a very dynamic utilisation of spawning grounds (Slotte, 1999, 2001). This is also supported by the finding that NSS found in Strandfjorden and Landvikvannet did not differ in otolith growth or vertebral counts. Consequently, only 3-year-old NSS sampled in 2015 could theoretically been spawned and hatched within the study area. However, NSS herring having their peak spawning in February-March (Eggers et al., 2014) experience the coldest ambient water temperatures, resulting in the lowest daily otolith growth at larval stages.

Back calculated water temperatures experienced during the larval stages for each year class of CSS and LV herring emphasised the importance of environmental conditions on otolith growth (Høie et al., 1999; Folkvord et al., 2004; Husebø et al., 2007). Larvae of LV herring spawned in May grew up under warmer conditions than CSS larvae spawned in March-April. Besides the differences in water temperature resulting in divergent daily otolith growth among the year classes, also the feeding conditions have an impact on the year class differences (Johannessen et al., 2000). In addition, the tendency to return to spawn under conditions they hatched themselves is supported by other studies (Brophy and Danilowicz, 2002; Husebø et al., 2005). Further, adult herring with largest otolith growth favour to spawn under warmer conditions. This indicates the adaptation and fidelity of adult individuals to a time and conditions of spawning resembling their own situation at the larval stage. An estimated Q_{10} value (calculated according to: Schmidt-Nielsen, 1990) of 4.2 for the otolith growth of CSS and LV herring corresponds to the average temperature differences of 1.15 °C during the larval stage. This value is in accordance with an otolith growth Q_{10} value of 3.4 at 8–12 °C (Folkvord et al., 2004) or an even higher Q_{10} value of 5.3 at lower temperatures from 6 to 10 °C (unpublished data, Folkvord).

Although, potential mixing during spawning and indications of a metapopulation exist in the study area (Eggers et al., 2014), the results of the present study clearly support that these three populations have grown up under differential environmental conditions resulting in phenotypic differences. This could be due to origination from different hatching times and locations, or both. In a recent study, Martinez Barrio et al. (2016) demonstrate that spawning time and location are implemented in the herring genome irrespective of each other. However, our analysis focused on average differences in otolith growth among these populations, and it is therefore not completely possible to exclude a potential mixing of populations in the spawning aggregations. Analysis on the individual levels indicated a small overlap between CSS and LV herring and a clear separation of individuals was not feasible. Still, the very specific characteristics of LV herring indicate that it is homing to spawn in its own hatching location inside Landvikvannet at nearly the same time of year as they were spawned themselves. For CSS herring, such a homing tendency to Strandfjorden is much more uncertain as the characteristics of this population in terms of growth and vertebral counts is generally found in herring all along the east coast of Norway with a similar timing of spawning. Hence, these CSS herring could have hatched at other locations, while the spawning time remains constant.

The relation between vertebral counts of adult fish and daily otolith

growth of the same individuals demonstrated in this study, even within populations, supports previous studies suggesting an environmental influence on the vertebral development (Tåning, 1952; Lindsey, 1988). Still, some of the differences in vertebral count among the populations cannot be attributed to the environment during incubation. Recent studies has demonstrated a higher parental effect on the number of vertebrae in herring compared as environmental conditions (unpublished data). This might explain why CSS herring with 58 vertebrae have similar otolith growth compared to herring with 56–57 vertebrae. Likewise, the heredity of vertebrae is well known for different marine species with a complex population structure (Christiansen et al., 1988; Løken and Pedersen, 1996).

One common result for CSS and LV herring was the decrease in daily otolith growth at the larval stage between 3 year olds compared to 4–6 year olds of the same year class. This indicates that growth already at the larval stage might be of importance for age at first spawning in a year class. When the year class is fully recruited to the spawning population, these differences are evened out. This is in accordance with herring in the Celtic Sea where first time spawners at age 1 had a significantly higher growth than first time spawners at age 2 (Brophy and Danilowicz, 2003) as well as the general trend of life history models predicting an earlier maturity of fast growing fish (Stearns and Koella, 1986). Besides the maturity effect, gear selectivity might also influence the results. If a higher otolith growth at larval stages also results in higher growth later, these herring might reach earlier a body length that is favourably selected by the fishing gears. If 3-year-old herring with slow otolith growth were not representatively caught, the otolith growth of the sampled herring is overestimated. However, since CSS herring have generally higher growth than LV herring (Eggers et al., 2014), also slow growing 3 year olds should have been found. However, the decrease was the same for both populations, therefore a fishing gear selection will have a minor impact.

To our knowledge, this is the first time that differences in daily otolith growth among overlapping herring populations has been shown within a restricted area over the same spawning season. These differences were seen in continuous year classes and over several years, with a clear link to environmental conditions experienced during hatching and successive spawning. The study signifies the importance of otolith growth history for understanding the dynamics of herring spawning time and location, and its effect on population discrimination. It further contributes knowledge to the interpretation of differences in vertebral counts, which might be of value for the continuous discussion on herring population structure, assessment and management.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2017.05.009.

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Supplementary material

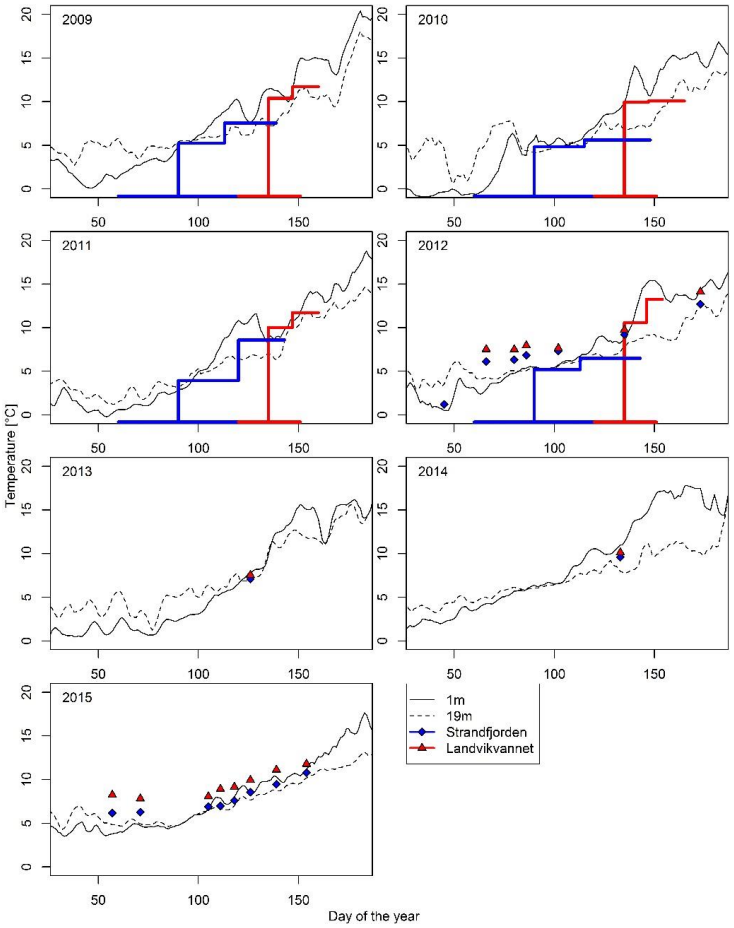

Figure S1. Water temperature at from the IMR Flødevigen marine stations in 1 m (black solid lines) and 19 m (black dashed lines) during the years 2009-2015. Points indicate the water temperature in Strandfjorden (blue diamond) and Landvikvannet (red triangle) from each sampling date. Coloured lines indicate the periods to estimate the average temperature during the larval period, including the spawning period (lowest level), incubation times of eggs (middle level) and the time until the otolith had a size of 20 µm (highest level), corresponding to the formation of the measured daily increments.

Paper IV

PLOS ONE

RESEARCH ARTICLE

Comparative biology and population mixing among local, coastal and offshore Atlantic herring (*Clupea harengus*) in the North Sea, Skagerrak, Kattegat and western Baltic

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Abstract

The population structure of Atlantic herring (*Clupea harengus*) from 13 local, coastal and offshore areas of the North Sea, Skagerrak, Kattegat and western Baltic (northeast Atlantic) was studied using biological and environmental data from 1970–2015. The objective was to identify distinct populations by comparing variability in the temporal and spatial phenotypic characteristics and evaluate the potential for mixing of populations in time and space. The populations varied in biological characteristics such as mean vertebral counts (VS), growth and maturity ogives. Generalized additive models indicated temporally stable VS in the North Sea and western Baltic, whereas intra-annual temporal variation of VS occurred in other areas. High variability of VS within a population was not affected by environmental factors such as temperature and salinity. Consequently, seasonal VS variability can be explained by the presence or absence of herring populations as they migrate between areas. The three main populations identified in this paper correspond to the three managed stocks in this area: Norwegian spring spawners (NSS), western Baltic spring spawners (WBSS) and North Sea autumn spawners (NSAS). In addition, several local populations were identified in fjords or lakes along the coast, but our analyses could not detect direct mixing of local populations with the three main populations. Our results highlight the importance of recognizing herring dynamics and understanding the mixing of populations as a challenge for management of herring.

Introduction

In many coastal and offshore areas, fish originating from different spawning populations mix during non-spawning seasons and can be targeted simultaneously by fisheries. Such mixing can be a challenge for fisheries managers who often prefer to use the term 'stock' in the management context instead of population. Throughout this paper, the term 'stock' is

used in the management context, defined by International Council for the Exploration of the Sea (ICES) [1] as "a part of a fish population (or several populations) usually with a particular migration pattern, specific spawning grounds, and subject to a distinct fishery. In theory, a 'stock' comprises all the individuals of fish in an area, which are part of the same reproductive process". This definition does not necessarily imply that a stock consists only of a single population. Here we use, a 'population' as a reproductive 'unit' of herring in the evolutionary context [2].

Herring (*Clupea harengus*) in the Atlantic are known for their complex population structure [3]. In the northeast Atlantic, several herring populations, which can be distinguished by spawning grounds and spawning times, otolith characteristics and number of vertebrae [e.g. 4, 5], have been identified [6, 7]. However, for management purposes, it is necessary to assign all herring catches to an appropriate stock. Herring caught in the North Sea or Skagerrak (Fig 1) are managed as three different stocks[8]. The majority of individuals are assigned to either the North Sea autumn spawners (NSAS) or the western Baltic spring spawners (WBSS). The third stock, the Norwegian spring spawners (NSS), occurs in smaller proportions along the Norwegian coast [9] and in the Skagerrak [10, 11]. The eastern North Sea and Skagerrak is known to be an important area where mixing of a number of herring population occurs [12], with important implications for fisheries management [13].

Whilst individuals are assign to one of the three main stocks (NSAS, WBSS and NSS), smaller populations which occur within the area are not assessed separately by ICES. These include smaller spring spawning populations in the North Sea [14], summer/autumn spawning populations [15] and several local fjord populations [16] along the Norwegian coast. Most local populations along the Norwegian coast encounter relatively uniform and stable environmental conditions within the fjords [17]. Other local herring populations (based on their geographical location and biological characteristics) have been identified in semi-enclosed coastal systems or even lakes. However, many of these local populations migrate at least once each year, e.g. Limfjorden [18] or Landvikvannet [10] and could occur in catches from fisheries targeting NSAS and WBSS herring. Despite some evidence of connectivity between local and offshore populations [5, 11], their interaction and connectivity have not been fully explored as yet. The maintenance of diversity by avoiding overexploitation of these local populations is an important objective in management [19, 20].

A number of individual characteristics can be used to assign or identify the population origins [4, 21, 22]. Here, we utilize the number of vertebrae as a means of distinguishing populations. Environmental conditions such as temperature and salinity can influence the number vertebrae, but it is fixed already during the embryonic development [23, 24]. However, differences in vertebral counts between populations also has a genetic basis [25]. Consequently, the range in numbers of vertebrae is population specific, which allowed Swain et al. [26] to delineated stocks of Atlantic cod (*Gadus morhua*) using vertebral counts. Herring populations have also been distinguished based on vertebral counts [11, 27], and stock composition in catches and surveys have been determined in this way for management purposes [8, 28, 29].

The main objective of this study is to investigate the complex population structure of Atlantic herring, by comparing historical data of the temporal and spatial variation in phenotypic characters of herring from 13 different geographical areas: four offshore areas (North Sea, Skagerrak, Kattegat and western Baltic), two coastal areas (west and east coast of Norway) and seven local fjords and lakes along the Norwegian and Danish coast (Fig 1). Distinct populations were identified based on phenotypic characteristics, and their maturity and potential mixing and interaction in time and space were investigated. Studies such as these are important for determining the extent of populations co-occurring in space and time, and thus being subjected to a fishery not recognizing the extent of mixed populations in catches.

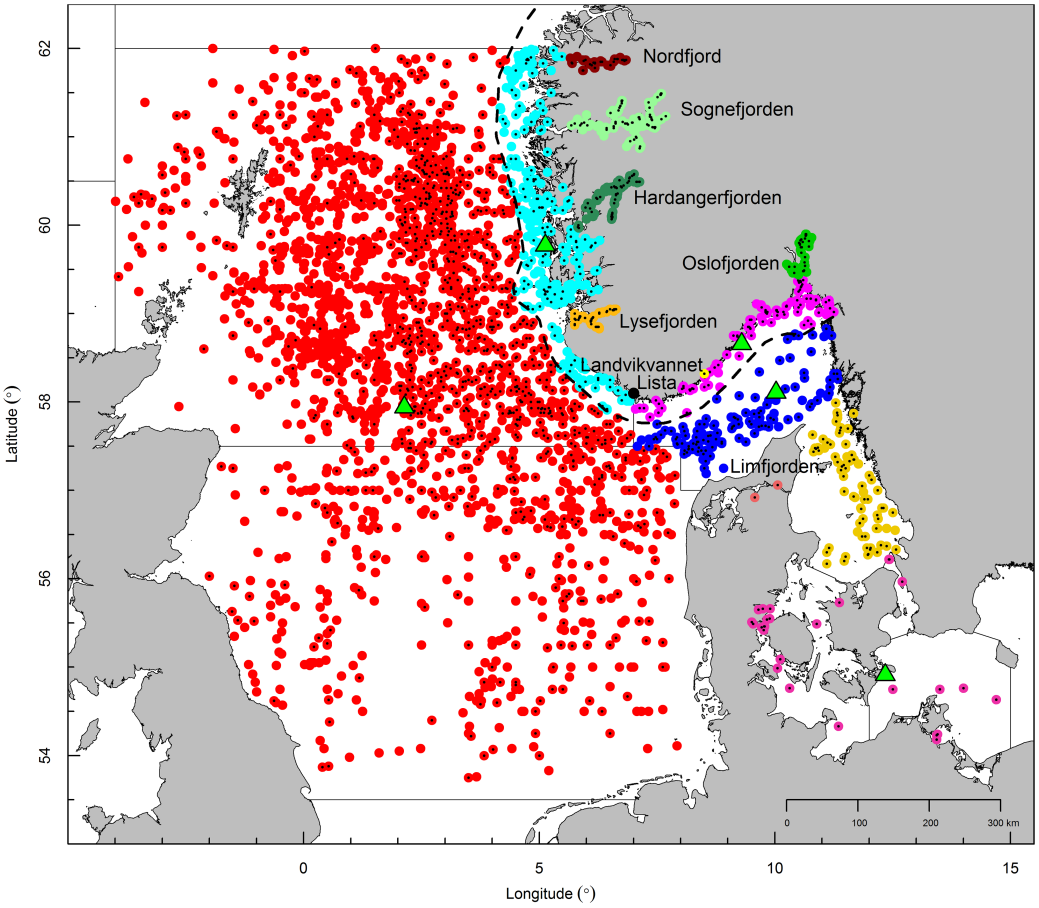

Fig 1. Map of the study area. Locations of sampling stations where biological data were collected from 1970–2015. There are 13 areas that include northern sections of the North Sea (red), west coast of Norway (cyan), east coast of Norway (purple), Skagerrak (blue), Kattegat (gold), western Baltic (pink) and adjacent fjords or lakes (also color-coded). Black dots indicate stations where the number of vertebrae were counted. Thick black stippled line = 12 NM zone. Mean position of hydrographic samples (green triangles) are shown for the North Sea, west coast, east coast, Skagerrak and western Baltic.

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Materials and methods

Study area

Samples from the northern North Sea, Skagerrak, Kattegat, western Baltic and southern Norwegian coast (including several fjords and lakes) were examined. To analyze spatial aspects of populations, the area was partitioned and fish were classified according to their location of capture (see Fig 1). The North Sea, Skagerrak, Kattegat and western Baltic were classified as offshore areas. The west and east coast of Norway were defined as coastal areas. These five major areas, except Kattegat due to data limitation, were included in the analyses to investigate the dynamics of maturation and spawning time and vertebral counts. A third geographical

classification was the 'local population area' that included fjords and some small lakes, such as Nordfjord, Sognefjorden, Hardangerfjorden and Lysefjorden (along the western coast of Norway). Local population areas also included Landvikvannet, Oslofjorden (along the eastern coast of Norway) and Limfjorden (Denmark). The local population areas are adjacent to the offshore areas and potentially inhabited by herring populations. S1 Supporting Information gives a detailed description of all the areas investigated.

Data sources

Biological data. Biological data from 428,773 herring, collected from the years 1970–2015, were extracted from databases at IMR (Norway) and DTU-Aqua (Denmark) and used for the analyses. All data from the IMR database were included, whereas only data with vertebral counts were extracted from the DTU-Aqua database. In general, samples from the same time and location which had less than 10 herring were excluded. The data originated both from regular scientific surveys and commercial catch sampling. The time and area of fish capture as well as the fishing gears used varied (S1–S3 Tables). Due to the survey design in the local fjords, there was a potential bias towards smaller fish in these areas. There was repeated annual sampling in fjords and it is generally assumed that the presence of older and larger fish demonstrates the presence of local spawning populations, whereas their absence would indicate that the area was a nursery area with no local herring population.

The usual standard sample size comprised 100 herring, but some samples were smaller (limited by small total catches). Biological parameters included total length (nearest 0.5 cm below), sex, stage of maturity, age (as determined by counts of winter rings (wr) from otoliths), and, for most samples, the number of vertebrae. Maturity stages were determined by visual inspections of gonads according to the following scale: immature = 1–2, maturing = 3–4, ripe = 5, spawning/running = 6, spent = 7 and recovering = 8 [30].

Physical data. Annual mean temperatures and salinities of sampling areas were estimated for each spawning seasons in each area. The spawning seasons were defined as August-October for the North Sea [6], February-March for the west coast [31], March-May for the east coast, Skagerrak, and the western Baltic [7, 32]. These periods correspond to the time when vertebral counts are fixed [23, 24]. Temperature and salinity data for the spawning seasons within each area were extracted from the ICES Dataset on Ocean Hydrography [33]. Values used for temperature and salinity were the means between depths of 20–150 m.

Ethical statement

Most samples were collected during standard scientific surveys or from commercial catches in national and international waters under international rights. Otherwise, the Institute of Marine Research (IMR), which is responsible for monitoring herring and giving advice to fisheries managers in Norway, has permission to sample herring at any location along the Norwegian coast by the Directorate of Fisheries, Bergen, Norway. The same accounts for the DTU-Aqua in Denmark. A special permission to sample herring with gillnets inside Landvikvannet was granted by the County Governor of Aust-Agder, Arendal, Norway. Our study did not involve any endangered or protected species.

Data analysis

All statistical analyses and figures were made using statistical software packages in R [34]. For all tests, we used 95% as the level of significance.

Length-at-age data, used as a proxy for growth of herring, were fitted to the von Bertalanffy growth model (VBGM) [35]:

$$L_t = L_\infty(1 - e^{-K(t-t_0)})$$

where L_t is the average length at age t, L_∞ is the asymptotic maximum length, K is the von Bertalanffy growth rate coefficient (i.e., the rate at which length approaches the maximum length asymptote) and t_0 is the intercept on the time axis.

Length-at-first-maturity was quantified by a generalized linear model [36] using a logistic regression to estimate the probability of herring to be mature at a certain length. Herring were grouped as immature or mature. The following model was used to calculate the length when 50% and 95% were mature:

$$logit(M) = \beta_0 + \beta_1 \times L$$

where β_1 describes the estimated effect of length L in the probability (M) to be mature.

For spatio-temporal comparison, the mean number of vertebrae (VS) was calculated for each ICES Statistical rectangle (1˚ Longitude, 0.5˚ Latitude) per quarter of the year. For purposes of illustration, these results were divided into five categories. The mean number of vertebrae of western Baltic spring spawners (<55.9) [28], North Sea autumn spawners (~56.5) [28] and Norwegian spring spawners (>57.2) [37] were used as reference points of the five categories. The variability of VS within each area was estimated as the difference of samples from the overall mean of the area (see Fig 2). In this study, a difference larger than ±0.25 was defined as highly variable (S1 Fig).

For the following analyses, only mature herring with three or more winter rings were used. In addition, only data from the five major areas (North Sea, west coast, east coast, Skagerrak and western Baltic) were included to investigate the dynamics in these areas. The data were fitted to generalized additive models (GAMs), since they allow flexible non-parametric effects of covariates [38]. Model selection was based on the generalized cross validation (GCV) score. Residual plots were used for checking model fits, and isotropic smoothing functions $s()$, uniform in all orientations, were used to define smooth terms (thin-plate regression spline) [39]. For further details on data exploration and model selection see SI Materials and methods.

The final GAM for dynamics among the areas in terms of vertebral count differences was:

$$VS_i = \alpha + \beta(Area) + s(Yclass_i) + s(Quat_i) + s(Mat_i) + \varepsilon_i$$

where VS is the number of vertebrae, $Area$ represents the five major areas, $Yclass$ is the calculated year of hatching (= sampling year minus the age (wr)). For autumn spawners (assumed for the North Sea) the actual age is wr + 1 since no winter ring is formed during their first winter. Aging methods were the same in all areas and did not affect the analyses. $Quat$ and Mat represent the quarter of the year when herring were sampled and the stage of maturity, respectively, and ε is the error term. Since the quarter of year and maturity stage were not correlated, differences in VS for pre-, post- or spawning herring would show the occurrence of different populations/stocks due to migration into and out of areas. Length, age, gear type, and the mean temperature and salinity during hatching of each year class were included in the initial model, but removed due to non-significance.

Spawning dynamics were analyzed by calculating the proportion of pre-spawning, spawning, and post-spawning herring per sample. For a comparison of intra-annual variations, the data of pre-spawning, spawning and post-spawning herring were fitted individually to a GAM:

$$Prop_i = \alpha + \beta(Area) + s(Month_i) + \varepsilon_i$$

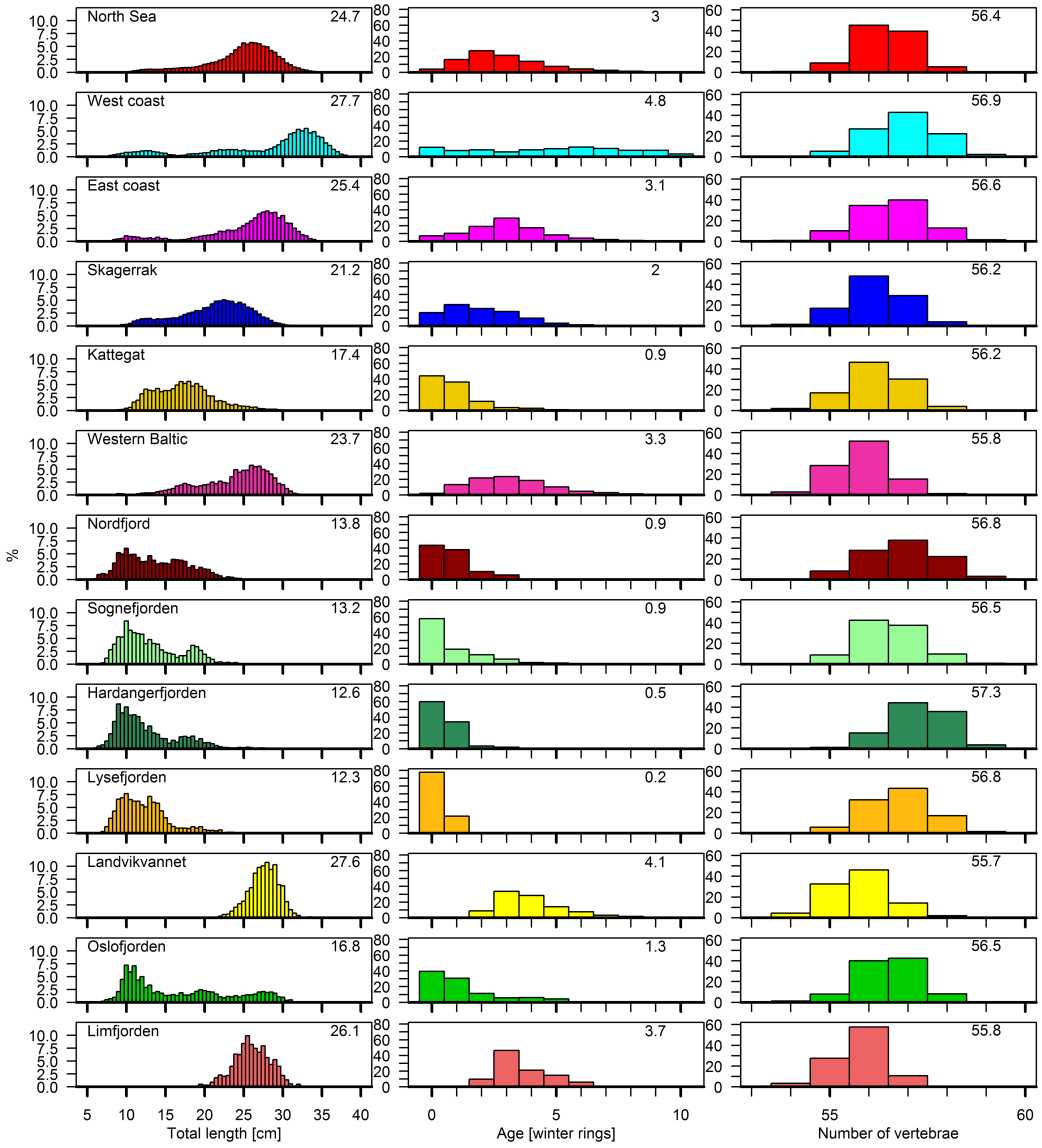

Fig 2. Distribution of biological characteristics. Histograms showing the total length, age and number of vertebrae (VS) by area (data from 1970–2015 pooled) including the mean value for all characters. The range of total length, age and VS was scaled to the amount of herring so very small values are not visualized.

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where *Prop* is the proportion of pre-, post- or spawning herring in percent and *Month* represents the sampling month.

Results

Area specific characteristics

The length and age of herring sampled ranged from 4.5–45.0 cm and 0–17 winter rings (wr). There were no significant sex differences in the biological characters analyzed in the data sets (ANCOVA; $p>0.05$). Therefore, all further analyses were carried out with sexes combined. Vertebral counts, the main population specific trait selected for this study, did not differ between scientific and commercial catches or fishing gears between and within each area (ANOVA; $p>0.001$).

A comparison among the five major areas, Kattegat and seven local fjords gave significant differences in length, age (wr) and vertebral counts (VS) (ANOVA; $p_{length}<0.001$, $p_{age}<0.001$, $p_{VS}<0.001$, Fig 2). The main tendency, based on Tukey-HSD tests, was an increase in length and age going from the fjords towards offshore (North Sea, Skagerrak and Kattegat) and back to the coast (western and eastern coast of Norway), with west coast herring being the largest and oldest of all groups (Fig 2). Landvikvannet and Limfjorden had larger and older fish compared to other fjords, because no juveniles were sampled. The highest VS were found at the west coast and inside the fjords along the west coast. One exception was the herring in Sognefjorden that had intermediate VS, similar to herring collected in the North Sea, east coast and Oslofjorden. The lowest VS were observed for herring in Landvikvannet, comparable to herring from the western Baltic and Limfjorden.

For most of the areas, high variability in VS occurred (S1 Fig). The largest variance was ±1.2 from the overall mean. Within the North Sea, western Baltic, Kattegat, Landvikvannet, Oslofjorden and Limfjorden nearly all samples had a variance lower than our threshold of 'high variability' (±0.25).

Body growth

The growth of herring, estimated from historical length-at-age data, differed among all areas (ANOVA; $p<0.001$, Panel A in S2 Fig). All coastal herring had higher growth rate than other offshore or fjord herring. Lowest growth rate occurred in Nordfjord and Sognefjorden. Fitting the historical observed length-at-age data to the von Bertalanffy growth model highlighted differences between all areas (ANOVA; $p<0.001$, Table 1, Panel B in S2 Fig). Again, coastal herring had the largest maximum asymptotic length (L_∞) of all groups. Similar maximum length and growth rate (K) were observed in the offshore North Sea and western Baltic as well as in Landvikvannet, Oslo- and Limfjorden. Herring in the Skagerrak and Kattegat had the lowest maximum length among the offshore areas. However, the overall lowest maximum length was for herring in the Nordfjord and Sognefjorden.

Maturity ogives

The maturity ogives of herring differed among all areas when comparing the length at 50% (L_{50}) or 95% (L_{95}) mature (ANOVA; $p<0.001$, Table 1, Panel C in S2 Fig). Herring in both coastal areas (west and east), as well as in the North Sea and Skagerrak, matured at larger body sizes compared to other areas. While 50% of herring in Oslo- and Hardangerfjorden were mature at approximately the same intermediate length, the L_{95} in Hardangerfjorden was the third smallest. Herring in Nordfjord and Sognefjorden were mature at the smallest lengths.

Table 1. Estimated parameters ± standard error for the von Bertalanffy growth model and the generalized linear model quantifying the length-at-first-maturity for each area. Length where 50% (L50) and 95% (L95) of herring were mature and numbers of observation (N) with valid data are given for each area. Growth data from Lysefjorden was not sufficient to estimate growth model parameters. Due to missing of mature herring (Lysefjorden) and immature herring (Landvikvannet and Limfjorden), no maturity probabilities could be calculated.

	North Sea	West coast	East coast	Skagerrak	Kattegat	Western Baltic	Nordfjord	Sogne-fjorden	Hardanger-fjorden	Lyse-fjorden	Landvik-vannet	Oslo-fjorden	Limfjorden
Growth													
L_∞	30.16 ±0.01	35.20 ±0.03	31.18 ±0.04	27.33 ±0.05	29.25 ±0.36	29.95 ±0.09	23.55 ±0.34	22.16 ±0.20	30.88±0.81	22.07 ±1.45	30.12 ±0.02	29.41 ±0.20	30.14±0.42
K	0.48 ±0.00	0.36 ±0.00	0.54 ±0.00	0.52±0.00	0.32 ±0.01	0.43 ±0.01	0.59±0.03	0.53 ±0.02	0.41±0.02	1.05 ±0.27	0.48±0.04	0.52 ±0.01	0.49±0.05
t_0	-1.15 ±0.00	-1.17 ±0.01	-0.97 ±0.01	-1.39±0.01	-2.08 ±0.05	-1.01 ±0.02	-0.95 ±0.03	-1.36 ±0.04	-1.08±0.03	-0.71 ±0.11	-1.48 ±0.28	-0.93 ±0.02	-0.66±0.26
N	208 348	34 142	27 646	34 813	7 018	10 382	2 069	4 635	2 729	2 683	2 386	2 404	1 177
Maturity													
Intercept (β_0)	-16.5 ±0.08	-18.1 ±0.28	-16.8 ±0.23	-14.3±0.18	-23.8 ±0.90	-13.1 ±0.27	-10.5 ±0.81	-14.7 ±0.82	-43.2±6.38			-24.9 ±1.51	
Length (β_1)	0.7 ±0.00	0.7 ±0.01	0.7 ±0.01	0.6±0.01	1.1±0.04	0.6±0.01	0.5±0.04	0.8 ±0.04	1.9±0.28			1.1 ±0.06	
L50	23.65	24.73	24.51	25.23	22.41	21.50	19.70	17.80	23.11			23.23	
L95	27.88	28.76	28.80	30.42	25.18	26.31	25.21	21.36	24.68			25.98	
N	195 905	30 850	26 397	28 808	3 780	10 509	818	1 505	522			1 127	

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Both western Baltic and Kattegat herring had an intermediate length-at-maturity, as compared to coastal and offshore herring (high) and fjord herring (low).

Maturation and spawning time

Based on the distribution of spawning herring by area and month, the herring from the five major areas included in these analyses could be separated into three main groups: autumn spawners (September-October), early spring spawners (February-April) and late spring spawners (March-June) (Fig 3). The proportion of pre-, post- and spawning herring differed within each area (Table 2). Autumn spawning herring were observed in the North Sea and along the west coast. Early spring spawners occurred solely in the west coast area, whereas late spring spawners dominated the east coast area, Skagerrak and western Baltic. In the North Sea, a small proportion of spawning herring also occurred in early spring.

The intra-annual variation of pre-spawning herring followed the opposite trend of spawning herring, with peak proportion shortly before spawning occurs. In the North Sea, very few pre-spawning herring occurred during spring, and the highest proportion was found in June-August. The changes for post-spawning herring were also related to the observed spawning time for each area. Along the western and eastern coast of Norway, high proportions of post-spawning herring were observed from April-July. In the western Baltic, barely any post-spawning herring were observed, except after the main spawning in June.

Population mixing

VS, the main population specific trait, were highly variable over the different quarters of a year in the coastal areas, eastern North Sea and Skagerrak (Fig 4). The VS of herring in the central North Sea was relatively stable throughout the year. Most variation occurred along the coast and the border between the North Sea and the Skagerrak. On the west coast, there was a clear

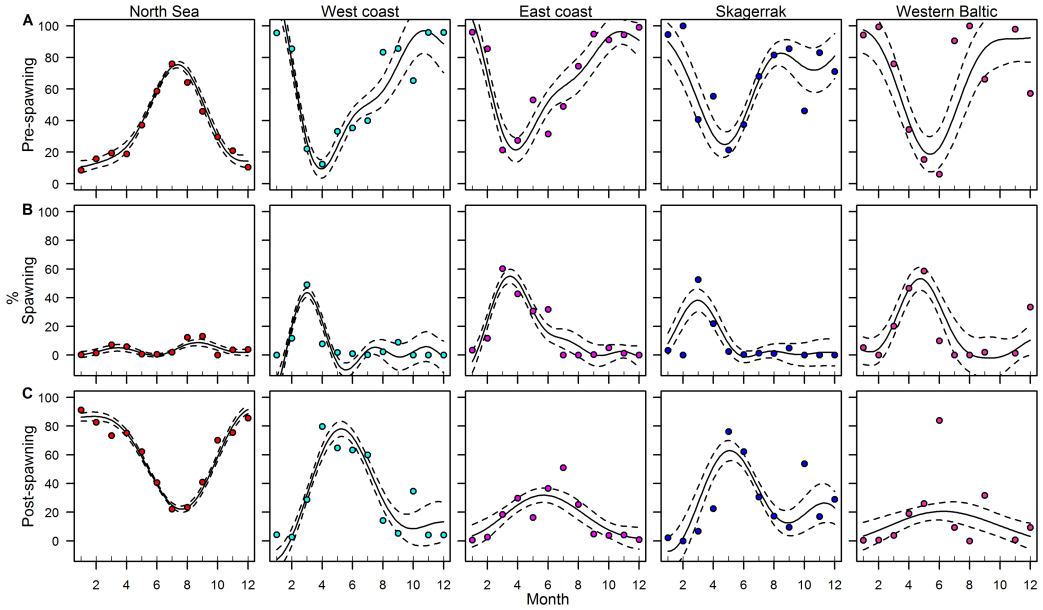

Fig 3. Annual variation of maturity. Fitted results for the GAMs on the proportion of pre-spawning (A), spawning (B) and post-spawning (C) herring per month. Solid lines indicate the fitted values, dashed lines the 95% confidence intervals and points the observed values for the five major areas.

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decrease from VS 57.2 or higher in the first quarter to 55.9–56.3 or lower in the third quarter. A similar decrease occurred on the east coast and the local area of Landvikvannet, but VS were never higher than 57.2. All herring migrated out from Landvikvannet by the end of the third quarter, as evidenced by fishing efforts resulting in zero catches. In Skagerrak and Kattegat,

Table 2. Estimates of GAMs for the effects on vertebral counts and the monthly spawning dynamics in terms of the proportion of pre-, post- and spawning herring. Modeled means together with each covariate's degrees of freedom (Ref.df, explaining the oscillations of the modeled trend, where 1 would be linear) and p-value for each area, and the deviance explained (R^2) by the model, are shown. For temperature and salinity, estimates were given before the variables were dropped from the model.

Model	North Sea			West coast			East coast			Skagerrak			Western Baltic			R^2 (%)
	Mean	Ref.df	p-value	Mean	Ref.df	p-value	Mean	Ref.df	p-value	Mean	Ref.df	p-value	Mean	Ref.df	p-value	
Vertebral counts																
Year class	56.5	8.6	<0.001	56.9	8.9	<0.001	56.6	9.0	<0.001	56.1	7.2	<0.001	55.9	5.5	<0.01	17.7
Quarter of the year		3.0	<0.001		3.0	<0.001		3.0	<0.001		2.9	<0.001		2.2	<0.001	
Stage of maturity		5.0	<0.001		4.6	<0.001		5.0	<0.001		4.8	<0.001		1.0	<0.001	
Temperature		2.6	0.18		5.6	0.06		5.3	0.54		4.5	0.08		1,0	0.05	
Salinity		1.5	0.43		6.4	0.07		1.0	0.75		1.1	0.69		1.0	0.05	
Spawning dynamics																
Pre-spawning	38.5	7.0	<0.001	61.6	7.0	<0.001	66.0	7.0	<0.001	65.8	6.6	<0.001	62.1	6.4	<0.001	38.4
Spawning	2.4	8.5	<0.001	4.5	9.0	<0.001	15.8	8.9	<0.001	7.7	8.3	<0.001	16.7	8.3	<0.001	39.8
Post-spawning	59.5	5.0	<0.001	34.9	5.0	<0.001	17.0	4.7	<0.001	28.6	5.0	<0.001	12.1	3.2	0.04	51.3

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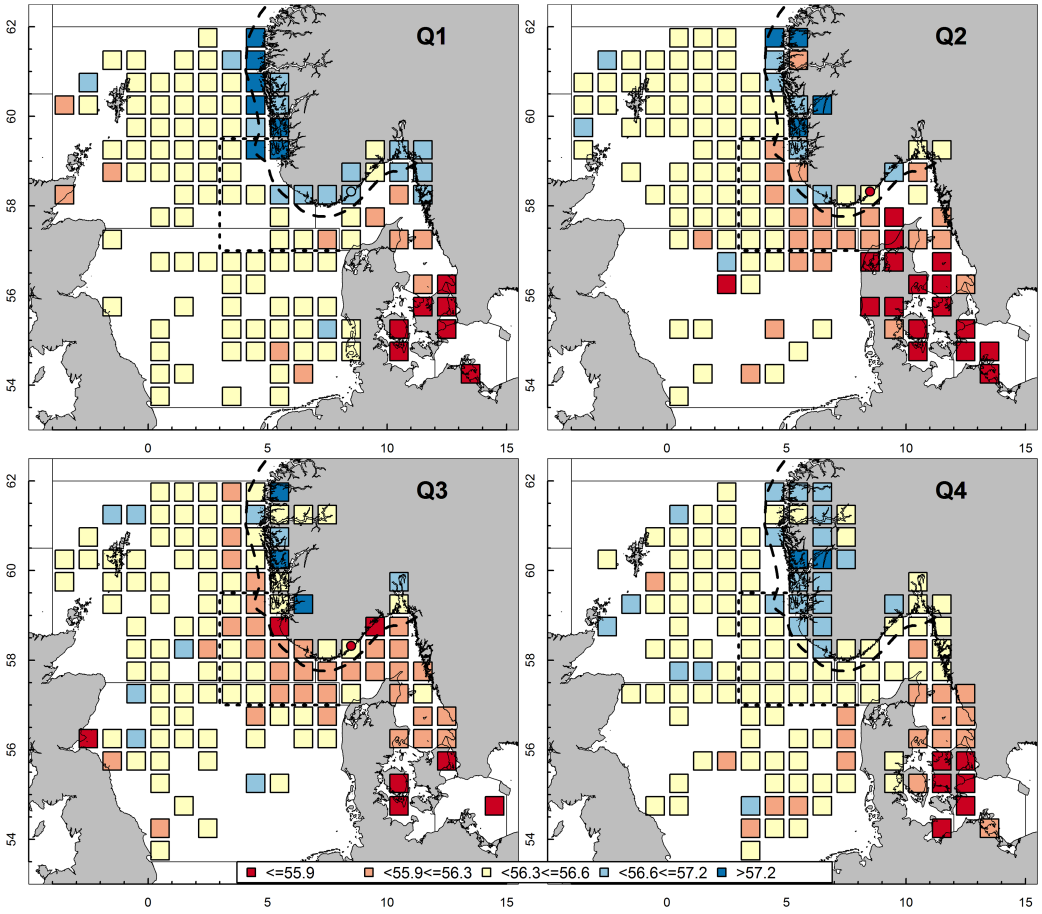

Fig 4. Spatial and temporal dynamics of mean vertebral counts. Mean vertebral counts (colored) for each geographic square (1˚ Longitude, 0.5˚ Latitude) and Landvikvannet (circle) per quarter (Q) of the year. ICES 'transfer area' is indicated by stippled line.

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herring with low VS occurred in the second quarter and dispersed into the eastern North Sea and up along the Norwegian west coast in the third quarter of the year (Fig 4). In the fourth quarter, the migratory populations with very high VS along the west coast and low VS in the Skagerrak and eastern North Sea disappeared and the VS became similar to the overall historic mean of these regions (see Fig 2). In the western Baltic, the low VS (55.9) was also stable throughout the year. Local fjords were not sampled continuously and consequently, VS data are sparse from those areas.

The GAM indicated significant variation of VS within each of the five major areas with respect to year class, quarter of the year and maturity stage (Fig 5; Table 2). Neither temperature nor salinity had a significant effect on the variation over time in VS. According to the GAM, VS in the North Sea and western Baltic varied significantly among different year classes, but still the variation was relatively stable around their general means of 56.4 and 55.8,

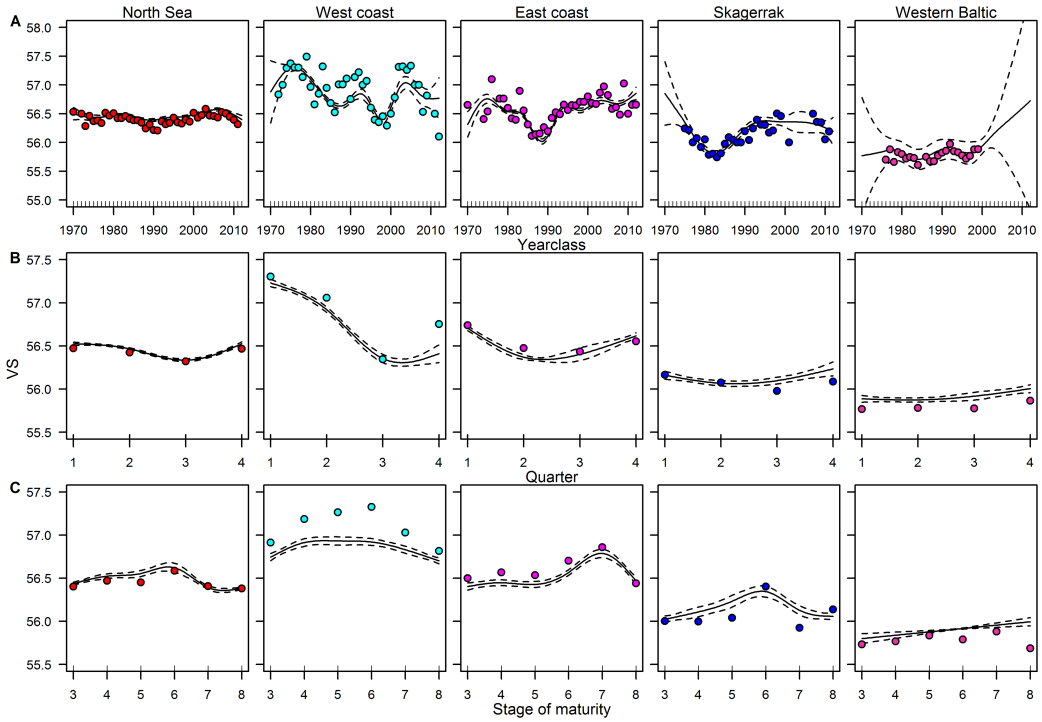

Fig 5. Variation and dynamic of mean vertebral counts. Fitted results for the GAM on mean vertebral counts (VS) in relation to year classes (A), quarters of the year (B) and stages of maturity (C). Solid lines indicate the fitted values, dashed lines the 95% confidence intervals and points the observed values for five major areas.

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respectively (Fig 5A), compared to the coastal areas and Skagerrak. Highest variation in VS occurred along the west coast; two periods with maximum VS were observed for the year classes around 1975 and 2005, while there was one period with minimal VS around 1997. At the east coast, maximum VS was found for the same periods as the west coast, but VS was only slightly higher than the overall mean. The year classes around 1990 had comparably very low VS after which it increased continuously. In the Skagerrak, VS have been stable for the last 25 years, after a minimum observed around year classes of 1983.

In addition to the inter-annual dynamics, seasonal dynamics could be seen in all areas (Fig 5B; Table 2). The highest seasonal dynamics were observed along the west coast, where VS decreased rapidly from 57.2 in the 1st quarter to 56.5 in the 3rd quarter, with a slight increase in the 4th quarter. In comparison, VS in the other areas were relatively stable with a maximum range of 0.4. However, the general trend was a decrease in VS with a minimum in the 2nd quarter (east coast, Skagerrak) or 2nd– 3rd quarter (North Sea) followed by an increase to the same level as observed in the 1st and in the 4th quarter of the year.

VS also varied for different stages of maturity in all areas (Fig 5C, Table 2). In general, spawning herring had the highest VS in all areas, except the western Baltic. In the western Baltic, VS was rather stable. In the North Sea, spawning herring only had slightly higher VS than other maturity stages. Along the east coast, spent herring (stage 7) had the highest VS.

A comparison of spawning herring solely indicated stable VS among the year classes in the Skagerrak and western Baltic. During the first quarter in the North Sea, spawning herring with VS above the general mean had been observed for the last 15 years (S3 Fig). Since 1990, an increasing VS during spawning in the first quarter was observed for the west and east coast areas.

Discussion

Analyses of historical data on the temporal and spatial variation in phenotypic characters of Atlantic herring demonstrate significant differences among 13 geographical areas. In some areas, vertebral counts (VS), the main population specific trait used in this study, are highly variable over time. Such variation indicates temporal changes in presence or absence of herring populations in an area. The temporal variation in VS was not related to environmental factors, but such factors can influence the development of meristic characters (e.g. VS) during the incubation period and early larval life [23, 24]. Therefore, one explanation for varying VS could be the co-occurrence of different herring populations in time and space, but without interbreeding. Alternatively, if herring from one population joined and interbreed with herring from another population, we would expect to see offspring with intermediate vertebral counts [26, 40].

Theoretically, the number of vertebrae is negatively correlated with temperature and positively with salinity conditions during the early larval life before vertebrae are developed [23, 24]. However, such a correlation was not apparent in our data. Also, the monotonic increase in temperature, during the study period, did not appear to have a direct influence on the number of vertebrae. Environmental factors can influence meristic characteristics, as demonstrated in laboratory studies. However, these may be masked by other factors resulting in differing vertebral counts in wild populations.

The historical North Sea data, showing stable vertebral counts over many decades, indicate no mixing of populations (Fig 5). One exception is the 'transfer area', as defined by ICES [8] (in the North Sea: east of 3˚E and between 57–59.5˚N, Fig 4), where herring with lower vertebral counts occur during the 2nd and 3rd quarter of the year. These herring are in pre-spawning condition and are presumably western Baltic spring spawners (WBSS) on their feeding migration [12]. Consequently, no connectivity between WBSS and North Sea herring is assumed. Mariani et al. [41] suggest that 'isolation-by-distance' would account for the North Sea herring being a homogenous population, genetically different from spawning aggregations in the English Channel (Downs) and along the south coast of Norway. Further, morphological differences between spawning herring in the Downs and the North Sea have been demonstrated [42] However, Downs herring, migrating into the North Sea during summer for feeding, could not in our study be identified based on biological characteristics in the North Sea. After excluding migrating WBBS herring, assessing North Sea herring as one stock, the North Sea autumn spawners (NSAS), appears reasonable. Changes in relative importance of the individual populations within a stock, like the potential increase in the Downs, could influence the perception of stock dynamics and thus management [8].

Even though the vertebral counts of herring in the North Sea have remained fairly stable, there are indications of mature herring with slightly higher VS in the 1st quarter of the year (S3 Fig). These herring have the characteristics of the Norwegian spring spawning (NSS) population, which has undergone dramatic changes in stock size and major shifts in migration routes [9, 43]. Prior to the collapse of NSS in the late 1960s, herring migrated from their wintering areas along the southern border of the east Icelandic Current towards the Norwegian coast [44]. After the stock collapse, NSS were confined to the coastal areas of western Norway. After

the recovery, the feeding migration extended further offshore to the more central part of the Norwegian Sea [31] and the stock also returned to their traditional spawning grounds south of 62˚N [45, 46]. In our results, the presence of spring spawners (March-April) in the North Sea (Fig 3) might be an indication of NSS migrating through the North Sea once again, although it is unclear whether these are periodic nomads of NSS or resident North Sea spring spawners [47].

Similar to the North Sea area, herring in the western Baltic area constituted a single population. In this area, the number of vertebrae were stable over many decades (Figs 4 and 5). It is therefore assumed that herring in this area were fish managed as WBSS. However, in recent years, an increasing fraction of herring from the central Baltic has migrated further westwards into the western Baltic [48, 49]. Herring from the central Baltic have an even lower VS [50]. However, our data do not suggest a decrease in VS associated with western ingress of central Baltic herring into the western Baltic.

The data from Skagerrak clearly revealed a mixture of several populations, indicated by highly dynamic intermediate VS, both within a year and inter-annually (Fig 5). These populations mainly represent mixtures of herring managed as NSAS and WBSS. NSAS dominated during the first quarter of a year, followed by an increased occurrence of WBSS in summer. Further, the observed dynamics in VS could also include a mixing of local spring spawners, which migrate into the Skagerrak for feeding, together with NSAS and WBSS during spring and summer [18, 45]. The occurrence of WBSS during summer can be traced to the North Sea, and even further north along the west coast of Norway (Fig 4). Our results support the assumption that all spring spawning herring caught after the 1st quarter of the year in the 'transfer area' are predominantly WBSS. Further, our data indicated the occurrence of different spring spawning populations in the Skagerrak and Kattegat, based on different growth rates and maturity ogives (Table 1), compared with herring in the western Baltic. These local populations originate from various spawning grounds along the Skagerrak and Kattegat coastal areas and are genetically distinct [51].

Extensive population mixing was also apparent through the high variability in VS. This indicates variability in the presence or absence of herring populations, especially during the spawning season and along the southern coast of Norway (Fig 5). There were differences in growth, length and VS between herring of the east and west coast (Fig 2 and Table 1). However, similar trends in inter-annual changes in these parameters suggest that migratory herring with higher VS occur regularly in coastal areas during spring. The migrating herring are most likely NSS, while the second population may be more stationary, coastal spring spawning populations with lower VS [11]. The occurrence of herring with high VS (above 57.0) combined with high growth along the west coast (Fig 4), is normally an indication of migratory NSS entering the area [37]. NSS reappeared at their traditional spawning grounds south of 62˚N in 1989 [45, 52, 53]. This is consistent with our results showing high VS during spawning along the west coast in the 1990s (S3 Fig). In addition, the VS along the east coast also increased after 1989 during the spawning season of NSS (S3 Fig), indicating that the migration of a proportion of NSS may have continued south and eastward into the Skagerrak area. However, whilst there have been years with low VS along the west coast, indicating a higher proportion of WBSS migrating further out of the Skagerrak, the proportion of NSS along the east coast has steadily increased. The lack of variability in the vertebral counts along the east coast indicate that a small proportion of WBSS migrate to this area. The majority of WBSS stayed in the Skagerrak during their feeding migration (Fig 4), as shown by Clausen et al. [12].

In addition to the relatively large migratory herring populations, there is the evidence of distinct local and stationary populations in Kattegat, Skagerrak and the coast of southern Norway [16, 18]. These populations differ in biological characters compared to populations in the

adjacent coastal areas, with for instance the presence of older individuals in Nordfjord, Sognefjorden, Landvikvannet and Limfjorden (Fig 2 and Table 1). In contrast, Hardangerfjorden and Lysefjorden seem to lack resident local populations, since there is an absence of older individuals. Lysefjorden appears to be a nursery area (only 0-1wr individuals) for mainly NSAS and small proportions of NSS according to VS, whereas Hardangerfjorden is clearly a nursery area for NSS. The historic data from Oslofjorden indicate a mixture of several populations. However, similar vertebral counts do not necessarily mean lack of a local population. Limfjorden is known to be genetically different from other local populations in the Skagerrak and Kattegat area [18] (Table 1). Our data indicate only one local population within Sognefjorden, even though two local populations were identified in the past: the Lusterfjord herring [54] and the Østerbø herring [55]. The VS in Sognefjorden indicate almost no occurrence of NSS, despite the high abundance of young-of-the-year. The small size-at-maturity and maximum length suggest the occurrence of at least one local population (Table 1). Similar results have been observed in Nordfjord, although these are not as clear as in Sognefjorden. Libungan et al. [56] demonstrated differences in otolith shape for herring from these fjords, supporting our notion of local populations. According to VS, Nordfjord is also a nursery area for NSS. Landvikvannet is completely different from the previous described local areas with much lower VS (Fig 2). No immature individuals have been observed and the local population within Landvikvannet is not stationary. Landvik herring only occur during spawning time and leave the brackish lake afterwards. This is supported by recent studies demonstrating differences in both vertebral counts [11], behavior [10] as well as genetics [16] in Landvik herring from its neighboring populations. However, local populations are included in the management of NSAS, NSS and WBSS stocks, without knowledge of the extent of mixing. Further, assessing and managing stocks close to areas inhabiting local populations is challenging even though combining biological characteristics demonstrated clear differences. This combination of biological characters can be used to identify areas with local populations and establish management regulations to ensure the maintenance and diversity of these local populations.

Despite the clear differences in VS between the areas, the variability did not allow for a clear separation of populations when mixing occurs. When only two populations are present, a discrimination in terms of proportion should be possible based on VS. However, no individual assignment to a population or even to a stock is possible by the number of vertebrae alone. Herring can be discriminated by spawning season dependent on otolith microstructure [4]. This is used currently in the study area to distinguish between NSAS and WBSS, but local populations are neglected.

Our results of stable VS, such as noted for the North Sea or western Baltic (Fig 5), might not be indicative of a single population. However, there could be a relatively stable mixture of multiple populations that could be comprised and managed as a single stock. In the North Sea for example, multiple populations have previously been defined by distinct spawning times and sites [57]. Further, these populations can be discriminated by small-scale differences in VS [27]. However, these small-scale differences could not be detected in our data, even though the high total sample size (N = 428 773) should allow for detection of small differences. The large sample size influences the deviance explained of the generalized additive model on the number of vertebrae, which was only 17%. The large sample size and use of individual vertebral counts (S8 Fig) breaks down the theory of the deviance explained for this type of model. Using individual vertebral counts resulted in similar ranges among the five major areas and no pattern was visible. Applying the GAM to mean vertebral counts per sample (S9 Fig) would increase the deviance explained, but not influence the significance of explanatory variables. However, a low deviance explained cannot necessarily be considered to be evidence of a poor fit [see pp. 118–119, 36].

In summary, the changes in VS, growth and maturity ogives observed in the extensive time series used in this study were independent of environmental effects such as salinity and temperature. Hence, along the south coast of Norway a clear mixture of more stationary coastal populations with lower VS and migratory herring with higher VS occurs during spawning. Such an overlap is a prerequisite for potential connectivity and interbreeding of populations [13], although no direct evidence for interbreeding exists in this study. High temporal variability in VS indicates mixing of herring from two or more populations and variation in intra-annular changes in their presence or absence. This mixing of populations should be considered when managing herring in this area. However, existing methods for assignment of individual herring to a population are in progress and need to be further developed.

Supporting information

S1 Fig. Variability of mean vertebral counts (VS) demonstrated by (left panel) the distribution of the standard deviation for each sample and (right panel) the variance for each sample from the mean VS of each area showed in the figure. Vertical stippled lines indicate ±0.25 variances from the mean which are defined as expected variation within each area.
(TIF)

S2 Fig. Length-at-age, estimated von Bertalanffy growth models and maturity ogives. Length-at-age (A), estimated von Bertalanffy growth models (B) and maturity ogives (C, proportion of mature herring at length) by area. Points and T-bars show the mean and the 95% confidence interval. Stippled and dotted lines indicate L_{50} and L_{95}, respectively, where 50% or 95% of the herring were mature. The legends are ordered according to the maximum asymptotic length or increasing L_{50}. Lysefjorden is not included in the estimation of the von Bertalanffy growth model because only data for age 0–1 winter rings were available. No complete data available for maturity ogives from Landvikvannet, Lysefjorden and Limfjorden.
(TIF)

S3 Fig. Mean number of vertebrae (VS) per year class for spawning herring caught in the 1st quarter of the year (A) in the North Sea, (B) west coast and (C) east coast. Only areas with significant differences were shown. Horizontal lines indicate mean VS for three herring stocks in the study area, stippled = western Baltic spring spawners, solid = North Sea autumn spawners, and dotted = Norwegian spring spawners.
(TIF)

S4 Fig. Validation plots for generalized additive model analyzing the number of vertebrae. See Table 1 for estimated parameters.
(TIF)

S5 Fig. Validation plots for generalized additive model analyzing the proportion of pre-spawning herring. See Table 1 for estimated parameters.
(TIF)

S6 Fig. Validation plots for generalized additive model analyzing the proportion of spawning herring. See Table 1 for estimated parameters.
(TIF)

S7 Fig. Validation plots for generalized additive model analyzing the proportion of post-spawning herring. See Table 1 for estimated parameters.
(TIF)

S8 Fig. Raw data of individual vertebrae counts (VS) of herring for each area used in the generalized additive model (GAM) analysis. The low explained variance of only 17% for the GAM is resulting from the similar range and variance of vertebrae counts for the different areas (red = North Sea, cyan = west coast, purple = east coast, blue = Skagerrak, pink = western Baltic).
(TIF)

S9 Fig. Mean vertebrae counts (VS) for each herring sample for each area. This data was not used in the generalized additive model (GAM) analysis, but would have increased the low explained variance of only 17% for the GAM, because the range and variance of vertebrae counts differs for the five areas (red = North Sea, cyan = west coast, purple = east coast, blue = Skagerrak, pink = western Baltic).
(TIF)

S1 Supporting Information. Further details on material and methods.
(PDF)

S1 Table. Total number of analyzed herring per year per area 1970–2015.
(PDF)

S2 Table. Total number of analyzed herring per month per area 1970–2015.
(PDF)

S3 Table. Total number of analyzed herring by fishing gear per area 1970–2015.
(PDF)

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S1 Supporting information

Materials and methods

Study area

The study area was divided and all fish classified as: North Sea, west coast of Norway, east coast of Norway, Skagerrak, Kattegat, western Baltic, Nordfjord, Sognefjorden, Hardangerfjorden, Lysefjorden, Landvikvannet, Oslofjorden and Limfjorden based on their catch locations. The North Sea constitutes the area classified by ICES as the North Sea management area, bounded for this study in the south by 53.5°N. In addition, any fish occurring within 12 nautical miles (NM) of the Norwegian coastline were excluded from this area. Coastal areas were defined as areas within 12 NM off the Norwegian coast. The division between west and east was at Lista (7°E). ICES Division 3a includes the Skagerrak (Subdivision 20) and Kattegat (Subdivision 21); again with the exception of any fish occurring within 12 NM of the Norwegian coastline. The western Baltic is defined by the ICES Subdivision 22-24. Nordfjord, Sogne- and Lysefjorden are bounded in the west by 5.5°E and Hardangerfjorden by 5.8°E. Landvikvannet samples were taken within the brackish lake. Oslofjorden is bounded in the south by 59.4°N. Limfjorden samples were collected within 9.4-10.3°E.

Data analysis

Prior the generalized additive model (GAM) analysis, we applied a data exploration including the variables: Total length, age, number of vertebrae, stage of maturity, catch area, quarter of the year, temperature, salinity and fishing gear. During the data exploration, response and explanatory variables were check for outliers and for collinearity, and relationships between response and explanatory variables investigated. Only outliers for the number of vertebrae were removed for the model analysis. No correlation between any of the used variables were identified.

The following steps of model selection and validation were applied for the GAMs. After estimating the model, covariate with largest and non-significant *p*-value was removed. New models were estimated until all non-significant variables were removed. The final models were validated (see Fig S4-7) by (i) assessing normality (QQ-plot and histogram), (ii) homogeneity (residuals versus fitted values), (iii) model fit (fitted values versus observed values), and (iv) still existing patterns in relation to covariates (scatter plot of residuals vs. remaining covariates). None of these four validations of the final models were violated.

S1 Table. Total number of analyzed herring per year per area 1970-2015.

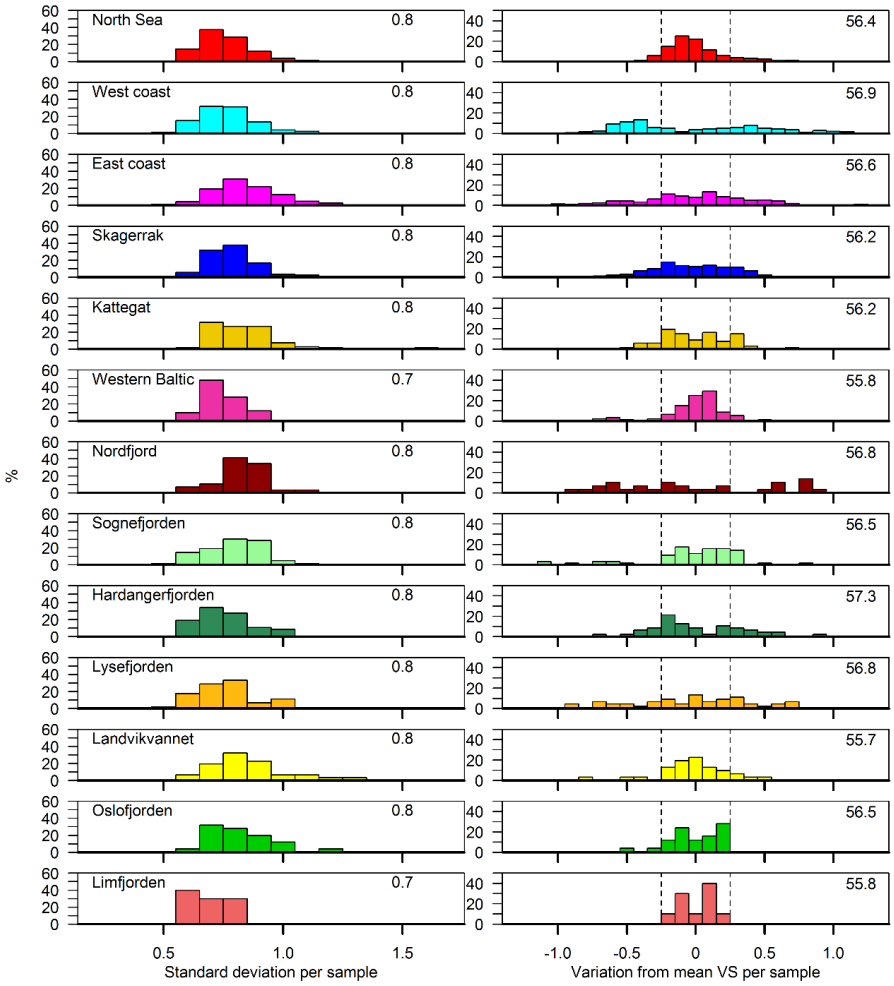
Year	North Sea	West coast	East coast	Skagerrak	Kattegat	Western Baltic	Nordfjord	Sogne-fjorden	Hardanger-fjorden	Lyse-fjorden	Landvik-vannet	Oslo-fjorden	Lim-fjorden
1970	3 000	77		477				100					
1971	1 370			179									
1972	1 684	217	91	301									
1973	2 100	53	95	200									
1974	2 455	257											
1975	2 910	100		57									
1976	1 883		194				100						
1977	900	315	300	100				24	17				
1978	100	278	190	300				100					
1979	563	616		591	158			58	40	80			
1980	1 120	1 095	1 999	541	969			87	45	142			
1981	3 171	1 220	1 200	449	668								
1982	2 219	1 362	1 800	1 683									
1983	5 451	1 658	2 627	2 411		1 035							119
1984	9 570	1 086	2 200	1 282	155	2 829					134		295
1985	8 911	697	1 545	3 041	931	904	100		100	79	100		
1986	10 051	675	700	3 080	427	424						95	231
1987	12 241	500	624	875	469	691		100	300	196			166
1988	9 824	405	900	1 100	500	48	195	482	237	400			
1989	10 761	1 256	400	781	300		156	172		200			
1990	8 698	1 435	500	1 466	400		96	247	316	200		100	
1991	10 000	1 725	600	1 842	469		41	135	174	159	100		
1992	7 407	3 967	940	2 823	550	100	213	1146	139	52		243	
1993	8 330	2 056	200	2 595	575		100	28	100	213	100	92	
1994	6 542	547	509	2 484	100	320		200	201	395	56	200	
1995	7 842	856	1 071	985	193	459	99	100	149	200	100	596	70
1996	3 993	794	548	544	231	341	100	171	146	399	100	210	
1997	6 260	2 106	558	338		580	100	300	275	200	100	46	
1998	5 050	2 915	476	400			50	236	52	100	100	100	
1999	5 405	2 057	1 261	999	100		55	258	547	506	50	326	
2000	5 139	1 861	804	719	38		210	485	815	700	100	399	96
2001	5 608	434	686	783			200	322	575	292	100		
2002	9 133	785	1 231	1 456	200	1 242	459	356	621	438		687	100
2003	7 826	665	1 213	1 016	300	1 348	116	441	476	29		82	100
2004	4 494	111	430	100		199		146	310	114		145	
2005	4 465	230	441					857	144	397		131	
2006	6 846	103	401	100			512	24	38	100		157	
2007	4 269		322	208			140	366	213	160		180	
2008	3 218	161	575				710	671	187	102		390	
2009	3 340	1 490	146						508		100		
2010	4 369	1 211	650						371		246		
2011	7 183	1 138	524					142			261		
2012	11 525	720	1 231								377		
2013	11 750	773	324	200							96		
2014	7 214	11	393	382			87	156			45	100	
2015	11 883	1 057	542				100	245	18		261		
Total	**268 073**	**41 075**	**31 441**	**36 888**	**7 733**	**10 520**	**3 939**	**8 155**	**7 114**	**5 853**	**2 526**	**4 279**	**1 177**

S2 Table. Total number of analyzed herring per month per area 1970-2015.

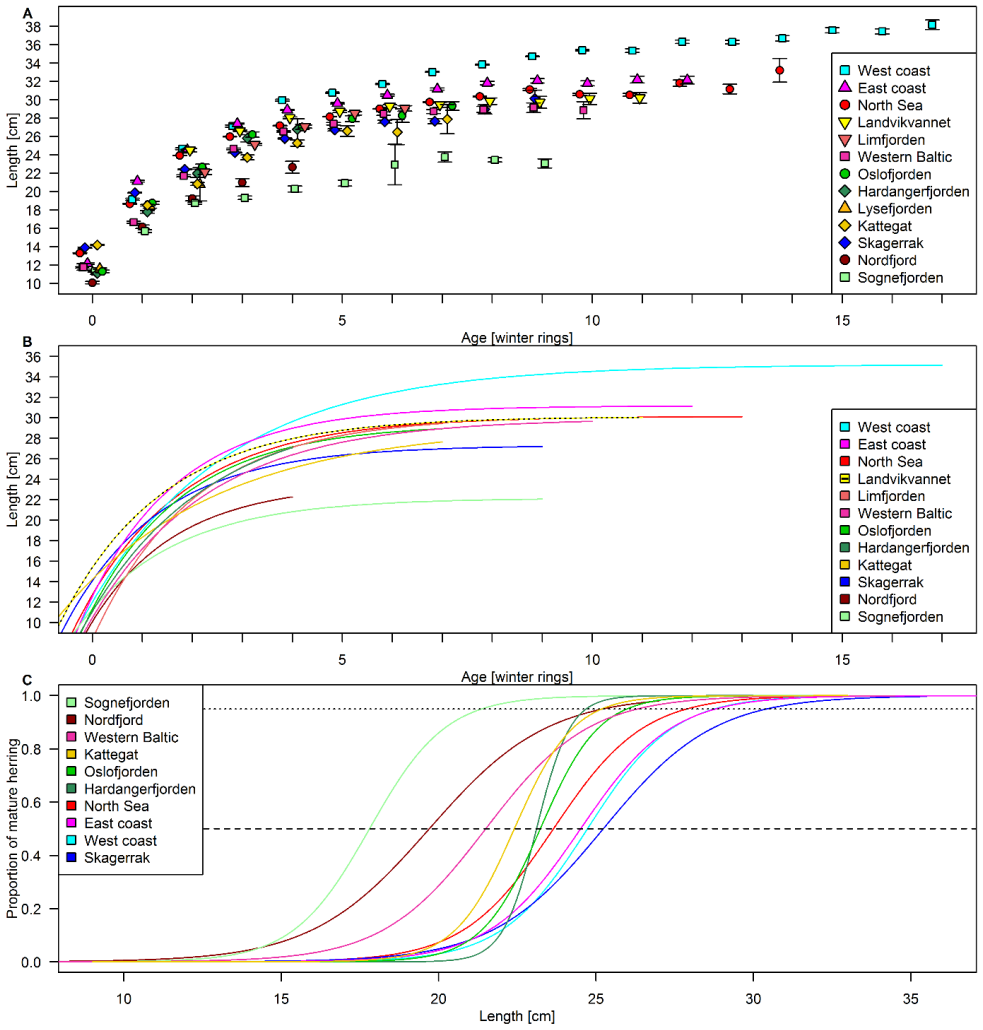
Month	North Sea	West coast	East coast	Skagerrak	Kattegat	Western Baltic	Nord-fjord	Sogne-fjorden	Hardanger - fjorden	Lyse-fjorden	Landvik-vannet	Oslo-fjorden	Lim-fjorden
1	14 597	240	525	1 761	231	1 131							
2	20 847	4 719	2 989	174		611							
3	7 269	14 558	6 137	993	218	1 033					109		
4	4 729	4 141	3 812	913	100	2 497			362		344		753
5	23 594	2 826	1 203	1 690	848	952			126	109	1 826	43	424
6	57 388	2 031	896	4 501	100	424		10	100		237		
7	72 829	4 365	567	10 999	138	230		500	44				
8	9 820	1 597	724	7 017	458	586					10		
9	8 354	716	1 078	1 230		658				50		100	
10	15 856	2 085	1 855	500			87	246	150	200		100	
11	25 118	3 214	10 338	4 708	2 577	2 001	3 383	6 050	5 955	5 163		3 599	
12	7 672	583	1 317	2 402	3 063	397	469	1 349	377	331		437	
Total	**268 073**	**41 075**	**31 441**	**36 888**	**7 733**	**10 520**	**3 939**	**8 155**	**7 114**	**5 853**	**2 526**	**4 279**	**1 177**

S3 Table. Total number of analyzed herring by fishing gear per area 1970-2015.

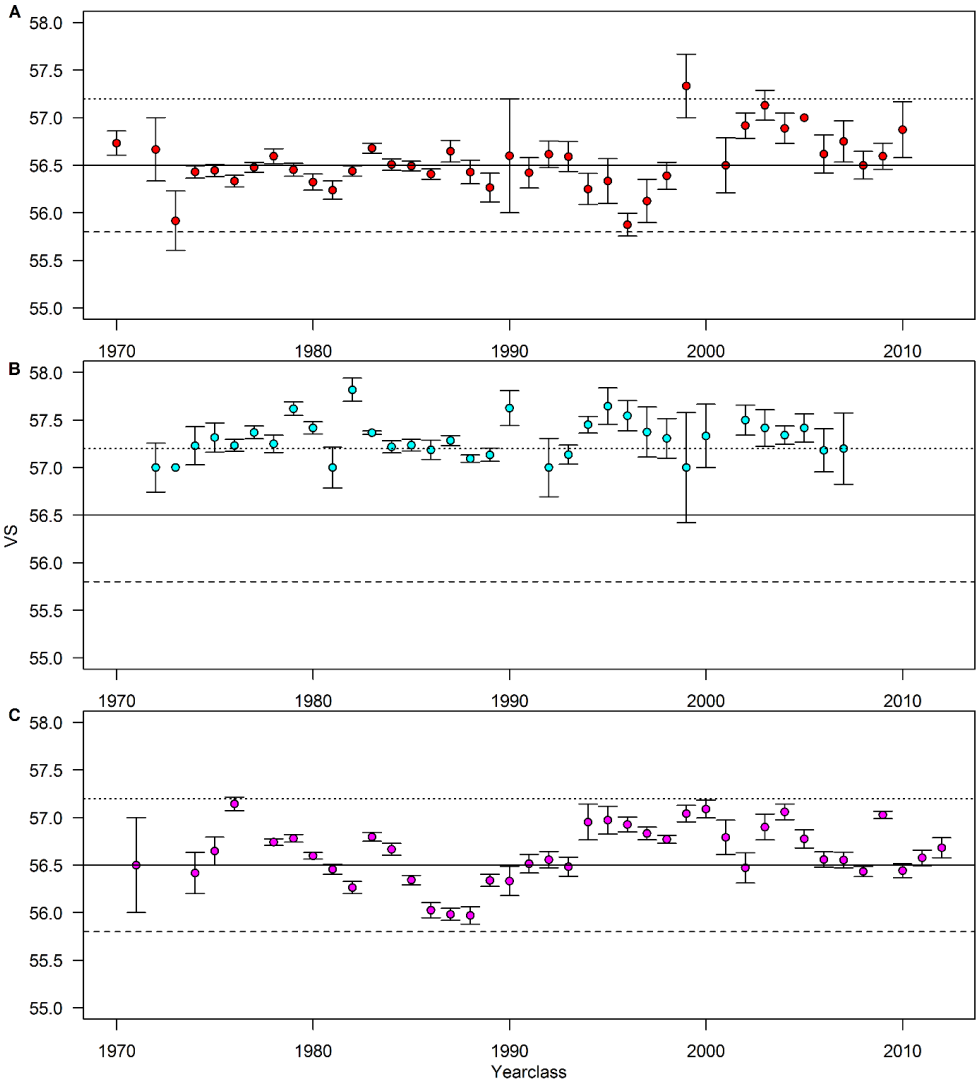
Fishing gear	North Sea	West coast	East coast	Skagerrak	Kattegat	Western Baltic	Nord-fjord	Sogne-fjorden	Hardanger-fjorden	Lyse-fjorden	Landvik-vannet	Oslo-fjorden	Lim-fjorden
Trawl	117 475	12 939	10 088	8 893	6 750	100	3 852	7 823	6 870	5 694		4 179	
Seine	114 160	24 026	7 127	7 055			87	256	244	159		100	
Gillnet	469	3 788	14 105	497				76			2 526		
Hook and line	32	77		108									
Unspecific	35 937	245	121	20 335	983	10 420							1 177
Total	**268 073**	**41 075**	**31 441**	**36 888**	**7 733**	**10 520**	**3 939**	**8 155**	**7 114**	**5 853**	**2 526**	**4 279**	**1 177**



S1 Fig. Variability of mean vertebral counts (VS) demonstrated by (left panel) the distribution of the standard deviation for each sample and (right panel) the variance for each sample from the mean VS of each area showed in the figure. Vertical stippled lines indicate ±0.25 variances from the mean which are defined as expected variation within each area.



S2 Fig. Length-at-age, estimated von Bertalanffy growth models and maturity ogives. Length-at-age (A), estimated von Bertalanffy growth models (B) and maturity ogives (C, proportion of mature herring at length) by area. Points and T-bars show the mean and the 95% confidence interval. Stippled and dotted lines indicate L_{50} and L_{95}, respectively, where 50% or 95% of the herring were mature. The legends are ordered according to the maximum asymptotic length or increasing L_{50}. Lysefjorden is not included in the estimation of the von Bertalanffy growth model because only data for age 0-1 winter rings were available. No complete data available for maturity ogives from Landvikvannet, Lysefjorden and Limfjorden.



S3 Fig. Mean number of vertebrae (VS) per year class for spawning herring caught in the 1st quarter of the year (A) in the North Sea, (B) west coast and (C) east coast. Only areas with significant differences were shown. Horizontal lines indicate mean VS for three herring stocks in the study area, stippled = western Baltic spring spawners, solid = North Sea autumn spawners, and dotted = Norwegian spring spawners.

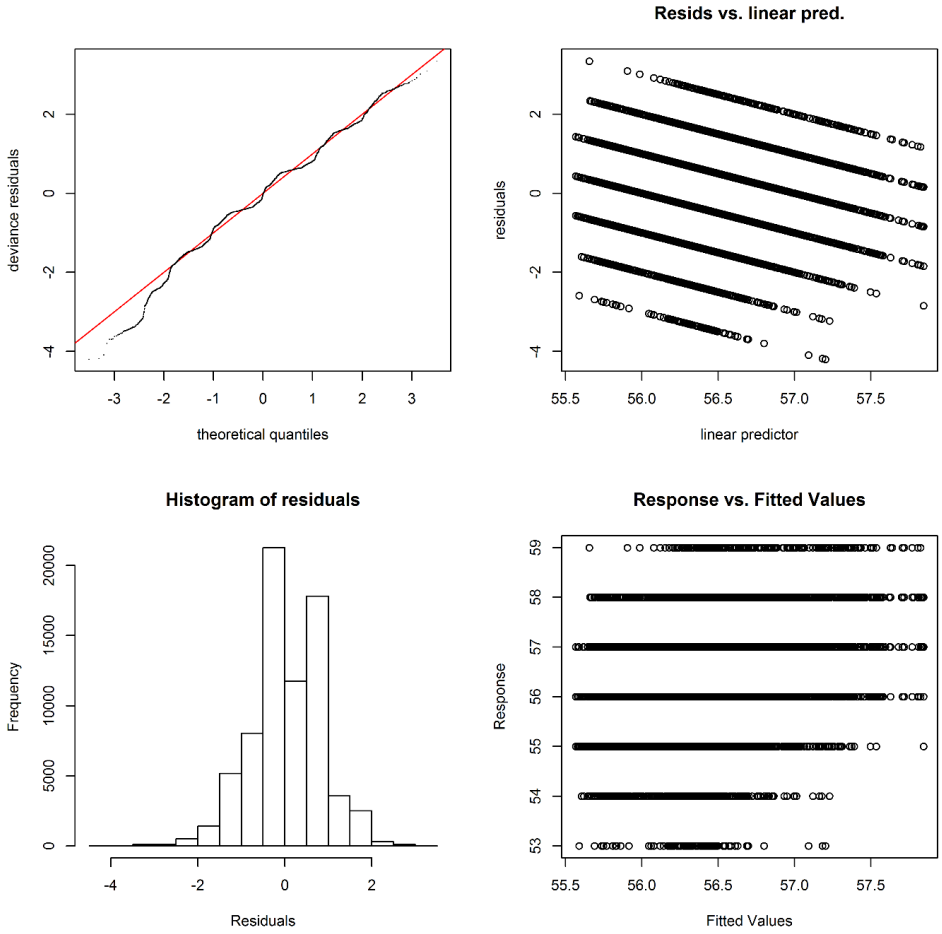


Fig S4. Validation plots for generalized additive model analyzing the number of vertebrae. See Table 1 for estimated parameters.

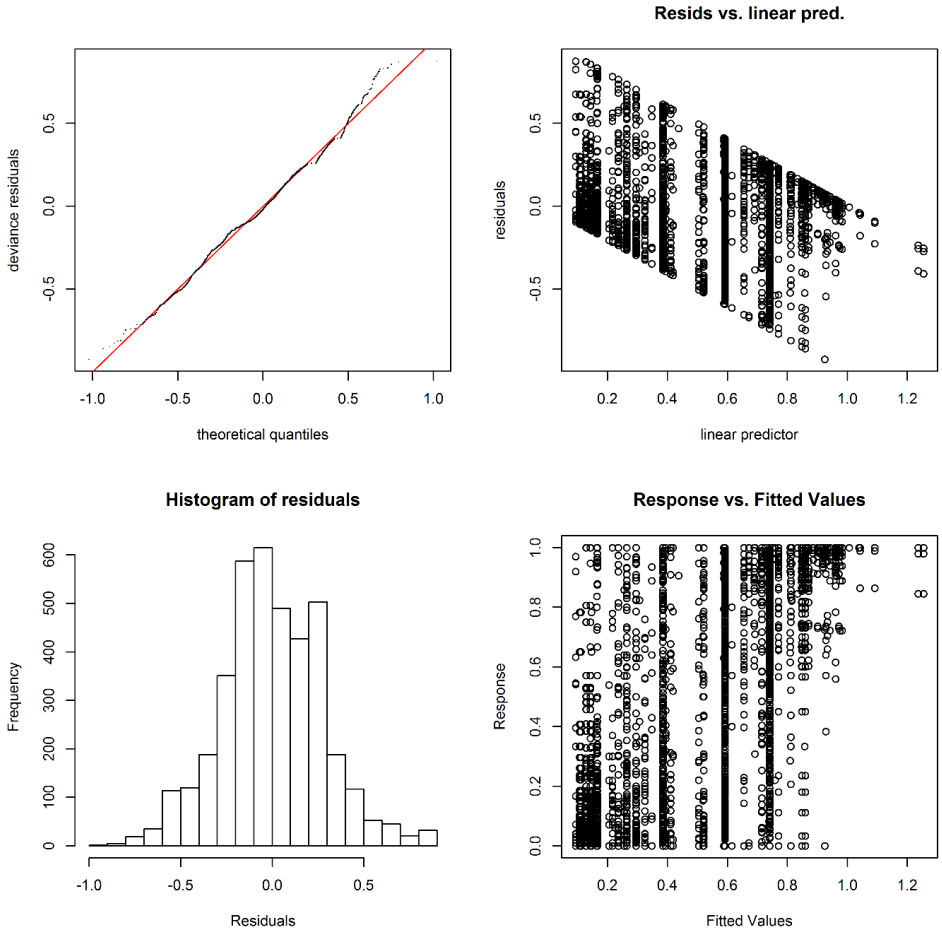


Fig S5. Validation plots for generalized additive model analyzing the proportion of pre-spawning herring. See Table 1 for estimated parameters.

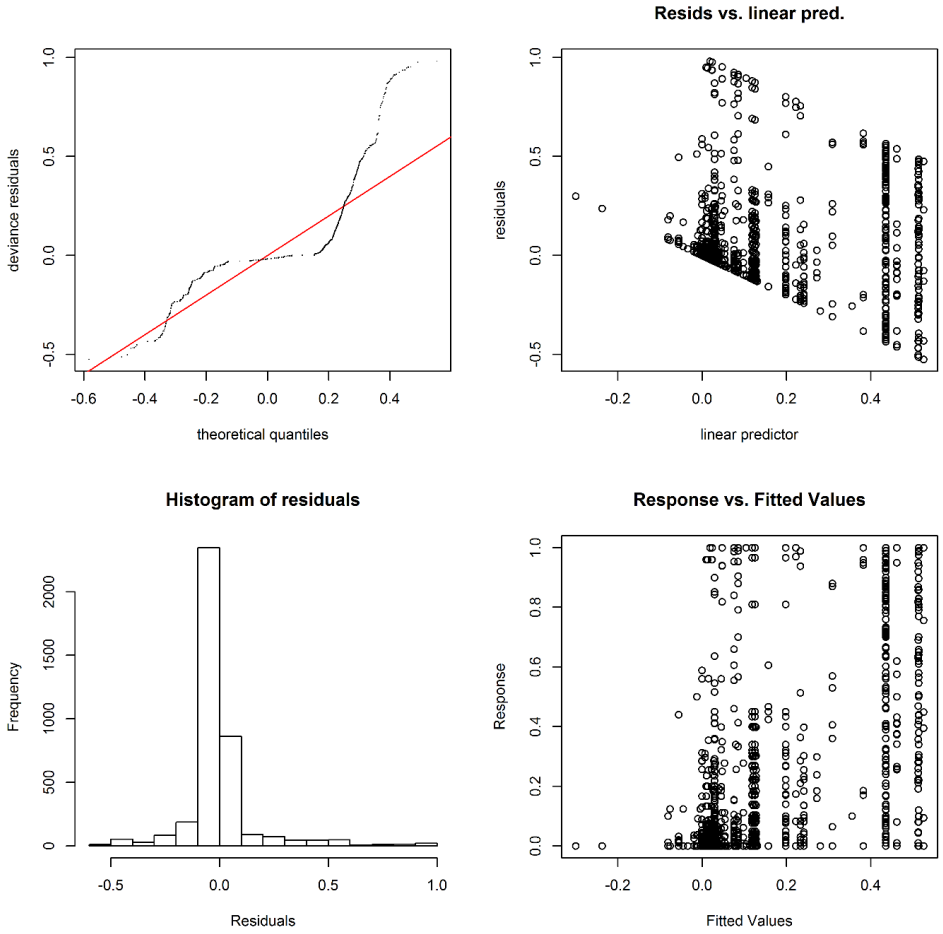


Fig S6. Validation plots for generalized additive model analyzing the proportion of spawning herring. See Table 1 for estimated parameters.

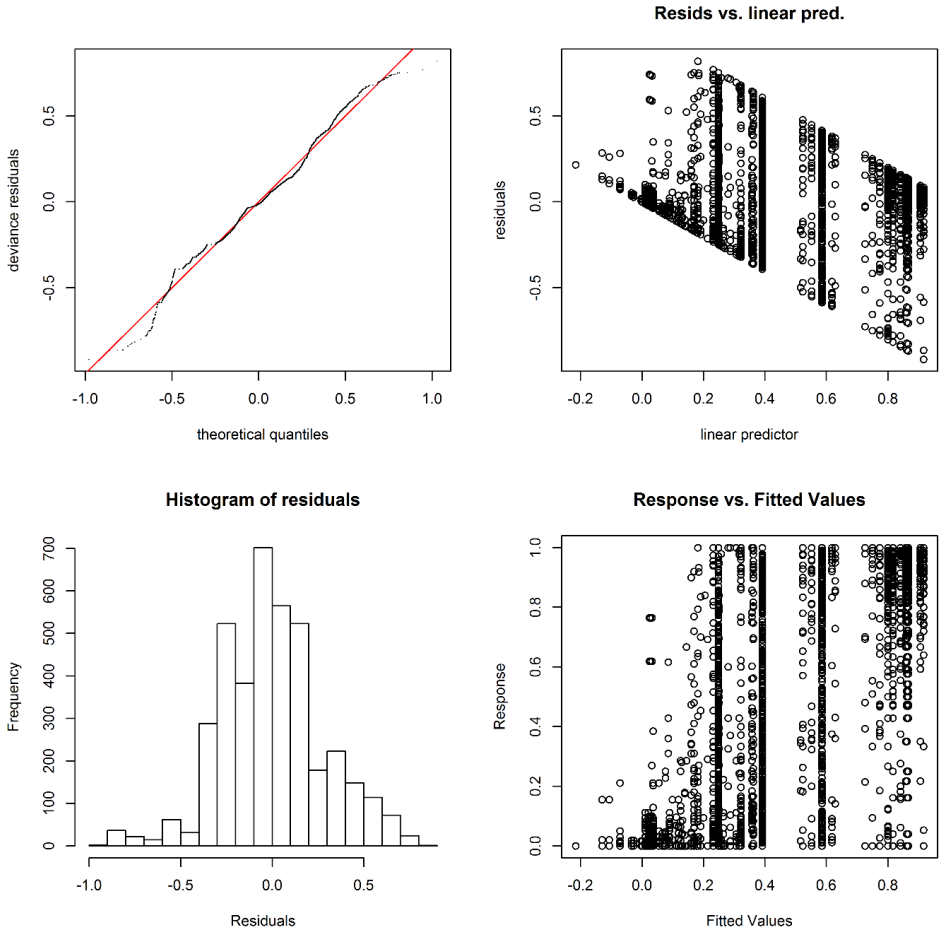


Fig S7. Validation plots for generalized additive model analyzing the proportion of post-spawning herring. See Table 1 for estimated parameters.

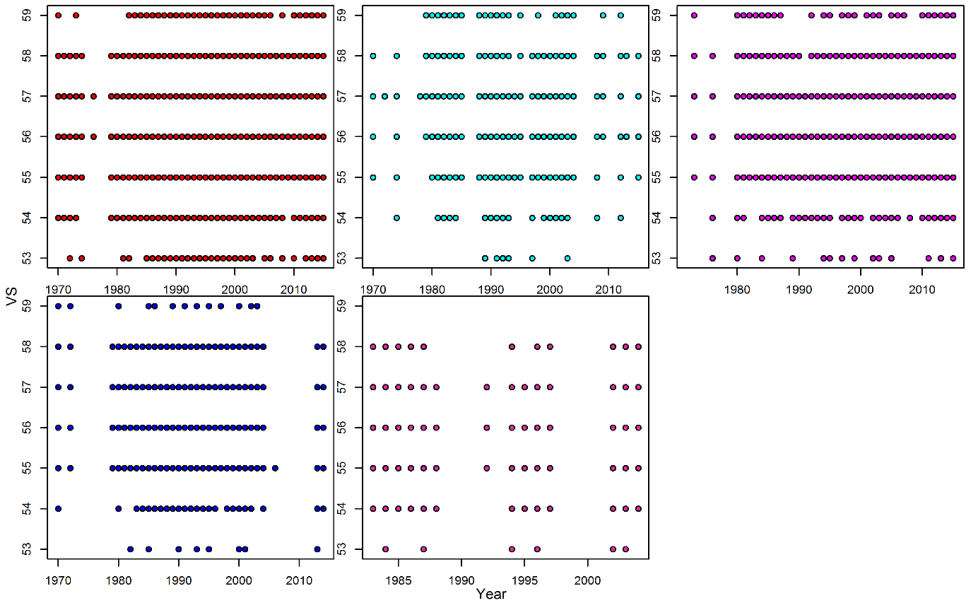


Fig S8. Raw data of individual vertebrae counts (VS) of herring for each area used in the generalized additive model (GAM) analysis. The low explained variance of only 17% for the GAM is resulting from the similar range and variance of vertebrae counts for the different areas (red = North Sea, cyan = west coast, purple = east coast, blue = Skagerrak, pink = western Baltic).

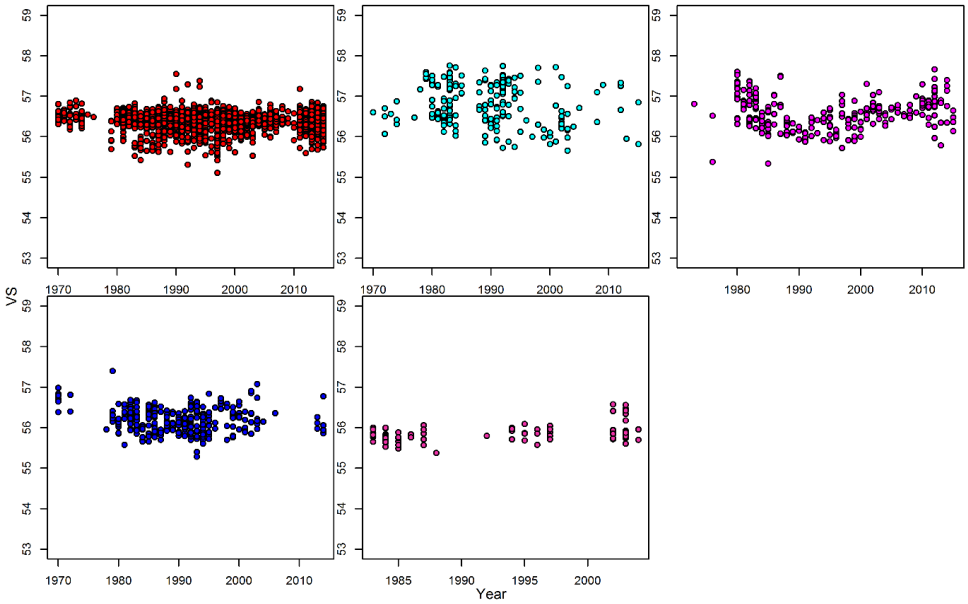


Fig S9. Mean vertebrae counts (VS) for each herring sample for each area. This data was not used in the generalized additive model (GAM) analysis, but would have increased the low explained variance of only 17% for the GAM, because the range and variance of vertebrae counts differs for the five areas (red = North Sea, cyan = west coast, purple = east coast, blue = Skagerrak, pink = western Baltic).

Paper V

Genetic Factors have a Major Effect on Growth, Number of Vertebrae and Otolith Shape in Atlantic Herring (*Clupea harengus*)

Short title: Genetic Effects on Phenotypic Traits in Herring

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Abstract

Atlantic herring, *Clupea harengus*, have complex population structures. Mixing of populations is known, but the extent of connectivity is still unclear. Phenotypic plasticity results in divergent phenotypes in response to environmental factors. A marked salinity gradient occurs from Atlantic Ocean (salinity 35) into the Baltic Sea (salinity range 2-12). Herring from both habitats display phenotypic and genetic variability. To explore how genetic factors and salinity influence phenotypic traits like growth, number of vertebrae and otolith shape an experimental population consisting of Atlantic purebreds and Atlantic/Baltic F1 hybrids which were incubated and co-reared at two different salinities, 16 and 35 respectively, for three years. The F1-generation was repeatedly sampled to evaluate temporal variation. A von Bertalanffy growth model indicated that reared Atlantic purebreds had a higher maximum length (26.2 cm) than Atlantic/Baltic hybrids (24.8 cm) at salinity 35, but not at salinity 16 (25.0 and 24.8 cm, respectively). In contrast, Atlantic/Baltic hybrids achieved larger size-at-age than the wild caught Baltic parental group. Mean vertebral counts and otolith aspect ratios were higher for reared Atlantic purebreds than Atlantic/Baltic hybrids, consistent with the differences between parental groups. There were no significant differences in vertebral counts and otolith aspect ratios between herring with the same genotype but raised in different salinities. A Canonical Analysis of Principal Coordinates was applied to analyze the variation in wavelet coefficients that described otolith shape. The first discriminating axis identified the differences between Atlantic purebreds and Atlantic/Baltic hybrids, while the second axis represented salinity differences. Assigning otoliths based on genetic groups (Atlantic purebreds vs. Atlantic/Baltic hybrids) yielded higher classification success (~90%) than based on salinities (16 vs. 35; ~60%). Our results demonstrate that otolith shape and vertebral counts have a significant genetic component and are therefore useful for studies on population dynamics and connectivity.

Keywords: common garden, otolith shape, phenotypic plasticity, population connectivity, salinity

Introduction

Phenotypic plasticity is the ability to display different phenotypes in response to environmental factors [1]. It has become increasingly important as the basic concept to discriminate marine fish populations [2] since individuals of a population are assumed to live under specific environmental conditions. Traditionally, fish populations have been identified based on phenotypic traits, although the relative importance of genetic and environmental factors on the determination of those phenotypic traits is generally unclear [3-5]. Therefore, the genetic and/or environmental mechanisms regulating phenotypic traits used for identification of fish populations need to be clarified and defined.

As one of the ecologically and commercially most important fish species in the northeastern Atlantic, herring (*Clupea harengus*) has been a key species for studies of population structure. Iles and Sinclair [6] proposed that Atlantic herring have complex population structure and much effort has been spent to resolve this structure. Phenotypic traits like growth [7, 8], numbers of vertebrae [9, 10], otolith microstructure [11, 12], as well as otolith shape [13-15] have been used to investigate the population structure of herring. Genetic studies have also become important during recent years. While no or very limited genetic differentiation was initially found between populations [16, 17], genome-wide analyses revealed clear genetic differentiation among Atlantic herring populations, but primarily at loci underlying ecological adaptation [18, 19].

Environmental factors have a strong influence on many phenotypic traits in herring. For example, temperature affects growth [20] and otolith microstructure [21, 22], as well as the number of vertebrae and can act in combination with salinity [23, 24]. While growth and otolith microstructure can vary over time with temperature, the number of vertebrae will be determined once during metamorphosis based on the experienced environmental

conditions [25]. In herring, there is also a co-variability between genetic differentiation and salinity [19, 26, 27].

A strong salinity gradient highly correlated with this genetic differentiation [19], occurs throughout the Baltic Sea from the inner Bothnian Bay (salinity <6) to the opening near the fully marine North Sea/Atlantic Ocean (salinity 35). Further, this salinity gradient is associated with differences in phenotypic traits of herring inhabiting these two environments. Beside the salinity, the temperature is often examined as the main factor determining variation in phenotypic traits. However, the average temperature difference between the Atlantic and Baltic (S5 Fig) is relatively minor compared to the vast salinity variation and subject also to marked seasonal variations. In addition, the lower average temperature in the Baltic is contradicting the common assumption of a negative correlation between the number of vertebrae and temperature [23].

To understand or resolve the genetic and environmental influences on phenotypic traits, we used offspring of two herring populations (Atlantic vs. Baltic) that were genetically different and living in contrasting salinities in a common garden rearing experiment. Common garden experiments are designed to rear offspring from different populations under identical environmental conditions. Both Atlantic purebreds and Atlantic/Baltic hybrids were reared under controlled conditions with fixed salinities of either 16 or 35. Our main objectives were to explore genetic and salinity influences on phenotypic traits like growth, number of vertebrae and otolith shape. Further, the experiment was conducted over a 3-year period to evaluate potential temporal variation in growth and otolith shape. Temporal variation in the number of vertebrae was tested to determine if it was subjected to selection.

Material and Methods

Spring spawning herring caught by gillnets on 21st May 2013 in the Atlantic, approximately 12 km west of Bergen, Norway (60°34'11.2"N 5°0'18.9"E) and Baltic, approximately 80 km northeast of Uppsala, Sweden (60°38'52.0"N 17°48'44.2"E) were used as parental fish in this study. Half of the eggs from one Atlantic female were fertilized and incubated on the day of capture with sperm of one Atlantic male; the other half was fertilized with sperm of one Baltic male. The Atlantic herring were 5 years of age, 30.5 (female) and 32.5 cm (male) in total length with 57 vertebrae. The Baltic male was 8 years of age, 20.5 cm in total length with 55 vertebrae. The age of herring was determined by counts of winter rings from otoliths. The experimental setup, including only one mother, was designed to avoid any environmental maternal effects. Those parental herring were from a subset of samples representing typical Atlantic and Baltic populations that exhibited huge phenotypic differences between groups (S6 Fig) and have been genetically characterized confirming population-specific differences [19]. The supporting information provides further details about the parental groups.

The fertilization and rearing experiment was conducted under common garden conditions at salinities 16 and 35, with values fluctuating during incubation between 15-17 and 34-35, respectively. Water temperatures varied with seasons with an average of 9.12±0.73°C and 9.04±0.71°C at salinity 16 and 35, respectively (S5 Fig) and the light intensities fluctuated according to the seasonal and daily cycle in Bergen (60°N). Fifty percent hatching, defined as day 0, occurred on 5th June 2013. Atlantic purebreds and Atlantic/Baltic F1 hybrids, hereafter called purebreds and hybrids, were co-reared at salinity 16 and 35 in two replicated 1 m circular tanks at each salinity, including in total 1000 larvae at an initial purebred/hybrid ratio of 1:2. Herring larvae were fed in excess, firstly with live natural zooplankton and cultured rotifers [28] and later with *Artemia* spp.

(23 days post hatching = DPH), until feeding on formulated feed started (71 DPH). On 3rd October (120 DPH), juveniles were transferred into two 3 m circular tanks, one with salinity 16 and one with salinity 35, where the herring were reared further for nearly 3 years until their first maturity. This experimental setup generated four groups (*Pop*, in statistical models) which can be distinguished genetically into purebreds and hybrids, as well as by salinity; H16 = hybrid at 16, H35 = hybrid at 35, P16 = purebred at 16 and P35 = purebred at 35.

During the three years, we sampled 690 otoliths out of 950 herring (Table 1). All sampled fish were measured to the nearest mm and fin clipped for DNA analysis, whereas the number of vertebrae was counted only for some samples (n = 522, Table 1). For the DNA analysis, a custom TaqMan® assay design tool was developed to discriminate purebred and hybrids by genotyping a diagnostic SNP [29].

Table 1. Total numbers of analyzed herring and otoliths (in brackets).

DPH	H16	P16	H35	P35	Sample
187	85 (73)	14 (13)	69 (61)	30 (26)	1*
297	36 (31)	4 (2)	31 (24)	19 (18)	2*
482	0	0	76 (24)	37 (19)	
524	0	0	56 (0)	34 (0)	
531	0	0	17 (0)	8 (0)	
618	27 (23)	3 (2)	16 (14)	14 (12)	3*
702	0	0	10 (8)	10 (8)	
861	11 (8)	1 (1)	19 (13)	12 (11)	4
960	7 (3)	1 (1)	23 (22)	9 (9)	4*
1055	0	0	16 (15)	14 (14)	
1079	0	0	31 (31)	8 (8)	
1098	33 (33)	5 (4)	38 (37)	14 (13)	5*
1106	23 (22)	8 (7)	18 (18)	12 (12)	5*
1120	17 (17)	4 (4)	16 (15)	14 (14)	5*
Total	**240 (210)**	**39 (34)**	**436 (282)**	**235 (164)**	

Samples from different sampling days (DPH = days post hatching) that were combined for otolith analyses were marked with identical numbers in the rightmost column. * Number of vertebrae was also counted. H16 = hybrids at salinity 16, P16 = purebreds at salinity 16, H35 = hybrids at salinity 35, P35 = purebreds at salinity 35.

A digital image of each otolith was captured using a Leica MZ95 stereomicroscope and reflected light with a Nikon digital sight DS-U1 microscope camera using the software NIS-elements F (Version 2.3). Following the method by Libungan et al. [30], otolith images were read into the R software [31], and otolith shape outlines were collected from the images using the shapeR package [32]. A discrete wavelet transformation to equally spaced radii from the otolith centroid to the otolith outline was conducted to obtain wavelet coefficients (unitless). Hereafter, the otolith shape refers to variation in the wavelet coefficients representing the otolith shape outline. An analysis of covariance (ANCOVA) was performed individually for each sample (Table 1) to determine the effect of fish length on the wavelet coefficients, as well as otolith length and width. Coefficients which showed an interaction between the four herring groups and total length were excluded from the analysis (S2 Table). In this study, fish length could also be used as a proxy for the growth rate, because all fish from a sample had the same age (days post hatching, DPH). Further, the remaining coefficients, as well as otolith length and width were adjusted for allometric relationships with fish length applying the normalization technique of Lleonart et al. [33].

For all model fittings, full and complex models were used as starting references and simplified in cases of non-significance. Length-at-age data, used as a proxy for somatic growth of individual herring, were fitted to the von Bertalanffy growth model [34]:

$$TL_t = L_{\infty_{Pop}}\left(1 - e^{-K(t-t_0)}\right)$$

where TL_t is the average length at age t, L_∞ is the asymptotic maximum length of each of the four herring groups (*Pop*), K is the von Bertalanffy growth rate coefficient, i.e., the rate at which length approaches the maximum length asymptote and t_0 is the intercept on the time axis.

The aspect ratio (otolith length/width) was calculated for comparison with the parental groups. Further ANOVA tests were used to evaluate the significance of genetic origin (*Gen*) and salinity (*Sal*) on otolith width (*OW*) or length (*OL*) at given age classes (*DPH* as factor):

$$OW\ or\ OL = \alpha + \beta_1 \times DPH + \beta_2 \times Gen + \beta_3 \times Sal$$

Differences in the otolith aspect ratio (*AR*) and the mean number of vertebrae (*VS*) among genetic origin (*Gen*) and salinity (*Sal*) were tested using a one-way ANOVA:

$$AR = \alpha + \beta_1 \times Gen + \beta_2 \times Sal$$

$$VS = \alpha + \beta_1 \times Gen$$

The initial starting model also included, in both cases, the age (*DPH*) as a predictor variable, but this was removed due to non-significance. Since the number of vertebrae is fixed during metamorphosis, temporal variation would indicate some kind of selection, either through sampling or mortality. Significant differences among the four herring groups were identified using Tukey-HSD tests. A significance level $\alpha = 0.05$ was applied for all analyses and statistical tests.

For a subset of the sampling days, otoliths had been obtained from fish in both salinities. Only these otoliths were used for the otolith shape analyses to allow for comparisons across salinities. In some cases (sample 4 and 5), adjacent sampling days (DPH) were combined due to low numbers (Table 1). Those samples were taken within 100 days of each other, and none of the analyzed characteristics differed. The temporal development of otolith shape outlines and general differences among the groups were examined visually by plotting the mean otolith shape outline of each group reconstructed of the wavelet coefficients (S1 Fig). To investigate which region of the otolith shape outline contributed most to the differences between the four groups, mean wavelet coefficients and their standard deviation was plotted against the angle of the outline.

Thereby, each mean wavelet coefficient indicates the variation of the otolith shape outline within their predefined region. Further, the correlation within each group along the outline was estimated with an intraclass correlation (ICC). Consequently, a combination of a high mean wavelet coefficient (>0.25) and a high intraclass correlation indicated the region along the otolith shape outline that differed most. For statistical analysis to demonstrate the variation in otolith shape represented by wavelet coefficients, Canonical Analysis of Principal Coordinates (CAP) [35] followed by ANOVA-like permutation tests were applied with 2000 permutations used to assess the significance of constraints. The CAP and ANOVA-like permutation tests were only applied to otoliths from herring of age 187 DPH and 1108 DPH (Table 1) because these samples provided enough otoliths from all groups to ensure reliable results. Finally, the ordinations of group averages were examined with the shape descriptors along the first two canonical axes. Using the CAP and the ANOVA-like permutation tests, otolith shape was compared among the four herring groups with an overall test, as well as by applying comparison between salinity and genetic groups. In addition, salinity effects on the otolith shape were investigated in the absence of any genetic differences by comparing hybrids originating from salinity 16 and 35 in isolation and purebreds in isolation. The genetic signal was examined in the same way in the absence of any salinity differences.

To validate if otolith shape analysis can be used for assigning individual herring to a given group, we applied a linear discriminant analysis (LDA) to the standardized wavelet coefficients of sample 1 and 5. The classification success into salinities, genetics, as well as the four groups was estimated using the leave-one-out cross-validation [36]. Thus, each otolith was removed individually from the dataset and assigned to one of the predefined groups.

Ethics statement

Herring samples of the parental Atlantic population were caught with permission of the Directorate of Fisheries, Bergen, Norway. The parental Baltic herring were purchased from a local commercial fisherman. The common garden experiment and rearing of the F1-generation was approved by the Norwegian national animal ethics committee (Forsøksdyrutvalget – FOTS ID-5072).

Results

Somatic growth of herring reared under common garden conditions over three years only differed in the maximum asymptotic length among the four herring groups (Fig 1A). Atlantic purebreds reared at salinity 35 (L_∞ = 26.2 cm) were larger (ANOVA: F = 194.5, $d.f.$ = 944, p<0.001) than the other three groups (purebreds at salinity 16 (L_∞ = 25.0 cm), Atlantic/Baltic hybrids at salinity 35 (L_∞ = 24.8 cm) and 16 (L_∞ = 24.8 cm)), which did not differ from each other (ANOVA: F = 0.69, $d.f.$ = 712, p>0.05).

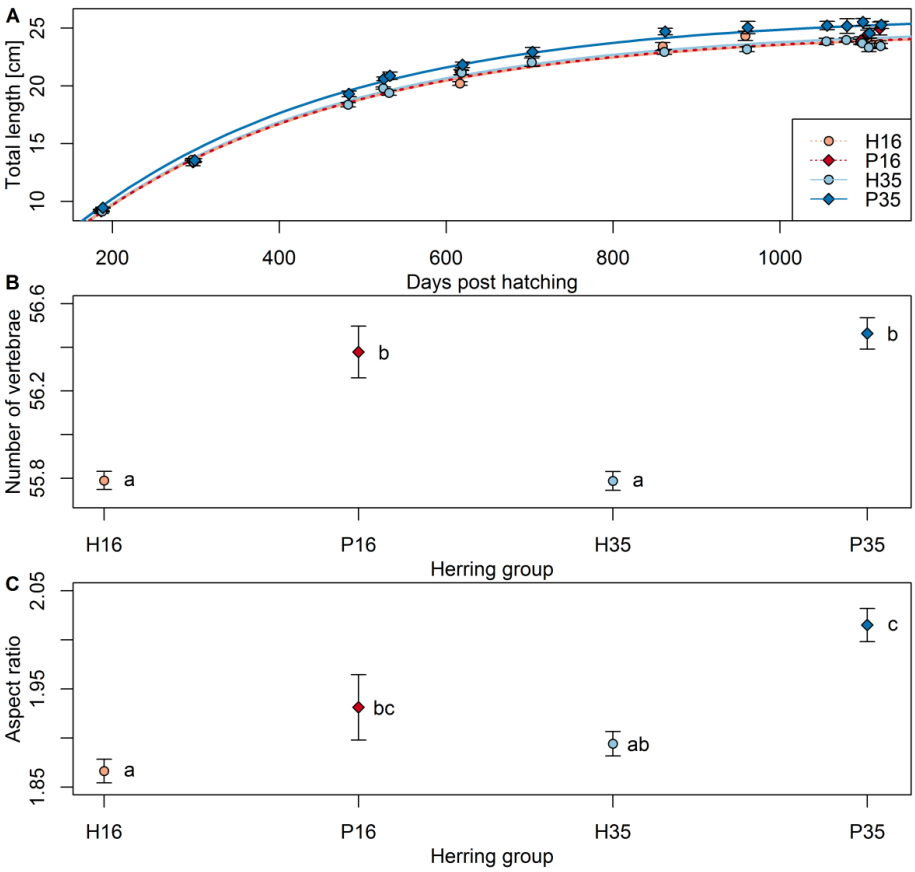

Fig 1. Comparison of phenotypic traits among the four herring groups. (A) length-at-age and von Bertalanffy growth models, (B) number of vertebrae, and (C) otolith aspect ratio were compared among H16 = hybrids at salinity 16, P16 = purebreds at salinity 16, H35 = hybrids at salinity 35, P35 = purebreds at salinity 35. Mean values and 1*SE are shown. Letters indicated posterior Tukey-HSD test results of all pair-wise comparisons. Groups which do not share a letter are significantly different to each other.

For the number of vertebrae, only a genetic effect could be demonstrated (Fig 1B; ANOVA: $F = 109.1$, $d.f. = 520$, $r^2 = 0.17$, $p<0.001$). Mean vertebral counts were higher for Atlantic purebreds compared to Atlantic/Baltic hybrids irrespective of salinity (Tukey-HSD tests: $p<0.001$). The number of vertebrae did not differ over time within each group, indicating that no selection in terms of sampling or mortality had occurred for this trait (ANOVA: $F = 0.97$, $d.f. = 517$, $p>0.05$).

In general, otoliths of hybrids were shorter but wider compared with purebreds, but there were no differences between otoliths of herring originating from different salinities within each genetic group (Table 2). The otolith aspect ratio was higher for purebreds than hybrids (Fig 1C; ANOVA: $F = 5.9$, $d.f. = 560$, $r^2 = 0.08$, $p<0.001$), and higher for herring reared at salinity 35(ANOVA: $F = 2.5$, $p<0.02$). Purebreds at salinity 16 had a higher aspect ratio than hybrids at salinity 16, but the ratio was not significantly different from hybrids at salinity 35 (Tukey-HSD tests: $p>0.05$). The aspect ratio did not vary significantly between samples of different ages (ANOVA: $F = 1.1$, $d.f. = 7$, $p>0.05$). Additional results of the development of the otolith shape outline can be found in the S1 Supporting information.

Table 2. Results from the ANOVA tests investigating the effects of age (days post hatching), salinity (16 vs. 35) and genetics (purebred vs. hybrid) on otolith width and length.

	Otolith width				**Otolith length**			
Variable	**d.f.**	**MS**	**F**	**p**	**d.f.**	**MS**	**F**	**p**
Days post hatching	11	23.46	3357.3	<0.001	11	105.09	4185.4	<0.001
Salinity	1	0.00	0.3	0.55	1	0.02	0.7	0.41
Genetics	1	0.10	13.6	<0.001	1	0.69	27.5	<0.001
Residuals	673	0.01			673	0.03		

d.f. = degrees of freedom, MS = mean square, F = *F*-value, p = *p*-value

For the following analyses, only otoliths from herring at age 187 days post hatching (DPH) and 1108 DPH were used due to a sufficient sample size. Significant differences in otolith shape, represented by wavelet coefficients, were observed between fish from the two salinities, between hybrids and purebreds, as well as among all four groups combined within both ages (Fig 2, Table 3). Comparing the salinity effect separated for hybrids and purebreds demonstrated slightly significant differences (ANOVA: $F = 2.0$, $d.f. = 1$, $p=0.049$) for hybrids from 187 DPH and purebreds from 1108 DPH, but not for purebreds from 187 DPH and hybrids from 1108 DPH (S3 Fig, S3 Table). While

separating herring based on the salinity, otolith shape was always significantly different between hybrids and purebreds originating either from salinity 16 or 35 at both sampling days (S4 Fig). Genetic differences had a higher impact on the otolith shape than salinity differences, as indicated by higher *F*-values (Table 3). CAP and ANOVA-like permutation tests combining both parameters revealed clear differences among the four groups. The first canonical axis explained most of the variation between genetic groups, both for otoliths from 187 DPH (CAP1: 86.8%) and 1108 DPH (CAP1: 81.0%, Fig 2). Differences between the two salinities were explained by the second canonical axis, but only for otoliths from older herring (CAP2: 14.1%). The otolith shape of younger herring also varied along the second canonical axis, but not significantly and without any distinct pattern.

Table 3. Results from ANOVA like permutation tests comparing the otolith shape (represented by wavelet coefficients) among salinities and genetic groups, as well as the four herring groups in the present study.

	187 days post hatching				**1108 days post hatching**			
Variable	**d.f.**	**Var**	**F**	**p**	**d.f.**	**Var**	**F**	**p**
Salinity	1	0.77	2.2	<0.05	1	1.87	2.3	0.03
Residuals	171	60.31			194	155.67		
Genetics	1	5.09	15.5	<0.001	1	11.89	15.8	<0.001
Residuals	171	55.99			194	145.65		
Genetics	1	5.09	15.7	<0.001	1	11.89	16.0	<0.001
Salinity	1	0.41	1.3	0.23	1	1.54	2.1	0.03
Genetics*Salinity	1	0.72	2.2	0.06	1	1.28	1.7	0.08
Residuals	169	54.87			192	142.83		

d.f. = degrees of freedom, Var = variance, F = *F*-value, p = *p*-value.

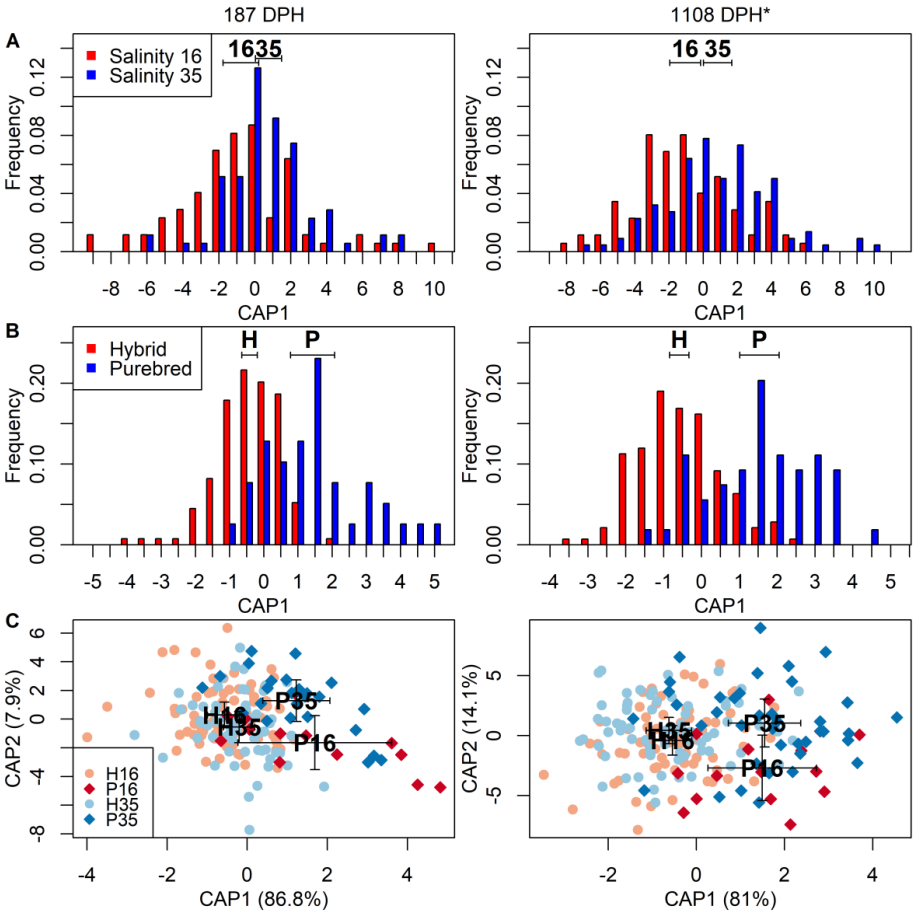

Fig 2. Canonical analysis of principal (CAP) scores of herring otolith shapes on discriminating axes. Scores of the first axis are shown for (A) salinity and (B) genetics, scores of the first and second axis for (C) all four groups. H16 = hybrids at salinity 16, P16 = purebreds at salinity 16, H35 = hybrids at salinity 35, P35 = purebreds at salinity 35. Black bold letters represent the mean canonical value for each character ± 1*SE. Individual fish are represented by frequencies (A, B) or symbols (C). * Mean day post hatching (DPH) for combined samples.

Otoliths from herring at age 187 DPH and 1108 DPH were classified based on their otolith shape. Classification success of otolith shape varied depending on the classifying character (Table 4). Otoliths of both ages had comparable results for each classifying character. Assigning otoliths based on salinities or the four groups had both a success rate between 50-60%, whereas based on the genetic groups ~90% of the otoliths were

classified correctly. Splitting the results into individual comparisons (e.g., hybrids and purebreds when comparing the salinity effect) gave similar classification successes as for combined samples (Table 4).

Table 4. Overall classification success (bold) of otoliths into salinity, genetic groups and the four groups based on a linear discriminant analysis. Further classification success was split up into individual possibilities. The analyses were conducted independently for otoliths from herring at different ages (187 and 1108 days post hatching = DPH).

	Classification success	
	187 DPH	**1108 DPH**
Salinity	**56.7%**	**60.7%**
Hybrid	58.2%	61.3%
Purebred	46.2%	55.6%
Genetics	**91.9%**	**87.2%**
Salinity 16	81.4%	77.0%
Salinity 35	81.6%	78.9%
All groups	**54.9%**	**49.5%**
Hybrid 16	58.9%	44.4%
Hybrid 35	54.1%	52.9%
Purebred 16	23.1%	33.3%
Purebred 35	61.5%	59.0%

Discussion

This study provides the strongest evidence reported so far that the number of vertebrae and otolith shape (represented by wavelet coefficients) in Atlantic herring have a clear genetic basis and genetics had a more profound effect on these phenotypes than salinity (16 or 35). In general, this study confirms the genetic regulation of otolith shape [37]. The clearly demonstrated genetic effects based on phenotypic data from offspring were consistent with the phenotypic difference between the parental populations (Atlantic vs. Baltic herring). Further, temporal variations over a 3-year period in these traits were not evident indicating that selection did not occur. The demonstrated differences in otolith aspect ratio or shape were genetically affected and independent of growth rate variations

because the allometric relationship was removed by scaling the otoliths according to Lleonart et al. [33].

So far, most studies on population discrimination in fish have largely been based on phenotypic traits without knowledge of the specific genetic background influencing these traits [see references in 38]. Yet, studies combining genetic and environmental impacts are essential to understand population structures in marine fish. In this study, the genetic effect was the main factor underlying the observed phenotypic variation, supporting the use of genetic markers for population discrimination. Additional factors, such as temperature, could not be assessed with the current experimental design of common garden conditions. Further, phenotypes are not as informative as direct genetic data because a lack of phenotypic differences does not prove a lack of genetic differentiation.

Despite the large salinity differences experienced throughout the entire lifecycle of the experimental fish, salinity had only a minor impact on the phenotypic variation. However, salinity is not only considered to reflect genetic distinctness [39-41] but also associated with rapid ecological speciation [42, 43]. Further, some fish species demonstrate higher growth at intermediate salinities than at a fully marine salinity [44, 45]. However, within this study Atlantic purebreds were growth retarded at salinity 16 compared with salinity 35, whereas the Atlantic/Baltic hybrids grew equally well in both salinities. This indicates the adaptation of Atlantic purebreds to high salinity. On the other hand, hybrids clearly outgrew the wild caught Baltic parental group within two years and had a much larger size-at-age (S6 and S7 Fig). It is possible that the genetic influence of the Atlantic parent contributed to this difference in growth conditions, but the captive F1-hybrids were also growing at strikingly different environmental conditions compared with their wild-caught Baltic herring parent. In herring, some of the loci underlying genetic

differentiation between Atlantic and Baltic herring show a strong correlation with average salinity conditions experienced by the different populations [18, 19]. However, there are also many other variables, for example in nutrition and temperature [21, 46], which could affect the growth of captive Atlantic/Baltic hybrids and wild Baltic herring. Herring in this study were fed in excess, and the water temperatures were generally higher than in the Baltic Sea (S5 Fig) which most likely promoted a higher growth of Atlantic/Baltic hybrids compared to the Baltic parental group.

Besides salinity, temperature is known to have a high impact on phenotypic traits, like growth [47] and number of vertebrae [48]. Temperature has been demonstrated to be the major determinant of otolith growth, and therefore, differences in otolith shape are essentially influenced by temperature [49, 50]. Further, environmental factors such as temperature and feeding conditions have impacts on otolith shape differences [14], even in the absence of genetic differences [51, 52]. However, in some cases, temperature does not affect otolith shape [53], or the genotype still has a higher impact than temperature on other phenotypic traits like growth [54, 55] or number of vertebrae [56]. Countergradient variation, i.e., the inverse relationship between environmental conditions and individual growth response [57], can maintain morphological similarity across populations and compensate for the effect of temperature [58].

Common garden experiments are ideally suited to dissect the relative importance of genetic and environmental factors affecting phenotypic traits [59, 60] and can play an essential role in resolving population structure [55]. Further, the exact knowledge of genetic and environmental origin is an enormous benefit, in contrast to natural samples where the origin can only be assumed. This knowledge was used for an individual assignment of otoliths achieving highest classification success when assigning the otoliths to their genetic origin (87.2-91.9%) being comparable to other classification studies

among populations using otolith shape [30, 61, 62]. Only a minor part of the otolith shape variation could be explained by salinity differences in the absence of genetic variation. Still, those differences are relevant for resolving the stock structure of herring, because otolith shape is also used to distinguish between groups that can so far not be separated using genetics [63].

This high classification success based on otolith shape is practically used to separate populations from mixed fisheries [see, e.g., 61, 64]. This could then be incorporated in fisheries management and assessment to allow more sustainable exploitation of the populations [65]. Also in herring, otolith shape is used to discriminate between mixing stocks with varying spawning seasons [66]. However, there are other examples where spawning components have been identified but are still assessed as a unit stock [50]. Based on our results demonstrating the effect of genetics we encourage the establishment of otolith shape baselines. This baseline can be further used in combination with machine-learning techniques [67] to assign individuals from mixed fisheries to populations.

In conclusion, our study revealed that the variation in several phenotypic traits, like growth, otolith shape and the number of vertebrae, was primarily controlled by genetic factors, while salinity played a minor role. To the best of our knowledge, this is the first time that hybrids of two herring populations were reared under common garden conditions until maturity. Finally, our results show that some of the phenotypic traits included in this study provide information to distinguish genetically differentiated herring. These phenotypic traits can be further used to study population dynamics and connectivity because they are to a large extent genetically determined. However, other factors, which were excluded due to the experimental design, might outplay the genetic response demonstrated within this study.

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Supporting information

S1 Supporting Information. Further details on the otolith shape outline development, as well as the parental group

S1 Table. Total numbers of analyzed parental fish and otoliths (in brackets) for each sample and parental group.

S2 Table. Number (N) and wavelet coefficients that were removed by adjusting otolith shape for allometric relationships with fish length individually for each sample.

S3 Table. Results from ANOVA like permutation tests comparing the otolith shape among salinities and genetic groups in isolation.

S1 Figure. An otolith shape outline example with lines indicating length and width going through the center of gravity. PoR = postrostrum, PaR = pararostrum, EMi = excisura minor, EMa = excisura major, R = rostrum, AR = antirostrum.

S2 Figure. (A) Mean otolith shape outline reconstructed from wavelet coefficients (unitless) and (B) their differences at respective otolith angle. Data are shown for each sampling date and the four herring groups (H16 = hybrids at salinity 16, P16 = purebreds at salinity 16, H35 = hybrids at salinity 35, P35 = purebreds at salinity 35). The mean and standard deviation (SD) of the wavelet coefficients represent otolith shape outline variation among all groups and the intraclass correlation (ICC, black solid line) represents the variation within each group. * Mean day post hatching (DPH) for combined samples. Note that the otolith outline for P16 at 279, 618 and 910 DPH is based on N = 2.

S3 Figure. Canonical analysis of principal (CAP) scores of herring otolith shapes indicating differences for salinity separated by the genetic groups (Hybrids and

purebreds). Data given for the samples A) 187 and B) 1098 days post hatching. Black bold letters represent the mean canonical value for each character ± 1*SE. Individual fish are represented by frequencies.

S4 Figure. Canonical analysis of principal (CAP) scores of herring otolith shapes indicating differences for genetics separated by salinity (16 and 35). Data given for the samples A) 187 and B) 1098 days post hatching. Black bold letters represent the mean canonical value for each character ± 1*SE. Individual fish are represented by frequencies.

S5 Figure. Daily water temperatures Atlantic purebred and Atlantic/Baltic hybrids were reared at their entire life in either salinity 16 (light blue) or salinity 35 (dark blue). Water temperatures of the Atlantic (light red) were measured at stationary hydrographic stations in Ytre Utsira and Sognesjøen. Daily temperatures were combined for both stations and average for depths from 20-120 meters. Water temperatures of the Baltic (dark red) were extracted from https://sharkweb.smhi.se/ and restricted to the area 16-23° E and 56.5-62° N. Daily temperatures were combined all stations within the area and average for depths from 20-50 meters. Mean±SD are given in the legend and lines represent a running mean.

S6 Figure. Weight-at-length data of the parental groups. Individuals used as parents for the F1-generation are marked (Atlantic male, Atlantic female, Baltic male).

S7 Figure. Comparison of mean length (left), number of vertebrae (middle), and otolith aspect ratio (otolith length/otolith width, right) among the parental fish. Mean values and 1*SE are shown.

S8 Figure. Average otolith shape outline for parental groups. The shown outline does not correspond to the actual size and ratio of the original otoliths.

S1 Supporting Information

Results

Otolith shape outline development

The development of otolith shape outline (see S1 Fig for nomenclature) over three years was similar among the four groups (S2 Fig A). At the early stage (187 days post hatching), the *excisura major* was between the *rostrum* and the *antirostrum*, but with increasing age of herring, the *antirostrum* became more prominent and was more *anterior* than the *excisura major*. A similar development was observed with the *postrostrum* and *pararostrum*. At early ages, the *pararostrum* was more developed, changing to a more prominent *postrostrum* over time.

Otolith shape outline differed among all four groups, as visually reflected in mean shape differences (S2 Fig A) and a high level of variation in the wavelet coefficients among the groups (S2 Fig B). Depending on the age of the otolith the specific regions showing the highest variation among the groups in combination with high within groups correlation (ICC) differed, but in general the main differences were found along the otolith outline at 200-240° (S2 Fig B).

Parental group

Spring spawning herring caught 21st May 2013 in the Atlantic (60°34'11.2"N 5°0'18.9"E) and Baltic (60°38'52.0"N 17°48'44.2"E) were used as parental fish in this study. An additional sample of the parental groups was taken during the spring spawning season (S1 Table). The same methods and analysis as for the F1-generation were applied for the parental groups. There were no differences between the samples. The age of parental fish was not completely identified. Therefore, no length-at-age data is available and only mean length for each group is given. Instead weight-at-length data are given (S6 Fig) to demonstrate the phenotypic differences between the two parental groups. Individuals used for crossing out the F1-generation are marked. All parental fish were in spawning conditions and older than 3 years. The results for the parental groups are summarized in S7 and S8 Fig.

S1 Table. Total numbers of analyzed parental fish and otoliths (in brackets) for each sample and parental group.

Date	Baltic	Atlantic
13.5.2013	42 (35)	9 (8)
21.5.2013	48 (47)	109 (63)
Total	90 (82)	118 (71)

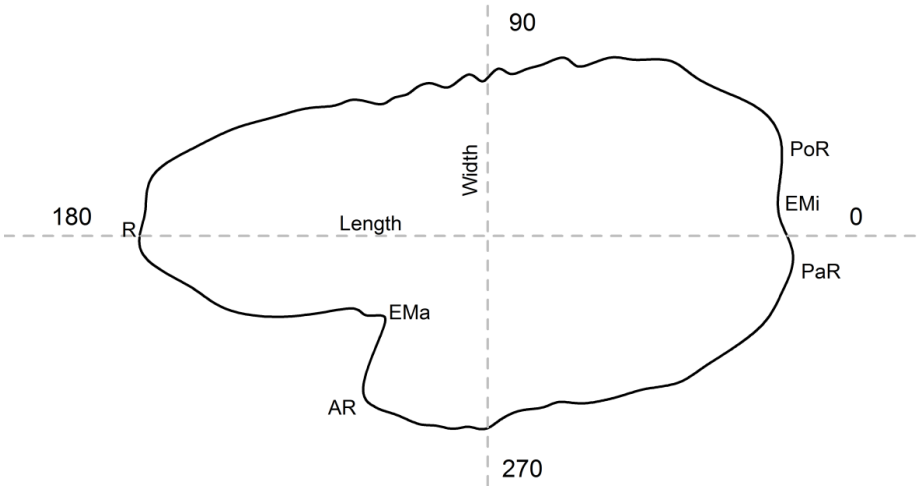
S2 Table. Number (N) and wavelet coefficients that were removed by adjusting otolith shape for allometric relationships with fish length individually for each sample.

Days post hatching	N	Removed coefficients
187	8	7, 8, 32, 34, 38, 44, 45, 54
297	2	56, 58
618	0	
910	4	8, 16, 53, 62
1108	0	

S3 Table. Results from ANOVA like permutation tests comparing the otolith shape among salinities and genetic groups in isolation.

		187 days post hatching				1108 days post hatching			
Isolation factor	**Variable**	**d.f.**	**Var**	**F**	**p**	**d.f.**	**Var**	**F**	**p**
Hybrid	Salinity	1	0.59	2.0	0.049	1	0.92	1.4	0.174
	Residuals	132	38.85			140	92.64		
Purebred	Salinity	1	0.54	1.2	0.237	1	1.90	2.0	0.049
	Residuals	37	16.01			52	50.19		
Salinity 16	Genetics	1	3.18	9.0	<0.001	1	4.52	6.5	<0.001
	Residuals	84	29.63			85	59.19		
Salinity 35	Genetics	1	2.27	7.6	<0.001	1	8.32	10.6	<0.001
	Residuals	85	25.23			107	83.64		

d.f. = degrees of freedom, Var = variance, F = *F*-value, p = *p*-value.



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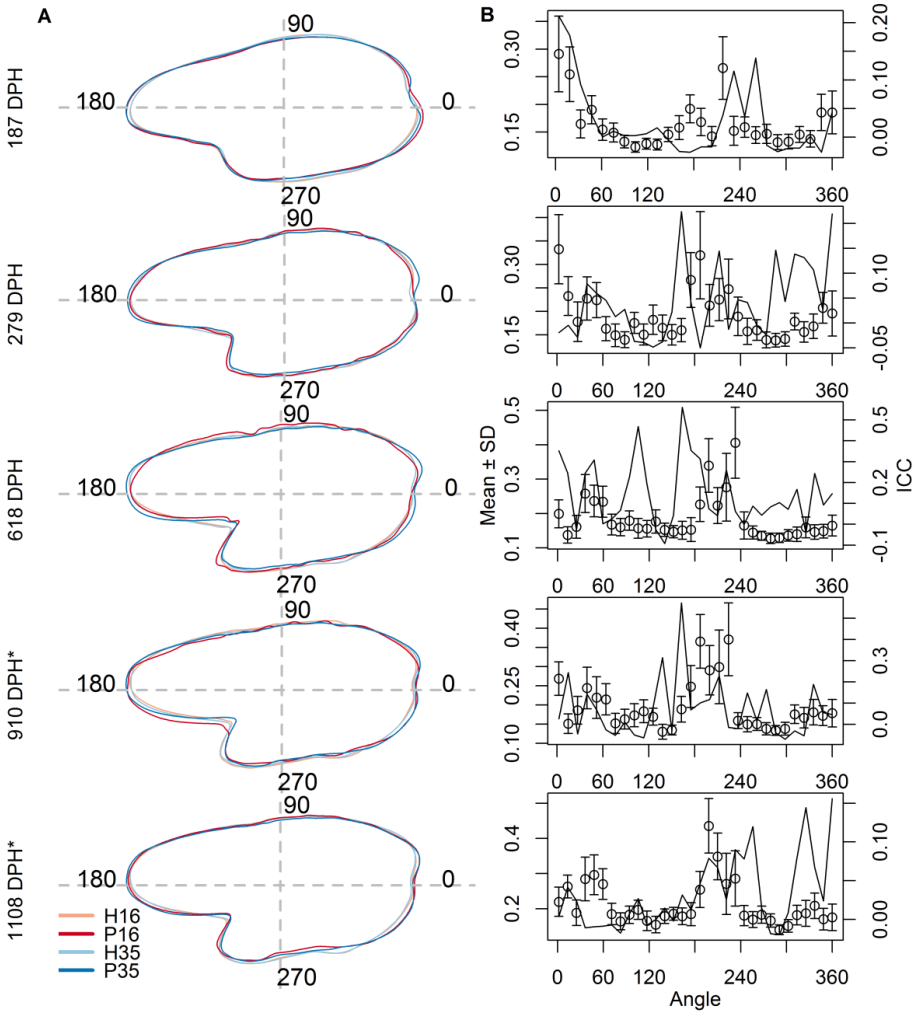

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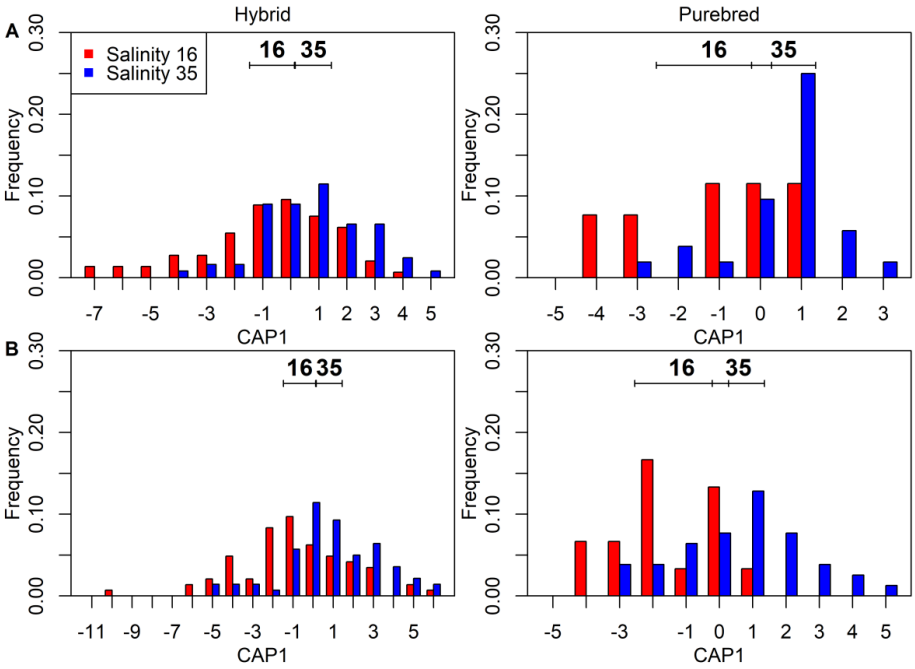

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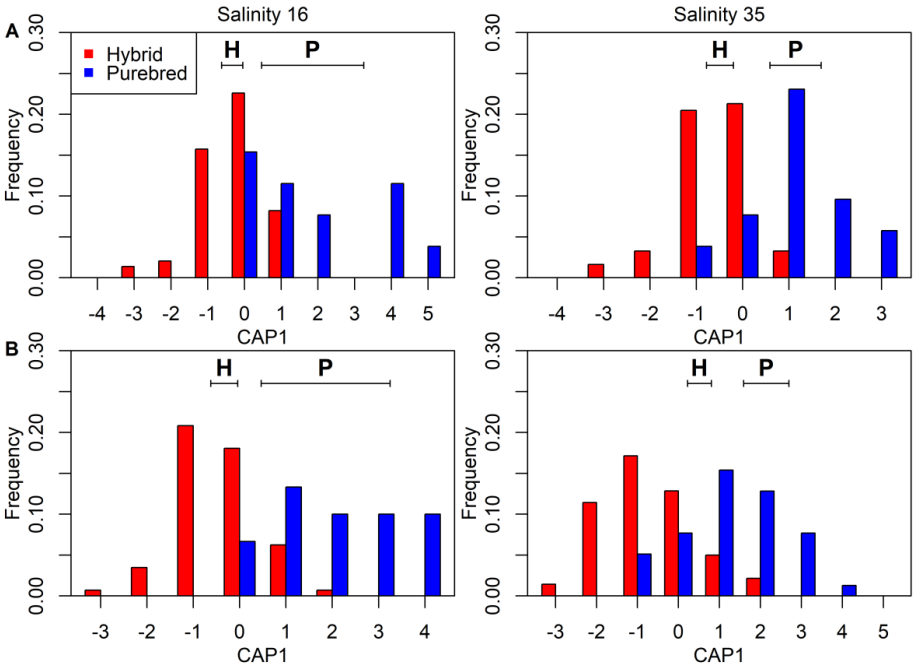

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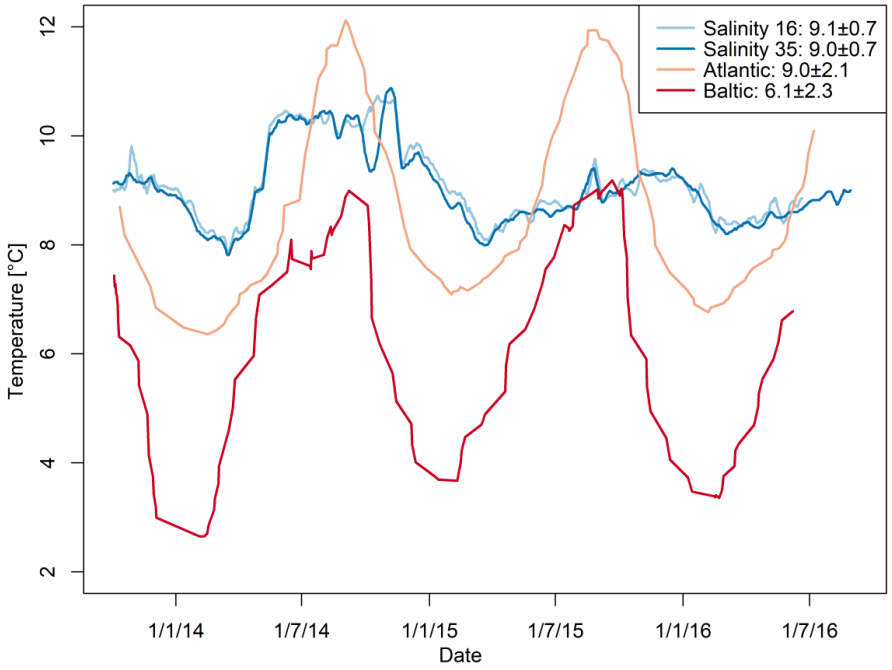

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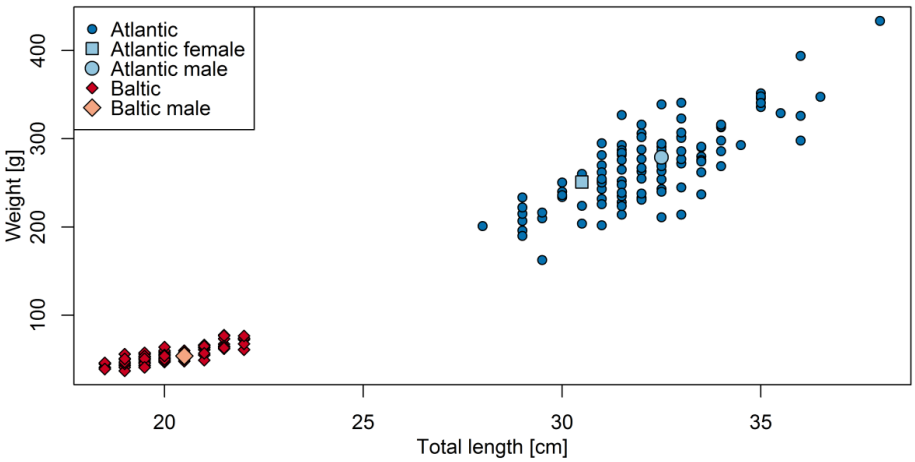

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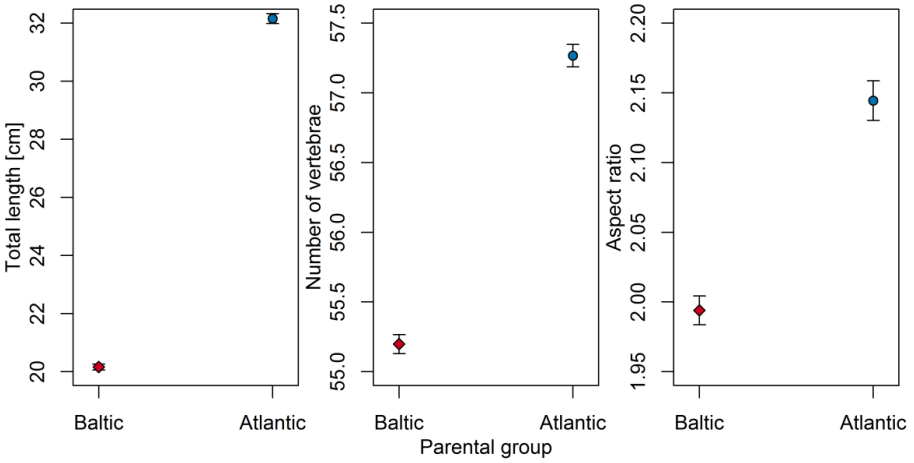

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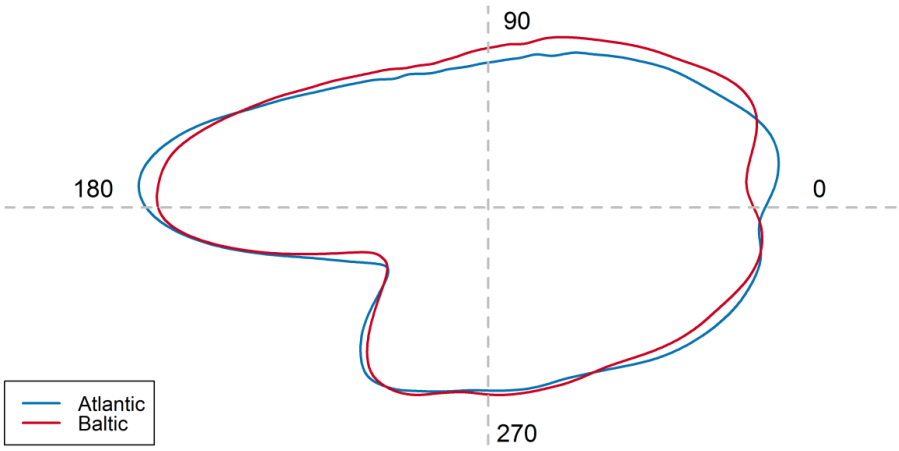

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