A combination of genetic and phenotypic characterization of spring- and autumn-spawning herring suggests gene flow between populations

Florian Berg[1,2*], Hedda D. Østgaard[1], Aril Slotte[2], Leif Andersson[3,4,5], Arild Folkvord[1,2]

[1]University of Bergen, Department of Biological Sciences, Post box 7803, 5020 Bergen, Norway

[2]Institute of Marine Research (IMR), Post box 1870 Nordnes, 5817 Bergen, Norway

[3]Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, SE-751 23 Uppsala, Sweden

[4]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden

[5]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas 77843-4458, USA

* Corresponding author: Mailing address: Post box 1870 Nordnes, 5817 Bergen, Norway; telephone: +47 94209887; e-mail: florian.berg@hi.no

ORCID

Florian Berg: 0000-0003-1543-8112

Arild Folkvord: 0000-0002-4763-0590

Abstract

Atlantic herring (*Clupea harengus*) has complex population structure and dynamics including diverse life histories and spawning times with spring- and autumn-spawning as the most common modes. Originally, spawning herring were phenotypically identified based on their maturity development or otolith microstructure by determining seasonal specific larval growth patterns. Recently, genetic markers have revealed clear genetic differentiation between spring- and autumn-spawning populations. All three methods were applied to herring caught at the same locations during spring and autumn to determine the coherence of methods. In a selected subset, most herring (~77%) had an otolith microstructure and genetic assignment coinciding with the phenotypically assigned spawning season. Non-spawning herring (<5%) that were classified as belonging to the current spawning season using genotyping and otolith-typing were assigned as skipped spawners. For ~8% of spawning herring, the genetic and otolith assignment contradicted the phenotypically assigned spawning season, characteristic of straying individuals. Otolith-typing contradicted the genetic and phenotypical assignment in ~7% of the cases, potentially representing individuals reuniting back to the spawning season favoured by their genotype. Although the viability of offspring from these individuals remains undocumented, it is suggested that the observed switching of spawning season may contribute to gene flow between herring populations.

Keywords: population structure, otolith microstructure, phenotypic plasticity, population discrimination, SNP, skipped spawning

Introduction

The general aim of fisheries management is the long-term maintenance of diversity of fish populations (Smedbol and Stephenson, 2001; Baguette and Schtickzelle, 2003). Conducting reliable stock assessments are absolutely dependent on correct population identification and discrimination (Begg et al., 1999). Still, many populations are separated based on a priori assumptions that fish populations rigidly follow artificial geographical boundaries. This might induce a mismatch between management areas and population distribution. Overexploitation of unique populations could be the consequences when population mixing is disregarded (Kerr et al., 2017). Therefore, population discrimination methods with high classification accuracy are essential to assign individuals from mixed fisheries to their original population (Cadrin et al., 2014).

Especially for marine fish species, population discrimination methods are continuously developing and are mainly based on morphology, behaviour, life history, or genetic differentiation (Cadrin et al., 2014). One major prerequisite of discrimination methods is the independence of a population as a reproductive group with a unique spawning timing and location (Iles and Sinclair, 1982). The most rapid development in recent years has occurred through genetic studies, where newly developed methods such as genotyping-by-sequencing (GBS), restriction site-associated DNA sequencing (RADseq), double digest RADseq (ddRAD; Andrews et al., 2016 and references herein) or whole-genome sequencing (Fuentes-Pardo and Ruzzante, 2017) can resolve the population structure of several species.

The interaction of an individual's genotype with the environment it experiences is commonly defining a set of observable characteristics known as the phenotype. If genetic methods fail to discriminate populations, other methods, e.g. based on phenotypic characteristics, are required (Svedäng et al., 2010; Imsland et al., 2014). In that case,

discrimination methods using phenotypic characteristics rely on the assumption that populations have experienced different environments throughout their life cycle. This ability of a genotype to have a set of phenotypes in response to varying environments is known as phenotypic plasticity (Via et al., 1995).

Atlantic herring (*Clupea harengus*) is one of the most abundant marine fish species on Earth (Feng et al., 2017) and is known for its phenotypic plasticity (Geffen, 2009). Since the days of Hjort (1914), the population structure and dynamics of herring have been investigated and are still debated (Reiss et al., 2009; Martinez Barrio et al., 2016). It has been documented that herring can consist of spatially discrete populations (Iles and Sinclair, 1982) or are comprised as metapopulations (Johannessen et al., 2009; Eggers et al., 2014). One of the major life-history traits of herring is their fidelity to a specific spawning season, mainly autumn or spring (Husebø et al., 2005; Brophy et al., 2006), although spawning can be observed throughout the year at various locations. Coherent genetic differences among spring- and autumn-spawning herring were recently documented at both sides of the Atlantic (Lamichhaney et al., 2017; Kerr et al., 2019). At the same time, mixing of different populations occur and these mixed aggregations are also targeted by fisheries (Stephenson et al., 2009; Clausen et al., 2015). Splitting of autumn and spring spawners in mixed catches is applied through various discrimination methods (ICES, 2019). Nonetheless, knowledge of coherence among discrimination methods, especially including newly developed genetic approaches, is missing.

Given the necessity of accurate discrimination methods, our aim was to compare three methods to distinguish between autumn- and spring-spawning herring. Herring were collected at the same locations during both autumn and spring spawning. Firstly, herring were discriminated based on maturity development, i.e. if herring were in spawning conditions or not. Secondly, we used genetic markers to discriminate autumn and spring

spawners. Thirdly, we applied otolith microstructure analysis, the major splitting method used in current assessment (ICES, 2019), to determine the season of hatching. Finally, we evaluated whether a combination of all three methods would improve discriminations and provide new insight into the underlying population structure and dynamics of Atlantic herring.

Material and Methods

Study area and sampling design

Atlantic herring were caught by gillnets in a semi-enclosed and rather shallow (6-25 m) area inside the fjordic coastline of Norway, approximately 26 km northwest of Bergen (60°34'11.2"N 5°0'18.9"E). Sampling was conducted during spring (March-May) and autumn (September-October), from autumn 2016 to autumn 2018 (Table 1, for detailed overview see Table S1). For each sample, we used gillnets with three different mesh sizes (29, 31, 34 mm) to ensure that spawning and non-spawning herring were caught. However, both non-spawning and spawning herring were collected simultaneously in gillnets of all three mesh sizes.

The total number of herring analysed was mainly limited by the total catch, but a maximum of 100 herring were analysed per sampling. For all herring, total length (to the nearest 0.1 cm below), total weight, and gonad weight were measured. Maturity stages were determined by visual inspection of gonads according to the following scale: immature = 1-2, maturing = 3-4, ripe = 5, spawning = 6, spent/recovering = 7-8, abnormal = 9 (Mjanger et al., 2017). Otoliths were extracted for age determination (counting winter rings) and microstructure analysis. Fin clips from each herring were stored in ethanol for genetic analysis.

Discrimination of spring and autumn spawners

In this study, we used three different methods to discriminate the spawning type of Atlantic herring. First, we discriminated herring using maturity development, to determine spawning season phenotype (hereafter spawning phenotype). Herring in maturity stages 5-8 were assumed to spawn in the season they were caught. Stage 8 herring were only found at the end of the spring spawning season (mainly May, Table S2), therefore, we interpreted these fish as early spring spawners rather than autumn spawners (see Discussion). The remaining herring (stages 3-4) were assumed to spawn in the opposite season as they were caught. In addition, herring in stages 5 with a gonadosomatic index (GSI) ≤15% were assumed to spawn in the opposite season of capture (Fig. S1). The GSI was calculated as follows:

$$GSI = \frac{100 \times gonad\ weight}{somatic\ weight}$$

where the *somatic weight* is the difference between *total weight* and *gonad weight*. Herring in stages 5 with a GSI ≤15% were solely found in autumn samplings. Usually, herring caught along the Norwegian coast in stage 5 caught in autumn (September-December) have a GSI ≥15% (Fig. S1B). We assume that these herring in stages 5 with a GSI ≤15% were misclassified and were actually in stage 4. Therefore, we used this as a threshold to discriminate herring to the opposite season. Immature herring (stages 1-2) or herring with abnormal maturity development (stage 9) were not included in this study.

Secondly, DNA samples were used to genetically identify spring- and autumn-spawning types of herring by genotyping two diagnostic SNPs using a Custom TaqMan® Assay Design Tool. The two SNPs (sequences used are given in Table S3) were identified by Lamichhaney et al. (2017) as the most differentiating in the spring- vs. autumn-spawning contrast. Spring-spawning herring tend to be homozygous T (thymine) or A (adenine) at a specific SNP locus on scaffold481_2824_F or scaffold1420_137_F, respectively, whereas autumn-spawning herring tend to be homozygous C (cytosine) in

both cases. Herring were classified as either spring or autumn type when both SNPs were homozygous for the associated SNP allele. If one SNP was homozygous and the second SNP heterozygous, herring were still assigned to the spawning type corresponding to the homozygous SNP. If both SNPs were heterozygous the herring were denoted heterozygous. If both SNPs were homozygous but not for the same spawning type the herring were referred to as ambiguous. DNA samples with low or poor DNA quality were dismissed from the following analysis (N = 4).

Thirdly, we used the otolith microstructure phenotype (hereafter termed otolith for short) according to Clausen et al. (2007) to discriminate herring of spring or autumn hatching origin. In contrast to the two other methods, the otolith microstructure revealed information of the hatching season of herring. The rationale is that otoliths of herring hatched in spring initially have wider increments that rapidly increase in width outwards from the nuclei (core) of the otolith, whereas autumn hatched otoliths have "close-to-constant" widths between increments (Clausen et al., 2007). This method can also be applied to discriminate winter spawners, which was not attempted in this study since no samples of winter spawning were available from the study area. However, during the discrimination process, we noted otoliths with potentially winter spawning microstructure pattern, but assigned them as autumn type (Table S4). Otoliths were ground and polished until the core was visible. A series of digital images was taken of each otolith during the grinding procedure with a Nikon DS-Fi2 digital camera attached to a Leica DMLB light microscope (Leica Microsystems, Wetzlar, Germany). Otoliths were investigated by two independent readers and assigned to either spring- or autumn-spawning/hatching type. In case of discrepancy between the readers, the second otolith was analysed. If the readers could not agree on one type (5.8%), the otolith was not included in further analysis. For quantitative documentation of the otolith discrimination method, daily increments were

detected, and widths measured using the Caliper function in Image Pro-Plus® version 7.0 (Media Cybernetics, USA) to reflect the underlying differences between potential populations. Daily increments were registered from the core up to a distance of 200 µm from the core.

Statistical analysis

All statistical analyses and plotting were conducted in the R software (R Core Team, 2019). For all tests, we used $p<0.05$ as the level of significance. In total, we analysed a random subset of 577 herring (Table 1), but we discriminated only a selected subset of 213 herring to spawning type using all three methods. The selected subset was limited by the number of herring analysed for otolith microstructure. In the selected subset, all potential autumn spawners (based on spawning phenotype and genetics) were analysed, but not all potential spring spawners. Potential spring spawners were randomly selected and limited to max. 20 individuals per sample. Therefore, the shown proportion of the selected subset will not reflect the real population proportions or dynamics. All statistical analyses were conducted using the selected subset of 213 herring that well represents a non-biased subset in terms of length distribution (Fig. S2).

To investigate the population dynamics during autumn and spring in the study area we estimated the catch per unit effort (CPUE = *Total catch/Number nets*). Further, we estimated the fraction of autumn and spring spawners among the 577 analysed herring. First, we used individuals with concordant assignment based on all three methods (N = 164). If the assignments were inconsistent, herring with homozygous genetics were used (N = 264). If genetics were heterozygous/ambiguous, we used assignments from otoliths (N = 20). For the remaining herring, we used the spawning season phenotype (N = 129). These resulting fractions of spring- and autumn-spawning fish were in the following

weighted with the CPUE of each sampling season and used to estimate the fraction (i.e. relative population size) in the area at time of sampling.

After discriminating herring with three methods we tested for their independence using a loglinear model. If the three discrimination methods were independent the frequency distribution would be equal (Fig. S3A). To visualize the frequencies between expected and observed counts we used a mosaic plot (Friendly, 1994). To corroborate the results from the visual inspection of otoliths, we estimated the mean increments widths corresponding to an early (at 35-65 µm otolith radius) and late (at 115-145 µm otolith radius) larval phase of each herring. According to Folkvord et al. (2009) the age of herring during the early larval phase would be 30-40 days post hatching. Considering the mean increment average for spring (~2.2 µm) and autumn (~1.8 µm) hatched larvae within the time between the two phases, herring would be approximately 36 and 45 days older, respectively, during the late larval phase. Further, we estimated the difference between the mean width of the early and late larval phase to indicate the assumed increasing or constant growth pattern for spring and autumn types, respectively. We also compared the relationship between mean increment widths for the early larval phase and the calculated differences between the early and late larval phase to confirm our initial visual assessment of hatching season.

To validate that herring discriminated as autumn and spring by all three methods are forming different populations, we compared additional biological parameters between concordant autumn and spring spawners. We compared the length-weight relationship of these two types using log-transformed values, and the common slope of both seasonal types was not different from 3 (ANCOVA: $p<0.001$). We, therefore, estimated Fulton's somatic condition factor K_s:

$$K_s = 100 \times \frac{somatic\ weight}{total\ length^3}$$

K_s of spring and autumn type herring was compared using an ANOVA, but only herring in spawning conditions (spawning phenotype coherent with sampling season) were included. Length-at-age data, used as a proxy for growth of herring, were fitted to the von Bertalanffy growth model (VBGM; Bertalanffy, 1934):

$$L_t = L_{\infty_{Type}}\left(1 - e^{-K_{Type}(t-t_0)}\right)$$

where L_t is the average length at age t, and t_0 is the intercept on the age axis. L_∞, the asymptotic maximum length, and K the von Bertalanffy growth rate coefficient were all specific for each spawning type (*Type*).

Results

Comparison of discrimination methods

Discriminating herring based on all three discrimination methods (spawning phenotype, genetics, and otolith) resulted in seven different combinations (Table 2). In the selected subset, the majority were discriminated as spring or autumn spawners by all three methods, hereafter referred to as concordant spawners. Concordant spring spawners included all herring in stage 5 affected by the threshold of a GSI ≤15% (Table S2). The smallest fractions were either genetically heterozygous/ambiguous or potential skippers (Table 2). Skippers were defined as non-spawning herring (stage 3-4) with coherent otolith type and genetics, but the spawning phenotype did not match. Otherwise, spawning herring with coherent otolith type and genetics but non-matching spawning phenotype had switched their spawning season and are defined as straying herring. In some cases, genetics and otoliths were inconsistent but spawning phenotype was always coherent with genetics; these herring are defined as reuniters (Table 2). We only found reuniters with autumn type otoliths. We found no herring with coherent spawning phenotype and otoliths but contrasting genetics. Herring in stage 8, only found in late spring, were mainly concordant or heterozygous spring spawners (N = 9) or autumn type

238 based on genetics and otoliths (N = 4). The loglinear model demonstrated that
239 discrimination methods were dependently favouring coherence between all methods for
240 both, spring and autumn types (Fig. S3B).

241 In general, the proportion of herring with discrepancies between methods was
242 slightly higher during spring sampling (Fig. 1), than during autumn sampling. When
243 herring were discriminated as the same type based on spawning phenotype and genetics
244 the probability that otoliths revealed the same type were highest, 100% and 90% for
245 autumn and spring type, respectively (Table 3). Herring discriminated based on spawning
246 phenotype and otoliths as autumn or spring type were always discriminated as the same
247 type or heterozygous/ambiguous based on the genetics. Coherent autumn assignments
248 based on otoliths and genetics resulted in relatively low agreement (74%) with spawning
249 phenotype assignments. Genetically heterozygous/ambiguous herring were always
250 characterised to the same spawning type based on spawning phenotype and otolith
251 analysis (Fig. 1, Table 3).

252 **Otolith analysis**

253 In general, for spring type otoliths the increment widths clearly increased with increasing
254 distance from the core, while they were rather constant for autumn type otoliths (Fig. 2A).
255 The increment widths of autumn type otoliths started to increase approximately at 130
256 μm from the core. At the same distance from the core, increment widths of spring otoliths
257 became more stable. The difference between mean increment widths during the early and
258 late larval phase was, as expected, larger for spring type than autumn type otoliths and
259 decreased for both otolith types when the mean increment width at the early larval phase
260 increased (Fig. 2B). Autumn type otoliths tend to have very limited differences between
261 late and early increments (overall mean differences = 0.01 μm; Fig. 3), while it was larger
262 for spring type otoliths (overall mean = 0.44 μm).

Biological parameters and population dynamics

Concordant autumn spawners had better condition factors compared with concordant spring spawners (ANOVA: $p<0.001$; Fig. 4A). Both types differed in their growth patterns, having a common theoretical age at size 0 (t_0 = -2.6). Concordant autumn spawners are characterised by a higher growth (K = 0.4) but smaller maximum length (L_∞ = 32.8) in comparison to spring spawners (K = 0.3, L_∞ = 36.9; Fig. 4B). Comparing the length-weight relationship demonstrated that autumn type herring were heavier at the same length than spring type herring (ANOVA: $p<0.001$; Fig. 4C). There were no obvious trends in the maturity stage composition within each spawning season (Fig. S4). The age distribution among herring sampled at different spawning seasons was similar (Fig. S5), and the mean age of concordant spring and autumn spawners did not differ (Table S2). However, herring with discrepancies between methods were in general older. The catch per unit effort (CPUE) was clearly higher in spring than in autumn (Table 4). Spring spawners dominated the catches in both sampling seasons and their total proportion is approximately 11.6 times larger than those of autumn spawners. This proportion was 3.8 and 15.3 in autumn and spring, respectively (Table 4).

Discussion

This is, to our knowledge, the first study comparing three different discrimination methods (spawning phenotype, genetics, and otolith data), to distinguish autumn- and spring-spawning Atlantic herring. The agreement between discrimination methods and the resulting spawning season fidelity is generally high and most herring are defined as either concordant spring or autumn spawners. Due to the combination of discrimination methods, discrepancies between the methods were identified allowing for additional ecological interpretations than concordant spawners. Non-spawning and spawning herring are characterized of skipped spawning or straying to another spawning season,

288 respectively, when genetic and otolith assignments were coherent with opposite spawning
289 phenotype assignment. Some herring were found to reunite back to spring-spawning
290 according to their genetic constitution although their otolith data showed that they hatched
291 in autumn. Further, herring with heterozygous/ambiguous genetics but coherent spawning
292 phenotype and otolith indicated interbreeding of genetically typed spring- and autumn-
293 spawning herring. These herring could potentially be offspring of straying fish suggesting
294 considerable gene flow between populations.

295 The benefit of combining several discrimination methods is the more precise
296 identification of a variety of herring spawning types. Even though each of the three
297 methods has its pitfalls that need to be considered when interpreting the results (Table 5),
298 the identified herring types are valid and not result of methodological issues. It is rather
299 an exception than the rule that the following described pitfalls affect the results.
300 Discriminating autumn- and spring-spawning herring by applying genetic approaches is
301 relatively new, but robust (Bekkevold et al., 2016; Martinez Barrio et al., 2016;
302 Lamichhaney et al., 2017). In a recent study using 66 SNPs, Kerr et al. (2019) could
303 discriminate autumn and spring spawners with a 100% cross-validation accuracy and
304 suggested that only six SNPs are needed to achieve such high accuracy. Further, Kerr et
305 al. (2019) also found a small number of heterozygous herring. Increasing the number of
306 SNPs in our study would increase accuracy to some extent but we have selected the loci
307 that show the strongest association with spawning type. Also, allele frequencies at these
308 loci are strongly correlated with other loci associated with spawning time (Lamichhaney
309 et al., 2017). Since all genetically heterozygous/ambiguous herring had coherent otolith
310 and spawning phenotype an increased number of SNPs is not expected to change the
311 results significantly. Further, we found no case where otoliths and spawning phenotype

312 were coherent but not the genetics, therefore, a misclassification as autumn or spring type
313 is unlikely in this dataset.

314 In contrast to the new genetic approach, otolith microstructure analyses have a long
315 history in discriminating autumn- and spring-spawning herring (Moksness and Fossum,
316 1991; Mosegaard and Madsen, 1996). An advantage of this method is that also winter
317 spawners can be discriminated (Clausen et al., 2007). Herring with potentially winter
318 spawning microstructure were discriminated as concordant autumn spawners, skippers,
319 strayers or reuniters (Table S4). Since we have not collected samples during winter, we
320 cannot confirm the existence of "real" winter spawners in this area. Also, no single SNPs
321 exist at the present to identify winter spawners. Whether the winter microstructure is
322 representing true winter spawning, or just a consequence of late autumn/early spring
323 spawning experiencing colder temperatures and having slower growth patterns needs to
324 be followed up. However, for this study we expect that herring with potential winter
325 microstructure and autumn genetics (Table S4) are correctly discriminated because we
326 did not observe a single herring with spring otolith but autumn genetics. In case of
327 reuniters with winter microstructure, misclassification might occur because their daily
328 growth patterns were closest to the spring type otoliths (Fig. 2B).

329 Discrepancies between spawning phenotype assignments and coherent otolith and
330 genetic assignments were largest (~12%). This visual maturity staging method is
331 dependent on a high level of experience because the stages will develop during the
332 spawning season and are not fixed like genetics or otolith microstructure. The additional
333 threshold of a GSI ≤15% has strengthened the spawning phenotype assignment since all
334 herring affected were concordant spring spawners (Table S2). Another source of
335 misclassification are recovering herring (stage 8) in the spring spawning season because
336 autumn spawners can also stay in stage 8 until summer and have a much faster maturation

curve than spring spawners (van Damme et al., 2009). We therefore have to be cautious when interpreting stage 5 or 8 herring as strayers solely based on incoherent spawning phenotype when genetics and otoliths were in accordance since a discrimination failure of spawning phenotype is more likely (Table S2).

The present study proposes the occurrence of at least two discrete populations in this local vicinity separated by their spawning times; either spring or autumn. The dynamic ratios and CPUE (Table 4) between sampling seasons are an indication of non-stationarity with varying proportions of local and migratory herring. Considering the higher CPUE in spring, the numbers of autumn-spawning herring in the two seasons are at comparable levels suggesting that this population is more stationary. Also, relatively many spring-spawning herring were found during autumn indicating non-migratory for some part of this component. The higher abundance of spring spawners during spring compared to autumn demonstrates the occurrence of a migratory component. Previous studies have also suggested the occurrence of two different "types" of spring-spawning herring in this area (Lamichhaney et al., 2017; Berg et al., 2019). Migratory individuals are presumably Norwegian spring-spawning (NSS) herring being the dominating population in the Norwegian Sea.

Overall, spring spawners are approximately 11-12 times more abundant than autumn spawners in the study area (~60° N). In higher latitudes (~67° N), Norwegian autumn-spawning herring (NASH) are recognized (Pampoulie et al., 2015) and its proportion is assumed to be 1:200 compared to NSS herring (Husebø et al., 2005). In the North Sea, south of the study area, an opposite situation with dominating autumn spawners is observed. Light is assumed to be a limiting factor for visual foraging planktivorous organisms such as larval herring during autumn in higher latitudes (Sundby et al., 2016). Warming under future climate change scenarios in light-limited conditions at high

latitudes may thus represent an additional metabolic challenge, favouring larger and higher condition larvae and early juveniles of spring spawners over autumn spawners during winter months.

Further, the measured increment widths of spring type otoliths are in accordance with other studies that analysed daily growth pattern of spring spawners along the Norwegian coast, but the growth is slower compared to herring spawned later in spring (Clausen et al., 2007; Berg et al., 2017; Slotte et al., 2019). On the other hand, autumn type otoliths had a larger growth compared to North Sea autumn spawners (Moksness and Fossum, 1991), but similar growth compared to Norwegian summer/autumn spawners (Husebø et al., 2005). This, in combination with the differences in biological characteristics, strengthens the existence of two or more discrete populations and the occurrence of migratory NSS in the study area.

Besides the majority of concordant spring and autumn-spawning herring, we observed herring where the discrimination methods were not in accordance and misclassifications due to potential pitfalls related to the discrimination methods are unlikely. Skipped spawning is known to occur in NSS herring, but with <2% not a common feature (Kennedy et al., 2011). In our study, herring with characteristics of skipped spawning occurred among both spawning types and accounted for <5% of the selected subset. Further, we observed few reuniting and straying herring, both defined by inconsistent hatching season (based on otoliths) and spawning phenotype, respectively. The majority of these herring shifted from autumn hatching to spawning in spring which is also more plausible considering the maturation development and reproductive strategies of herring (van Damme et al., 2009; dos Santos Schmidt et al., 2017). Also, other studies demonstrated high spawning season fidelity with a limited amount of straying from hatching to spawning season (Husebø et al., 2005; Brophy et al., 2006).

McQuinn (1997), however, found that a relatively large proportion of herring hatched in spring (based on otoliths) ended up spawning in autumn (based on maturity development). This potential straying of herring and consequently interbreeding could explain the appearance of genetically heterozygous herring. The effect of these heterozygous herring on the population structure and the following biological and ecological consequences are unclear (Lamichhaney et al., 2017; Kerr et al., 2019). However, switching of spawning season and interbreeding will contribute to the complexity and diversity of herring populations. Experimental common garden studies have revealed that autumn-spring hybrid larvae had higher overall survival than concordant autumn spawned offspring, especially at relatively poorer feeding conditions (Folkvord et al., 2009). These results suggest that hybrid offspring of spring- and autumn-spawning herring do not have impaired survival potential.

Knowing the population structure and dynamics of marine fish and how to discriminate them is important for their assessment and management. At present, herring management units (stocks) are mainly separated by geographical areas and discriminated based on otolith microstructure or numbers of vertebrae in case of mixing (ICES, 2019). According to the results of this study, a change to more objective and precise methods, like genetics, can potentially increase the discrimination accuracy. However, the results combining genetics and otolith microstructure analyses will be even more reliable and informative. “Real-time” assessment could improve the estimation of population proportions in mixed catches in a time-efficient manner (Dahle et al., 2018). Thus, genetic tools are expected to become increasingly important in the future when applying population discrimination for fisheries assessment.

Considering the pitfalls of different discrimination methods, their comparison still reveals new insight into the population structure and dynamics of spring- and autumn-

spawning herring in a coastal area of the northeast Atlantic. Herring showed high spawning season fidelity, however, low rates of straying could be demonstrated. Further, skipped spawning was observed to a limited extent for both spawning types as well as potentially reuniting of individuals back to the spawning season in line with their genetic constitution. A consequence of straying herring is the occurrence of spring/autumn heterozygous herring. The evidence of straying between spawning types suggest gene flow consistent with the observed lack of genetic differentiation between spring and autumns spawners at selectively neutral loci (Martinez Barrio et al., 2016; Lamichhaney et al., 2017). However, a clear coherence is confirmed between the spawning phenotype and genotype associated with spawning season.

Supplementary material

The following supplementary material is available at ICESJMS online. The material includes further information on the selected subset, the loglinear model, the discrimination of herring based on maturity stages, and the age distribution.

Acknowledgments

We are grateful to Christel Krossøy, Frank Midtøy, Heikki Savolainen and Julie Skadal from the UiB for their efforts in sampling the data material. This work was funded by the RCN project 254774 (GENSINC).

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569

570 **Table 1** Overview of samples collected from autumn 2016 to autumn 2018. Total number
571 of samples, gillnets used, total catch per sampling time, number of herring that were
572 randomly selected from the catch and analysed (length-weight), and selected herring from
573 length-weight samples discriminated based on all three methods are presented.

Sampling time	No samples	No nets	Total catch	Length-weight sample	Discrimination sample
Autumn 2016	4	14	53	53	39
Spring 2017	2	8	210	133	37
Autumn 2017	4	20	119	119	54
Spring 2018	2	7	620	176	34
Autumn 2018	1	4	164	96	49
Total	**13**	**53**	**1166**	**577**	**213**

574

Table 2 Number of herring types within each sampling season and year based on all three discrimination methods. 1st letter = spawning phenotype, 2nd letter = genetic, 3rd letter = otolith. A = autumn, H = heterozygote/ambiguous, S = spring. There are in total seven different three-letter combinations, with ASS and SAA represented twice but interpreted differently depending on sampling time. Concordant means that agreement between all methods existed; Skippers means that genotype and otolith type agree but they do not spawn as expected based on the classification. Strayers denotes herring with coherent otolith type and genetics switch to a new spawning season. Reuniters denotes herring changed from their hatching season (otolith) to a new spawning season that is in accordance with their genetics. Terms in quotation marks represent biological categories not excluding other classifications and interpretations.

Category	**Concordant**		**"Skippers"**		**"Strayers"**		**"Reuniters"**		**Heterozygous**	
Sampling time	AAA	SSS	ASS	SAA	ASS	SAA	AAS	SSA	AHA	SHS
Autumn 2016	6	25		2	3			3		
Spring 2017	1	25	3			3		3		2
Autumn 2017	9	29		2	4			6	3	1
Spring 2018	1	24	1			6			1	1
Autumn 2018	22	22		1				2	1	1
Total	**39**	**125**	**4**	**5**	**7**	**9**	**0**	**14**	**5**	**5**

587 **Table 3** Agreement and discrepancy between discrimination methods estimated for A) otoliths, B) genetics, and C) spawning phenotype. Hetero

588 represents genetically heterozygous or ambiguous results.

A)

Spawning	Genetic	Otolith (%)		N
		Autumn	Spring	
Autumn	**Autumn**	100	0	39
	Hetero	100	0	5
	Spring	0	100	11
Spring	**Autumn**	100	0	14
	Hetero	0	100	5
	Spring	10	90	139

B)

Spawning	Otolith	Genetic (%)			N
		Autumn	Hetero	Spring	
Autumn	**Autumn**	89	11	0	44
	Spring	0	0	100	11
Spring	**Autumn**	50	0	50	28
	Spring	0	4	96	130

C)

Otolith	Genetic	Spawning (%)		N
		Autumn	Spring	
Autumn	**Autumn**	74	26	53
	Hetero	100	0	5
	Spring	0	100	14
Spring	**Autumn**	-	-	0
	Hetero	0	100	5
	Spring	8	92	136

589

Table 4 Estimates of catch per unit effort (CPUE = Total catch/No nets), N in length-weight sample, fraction (%) of spring- and autumn-spawning herring caught each season and estimated total number (N_{tot}) of autumn- and spring-spawning herring per sampling season with corresponding ratios of spring:autumn type herring. The total catch was discriminated in autumn or spring spawners, based on available genetic, otolith, spawning phenotype assignments. ***Numbers in italics*** in the total row are weighted with the CPUE for each sampling season, representing overall average values.

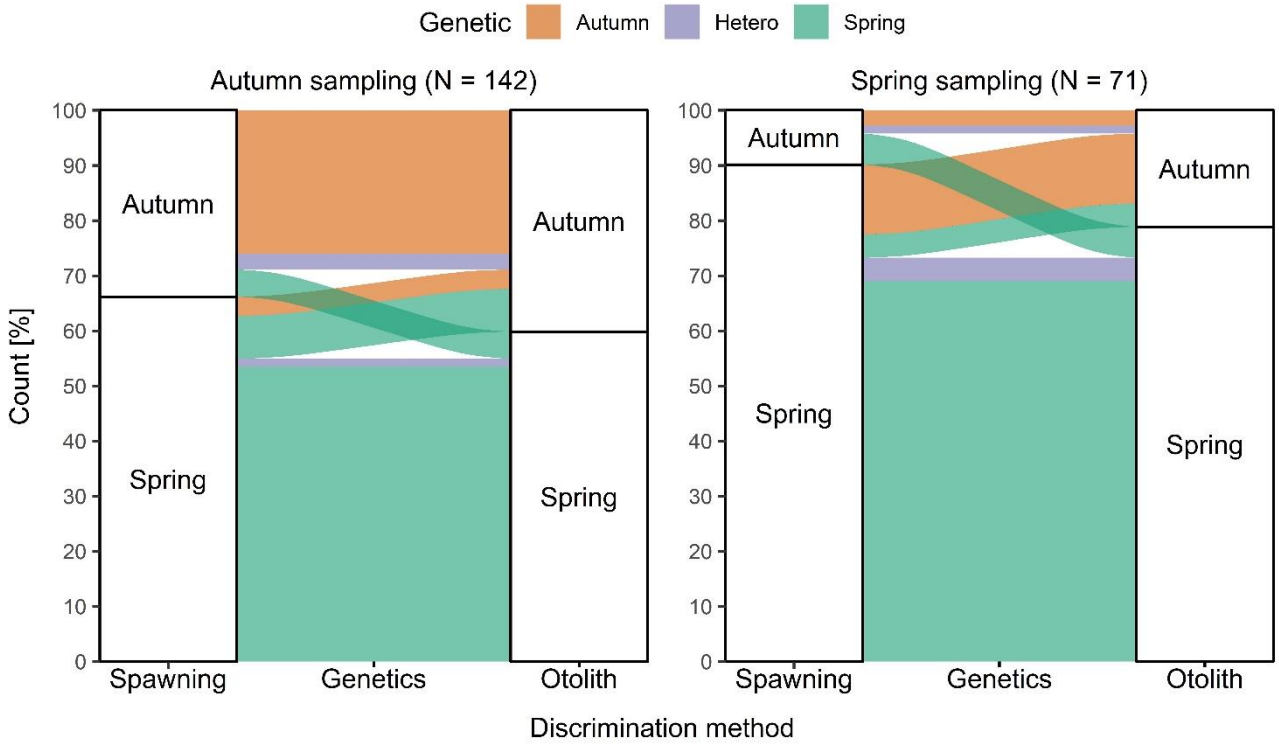
Sampling season	No nets	Total catch	CPUE	N Autumn	N Spring	% Autumn	% Spring	N_{tot} autumn	N_{tot} spring	Ratio
Autumn	38	336	8.8	56	212	20.9	79.1	70	266	3.8
Spring	15	830	55.3	19	290	6.1	93.9	51	779	15.3
Total	**53**	**1166**	**22.0**	**75**	**502**	***7.9***	***92.1***	**121**	**1045**	***11.6***

596 **Table 5** Summary table of the main advantages and pitfalls of the three methods
597 (spawning phenotype based on maturity stages, otolith microstructure analysis, and two
598 SNPs as genetic tool) used to discriminate spring- and autumn-spawning herring, as well
599 as the advantages of combining the results of different methods if the results of each
600 individual method are reliable.

Discrimination methods	Advantages	Pitfalls
Spawning phenotype	- Easy to discriminate when running/spawning - Fast, no extra analysis needed	- Subjective method - High level of experience needed - Developing during the spawning season - GSI as additional information needed - Same maturity stage (8 = recovering) for autumn and spring herring after spring spawning
Otolith microstructure	- Partly objective method - Widely used and excepted method - Fixed microstructure - Identification of winter spawners	- Experienced readers necessary - Large variation between early and late spring/autumn spawners - Hard to define exact objective criteria
Genetics	- Objective method - Robust and temporal stable - High accuracy	- Interpretation of heterozygous results
Combination of methods	- **Identification of ecological important events, like skip-spawning, switching of spawning season, or reuniting**	- **Increased complexity in interpretation**

601

Fig. 1 Alluvial plots visualizing the discrimination results for all three discrimination methods of each herring sampled in autumn (left panel) and spring (right panel). The columns represent the percentage of herring discriminated as spring- or autumn-spawning type based on the spawning phenotype (left) and otolith microstructure (right). The genetic spawning type is indicated by colour between the two columns. Hetero includes both, heterozygous and ambiguous genetic assignments.



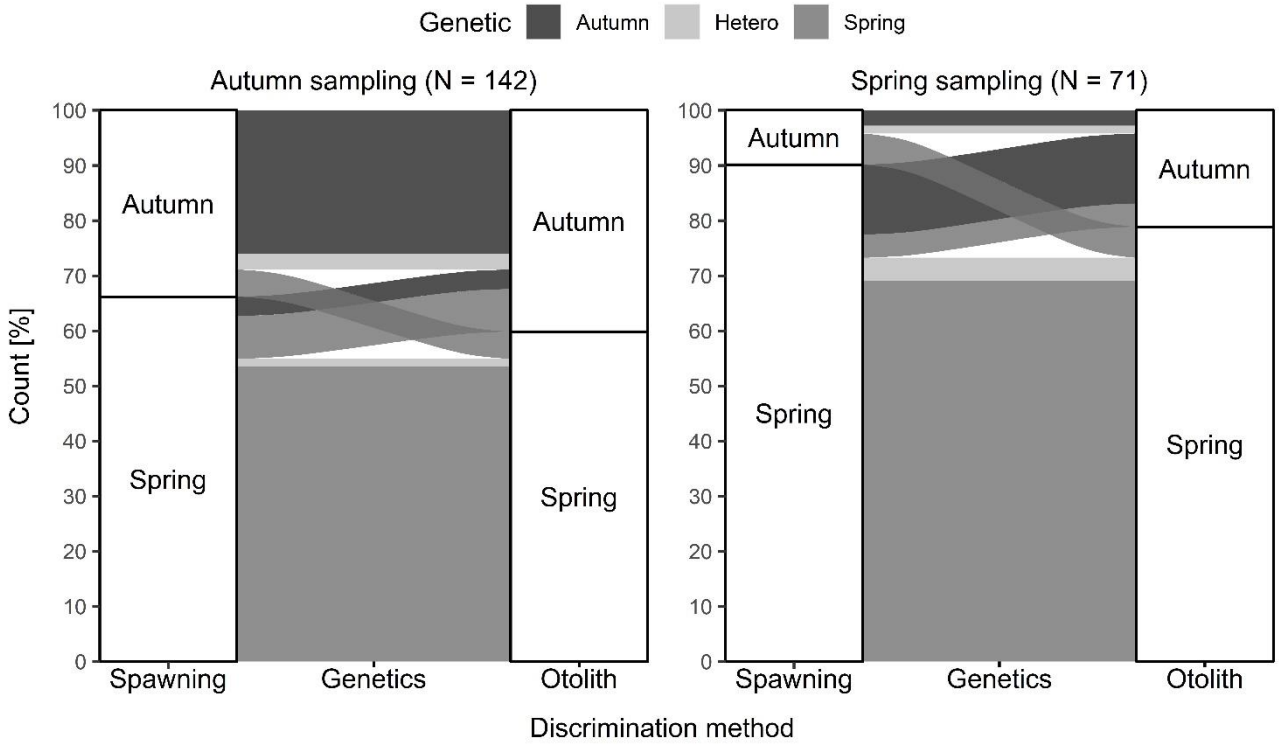
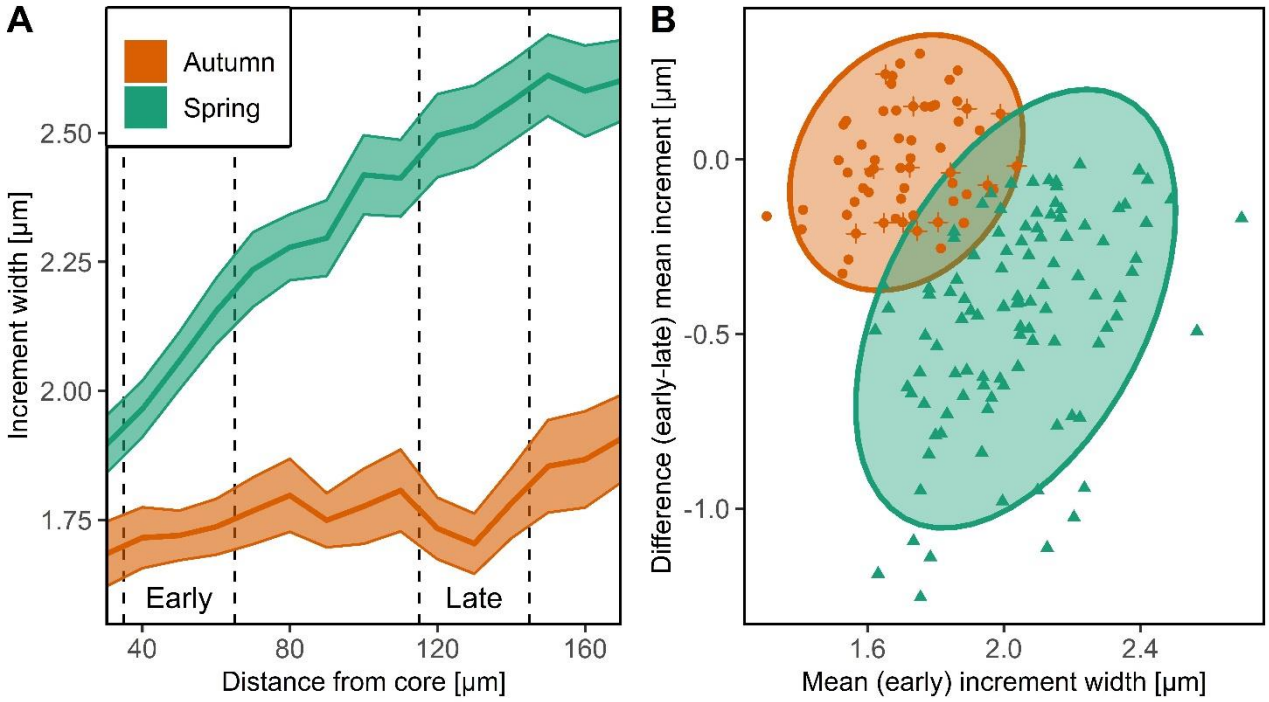


Fig. 2 A) Mean daily growth of autumn and spring discriminated otoliths with 95% confidence intervals. Dashed lines indicate intervals used as early (left, approximate age 30-40 days post hatching) and late (right; approximately 36 to 45 days older) larval phase. B) Mean increment width during the early larval phase and the difference between mean daily increment width between early and late larval phase for autumn and spring type otoliths with 95% confidence ellipses. SSA type herring (see Table 2) are marked with a cross.



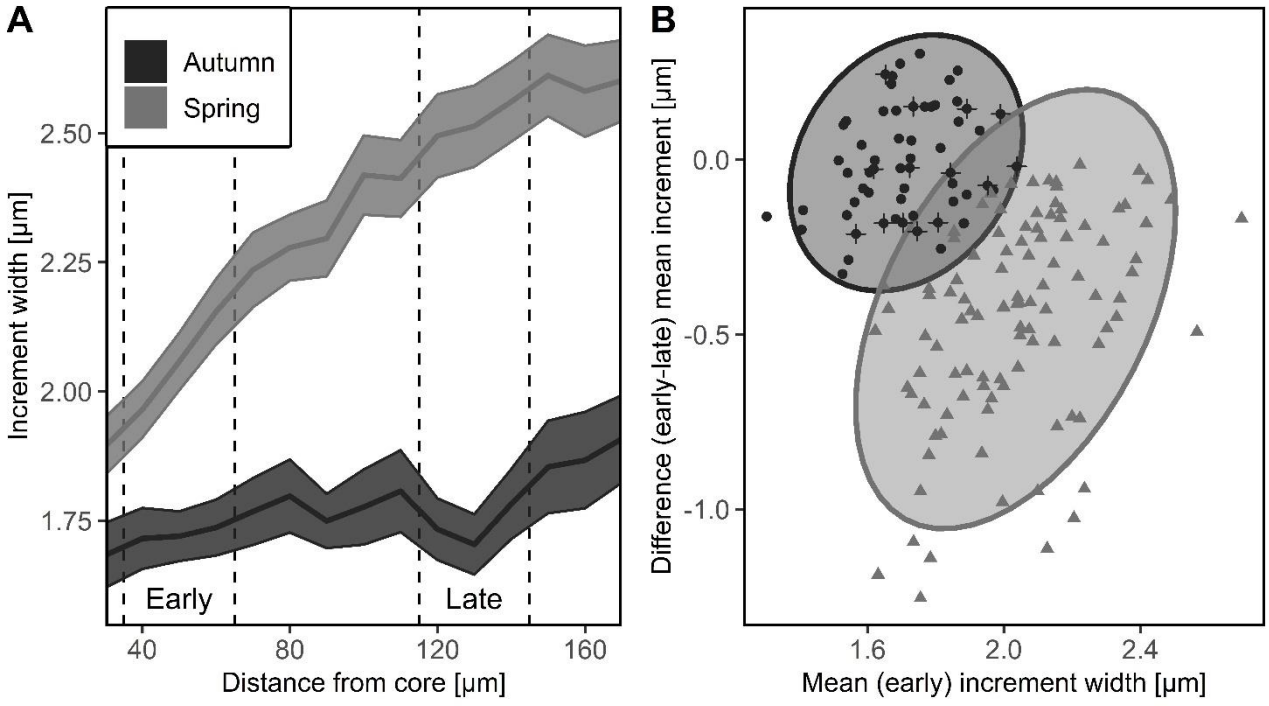
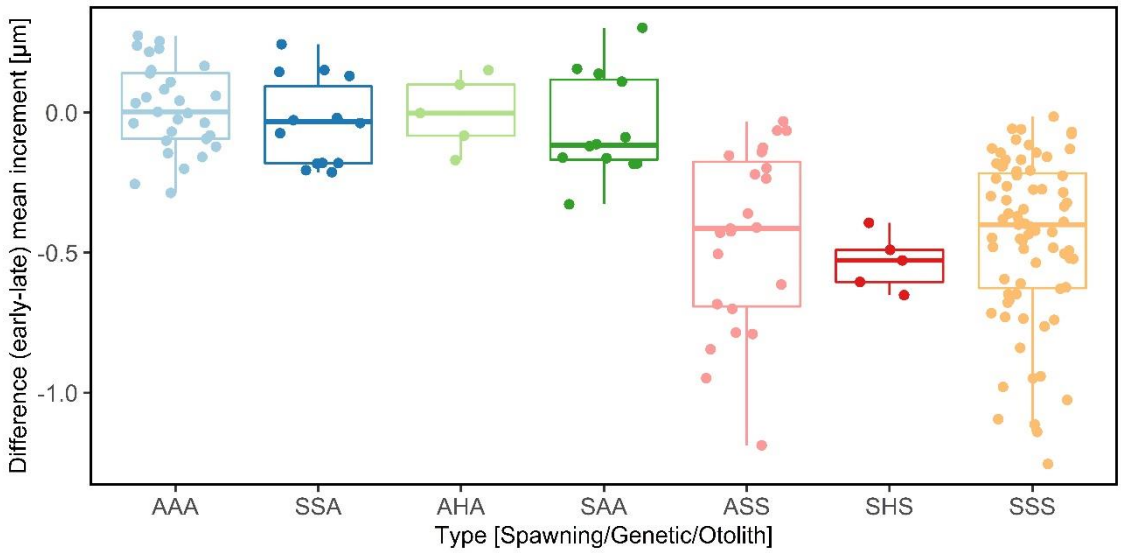


Fig. 3 Differences between mean daily increment width between early and late larval phase for all discrimination methods (Type). 1st letter = spawning phenotype, 2nd letter = genetic, 3rd letter = otolith. A = autumn, H = heterozygote/ambiguous, S = spring.



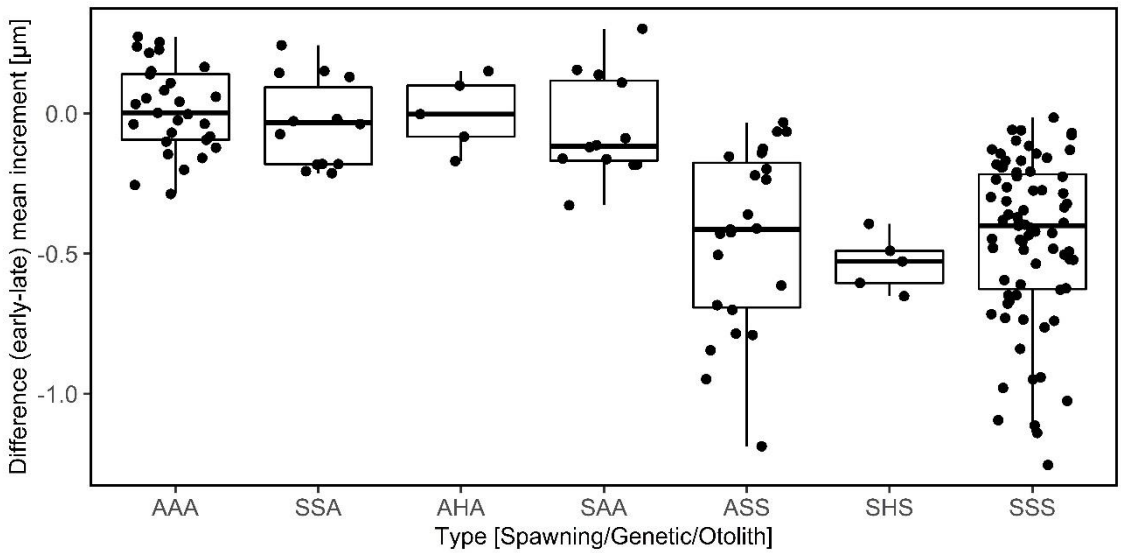


Fig. 4 Differences between herring discriminated as autumn (AAA) and spring (SSS) type by all three methods for A) Fulton's somatic condition factor, and B) length-at-age data (mean ± 95% confidence interval) fitted to the von Bertalanffy growth model. A) includes only herring in spawning conditions.