Annex 6: Summaries of presentations from Stock ID mini symposium

A6.1 Genetic Stock Identification of 6a/7bc Herring

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Commercially important seasonal fisheries for Atlantic Herring (*Clupea harengus*) take place in many different areas around the coasts of Ireland and Britain. The definition of these western stocks has changed considerably over the last five decades (see ICES 2015) and the putative stocks are currently recognised as: 6aN; 6aS/7bc; Irish Sea; Celtic Sea & 7j (ICES 2014). This separation is largely based on information from commercial fisheries and the recognition of temporal and spatial differences in spawning season and grounds (ICES, 2015); the 6aN and Irish Sea herring spawn in Autumn (Sept/Oct), the 6aS/7bc and Celtic Sea herring in winter (Nov-Jan) and there are small groups of herring (6aN and the Clyde) that spawn in spring (Feb-May). However, herring from separate stocks are believed to form mixed aggregations on common feeding grounds (Hatfield et al., 2005). Potentially mixed stock fisheries and surveys operate in these areas and the inability to assign catches to their stock of origin prevents accurate assessment, and has hampered the development and implementation of effective management strategies.

In an effort to resolve this issue a genetic stock identification project was initiated in 2016 at University College Dublin, Ireland. The project was funded by a collaboration of the Irish, Scottish and Dutch industries and the Irish Marine Institute and Marine Scotland Science. In December 2018 the project partners secured funding from the European Commission's Executive Agency for Small and Medium Enterprises (EASME) to extend the project until December 2020 and to also include morphometric analyses. The primary objectives of the project are to assess the genetic population structure of herring stocks in ICES 6a/7bc and to develop genetic baselines of the 6aN and 6aS/7bc stocks, which can be used to discriminate mixed aggregations of non-spawning herring in area 6a.

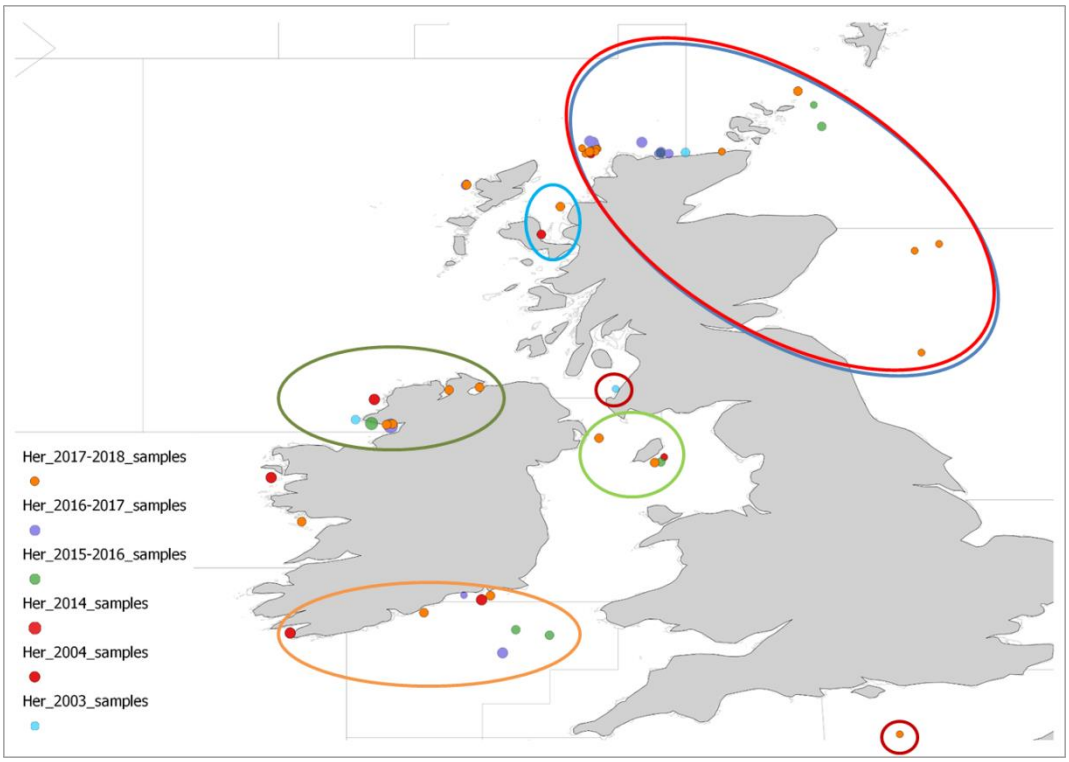

Figure 1. Baseline spawning samples analysed to date. The clustering of samples is indicated by the coloured circles.

To date, baseline spawning samples from 6 spawning seasons, comprising 56 samples and c.4,442 herring have been genotyped at 38 SNPs and 38 microsatellite markers (Figure 1). Results indicated that the 6aN autumn spawners and 6aS/7bc winter spawners represent at least 2 genetically distinct populations. No genetic differentiation has been found between the 6aN autumn and North Sea autumn spawners and the samples from these areas indicated a high degree of temporal stability. The 6aN spring spawning samples from the Minch and the Clyde areas were genetically distinct from the other 6a populations. The 6aS, Celtic Sea and Irish Sea samples all showed significant genetic differentiation between each other and were more significantly different to 6aN samples than they were from each other. Though more similar to each other than to the surrounding populations, the 6aS samples displayed a higher level of genetic variation among themselves than the other populations did. This is not unexpected as it has been well documented than the spawning time in 6aS has changed from being dominated by spring spawning in the 1920's, to autumn spawning from 197-1994 and to winter spawning from 1995 onwards (ICES, 2015). This appears to be reflected in the genetic diversity of the herring in this area. In order to improve the baseline dataset and increase the accuracy of future assignment testing of mixed samples, an additional year of baseline samples were collected (Figure 2) and are currently being analysed with the same marker panel as the previous samples.

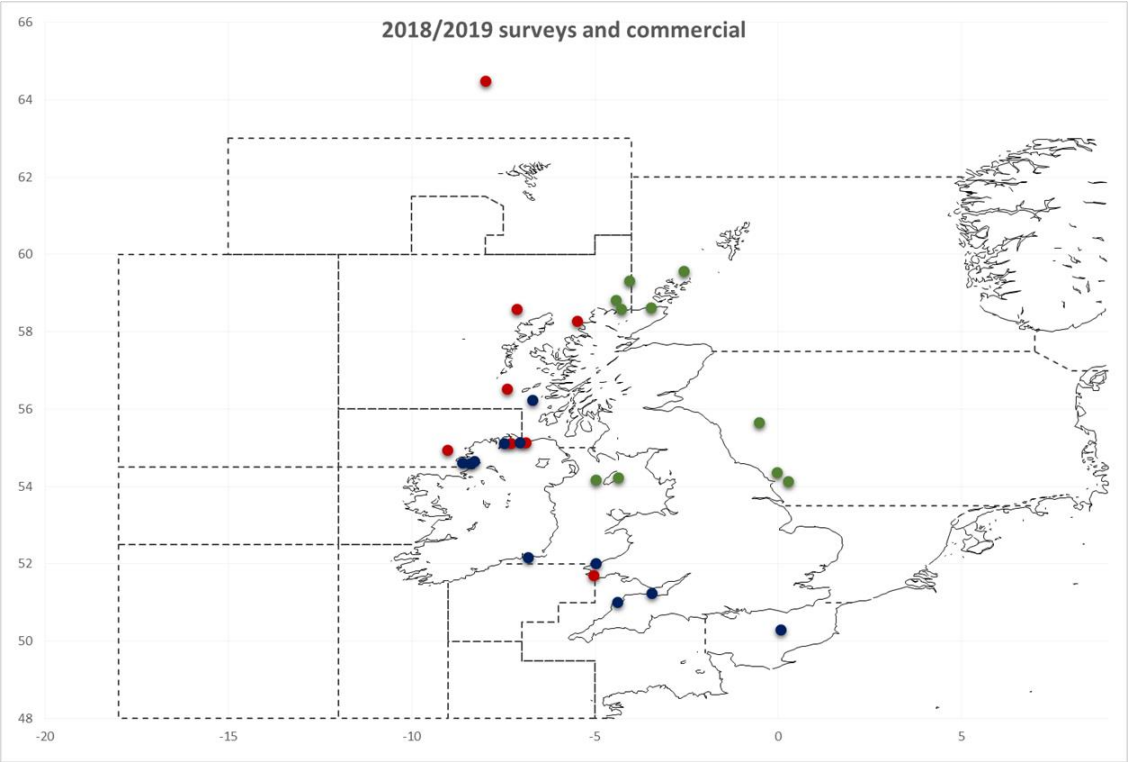

Figure 2. Baseline spawning samples analysed to date. The clustering of samples is indicated by the coloured circles.

In addition to the extra baseline samples, the marker panels being used to genotype the herring samples are also being further analysed. Collaborations are underway with DTU-Aqua and also the GENSINC project to determine if there are additional informative SNP markers that many be useful for discrimination the herring population west of Ireland and Britain from each other and from surrounding populations. Once the these analyses are completed and the additional baseline samples analysed the project aims to discriminate the samples collected during the Malin Shelf Herring Acoustic Survey (MSHAS) using both genetic methods, for samples 2014-2018 (Figure 3), and morphometric methods, for samples collected 2010-2018. The combined analyses will provide separate survey indices for the herring in 6aN and 6aS/7bc, thus enabling separate assessments to be performed on these stocks.

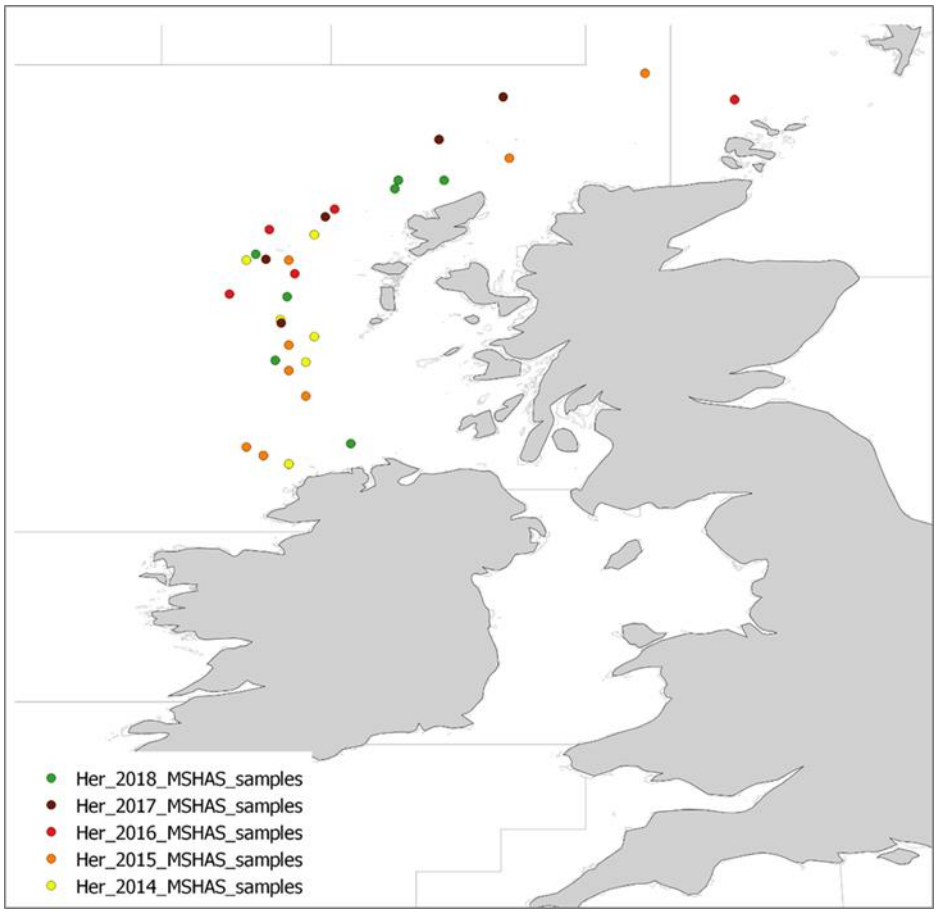

Figure 3. The genetic samples collected on the MSHAS from 2014-2018.

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A6.2 Genetic stock determination in Atlantic herring: New possibilities for accurate stock discrimination

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Genetic marker based methods to determine biologically coherent units of herring and to classify individuals in mixed samples have undergone a paradigm shift since the first genetic study by Andersson et al. (1981). Application of newly developed genomic resources for herring (e.g. Bekkevold et al. 2015; Lamichhaney et al. 2012; Barrio et al. 2016) has enabled a much improved understanding of the degree of reproductive separation among stocks and of the local selective pressures acting on them. Validation of improved accuracy marker panels to trace individuals in time and space is in development but for a number of stocks genetic methods are fully available for use within a routine monitoring framework. A Single Nucleotide Polymorphism (SNP) marker classification tool is thus applicable for distinguishing, at high statistical accuracy, among major stocks and sub-stocks mixing in areas SA4, SA3a and DIV22-25. Extended sample analyses are required to compare information from genetic markers with morphological traits.

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A6.3 Tools to split herring populations

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Discrimination and splitting of mixed stocks are essential for stock assessment and advice. Herring stocks assessed by HAWG are mainly separated based on a priori assumptions that fish stocks rigidly follow artificial geographical boundaries. Currently, splitting methods are only applied for the separation of North Sea autumn spawning herring (NSASH, her.27.3a47d) and western Baltic spring spawning herring (WBSSH, her.27.20-24). However, the splitting is limited to Danish and Swedish samples from commercial landings and scientific surveys in division 3.a, Norwegian samples from scientific surveys, and samples from commercial landings in the "transfer area" in subarea 4. Further, applied splitting methods are not consistent between labs and countries.

One of the used splitting methods to separate NSASH and WBSSH is otolith shape analysis. In recent years, the use of otolith shape analysis to discriminate fish stocks increased rapidly. Open-access packages like shapeR (Libungan and Pálsson, 2015) allow scientist to easily extract otolith outlines for further analysis. Otolith shape analysis of Atlantic herring reveal clear differences between populations in the north-eastern Atlantic (Libungan *et al*., 2015). Further, there is a clear genetic effect on the otolith shape of Atlantic herring (Berg *et al*., 2018). Using otolith shape analyses also allow to discriminate more than two stocks and assign individual fish to one of the discriminated stocks. In the greater North Sea ecoregion, there is evidence that Norwegian spring spawning herring (NSSH) might occur in this management area and can even migrate into the Skagerrak (Eggers *et al*., 2014, Berg *et al*., 2017). It is also debated if herring from division 6.a migrate into subarea 4 and mix with NSASH. Furthermore, comparisons of historical vertebral counts demonstrate that WBSSH occur outside of the "transfer area" (Berg *et al*., 2017).

In a preliminary analysis, a baseline was build-up including otoliths from herring collected at spawning grounds as well as herring of all three stocks (NSASH, WBSSH, and NSSH) and samples from spawning herring in division 6.a. Spawning herring representing NSASH were collected on spawning grounds near Shetland. NSSH were collected during the spawning survey along the western Norwegian coastline. Otoliths of WBSSH were sampled at the main spawning ground in Greifswalder Bodden. In addition, otoliths assigned as spring (WBSSH) and autumn (NSASH) spawning herring from the Skagerrak and Kattegat based on otolith microstructure were included. Ideally, this baseline should be updated by annual samples, instead of rebuilding it from year to year.

The otolith shape of herring was transformed into 64 wavelet coefficients for further testing. There were no differences between otoliths from NSASH and spawning herring in division 6.a. Therefore, these herring were merged for the following analyses. Monte-Carlo and k-fold cross-validations, provided by the assignPOP package in R (Chen *et al*., 2018), were conducted on this baseline using support vector machine as classification method. In addition to the wavelet coefficient, length data was included as an extra variable in the analysis. Analyses were conducted on a cohort basis comparing only individual of similar age. In general, the overall assignment accuracy was relatively high (>80%). The miss classifications occurred mainly between NSASH and WBSSH. These results indicate that our baseline is suitable for assignment of individuals from unknown catches.

Unknown catches were collected during the Norwegian part of the Herring Acoustic (HERAS) survey in the North Sea where the proportion of spring and autumn spawning herring is currently calculated based on vertebral counts. The benefit using the otolith shape is an individual

assignment, while only proportions are estimated on mean values using vertebral counts. Comparing the assignment of the same data demonstrate that both methods results in similar assignments.

Such an individual assignment will be also beneficial for the stock assessment since more reliable data as weight-at-age can be estimated than using only proportions. Another advantage is the incorporation of NSSH as a third component. Using the vertebral counts, only to groups can be separated. The most western station resulted e.g. in 100% NSASH that means the vertebral counts were higher than the overall mean for NSASH. The same stations also indicate the occurrence of some NSSH which could explain the high mean vertebral counts, because the overall mean of NSSH is even higher. Consequently, the use of otolith shape can provide a better and more accurate assignment of individual on the stock levels than using vertebral counts.

In addition to otolith shape and vertebral counts, genetic samples were collected for two station outside the "transfer area" during the HERAS 2018. The general trend for all the methods was, that the northern station consisted mainly of NSASH, while the southern station included mainly WBSSH. The individual assignment based on otolith shape and genetics allowed for more detailed comparison. Overall, >70% of the herring were assigned to the identical stock using genetics and otolith shape (Table 1). The biggest discrepancy is that some herring were assigned as NSSH using otolith shape analysis. However, the genetics did not assign a single herring as NSSH. This discrepancy needs to be further investigated.

All in all, otolith shape analysis provide a useful tool to discriminate and assign unknown herring catches to a given stock. Further, the preliminary results indicate that the geographical boundaries, not only for stocks, but also for the "transfer area", should be discussed. Potential readjustments or the implementation of splitting several stocks might improve the assessment and advice of herring stocks in the greater North Sea ecoregion.

References

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Table 1. Assignment results of individual herring to one of the three stocks (NSASH = North Sea autumn spawning herring, WBSSH = Western Baltic spring spawning herring, NSSH = Norwegian spring spawning herring) occurring in the greater North Sea ecoregion based on otolith shape analysis and genetic markers.

		Genetics	
		NSASH	WBSSH
Otolith shape	NSASH	34.4% (n = 52)	14.6% (n = 22)
	NSSH	6.6% (n = 10)	2.7% (n = 4)
	WBSSH	5.3% (n = 8)	36.4% (n = 55)

A6.4 Herring otolith microstructure – analysis and calibration

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Herring otolith microstructure (OM) analysis is carried out at DTU Aqua (Denmark) and SLU (Sweden) on samples from commercial landings in ICES areas 4.a, 4.db, 3.aN, 3.aS, SD22, SD23 and SD24 and from scientific surveys in ICES areas 3.aN and 3.aS. The aim is to determine the spawning type or stock ID's of the fish; North Sea autumn spawners (NSAS or 9's), Downs winter spawners (Downs or 12's) and Western Baltic spring spawners (WBSS or 4's). The samples form the baseline for the otolith shape/stock ID analysis conducted on the combined commercial catches from Denmark and Sweden prior to the annual stock splitting of the WBSS component from the North Sea component in the 3.a and 4.a.E. and 4.b.E "transfer area" and the Danish HERAS samples. Calibration exercises have been ongoing since 1999 to ensure consistency in agreement between readers and laboratories and for training new readers.

The 2019 exchange utilised samples with genetically assigned stock ID. The genetic methods applied have a very high statistical power for stock assignment using SNP markers (> 95% of fish classified correctly) with the added possibility to identify likely sub stocks (Rügen, Kattegat, Skagerrak, Central Baltic autumn and spring spawners). Readers results were compared against the genetically validated stock ID for each sample and the percentage agreement (PA) and a comparison matrix of each reader versus validated ID was calculated, DK01 and DK02 reached a PA of 87%, SWE01 reached a PA of 84% and SWE02 reached a PA of 80%. These results show a huge improvement from the 2016 workshop and the 2018 exchange where there were few samples where all 4 readers were in agreement. The correct identification of the Down winter spawning component was problematic is all recent calibration events. In the 2018 exchange, the inclusion of a subset of genetically validated samples provided the opportunity to calibrate against material with known stock ID. Following the exchange images were discussed with the readers and guidelines agreed upon based on these validated samples. This material has certainly contributed enormously to the improved 2019 results.

It is likely that there has been a change in increment width (IW) patterns observed in the otoliths of herring caught in this area overtime considering otolith microstructure is under the influence of growth, spawning time variation and environment. In addition, other sub stocks of herring (Rügen, Kattegat, Skagerrak, Central Baltic autumn and spring spawners) caught in this area are amongst the samples being analysed. An updated baseline set of samples is needed so that guidelines for IW measurements and an image library can be included in an updated OM reader protocol. This requires that samples from both spawning fish and 0-group fish covering the three main stock ID's plus the sub stocks are collected for combined genetic, OM and otolith shape analysis. These samples can potentially be used in the future to test the validity of the various stock identification and splitting methods and be used for quality assurance exercises within and between national laboratories.

A6.5 Herring otolith classification

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Background

The separation of different herring stock components is an issue in several of the surveys coordinated in WGIPS. Recently concerns have been raised by the survey groups for the International ecosystem surveys in the Nordic Seas (IESNS and IESSNS) on mixing issues between Norwegian spring-spawning herring (NSSH) and other herring stocks (e.g. Icelandic summer-spawning ISSH, Faroese autumn-spawning FASH and North Sea type autumn-spawning herring NASH) might have occurred in some of the fringe regions in the Norwegian Sea. Up to date fixed cut lines have been used to exclude herring of presumed other types than NSSH, however this simple procedure is thought to introduce some contamination of the stock indices of the target NSSH.

Summary

Havstovan (the Faroe Marine Research Institute) uses a combination of maturation stage and otolith microstructure (nucleus or hatch type and otolith shape/growth pattern) to separate autumn-spawning herring from the Norwegian spring-spawning herring. The nucleus (hatch) type and the width of first winter ring/summer growth (L_1) combined with maturity stage (GSI) apparently gives a high degree of separation power if employed by experienced personnel.

Method

Mixing of herring occurs in the Faroese area and neighbouring areas during the IESNS and IESSNS. The NSS herring is found feeding in the northern part of the Faroese EEZ, usually north of 62°N. However, in this fringe area NSSH is found mixed with the local Faroese herring (FASH). Similarly east of the Faroes and into the northern part of the EU EEZ, herring of the autumn-spawning type is found mixed with NSSH to a varying degree. In the Faroe zone they are believed to be autumn-spawning herring of Faroese origin while further east they might originate from the northern North Sea (IVa or VIa north).

There are many ways to classify herring e.g. by

observing the spawning site

otolith microstructure, e.g. nucleus type (opaque or hyaline) or shape analysis, intercirculi spacing in otoliths (and scales)

morphological (fenotypical) differentiations of the herring such as gillraker spacing, vertebrae counts, maturity stage at time of capture

genetic methods (microsatellite or SNPs)

chemical and fatty acids analysis.

The current method used by the Faroese Marine Research Institute to split herring samples into NSSH and other herring types consists of two parts: otolith micro structures (nucleus type and annual growth patterns) together with gonad development indices.

The measurement is the growth in the first year (L_1 radius from centre to first winter zone or diameter D_1). The L_1 measure has been reported in literature (Geffen 1982, Husebø et al. 2005). The observed smaller width of the first winter ring in the opaque type NSSH might be attributed to the nursery area in northern Norway/Barents Sea, i.e. in colder environment than the herring grown up on the Faroe Plateau of further south in the northern North Sea. Thus the hyaline types have a wider diameter to the first winter ring compared to the NSSH. A further indication is the hyaline nucleus in the autumn-spawning herring, where the transparent nucleus indicate that the larvae was spawned the autumn before (with poor growth as larvae during winter) prior to the first years growth. Geffen (1982) found a positive relationship between herring growth rate and daily ring deposition rate in herring. Husebø et al. (2005) also used otolith microstructure and gonad development indices to demonstrate spawning season fidelity in autumn- and spring-spawning herring in mixed overwintering aggregations.

Initially otoliths from herring of both types (NSSH and FASH) were photographed and measured (L_1) to determine their origin. This analysis showed a clear separation between the two types of herring. This method is time consuming and cumbersome, however, it appeared that it was relatively easy to distinguish the two otolith types by visual inspection only by trained personnel.

Visual discrimination:

Opaque (spring-spawning) type: shorter, shorter rostrum, wider appearance, more square winter zones, L_1 (or D_1) shorter than for hyaline otoliths

Hyaline (autumn-spawning) type: opposite to above.

The L_1 measurements and nucleus type together with maturity stage transformed to gonosomatic index GSI (gonad weight in relation to body weight) apparently gives a very high degree of separation power if employed by experienced personnel.

Examples of L_1 measurements from a Faroese sample in May 2008 in the northernmost part of the EU zone (where mixed concentrations of herring was found). Most likely the opaque type herring were NSSH and the hyaline types with larger first summer growth were FASH or NASH: Hyaline type $L_1 = 0.71$ mm and Opaque type $L_1 = 0.50$ mm (small sample in the ppt presentation). The method has been tested by comparison of "known" samples of NSSH and FASH.

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A6.6 Morphometric discrimination of herring in 6a,7bc

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Identifying stocks is one of the primary prerequisites to perform an assessment. In the divisions 6a and 7bc, ICES recognise that two stocks of herring are assessed as one. As both stocks are not the same size and have different dynamics, using one fishing mortality for both could lead to the overfishing of one stock. In December 2017, an EASME funded project led by University College Dublin with the Marine Institute and Marine Science Scotland as partners started. The objective of the project is first to develop tools that enable the identification of the herring stocks that occur in the ICES divisions 6a and 7bc and second to use the developed tools to identify the origin of fish sampled in a putative mix. The stock identification will be based on genetics, body morphometrics and otolith shape. The objective of the presentation is to give the HAWG an update about the work undertaken on body morphometrics and otolith shape. Regarding body morphometrics, 20 landmark points were digitised and enabled the derivation of 40 body morphometric measurements. Among these, 8 were excluded due to correlation with maturity stages. Regarding otolith shape, each otolith was photographed and the R package ShapeR (Libungan and Palsson, 2015) used to derive either the coordinates of the Fourrier ellipses or the wavelet coefficients. Both of them can describe the shape of an otolith and can be used to identify fish of different stocks. Baseline data collection started in 2003–2005 as part of the WESTHER project and further samples were collected in 2014 and 2016–2017. In total, 1900 fish were sampled for baseline morphometrics. Regarding mix samples, from 2010 to 2018, 9,700 fish were sampled on mix aggregations. Some preliminary cross validation tests using the R package AssignPOP (Chen et al., 2017) showed 87% success in allocation. Although morphometric data are more labour intensive than genetic data to collect, the work continues as the time series is longer (time series on the mix starts in 2010 for morphometrics vs 2014 for genetics). The data collection will carry on until the end of the project in 2020. Comparisons between morphometric and genetic tools will be made to choose the most efficient and less costly method that will be used on the long term to monitor the mix aggregations.

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