Second Workshop on Stock Identification and Allocation of Catches of Herring to Stocks (WKSIDAC2; outputs from 2023 meeting)

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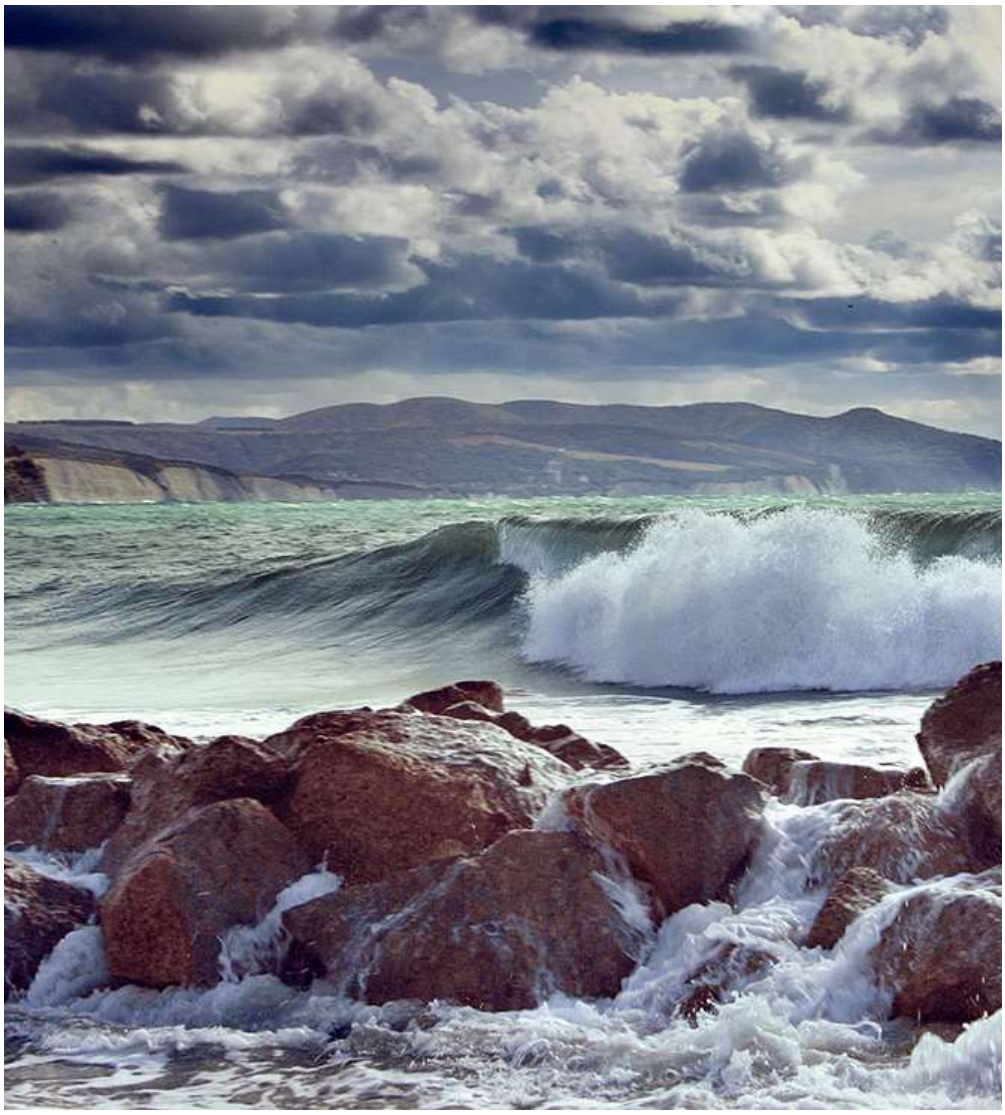

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Contents

i Executive summary

Population identification plays an important role in the assessment of Atlantic herring (*Clupea harengus*). Several morphological/traditional methods have been applied in the past to split catches (both within survey and commercial samples) and to identify herring populations. However, in recent years advances in genomics have provided a robust and precise method to genetically identify herring populations. WKSIDAC2 reviewed the current status of genetic population identification for herring and outstanding issues affecting identification accuracy/success. The genetic methods using single nucleotide polymorphism (SNP) as genetic markers to identify herring population are considered appropriate. Depending on the region of interest, different sets of markers are used to identify herring populations. The differences in the set of markers have only minor influence on the population identification and can be negligible. However, it is recommended to choose sets of markers which will allow a direct comparison between institutes undertaking the classification. Furthermore, the review concluded that 26 different herring spawning populations can be currently identified using genetic methods in the northeastern Atlantic. However, future genetic studies may increase the number of identified populations and also provide a greater resolution for the spatial distributions. For example, from the genetic markers presented during WKSIDAC2, we are not able to genetically differentiate between Icelandic summer spawners, Faroese autumn spawners, and Norwegian autumn spawners. These three populations are currently combined as a single genetic unit. Future work incorporating whole genome sequencing data, is recommended to investigate this issue in more detail. In addition, of the 26 identified genetic populations only some can directly and uniquely be assinged to a particular stock (management unit) and for this reason WKSIDAC2 refrained from establishing such correspondence which is left for future work. Baseline samples of these 26 populations have been analyzed based on different sets of markers. Therefore, the baseline information cannot be shared directly between institutes. Also, it is recommended that a common method for analyzing baseline samples, as well as storing the genotypes in an open and public database is agreed. During WKSIDAC2, a general description of prerequisites for the implementation of population identification of herring was discussed and several options have been presented. A series of presentations provide potential solutions on how to implement population identification in current stock assessment models or during survey estimates. However, analyses of the optimal baseline requirements for stock assessment purposes, both for specific surveys as well as commercial catches is to be the subject of a new workshop WKSIDAC3, proposed for 2024. The aim of the workshop is to establish a simulation framework for estimating stock compositions based on genetics 1) to investigate differences between random or stratified sampling, and 2) to investigate inter-haul variability in surveys.

ii Expert group information

Expert group name	Second Workshop on Stock Identification and Allocation of Catches of Herring to Stocks (WKSIDAC2)
Expert group cycle	Annual
Year cycle started	2023
Reporting year in cycle	1/1
Chairs	Richard Nash, UK
	Florian Berg, Norway
Meeting venue and dates	19-23 June 2023, ICES HQ; Copenhagen, Denmark (31 participants (12 online))

1 Introduction

Most herring populations (biological unit) are migratory and often congregate on feeding and wintering grounds where aggregations may consist of mixtures of individuals from several populations, thus the standard concept of a 'herring stock' within a geographical area such as a management unit is not straight-forward to assume. Throughout the report the term 'stock' refers to a management unit which is defined within a geographical area, currently assessed by ICES and advice provided. Whereas the term 'population' is defined as a distinct biological unit exhibit reproductive isolation. Thus, several populations can exist within a defined management area of a stock. These definitions are within the ICES Glossary of terms (Glossary - ICES Glossary).

Previously, morphometric analyses, analysis of calcified structures, tagging and analysis with a small number of genetic markers were among the most widespread tools available for population identification of bony fish and a wide variety of such methods have been used to separate herring into populations (ICES, 2017). These methods have been developed for specific subsets of populations where each method yielded various degrees of classification accuracy among populations. However, the recent advent of genomic characterization of herring populations (Martinez-Barrio et al., 2016; Lamichhaney et al., 2017; Pettersson et al., 2019; Han et al., 2020; í Kongsstovu et al., 2022) has the potential to provide more detailed and reliable identification and partitioning of individuals to populations. The analysis of the genetic composition is becoming a widely and cost-effective tool for separating herring into populations. However, the genetic identification is not able to identify where an individual actually hatched (e.g., which spawning ground in the North Sea, or where along the Norwegian coast), so other analytical techniques are needed to provide this information (see ICES, 2017). However, it needs to be investigated if this knowledge is needed for stock assessment purposes. Currently, two genetic baselines are published to identify herring populations northwest of Ireland and west of Scotland (Division 6.a, 7-b-c, Farrell et al., 2022) and the North Sea and Baltic Sea focusing on the transition area between them (Bekkevold et al., 2023).

This led to the implementation of genetic population identification of individuals in both surveys and commercial catch samples for the assessment of a number of herring stocks. Genetic population identification is currently utilised to separate autumn- and winter-spawning herring originating from the North Sea and eastern English Channel (her.27.3a47d) from spring-spawning herring originating from the western Baltic Sea, Skagerrak and Kattegat (her.27.20-24) (Berg et al. 2023). Further, a benchmark for the herring stocks in divisions 6.a, 7.b, c was held in 2022 and accepted the applied genetic population identification method (ICES, 2023). The genetic method is currently able to separate the individual 6.a.N autumn-spawning and 6.a.S winter-spawning stocks, and also spring-spawning herring from division 6.a.

Given these developments, it was deemed timely to revisit the genetic herring population identification method, following on from the conclusions of WKSIDAC in 2017 (ICES, 2017). The workshop was set up to include ICES Subareas 2, 3, 4, 5, 6 and 7.

The report is structured in line with the terms of reference with each section covering one of the four terms of reference. There is also an overall Workshop discussion and conclusion section, a references section and annexes, one of which provides relatively detailed rapporteur notes.

1.1 References

Bekkevold, D., Berg, F., Polte, P., Bartolino, V., Ojaveer, H., Mosegaard, H., et al. (2023). Mixed-stock analysis of Atlantic herring (Clupea harengus): a tool for identifying management units and complex

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ICES. 2023. Benchmark Workshop on North Sea and Celtic Sea stocks (WKNSCS). ICES Scientific Reports. 4:85. 324 pp. https://doi.org/10.17895/ices.pub.21558681

Lamichhaney, S., Fuentes-Pardo, A.P., Rafati, N., Ryman, N., McCracken, G.R., Bourne, C., et al. (2017). Parallel adaptive evolution of geographically distant herring populations on both sides of the North Atlantic Ocean. Proceedings of the National Academy of Sciences 114(17), E3452-E3461. https://doi.org/10.1073/pnas.1617728114

Martinez Barrio, A., Lamichhaney, S., Fan, G., Rafati, N., Pettersson, M., Zhang, H., et al. (2016). The genetic basis for ecological adaptation of the Atlantic herring revealed by genome sequencing. eLife 5, e12081. https://doi.org/10.7554/eLife.12081

Pettersson, M.E., Rochus, C.M., Han, F., Chen, J., Hill, J., Wallerman, O., et al. (2019). A chromosome-level assembly of the Atlantic herring genome—detection of a supergene and other signals of selection. Genome Research 29, 1919-1928. https://doi.org/10.1101/gr.253435.119

2 ToR a: Review recent status of genetic population identification for herring and outstanding issues affecting identification accuracy/success

In total, eight presentations were given providing information about the recent status of genetic identification of herring populations. Presentation abstracts, and rapporteur's reports covering each presentation, which include questions, answers, and comments, are given in the Annex. All presentations are retained on the WKSIDAC SharePoint.

Based on these presentations, 26 genetically distinct populations can be currently identified across the north-eastern Atlantic Ocean (Table 2.1). Several laboratory-dependent baselines were presented using different sequencing methods to identify the 26 populations. The idea of a "universal assignment model" able to separate herring populations across the whole north-east Atlantic was presented and positively perceived by participants. Outstanding issues to achieve such a baseline were discussed.

2.1 Identified populations and available baseline samples

The knowledge about herring genetics has increased rapidly during the last few years. Following the road map described by Andersson et al. (2024) the current extensive genomic data resource has been established. First, local and ecological adaptation in herring populations were revealed by genome sequencing (Lamichhaney et al., 2012; Martinez Barrio et al., 2016), followed by a chromosome-level assembly of the genome (Pettersson et al., 2019), and finally, the identification of single nucleotide polymorphisms (SNPs) associated with selective outlier regions (Han et al., 2020). This is the basis for all the genetics studies presented at the workshop. Selected markers from that initial genome sequencing have been applied through the years in different areas, mainly with the aim to split survey and commercial samples, but also as a mean to determine the distributions of spawning populations on local to regional scales. Each study focuses on slightly different population mixing scenarios, geographical areas, and management issues. Each, therefore, applies different high-graded genetic marker panels specifically designed to detect genetic variation among the identified populations believed to be mixing in the area of interest, while keeping marker numbers down in order to minimise the costs of genotyping. It should be noted that unknown populations or unknown mixing may not be detected by these specified marker panels.

The main results of the first presentation by Dorte Bekkevold are published in Bekkevold et al. (2023). The presentation focussed on issues of population classification, with 59 SNPs selected to maximise resolution among populations spawning and feeding in the North Sea-Baltic Sea area. A baseline dataset with collections from more than 45 spawning locations has been used. All analyses with the 59 SNP tool agree with genome-wide sequencing results generated for population samples collected across larger geographic scales and thus support the notion that the tool is an accurate population classification method. Nonetheless, there are examples of local, genetically distinct populations that only, to some degree, can be accurately classified with the tool. The design of the tool is, therefore, under continued development (by adding additional SNPs to the array) with the attempt to increase classification power for specific populations in the Western Baltic/Baltic Sea area. In total, the study identified 12 genetic populations. All analyses to date confirm the notion that the western Baltic spring-spawning (WBSS, her.27.20-24) stock consists of multiple, genetically divergent populations (Table 2.1), which can be divided into four

main populations; Rügen herring (her_rug_sp), western Baltic herring (her_balW_sp), inner Danish water herring (her_idw_sp), and Skagerrak herring (her_skag_sp). Spring-spawning herring from Norwegian and Swedish Skagerrak coasts are genetically highly divergent from other WBSS populations, showing closer genetic relationships with spring-spawning herring from the westcoast of Norway (her_nor_sp). No less than two populations of genetically distinct autumn-spawning herring are revealed to spawn in several local areas in the western Baltic Sea (her_balW_au) and Baltic Sea basins (her_balE_au). Further, a Southern (her_balS_sp) and a Northern Baltic Sea (her_balN_sp) spring-spawning population have been identified in the central Baltic. Only the Southern populations migrates out of the Baltic Sea with a genetic gradient of differentiation characterising populations from the southern Baltic Sea coasts. The 59 SNP results also demonstrate that North Sea autumn-spawning herring (her_ns_au) and Downs winter-spawning herring (her_down_wi) are genetically highly distinct and can easily be classified in scientific survey and commercial catch data. Lastly, summer/autumn-spawning herring from the North Atlantic (her_nea_au), in this case from waters in the vicinity of the Faroes Islands, to be more specific, are also genetically distinct from all other populations.

Parts of the presentation by Edward Farrell are published in Farrell et al. (2021; 2022). The presentation focussed partly on issues of population identification with 45 SNPs selected to maximise resolution among populations occurring in ICES divisions 6.a, 7.b-c. The panel of 45 SNPs combined with a hierarchical approach to population assignment can discriminate between the herring populations that spawn northwest of Ireland in winter (her_irlNW_win_sp), north of Scotland in autumn (her_ns_au). The panel cannot currently distinguish between the 6.a.N spring-spawning herring (her_wos_sp) or the herring that spawn northwest of Ireland in the spring (her_irlNW_win_sp). The analyses also highlighted that the 6.a.N autumn-spawning herring are genetically identical to the North Sea autumn-spawning herring and could be considered together for assessment purposes. Further results from a number of projects discussed during the presentation using a "MultipFishSNPChip" array, which have investigated the population structure of herring in the Irish Sea, Celtic Sea, and the Bristol Channel, are summarized below (Davies et al., 2020; Gwilliam et al., 2020).

Guðmundur J. Óskarsson presented work on herring in the Norwegian Sea and adjacent waters using a panel with 120 SNPs to mainly identify Icelandic summer-spawning (ISSH), Norwegian autumn-spawning (NASH), Faroese autumn-spawning (FASH), Faroese spring-spawning (FSSH), North Sea autumn-spawning (her_ns_au), and Norwegian spring-spawning (her_nor_sp) herring based on analyses of samples from a previous study (Pampoulie et al., 2015). Based on the 120 SNP panel, only Norwegian spring-spawning and North Sea autumn-spawning herring could be genetically discriminated as distinct populations (Pampoulie et al., 2022). The other expected groups (ISSH, NASH, FASH, FSSH) could not be uniquely identified and are referred to in the following as North Atlantic summer/autumn-spawning (her_nea_au) herring. However, North Atlantic summer/autumn-spawning (her_nea_au) are genetically distinct from Norwegian spring-spawning (her_nor_sp) and North Sea autumn-spawning (her_ns_au) herring.

Florian Berg presented results based on a 76 SNP panel to discriminate herring populations in the North Sea, Norwegian Sea and along the Norwegian coast. The SNP panel was established following a similar set of criteria as described in Bekkevold et al. (2023). Additional markers to discriminate populations along the Norwegian coast and inside the fjords were included. In contrast to Bekkevold et al. (2023), the SNPs chosen were selected to primarily differentiate populations likely present in the North Sea, Norwegian Sea and along the Norwegian coast. Therefore, the number of SNPs to discriminate among populations within the Baltic Sea was reduced to only identify Baltic vs non-Baltic herring. In total, 23 baseline samples from spawning aggregations were sequenced, resulting in 13 genetically distinct populations. Many of them have been described previously in Bekkevold et al. (2023). Newly genetically identified populations were

autumn-spawning herring from Sykkylven (her_sykk_au), spring-spawning herring from Trondheimsfjorden (her_thf_sp), hybrids of Atlantic and Pacific herring in Balsfjorden and Rossfjordvannet (her_pachy_sp, see also Pettersson et al. (2023)), and local fjord herring (her_norfj_sp). Local fjord herring can be found in Gloppenfjorden, Lindåspollen, Lustrafjorden and Sognefjorden and cannot be genetically differentiated. Also, genetically distinct herring from Landvikvannet (her_lndv_sp) along the Norwegian Skagerrak coast have been identified, differentiating from other spring-spawning herring in the Skagerrak (her_skag_sp). Moreover, an additional genetically distinct spring-spawning population is detected in the North Sea (her_ns_sp). However, spawning individuals of these populations have not been found as yet. All spring-spawning individuals collected along the Norwegian coast have been assigned as Norwegian spring-spawning (her_nor_sp) herring. There is a necessity to check if these spring-spawning herring from the North Sea are genetically distinct from spring-spawning herring in 6.a.N (her_wos_sp) identified by Farrell et al. (2022) to ensure if these can be treated as two distinct popualtions or should be merged as one genetic unit. Similar to the results presented by Guðmundur J. Óskarsson, groups included in the genetic unit of North Atlantic summer/autumn-spawning herring (her_nea_au) could not be differentiated (although a SNP study from í Kongsstovu et al. (2022) indicates that genomic differences are present within this set of populations). As indicated earlier, this panel consisting of 76 SNP is only able to identify Baltic autumn-spawning herring as well as central Baltic spring-spawning herring but cannot distinguish between her_balW_au and her_balE_au nor her_balN_sp and her_balS_sp. Further, the panel cannot differentiate between the western Baltic spring-spawning populations described by Bekkevold et al. (2023) her_rug_sp, her_balW_sp or her_idw_sp, except for her_skag_sp.

Ian Richardson presented IdentiGEN Ltd. (Dublin, Ireland) which has its core business in food traceability, which was then expanded to genomics, and much more. IdentiGEN works on more than 10 species, e.g., cattle, pigs, chicken, sheep, horses, salmon, shrimp, and wild fish. They also conduct DNA traceback studies. In partnership with a team led by Leif Andersson, IdentiGEN Ltd. (Dublin, Ireland) developed a MultiFishSNPChip_1.0 array containing 3,000–4,000 SNPs per species. The species included are Atlantic herring, European sprat (*Sprattus sprattus*), Atlantic horse mackerel (*Trachurus trachurus*), brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), European perch (*Perca fluviatilis*), and Atlantic cod (*Gadus morhua*). The SNPs included within the array design were carefully selected to include SNPs that, based on previous data, showed genetic differentiation between populations. However, this does not guarantee the local SNP panels used by several institutes (see studies above) are represented in full on the current MultiFishSNPChip_1.0 array, as technical issues may limit the inclusion of a small fraction of SNP. Work is ongoing to double-check if these missing local SNPs (<10%) need to be added to an updated array version, or whether the redundancy inherent to the array's design (which ensures an equivalent, neighbouring SNP is typically present) sufficiently compensates for any dropout. In addition, sets of random selected putatively neutral markers were also included. The same array is thus used across species, but only data generated for the species in question are considered in the bioinformatic analysis. Validation and analysis of the MultiFishSNPChip_1.0 array are ongoing. Early findings from use of the SNP array in Atlantic herring are presented in the following paragraphs and in Andersson et al. (2024) showing that the array is scalable and has strong discriminatory power to distinguish between known populations.

David Clarke and Edward Farrell further presented results from a number of projects where the MultiFishSNPChip_1.0 array from IdentiGEN was used for the genotyping to investigate the population structure of herring in the Irish Sea, Celtic Sea, and the Bristol Channel (Davies et al., 2020; Gwilliam et al., 2020). In addition to previously described populations, these projects have identified a genetically distinct autumn/winter-spawning herring population in the Celtic Sea (her_celt_wi) and the Bristol channel (her_bc_au_wi), autumn-spawning herring in the Irish Sea (Douglas Bank/Mourne, her_irs_au), winter-spawning herring in the western English Channel

(her_wch_wi), and two populations of spring-spawning fish; a low salinity spring-spawning group spawning within Milford Haven (her_mlfh_sp), and a group thought to be spawning in the marine environment off the South Pembrokeshire coast (Freshwater East, Bristol Channel, her_bc_sp).

Further, the idea of a "Universal Assignment Model" was introduced. There has been significant progress in the development of area and population specific genetic assignment models, both to the west of Ireland, Britain and also in the western Baltic and North Sea areas. However, there are unresolved questions of what assignment models to use where these areas meet. First analyses were conducted by IdentiGEN and preliminary results were presented by Ian Richardson. In total, 70 baseline samples from 38 geographic locations and 13 ICES areas were genotyped using the MultiFishSNPChip_1.0 array. These samples could be linked to 11-12 distinct genetic populations described above. In the end, less than 200 SNPs were used yielding in a self-assignment accuracy of 66-68%. Refinements of populations, e.g. for her_bc_sp and her_irlNW_wi_sp, increased the accuracy to 80%. A machine learning approach is used to find the optimal combination of populations, SNP markers, and algorithms (i.e., testing different classifiers, feature selection, data sampling) resulting in the highest self-assignment accuracy of 88.5%. Future work is needed, and a new workshop is proposed to tackle this issue (see roadmap).

Jake Goodall presented the ongoing development of population assignment models in Atlantic herring using the MultiFishSNPChip_1.0 array, with a focus on population genetic studies in the Baltic Sea. Initial trials utilizing 4,355 SNPs showed strong discriminatory power, clearly differentiating Baltic, Norwegian, and North Sea/British individuals and spring- and autumn-spawning populations. Broader applications of the SNPChip-based datasets were discussed, as were some of the current limitations of the technology (See Annex for more details). The MultiFishSNPChip_v1.0 array contains many redundant SNPs in regions of high biological interest, which have the potential to skew visualization population trends. Methods to account for this redundancy were proposed and demonstrated primarily via the reduction of specific structural regions (i.e., chromosomal inversions) to a single representative SNP. These 'haplotype reduction' methods were particularly useful for characterizing genetic variation in Baltic herring populations and allowed for the identification of seemingly novel herring ecotypes. Preliminary studies of various novel herring ecotypes were discussed, as was the genetic variation putatively underlying said ecotypes. However, the characterization of novel ecotypes in the Baltic Sea remains in its infancy and, therefore, requires further screening and scientific assessment. The dataset used for this study was presented by Lovisa Wennerström. This was a collaborative effort of herring sampling along the Swedish coastline. The selection of stations was influenced by the Swedish management and was often due to practical reasons.

Aaron Brazier presented a case study where genetic population identification is not yet applied, but potentially needed. Blackwater (Thames) herring represent a localized, spring-spawning population in the Blackwater Estuary (Essex, United Kingdom). This population is subject to a sentinel fishery, operating from September through January. The fishery is closed when the herring are known to spawn (late February – April). Whilst no genetic samples have been taken or analyzed, the population can be identified from North Sea herring through its spawning period. A, now discontinued, fisheries-independent survey quantified the mixing between the Blackwater and North Sea (winter spawning, Downs component) populations with 15% of catches being Downs fish.

Table 2.1 Spatial distributions baselines (to view table in full, please go to https://doi.org/10.17895/ices.pub.24998747).

Description	Code	Location baseline samples	Lat	Long	Contact person	Genotype method/panel	whole-genome resources in
Central and Northern Baltic Spring spawning	her_balN_sp	Gulf of Finland	60.40	26.70	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	
Central and Northern Baltic Spring spawning	her_balN_sp	Bothnian Bay (Oulu)	65.05	24.58	Dorte Bekkevold	Bekkevold et al. (2023)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076 for site Kalix
Central and Northern Baltic Spring spawning	her_balN_sp	Gulf of Riga (Gulf of Pärnu)	58.16	24.26	Dorte Bekkevold	Bekkevold et al. (2023)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Central and Northern Baltic Spring spawning	her_balN_sp	Baltic proper southwest (Hanö)	55.57	15.18	Dorte Bekkevold	Bekkevold et al. (2023)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Eastern Baltic Autumn spawning	her_balE_au	Gulf of Riga (Saaremaa)	58.02	23.50	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Southern Baltic Spring spawning	her_balS_sp	Curonian Lagoon (Smiltyne)	55.60	21.13	Dorte Bekkevold	Bekkevold et al. (2023)	Fuentes Pardo et al. (unpublished)
Southern Baltic Spring spawning	her_balS_sp	Vistula Lagoon (Gdansk)	54.37	19.67	Dorte Bekkevold	Bekkevold et al. (2023)	Fuentes Pardo et al. (unpublished)
Rügen Spring spawning	her_rug_sp	Roedvig, Denmark	55.24	12.39	Dorte Bekkevold	Bekkevold et al. (2023)	
Rügen Spring spawning	her_rug_sp	Western Baltic Sea (Greifswald Bay, Rügen)	54.21	13.62	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Rügen Spring spawning	her_rug_sp	Western Baltic Sea (Warnow estuary)	54.13	12.09	Dorte Bekkevold	Bekkevold et al. (2023)	
Western Baltic Autumn spawning	her_balW_au	Bornholm Basin (Christiansø)	55.26	15.33	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076

Description	Code	Location baseline samples	Lat	Long	Contact person	Genotype method/panel	whole-genome resources in
Western Baltic Autumn spawning	her_balW_au	HolbaekFjord, Denmark	55.73	11.71	Dorte Bekkevold	Bekkevold et al. (2023)	
Western Baltic Autumn spawning	her_balW_au	Western Baltic Sea (Greifswald Bay, Rügen)	54.21	13.62	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	
Western Baltic Spring spawning	her_balW_sp	Western Baltic Sea (Kiel Bight)	54.36	10.16	Dorte Bekkevold	Bekkevold et al. (2023)	
Western Baltic Spring spawning	her_balW_sp	Western Baltic Sea (Lübeck Bight/Trave)	53.92	10.85	Dorte Bekkevold	Bekkevold et al. (2023)	
Western Baltic Spring spawning	her_balW_sp	Western Baltic Sea (Schlei)	54.60	9.76	Dorte Bekkevold	Bekkevold et al. (2023)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Inner Danish waters Spring spawning	her_idw_sp	Kattegat (Isefjord)	55.73	11.37	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Inner Danish waters Spring spawning	her_idw_sp	North Sea (Ringkøbing Fjord)	55.97	8.24	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Inner Danish waters Spring spawning	her_idw_sp	North Sea (Wadden Sea)	55.44	8.54	Dorte Bekkevold	Bekkevold et al. (2023)	
Inner Danish waters Spring spawning	her_idw_sp	North Sea/Kattegat (Limfjord)	56.96	9.14	Dorte Bekkevold	Bekkevold et al. (2023)	Fuentes Pardo et al. (unpublished)
Skagerrak Spring spawning	her_skag_sp	Oslofjorden	59.82	10.57	Florian Berg	IMR (Norway)	
Skagerrak Spring spawning	her_skag_sp	Skagerrak East (Öckerö)	57.60	11.40	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076 for site Björköfjorden
Skagerrak Spring spawning	her_skag_sp	Skagerrak West (Høvåg)	58.15	8.27	Florian Berg	Bekkevold et al. (2023), IMR (Norway)	Fuentes Pardo et al. (unpublished)

Description	Code	Location baseline samples	Lat	Long	Contact person	Genotype method/panel	whole-genome resources in
Landvik Spring spawning	her_lndv_sp	Landvikvannet	58.32	8.50	Florian Berg	IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
North Sea Autumn spawning	her_ns_au	Banks, East of England	54.13	0.28	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
North Sea Autumn spawning	her_ns_au	Banks, East of England	55.67	-0.53	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
North Sea Autumn spawning	her_ns_au	Banks, East of England	54.2	3.3	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	
North Sea Autumn spawning	her_ns_au	Buchan, Scotland	57.22	-0.45	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
North Sea Autumn spawning	her_ns_au	Orkney, Scotland	59.39	-2.38	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
North Sea Autumn spawning	her_ns_au	Orkney, Scotland	58.63	-3.47	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
North Sea Autumn spawning	her_ns_au	Cape Wrath, Scotland	58.62	-4.40	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
North Sea Autumn spawning	her_ns_au	Northwest Cape Wrath, Scotland	58.67	-5.38	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Fuentes Pardo et al. (unpublished)
North Sea Spring spawning	her_ns_sp	Eastern North Sea	59.06	5.19	Florian Berg	IMR (Norway)	
Southern North Sea Winter spawning	her_down_wi	Eastern English Channel	50.18	-0.39	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	

Description	Code	Location baseline samples	Lat	Long	Contact person	Genotype method/panel	whole-genome resources in
Southern North Sea Winter spawning	her_down_wi	Southern Bight (Downs)	51.63	1.68	Dorte Bekkevold	Bekkevold et al. (2023), IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
West of Scotland Spring spawning	her_wos_sp	The Minch, Scotland	58.28	-5.49	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
West of Scotland Spring spawning	her_wos_sp	The Minch, Scotland	57.81	-5.87	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	Bruckless Bay, Donegal, winter spawning	54.61	-8.41	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Fuentes Pardo et al. (unpublished)
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	Drumanoo Head, Donegal, winter spawning	54.61	-8.49	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	Glen Head, Donegal, winter spawning	54.65	-8.78	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	Lough Foyle, Donegal, winter spawning	55.16	-7.04	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	Lough Swilly, Donegal, winter spawning	55.12	-7.49	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	Teelin Bay, Donegal, winter spawning	54.63	-8.63	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	Teelin Bay, Donegal, winter spawning	54.61	-8.41	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076

Description	Code	Location baseline samples	Lat	Long	Contact person	Genotype method/panel	whole-genome resources in
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	Limeburner, Donegal, spring spawning	55.31	-7.75	Edward Farrell	MultiFishSNPChip_1.0 (IdentiGEN)	
Northwest of Ireland Winter/Spring spawning	her_irlNW_wi_sp	West of Donegal, spring spawning	54.95	-9.02	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
Irish Sea Autumn spawning	her_irs_au	Douglas, Isle of Man	54.06	-4.45	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Irish Sea Autumn spawning	her_irs_au	Douglas, Isle of Man	54.06	-4.45	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Irish Sea Autumn spawning	her_irs_au	Portavogie, Northern Ireland	54.41	-5.28	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
Irish Sea Autumn spawning	her_irs_au	Mourne, Northern Ireland	54.04	-5.97	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	
Celtic Sea Winter spawning	her_celt_wi	Llyn North, N Wales	52.88	-4.67	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	
Celtic Sea Winter spawning	her_celt_wi	Aberdaron, Cardigan Bay	52.80	-4.71	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	
Celtic Sea Winter spawning	her_celt_wi	Aberystwyth, Cardigan Bay	52.40	-4.10	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	
Celtic Sea Winter spawning	her_celt_wi	Fishguard, Cardigan Bay	52.01	-4.98	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	
Celtic Sea Winter spawning	her_celt_wi	Hell's Mouth, Cardigan Bay	52.82	-4.60	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	

Description	Code	Location baseline samples	Lat	Long	Contact person	Genotype method/panel	whole-genome resources in
Celtic Sea Winter spawning	her_celt_wi	Knockadoon, S Ireland	51.84	-7.86	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
Celtic Sea Winter spawning	her_celt_wi	Baginbun, S Ireland	52.17	-6.83	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	
Celtic Sea Winter spawning	her_celt_wi	Dunmore East, S Ireland	52.09	-6.88	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Fuentes Pardo et al. (unpublished)
Bristol Channel Autumn/Winter spawning	her_bc_au_wi	Minehead, Bristol Channel	51.21	-3.47	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	Fuentes Pardo et al. (unpublished)
Bristol Channel Autumn/Winter spawning	her_bc_au_wi	Clovelly, Bristol Channel	51.00	-4.40	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	Fuentes Pardo et al. (unpublished)
Bristol Channel Autumn/Winter spawning	her_bc_au_wi	Swansea Bay, Bristol Channel	51.59	-3.84	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	
Bristol Channel Spring spawning	her_bc_sp	Freshwater East, Bristol Channel	51.64	-4.86	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	Fuentes Pardo et al. (unpublished)
Western English Channel Winter spawning	her_wch_wi	Mevagissey	50.28	-4.70	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	
Milford Haven Spring spawning	her_mlfh_sp	Milford Haven	51.73	-4.89	David Clarke	MultiFishSNPChip_1.0 (IdentiGEN)	Fuentes Pardo et al. (unpublished)
Northeast Atlantic Autumn spawning	her_nea_au	Icelandic summer-spawning herring (ISSH)	63.75	-16.38	Guðmundur J. Óskarsson	MFRI (Iceland), IMR (Norway)	Kongstovu et al. (2022) https://doi.org/10.1016/j.fishres.2022.106231
Northeast Atlantic Autumn spawning	her_nea_au	Lofoten, Norwegian autumn-spawning herring (NASH)	68.15	14.65	Florian Berg	MFRI (Iceland), IMR (Norway)	

Description	Code	Location baseline samples	Lat	Long	Contact person	Genotype method/panel	whole-genome resources in
Northeast Atlantic Autumn spawning	her_nea_au	Faroes autumn-spawning herring (FASH)	61.02	-6.38	Dorte Bekkevold	Bekkevold et al. (2023), MFRI (Iceland), IMR (Norway)	Kongstovu et al. (2022) https://doi.org/10.1016/j.fishres.2022.106231
Norwegian Spring spawning	her_nor_sp	NSS spawning survey	67.12	11.74	Florian Berg	IMR (Norway), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076; Kongstovu et al. 2022
Norwegian Spring spawning	her_nor_sp	East of Shetland, Scotland	60.72	0.02	Edward Farrell	Farell et al. (2022), MultiFishSNPChip_1.0 (IdentiGEN)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Norwegian Spring spawning	her_nor_sp	North Sea (Haugesund/Karmøy)	59.35	5.19	Dorte Bekkevold	Bekkevold et al. (2023)	
Norwegian Spring spawning	her_nor_sp	North Sea (Telavag)	60.2	4.98	Florian Berg	Bekkevold et al. (2023)	
Norwegian Spring spawning	her_nor_sp	North Sea (Askoy)	60.57	5.00	Florian Berg	Bekkevold et al. (2023)	Fuentes Pardo et al. (unpublished)
Norwegian North Sea fjords	her_norfj_sp	Gloppenfjorden	61.78	6.17	Florian Berg	IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Norwegian North Sea fjords	her_norfj_sp	Lindas	60.73	5.13	Florian Berg	IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Norwegian North Sea fjords	her_norfj_sp	Lustrafjorden	61.34	7.37	Florian Berg	IMR (Norway)	Han et al. (2020) DOI: https://doi.org/10.7554/eLife.61076
Norwegian North Sea fjords	her_norfj_sp	Sognefjorden	61.11	6.38	Florian Berg	IMR (Norway)	
Hybrids of Atlantic and Pacific herring	her_pachy_sp	Balsfjorden	69.18	19.22	Florian Berg	IMR (Norway)	Petterson et al. (2023) https://doi.org/10.1093/gbe/evad069

Description	Code	Location baseline samples	Lat	Long	Contact person	Genotype method/panel	whole-genome resources in
Hybrids of Atlantic and Pacific herring	her_pachy_sp	Rossfjordvannet	69.32	18.27	Florian Berg	IMR (Norway)	
Sykkylven Autumn spawning	her_sykk_au	Sykkylven	62.40	6.56	Florian Berg	IMR (Norway)	
Trondheimsfjord Spring spawning	her_thf_sp	Trondheimsfjord	63.80	11.00	Florian Berg	IMR (Norway)	
Total	**26**						

2.2 Genetic SNP panels currently used

During WKSIDAC2 several SNP panels were presented, which are currently used for population identification of Atlantic herring. There is a relatively large overlap between the different SNP panels enabling a certain level of comparative studies of resolution.

1. Bekkevold et al. (2023): A panel consisting of 59 SNPs (out of 96 SNPs genotyped per fish) is applied in the Danish part of the Herring Acoustic Survey (HERAS) and Danish commercial catches since 2019 focusing on populations in the North Sea-Baltic Sea area.
2. Farrell et al. (2022): A panel of 45 SNPs that is capable to discriminate between the herring populations that spawn northwest of Ireland in winter (her_irlNW_wi_sp) and north of Scotland in autumn (her_ns_au). The panel cannot currently distinguish between the 6.a.N spring-spawning herring (her_wos_sp) or the herring that spawn northwest of Ireland in the spring (her_irlNW_win_sp). This is currently applied (2014-onwards) to discriminate populations during the Malin Shelf Herring Acoustic Survey (MSHAS; ICES, 2023) and the commercial fishery in 6.a.S. It has previously been applied to herring sampled during the Scottish West Coast groundfish survey (SWC-IBTS), The Irish Groundfish Survey (IGFS) and the Spawning Herring Acoustic Survey in Division 27.6.a (6aSPAWN) (see Farrell et al., 2021). Since 2022 the MSHAS and 6.a.S commercial samples have been genotyped with the MultiFishSNPChip_1.0 array and only the 45-SNPs used for assignment, whilst the remaining data us archived for use in a future universal assignment model.
3. MFRI, Iceland: A panel of 120 SNPs to identify herring populations in the Norwegian Sea and adjacent waters.
4. IMR, Norway: A panel of 76 SNPs to discriminate populations in the North Sea, Norwegian Sea, along the Norwegian coast, and inside Norwegian fjords. However, in contrast to Bekkevold et al. (2023), the number of SNPs to discriminate among populations within the Baltic Sea was reduced to only identify Baltic vs non-Baltic herring. This panel is applied in the Norwegian HERAS and Norwegian commercial catches since 2019 and 2021, respectively. Samples from the International Ecosystem Survey in the Nordic Seas (IESNS) in May and International Ecosystem Summer Survey in the Nordic Seas (IESSNS) in July since 2019, both in the Norwegian Sea, have been genotyped, but not implemented in their assessment.
5. SLU Sweden: a panel of 73 SNPs from an initial selection by Bekkevold (DTU) has been used to identify herring populations in the Q1,3 International Bottom Trawl Survey (Q1,3 IBTS) and Swedish commercial catches since 2022.
6. IdentiGEN (Andersson et al., 2024): A MultiFishSNPChip_1.0 array including >4,000 SNPs of herring to discriminate populations throughout the north-east Atlantic. This panel has been used by SLU (Sweden) to genotype herring. The array has also been used to genotype all of the baseline and mixed Irish Sea, Celtic Sea and Bristol Channel herring samples analysed by Swansea University to date and also baseline samples from 6.a and 4.a, 4.b, 4.c and 7.d from Farrell et al.(2021).

2.3 Outstanding issues

2.3.1 Missing markers to discriminate populations

The presented studies have demonstrated that genetic analysis is a powerful tool to identify herring populations across the north-east Atlantic. However, examples were also shown where current markers are not able to discriminate expected populations. An example is the genetically

identified cluster of North Atlantic summer/autumn spawners (her_nea_au) consisting of spawning samples of summer spawners from Iceland (ISSH), autumn spawners from Norway (Lofoten, NASH) and autumn spawners from the Faroese Islands (FASH). These potentially distinct groups were not included in the previous whole genome-sequencing. Therefore, it is not surprising that differentiating markers have not been selected on the current panels. There is an urgent need to undertake pooled whole genome sequencing (Pool-Seq) for Icelandic, Faroese, and Norwegian autumn spawners and add their data to the existing Pool-Seq data maintained by Uppsala University. This will enable confirmation of whether or not these are distinct populations of herring and if so, should also allow identification of informative markers for each. í Kongsstovu et al. (2022) found low, but statistically significant, levels of genetic separation between ISSH and FASH samples and this needs to be further investigated before genetic population identification can be fully implemented in the Norwegian Sea and adjacent waters.

2.3.2 Missing populations in a baseline and robustness of panels

None of the presented studies has used a baseline including all identified populations. Potential consequences of missing populations in a baseline were discussed during the workshop, but further analyses are needed to verify the consequences. For example, it has been discussed to only assign fish to a given population if the assignment rate is above a certain threshold. It might be expected that if a population is missing in the baseline, the overall assignment accuracy should be lower for individuals of that population. Again, these are theoretical assumptions that need to be tested. A similar issue was discussed for the robustness of panels with selected makers. All local panels have selected markers for specific areas/populations. Using the MultiFishSNPChip_1.0 array across institutes might help to align genetic assignments. Whilst there has been significant progress in the development of area and population specific genetic assignment models, both to the west of Ireland and Britain and in the western Baltic and North Sea areas, there are unresolved questions of what assignment models to use where these areas meet. If the baseline used to develop a specific assignment model does not contain baseline samples for a potential population, then individuals from this population will be assigned to the most genetically similar population. This could lead to errors in the assignment of mixed survey and commercial samples. To avoid this issue, subjective area-based decisions on where to use particular assignment models could be applied or more appropriately a universal assignment model, incorporating all potential populations in a single model, should be considered. Work is ongoing to develop an exploratory universal assignment approach for the herring populations around Ireland and Britain. However, in general all the genetic methods applied to date offer a much-improved accuracy and precision on population splitting data and pave the way for implementing population-specific data into stock assessment.

2.3.3 Other issues

A clear outcome of the workshop is that we can now identify genetic herring populations, however, several of them cannot be uniquely assigned to any a specific stock (management unit). Allocation of individuals to a stock is currently not always possible because there are several identified populations which are not included in the present stock definitions.

Such an example is represented by autumn-spawning herring from the Baltic Sea (her_balE_au) outside the Gulf of Riga, and to date it is ambiguous if these autumn spawners should be allocated to the western Baltic stock (her.27.20-24) or the central Baltic stock (her.27.25-2932), which are both dominated by spring spawners, or if they should represent a separated unit.

Another example are the spring-spawning herring from the North Sea (her_ns_sp), where currently the spawning grounds and their population dynamics are unknown, and spawning

individuals have not been sampled. Following today's methods, they are allocated to the North Sea stock (her.27.3a47d) when splitting survey data and commercial catches. In the case where this population is not part of the baseline it will most likely be allocated either to the western Baltic stock (her.27.20-24) or the Norwegian Sea stock (her.27.1-24a514a).

In the opposite case, the populations North Sea autumn-spawning herring (her_ns_au) and Downs winter-spawning herring (her_down_wi) are genetically highly distinct and can easily be classified in scientific survey and commercial catch data with appropriate sampling. However, they are both combined within the assessment of the North Sea stock (her.27.3a47d), without knowing the individual dynamics of these populations, and treated as a single stock unit.

Further, for the assessment of the North Sea stock (her.27.3a47d) and the western Baltic stock (her.27.20-24), all identified biological populations are assigned to one of these two stock mimicking traditional methods such as otolith microstructure or mean vertebral counts (see ICES, 2017 for details). Thus, at present, individuals that are confirmed genetically to be from the same population are assigned to different stocks because of this adherence to historical methods.

At present, we lack an understanding of the life cycle for many of these herring populations. Ecological differences should be interpreted in the light of the population diversity presented by the genetics. Moreover, resolving the migratory patterns and distribution in space and time and throughout the ontogeny of the different populations would help to address the issue of stock allocation and could potentially lead also to a revision of the present stock definitions.

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3 ToR b: Analysis of the optimal baseline requirements for stock assessment purposes, both for specific survey as well as commercial catches

3.1 Principles

In order to assess the proportions of natal populations in both scientific surveys and commercial catches, genetic baselines are required which include all likely populations which will be exploited in the area in question. By definition, a baseline sample is a sample of fish that is representative of the local biological (reproductive) unit. In practice, this entails samples of spawning or near spawning (Maturity stage 3 on ICES 6-point scale) herring collected at spawning time on or adjacent to known spawning grounds. The use of genetic tools requires a systematic approach to the collection of baseline samples and baseline samples are needed from all populations across the Northeast Atlantic area.

It is clear from many studies that genetically (biologically) discrete populations exist with separation determined by a combination of geography and season. Different populations spawn in autumn, winter, and spring in most areas, with differentiation between autumn and winter populations in some cases. Analysis of data from several studies (Farrell et al., 2021;2022; Bekkevold et al., 2023) suggest that subtle changes in genetic composition may occur, even within a spawning season in the same area. Therefore, to reflect the full genetic diversity within a population in a baseline sample, multiple samples are needed from different times across the spawning season, and from several geographic locations within the area the population spawns in, rather than a single large sample taken from a single location at a single time. Baseline samples should also be collected across different years to verify temporal stability, ensuring that progressive year classes are sampled over years to, assignments of year classes should be sufficient to detect temporal drifts or abrupt changes.

These principles underpin the recommendations in this section.

3.2 Sampling requirements

The study group discussed a number of key factors that need to be considered. These include:

- The nature of samples, e.g., maturity state
- Geographic and temporal distribution of samples
- The number of samples required to underpin assignment models
- Tissue types, Sample handling and storage
- Genetic processing
- Analytical tools

The following recommendations are intended as an initial starting point, for individual countries or laboratories to build into their sampling programmes. They focus on baseline samples (though methods and metadata requirements for potentially mixed population survey and commercial catch samples would be similar).

3.3 Sample sources for baselines

Generally, mature adult fish should, and have been used for this purpose. Ideally these are taken in the immediate vicinity of spawning grounds, and where practicable we recommend the use of fish which are actively spawning. Realistically that is not always practical, and near spawning 'prepared to spawn' fish have been used in many baseline samples. These can be considered viable baseline samples if they are captured alongside spawning fish. For example, in the stock identification of herring in 6a, 7b-c, only individuals of maturity stage 3 on the 6-point scale were included in the baseline analysis (Farrell et al., 2021). Macroscopic maturity staging can be unreliable (ICES, 2011) and even with strict criteria regarding the use of baseline samples, misclassification can occur. It is important that the maturity scales used by different institutes are documented and are comparable. However, transient migrants *en route* to other spawning locations may also be captured in mixture with local spawning contingents and sampling designs should seek to minimise this risk, e.g., by maximising sample sizes and using temporal replicates.

Samples may be collected from a range of sources including research and commercial vessels undertaking fisheries surveys, target commercial fisheries or opportunistic bycatch. This will depend on local circumstances and availability. The only requirement is that the samples are preserved in optimum condition prior to collecting the genetic samples. To this end genetic samples are ideally collected immediately upon capture of the fish. If this is not possible then samples may be chilled for a number of hours before returning to port e.g., on ice or on pelagic vessels in Refrigerated Sea Water (RSW) tanks. Samples may also be frozen prior to collecting genetic samples. Yolk-sac or early-stage larvae could also be considered as baseline samples. Sampling larvae for use as baselines offers additional sampling opportunities within close proximity to spawning grounds. Larval samples have also been shown to assign to the expected groups (Davies et al., 2020; Bekkevold et al., 2023).

The group also recognised that in some instances, fish which are not in spawning condition may be of known origin, because of specific local knowledge. Expert judgment can be applied, and such samples could also be considered as baseline samples, provided a clear rationale for their inclusion is available, linking them to the spawning population they are expected to represent.

3.4 Metadata

The following data should be collected for each adult /fish used for baseline samples. Age, length, weight, sex, spawning condition (specify maturity scale used), capture location (latitude and longitude). If larval samples are used, individual length and development stage (e.g., yolk sac) should be recorded, in addition to capture location. The location of the sample is very important as its proximity to spawning grounds provides additional spatial information. Samples need to be representative of the populations of interest.

Similar data should be collected for potentially mixed population survey and commercial catch samples which are going to be genetically assigned to population of origin. Ideally samples would include accurate grid references, but we recognise that may not always be possible for commercial catches.

3.5 Tissue types, sample handling and storage

Sampling for genetics in individual fish should follow current best practices regarding extraction, storing, and avoiding cross-contamination of samples to ensure the highest possible quality. Both baseline and mixed population genetic samples require the collection of biological material from individual fish. Fish selected for sampling should follow a pre-determined sampling

protocol. The protocol should ideally account for population and age distributions, cohort strength, sampling variability and the effect on subsequent survey indices, stock assessments and biological advice. Detailed protocols are required to prevent cross contamination between individual samples and to ensure high quality samples are collected, even though it seems to be a minor issue in presented studies so far.

For adult samples we recommend tissue or fin clip samples taken from each fish. Storage should be in molecular grade 95-100% ethanol. If larvae are used, they should be washed in alcohol before storage and stored in individual tubes. Both fin and muscle tissue may be used for genetic analyses, but fin tissue is more susceptible to short term degradation if samples are not processed immediately upon capture. Fin tissue and muscle samples which contain some skins are also more prone to surface contamination. Therefore, it is preferable to collect a tissue sample from beneath the skin of the fish being sampled, thus avoiding surface contamination. Adequate preservation and tracking of the collected samples must also be ensured, particularly for those samples that may be held in archive for future analyses. Any tissue sampling protocol should aim to minimise the time and effort needed per individual fish due to the number of other biological parameters that must be recorded and the large volume of fish that are processed in a typical survey or port sampling event. One approach that addresses these needs is the genetic sampling tool (GST), which utilises individually barcoded, screw-top vials and a manual decapper that is operational with one hand. A full description of the tool and a sampling protocol is presented in Annex 6. Briefly, the cap of each pre-filled vial incorporates a barb or window that collects an ideal amount of tissue (c. 30 mg) when it is inserted into the muscle of the fish. A specialised sampling tool/decapper means that the cap can be opened, inserted into the fish, and returned to the vial in one motion without the sampler making direct contact with the sample tube or sampling tool, Figure 3.5.1. This greatly reduces the chances of cross-contamination as the need for decontaminating blades between specimens is eliminated. Barcodes on both the racks and the individual vials mean the samples can be reliably traced from collection through to sequencing.

Collection of genetic samples on scientific surveys generally occurs very soon after the fish have been caught, so degradation of DNA due to handling times and method of preservation is usually not an issue. However, for specimens being taken from the catch of commercial fishing vessels, such as port-based sampling events, this may need to be considered. For example, some commercial samples will have been in a holding tank for a length of time before being landed and genetically sampled, others are frozen and then thawed, and others still may be left overnight before freezing. Certain batches of samples in the past have had a high error rate and commercial samples seemed to show lower quality, which may reflect their handling before sampling. To investigate if there was a need to determine a quality cut off point after which a sample is not worth genotyping, the Marine Institute and EDF Scientific ran a small treatment experiment in 2021. A brief description of the methods and results are presented here.

The experiment aimed to look at different handling treatments of herring after being caught and to investigate what effect this has on the quality of the genetics collected from these fish. Six different treatments were investigated here, including sampling intervals and thawing methods.

The treatments were:

- Thawing duration – fish frozen immediately and then thawed and sampled at intervals
- Thawing method – fish frozen immediately then thawed using either hot or cold water
- Sampling interval – fish left on deck with no ice and sampled at intervals
- Sampling interval – fish left on deck with ice and sampled at intervals
- Frozen after time – fish left on deck with no ice, then frozen at intervals and thawed for tissue samples

- Frozen after time – fish left on deck with iced, then frozen at intervals and thawed for tissue samples

Twenty-five herring in total were randomly assigned between each of the treatments and standardised genetic tissue samples were taken (using the GST described above) at intervals of 2, 4, 8, 12, 18 and 24 hours. All genetic samples were genotyped by IdentiGEN for the 45 SNP panel used in the discrimination of 6a herring. The results showed that almost all the samples worked well. Only one individual had less than 80% of the genotypes and the majority were over 89% (40/45 successful genotypes), which is the threshold for baseline samples. No patterns were apparent within treatments in terms of the return of genotypes. Further statistical analysis is required but everything suggests that sample quality is not adversely affected by storage and handling in the 24 hours prior to tissue collection. Previous issues may therefore have been caused by cross-contamination, something which has been alleviated by the adoption of the GST.

Figure 3.5.1. An extracted Genetic Sampling Tool (GST) showing the tissue sample retained within the sample collection window. See Annex 6 for further illustration and operating procedure.

3.6 Baseline sample sizes

For a sample to be considered a baseline sample, it needs to be representative of the biological unit, i.e., spawning population that it is expected to represent. During the workshop we did not conclude any specific number for the baseline sample size. The baseline sample size is specific to areas and depends on the number of possible populations in that area. This is often the unknown when starting baseline sampling. The same resolution of sampling is not needed in all areas. Additional effort may be required to monitor smaller populations where mixing occurs and population sizes vary (Hintzen, et al., 2015). Ideally baseline samples would be taken over at least two seasons, with ongoing checks to update baselines over time, to account for any drift in genetic composition over time. We note that not all samples have to be processed. Once the sample is fixed in alcohol, storage is straightforward (and low volume) so collecting and storing surplus samples which can be used later has relatively low cost. Over-sampling is therefore preferable to under-sampling.

3.7 Commercial catch sample sizes

Sampling programme should be developed in conjunction with the existing catch sampling programme to ideally result in a genetic assignment for each aged fish used in the assessment. This applies equally to survey samples. The number of samples and their distribution is beyond the scope of WKSIDAC2 but should be considered by WKSIDAC3.

3.8 Genetic processing

As described in detail in ToR a, different laboratories have used different marker panels in developing assignment models for different areas. No single processing method currently accounts for all markers, with the most extensive coverage provided by the MultiFishSNPChip_1.0 array. There is clear longer-term advantage in moving to a common genotyping approach, probably based on the MultiFishSNPChip_1.0 array, but this will only be practical if the existing array can be developed to include key markers from all areas, and to further include markers for populations which remain difficult to resolve with current panels. Work is ongoing to update the array to include missing markers, identify additional markers for poorly resolved populations, and move toward a more comprehensive marker set. We support the need to address that issue quickly.

3.9 Analytical tools and approach

There are several packages available for analysing these data including RUBIAS, ASSIGNPOP and GENECLASS2, combinations of these and other bespoke work including some commissioned by the MI with IdentiGEN. Whichever package is used, to optimise resolution, some hierarchical or stepwise analysis may be needed, identifying, and removing seasonal or geographic populations from analysis and then focussing on use of markers which better characterise the remaining fish. This work is ongoing through the Universal assignment approach and needs to be developed further.

3.10 Baseline samples for stock assessment

Robust baseline samples for individual populations across multiple years are required if population identification methods are going to be incorporated in stock assessments. If these baseline

samples achieve high levels of assignment accuracy, they can then be used in assigning unknown or mixed samples back to their population of origin. Data analysis is carried out using the agreed assignment approaches. Work is ongoing to develop a universal assignment model. Following on from this analysis it is possible to split either survey indices or catch data into the different populations. This data can then be used in stock assessments, either full analytical assessment models or assessment methods that use indicators of stock size.

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4 ToR c: Outline a general description of prerequisites) for the implementation stock identification of herring

With the recent advances in genetic analyses of fish, it is becoming evident that stock areas used in assessments are often not aligned with biological populations and often comprise a mixture of fish from different populations. Understanding and forecasting population dynamics, including recruitment, are central to fisheries stock assessment and subsequent advice. Therefore, if a stock complex is assessed and managed as a single homogeneous biological population, the resulting scientific advice will be biased, and can lead to overfishing some populations while being over-precautionary for other populations (e.g., Stephenson 1999, Ying et al. 2011, Kerr et al. 2016, Cadrin 2020, Morse et al. 2020).

While genetic assignment analyses to resolve population mixing is partly implemented for some herring stocks already, several prerequisites need to be met for a full implementation of population identification of herring. As described under ToR b, a reliable baseline collection of representative genotypes from the different herring populations is the foundation for implementing population identification. In turn, protocols for routine collections of genotype data from scientific surveys and commercial catches should be in place before population identification of herring can be implemented in survey indices and assessments.

As a prerequisite for implementing routine data collections for population identification based on genetics, sampling strategies, such as length stratified or random sampling, should be investigated and acceptable sample sizes should be determined. While the general considerations are similar to the currently implemented sampling programs for other biological parameters, sampling variability, within-haul correlation, misclassification risk, and effect on survey index and assessment output confidence intervals and estimates will be specific to genetic samples and vary by area. In turn, these theoretical considerations should be aligned with practical considerations during sampling operations when accounting for splitting populations.

4.1 Implementation in survey sampling

The implementation of population identification of herring in sampling programs from scientific surveys must be aligned with current sampling protocols. These will vary by survey type i.e., acoustic or groundfish, from survey to survey within type and even within a single coordinated survey such as the HERAS, which employs different sampling methods in different parts of the survey. Therefore, the genetic sampling protocols must be adapted to fit within a wide variety of survey sampling types. Further the number of fish to genetically sample per haul will be dependent on how the analysis of the survey data is undertaken in order to derive an age-based index. Converting the number of winter rings in the otolith to a corresponding age depends on the population origin of the fish; in particular, the spawning period. Therefore, it may be an advantage to genetically assign each aged fish for use in age-based herring assessments, while this is not a concern for winter-ring-based assessments, such as the North Sea autumn-spawning (her.27.3a47d) and Western Baltic spring-spawning (her.27.20-24) herring assessments. In general, it may also be more cost efficient to genetically assign a subset and rely on population-winter ring keys in the estimation procedures of catches and survey indices, similarly to the frequently used age-length keys. The optimal strategy will depend on the trade-off between

analysis costs and additional information by increasing sample sizes, which should be statistically investigated further.

As a prerequisite for successful integration of protocols, case-specific information on sample numbers, most urgent areas and populations, as well as best practice for sample storage, practice, materials and sampling tools are needed. Further, the sample strategy considerations should account for the time available for sampling in combination with the current sampling program, as well as funding needs.

To ensure a cost-effective genetic sampling program, the design of a sampling program should consider the optimal allocation of samples between hauls. Often within-haul correlation is seen, such that fish from the same haul are more likely to be from the same age-group, length-group, or population than fish from different hauls. This correlation will limit the added value of additional composition samples from the same haul after a certain number of samples. Likewise, the sample program should incorporate the best available knowledge about the spatial distribution and mixing of herring populations. However, the programs should allow for continued testing and updating of population distribution assumptions.

4.2 Implementation in commercial sampling

The prerequisites for implementing population identification of herring in commercial sampling programs are similar to those for survey sampling. Further, protocols should ensure that the sampling covers different metiérs to be representative of the catches. Due to the granularity in landings and multiple catches constituting a haul, exact haul locations are often not available. Since the spatial distribution is important for accurately raising population compositions to areas, the preferable sampling protocol, where possible, is to collect genetic samples from onboard sampling or sample retention programs. Likewise, fishery self-sampling can allow for fine scale sampling of, for example, specific hauls.

4.3 Implementation in survey and assessment models

Population identification can be implemented in survey and assessment models with different levels of complexity. In the simplest approach, individual classifications of population origin are used to split input data before the estimation of survey indices or assessments. At the other end of the complexity scale, individual genotypes can be incorporated in integrated models. Irrespective of the model complexity, the implementation of population identification in survey index and stock assessment models must account for the risk of misclassification as well as the sampling scheme. If individual classifications are not 100% accurate, population compositions calculated from the number assigned to each population will be biased. The bias increases with the misclassification rate but can be corrected for when known. Therefore, classification accuracy should be quantified and accounted for in the analyses. Further, and similar to other biological information, stratified sampling schemes must be accounted for to avoid biased results.

Understanding, estimating, and forecasting fish population dynamics requires historical data. Therefore, there may be a transition period where the implementation of population identification of herring is limited by the length of genetically accurate time-series. Therefore, methods for correcting historical time-series may be considered. Alternatively, the minimum time-series length of, for example, a survey index needed before integration in assessment, together with the consequences of changing assumptions about population mixing, should be investigated.

To facilitate the implementation of population identification of herring in survey index estimation and stock assessment models, digitized samples should be stored and become available to researchers in relational databases with links to biological and sampling information. Preferably,

genetic information should be stored at genotype levels, since individual classifications will be a model output that depends on the baseline and classification method used. Additionally, assignment outputs based on a particular baseline should be stored with the raw genotype data to enable comparison once baselines have been updated and assignment models reconfigured.

4.4 References

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5 ToR d: Provide guidance on a retrospective correction of herring survey and catch time-series where necessary

The objective of a retrospective change of assessment input data would be to sort out populations which do not belong to the specific stock and adapt survey indices back in time. This could improve the internal consistency in assessment models because single populations could be followed over time rather than a mixture of different populations. In order to modify both survey and catch time-series back in time, time-series of population assignments need to be available and methods for the revision need to be transparent and reproducible.

At the WKSIDAC2 meeting, recent developments in genetic population analyses were presented. The results are mostly based on individual genetic population assignments. They mirror the occurrence of different populations in an area at a given time quite well (see sections 2 and 3). The contribution of these populations to the overall stock quantity can, however, still not be given due to a lack of modelling methods. These stock-specific quantities are an important pre-requisite before the development of population-based time-series.

In an historic perspective, it is very important that the population's distribution has been monitored adequately over time. Otherwise, interannual variation in the separation of populations may be hard to track. To be able to follow the proportion of different populations back in time, a mixture of different methodologies could be applied to cover years for which genetic samples are not available. Well-established methods, such as otolith microstructure and/or vertebrae counts combined with spatial modelling may help in this context. When introducing genetic data collection, both methods should be applied in combination for at least 2-3 years to ensure comparability. There is a need for further investigation as to which kinds of models and statistical analyses are useful for detecting systematic changes in the distribution of populations over time.

Many of the aspects discussed under ToR d are very case-specific and depend on the available data in that area. Importance and feasibility of index adaptation or alteration have to be prioritized. Additionally, prior to adapting and adopting time-series back in time, the possible impact on the assessment should be analysed due to the high effort for data submitters and stock coordinators involved in altering the database and subsequent processes.

The overall agreement of the group was that the development of population-based time-series for upcoming years should start as soon as possible and a spatially-resolved internationally standardized data collection should be implemented. For the time being, changes to historic survey and catch time-series may be regarded as of minor importance due to a lack of available representative data and stable and reproducible methods.

6 Roadmap/Way forward

The roadmap for moving forward has been brigaded into four general themes. 1. Population identification, 2. Surveys and sampling, 3. Universal assignment panel/model, and 4. Database and sample storage (see Table 6.1). This Table outlines the agreed set of research needs and actions discussed during the Workshop, roughly ordered by priority, and including an indicative timeline. There is a greater likelihood that those of higher priority and shorter deadlines will occur simultaneously, and dependencies on other research items are identified. This table is by no means final and should be updated when necessary. The table also includes a timeline with years. The Milestones are years when updates should be available, and completion years are when an update report or completion report should be provided.

Currently, there are a number of populations targeted for further work. These include populations in the Celtic Seas (6aS spring included, western English Channel), Icelandic summer-spawning herring (ISSH), Faroese autumn-spawning herring (FASH), Norwegian autumn-spawning herring (NASH), Thames/Blackwater herring (TBW), herring on the West Coast of Sweden and a number of populations occurring in the eastern Baltic Sea.

One central issue with moving forward with all the work on the identification of herring and assigning them to populations to the level of being useful for stock advice and then management is the funding. This includes both the routine sample collection and the processing of the genetic material.

To take this Roadmap forward over the next three years, interested scientists and parties should meet in ICES Workshops: WKSIDAC3 2024, WKSIDAC4 2025 and WKSIDAC5 2026.

6.1 Population identification

It is apparent that currently there are gaps in the genetic information and there is a necessity for further genome sequencing to be undertaken (Table 6.1; 1.1). Further investigations need to be made to determine which populations need to be targeted for these studies. Allied to this, further sampling and analyses need to be targeted at spawning populations over a range of locations to compile a robust and comprehensive baseline for the identification and classification of all the major populations in the Northeast Atlantic (Table 6.1; 1.2). Examples include some of the spring-spawning fish that have been found in the northern North Sea and summer/autumn-spawning herring found in Icelandic, Faroese, and Norwegian waters.

Currently, there is evidence of temporal stability in the genetic markers used for the allocation of individuals to populations, however, this needs to be periodically examined to ensure the baselines are appropriate for use (Table 6.1; 1.3). It has been seen that genetically distinct populations occur in close proximity. Whilst this does not impact on any assignment results it is useful to understand the mechanisms which maintain any discreteness (Table 6.1; 1.4).

The current sampling and genetic investigations are uncovering a complex array of herring populations representing an unprecedented level of biological diversity not recognised in the ICES stock codes (Table 1.6; 1.5). There is a need to account explicitly for this population diversity by adding population codes into the ICES scheme. Allied to this follows a re-evaluation of the definitions of each of the stock codes and their relationship to population codes.

The recent genetic studies have been highlighting the existence of a multitude of distinct populations. To recognise this population diversity is central from a biodiversity perspective, and links to two main aspects: i) to understand how this population diversity plays in relation to the

resilience of the species and the stocks, ii) to harmonise stock definitions and operational needs of fisheries management with the high population diversity revealed by the genetics (Table 1.6; 1.6).

As the location of genetically identified individuals from each of the recognised populations is discerned then these data should be visualised on maps (Table 1.6; 1.7). Furthermore, the location of the various populations over all their life stages should be determined. This information is important for understanding the dynamics of the populations and the life cycle connectivity and closure.

The logical extension of these distributional maps is to make recommendations on where stock boundaries should be investigated for change (Table 1.6; 1.8).

6.2 Surveys and sampling

Genetics data need to be obtained from samples collected both from the commercial fishery and scientific surveys. In both cases the sampling protocols, i.e., stratification of the sampling, numbers of samples, etc need to be investigated and evaluated to determine appropriate levels and coverage (Table 6.1; 2.1). There is urgent need to develop and evaluate sampling designs for genetic data which integrate with current sampling programmes which will be considered by WKSIDAC3 in 2024. It may also be desirable to investigate extensions or minor alterations to surveys so as to obtain the required data coverage to inform population distributions.

6.3 Universal assignment panel/model

There is a need to decide on appropriate assignment panels or models that can be used by all the institutes using genetic identification of individuals and their assignment to populations (Table 1.6; 3.1). The objective is to get consistency across all laboratories assigning individuals to populations across the Northeast Atlantic. This should be under the auspices of ICES and considered a priority where data are incorporated into stock assessment and for scientific advice, and we foresee the need for a designated Workshop or Working Group to provide governance on this matter.

It is anticipated that the technology and understanding will advance with time, as such there will probably be ongoing refinements of a UAM. These advances should be referred back to the appropriate Workshop or Working Group (see above) for incorporation into any standard protocols that have been established (Table 6.1; 3,2).

6.4 Database and sample storage

There are a number of different institutions undertaking research on the genetic identification of individual herring and assignment to their parent population. Collectively these data are more valuable than isolated in national databases. The identification of a location for a central database, its curation and maintenance are vitally important for the scientific community to make progress (Table 6.1; 4.1). This also requires the definition of standard formats and data structures that could serve the multiple purposes of genetic data.

Collectively the institutes have samples that have been used for genetic analyses (part of the sample is often not used and can be used again at some later date) or have been collected for potential future use (Table 6.1; 4.2). Further samples are still being collected. There is a need for storage of these samples along with an inventory of what could be available from storage. Most institutions do not have adequate storage space for the long-term storage of these samples,

therefore there is a need to explore possible solutions. The long-term storage would allow for investigations of historical material and/or taking advantage of new analytical techniques or more advanced analytical models.

Table 6.1 Summary of themes and topics for a roadmap going forward for studies and collection of genetic data on herring, which will inform on the designation and delineation of stocks in the Northeast Atlantic.

Item	Description	Timeline		Notes
		Milestone	Completion	
1	Population identification			
1.1	Identify which populations (samples) need to be genome sequenced.		2024	These include present and new samples.
1.2	New baseline samples (including additional sampling).	2024	2025	Collection of samples of herring in spawning condition (running ripe) and/or relatively newly hatched larvae.
1.3	Address the potential issue of temporal stability.	2024	2025	Systematic resampling of herring in spawning condition from a selection of genetically identified populations for verification of the efficacy of baselines used for the assignments
1.4	Assess the underlying reasons for differences in adjacent populations.	2025	2026	Investigations for understanding the underlying reasons why adjacent populations maintain their integrity and remain genetically distinct.
1.5	Population codes/names.	2024	2024	Recommend new population codes (see Table 2.1) to be implemented in ICES databases. Determine whether the current ICES stock codes are sufficient for usage with the current and evolving suite of genetically identified herring populations.
1.6	Work with stakeholders (fishers) to determine locations of e.g., small-scale spawning locations. Furthermore, how to use information of small (still to be defined) populations e.g., growth rates etc.	2024	2025	There are a number of small herring populations that should be added to the baseline as these may appear in fisheries and surveys. These populations may create uncertainty in any assignments.
1.7	Expanding distribution maps to cover occurrences of the locations of each population over life stages etc.	2024	2026	Provide a map showing the locations of confirmed individuals for all of the identified populations. These maps should also give some indication of the abundances, at least relative to the overall population size.
1.8	Highlight where the potential revision of stock units based on populations may occur.	2025	2026	Make recommendations as to where stock boundaries should be examined with respect to any changes from the status quo.
2	Surveys and sampling			
2.1	Do changes need to be made to surveys and sampling techniques to answer our questions?	2024	2026	Determine whether the current surveys have sufficient spatial and temporal coverage to investigate the potential stock distributions. Consider current sampling techniques and investigate future sampling techniques for the efficacy in determining population distributions.
3	Universal assignment panel/model			
3.1	WKSIDAC Group responsible for data and baseline availability and updating the Universal Assignment Model (UAM).	2024	2025	Decision as to best practice for assignment panel/model for assigning individuals to populations. This is to be under the auspices of ICES and have a designated Workshop or Working Group to provide governance.

Item	Description	Timeline		Notes
		Milestone	Completion	
3.2	Any further issues with UAM?		2026	On going refinement of a UAM which can incorporate advances in the science underpinning the genetic identification of populations.
4	Database and sample storage			
4.1	Database for genetic samples/populations.	2024	2025	Investigate where a data base can be housed over the long-term. Matters such as format and access need to be considered.
4.2	Where to store physical samples for future use.		2024	Should this be centrally located, in a limited number of locations or the responsibility of each individual laboratory that collected the samples?

7 Recommendations

Throughout the duration of the Workshop, participants discussed additional topics and recommendations to investigate further in any subsequent workshops. The following list provides an overview of the main points from these discussions which may facilitate focus points in later meetings. These recommendations are made in the light of the focus of this Workshop – all herring in the north-east Atlantic:

1. Set up a subgroup to try to and develop a universal assignment model, initially for genetics and then incorporating other relevant characteristics.
2. Set up a study group to investigate sampling strategies for genetic information to inform on the allocation of individuals to their genetically identified population and integration of that information into the current and next generation of stock assessment models.
3. Analyse the currently available survey samples, especially those which will provide significant information on the location of individuals and their assignment to populations.
4. A group of experts (covering expertise on biology/survey/sampling/statistics/stock assessment) to assess the impacts of not accounting for multiple population data being incorporated into a single stock assessment.
5. Data management at the institute level: convene meetings to discuss present, future directions, and co-operation.
6. Promote publication, sharing and open access of baseline genetic samples, at least among institutes involved in the definition of the assignment models.
7. Convene a subgroup to work with ICES on the housing of genetic data in ICES databases.
8. Compile the historical and current information on spawning locations for all populations (widen to other information).
9. The compilation of distributional maps of each population and highlight gaps in the data coverage.
10. Alignment of stock units and populations with recommendations to ICES.
11. Reach out to the community working with other techniques/methodologies to enhance the population perceptions raised by the genetic studies (biological inference). Potential methods include:

    a. Otolith microstructure for historical data
    b. Otolith micro-chemistry (explanations and understanding)
    c. Fatty acids
    d. Stable isotopes
    e. Tagging

8 Conclusion

The analysis of genetic markers is becoming a widely applied and cost-effective tool for separating herring into biological populations and potential management units at accuracies greatly surpassing previously applied methods. Genetic population identification is currently used routinely for the classification of individuals captured in commercial fisheries and scientific surveys for stock assessment purposes in some but not all herring stocks. The scrutiny of available genetic data showed that at present 26 distinct populations can be identified in the north-eastern Atlantic Ocean. However, knowledge needs to be updated as additional samples improving geographical coverage of spawning units are progressively being analysed. Genetic tools used for the identification of herring populations in different management areas were identified during the workshop and their individual merits were discussed. These tools vary in terms of the number of genetic markers in the panels and the genome regions they cover. The unification of genetic techniques and the creation of a "universal assignment model" that allows for a consistent and accurate differentiation among herring populations throughout the northeast Atlantic are therefore suggested to be an asset. The creation of a universal assignment model is still in progress. To achieve a goal of access to a tool that is fully validated across populations and management areas, there is a need for evaluating the potential inclusion of missing populations, the necessity to undertake whole-genome sequencing of yet not analysed populations, and the identification of informative markers for all populations. Analyses can subsequently be tailored to address local operational management issues. To take full advantage of the increased biological information content in genetically analysed data, there is a need to ensure the appropriate quality of the samples and agree on a sampling protocol (these tasks are included in the ToRs of WKSIDAC3). Genetic data and sample repositories should follow general requirements under the Data Collection Framework (DCF) or equivalent, and metadata, including on the applied baselines, analysis protocols, tissue type, samples size and statistical approach used, should be fully transparent and accessible. To achieve functional information for stock assessment purposes, both the specific survey and commercial catches need to be analysed using fully developed methods, and the contributions of different populations in mixed catches need to be estimated on a basis of individual fish. Information on the contributions of individual populations is only partly incorporated into the current stock dynamics models necessitating model developments to accommodate this information. Further, and methods for correcting historical time series should also be considered. There is a need for further investigation as to which kinds of models and statistical analyses are useful for detecting systematic changes in the distribution of populations over time. The available tools for the population identification of herring and assigning them to populations within a framework useful for stock advice and management have in recent years transformed from an emergent to a mature stage although further fine tuning and operationalization of genetic tools is expected for specific management applications.

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Annex 2: Resolutions

WKSIDAC2 - Second Workshop on Stock Identification and allocation of catches of herring to stocks

2022/2/FRSG42 A **Second Workshop on Stock Identification and allocation of catches of herring to stocks (WKSIDAC2)** chaired by Richard Nash, UK, and Florian Berg, Norway, will meet at ICES HQ in Copenhagen, Denmark, 19–23 June 2023 (start and end at 13:00), to:

a) Review recent status of genetic stock identification for herring and outstanding issues affecting identification accuracy/success
b) Analysis of the optimal baseline requirements for stock assessment purposes, both for specific survey as well as commercial catches
c) Outline a general description of prerequisites for the implementation of stock identification of herring
d) Provide guidance on a retrospective correction of herring survey and catch time-series where necessary

WKSIDAC2 will report by 15 August 2023 for the attention of ACOM and WGBIOP.

Key stocks to focus on:

Stock / component	Abbr.	Area
Icelandic Summer Spawning Herring	ISSH	27.5.a
Norwegian Spring Spawning Herring	NSSH	27.2.a
North Sea Autumn Spawning Herring	NSAS	27.4, 27.3.a, 27.7.d
Downs Winter Spawning Herring		27.4.c,27.7.d
Western Baltic Spring Spawning Herring	WBSS	27.3.a, etc.
Central Baltic Herring	CBH	27.3.d
Irish Sea Herring	NIRS	27.7.aN
Celtic Sea Herring	CSH	27.7.aS, 7.g-h, 7.j-k
6a North Autumn spawning herring		27.6.aN
6a South 7bc herring		27.6.aS, 7.bc

In addition, the following stocks may be considered as part of WKSIDAC 2.

Norwegian Autumn Spawning Herring	NASH	27.2.a
Faroese Autumn Spawning herring		
Baltic Autumn spawning herring	BASH	27.3
6a North Spring spawning herring		27.6.aN
Thames/Blackwater herring		
Clyde herring		27.6.aN
Herring in Divisions 7ef		27.7.e, 27.7.f

Supporting information

Priority	High
Scientific justification	Most herring populations are migratory and often congregate on feeding and wintering grounds where aggregations may consist of mixtures of individuals from several populations, thus the standard concept of 'a herring stock' within a geographical area such as a management unit is not straight-forward to assume. The analysis of the genetic composition is becoming a widely and cost-effective tool for stock identification for separating herring into populations or stocks. Recent advances include Next Generation Sequencing (NGS) and Genotyping by Sequencing (GBS) based approaches have recently been developed and applied to e.g., herring (*Clupea harengus*), cod (*Gadus morhua*), boarfish (*Capros aper*) and horse mackerel (*Trachurus trachurus*) for marker development and screening of spawning samples. Given these developments, it is now timely to revisit this herring stock identification method after the conclusions of WKSIDAC in 2017 (ICES, 2017). The objectives of the workshop are to • improve the accuracy and precision of the methods currently applied across laboratories • outline a common generic approach in terms of methods • draft guidelines for conducting stock-splits for assessment purposes • provide new insights about spatio-temporal distribution areas of herring stocks. The workshop will cover the ICES SubAreas 2, 3, 4, 5, 6 and 7. Manual descriptions will be drafted, specifying the areas/surveys relevant for the given method, hold details on minimum sampling size, stratification, and other sampling related issues. Undertaking 'stock separation' for the most recent time period i.e. going forward with a new sampling protocol using e.g. genetics is possible where appropriate samples have or are being obtained. Stock assessment, however, is reliant on a time-series of data where the stock information is known. Having the ability to retrospectively separate both survey and catch data to stock is important and this needs to predate any new genetic protocols which may be implemented. The Workshop will also consider a number of available data datasets that are available which could be used to separate the historical survey and catch data into the various stocks. These could include otolith archives, age and growth data etc.
Resource requirements	
Participants	20-30
Secretariat facilities	Meeting rooms
Financial	None
Linkages to advisory committees	ACOM
Linkages to other committees or groups	WGBIOP
Linkages to other organizations	

Annex 3: Agenda Resolutions

Monday 19th June

13:00 Start
Introduction, ICES etc
ToRs and agenda
Short recap on what was done in WKSIDAC 1 and recommendations.
Background to the problem – all the herring stocks of interest
14:30 Break
15:00 Plenary: Open ToR a. Review recent status of genetic stock identification for herring and outstanding issues affecting identification accuracy/success
Presentations (to be continued on Tuesday morning):

1. Dorte Bekkevold ("Keynote")
2. Jake Goodall (Herring genetics in the Baltic Sea)
3. Aaron Brazier (Thames herring fishery)
4. Florian Berg (Herring genetics in Norwegian waters)
5. Ed Farrell (Herring genetics in 6.a and in the Irish and Celtic Sea)
6. Ian Richardson (Introduction to IdentiGEN, first on Tuesday morning 09:00)

18:00 Close

Tuesday 20th June

09:00 Start
Plenary: Resume ToR a. Review recent status of genetic stock identification for herring and outstanding issues affecting identification accuracy/success
Open discussion on perceived status
12:00 Lunch
13:00 Plenary: ToR c. Outline a general description of prerequisites for the implementation of stock identification of herring

1. Christoffer Moesgaard Albertsen ("Keynote" – Overview statistical modelling and sampling)

Break-out groups
18:00 Close

Wednesday 21st June

09:00 Open
Continue ToRc – report back from breakout groups
12:00 Lunch
13:00 Plenary ToRc – mixed stocks in assessments

1. Benoit Berges ("Keynote" – Implications for stock assessment)

Discussion on which areas this may affect.
18:00 Close

Thursday 22nd

09:00 Open
Plenary: ToR b. Analysis of the optimal baseline requirements for stock assessment purposes, both for specific survey as well as commercial catches
18:00 Close

Friday 23rd

09:00 Open
Plenary: ToR d. Provide guidance on a retrospective correction of herring survey and catch time-series where necessary
13:00 Close

Annex 4: Presentation abstracts

Abstract for Monday afternoon "Keynote" by Dorte Bekkevold

Building on recent whole genome sequencing studies there have been multiple parallel activities to develop genetic stock/population classification tools in Atlantic herring. The systems have been routinely applied in parallel for years, mainly with the aim to split survey and commercial samples under the DCF, but also as a means to determine the distributions of spawning populations on local to regional scales. Each system focuses on slightly different stock mixing scenarios, geographical areas and management issues. Each therefore apply different genetic marker arrays designed to high-grade genetic variation among the populations/stocks mixing in the area of interest, while keeping marker numbers down in order to minimise the costs of molecular work when done in-house. The presentation focussed on issues of population classification with 59 SNPs selected to maximise resolution among populations spawning and feeding in the North Sea-Baltic Sea area. A baseline dataset with collections from more than 45 spawning locations is published and available upon request. All analyses with the 59 SNP tool agree with genome-wide sequencing results generated for population samples collected across larger geographic scales and thus support that the tool is an accurate stock classification method. Nonetheless, there are examples of local, genetically distinct populations that only to some degree can be accurately classified with the tool. The design of the tool is therefore under continued development (by adding additional SNPs to the array) with the attempt to increase classification power for specific populations in the Western Baltic/Baltic Sea area.

All analyses to date confirm the notion that the WBSS stock consists of multiple, genetically divergent populations. Spring-spawning herring from Norwegian and Swedish Skagerrak coasts are genetically highly divergent from WBSS, showing closer genetic relationships with spring-spawning herring from the west coast of Norway. No less than two populations of genetically distinct autumn-spawning herring are revealed to spawn in several local areas in the western Baltic Sea and Baltic Sea basins. Genetic split analyses of HERAS survey data revealed that these Baltic autumn spawners migrate out of the western Baltic to feed in the Kattegat, Skagerrak and the North Sea. There are indications that their relative contributions to survey data are increasing, but this needs to be further determined. Genetic split analyses of HERAS data also revealed that the Baltic Sea spring-spawning herring migrating out of the Baltic Sea and into 3a and 4a to feed mainly belong to the Southern Baltic Sea spring-spawning population. In contrast, very few fish with a Northern Baltic Spring-spawning gene profile were encountered in HERAS samples (outside of the Baltic Sea). The 59 SNP results also demonstrate that North Sea autumn-spawning herring and Downs winter-spawning herring are genetically highly distinct and can easily be classified in scientific survey and commercial catch data. The 59 SNP tool is since 2019 implemented in data collection for Danish HERAS, IBTS 1-2 and commercial catches. There is a relatively large overlap between the 59 SNPs and SNP panels currently used in Norwegian analyses, as well as SNPs on the IdentiGEN SNP array, enabling comparative studies of resolution.

Abstract of Genetic stock identification of herring around Ireland and Britain by Edward Farrell (Killybegs Fishermen's Organisation, Ireland) and David Clarke (Swansea University, Wales)

The presentation discussed results from a number of projects, including the Whole Genome Sequencing (WGS) GENSINC project (Han et al, 2020; Martinez Barrio et al., 2016; Pettersson et al., 2019), the 6.a, 7b-c herring stock identification project (Farrell et al., 2020; 2021) and the Swansea University SEACAMS, EMFF and AFBI projects which have investigated the population structure of herring in the Irish Sea, Celtic sea, and the Bristol Channel (Davies et al., 2020; Gwilliam et al., 2020).

GENSINC has undertaken whole genome sequencing to identify distinct herring populations, signatures of local adaptation and informative genetic markers (single nucleotide polymorphisms [SNPs]) capable of discriminating between most herring populations in the Northeast Atlantic and Baltic Sea. The outputs of the GENSINC project were used to identify a panel of 45 genetic markers (SNPs) capable of discriminating between the herring populations that spawn northwest of Ireland in winter (6.a.S, 7.b-c winter-spawning herring) and north of Scotland in autumn (6.a.N autumn-spawning herring). There was a long-standing issue of stock identification in this area which led to the development of an inappropriate combined assessment and poor catch advice on which to base management decisions. In order to resolve this over 4,800 spawning herring from these and adjacent populations were genotyped with the marker panel to develop a genetic baseline. Based on this a genetic assignment model was developed for the populations in division 6.a, which enabled individual herring to be assigned to their population of origin with and accuracy of greater than 90%. The assignment model was then used to assign herring samples (c.6,000 individuals) collected during the Malin Shelf Herring Acoustic Survey (MSHAS) from 2014-2021 to their population of origin, thus enabling separate survey indices to be developed for the 6.aS, 7.b-c and 6.a.N stocks. As a result these stocks are now assessed separately which is more appropriate and will lead to improved advice and management. The analyses also highlighted that the 6.a.N autumn-spawning herring are genetically identical to the North Sea autumn-spawning herring and should likely be combined for assessment purposes. Further there was evidence of the 6.a.S, 7.b-c herring crossing the 4°N line of longitude and being surveyed as part of the North Sea Herring Survey (HERAS).

In parallel, the full GENSINC dataset was utilised by Uppsala University to develop a larger panel of over 4,000 informative SNPs, distributed across the herring genome, capable of discriminating between most of the known herring populations identified to date. In collaboration with MSD Animal Health/IdentiGEN this panel of markers was included on a SNP genotyping array (DNA TRACEBACK® Fisheries platform), which also includes panels of markers from multiple other marine fisheries species including sprat, horse mackerel and cod. The array enables large scale and consistent genotyping of selected SNP's for multiple species and is now being used by the Marine Institute, Ireland for genotyping the herring samples from the MSHAS and commercial catches. Swansea University have also recently used the array to genotype over 5,000 herring from the Celtic Sea, Irish Sea, Bristol Channel and Cardigan Bay areas.

Work undertaken by Swansea University in the Celtic Sea includes morphological sampling alongside genetic studies. This has demonstrated the presence of spawning fish from October to early January from the Lleyn peninsula to Pembrokeshire in West Wales, and along the North Devon and Somerset coasts in the Bristol channel. Two populations of spring-spawning fish have also been identified; a low salinity spring-spawning group spawning within Milford Haven, and a group thought to be spawning in the marine off the South Pembrokeshire coast. Morphologically the spring-spawning groups exhibit lower vertebral counts (ca. 55, compared to 56.5-57.5 in the Autumn winter populations), and the marine spring spawners exhibit a higher growth rate than the low salinity spawners in Milford Haven.

Genetic analyses undertaken as part of an EMFF project, and in a project for AFBI, demonstrated clear genetic differentiation between spring spawners and autumn/winter spawners; as well as differentiation of the two spring groups. For the autumn/winter spawners the 6.a.S, 6.a.N, Irish Sea (Douglas Bank/Mourne) and the Celtic Sea (Dunmore East) are also clearly differentiated, with most of the West Wales and Bristol channel samples grouping with the Dunmore East spawning samples. Some fine scale differentiation may also be present and is currently being examined further.

Based on the initial genetic baseline an assignment model was developed with 38 samples comprising 2,210 Celtic and Irish sea fish, which was capable of discriminating between spring spawners, Irish Sea autumn spawners and Celtic Seas autumn/winter spawners with a self-assignment accuracy greater than 95%. The assignment model was further validated by assigning Irish Sea larval samples from 2021 and 2022 (a 'known/unknown') back to their population of origin with an accuracy greater than 90%. Eighteen mixed non-spawning samples from the Irish Sea Herring Acoustic survey conducted in early September 2021 (n=280) and 19 samples (n=694) from early September 2022 were also assigned, which indicated the presence of 40% Celtic Seas fish in 2021 and 35% in 2022. In both years the proportion of Celtic Seas fish was higher in samples from the Western Irish Sea.

Whilst there has been significant progress in the development of area and population specific genetic assignment models, both to the west of Ireland Britain and also in the western Baltic and North Sea areas, there are unresolved questions of what assignment models to use where these areas meet. If the baseline used to develop a specific assignment model does not contain baseline samples for a potential population then the likelihood is individuals from this population, if present in mixed samples, will be assigned to the most genetically similar population. This could to significant errors in the assignment of mixed survey and commercial samples. In order to avoid this one may apply subjective area based decisions on where to use particular assignment models or more appropriately develop a universal assignment model, incorporating all potential population in a single model. Work is ongoing to develop an exploratory universal assignment approach for the herring populations around Ireland and Britain.

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Abstract of Norwegian genetic baseline used for population identification in the North Sea, Norwegian Sea and along the Norwegian coast by Florian Berg

In this study, we developed and used a genetic baseline for Atlantic herring collected during scientific surveys and commercial catches in the North Sea and Norwegian Sea to investigate the validity of the current management boundaries. This was achieved by genotyping >10,000 herring from the northern European seas, including samples of all the known herring populations in the area, with a panel of population-informative SNPs mined from existing genomic resources. The final baseline consisted of ~1000 herring from 13 genetically distinct populations, including newly identified populations. A panel consisting of 76 SNP was established to discriminate herring populations in the North Sea, Norwegian Sea and along the Norwegian coast. The SNP panel was established following a similar set of criteria as described in Bekkevold et al. (2023). Additional markers to discriminate populations along the Norwegian coast and inside the fjords were included. In contrast to Bekkevold et al. (2023), the SNPs chosen were selected to primarily differentiate populations likely present in the North Sea, Norwegian Sea and along the Norwegian coast. Therefore, the number of SNPs to discriminate among populations within the Baltic Sea was reduced to only identify Baltic vs non-Baltic herring. In total, 23 baseline samples from spawning aggregations were sequenced, resulting in 13 genetically distinct populations. Many of them have been described previously in Bekkevold et al. (2023). Newly genetically identified populations were autumn-spawning herring from Sykkylven (her_sykk_au), spring-spawning herring from Trondheimsfjorden (her_thf_sp), hybrids of Atlantic and Pacific herring in Balsfjorden and Rossfjordvannet (her_pachy_sp, see also Pettersson et al. (2023)), and local fjord herring (her_norfj_sp). Local fjord herring can be found in Gloppenfjorden, Lindåspollen, Lustrafjorden and Sognefjorden and cannot be genetically differentiated. Also, genetically distinct herring from Landvikvannet (her_lndv_sp) along the Norwegian Skagerrak coast have been identified, differentiating from other spring-spawning herring in the Skagerrak (her_skag_sp). Moreover, an additional genetically distinct spring-spawning population is detected in the North Sea (her_ns_sp). However, spawning individuals of these populations have not been found as yet. All spring-spawning individuals collected along the Norwegian coast have been assigned as Norwegian spring-spawning (her_nor_sp) herring. There is a necessity to check if these spring-spawning herring from the North Sea are genetically distinct from spring-spawning herring in 6.a.N (her_wos_sp) identified by Farrell et al. (2022) to ensure if these can be treated as two distinct popualtions or should be merged as one genetic unit. Icelandic summer-spawning herring, Faroes autumn-spawning herring, and Norwegian autumn-spawning herring included in the genetic unit of North Atlantic summer/autumn-spawning herring (her_nea_au) could not be differentiated. As indicated earlier, this panel consisting of 76 SNP is only able to identify Baltic autumn-spawning herring as well as central Baltic spring-spawning herring but cannot distinguish between her_balW_au and her_balE_au nor her_balN_sp and her_balS_sp. Further, the panel cannot differentiate between the western Baltic spring-spawning populations described by Bekkevold et al. (2023) her_rug_sp, her_balW_sp or her_idw_sp, except for her_skag_sp.

Finally, after the baseline was established, 8,302 herring from mixed-population samples captured during commercial fisheries and scientific surveys between 2019-2022 were assigned using different assignment models to the baseline samples with an average posterior probability of 98.0% and 72.2% for rubias and assignPOP, respectively. The application of two different assignment methods resulted in a generally high overall agreement (77.8%). The biggest discrepancy between the two methods occurred when assigning herring as North Sea autumn-spawning herring (her_ns_au) and Downs winter-spawning herring (her_down_wi), North Atlantic summer/autumn-spawning herring (her_nea_au) or Sykkylven herring (her_sykk_au) which also clustered according to the DACP, where rubias is clearly favouring NSAS (Table S10). The assignments of mixed-population samples demonstrated that populations were identified outside their geographical defined management areas, such as North Sea autumn-spawning herring or Downs herring north of 62°N in the Norwegian Sea or western Baltic spring-spawning herring outside the 'transfer-area' in the North Sea. However, populations associated with the Baltic Sea, e.g., Baltic autumn-spawning herring, central Baltic spring-spawning herring, or western Baltic spring-spawning herring, were not at all identified north of 62°N.

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Preliminary results obtained using the MultiFishSNPChip_1.0 in Atlantic herring by Jake Goodall and Leif Andersson (Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden)

Abstract:

MultiFishSNPChip_1.0 is a novel multi-species SNP array that facilitates the development of enhanced population assignment methods in fish. However, many of the target fish species lack the baseline reference population frameworks required to utilize such methods fully. Here we discuss the ongoing development of population assignment models in Atlantic herring using the MultiFishSNPChip_1.0 array, with a focus on population genetic studies in the Baltic Sea. In particular, we discuss the coopting of existing and simulated datasets into assignment models, in lieu of baseline reference frameworks, and the subsequent potential for developing population assignment models. Additionally, we discuss haplotype assignment methods as a means of managing structural variation, such as inversions.

Presentation Summary:

Jake Goodall's presentation focused on preliminary applications of the Atlantic herring SNP chip, MultiFishSNPChip_v1.0 (Andersson et al., 2024). Initial trials utilizing 4,355 SNP showed strong discriminatory power, clearly differentiating Baltic, Norwegian, and North Sea/British individuals and spring and autumn-spawning stock. Key genes differentiating spring and autumn spawning were also characterized using the MultiFishSNPChip_v1.0, with GWAS profiles showing consistency with published works by Han et al. (Han et al., 2020).

Broader applications of the SNPChip-based datasets were discussed, as were some of the current limitations of the technology. Two key considerations were identified, the first of which pertains to the structure of current baseline reference datasets. Reference datasets such as Han et al. 2020 typically comprise a singular pooled sample per location. However, downstream population assignment tools such as AssignPOP (Chen et al., 2018) typically require multiple replicate samples (per location) for model optimization and training, creating a disconnect between the current and required data structure. Data simulation was proposed to bridge this disconnect, with individual replicates re-simulated from pools to fulfill the requirements of population assignment models. Application of simulated datasets, such as tracking shifts in stock composition within static regional boundaries and assigning individuals to proximate locations of origin, was demonstrated. Still, it was emphasized that simulated datasets are limited by the coverage implicit to reference datasets from which they are derived. Therefore, broader sampling and development of reference datasets should remain a priority.

The second key consideration focused on characterizing broad population trends in herring using SNP Chip data. The MultiFishSNPChip_v1.0 array, for example, contains many redundant SNP in regions of high biological interest, which have the potential to skew visualization population trends. Methods to account for this redundancy were proposed and demonstrated primarily via the reduction of specific structural regions (i.e., chromosomal inversions) to a single representative SNP. These 'haplotype reduction' methods were particularly useful for characterizing genetic variation in Baltic herring stock and allowed for the identification of seemingly novel herring ecotypes. Preliminary studies of various novel herring ecotypes were discussed, as was the genetic variation putatively underlying said ecotypes. However, the characterization of novel ecotypes in the Baltic Sea remains in its infancy and, therefore, requires further screening and scientific assessment.

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An update on the Blackwater (Thames) herring fishery and research on the population by Aaron Brazier (Cefas, United Kingdom)

Blackwater (Thames) herring represent a localised, spring-spawning population in the Blackwater Estuary (Essex, United Kingdom). This population is subject to a sentinel fishery, operating from September through January, with a TAC of 10 tonnes. The fishery is closed when either this TAC is taken, or temporally, when the herring are known to spawn (late February – April). Spawning takes place on Eagle Bank at the mouth of the River Blackwater, however historical spawning is known to have occurred in Herne Bay (Kent) and Osea Island (Essex). Whilst no genetic samples have been taken or analysed, the population can be identified from North Sea herring through its spawning period, whilst also being generally smaller with (on average) one fewer vertebra. A, now discontinued, fisheries-independent survey quantified the mixing between the Blackwater and North Sea (Downs component) populations with 15% of catches being Downs fish. Genetic analyses would allow for further confirmation of population independence; however no plans have been finalised for any research on the subject to continue.

Workshop on a Research Roadmap for Bristol and Western Channel Herring (WKRRBWCH; 4–6 December 2023) by Aaron Brazier (Cefas, United Kingdom)

Following a request to present advice for herring in ICES 7e (western English Channel) and 7f (Bristol Channel), an ICES Workshop (WKRRBWCH) is being held in December 2023 with the following terms of reference:

a) Identify methods and data available for the identification of herring stock structure in the western English Channel (Division 7e), Bristol Channel (Division 7f), and adjacent waters.
b) Identify potential and existing data sets (including environmental parameters) for the assessment and management advice for herring occurring in Divisions 7 e and f.
c) Produce a roadmap for the delivery of future research needs for the scientific advice that underpins management of herring in the western English Channel and Bristol Channel, either together or independently.

Genetic analyses on herring in these areas indicate local populations in these areas, therefore gaining attention of the Stock Identification Workshop (WKSIDAC). WKRRBWCH will utilise this material, whilst also attaining information from other sources to provide a roadmap on how to manage the population/s with a view of producing advice in the future.

Abstract for Tuesday afternoon presentation by Christoffer Moesgaard Albertsen "Implementation of stock identification for assessments - Statistical challenges"

The increasing collection of genetic samples and classification of herring opens many possible applications. However, there are also challenges to consider when using individual classifications. The presentation discussed two potential pitfalls when using individual classifications to infer stock compositions and how to avoid them using integrated models. This was followed by considerations on sampling strategies for mixed samples. Finally, three applications of genetic samples in integrated models were presented.

When using individual classifications to estimate stock composition, the risk of misclassification must be accounted for. If not, the resulting stock composition estimates will be biased. By construction, misclassification will move individuals to the wrong category in an asymmetric way, thereby giving a biased perception of the stock composition. This issue is independent of the type of classifier and data source used as long as there is a risk of misclassification.

Further, fish caught together are often more similar than fish collected in different hauls. This is seen for length, age, and stock compositions. This must also be accounted for to give proper weight to different hauls in the analysis. In essence, 1000 fish from one haul will most often not be as informative as 1000 fish collected with 100 fish in each of 10 hauls.

Besides the risk of misclassification and intra-haul similarity, data collection strategies can influence the estimation of stock compositions. A simulation study was presented to illustrate the difference between length stratified and random sampling of stock origin. The study illustrated the potential bias that arises if length stratified sampling is not accounted for. In this case, the stock composition was estimated to be 55%-45% instead of the true 70%-30%. In contrast, assuming randomly sampled observations arose from a length stratified program is less problematic and gave unbiased results, similar to the scenarios accounting for the sampling procedure. However, finding an optimal sampling strategy will be case specific and depend on the subsequent method of analysis, the degree of intra-haul similarity, as well as practical and financial considerations.

Three examples of using genetic samples in integrated models were presented. The first example calculated catch numbers-at-age. Often, catch numbers are derived from a combination of total catch weight, length distribution samples, and age-length samples and - when relevant - subsequently split into stocks using stock keys or age-stock keys. As an alternative to the stepwise procedure, the example presented an integrated model estimating stock-wise catch numbers-at-age. The integrated model included age, length, weight, and genotype observations. Individual weight was modelled as a function of stock and length, length was modelled as a function of age and stock, age was modelled as a function of stock, and genotype was modelled as a function of stock. In the fitting, missing observations were accounted for. As a result, catch numbers-at-age could be calculated from total weight and estimated stock-wise weight-at-age and age compositions. The results affected both the estimated age and stock compositions compared to a stepwise approach. This was partly because the stepwise approach did not sufficiently account for stock-wise differences in length- and weight-at-age.

The second example presented, illustrated a conversion of otolith-based hatch month to genetic stock. Until recently, stock origin in, e.g., the HERAS survey was determined from otolith microstructure readings giving a hatch month classification, 4 four spring spawners, 9 for autumn spawners, and 12 for winter spawners. In the example, Danish samples from HERAS in subdivision 3.a.20 was used. In 2019, both genetic and hatch month classifications were available, while only hatch months were collected in 2020. The aim was to use the 2019 data to convert 2020 hatch months to genetic stock origin, Baltic Autumn Spawners, Downs winter spawners, North Sea Autumn spawners, or Western Baltic Spring Spawners. For simplicity, 5 Central Baltic herring were converted to 'unknown'. Further, Norwegian Spring spawners were not accounted for

because there were none classified in the 2019 data. The model was similar to the model in the previous example, but also included the probability of observing a given hatch month classification given the true stock origin. The model provided reasonable estimates of genetic stock origin using the partial observations available. Further, the model estimated a confusion matrix for hatch month classifications. True Western Baltic Spring spawners were estimated to have 88% probability of being correctly classified as hatch month 4, North Sea Autumn spawners had an 86% probability of being classified as hatch month 9, Downs winter spawners had a 69% probability of being classified as hatch month 12, while Baltic autumn spawners had 44% probability of being classified as hatch month 9.

The final example presented extended the models from the two first examples to split acoustic survey data into stocks. Building on the two previous examples, an integrated model of nautical area scattering coefficients (NASC), trawl catch compositions, and spatio-temporal stock/species abundance was developed with the aim of estimating a smooth split of NASC values into species and stocks, while accounting for differences in growth, length distribution, and spatial abundance.

Annex 5: Rapporteur reports

Monday afternoon session, 19.06.23

- Meeting takes place in hybrid format
- Introduction to ICES and house rules
- Followed by who is who

What do participants expect from the meeting:

- Mapping of herring spawning grounds, stock-distribution, mixing of populations, all based on evidence!
- Describe progress in align processes/methodology/sampling/procedures
- Guidelines to optimal genetic sampling and usage of markers for stock ID
- Preserve productivity of stocks and management units
- Update WKSIDAC1-meeting tables on spawning areas and stocks
- Priorities tasks for the coming years (produce a Roadmap)
- Identify what we cannot deliver yet to management
- Issues and roadmaps how to separate/allocate catches to populations and implications for management
- Incorporation of WK results into ongoing work in other ICES groups
- Protocol and procedures how to sample the surveys
- How can we do better? Where is improvement possible, where is progress already made?
- How to deal with the data? Where to store them? Restricted or open access? Specific rules when using baselines and genotypes?
- Develop of a database needed? What data level to be stored (Metadata/raw data/some in between)?
- Where and how to store physical samples?
- Keep in mind variability and dynamic of results already made.
- Some for variability in space and time (How often do we need to update maps and tables?)
- Inclusion of surveys on national basis wherever possible.
- ToR d – Provide guidance on a retrospective correction of herring survey and catch time-series with regards to changes in population structure. Is this relevant and necessary?
- Implementing new methods and techniques is yet more relevant than changing data in the retrospective view. First produce stable and reproduceable results.

Subgroups will be implemented as needed

Presentations

- All presentations can be found at the SharePoint.
- The minutes focus on the discussion

<u>Dorte Bekkevold ("Keynote"): Project results from GENBYGSILD</u>

– Rebuilding WBSS herring –

- Where do the genetic sample associate to Bohüslan herring originates from? -> they are from actual catches in the area where no mixing with other stocks was expected.
- How strong is homing in herring? Winter spawners were found in Danish waters, showing the same genetical pattern as Downs herring. How much spatial plasticity is in herring spawning behaviour? Are our expectations right?

- Are some NSAS on the edge of the distribution cloud? ->No, they are all in the middle. This may be due the restriction to usage of 59 SNP.
- How were the 59 SNPs selected? Through maximisation of genetic distance (select fishes resp. SNPS that produces best results).
- Where does the Baltic herring stock begin? Suggesting line between southern tip of Sweden trough the Belt. It is unclear how we should redefine our stock definitions.
- As one result, it may be better to separate Baltic herring in a western, southern and northern component.
- Downs herring is genetically more linked to Celtic Sea than NSAS.
- Is real time monitoring meaningful? It can be a useful management tool for closed areas.

Jake Goodall (Herring genetics in the Baltic Sea)

- Herring can switch spawning times.
- Indication that they spawn at the "wrong" time? -> Usage of otoliths increments to resolve it.
- How many proportions have switched? Only a small fraction, not many. Most spawn in spring. They are associated to specific locations. There is yet no biological explanation around.
- There must be a way how the different group separate. Spring and autumn spawners sometimes may use of the same spawning grounds.
- The sampling depends also on the interest of the involved fishermen. Thus, statistics on proportion etc may be not too informative.
- Behaviour of herring shoaling should be kept in mind when it comes to sampling protocols

Lovisa Wennerström (Collaborative effort of herring sampling)

- Large sampling along the Swedish coastline. The selection of stations was influenced by the Swedish management and was often due to practical reasons.

Aaron Brazier (Thames herring fishery)

- 10 t TAC, but only small uptake (ca 50 kg)
- The fishery is closed at spawning time, so it is hard to get samples
- Fishing outside the driftnet area yield much more herring
- Advice has not changed in recent years

Tuesday morning session, 20.06.23

Genetic Stock Identification of Herring around Ireland Britain

West of Scotland: Edward Farrell:

- GENSINC - whole genome sequencing project on herring was a milestone, from that it was possible to select markers of interests for different areas
- multiple populations occur in 6a, 7b-c but boundaries difficult to be defined as suggested by difficulties with the stock assessment
    - central task for the use of genetics in the area so far has been to split the survey
    - 45 SNP from GENSINC initially but genotyped slightly differently recently

- aim for a model able to split several adjacent populations throughout the area
- hierarchical approach to stock separation, first separate the Autumn spawning, only after 6aS and spring spawning. It is successful
- Sampling from 2014 to present
- The genetics showed that the 56N (old cutoff) was largely inaccurate

Implementation - Irish Sea v Celtic Sea/Bristol Channel: Dave Clarke:

- results from three main projects, SEACAMS, Swansea Univ EMFF, AFBI-Swansea tender
- initially used the same 45 markers from the Division 6a project, recently moved to DNA TRACEBACK

SEACAMS2

- SW Wales spawning ground in spring
- aim was to evaluate the impact of establishing a marine energy area
- 45 SNP + morphological data
- numerous sampling locations
    - green in pie are spawning fish
    - Autumn-Winter and also Spring spawners are found, about in the same grounds, but differences at small scale may exist (i.e. patches of gravel for Autumn spawners)
- vertebral count span over a wide range, with typical low count for spring spawners
- SNP shows three main groups
    - Celtic Sea, Irish Sea and Clovelly
    - Milford Haven genetically separated from freshwater group but also a distinct growth

AFBI/EMFF project

- 3217 SNP
- two runs with ~3000 and 1900 individuals respectively, mainly from acoustics
- assignment model with four main groups (based on SVM)
- larvae mainly from Irish Sea but with some exceptions
- A good proportion of fish in the Irish Sea is in fact found to come from the Celtic Sea
    - West of Isle of Man large proportion of Celtic Sea fish and east of Isle of Man large proportion were of Irish Sea origin
    - possibly linked to circulation but it is a snapshot in time over the year, and seasonal variability remain to be understood

Universal Assignment Model: Edward Farrell:

- We start to have multiple good models for a number of different areas
    - instead of patching them we should look at a Universal Assignment Model (UAM)
    - UAM allows to assign any herring throughout the large domain of HAWG and beyond

Discussion

- Importance to get fishermen knowledge in on coastal spawning grounds
- Celtic Sea data suggest substructures but more baselines from Irish and Celtic Sea are needed
- There's a need of mapping spawning grounds and their characteristics
- Splitting commercial catches in 6aS a bit behind but rolling in from 2022. There are still a lot of questions on sample size and design

- Sub-models were design to work in specific areas so they are only valid within their spatial domain
- What if we aim for a lower accuracy assignment by compensating with a space-time model
- A space-time model will be the best way to fill data gaps (i.e. catches which are not sampled) and eventually do inference on future mixing

IdentiGEN and DNA Traceback fisheries: herring genetic assignment: Ian Richardson:

- work towards a Universal Assignment Model (UAM) for herring
- HQ in Dublin with labs all over
- core business food traceability then expanded to genomics, and much more
- work on 10+ species, ie cattle, pigs, chicken , sheep,/goats, horses, salmon, shrimp + wild fish
- DNA traceback
    - salmon, brown trout, cod, herring, horse mackerel, perch, sprat, pacific shrimp

Herring work

- 70 samples for baselines, 38 locations, 13 ICES stat areas
- export assignPOP approach with SVM with state-of-the-art machine learning approach
- 11-12 agreed populations
    - <200 SNP
    - accuracy 0.68-0.66
- finer refinement (grouping) needed of FreshWater with 6aS increased accuracy to 0.8
    - see confusion matrix
- machine learning approach, looking for an optimal combination among
    - test different classifiers
    - feature selection
    - data sampling
- some improvement (Accu 0.885) but still
- more work on collinearity required

Discussion

- The algorithm could be helped with geographical info?
    - Yes
- Simulations are used for balancing samples but need of increasing sample size for some of the groups
- A power analysis would be useful to understand the number of samples required per group to reach a certain accuracy
- There's a lot of linkage
- Selecting method possibly too aggressive in this preliminary approach
- possibility of strayers polluting the baselines, but hopefully not too much
- all fish used were spawning (stage5-6) and we preferred not removing individuals
- considerable computation power is needed
- need to expand the sample to include the Baltic for an UAM
- Accuracy of assignment towards some groups of specific interest may be necessary for conservation and management
- among the data sampling methods tested, some may price small groups and other large groups and in this way privilege some groups vs others

- The testing strategy with resampling and simulation is quite sophisticated and should make the approaches quite comparable
- Cost per sample and volume are strictly related?
  - confirm, for large volumes like 10000 samples ~20 EUR/per sample but it can get cheaper
- Time for processing?
  - few weeks
- many small or one large contract?
  - Join submission as a single proj might reduce the coast and admin burden
  - but in practice we still have to work with our single institutes admins
  - Maybe individual surveys can at last be presented as a "single proj" to IdentiGEN so they could be dealt in that way in the portal but the admin with stay at institutes level
- IdentiGEN has local labs that might help some of the logistics

Assessing the composition of mixed fisheries of Atlantic herring (*Clupea harengus* L.) in the Norwegian Sea and adjacent waters using a single nucleotide polymorphism (SNP) panel: Gudmundur:

- Three managed herring stock NSSH, ISSH, NSAH, but many more units occur in the area incl a number of local populations
- prop of ISSH and NSSH in the catches approximately 12-88%//16-84% in the period 2018-2021, estimated 25-75% in 2022, are calculated based on maturity stages
- 5-10% recorded as NSSH based on maturity are most likely not NSSH. What are they? it remains still unanswered probably because some of these pops were not included in the initial poolSeq work
- 120 SNP panel selected from Uppsala Univ work
- samples include spawning fish from many of these populations and mixed samples, but in both cases number of individuals doesn't seem very high
- Results show that FASH, FSSH, ISSH as well as NASH are difficult to separate with this panel
- mixed samples are assigned to NSSH and [FASH, FSSH, ISSH] group but none to the NSAH
- west Iceland all ISSH while east Iceland mixing dominated by NSSH with ISSH
- Conclusion
  - SNP separate NSSH, NSAH and ISSH but not between ISSH,FASH,FSSH,NASH
  - maturity overall supported
  - more powerfull discrimination tool needed for summer/autumn-spawning stocks. Need of better baselines for some of these components
- Implications
  - FASH and FSSH isolated pops? it seems so but strong relationship between FASH and ISSH with low genetic separation

Discussion

- have you tried directly/formal comparison of maturity with genetic assignment?
  - not yet
- Presence of FASH on the Icelandic shelf?
  - cannot be excluded but it's expected to be small

- what about mixing in the survey
  - not expected to be a problem in the survey time
- limited ability to separate some of these units (ie FASH and ISSH) are likely linked to the present baselines

Norwegian genetic baselines: Florian Berg:

- 23 baseline samples frompaaggregations
- 13 biol units/pops
- large mixed sample
- 76 markers
- samples from surveys, HERAS, IESNS, IESSNS
  - NASS seems to stay close to the NOR coast at least during summer
- assignment of mixed samples by assignPOP and rubias overall similar
- HAWG has been using genetics in the last few years and for comparability has been tried to mimic assignment by previous methods (vertebrae and otolith) to current stocks
- New project funded: Assigning spatiotemporal dynamics in HERring POPulation Structure under climate change (HERPOPS)

Discussion

- In ICES we have stock code but not population codes, strong need for population codes which may be a recommendation from WKSIDAC2

Tuesday afternoon session, 20.06.23

ToR a. Review recent status of genetic stock identification for herring and outstanding issues affecting identification accuracy/success

Potential table of genetic units

1. A lovely map/table of herring populations (spawning grounds, populations, mixing (based on evidence))
2. Do progress in align processes/methodology/sampling/procedures etc.
3. Guideline for the optimal sampling and use of markers for stock ID
4. Priority list of what we do not know (next 5-10 years → roadmap)
5. What we cannot be asked by the managers
6. Provide roadmap how to implement stock ID in assessment/survey/catches
7. How can we do things better/improve
8. Data management/storage/availability (baseline/genotypes) → Which data goes into a database, which level (final assessment vs. genotypes)
9. Where and how to store physical samples
10. Variability in space and time (How often do we need to update maps/tables)

Optimal sampling strategy

- as long as know what you want to know something about the strategy may be adopted
- Is sampling of all age classes important
- inform the assessment important
- stratify vs random
- threshold of assignment 2 per mille
- ascertain future estimations of the proportion

- estimate F opt. for 2024
- something to have in the back of our mind
- ages are important
- no need for new sampling schemes
- that's not how it works in reality – x ships x protocols, why do you need less genetics than ages
- if signal is weak, you need less samples
- we have protocols but are not able follow – one sample to raise to thousands of tons – if crew is asked for more sampling, they want a prioritisation
- genetics for each fish – quick
- it's a matter of resources
- budget = 200-300 fish limit
- need to see what's out there - start big
- as many hauls as needed, Scottish survey change strategy at 4o W
- MIK samples, 50 p/herring for baselines
- what to do with the herring age or genetics – where do fish come from
- different reasons for diff Inst having diff strategies
- Baltic some countries take less than others
- documentation to take back to Inst
- common sampling design will help modelling over boundaries
- two purposes precision or population of interest
- simulation exercise will not be fair without consulting the survey people
- Survey people want to know how many samples do you need?
- prior analyses would give you one answer
- specific for a survey
- IBTS – if you can wait 4 hours before sampling ok – otherwise extra person production line
- procedure is years behind
- standard protocols!
- buy from trusted fishers is convenient
- opportunistic samples is sometimes a waste of time and money
- Developed from WESTHER – carried on with genetics down to 96 fish per haul
- any thoughts from the North how many samples?
- coastal fishery with lower amount of samples
- question – what is the question, required number of genetic samples for specific questions
- we haven't looked at the bulk of fishes – what they are
- temp distr. of genetic samples
- two stage process sampling and analysing deciding on analysis of covariates
- started to sample before we knew how – bank the samples.
- we are in an initial phase where intensive sampling is needed to understand the structure/dynamics – start with better baselines
- collect genetics of all aged fish – and decide later which to analyse
- are there samples hanging around
- most are analysed
- most HERAS have been processed
- IBTS Q1 MIK in alcohol
- SWE stored in alc
- opportunity for working up by other parties
- what questions are we going to answer
- is there something about markers, how many, which etc.

- it's an ultimate goal – we have an idea of where the holes are
- update of chip with more species e.g. sprat
- is the general approach to store the physical samples // yes // DNA in fridge, tissue in alc.
- you need to stab twice if you want your own sample

Discussion on the map

- Define good baseline samples and good baseline pops
- fishers tell something about herring behaviour
- good idea of what we have
- there are holes in the NS and East Baltic
- design protocols for baseline sampling
- everything has be to aligned for "industrial" processing

WHERE HAVE WE GOT TO?

1. A lovely map/table of herring populations (spawning grounds, populations, mixing (based on evidence)) *Done*
2. Make progress in *align* processes/methodology/sampling/procedures etc. *Are we willing to undertake the alignment? (only one + one)*
3. Guideline for the optimal sampling and *use of genetic markers* for stock ID (case specific)
4. Priority list of what we do not know (next 5-10 years → roadmap)
5. What we cannot be asked by the managers *(genetic unit yes to stock no)*
6. Provide roadmap how to implement stock ID in *survey/catches/assessment*
7. How can we do things better/improve *(Universal method etc moving forward, baselines, methodologies and trade-offs)*
8. Data management/storage/availability (baseline/genotypes) → Which data goes into a database, which level (final assessment vs. genotypes)
9. Where and how to store physical samples
10. Variability in space and time (How often do we need to update maps/tables)

Wednesday morning session, 21.06.23

Not available

Wednesday afternoon session, 21.06.23

<u>Presentation from Benoit Berges ("Keynote" – Implications for stock assessment)</u>

Benoit presented modelling analyses directed towards a number of scenarios:

1. Practicality of breaking down the index into two stocks, using NSAS & WBSS (to a lesser extent) assessment data in StoX (IMR developed) underpinning index calculations. Main variables were: age, length, weight, maturity level, stock. Some variation in data collection strategy: Before 2015 data were collected by ices SQ, and from 2016 onwards by strata. In 2018-20 there was some overlap in vertebrae, otolith microstructure and genetic assessment of stock affiliation and this was exploited in analyses. Results indicated both consistency across data types and in others some potential dramatic differences.

    - there is additional data available for HERAS 2019.
        - If it is useful to include these data in analyses.

2. Testing different stock mixing scenarios on biomass estimation. Showed different outcome for different mix scenarios.

- Asked whether stock specific effects depend on size of stock.
  - Commented that the effect of splitting approach will vary among areas with larger or smaller stock densities, not just stock size per se.
- Asked if this was an effect in HERAS data only.
  - Replied that there should be little difference.
- Asked whether (based on slide 17) analyses can be extended further back in time.
  - Replied that it could be done for two additional years but before then the strata were different.
- Asked if sample sizes were sufficient.
  - Replied that sample sizes appear to be adequate. In the model missing stock ID is imputed by age class/strata or length.
- Commented that the Downs herring cause problems in the MIK survey and that Downs index appears to get extremely large (few NSAS).
- Scottish data not included in this.
- Mentioned preliminary genetic data from Scottish data (shown at HAWG 2023) showing potential huge mixing and many Downs.
- Asked if sampling design affect estimates.
- Commented that just because StoX produces an estimate it does not mean we have the best design.
- Asked if it is possible to run a variance analysis.
- Replied that he could run a bootstrap analysis to look into this.
- Suggested that the Norwegian data set can be used to simulate effects of different sampling strategies. E.g. by reducing numbers of fish per sample.
- Commented that is it difficult to judge effect size yet, but by definition the genetic stock split should improve stock estimates – as long as it doesn't increase noise as well
  - Commented: Internal consistency for WBSS cannot get worse! Therefore including genetic stock splitting should only get better. Should improve Downs relationships.
- We need to consider all aspects of including this information and whether it does in fact a priori improve the index.
- Asked if we could look into effects of imputing different data types, especially for the rarer populations where there will typically be more missing data per age/length/stock class.
  - I did try to do this with the model, using a von Bertalanffy model to impute data, but there is not that much missing data.
- Asked about Downs data
  - showed examples from preliminary data using SNP array data in Scottish areas where several stocks mix (NSAS/6AN; ASH, WBSS/BAS, Downs, 6aS+spring) in an area where the expectation is there are only NSAS fish. The data represent surveys and may not reflect the fishery. Further, there may be a large effect of the specific samples if/when specific stocks tend to school together.
- We have to be very aware of specific sampling approaches that may cause bias when we want to estimate numbers and we cannot assess all populations anyway.
- Mentioned evidence of otolith growth differences in first winter-ring between NSS and NSAS/WBSS not including Downs.
- Briefly reported on herring in 7e-f (Bristol Channel and western English Channel – and adjacent areas). There is no recognized stock and recent landings have been small (80t).

- Flagged the upcoming workshop WKRRBWCH December 2023.
- Commented that although it is not a stock, it is in management and given we now have two parties (EU/UK) who have to agree on management local exploitation of the 800t TAC could be potentially harmful to local populations. No advice is given at present but it is an ICES request. Locally caught fish are currently mainly sold on local markeds.
- Reminded of the PELTIC survey, targeting sardine, sprat (with few herring) and some samples have been collected. Perspectives for advice to ICES were discussed and it was commented that ultimately, herring could become choke species for sprat and sardine if the full 800t are actually fished out.

"Roadmap for WKSIDAC 2"

1. Baselines

    - We should identify (baseline) populations to be prioritized for whole genome sequenced. Whole genome analysis by PoolSeq of 12 spawning locations would cost approximately 16-20000 Euros. We should pinpoint areas based on genetic and biological data that we expect to be of importance and should be prioritized for genotyping, and even small populations can be important if they are threatened by overexploitation in mixtures with other, larger stocks. Temporal replicates of baselines need to be prioritised. Suggested to be at approx. 5 year intervals.

2. Surveys and sampling:

    - We can produce maps with presence/absence data (including where data are missing) by area, season, life-stage and population. Participants are encouraged to send information to Florian.

3. Universal assignment panel and -model.

    - We need continued collaboration.

4. Database and sample storage issues:

    - In databases transparency is important.
    - Used acoustic data from trawl database and work with different databases depending on type data (acoustic, trawl, etc).
    - different countries have aggregated data in different formats and it is quite complex. Some store genotypes and bio data separately, others store them together but with different types of metadata involved, depending on methods (markers, assignment methods, etc.).
    - remarked that we should decide whether suggested new population codes and names can be matched with ICES stock codes.
    - commented that stock units cannot be changed (but can comprise multiple populations) but the information on which stocks are in which management units can be amended.
    - whether it is within the remit of the group to advice on which populations and assessment units should be split (Gulf of Riga autumn spawners and western BAS; Downs vs NSAS) or combined (6aN/NSAS)
        - replied that we can recommend for a group (which group? – this needs to be determined) to look at what we suggest and consider whether the two stocks can be assessed as one (in a benchmark or similar).
        - commented that we need both general advice to assessment groups to look into revision of stock assessment units incorporating the appropriate population information, as well as specific advice on which units should be split vs combined.

- it is an inherent goal to identify the bounds of individual stocks to perform an assessment. You need to be able to remove smaller populations/stocks from the data used for the stock you are assessing.
- suggested that we produce a roadmap with priorities for alterations in specific areas.
- cautioned that we have not yet taken into consideration the consequences for managers of the changes we suggest; also how these changes should be dealt with internally in ICES.

Thursday morning session, 22.06.23

Subgroup on population names, codes and descriptions based on the baseline spawning samples.

- Baseline samples overview map is the starting point.
- What genetic units have been identified by papers, working groups, genetics etc.
- Excel table with the population names, potential codes and descriptive information
- These are population codes and don't necessarily match the stock codes used for assessment purposes.
- These codes should fit in with the data centre codes – need to contact the data centre and find out what would work.
- Agreement on the use of species and spawning time. Descriptive information in between needs some more agreement.
- Remove ICES divisions and boundaries from maps. Information is based on the sample information only.
- Need a table with a description of what each of the codes mean.
- Could a number be used instead of a code for populations in the database? The disadvantage of using numbers instead of codes is there is more room for error. Once the numbers go above 10 you could miss a digit and still find a valid population. Would letters be better? Error checking is easier when using codes and codes are easy to enter.
- Need to decide what we want from this and find out what will work with the existing databases. Will numbers or codes work better?
- Need to think about how this fits into stock assessment and management units. Can't set up assessments for all of the populations listed.
- What we do has to fit in with the existing framework In assessment working groups etc
- Database feedback. Population code – no limitations on requirements
- We are not using the stock codes that are used in the assessment.
- Talked to ICES database people: A new code for each population can be added to the database. He will take up the issue with the acoustic governance group –Also, for egg and larval database if possible. To discuss at WGSINS.
- This is not an open field. A basis is needed for the vocabulary. Codes that are put in are up to the group.

Thursday afternoon session, 22.06.23

- Distribution of report responsibilities, discussion on report structure, deadline 6th of October 2023 (soft submission date? Needs to be discussed with ICES)
- WGBIOP would be the ICES working group where genetic sampling would most naturally fit in; FB will bring this up again at this year's meeting; last time this was discussed it was more a lack of expert knowledge than interest

- visualize the distribution of genetically assigned commercial and survey samples → we should not use proportions as they are dependent on sample size; split into methods?
- prepare a table of unanalysed samples? Difficult to get a complete list but we can prepare a recommendation to collect them in the future

ToR d: Provide guidance on a retrospective correction of herring survey and catch time-series where necessary

- Aim: correct survey index back in time and sort out populations which do not belong to the stock. This could improve the internal consistency in stock assessment models; stock assessment will show a much better consistency if populations are followed over time and not a mix of different populations (consistency in age classes)
- Are we able to correct the time-series back in time with the currently available data/ samples?
- Presentation of Christoffer: not impossible, otolith microstructure and vertebrae count; 2 years for calibration → combination of different methods.
- Did the distribution of the fish change over the period? It is necessary to consider inter-annual variation. Can we pick this up? When we are going back in time and are able to use several techniques but know that the system has changed over time, is it possible to use other methods to fill in the gaps? Which models and statistical analysis are needed for this?
- Better not phrase it "time-series correction" but something like "development of population-specific time-series"
- Consequence: Alter the databases –> long process with additional workload for the contributing countries
- Study on uncertainty and variance in the index
- StoX cannot calculate uncertainty about genetics but just in haul variation and acoustic transects
- Gudmundur showed an example from the herring distribution during the May survey between Iceland and Norway. Group of fish close to the Norwegian coast far away from the core distribution. What is the actual influence/ impact of this? Can/ should this be corrected?
    - Is there a difference in the acoustic properties? Combine acoustic descriptors (density of school, shape of school) with haul information?
- Correction is more likely for surveys than for commercial catches (maybe proportions)
- A project is needed to further investigate possibilities, this cannot be done in between years (a benchmark is needed when the approach of calculating the survey index is changed anyway)
- Does it change the perspective of the stock? Is it worth the effort and money to do so?
- → combining methods with an overlap; also when applying new methods, make sure to have holding points between the methods back in time!
- Very area specific, depending on the stock size in that area
- → yes it needs to be done, but it does not need to be done everywhere, some areas are more important → prioritization
- What is needed for an improvement of the assessment, what is needed as data input?
- Wrap up: Suggestion to look at pursuing the idea to be able to look back in time, we need to figure out if it is valuable for money invested; what are the resources necessary to take us back in time vs what we are gaining.

Revisit WK expectations defined on Monday

- Are we willing to undertake the alignment of different methods between labs? We do not want to align our methods as different methods work fine for different labs. Either methods are fine.
- Guidelines for the optimal sampling and use of genetic markers and stock ID → this is very case specific
- Roadmap is a section of the report and has been discussed
- What we cannot be asked by the managers → Within this workshop we focused on talking about genetic units but not stocks
- many points are very area or case specific. We need a meeting (coming up with a benchmark) to investigate details and get into work for individual stock units/ areas.
- How can we do better/ improve? Work on the universal assignment method → discussions continue, also on the baseline analysis, methodologies and trade-offs.
- Trade-off between cheap & numerous vs. precise & costly
- Several institutes currently develop databases and collaboration among institutes would be very beneficial
- Where and how to store physical samples → companies offer possibilities but this is a rather local problem

Finalise any ToR a open topics

- no open topics

Roadmap with direction of travel

- drafted and will be revised after report writing

Recommendations (to ourselves)

- document on the SharePoint

Section in the report about "Where can genetics not help us, there are other methods necessary?"

Annex 6:

Standard Operating Procedure (SOP)
Genetic sample collection for WESPAS 2022

Edward D. Farrell, EDF Scientific Limited.

March 2021

The following SOP describes the recommended sample collection approach using the LVL Genetic Sampling Tool (GST). The genetic sampling kits comprise a standard SBS format barcoded rack with 96 individual 2D barcoded 1ml tubes each with a GST incorporated into the tube cap. Racks come pre-assembled with the tubes in place and have been pre-scanned. The barcodes of the tubes in each rack consist of a two letter and unique 10 digit number (e.g. LV**********) and are arranged in sequential order from A1-12, B1-12, C1-C12 etc and as such the tubes should not be removed from the racks and should not be reordered within the racks. The racks have been supplied prefilled with molecular grade EtOH.

The genetic sample collection step should be incorporated into the standard sample collection workflow. In order to minimise the occurrence of cross contamination the genetic sampling step should be performed prior to opening the abdominal cavity for maturity assessment or the head for otolith extraction.

1. Clean down the workspace and equipment with Microsol 4 Decontaminant or a 10% bleach solution.
2. Set up workspace with required equipment, including measuring board, weighing scales, otolith boxes, genetic boxes, knife, forceps, water and sponge/cloth for cleaning otoliths etc.
3. Place the genetic box in a suitable position where it will not become contaminated and will remain clean and dry.
4. For onboard sampling the genetic box can be stabilised by placing it a bracket that is securely attached to the bench (Figure 1) or through the use of non-slip mats.
5. Ensure that the rack is orientated in the correct position before placing in the bracket or starting sampling. The upper left-hand corner has a cut away and the letters A-H are embossed on the left-hand side of the rack, labelling the rows of tubes. The numbers 1-12 are embossed on the top of the rack, labelling the columns of tubes (Figure 2).
6. The first tube in each box is therefore designated position A1.
7. The direction of use should **always** follow A1 to A12 then B1 to B12 and so on, in order to follow the sequentially barcoded tubes. There should be **no** deviation from this pattern and tubes should not be skipped and left empty.

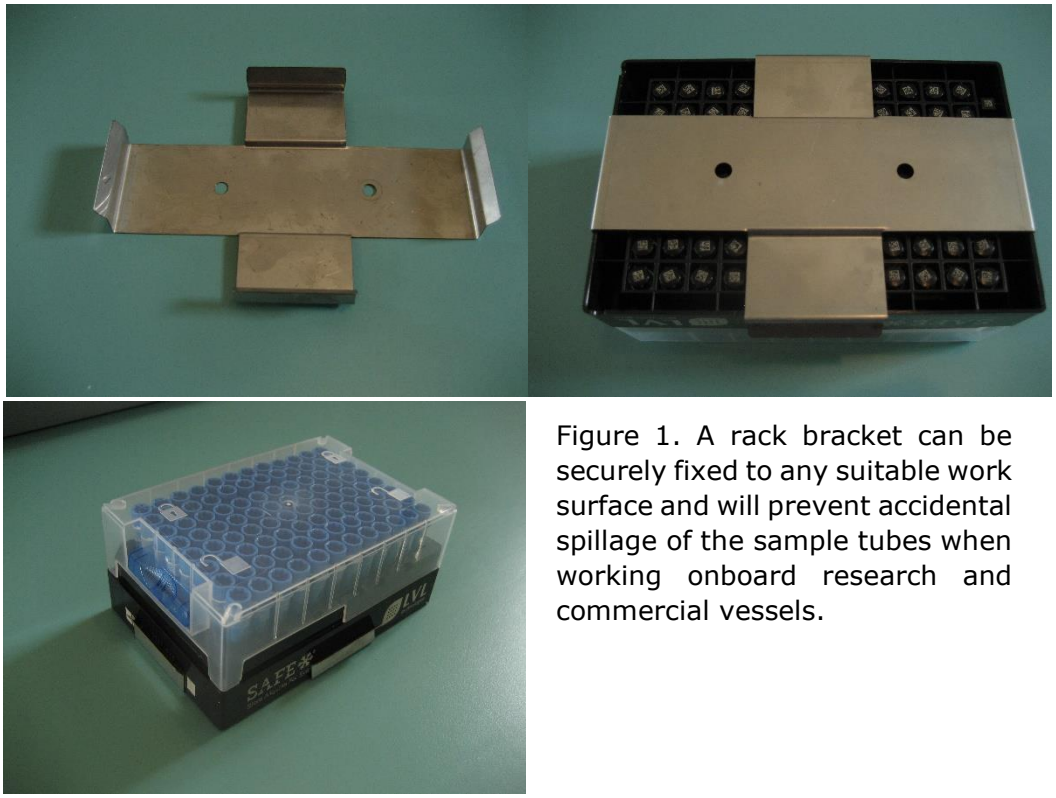

Figure 1. A rack bracket can be securely fixed to any suitable work surface and will prevent accidental spillage of the sample tubes when working onboard research and commercial vessels.

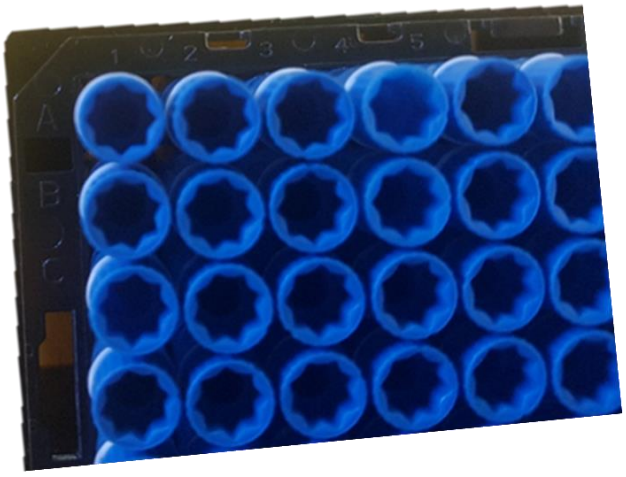

Figure 2. A close up of the upper left-hand corner of the rack showing the cutaway and the start of the row letters and column numbers.

8. Remove the rack lid and place in a clean and safe place until sampling is completed. There is no need for further contact with the rack with contaminated gloves. During sampling all interactions with the rack and tubes should be done using the single channel manual decapper tool, which will prevent contamination of the tubes and rack. **Do not** remove tubes from the rack unless absolutely necessary.
9. Record the survey name, date, haul number, catch position and rack number in the data sheet. If using electronic data capture software scan the rack barcode.
10. Lay out fish in a line and wash down their surface with water to remove excess surface contamination e.g. blood, slime and loose scales.
11. Start sampling the first fish.
12. Measure the total length and weight of the fish and record on data sheet beside the relevant sample no.

Figure 3. The GST in place on the manual decapper tool, which functions as a handle.

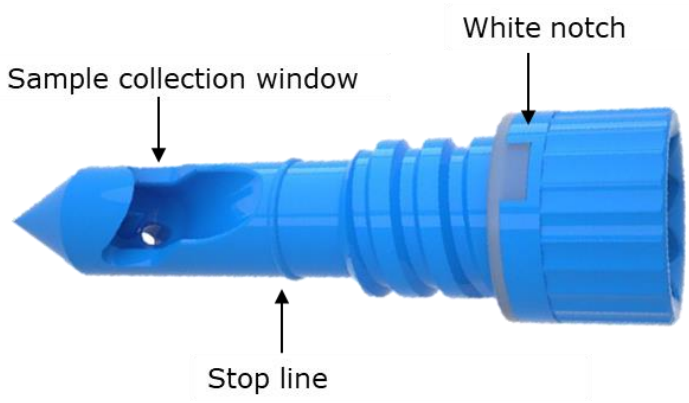

Figure 4. Close up of the genetic sampling tool (GST) with key features highlighted.

13. Using the manual 1-channel decapper (Figure 3) push the tool into the cap to select the first tube in the rack in the A1 position. Unscrew the cap. Note the sample collection window behind the tip of the GST and the marker line on the shaft (Figure 4).
14. Orientate the GST with the sample collection window facing down towards the fish (Figure 5). The white notches on the GST cap seal can help to orientate the GST correctly (Figure 4). One white notch is in line with the sample collection window and the second notch is directly opposite on the reverse side.
15. Push the GST at an angle of c.45° into the dorsal musculature of the fish on the area indicated in Figure 5. The area adjacent to the dorsal fin is the thickest part of the body and yields the best samples. **Do not** insert the GST deeper than the stop line (Figure 4) in order to avoid contamination of the screw threads. Take care to avoid puncturing the body cavity, which can happen when sampling small specimens.
16. Once the sampling tool has been inserted up to the stop line on the GST, rotate the handle 180° so the tissue collection window is facing upwards and withdraw from the fish, whilst exerting an upward force on the underside of the fish skin with the GST. This will ensure a c.30mg sample of white muscle tissue will be cleanly cut from the fish and will be firmly retained within the tissue collection window (Figure 6).

Figure 5. With the sample collection window facing down towards the fish insert the GST at an angle of 45° into the dorsal musculature up to the stop line.

17. Return the GST to the relevant tube, screw closed and depress the plunger on the manual decapper to eject the tube. **Avoid any contact with the rack or the tube with contaminated hand or gloves**.
18. Continue sampling the fish for sex and maturity and extract the otoliths as required.
19. Move to the next fish and the next sample tube (A2) and repeat steps 12-18. **Always work in the order A1-A12, B1-B12 etc.**
20. Once sampling is completed store the racks upright in a fridge (4°C) or freezer (-20°C) until further processing.

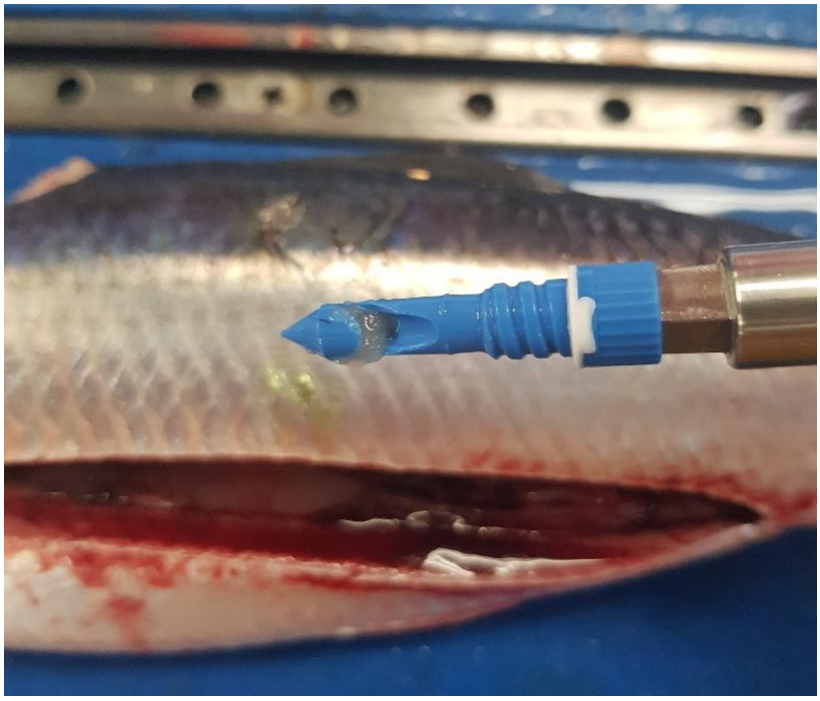

Figure 6. The extracted GST showing the tissue sample retained within the sample collection window.