Herring in Divisions 6.a, 7.b and 7.c: Scientific Assessment of the Identity of the Southern and Northern Stocks through Genetic and Morphometric Analysis

European Maritime and Fisheries Fund (EMFF)

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April – 2021

This report should be cited as:

Farrell, E.D., Campbell, N., Carlsson, J., Egan, A., Gras, M., Lusseau S.M., Nolan, C., O'Connell, S., O'Malley, M., White, E. 2021. Herring in Divisions 6.a, 7.b and 7.c: Scientific Assessment of the Identity of the Southern and Northern Stocks through Genetic and Morphometric Analysis. Final Report. European Commission. Service Contract EASME/EMFF/2017/1.3.2.1/SI2.767459. 251 pp.

EUROPEAN COMMISSION

European Climate, Infrastructure and Environment Executive Agency
Unit D.D 3.1 — Sustainable Blue Economy

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Herring in Divisions 6.a, 7.b and 7.c: Scientific Assessment of the Identity of the Southern and Northern Stocks through Genetic and Morphometric Analysis

Final Report

Service Contract
EASME/EMFF/2017/1.3.2.1/SI2.767459

Luxembourg: Publications Office of the European Union, 2021

PDF	ISBN 978-92-9460-583-2	doi: 10.2826/208498	EA-09-21-113-EN-N

Executive Summary

The primary objective of this study was to assess the identity of herring stocks in ICES Divisions 6.a, 7.b and 7.c, through genetic analysis, in order to develop genetic profiles of the northern (ICES Division 6.a North) and southern (ICES Divisions 6.a South, 7.b and 7.c) stocks, which could be used to discriminate the two stocks during times of mixing, such as, in the summer acoustic surveys. In addition, body and otolith morphometric methods were developed to test if morphometric methods were also able to discriminate the stocks in these areas. The study comprised an extensive review of the history of the existing stock delineations, comprehensive sampling for both the genetic and morphometric components of the project, genetic marker development, genetic screening of samples, the establishment of a genetic protocol for large scale sample screening, morphometric analyses and comparative analyses of both methods.

Baseline spawning samples were collected over five spawning seasons (2014-2019) and archive samples from the WESTHER project (2003-2004) were also reanalysed. In total c.4,900 individuals from Divisions 6.a, 7.b and 7.c, 1,860 individuals from outgroup populations, 650 individuals from the WESTHER samples and 3,665 individuals from the MSHAS samples were analysed as part of the genetic analysis tasks. A total of 1,815 baseline spawning individuals from Division 6.a were analysed for the morphometric component of the project and data from more than 10,000 individuals from the MSHAS samples were collected.

The genetic analyses indicated that herring in ICES Division 6.a comprise at least three distinct populations; 6.a.S herring, 6.a.N autumn spawning herring and 6.a.N spring spawning herring. The 6.a.S herring are primarily a winter spawning population though there is a later spawning component present in the area also. These components are currently inseparable and for the purposes of stock assessment should be combined as 6.a.S herring. No baseline spawning samples could be collected in Divisions 7.b or 7.c therefore the relationship between the herring that spawn in this area and those that spawn in 6.a.S is unknown. Non-spawning herring caught in Division 7.b assigned genetically to the 6.a.S population.

There is no historical or contemporary evidence to support the differentiation of 6.a.N autumn spawning herring and North Sea autumn spawning herring. The term ‘west of Scotland herring’ originally referred to populations of spring spawning herring that spawned in the Minch area. It now refers to autumn spawning herring that occur west of the 4°W boundary during the period of the MSHAS.

The Celtic Sea herring and Irish Sea herring are distinct from each other and from the populations in ICES Divisions 6.a however the current genetic marker panel is not optimised for their inclusion in the baseline assignment dataset. This is not considered to be a significant issue as there is no robust evidence that Irish Sea herring are found in large abundance west of the Hebrides during summer. Historical evidence does suggest that they may be found in the Clyde area at this time before returning to spawn in the Irish Sea in autumn.

The morphometric methods used in the current study indicated significant differences between the 6.a.S herring and the 6.a.N autumn spawning herring, however they did not show sufficient power to discriminate mixed survey catches in ICES Division 6.a.

The genetic markers and assignment methods presented in the current study constitute a tool that can be used for the assignment of herring caught in mixed survey and commercial catches in Division 6.a into their population of origin with a high level of accuracy (>90%). This approach should be used for regular monitoring of MSHAS and commercial catches of herring in this area and further efforts should be made to expand the genetic screening to the North Sea also.

Résumé

L'objectif principal de cette étude était de définir l'identité des stocks de hareng au sein des divisions CIEM 6.a, 7.b et 7.c, à l'aide d'analyses génétiques, afin de développer des profils génétiques pour le stock de hareng du nord (Division CIEM 6.a nord) et le stock de hareng du sud (Division CIEM 6.a sud, 7.b et 7.c), qui pourraient être utilisés pour identifier l'origine des poissons des 2 stocks lorsqu'ils se mélangent en été à la période durant laquelle la campagne acoustique est organisée. Des méthodes basées sur les morphologies corporelle et otolithique ont été développées et testées pour évaluer leur capacité à séparer les stocks dans cette zone. Cette étude inclut une revue bibliographique approfondie de l'historique de la définition des stocks de hareng dans cette zone, un échantillonnage exhaustif des composantes génétiques et morphométriques du projet, le développement de marqueurs génétiques, l'identification génétique des échantillons, l'établissement d'un protocole à grande échelle pour l'identification des échantillons, l'analyse morphométrique et l'analyse comparative des 2 méthodes.

Les échantillons de référence de poissons frayeurs ont été collectés durant cinq saisons (2014-2019) et des échantillons archivés par le projet WESTHER (2003-2004) ont aussi été ré-analysés. Un total d'environ 4500 individus provenant des divisions 6.a, 7.b et 7.c, 1860 individus provenant de populations extérieures à la zone d'étude, 650 individus provenant des échantillons du projet WESTHER et 3665 individus provenant des échantillons de la campagne MSHAS ont été génétiquement analysées. Un total de 1815 individus frayeurs de référence provenant de la division 6.a ont été analysés pour la composante morphométrique du projet et les données provenant de plus de 10 000 individus échantillonnés par la campagne MSHAS ont été collectés.

Les analyses génétiques ont indiqué que les harengs des divisions CIEM 6.a, 7.b et 7.c forment au moins trois populations distinctes ; le stock de hareng de 6.aS, le stock de hareng d'automne de 6.aN et le stock de hareng de printemps de 6.aN. Le stock de hareng de 6.a.S fraie principalement en hiver même si une composante de la population présente dans cette zone fraie plus tard dans l'année. Il n'est actuellement pas possible de séparer cette composante et en ce qui concerne l'évaluation de stock elle devrait être combinée avec le hareng de 6.aS. Aucun échantillon frayeur de référence n'a pu être collecté dans les divisions 7.b et 7.c, donc les relations entre les populations qui fraient dans cette zone et ceux qui fraient dans la division 6.aS sont actuellement indéterminées. L'origine génétique des stocks de hareng non frayant et capturés dans la division 7.b a été déterminée comme peu différente de celle des stocks de hareng de 6.aS.

Il n'y a pas de preuve historique ou contemporaine en ce qui concerne la séparation du stock de hareng d'automne de 6.a.N et du stock de hareng d'automne de Mer du Nord. Le terme hareng de l'ouest de l'Ecosse fait référence au stock de hareng qui frayait au printemps dans la zone du Minch. Il fait maintenant référence au hareng d'automne qui se trouve à l'ouest du méridien 4°W durant la période pendant laquelle la campagne MSHAS est organisée.

Les stocks de hareng de Mer Celtique et de Mer d'Irlande sont distincts entre eux et sont distincts des populations de la division 6.a, cependant les marqueurs génétiques actuellement sélectionnés ne sont pas adaptés pour inclure ces populations dans la base de données de référence. Cela n'est pas considéré comme un problème majeur étant donné qu'il n'y a pas de preuve solide que le stock de hareng de Mer d'Irlande se trouve en abondance à l'ouest de l'archipel des Hébrides en été. Des preuves historiques suggèrent qu'ils peuvent migrer dans le *Firth of Clyde* à cette période avant de retourner frayer en Mer d'Irlande en automne.

Les méthodes morphométriques utilisées dans cette étude indiquent que des différences significatives existent entre le stock de hareng de 6.a.S et le stock automnal de hareng de

6.aN, cependant leurs capacités à identifier chaque stock dans les échantillons estivaux collectés par la campagne scientifique en 6.a n'ont pas été démontrées.

Les marqueurs génétiques et les méthodes d'identification présentés dans cette étude constituent un outil précis (précision supérieure à 90%) qui peut être utilisé pour l'identification des harengs capturés par la campagne acoustique annuelle en été et la flottille commerciale qui pêche sur les zones de nourrissage et mélange des stocks. Cette approche devrait être utilisée de manière opérationnelle durant la campagne acoustique MSHAS et durant la saison de pêche dans cette zone et des efforts supplémentaires devraient être entrepris afin d'augmenter le nombre de marqueurs sélectionnés pour inclure le stock de la Mer du Nord.

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1. Introduction

In December 2017 the Executive Agency for Small and Medium-sized Enterprises (EASME), acting under the powers delegated by the European Commission, contracted University College Dublin (UCD), The Marine Institute (MI) and Marine Scotland Science (MSS) to undertake a 36-month study to assess the identity of the southern and northern herring stocks (within ICES Divisions 6.a, 7.b and 7.c), using genetic and morphometric analyses.

The general objective of the study, as detailed in the tender specifications (EASME/EMFF/2017/013), was to contribute to the achievement of Maximum Sustainable Yield (MSY) assessment and to the development of rebuilding or management plans for the herring stocks in ICES Divisions 6.a, 7.b and 7.c. The herring in this area is considered to consist of two separate stocks, but, due to unknown amounts of mixing outside of their respective spawning periods, it has not been possible so far to specify separate catch advice.

The specific objectives, as defined during the "kick-off" meeting and in the *Inception Report* were as follows:

- Genetically analyse spawning herring to determine if 6.a (North) and 6.a (South), 7.b and 7.c herring stocks comprise different populations.
- Ensure the genetic marker panel can discriminate 6.a.N and 6.a.S, 7.b and 7.c from adjacent stocks.
- Develop a robust genetic baseline of the 6.a.N and 6.a.S, 7.b and 7.c populations based on spawning samples collected over multiple years (at least 2014-2018).
- Optimise the genetic marker panel for screening of non-spawning putatively mixed samples.
- Screen mixed samples collected during the Malin Shelf Herring Acoustic Survey (MSHAS 2014-2019) and split them into their constituent stocks of origin.
- Conduct whole body and otolith morphometric analyses on the spawning herring from the first objective above and determine if these methods can split them into two groups.
- Compare the baseline classification based on genetics and morphometrics.
- If morphometric analyses can discriminate baseline samples then analyse the MSHAS morphometric samples (2010-2019).
- Compare the MSHAS sample classification based on genetics and morphometrics.
- If congruent, then attempt to retrospectively split the MSHAS time series.

These primary objectives were broken down into detailed tasks in sections 3.2-3.9 of the *Inception Report*. The description of each task and the deliverables were also detailed in the original *Full Technical Tender* submitted by the applicants. The final results of this study, divided by the specified tasks, are provided in the following report. The results of each task are preceded by the specified objectives and the deliverables of that task as defined in the original *Full Technical Tender* and the *Inception Report*. The preliminary analyses and results presented as evidence of progress in the *1st Interim Report* in November 2018 and the *2nd Interim report* in November 2019 are not presented here as they are superseded by the final analyses, which includes all the relevant information regarding the approaches used and the final results. Copies of the *1st* and *2nd Interim Reports* are available upon request from EASME or from the lead author of the current report.

Please note that the UCD research group commenced studies on the genetic stock identification of herring around Britain and Ireland in 2015 through a series of industry funded projects (see Section 2.7). The data for the samples collected and analysed prior to the initiation of the EASME funded project in December 2017 have been included in the final analyses presented in this report.

2. Task 1 – Literature Review

2.1. Objectives

- Review available publications regarding the study topic.
- Summarise the advantages and disadvantages of different methodologies and lessons learned.

2.2. Deliverables

- Literature review as part of the inception report*:* ***Completed***
- Peer reviewed publication on the history of herring in ICES Divisions 6.a, 7.b and 7.c: ***In preparation***

2.3. Stock assessment and management

There are two basic assumptions of stock assessment. Firstly, that the stock is a closed unit i.e. there is no immigration or emigration; and secondly, that the data used in stock assessments are representative of the entire stock i.e. catches are not removed from certain components only and survey data are a relative measure of the entire stock throughout its geographical distribution. For this reason, stock identification has been an important prerequisite for fish stock assessment throughout its history (Cadrin and Secor, 2009). However, the central fundamental weakness that remains in many existing stock assessments is the inaccurate recognition, definition and delineation of 'stocks' for data collection and aggregation. Traditionally, exploited stocks have been defined, assessed and managed according to geographical and political features or regions. As more information becomes available, it is evident that the temporal and spatial distributions of most fisheries resources are not aligned to these artificial divisions (Kerr *et al*., 2016). It is also evident that biological populations, defined as groups of organisms of one species that interbreed and live in the same place at the same time, are more dynamic and complex (Reiss *et al*., 2009; Stephenson, 2002). The mismatch between the scale of a biological population and that of a management unit or 'stock' can hamper the development and implementation of effective management (Cope and Punt, 2011).

In EU fisheries management the term 'stock' is defined as 'a marine biological resource that occurs in a given management area' (Anon, 2014), hence it is a management definition rather than a biological definition. In EU terms a single 'stock' may comprise a number of reproductively isolated biological populations of the same species that are caught in mixed aggregations and are assessed and managed as one complex, such as is the case with Atlantic herring (*Clupea harengus* Linnaeus, 1758) west of Ireland and Scotland (ICES, 2015a). Whilst delineation by predefined area may be more convenient for management and regulation purposes, accurately assessing the status, biomass and sustainable exploitation rates of mixed 'stocks' is inherently difficult if not impossible as they do not correspond to biological units. Fisheries dependent and independent data may be confounded in such mixed 'stock' scenarios, which may mask changes in the abundance of individual populations and lead to biased estimates of population abundance, and consequently overexploitation of smaller populations (Hintzen *et al*., 2015). It is, therefore, critical to be able to assign individuals in mixed survey and commercial catches to the biological population unit to which they belong (Casey *et al*., 2016; Hintzen *et al*., 2015).

2.4. Atlantic Herring

The Atlantic herring is arguably the most important fish in human history, particularly in northern European waters where they have been targeted by large scale commercial fisheries for centuries (Mitchell, 1864). It is a pelagic species of the Subfamily Clupeinae and is distributed in continental shelf waters in the Northeast Atlantic from the northern Bay of Biscay to northern Norway, including Iceland and the Baltic Sea and in the Northwest Atlantic from southern Greenland to the coast of North Carolina. Herring are demersal spawners (Blaxter and Hunter, 1982) and appear to favour gravel type substrate

or seaweed for spawning (De Groot, 1980). Herring typically congregate near their spawning grounds for several weeks to months prior to spawning (Haegele and Schweigert, 1985). In the northwest Atlantic, herring spawning grounds have been observed to be located in high-energy environments, either nearshore for spring spawners or in tidally active areas for fall spawners (Haegele and Schweigert, 1985). Herring are known to spawn somewhere in the northeast Atlantic during every month of the year (Hodgson, 1951) and individual population units are believed to display fidelity to spawning season and site (Iles and Sinclair, 1982).

There is a long history of research into the characterisation of these herring populations, using a wide variety of different techniques: life-history characteristics, morphometric and meristic characters, of whole body and otoliths, parasite analyses, physical tagging and genetic techniques (see McQuinn, 1997). The focus of much of the early research was on the North Sea and Baltic areas, where the earliest commercial fisheries for herring were centred. Whilst many of the approaches recognised a distinction between autumn and spring spawning herring and purported to offer reliable methods of discrimination between different populations, the reality is that confusion surrounding the population structure in herring across its distribution has persisted. This included, until recently, a lack of consensus over the distinctiveness of herring spawning types and population structure. Two opposing theories of Atlantic herring structure were initially developed, the discrete population concept that suggested the timing of spawning in herring represents an adaptation to the quality of larval retention areas and is thus a predetermined trait that maintains segregation of seasonal spawning populations (Iles and Sinclair, 1982); and the dynamic balance concept that considers herring populations to be a heterogenous group of individuals of numerous genetic combinations (Smith and Jamieson, 1986). Neither of these concepts is believed to adequately explain herring population structure and dynamics. More recently it has been suggested that the population structure and dynamics of Atlantic herring are better described within the metapopulation concept (McQuinn, 1997), where population structure in a given area can be considered as an array of local populations linked by variable degrees of gene flow. Whilst Iles and Sinclair (1982) considered that the genetic isolation of population units of herring must involve the spawning stage and may also involve the pre-spawning aggregatory stage, McQuinn (1997) stated that population affinity was established at the time of first maturation and was fixed for all subsequent spawning by adhering to an annual maturation cycle.

The latest research, utilising genome sequencing analyses (Lamichhaney *et al*., 2017; Martinez Barrio *et al*., 2016), has confirmed that autumn spawning and spring spawning are two genetically distinct reproductive strategies in herring and that Atlantic herring populations are characterised by a high level of local ecological adaptation and strong population structure. Further, genetic markers associated with loci under selection have been proven to provide a much better resolution to distinguish population structure than random neutral genetic markers (Han *et al*., 2020). Gene flow or 'straying' between sympatric populations of autumn spawning and spring spawning herring has been observed in the northeast Atlantic and northwest Atlantic herring (Berg *et al*., 2020; Kerr *et al*., 2019) though the overall rates were low. The viability of hybrid offspring is unclear.

There are approximately 30 populations of herring described in the North Atlantic (Hay *et al*., 2001) and two of the main factors that define these populations is their adaptation to using discrete spawning periods at specific spawning locations (Geffen, 2009). The waters around Ireland and Britain are inhabited by the southernmost ecomorphs of herring in the Northeast Atlantic that spawn in warmer seawater than other herring populations (Han *et al*., 2020). They are genetically distinct from the oceanic herring, including Norwegian Spring Spawning herring, to the North and the Baltic herring to the east (Han *et al*., 2020). It is now possible to resolve the finer level population structure of herring in the waters around Ireland and Britain and to at least identify biological populations before choosing to aggregate or separate them for the purposes of data collection and stock assessment.

2.5. Herring around Ireland and Britain – Current stock definitions

Commercially important seasonal fisheries for Atlantic herring take place in many different areas around the coasts of Ireland and Britain. The herring in these areas are divided into five stocks for assessment and management purposes (Figure 2.1). The North Sea autumn spawning stock (ICES Subarea 4, Divisions 3.a and 7.d.) is the most abundant and well-studied of these (Simmonds, 2009) and considered to be a complex of four spawning components (Annex 2: the autumn spawning Shetland/Orkney, Buchan, Banks components and the winter spawning Downs component), which are largely managed as one unit (Dickey-Collas *et al*., 2010; Simmonds, 2009). A sub-TAC (Total Allowable Catch; 11% of total North Sea TAC) is set for the Downs component in divisions 4.c and 7.d to protect Downs herring on their spawning grounds. Provision is also made for potential mixing with Western Baltic Spring Spawning Herring in division 3.a though no consideration is given to potential mixing with 6.a.N autumn spawning herring.

The definition of the western stocks has changed considerably over the last five decades (see ICES 2015a; Molloy, 2006) and the main stocks are currently recognised as: 6.a.N; and 6.a.S, 7.b and 7.c; Division 7.a North of 52°30'N (Irish Sea); Divisions 7.a South of 52°30'N, 7.g, 7.h, 7.j and 7.k (Irish Sea, Celtic Sea, and southwest of Ireland) (Figure 2.1; ICES, 2014). The 6.a.N herring spawn in Autumn (late Aug to early Oct) off Cape Wrath on the northwest coast of Scotland, the 6.a.S, 7.b and 7.c herring spawn in winter (Nov-Jan) primarily off the coast of Donegal, Irish Sea herring spawn in Autumn (Sept/Oct) mainly on the Douglas Bank east of the Isle of Man and Celtic Sea herring spawn in winter (Nov-Jan) off Cork and Waterford on the south coast of Ireland. A number of small groups of spring spawning (Feb-May) herring are recognized in the Minch, the Clyde and Milford Haven (Annex 2), though these are not currently assessed and only the Clyde herring stock is managed, with TAC setting responsibility delegated to Scotland under the provisions of the European Commission TAC and Quota Regulations (http://data.europa.eu/eli/reg/2019/124/oj). There have been no catches reported from the Clyde since 2013 (ICES, 2019a), the Minch spring spawning herring is reported to have collapsed in the 1950s (Baxter, 1958), though in March 2019 herring spawning was observed near Gairloch on the west coast of Scotland. Other autumn/winter spawning herring groups are found in the western English Channel and Bristol Channel (Divisions 7e and 7f, respectively), though no assessments are made of these groups.

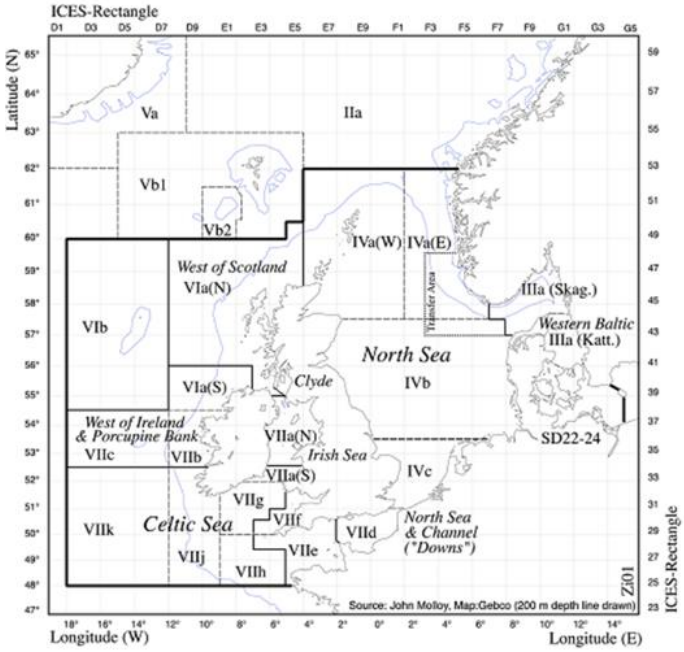


Figure 2.1. Current defined assessment and management areas for herring stocks around Ireland and Britain. From ICES, 2014.

The management stock divisions described above are largely based on the recognition of temporal and spatial differences in spawning season and grounds and are believed to broadly align with biological population structure (ICES, 2015a), though some geographic and political boundaries are still in place and the mixing across these boundaries is unclear. This is particularly evident in ICES Division 6.a, where the 6.a.N autumn spawning stock is separated by the 4° west line of longitude from the North Sea autumn spawning stock. Within Division 6.a the herring are subdivided into two stocks (Figure 2.1) by the 56° north line of latitude and 7° west line of longitude (ICES, 1982). Herring caught or surveyed to the north or east of this boundary in Division 6.a (excluding the Clyde area) are included as part of the 6.a.N autumn spawning stock regardless of their population of origin or their spawning type. This includes herring caught in Lough Foyle, whose waters are bisected by the 7° west line. Most of Lough Foyle is west of this line, however the mouth of Lough Foyle is east of this line and hence the herring in the whole lough are considered to be part of the 6.a.N autumn spawning stock despite having no obvious biologic or geographic proximity to this population. Herring caught to the south and west of the 56° and 7° lines are considered to be part of the 6.a.S stock in combination with herring in Divisions 7.b and 7.c.

Herring from the separate stocks, both within Division 6.a and possibly from adjacent stocks are believed to form mixed aggregations on common feeding grounds to the west of the Hebrides during summer (Hatfield *et al*., 2005). It is during this time that they are surveyed by the Malin Shelf Herring Acoustic Survey (MSHAS), which is part of the internationally coordinated Herring Acoustic Survey (HERAS). The inability to assign herring catches from the MSHAS into their population of origin prevents the development of separate indices of abundance for the two stocks. At present ICES is unable to distinguish between the herring stocks in Divisions 6.a.N and 6.a.S, 7.b and 7.c in commercial catches or research surveys. This means that ICES has to conduct a combined assessment of these populations and provide combined management advice, leading to a combined quota and management. However, based on survey and commercial data and previous research (ICES, 2015a), ICES and the scientific community all agree that the two stocks are indeed discrete populations. Combined management of what amounts to an amalgam of separate populations is a dangerous situation, as such management can only be precautionary if the two populations are of similar size and are homogeneously distributed together in commercial catches. If these conditions are not met, the smaller population will suffer a higher mortality than would be sustainable and will eventually decline (Hintzen *et al*., 2015).

2.6. Early research and origin of the stock definitions

Extensive research on the identification of populations and delineation of stocks has been conducted on herring since the 19th century and numerous methods applied. It is informative to present a brief synopsis of this research in order to identify historical trends in distribution and also potential sources for the confusion regarding herring in ICES Divisions 6.a, 7.b and 7.c. This is not intended to be an exhaustive review but is intended to highlight the origin of the stock boundaries, which are used to segregate catch and survey data for stock assessment purposes.

Whilst much of the early research focussed on the Baltic herring, where the most important early fisheries were located, interest soon spread across the North Sea as fisheries expanded in British waters. Mitchell (1864) documented the periodical visits of the herring to the areas around Ireland and Britain. On the north and west coasts of Scotland, herring were noted to arrive on the coast and into the lochs in two periods, from July to September and from November to January or later. At Killybegs the herring were noted to be in abundance from the end of December to the beginning of March. Further south along the coast the herring were less predictable and appeared in winter in some years but not in others, though in Galway Bay the fishery was reliable and started in September. Andrews (1866) described two seasons of herring in Ireland, the summer or 'harvest' herring that approached the coast in late summer and spawned in September and October and the

winter herring that spawned from January to February. The west coast bays of Donegal, Galway and Bantry were noted for abundant winter fisheries. Huxley (1881) stated '*Taking all parts of the British coast together, February and March are the great months for the spring spawning, and August and September for the autumn spawning. It is not at all likely that the same fish spawn twice in the year; on the contrary, the spring and the autumn shoals are probably perfectly distinct*'. Green (1884) noted that as a general rule winter herring spawn in February and March and summer or autumn herring in September and October. Munro (1884) stated that the best quality herring were to be found on the west coast of Scotland in Loch Broom, Loch Hourne and Loch Fyne, which were fished in the early part of the year. The Clyde herring were noted to arrive regularly in February to spawn on the Ballantrae Banks, however the main season for herring was from July to September on the east coast of Scotland and England.

In the northern part of Division 6.a the earliest systematically documented references to the herring fisheries are the annual Fishery Board for Scotland reports. These reports provide detailed accounts of the landings from the herring ports around the Scottish coast and also provide details of early scientific studies. The reports from 1885 to 1920 are available on the Biodiversity Heritage Library (https://www.biodiversitylibrary.org/). Ewart (1884) noted two spawning periods of herring in Scottish waters and concluded that the autumn spawning was primarily on the east coast and the winter or spring spawning primarily on the west coast. Matthews (1885) also described the spawning times of the herring caught in the different areas around Scotland. On the west coast the herring in Loch Broom, in the north Minch, and Girvan, in the Clyde, were observed to be in ripe condition from January to March and in Stornoway, in the North Minch, and Campbelltown, in the Clyde area, in April (Annex 2). Further spring spawning fish were also caught in a number of places along the east coast including the Firth of Forth. Spawning fish were observed along the whole of the east coast in August and September and later in October and November in Loch Fyne in the Clyde area. Matthews concluded that spawning fish were to be found in every month of the year in some part of the Scottish coast but that there were two principal spawning periods, which could generally be called the winter and summer periods. The months of April to July were noted for fewer spawning fish and a large proportion of immature and spent fish. By studying these early reports, a clear picture emerges about the prevalence and wide distribution of herring in the waters to the west and east of Scotland, even as late as the 19th century, and also of the general differences between the different spawning areas and times.

Baxter (1958) detailed the composition of the Minch herring stocks and identified the main spawning and fishing grounds in the area. Baxter (1958) stated that the Minch herring population was composed of a mixture of spring and autumn spawning herring. The spring spawners were resident and spawned primarily on the northwest coast where they were targeted in a spring fishery in February and March (Figure 2.2). Between June and August, the fishing was primarily in the North Minch and catches comprised maturing autumn spawners and immature adolescents of spring and autumn spawners. Between September and May the grounds along the east coast of the Hebrides were fished for spent and recovering autumn spawners, with catches also containing some maturing spring spawners and adolescents of both groups. In April and May there appeared to be a movement of adult herring out of the Minch. The autumn spawners in the Minch were not targeted at spawning time. Autumn spawners were suggested to be comprised of two groups; a resident Minch autumn spawning group and a group which does not spawn in the Minch but spends winter there as recovering spent fish. The latter group was suggested to arise in part at least from the north western North Sea because of similarities in many of the biological characters such as growth, otolith characters, vertebral counts etc. Baxter (1963a) reported that the abundance of spring spawning fish fluctuated in the north Minch with periods of high abundance in 1920s, late 1940s and early 1950s and periods of low abundance in the 1930s, early 1940s. Since 1955 there was almost no fishery for spawning herring in the north Minch in February and March and instead spent autumn spawning herring dominated the catches though they were not observed to spawn in the area.

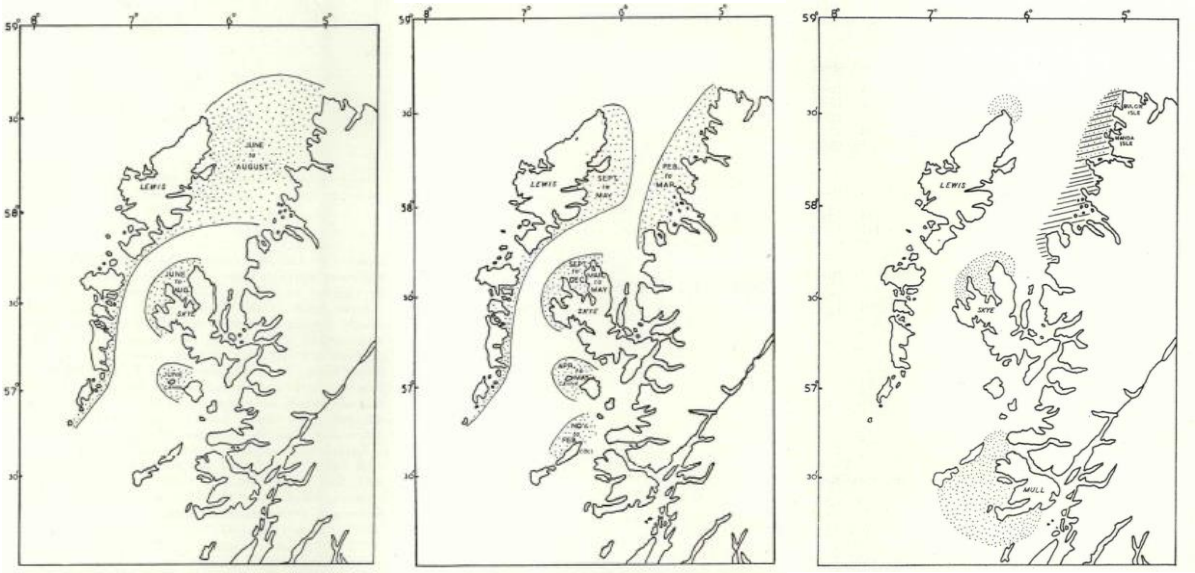


Figure 2.2. Herring fishing grounds in the Minch (left panel) between June and August. (middle panel) between September and May. (right panel) Spring (lines) and autumn (dots) spawning grounds in the Minch. From Baxter, 1958.

The fluctuations in the abundance of the spring spawning herring in the Minch was also observed in the waters around Donegal during the same period (see Clarke *et al*., 2011). Farran (1930; 1937; 1944) reported on the herring fishery in Donegal for the period 1921 to 1941. The main fishery was in winter or early spring and started in December or January when the herring were shoaling for spawning. The majority of fish would be spent by March however and they would not be targeted until they regained condition in May and June, after which they moved offshore beyond the reach of the fishery. A small autumn fishery was also noted to occur in the area in September and October and this was closer to shore and had a shorter season. As was seen in the north Minch area the fishery in Donegal also underwent a composition change and Bracken and Phillips (1970) reported that since 1955 the catches were dominated by autumn spawning herring that were caught from October onwards as spent and recovering fish. Elsewhere estuarine spring spawning herring fisheries in the Firth of Forth collapsed in the 1940s and in the Clyde suffered serious declines around this time (Saville, 1961).

In an effort to further elucidate the movements of herring between the different areas tagging experiments were carried out both in the South Minch in 1961 and in the Clyde in 1961-1962 (Saville, 1962; Baxter, 1963b). At the time the herring fishery in the Clyde was based almost entirely on spring spawning herring which spawned locally at Ballantrae Bank in the Clyde between February and mid-April. Prior to the tagging experiments it had been suggested that Clyde spring spawners left the Clyde after spawning as there was a lack of spent fish and a pre-dominance of immature fish in post spawning catches (Wood, 1960). In the experiments some herring tagged in the South Minch were recovered in the Clyde, and some herring tagged in the Clyde were recovered in the Minch, to the north-west of Ireland, and in the Irish Sea, indicating a degree of movement between these different areas.

Saville *et al*.'s (1966) review of the herring fisheries and stocks off the west coast of Scotland largely agreed with the aforementioned research. The Scottish fishery took place primarily in the Minch and in the Firth of Clyde. The Minch fishery occurred in all months of the year but had two main seasons; summer fishery (May to September) on maturing autumn spawners in the open waters of the Minch and in sea lochs and late 'autumn-winter' fishery (November to March) on spent and recovering herring. The authors noted

that there used to also be a fishery in February and March on spring spawning herring but that this fishery had ceased since the early 1950s due to the disappearance of the spawning shoals. The autumn spawned herring were the major component of the Minch catches throughout the year with spring spawners rarely exceeding 20% of catches (discriminated by otolith analysis). Similar to the findings of Baxter (1963a) spawning fish were absent from the catches in the north Minch, suggesting that autumn spawning was taking place outside of the area. Saville *et al*. (1966) also noted that the Clyde fishery took place throughout the year with seasonal peaks in summer (June-September) on adolescent herring and in winter (January to March) on pre-spawners and spawners. The scarcity of autumn spawners amongst older fish in the Clyde catches suggested that the area was simply a nursery area for an autumn spawning stock whose main adult distribution was outside the Clyde. During the adolescent stage the fish appeared to be retained wholly within the Clyde estuary and chiefly within its inner reaches.

By the mid-1970s the racial composition of herring landed in the Clyde had changed very considerably from that at the time of the early tagging experiments in the early 1960s (ICES, 1978). The presence of autumn spawning herring in the Clyde during this period was confirmed and, in this period, they formed a major component of the catches. A new tagging experiment (1975-1977, 1979) was established to investigate the relationship between the Clyde and adjacent areas. The majority of tag returns from the four tagging experiments were recorded from the Clyde itself, but recaptures were also reported from the Irish Sea, the Minch, and from the Donegal and Mayo coasts to the north-west of Ireland. From those returns outside of the Clyde, the majority came from the Irish Sea. In particular herring tagged in the Clyde in Summer (May and July) in 1977 and 1979, were recovered on the Douglas Bank and in the Mourne area in spawning condition in autumn (Morrison and Bruce, 1981). This clearly demonstrated that the Irish Sea autumn spawning herring provided a component of the herring population in the Clyde during the summer months. Further tagging studies also revealed movement between Donegal and the Celtic Sea, the Irish Sea and the Celtic Sea, Donegal and the Irish Sea and also between the north Minch and the North Sea (Molloy *et al*., 1991; Morrison and Bruce, 1981; Saville and Bailey, 1980). Perhaps the most extensive tagging programme was the Bløden tagging experiment undertaken between July 1969 and March 1970, during which 57,496 juvenile herring were tagged in the south-eastern North Sea (ICES, 1975). Approximately 12% of the tagged herring were recaptured over the following four years and the recovery of tags from areas to the west of Orkney and Shetland and in 6.a.N was a notable feature in all years. The results demonstrated that the south-eastern North Sea was a nursery area for fish which may subsequently recruit to the areas west of Shetland and the north Minch (ICES, 1975).

Around this time there was increased focus on the autumn spawning herring in 6.a.N due to the decrease in catches of North Sea autumn spawning herring and a redirection of effort to Division 6.a. Trawl surveys carried out in Division 6.a during late summer and autumn from 1962-1964 indicated that both spring and autumn spawning herring were to be found in the area but 75% of the herring caught were considered to be autumn spawners (Wood, 1971). It should be noted that trawl surveys were not conducted at a time of year when spring spawners would have been found inshore preparing to spawn. As a result, the primary focus for subsequent scientific studies in Division 6.a shifted almost exclusively to autumn spawning herring and to the identification of spawning areas. Efforts were made to locate the spawning grounds of the autumn spawning herring through larval surveys first undertaken in September and October 1965 (Wood, 1972). Significant abundances of small larvae were detected off Cape Wrath, west of the Hebrides and off Donegal suggesting that spawning had occurred recently in these areas. The sampling was not however extended toward the 4°W boundary and as such an incomplete picture of the distribution of larvae was observed. The survey was repeated in 1971 and the results indicated a similar picture of larvae distribution as the 1965 surveys (Wood, 1972). One notable difference was the extension of survey stations up to the 4°W boundary with the North Sea stock and the observation of high-density patches of larvae in this area. Wood

(1972) stated that this '*patch was almost completely separate from the high-density patch just west of Orkney which had been surveyed during the preceding two days*' as part of the International survey of herring larvae in the North Sea. Wood (1972) did not present the data from the North Sea survey and the distribution maps were bounded by the 4°W line of longitude. Examination of the complete distribution map from the Report of the International Survey of Herring Larvae in the North Sea and Adjacent Waters 1971/1972, illustrates a continuity of distribution across the boundary (Schnack, 1972). Wood (1972) did however concede that '*it is quite probable that many of the larvae which originate from the Butt of Lewis – Cape Wrath area in early September are carried eastwards where they would later be included in the estimates of abundance for northern North Sea Herring larvae*'.

The first International survey of herring larvae in the North Sea was conducted in 1967 and in the early years of the survey sampling was sometimes extended across the 4°W boundary. It is somewhat surprising that Wood (1972) did not discuss the putative boundary between Divisions 6.a and 4.a in more detail, as in the 1970/1971 survey the centre of distribution of larvae to the north of Scotland traversed the boundary and a very high density of larvae were found to the west (Figure 2.3; Zijlstra, 1971). This pattern of high density larvae patches in the Cape Wrath area west of the 4°W boundary was also evident in the 1973/1974 survey (Wood, 1974), 1974/1975 survey (Pommeranz, 1975) and the 1975/1976 survey (Jaconsen and Hansen, 1976). This area was not surveyed in the 1972/1973 survey (Saville and McKay, 1973) however a high density of larvae immediately east of the 4°W boundary was observed. This area was not surveyed from 1976 to 1980 and as this was a period of documented collapsed of North Sea herring, larvae abundances were low throughout the North Sea in general and during the survey they were primarily recorded around Orkney and Shetland.

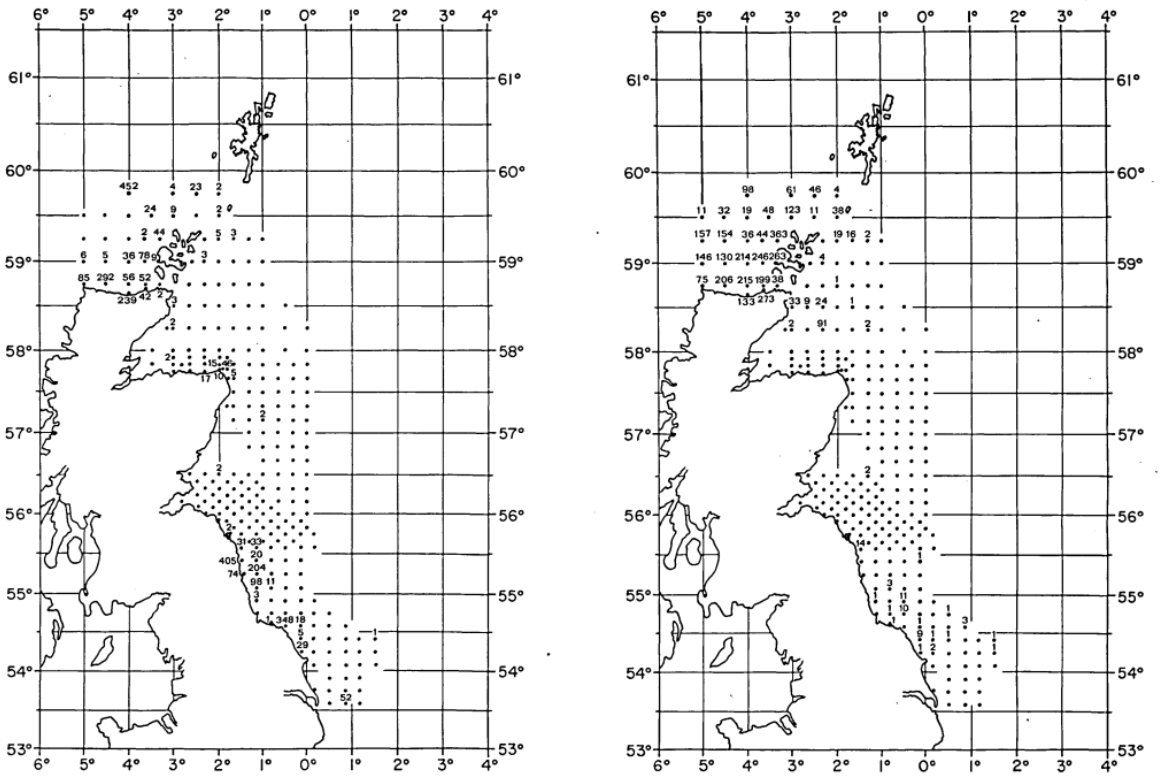


Figure 2.3. Numbers of herring larvae (left panel) <10mm long and (right panel) 10-15mm long below 1 m^2 surface in the western North Sea, 8– 29 Sept 1970. From Zijlstra, 1971.

Saville (1970) reported that in the late 1960s a large proportion of the Scottish herring catch from the Shetland area (North Sea autumn spawning herring) had been taken from grounds situated to the west of Shetland. In the years 1960-65 the proportion of the Scottish catch taken on west Shetland grounds was always less than 22% of the total from the Shetland area and averaged about 12.5%. Since 1965 the proportion taken on these western grounds was much higher in every year except 1968 and has averaged about 34% of the total Shetland catch. It is also clear that in the earlier period, except in 1960, the western grounds gave a significant proportion of the total monthly catch only in May. Since 1965 the fishery west of the islands made its biggest contribution to the Shetland catches in June and was also productive in July. The fisheries data from this area were confounded by the presence of significant quantities of spring spawning herring (up to 38%), which were more abundant in the western area and in the North Minch as previously described (Saville, 1970). As the autumn spawning component was more abundant the analyses were restricted to that group and efforts were made to separate the spring spawners out of the data for autumn spawning herring. This was an imperfect process though and Saville (1970) noted that '*the data available for stock identification are inadequate for discriminating between closely related stocks and the various criteria used in this paper give rather conflicting results.*' The resulting data assumed to be for autumn spawning herring was contaminated to varying degrees, depending on area, by spring spawning herring. Despite this Saville (1970) concluded that '*the data available would suggest that there is a distinct possibility that the stock fished to the west of Shetland is distinct from both the east of Shetland and the Minch herring*'. Hence the 6.a.N autumn spawning herring stock was introduced as a distinct stock from the North Sea autumn spawning stock.

Whether the increase in catches west of Orkney and Shetland (Savile, 1970) indicated a shift in distribution of the herring, a contraction of the stocks or a shift in distribution of fishing effort is unclear and it was perhaps a combination of these factors. The results of the herring larvae surveys in the late 1960s and 1970s support the assertion that these were still primarily North Sea autumn spawning herring that were being fished in the area west of Orkney and Shetland and that the centre of distribution of spawning of North Sea herring underwent temporal spatial changes. In response to the changes in the fisheries and the decrease in the North Sea stock, the Liaison Committee of ICES requested the North Sea Herring Assessment Working Group to meet and consider the state of the herring stocks around Ireland and northwest of Scotland for the first time (ICES, 1970). As this was undertaken during a period when autumn spawning fish dominated the catches in Division 6.a and had already been described by Saville (1970) these were the primary focus of the work. It should be noted that during this period major changes were also underway in the herring fisheries around Ireland and Britain with a shift away from drift and ring netting towards trawling and pair trawling (ICES, 1970). This meant the fisheries were more concentrated further offshore and taking place in areas that had not previously been targeted heavily. The group considered both the Celtic Sea herring and the Division 6.a herring and concluded that the Celtic Sea herring were a separate winter spawning stock. The autumn spawning herring in Division 6.a were considered to be one unit. Stock abundance estimates were made based largely on comparison of larvae abundance from the area with that of the North Sea and were not considered robust. Further assessment work was undertaken in the following years, though confusion regarding the boundary between the 6.a stock and the North Sea stock in Division 4.a seems to have persisted with the ICES Report of the North Sea Herring Assessment Working Group (ICES, 1972) concluding that '*on the basis of the available data it is not possible to state categorically where the western boundary of the North Sea herring stocks should be drawn*'.

Saville and Morrison (1973) presented a reassessment of the herring stocks west of Scotland and also noted that '*..the validity of the 4°W boundary as the dividing line between the herring stocks in these two areas is open to question*' and that there was '*increasing evidence that an appreciable part of the recruitment to the herring stock in area VIa spend their juvenile stage in nursery areas within the North Sea*', a conclusion

based largely on the findings of Saville (1971). In more recent times this particular issue has been confirmed as larvae from known spawning grounds in the north of Division 6.a (Rankine, 1986) have been observed to be transported in an easterly direction by the Scottish Coastal Current into the North Sea (Heath, 1989; Heath, 1990; Heath *et al*; 1987). Regardless of available evidence, a separate assessment was conducted for Division 6.a by Saville and Morrison (1973) and catches in the area were observed to increase from approximately 65,000 tonnes in the period 1957-1965 to over 200,000 tonnes in 1971 and 175,000 tonnes in 1972. Mention is made of a Scottish tagging experiment in the area around the 4°W boundary that appears to show a split in the movement of herring between Divisions 6.a and 4.a but it has not been possible to locate any primary material with details of this tagging study.

In 1974 ICES decided to increase the remit of the North Sea Herring Assessment Working Group to include the '*Celtic Sea Herring*' and the '*Herring in Division VIa*' as separate stocks and thus the Herring Assessment Working Group for the Area South of 62°N (HAWG) was born. Molloy (2006) provided a detailed account of the working groups main findings and invaluable insights into discussions up to 2005 and as such only the key references will be included here. Molloy (2006) summed up the experience of the working groups succinctly by saying '*I often wonder at what I achieved during the period that I spent attending HAWGs and ACFM! After thirty years of assessments the state of all the stocks around Ireland is unknown! There is still a lack of biological data, a lack of survey data but no lack of uncertainty about the catches. There is an absence of recruitment information, maturity ogives have remained constant for thirty years despite major changes in growth rates, and values of natural mortality are still assumed to be as they were in the 1960s*'. The herring in Division 6.a continued to be assessed as a single stock within HAWG over the following years, despite Irish scientists noting differences between the herring in 6.a.N and those off the northwest coast of Ireland (Molloy, 2006). In 1978 HAWG recommended that all herring fishing in Division 6.a should stop in 1978 and 1979 due to a sudden decline in the stock. This mirrored what had happened previously with the North Sea stock (Simmonds, 2009), which is not surprising as the majority of 6.a catches were taken on the boundary with the North Sea and as described above were very likely the same population. Also of note in 1978 were the discussions about the relationship between herring in Divisions 7.b and 7.c and 6.a, as there was an increase in catches in the more southern areas. The Irish scientists had suggested that two separate stocks existed in Division 6.a and that the southern stock was closely aligned with the herring in Divisions 7.b and 7.c (Grainger, 1978). It was eventually decided to conduct a separate assessment for Divisions 7.b and 7.c and the southern part of Division 6.a, south of 57°N and west of 7°W, as the fisheries in these areas and those off the northwest of Scotland in 6.a.N were largely separate. This indicated that there was an urgent need to restrict catches in the area however the newly proposed assessment area was not accepted and further research was recommended in the area.

With both the North Sea and Division 6.a herring fisheries closed due to significant declines in stock biomass, the boundaries between the different stock areas were again revisited. Saville and Bailey (1980) reviewed the assessment and management of the herring stocks in the North Sea and to the West of Scotland and noted that '*the dividing line between VIa and the North Sea (sub-area IV) at 4°W longitude was not chosen on any criterion of herring stock differentiation but for convenience in statistics collection. In recent years there have been major herring fisheries during summer on either side of this dividing line, with catches taken east of 4°W being allocated to the North Sea and those west of 4°W to VIa. It is unlikely that this arbitrarily chosen boundary firmly demarcates the distributions of the two stocks. If the two populations are to be assessed separately, however, some demarcation line must be drawn, and such evidence as is available would suggest that 4°W is as good a boundary as can be found*'. The evidence cited for this boundary was again a Scottish tagging experiment in the area around the 4°W boundary that appeared to show a split in the movement of herring between Division 6.a and 4.a but no citation is provided for this study and no detail is provided on the number of fish tagged or the time

between tagging and recapture etc. Therefore, the validity of the data cannot be evaluated. The second evidence provided by Saville and Bailey (1980) states '*Sampling of herring in the area west of Shetland for parasite infestation rates, using McKenzie's technique, has shown that the adult herring east of 4°W have rates typical of North Sea herring. Inevitably VIa recruits which have spent their nursery period in the North Sea must, on their return migration, travel through the area of the Shetland fishery. The few returns of herring tagged in sub-area IV which have been recaptured in VIa can probably be explained in this way.'*. It is unclear what is meant by this statement, but the authors may be suggesting that the recruits pick up infection as they pass by North Sea herring. This assertion is not supported by the extensive parasitological work undertaken by MacKenzie (1985). It is evident that there was still little support for retaining the 4°W boundary delineating the North Sea autumn spawning herring and the purportedly separate 6.a.N autumn spawning herring. There appeared to be a reluctance to remove it though and this may also be related to the inability or lack of impetus to further subdivide the northern and southern parts of 6.a, despite mounting evidence for such a division. Saville and Bailey (1980) noted that within Division 6.a the '*adult fisheries can be divided into two clearly demarcated components: a summer/autumn fishery in the period May-October on the offshore feeding and spawning areas*' and '*a winter/spring fishery in the coastal areas to the northwest of Ireland and off the west coast of Scotland'*. However, they argued against the subdivision into northern and southern components for assessment and management purposes as there would be a '*distortion caused in the assessments if there is an emigration of fish between them.'*. This is somewhat surprising as despite the strong evidence of connectivity across the 4°W boundary they still fervently defended the retention of two separate assessments in that area. Further analyses were presented in an attempt to explain the rapid collapse of the two stocks but it must be remembered that all the input data for Division 6.a, at least, were confounded by the lack of account for the significant proportion of spring spawning herring and also the temporally variable movement of North Sea herring across the 4°W boundary. It was tentatively suggested that there may be significant emigration, increasing with age, from nursery areas in the North Sea to the 6.a stock, which cannot be ignored if realistic assessments are to be conducted.

At the 1981 HAWG (ICES, 1981) a joint assessment of herring in Divisions 7.b and the southern part of 6.a was again attempted as the fishery in the area was taking place across the border of the two divisions and the catch composition was determined to be the same. Molloy (2006) noted that the '*more conservative members of the group were reluctant to carry out any assessment for this new area because it meant that the existing data sets on ages and catches would have to be reconstructed for the remainder of Div. VIa*'. It was ultimately decided that a new assessment of a combined stock of Divisions 7.b and 6.a, south of the 56°N and west of the 7°W (Figure 2.1) would be presented at the 1982 meeting. Therefore, in 1982 HAWG conducted assessments on '*West of Scotland Herring*' and '*Herring in Divisions VIa (South) & VIIb,c*' for the first time (ICES, 1982). The separation of these areas was to persist in HAWG up until the 21st century when they would again be questioned as a result of the WESTHER project (Hatfield *et al*., 2005). It is important to highlight that the definition of the '*West of Scotland Herring*' stock and consequently the fishery changed considerably over the course of the 20th century as illustrated in the summary of the research above. What was, prior to the 1960s, a fishery based in the Minch primarily using drift nets and ring nets and targeting spring spawning herring in the winter and also spent and recovering autumn spawning herring, became an offshore trawl fishery conducted mainly off Cape Wrath and was for all intents and purposes the same as the North Sea fishery. The 6.a.S, 7.b and 7.c fishery remained for the most part a coastal fishery and fished the same grounds as were fished in the early 20th century (Molloy, 1983) though changes were noted in the proportions of the spawning types that dominated the catches. Having shifted from spring spawning in the early 20th century to autumn spawning in the 1960s there was a further shift noted in the 1980s when non-autumn spawning fish began to compose an increasing proportion of the catch (Molloy, 1983). These fish were mainly caught from December to March as spawning and

pre-spawning fish (Molloy, 1983). In the 1990s winter spawning herring began to dominate the catches (ICES, 2015a). It was not clear if these shifts represented changes in the proportion of distinct spawning components or simply a shift in the spawning time of a single population.

Despite improving the definition of the stocks in Division 6.a, by splitting the area into northern and southern stocks, the lack of a reliable survey index in both areas was causing issues for the assessments. The larvae survey, which resumed in Division 6.a in 1982 (Wood, 1982), continued to show a continuity in the distribution of larvae across the 4°W boundary. In 6.a.S, 7.b and 7.c in particular, the larvae survey was not providing an accurate picture of the abundance in the area as a significant proportion of spawning was now taking place after the surveys had finished in November (Molloy, 1983). Larvae surveys in this area, which had been conducted by Ireland from 1981-1988 were abandoned in 1989 (ICES, 1990).

An international coordinated summer acoustic survey was initiated in the North Sea in June and July 1980 (ICES, 1980) in order to estimate the abundance of adult herring as they aggregated on the summer feeding grounds prior to spawning in the autumn. An exploratory acoustic survey was also conducted in a small area of 6.a.N in 1983 though this was undertaken in November as it was combined with an acoustic survey for mackerel aggregations that had been found near the shelf edge to the northwest of Scotland (Heath, 1984). The 6.a.N survey also used parasitology to attempt to differentiate the herring caught into their stock of origin. The main conclusion was that '*fish originating from nursery grounds in the sea lochs on the west coast of Scotland do not appear to make any significant contribution to the adult stock in VIa(N) and it is suggested that these fish may join the adult population distributed along the north coast of Ireland (VIA(S)).'*. The survey was repeated in November 1985 (Heath and Copland, 1986) and extended to cover the complete area of the 6.a.N stock. The majority of herring were observed off the south west of the Hebrides in close proximity to the 56°N boundary with the 6.a.S, 7.b and 7.c stock. The November survey was repeated annually in 1986 and 1987 and then in December in 1988 and January in 1990 however all but one of the surveys were curtailed by bad weather and the survey series was subsequently abandoned (ICES, 1991a). A decision was made to carry out future surveys in the 6.a.N area in the summer concurrently with the North Sea acoustic survey. This would serve two purposes in that it would provide a new survey index for the 6.a.N autumn spawning herring and would also allow the North Sea acoustic survey group '*to evaluate the possibility that North Sea herring were present in Division VIa. This has not been evaluated, but there was no evidence of large concentrations of herring along the 4 degree W boundary.'* (ICES, 1991b). This statement is somewhat surprising given that in the 1988 herring acoustic survey in the North Sea the highest biomass detected in the Shetland/Orkney area was immediately adjacent to the 4°W boundary despite limited coverage in this area (Kirkegaard *et al*., 1989). This was also the case in the 1989 survey (Kirkegaard *et al*., 1990) and in fact, in previous years when the transects had traversed the 4°W boundary, for example in the 1985 survey (Simmonds *et al*., 1986), a large biomass of herring was detected to the west of the boundary and there was no break in the continuity of herring distribution between the two areas.

Regardless, the 1991 survey (Simmonds *et al*., 1992) aimed to resolve these discrepancies by surveying the combined areas during the same period. However, the survey plan indicated that the 6.a.N part of the survey did not extend transects into the area between 4°W and 5°W, south of 59.5°N (Figure 2.4). These areas were partially covered by the North Sea survey although gaps in the data collection were still present, which gives the erroneous impression that there was a gap in the distribution of herring across this area. The 6.a.N data were further confounded by the lack of trawl samples due to technical gear issues and as such the biomass estimates for this area could not be used. The data from both areas was presented separately and analysed to a different spatial level making comparisons more difficult (Figure 2.4). The transect coverage issue was resolved in the

following year with the 6.a.N transects extending across the 4°W boundary and data were presented in a single figure to allow easy interpretation of the centres of distribution of the herring stocks during the survey.

In 1994 Ireland initiated a survey in Division 6.a.S as part of the herring summer acoustic survey (Simmonds *et al*., 1995). This enabled a more comprehensive picture of the distribution of herring around Ireland and Britain to be developed, though the Celtic Sea and Irish Sea were excluded. It should be noted that Divisions 6.a and 4.a were treated separately and, in most years, the transects did not traverse the 4°W boundary and instead ended either side of it. Further, all herring surveyed in Division 6.a were assumed to be autumn spawning herring without any evidence to support this assertion. The combined figures of herring abundance and biomass observed during the surveys provide an illustration of the annual temporal variation in the centre of distribution of the herring in the area (Figure 2.5). In the 1994 survey the main centres of biomass were around Shetland in the North Sea, in the Buchan area in the North Sea, on the south west boundary of Division 6.a.N and off the northwest of Ireland (Simmonds *et al*., 1995). In the 1995 and 1997 surveys the centre of distribution of the North Sea herring was spread across the 4°W boundary (Figure 2.5). The Irish survey in 6.a.S, 7.b and 7.c was not conducted in 1997 and 1998 but returned in October 1999. Between 2000 and 2007 the Irish survey was conducted in winter between November and January and as such the data were not combined with the summer surveys in 6.a.N and the North Sea. The winter survey was aimed at surveying the winter spawning herring in Divisions 6.a.S, 7.b and 7.c as these were the dominant type in this stock since the 1990s (ICES, 2015a). The survey was often disrupted by bad weather and eventually reverted to a summer survey in 2008 at which point it again became part of the combined summer acoustic surveys for herring. At the time of the survey the vast majority of adult fish are observed in 6.a.N, thereby providing little information on the abundance of the stock in 6.a.S, 7.b and 7.c without a quantitative splitting method (ICES, 2015a).

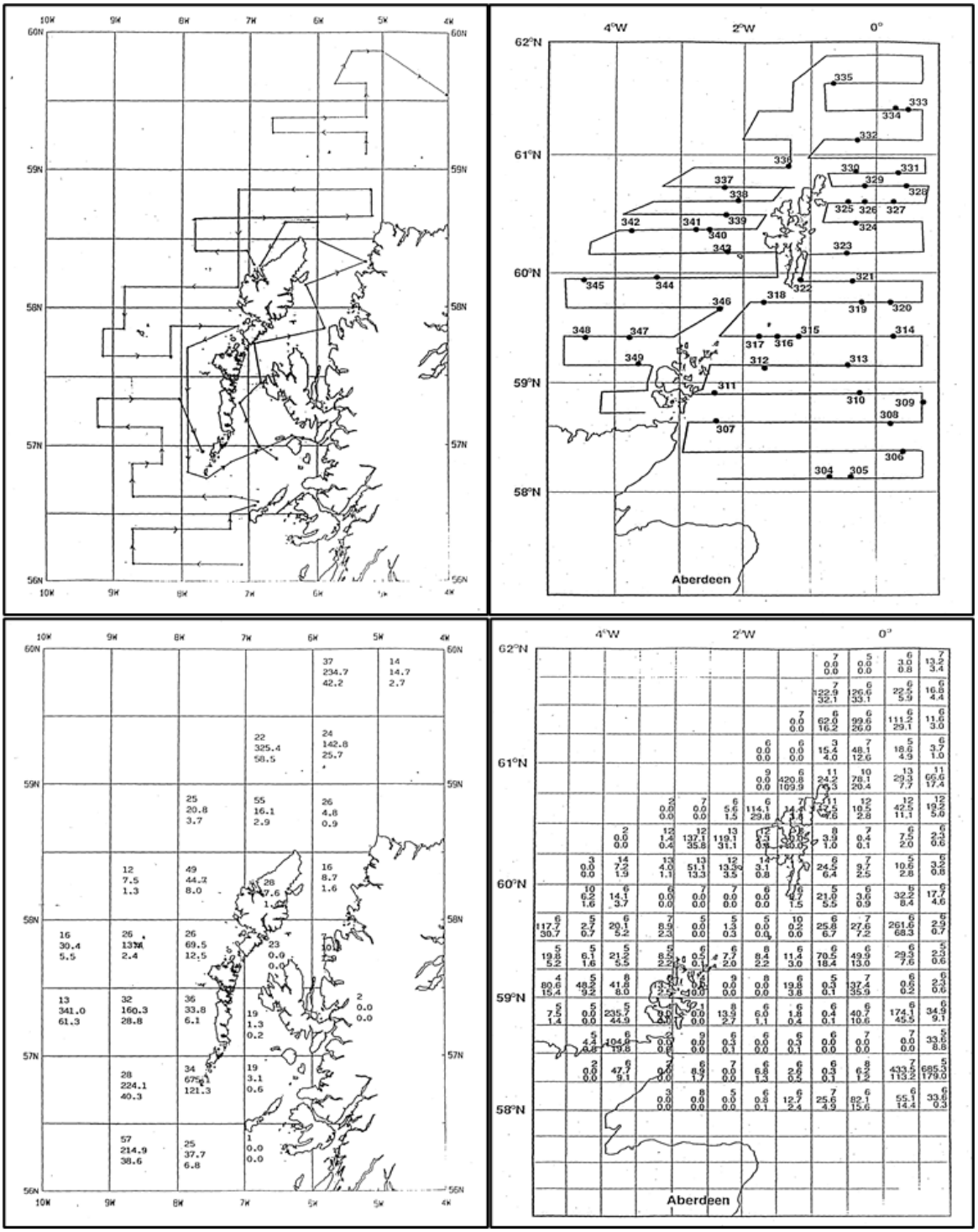


Figure 2.4. The survey track for the 1991 herring acoustic survey in (top left) 6aN, 13-26 July (top right) the North Sea, 12 July – 1 August. The number of 15 minute integrator runs, herring, numbers and herring biomass (* 10^{-3} tonnes) for (bottom left) 6aN by lCES rectangle and for (bottom right panel) the North Sea by quarter ICES rectangle. From Simmonds *et al*., 1992.

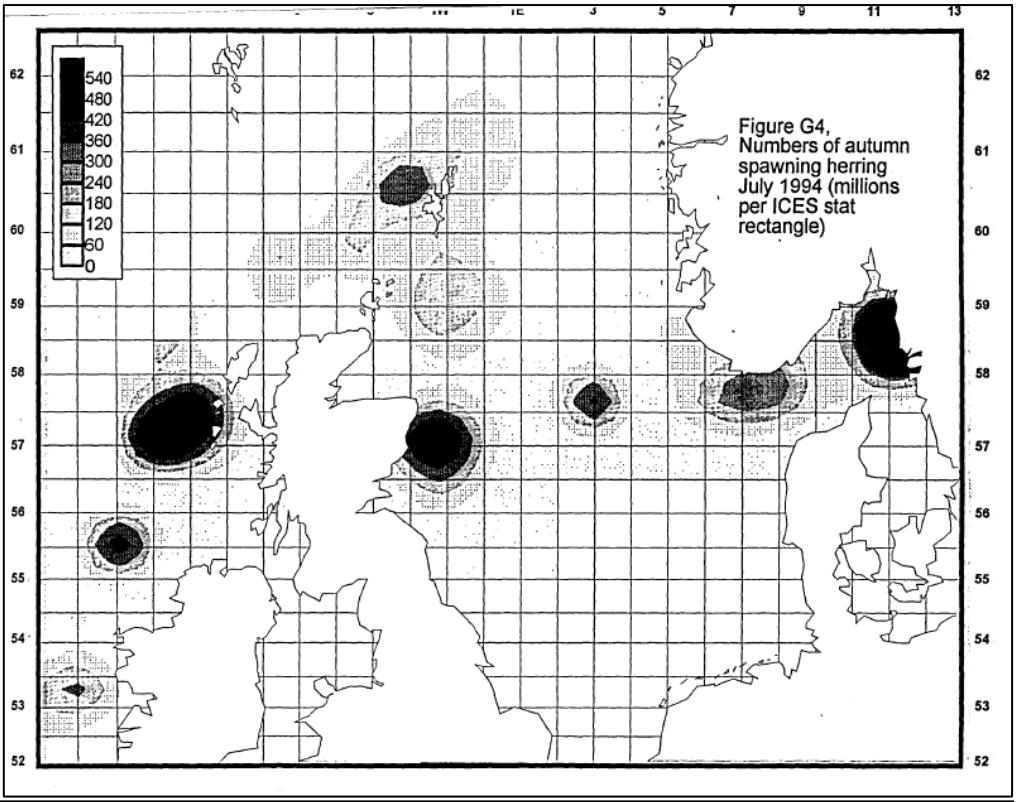


FIGURE 23 **Numbers (millions) of mature autumn spawners (1997).**

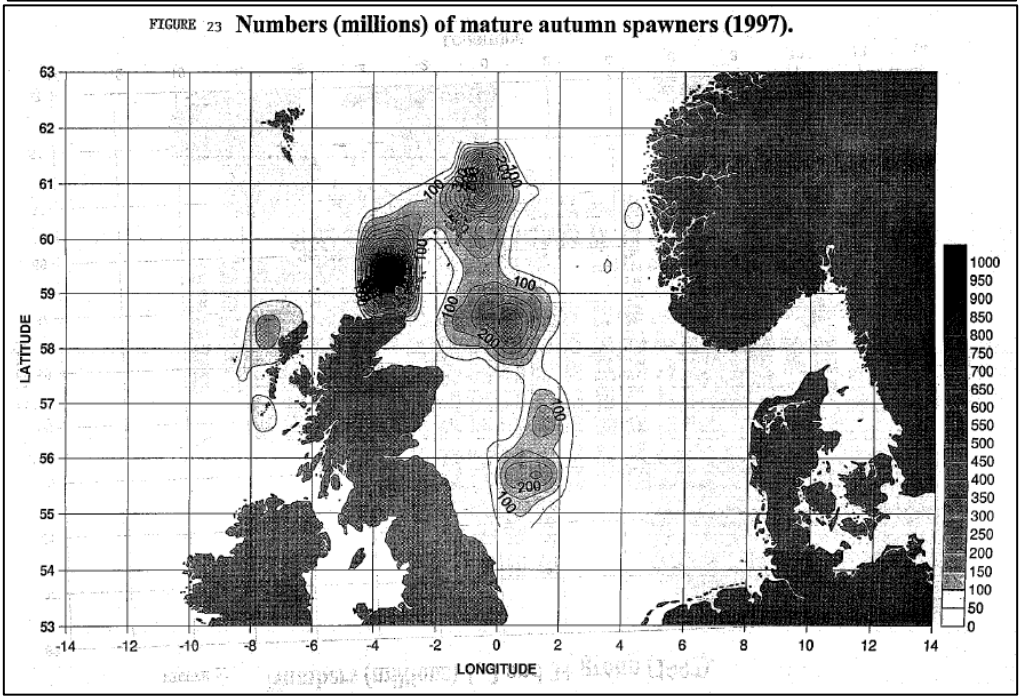


Figure 2.5. (top panel) Numbers (millions) of autumn spawning herring in the 1994 North Sea and 6a acoustic survey, from Simmonds *et al*., 1995. (bottom panel) Numbers (millions) of mature autumn spawning herring in the 1997 North Sea and 6a acoustic survey, from Simmonds *et al*., 1998.

2.7. Revision of the stock definitions

As more catch and survey data was collected the stock assessments of the various stocks became increasingly complex. However, considerable confusion still persisted about the composition and delineation of the herring stocks defined by ICES and how the data should be aggregated. This prompted the initiation of two large international collaborative EU-funded projects to investigate the population structure of herring in the northeast Atlantic

at a range of scales using a variety of methods. HERGEN (2002-2004, FP5-LIFE QUALITY Q5RS-2001-01370) focussed on herring in the North Sea using genetic and otolith-based methods and the WESTHER project (2003-2005, FP5-LIFE QUALITY Q5RS-2002-01056) focussed on the western herring stocks including 6.a.N and 6.a.S, 7.b and 7.c, and applied a multidisciplinary approach to investigate the putative stocks for assessment and management. It is not clear why the issue of stock identification was approached separately in two temporally overlapping projects for the two areas despite the strong evidence of the lack of appropriateness of the boundary between them. It would however have consequences for the sampling undertaken in the projects and for the interpretation of the results.

The overall goal of the HERGEN project was to provide guidelines for the conservation and management of biodiversity of Atlantic herring in the North Sea and adjoining waters in the Norwegian Sea, the Skagerrak, Kattegat and Western Baltic Sea (Figure 2.6) by identifying genetic population structure, and by quantifying relative stock contributions to the fishery. Much of the focus of the project was on the discrimination of the western Baltic spring spawning herring from the North Sea autumn spawning herring and as such this is not discussed here. Of relevance to the current project was the inclusion of 4 samples from the 6.a.N autumn spawning herring. Both genetic and morphometric methods were used to distinguish the samples including mitochondrial DNA, nine microsatellites, eleven allozymes, major histocompatibility complex (MHC) analyses, otolith shape analysis and otolith microstructure. Of the molecular methods, microsatellites were considered to be the most powerful genetic marker to distinguish between different stocks of herring. The microsatellite analyses of the North Sea, English Channel and Norwegian Atlantic baseline samples (Figure 2.6) indicated the presence of a genetically homogeneous autumn spawning unit off Northern Scotland with weak but significant differentiation between this group and the English Channel winter spawning herring and the Norwegian Spring Spawning herring (Mariani *et al*., 2005). There were no detectable differences between the 6.a.N and northern North Sea populations (HERGEN, 2004).

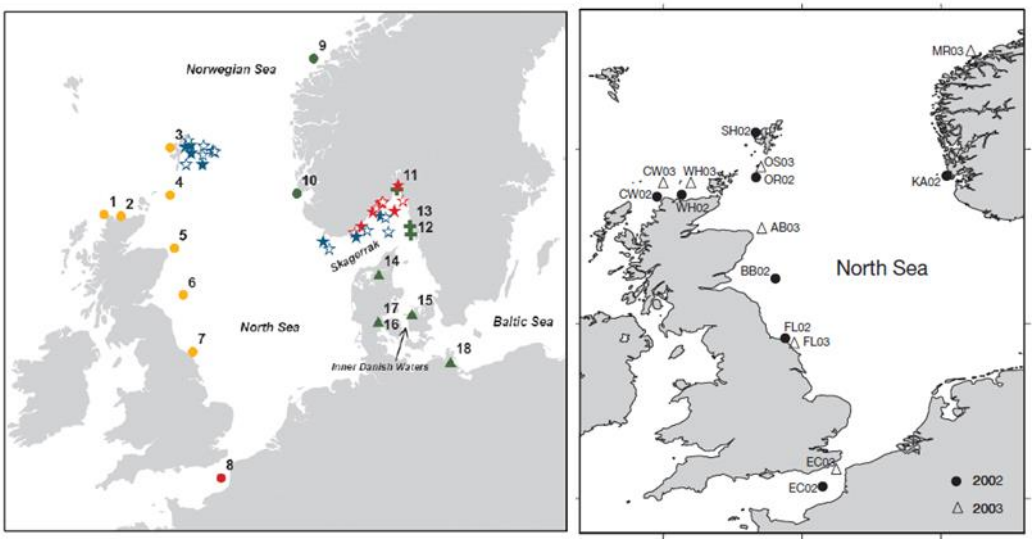


Figure 2.6. (left panel) The sampling locations in the HERGEN project. Spawning samples are numbered and couloured yellow for autumn, green for spring and red for winter. Mixed aggregations are denoted by solid stars for 2002 and open stars for 2003 (right panel) The HERGEN samples analysed in Mariani *et al*. 2005.

The WESTHER project applied a wider range of multidisciplinary approaches to the identification of herring stock components west of Ireland and Britain. The project had four main research objectives: (1) Estimation of genetic and phenotypic differentiation between spawning aggregations (2) Determination of stock origins and life history of juveniles (3) Determination of composition of feed aggregations (4) Improved guidelines for the

conservation and management of biodiversity and stock preservation. These objectives were approached through analyses of spawning, juvenile and mixed samples using a number of techniques, including body and otolith morphometrics, meristics, internal parasites, otolith microstructure, otolith core microchemistry and genetics, each of which was carried out on the same individual fish. The sampling was extensive in core areas west of Ireland and Britain (Figure 2.7) and included temporal replicates for most of the areas sampled. Otolith and morphometric data were not collected from the outgroup eastern North Sea samples or the western North Sea samples. Genetic samples were collected but were not included in the analyses. Further, the western North Sea samples were collected in January 2004 and as such were not baseline spawning samples for that area. The results of each of the methods are reviewed below.

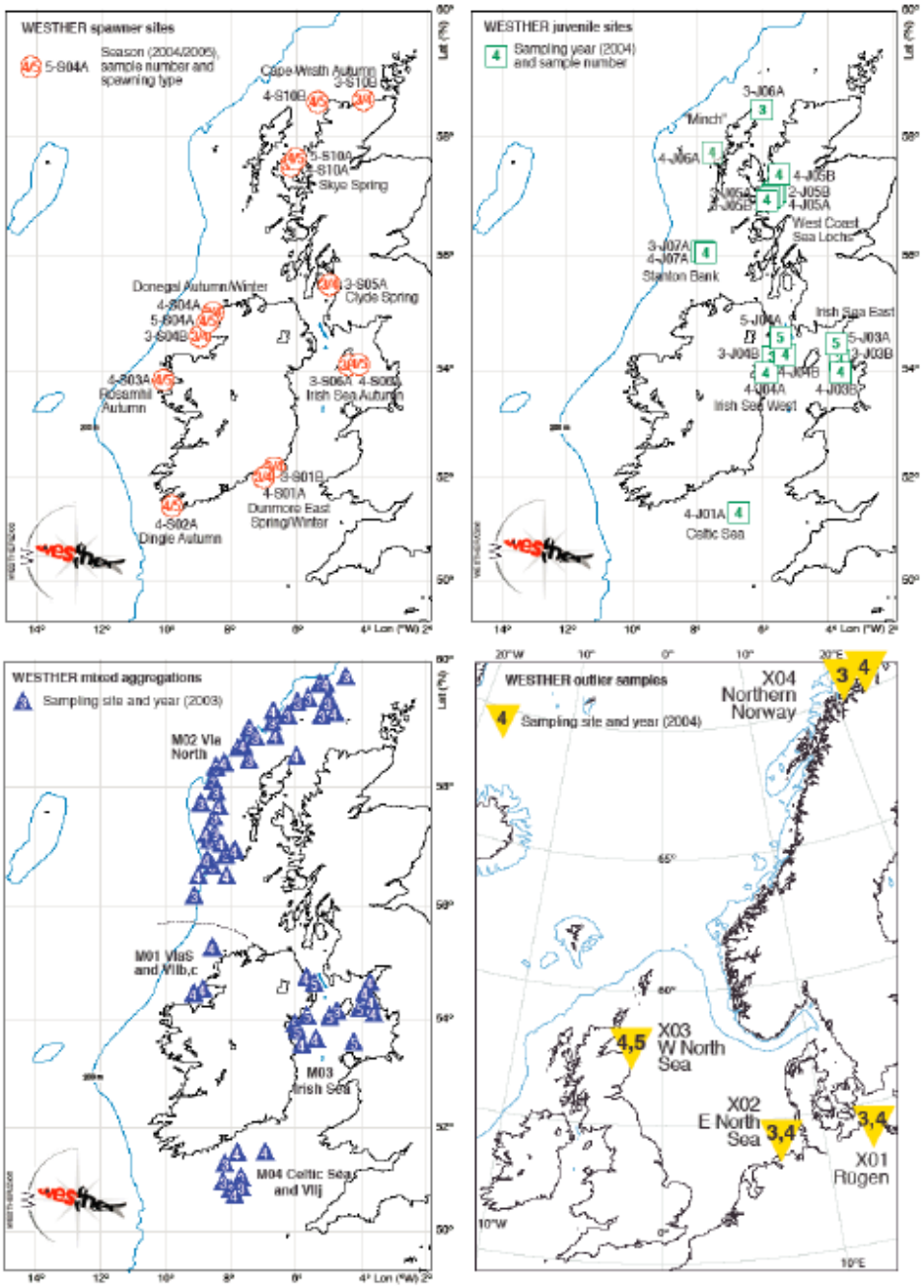


Figure 2.7. Sampling conducted during the WESTHER project from Hatfield *et al*. 2005. (top left panel) spawning samples (top right panel) juvenile samples (bottom left panel) mixed samples, and (bottom right panel) outgroup samples.

The meristic method of counting pyloric caeca, which is an organ with fingerlike projections located near the junction of the stomach and the intestines, did not provide useful information to discriminate between spawning stocks, link juveniles to spawning adults, or identify composition of mixed, non-spawning aggregations.

The whole-body morphometric methods achieved a classification success of c.70-80% for self-classification of spawning fish back to the baseline of spawning fish from which they came. Whilst this classification level appears high relative to the other methods detailed below, it is considered low overall. There was a high level of overlap in body morphometric variables between some spawning locations and a lack of discriminatory power. Therefore, the further classification of assumed mixed non-spawning samples back to their spawning stock of origin was less successful and indicated potentially high levels of mixing of stocks with little if any geographic pattern, when this may not actually have been the true situation. During hierarchical analysis of assigning mixed samples back to ICES management area it was noted that a higher proportion of the mixed samples were assigned to the 6.a.N and the 6.a.S, 7.b and 7.c groups than the Irish Sea and Celtic Sea groups, however this was likely an artefact of the larger sample size of the aforementioned groups and the degree of overlap in the discriminating characteristics rather than a true indication of origin. Using unequal number of baseline individuals in each source group can introduce assignment biases (e.g. Wang, 2017). Otolith shape analyses achieved similar levels of self-classification of spawning fish to stock of origin and also indicated similar potentially high level of mixing of non-spawning fish with the same issue regarding baseline sample size. If these issues are resolved it may be worth investigating the use of body and otolith morphometrics further.

Analyses of otolith microstructure indicated that it was not possible to separate samples purely on the basis of increment widths. However, if the spawning period across the whole area, August to March, was split into two 'seasons' i.e. 'Autumn' (August to November) and 'Spring' (December to March) then it was possible to allocate the single fish to one of two seasonal patterns with a classification success of c. 60-91%, indicating fish in the spawning aggregations were not entirely homogenous as to the timing of their own hatch. It must be noted also that the number of fish analysed per spawning baseline samples was low (n=10), and only 86% of which were used to build the classification model. It appears more effort was directed towards analysing juvenile samples (average per sample = 25) and mixed samples (average per sample = 55). Regardless, results indicated that this method may not be sensitive enough for discriminating the stocks in these areas.

Otolith microchemistry was used to analyse the composition of the core of herring otoliths, as otolith growth in herring begins before hatching, and the core material represents otolith growth during the first 3 – 6 months of life. Therefore, the results, detailed in Hatfield *et al*., 2005 and Geffen *et al*., 2011, relate to the similarity in early life history or young-of-year distribution and environment. There was significant inter-annual variability in the concentrations of a number of elements in most spawning areas indicating a lack of temporal stability in these markers. There was considerable overlap in the multi-element signal from each group, which resulted in variable rates of classification success. Hatfield *et al*. (2005) reported classification success, of assigning spawning samples back to spawning stock of origin, in period 1 ranging from 15 to 87% with an average of 48%, and in the second season ranging from 36 to 65%. Discriminant analysis of the second season sample did however indicate some ability to distinguish between the Cape Wrath and Irish Sea autumn spawners from the Minch spring spawners and Donegal spawners. When the spawning group samples were combined across years the classification success ranged from 3 to 60%. The lowest level of self-classification success was observed in the sample with the smallest baseline sample size, c. 30-50% size of the other samples, again illustrating the potential assignment bias with unequal baseline sample size. Overall, there was a low level of classification power and statistical analyses confirmed that generally less than half of the individual herring from any sample were assigned with high probability to any group. Given the low level of classification success between the spawning samples,

these results indicate that otolith microchemistry was likely not a suitable method for classifying mixed or juvenile samples of herring into spawning stock of origin. However further analyses were performed on the juvenile and mixed samples, some of which proved interesting. Discriminant analysis of the mixed samples from 6.a.N, Irish Sea and Celtic Sea indicated a degree of clustering of the Irish Sea and Celtic Sea mixed samples and a separation of these from the 6.a.N mixed samples in season 1. This pattern was also seen in the season 2 samples, which included the 6.a.S mixed sample that clustered with the 6.a.N sample. Further attempts to assign the mixed samples to spawning groups were unwarranted given the low level of self-classification success of the baseline samples. As a result, analyses indicated significant mixing of all baseline groups within each of the mixed samples. Discriminant analysis of the juvenile samples also indicated some clustering with the Celtic Sea and Irish Sea being aligned and the samples from 6.a forming a second group. There was however a large degree of overlap between the samples. As was the case with the mixed samples, further attempts to assign the juvenile samples to spawning groups were unwarranted given the low level of self-classification success of the baseline samples. Despite the proven inability to accurately distinguish the baseline spawning samples and the demonstration that less than half of the individual herring from any sample were assigned with high probability to any group, it was concluded that all of the mixed and juvenile samples were heterogeneous as to the origins assigned.

It is well documented that the larvae of autumn spawning herring off the northwest of Scotland are carried in easterly flowing currents and spend their juvenile phase in the North Sea (Heath, 1989; Heath, 1990; Heath *et al*., 1987; MacKenzie, 1985; Saville and Morrison, 1973) before apparently returning to the west of Scotland to spawn as adults. Given that this area was not sampled for otoliths as part of the WESTHER project, it is likely that the Cape Wrath autumn spawners were not represented in the otolith data in any of the juvenile samples, adding further doubt to the significant assignments made to this group. Despite this a final attempt at assignment was made in the discussion section (Section 3.7.4) of Hatfield *et al*. (2005). In this instance the assignment process was performed in reverse and the composition of the otoliths in juveniles on the nursery grounds was used as the baseline to which to assign spawning samples. It is noted that '*due to possible mixing of fish from different spawning areas on different nursery grounds there will inevitably be a certain degree of blurring between areas*' and the analyses is not discussed in detail. This final approach seems counterintuitive, as assigning weakly differentiated baseline spawning samples back to an incomplete set of individuals of unknown origin is unlikely to yield a robust result.

Geffen *et al*. (2011) developed this second approach further and presented the reanalysis in more detail, based on the same samples. The primary aim was to identify the nursery ground sources of the various spawning ground components. No new juvenile samples from the Cape Wrath autumn spawning grounds, the western North Sea or the eastern North Sea were included to fill the sampling gap identified in Hatfield *et al*. (2005). A single year (2004) of juvenile samples was used to develop a linear discriminant analysis (LDA) model as a baseline and then spawning samples were assigned back to these to attempt to determine their origin as juveniles. Similarly, an LDA model for the spawning samples was then used as a baseline to which to assign mixed samples. The juvenile baseline was not temporally stable, and the 2003 juvenile samples assigned to the 2004 juvenile samples with a self-assignment success rate ranging from 12.5 – 66%. Despite the high level of misclassification and the lack of temporal stability, the 2004 juvenile baseline was considered robust enough to perform further assignments of spawning samples to it. As expected, further assignments including spawning samples and mixed samples indicated mixing of all groups. There are two caveats noted in the text in relation to these assignments. The first states that '*a spawning herring that is classified as coming from a particular juvenile sample is an adult fish that shares the same relative elemental composition in the otolith core as juvenile fish sampled in that location. It is not a direct measurement of a link from a juvenile source group to an adult spawning group. However, interpretations about connectivity can be made*.' and the second relating to the mixed

samples states '*this is not a direct measurement of the link between a mixed population group and a spawning group, but the results imply likely connections between groups*.' Furthermore '*the discriminant analysis models did not identify distinctive otolith signatures that could yield unambiguous classification of each spawning location for the mixed stock analysis' and 'the juvenile samples failed to produce clear unambiguous signals.'*. Essentially the results of this analysis should not be used to define baselines from the putative herring populations and as such no meaningful conclusions can be drawn regarding further assignments or the potential levels of mixing between populations. However, Geffen *et al*. (2011) concluded that '*spawning groups consist of individuals from a number of different nursery areas and originate from several different management areas. Each of the mixed aggregations contained at least three spawning components. Results suggest that most west coast herring belong to interconnected populations subject to mixing and that populations are not discrete, so the current practice of assessments based on individual spawning components will probably not provide sufficiently robust information for management advice*.' These unsupported conclusions have persisted in the research concerning the herring west of Ireland and Britain and have confounded further the attempts to resolve the stock identification issues (ICES, 2015a).

Figure 2.8. Key parasitological tag species identified during the WESTHER project (left) *Cercaria doricha* (L) and *Cercaria pythionike* (R), (middle) *Anisakis simplex sensu stricto,* (right) *Lacistorhynchus tenuis* (N. Campbell, pers. comm.).

The analyses of the occurrence of three out of four parasites; the trematodes *Cercaria doricha* and *Cercaria pythionike,* the nematode *Anisakis simplex sensu stricto* and the cestode *Lacistorhynchus tenuis* was perhaps the most successful approach for distinguishing between herring from different areas in the WESTHER project (Figure 2.8) (Campbell *et al*., 2007; Hatfield *et al*., 2005). It was noted that *Anisakis simplex* infections are of limited use as biological tags as infections are cumulative with host age and as such the results were based on the other three parasites. The metacercariae of *C. doricha* and *C. pythionike* are valuable as biological tags as herring are only susceptible to infection in their first year of life after which no further infection occurred. These parasites also have life spans in herring extending to several years and possibly as long as that of the host itself. The value of *L. tenuis* as a biological tag is also limited by the fact that further infection of herring as adults is possible. Confirmation of the long-term (30 years) temporal stability of *C. doricha* and *C. pythionike* prevalence was possible in two of the areas sampled; Stanton Bank to the south of the Outer Hebrides and a number of Scottish west coast sea-lochs, by comparison with the data from MacKenzie (1985). Temporal replicates were also available from the eastern North Sea, Moray Firth and Irish Sea, although only the former were compared by Hatfield *et al*. (2005) and temporal instability was evident in the prevalence of *C. doricha* and *C. pythionike* in the Moray Firth samples.

Parasite analyses of WESTHER samples indicated significant differences in the prevalence and intensity of infection between some of the different areas sampled. Spawning herring from Donegal, north-western Ireland and spring spawning fish from Skye had very similar parasite infections. Whereas there was a marked difference between infections in Skye spring spawners and Cape Wrath autumn spawners, both taken in 6.a.N, west and northwest of Scotland. The absence of three out of four tag parasites from the Cape Wrath

autumn samples was considered to be an indication that Cape Wrath spawners did not recruit from any Scottish coastal nurseries, but probably from the eastern North Sea. This agrees with the results of the Bløden herring tagging project (ICES, 1975), which showed conclusively that there was a migration of some adult herring from the North Sea to the west coast of Scotland and also with larvae tracking studies that have observed larvae in Division 6.a.N to be transported in an easterly direction by the Scottish Coastal Current into the North Sea (Heath, 1989; Heath, 1990 Heath *et al*; 1987). Campbell *et al*. (2007) noted that '*other differences are less significant or produce confusing patterns, suggesting that the rather low number of species recorded may make this a rather blunt tool for identifying these populations*.' The presence of *C. doricha* and *C. pythionike* in Irish Sea juvenile samples but not in Irish Sea spawning samples indicated that these juvenile fish potentially belonged to the Celtic Sea spawning population as these parasites occurred there with similar prevalence. Further intermediate prevalence of these parasites in the Irish Sea mixed stock sample suggested that these samples comprised a mixture of herring spawning both in the Irish Sea and Celtic Sea.

The most significant conclusion of the WESTHER parasite analyses was that adults that spawn in the Irish Sea were present in the mixed 6.a.N feeding area. This was based on the identification of *L. tenuis* in mixed samples collected in Division 6.a and the presence of this parasite in the Irish Sea spawning samples and the apparent lack of this parasite in the other spawning areas. Whilst this seems to be conclusive, on closer examination it is not very well supported. Firstly *L. tenuis* was also detected in two spawning samples from the Celtic Sea, one from Waterford and one from Baltimore, albeit at a lower prevalence than in the Irish Sea. MacKenzie (1985) also reported that *L. tenuis* was present in juveniles caught in the eastern North Sea, an area with a proven connectivity to Division 6.a. Whilst Campbell *et al*. (2007) did not find any *L. tenuis* in the eastern North Sea juvenile sample it should be noted that this sample only comprised 50 juvenile herring. Given the expected prevalence of 1% (MacKenzie, 1987) it is entirely plausible that this sample size was insufficient to detect the presence of this parasite. The mixed samples from Division 6.a analysed for parasites comprised 116 herring from 6.a.S in 2004, 110 herring from 6.a.N in 2003 and 98 herring from 6.a.N in 2004. In total four herring, two from each year, were identified as having a single *L. tenuis* in each of them (Figure 2.9). The 6.a.S sample was caught in January and was a stage 6 (spawning) female, which was also infected with *C. pythionike*. This 6.a.S sample was collected in an area of known spawning, at a known spawning time during acoustic surveys of spawning herring (Hatfield *et al*., 2005) and could therefore equally have been classified as a spawning sample, given the high proportion of stage 5 and 6 fish present in the total sample. Therefore, the presence of *L. tenuis* and *C. pythionike* indicates that this parasite is also present in the 6.a.S spawning herring and that this fish was unlikely to have originated in the Irish Sea, where spawning fish were observed to have no infection by *C. pythionike.* The three herring in 6.a.N with an *L. tenuis* infection could thus have originated from the 6.a.S herring or equally they could have originated from the Celtic Sea, Irish Sea or eastern North Sea. Further, as infection by *L. tenuis* can occur at any life stage of herring (MacKenzie 1985) it is impossible to know when the four herring were infected. Members of the houndshark family triakidae, including the starry smooth-hound *Mustelus asterias* Cloquet, 1819 and the tope *Galeorhinus galeus* (Linnaeus, 1758) are known intermediate hosts of *L. tenuis*. These species are abundant in the Irish Sea, however both species are also regularly recorded off the Irish coast in Division 6.a and in the eastern North Sea (ICES, 2019b). As a result, it is not possible to conclude with any degree of certainty that the four herring, caught in Division 6.a during the three years of sampling in the WESTHER project, that were infected with *L. tenuis* originated from the Irish Sea population. The overall low prevalence of *L. tenuis* across the study area and the fact that infection can occur at any life stage also makes it likely to be unsuitable as a stock identification marker, as it is easy to misidentify a sample.

Figure 2.9. The distribution of Division 6.a mixed samples from the WESTHER project that were infected with *L. tenuis*. Reconstructed from original WESTHER data.

In an effort to assess the genetic composition of the herring stocks in the study more than 4,000 herring were genotyped at 12 microsatellite loci. Genotyping was undertaken in two different laboratories (University College Cork, Ireland and Liverpool University, UK) using two different platforms, a LiCor 4200 automated DNA sequencer and ABI 3100 automated capillary sequencer, respectively. Results indicated a lack of significant population structure between all spawning samples collected in the core study area (West of Britain and Ireland). Significant differentiation was found between these samples and the outgroup samples from the Baltic, however no differentiation was detected within the core sampling area or between it and an additional outgroup in the northwest Atlantic, suggesting that the genetic markers used had limited power to detect genetic population structure if present.

Of primary interest from the WESTHER results are those concerning the spawning samples. If it is not possible to discriminate between individual spawning samples collected from different putative populations then the conclusion is that either the methods do not have sufficient power or there is no difference to be found. Without a robust spawning baseline on which to test unknown or mixed samples there is no value screening additional juvenile or mixed samples as this will likely result in misleading results. Of the methods utilised in the WESTHER project both otolith microstructure and microchemistry appear to have the least power. Whole body morphometrics and otolith shape analysis appeared to be limited in their classification power, though many of the issues appeared to be related to the effect of unequal sample size on the assignments, which may be resolved through applying different methods to the analyses. Parasite analysis appeared to show potential in discriminating the most differentiated populations (autumn spawners from spring spawners in 6.a.N), however it requires an in-depth knowledge of the life history of the parasites in question and is also more prone to temporal changes given climate induced changes in the distribution of many fish species and their parasites and prey. Discrimination of biological populations through genetic analyses is potentially the most robust method to uncover the underlying populations that comprise the stocks in question. However, at the time of WESTHER this was not possible due to technological limitations. The genetic component of the project failed to resolve stock structure largely due to the low number and low power of genetic markers employed. These issues have now been resolved and Next Generation Sequencing (*NGS*) based methods are likely to improve on the genetic identification of these stocks. One notable exclusion in the WESTHER project

is the analysis of North Sea herring in all but the parasite analyses, which indicated no difference between these samples and those collected at Cape Wrath in 6.a.N. Genetic samples were collected in both the Eastern and Western North Sea however there is little reference to them in the final report and no indication that they were genetically analysed. Considering the scale and proximity of the North Sea stock to the study area, the fact that the 4°W boundary is purely a political/statistical separation, the fact that the 6.a.N autumn spawners and North Sea autumn spawners spawn at the same time either side and sometimes on top of the boundary and that this division does not represent any hydrological features, it is surprising that more focus was not put on the potential links with this stock.

Despite the obvious limitations and limited support for the conclusions of the WESTHER project the primary recommendation of the WESTHER project was to assess the western herring as two stock units (Figure 2.10). However, this was not incorporated into assessment or management advice as the ICES Herring Assessment Working Group (HAWG) considered that it was unclear what management regime would provide the most cost-effective method for successful management and what data would be needed to support this management (ICES, 2015a). Given the limitations of the WESTHER project, the existing separation of management units (6.a.N, 6.a.S, 7.b and 7.c, Irish Sea and Celtic Sea) was considered to afford the best possible protection for local spawning stocks but it did not afford protection to the fish of one stock distributed in another management area at feeding time (ICES, 2015a). In response to these issues, the ICES Study Group on the evaluation of assessment and management strategies of the western herring stocks (SGHERWAY) was established in 2008.

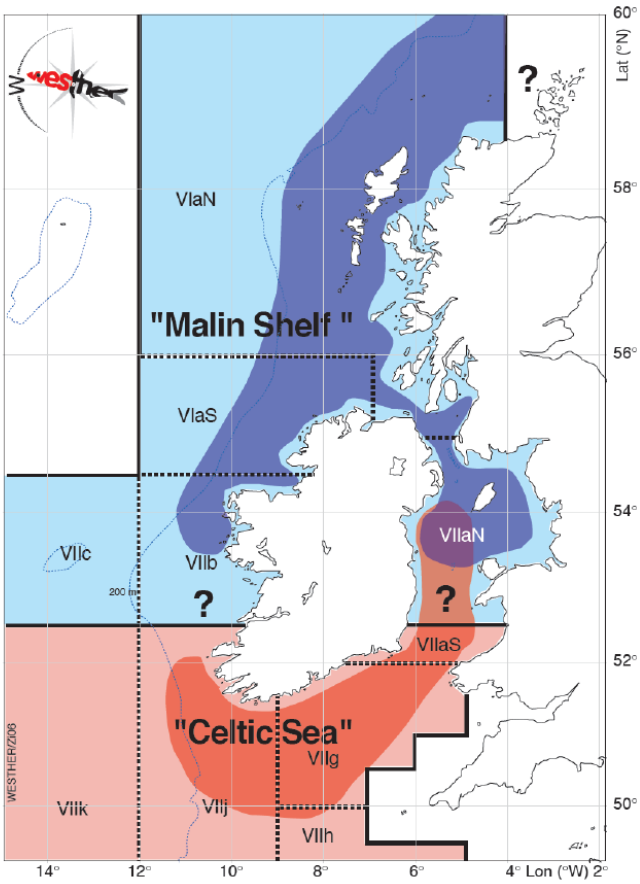


Figure 2.10. Proposed assessment units for assessments of western stocks, based on grouping suggested from WESTHER. Darker colours indicate known distribution of herring in those areas. From Hatfield *et al*. 2005.

SGHERWAY was asked to evaluate the utility of a synoptic acoustic survey in summer for the Hebrides, Malin and Irish shelf areas. In 2010 The Scottish and Irish research vessels both began collecting biological information on herring caught during the survey for use in the body and otolith morphometric analysis for stock separation (ICES, 2010). A combined assessment of the three stocks 6.a.N, and 6.a.S, 7.b and 7.c and 7.a.N (Irish Sea) was explored and its utility for advisory purposes investigated. It was found that the combined assessment provided important information on the proposed Malin Shelf metapopulation, though it was unlikely to be useful for management advice purposes. SGHERWAY showed that managing meta-populations was only possible with detailed information on fisheries independent data. However, whenever subcomponents of the metapopulation differ considerably in abundance, sustainable management is impossible for the smallest subcomponent (ICES, 2015a). Where there is uncertainty of stock identification fishing mortality should be kept at low levels.

At the 2014 HAWG meeting, preliminary analyses were performed to provide a split of the Malin Shelf survey time-series (2008–2013) to derive an age-based abundance index for the 6.a.S, 7.b and 7.c stock (ICES, 2014). Data were derived from analyses of otolith and body morphometry from the 2010 to 2013 surveys, from hauls across the entire surveyed area, as the basis for a quadratic discriminant analysis (*QDA*). The WESTHER baseline dataset of spawning herring from 2003 to 2005 (Figure 2.7) was used as the training set for the 30 variable QDA and the resultant model was applied to the 2008–2013 MSHAS numbers-at-age data to derive an age-based abundance index for the 6.a.S, 7.b and 7.c stock. However, the validity of the WESTHER baseline was unproven at the time of the analyses. It was agreed to collect new baseline spawning samples from 6.a.N and 6.a.S., 7.b and 7.c stocks in 2014. Analyses of these samples indicated a lack of temporal stability in the baseline samples and as such further assignments of the mixed MSHAS samples were uncertain. An ICES benchmark assessment of western herring (WKWEST) was conducted in 2015 (ICES, 2015a). Due to high levels of uncertainties in the ability of the morphometric methods to assign fish in either catches or surveys to their spawning population, it was not possible to analytically assess the 6.a.N and 6.a.S, 7.b and 7.c stocks separately. Therefore, a combined assessment was conducted for the 6.a, 7.b and 7.c stocks. WKWEST noted '*There is a clear need to rapidly develop robust methods of being able to identify individuals to their spawning population, both in the catches and surveys. The development of the methods is a matter of priority and this recommendation should be addressed to the EU, national governments, ICES, National laboratories and the prosecutors of the fisheries (fishers and processors etc). It is clear that a combined effort is needed to provide management advice for the herring stocks in this area*.'.

Since 2010 the summer acoustic surveys, undertaken in quarter three, have indicated annual variability in the distribution of herring in Division 6.a and also in the North Sea at the time of the survey (Figure 2.11 and Annex 6). It should be noted that the survey only provides a snapshot of the distribution of herring over the course of a c. three-week period. In years where the centre of distribution of North Sea herring is further west, they are often observed to traverse the 4°W boundary, whilst in other years the centre of distribution may be further to the east of Shetland (Figure 2.11 and Annex 6). In 6.a.S, 7.b and 7.c the centre of distribution is sometimes north of the 56°N boundary and may be located west or north of the Hebrides. Adult herring have rarely been observed within the Minch in recent years, however, juvenile/immature herring are sometimes found. The survey is not designed to comprehensively cover juvenile herring nursery areas. Annual catch data from fisheries also provide an insight into the annual distribution of herring though since 2016 the catches in 6.a, 7.b and 7.c have been taken under a monitoring TAC and as such the distribution of catches is dictated by sampling needs. Prior to this the 6.a.N catches were primarily taken in quarter three in the area immediately adjacent to the 4°W boundary with the North Sea (Figure 2.12). It is informative to also study the North Sea catches for the same year and note that in quarter three significant catches were also taken to the east of the 4°W boundary during the same period (Figure 2.13). The catches in both areas reflect the observed distribution of the herring during the

summer acoustic survey in that year (Figure 2.11). The 6.a.S, 7.b and 7.c catches in the same year were mainly taken in quarters one and four (Figure 2.12). The quarter four catches were distributed closer inshore, which is likely due to this being the peak spawning period in the area. Whilst the quarter one catches were taken further offshore.

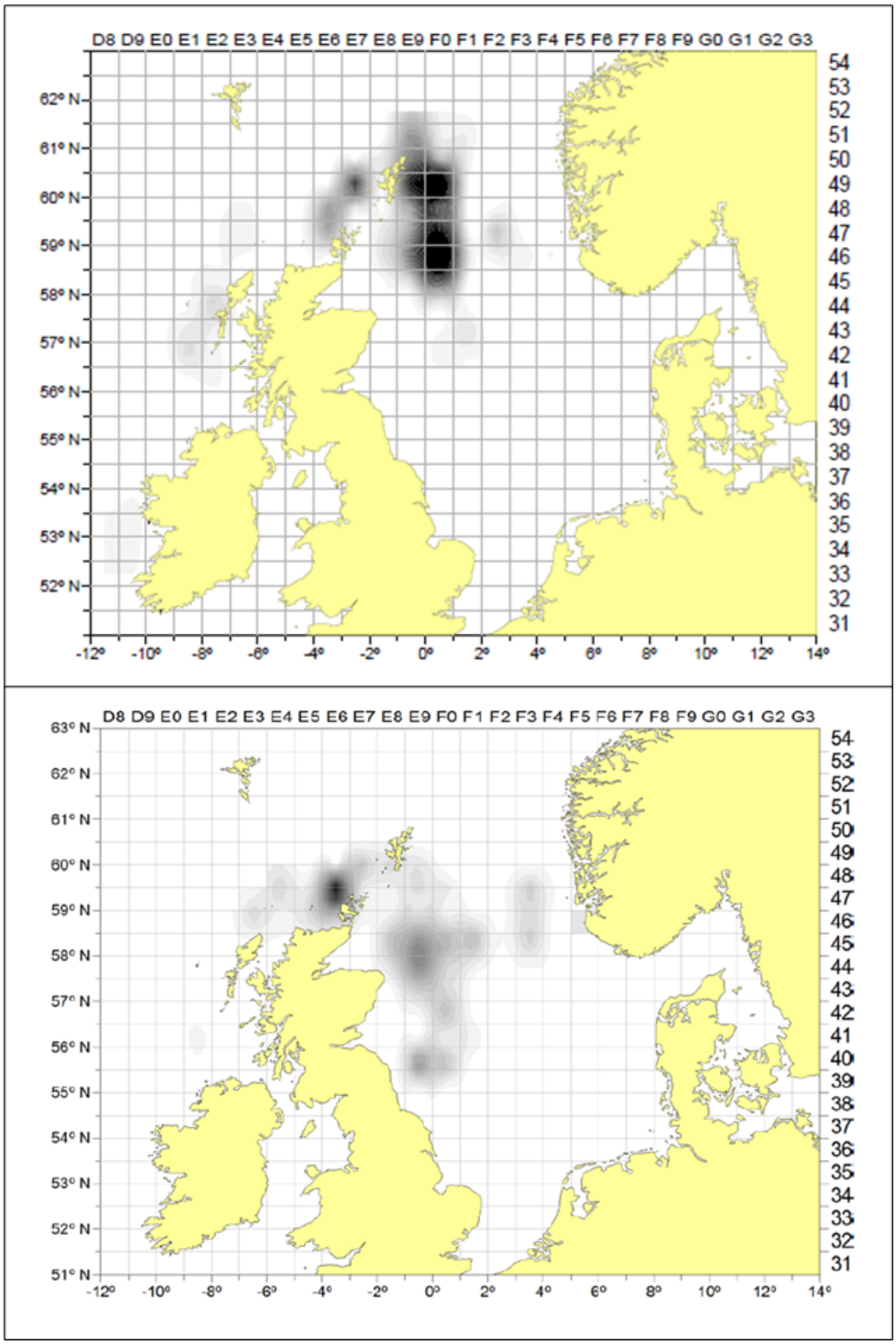


Figure 2.11. Biomass of mature autumn spawning herring from the combined acoustic survey in June-July (top panel) 2010 and (bottom panel) 2013. From ICES, 2011a and ICES, 2014.

In an effort to resolve some of the issues with stock mixing during the summer acoustic surveys an industry-led survey series was initiated in Divisions 6.a, 7.b and 7.c in 2016 (Mackinson *et al*., 2016). The overall aim was to improve the knowledge base for the spawning components of herring in 6.a.N and 6.a.S, 7.b and 7.c and submit relevant data to ICES to assist in assessing the herring stocks and contribute to establishing a rebuilding plan. Following agreement on a monitoring fishery TAC of 5,800t (EU 2016/0203), the scientific survey was designed based on ICES advice for the timing, location and number of samples required to collect assessment-relevant data from the monitoring fishery. The survey has been conducted annually from 2016 to 2019 in 6.a.N in September/October and in 6.a.S, 7.b and 7.c in November/December and both genetic and morphometric samples have been collected where possible for stock identification analyses (Mackinson *et al*., 2016, 2017, 2018, 2019). In 6.a.N, spawning herring have been primarily distributed north and northwest of Cape Wrath during the survey period. Little if any spawning activity has been observed west of the Hebrides. A high abundance of juvenile herring has been recorded in the north Minch on the east side of the Isle of Lewis. In 6.a.S, 7.b and 7.c herring have been found close inshore with the overall distribution dominated by aggregations of herring in a few discrete areas, including Donegal Bay, Lough Swilly and Lough Foyle (Annex 2).

In addition to the spawning surveys, two exploratory surveys have been undertaken. In February 2018 an industry-led survey was undertaken in 6.a.N to try to find spawning aggregations of spring spawning herring. Very few herring were found across the area and only one spawning sample of herring was located in the Minch close to Gairloch, which was historically an important area of spring spawning. In July 2019 a survey was conducted in the Minch to address the question whether the limited coverage in the Minch by the International acoustic survey might be missing herring aggregations outside of the survey track. The acoustic survey did not record any herring marks, and trawl samples found very few herring that were mixed in with catches dominated by other species.

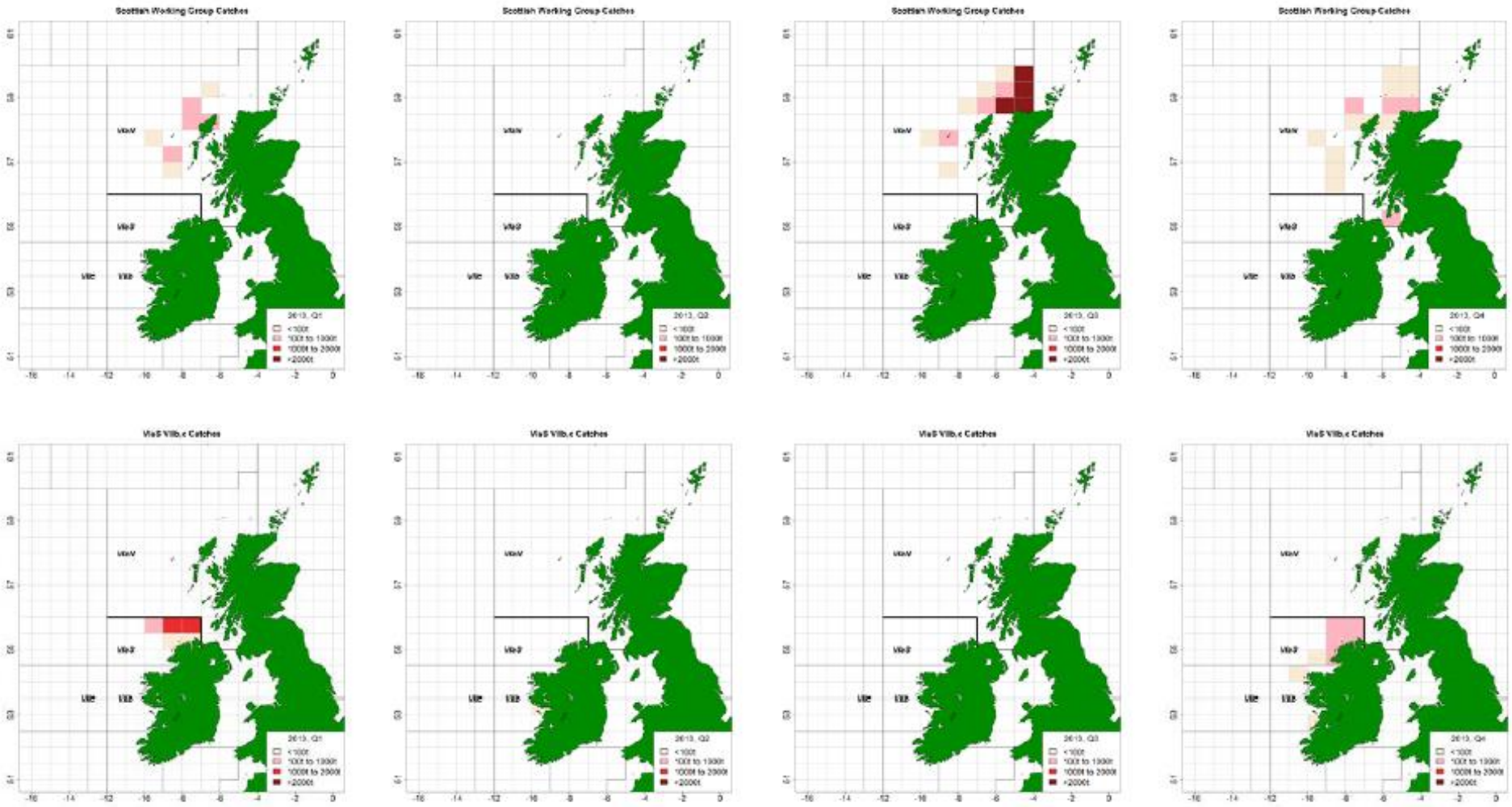


Figure 2.12. Working group catches of herring in 6.a, 7.b and 7.c by quarter in 2013. From ICES, 2015a.

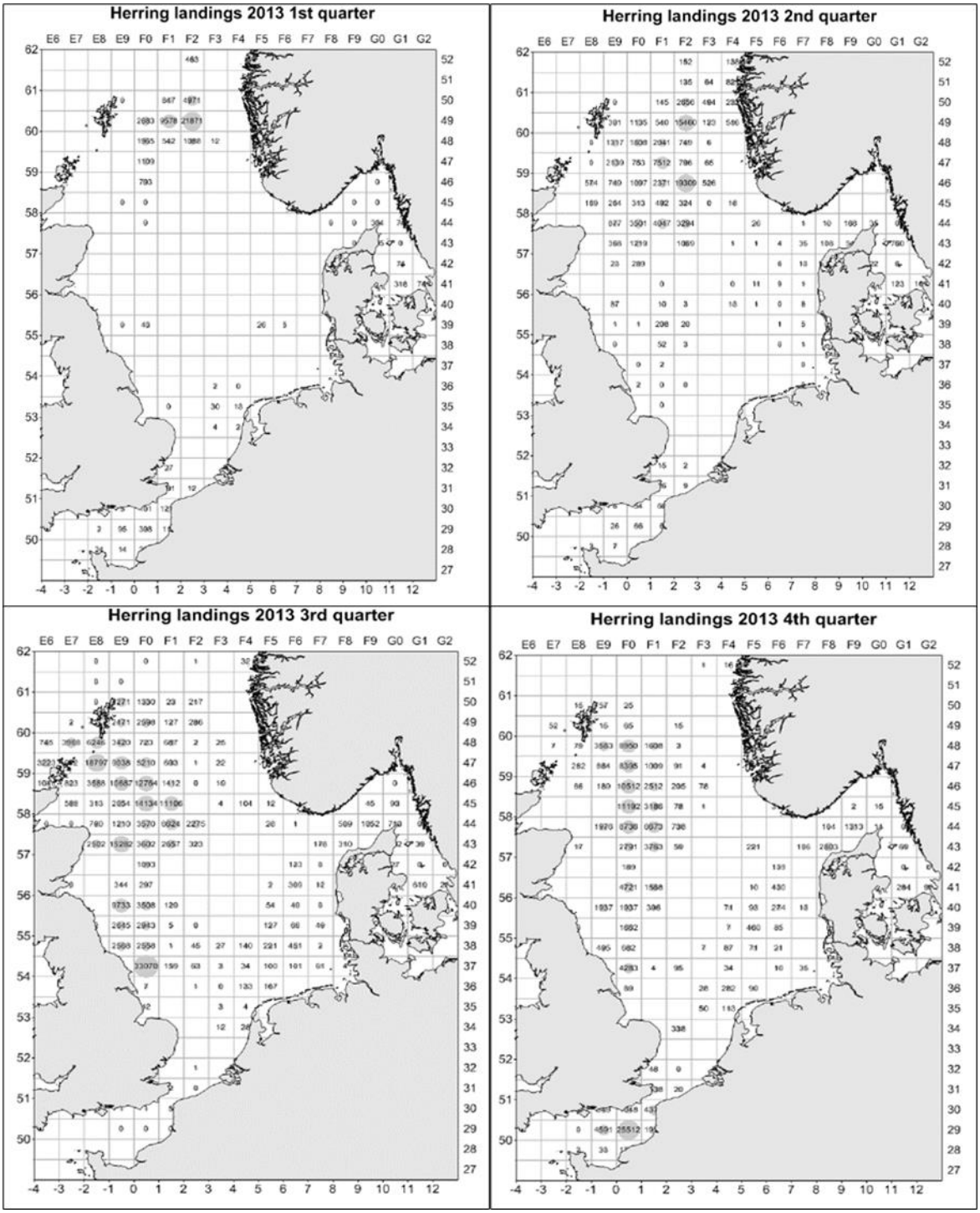


Figure 2.13. Herring landings in the North Sea in 2013 in quarters 1-4. From ICES, 2014.

2.8. Stock identification methods

The population structure of marine fish is temporally and geographically dynamic, and largely driven by environmental factors. As such it requires ongoing monitoring, particularly in the mixing zones between stocks. There may be temporal or geographic changes in the levels of mixing which will be reflected in the catch and which should be identified and accounted for in the sampling, stock assessment and management. An ideal method of stock identification would allow continued monitoring of the spatial and temporal integrity of a stock, allow individual fish to be assigned to a given population on an ongoing basis and should be reproducible among laboratories. The 'state of the art' in stock identification is considered to be the application of multiple approaches, to the same biological samples, with comparison of results to achieve an interdisciplinary perspective and consensus (Cadrin *et al.*, 2013). These methods include genetics, body

morphometrics, otolith shape and chemistry analyses, tagging, parasite analysis and life-history analyses (Cadrin *et al*., 2013). Conventional tagging methods rely on recovery of tags and only provide release and recapture positions without knowledge of the intervening period. Electronic tags provide more detailed information however they are costly, rely heavily on computer modelling to interpret results and are often unsuitable for smaller species. Parasites have been used as biological tags though their own variable life-history characteristics, changing distribution and persistence in the host add further complexity to an already complex issue (Lester and MacKenzie, 2009). Life-history analyses may provide useful information for stock identification, as distinct populations should exhibit temporal and spatial variation in key processes such as spawning. However, identification of mixed stock samples is still problematic. Whilst a multidisciplinary approach may be recommended this is rarely possible given time and funding constraints and can lead to a lack of sufficient time and resources being allocated to individual methods. A multidisciplinary approach may be useful for establishing baseline data in stand-alone projects yet, it is unlikely to be applicable to rapid large-scale annual screening of fisheries dependent and independent samples.

Morphometry is the quantitative description of biological shape, shape variation and covariation of shape with biotic and abiotic variables. It is the process of measuring external shape and dimensions of objects, including living organisms and their organs (Reyment, 2010; Webster and Sheets, 2010). Morphometric methods are based on analyses of phenotypic characters, which are influenced by various factors, including, but not limited to, genetics, the environment and geographic isolation (Begg and Waldman, 1999; Berg *et al*., 2018; Turan, 2000) but in populations where spatio-temporal mixing occurs these differences between neighbouring populations may be more difficult to identify (Hüssy *et al*., 2016; Turan, 2000). The complex population structure of herring is underpinned by larval drift and adult migration, causing significant mixing of different populations in different areas (Molloy, 2006). As a result, numerous studies have been carried out, testing different methods to separate mixed herring stocks, including body morphometry (Armstrong and Cadrin, 2001), otolith shape analysis (Libungan *et al*., 2015a,b), otolith microstructure (Brophy and Danilowicz, 2002), parasite prevalence (Campbell *et al*., 2007) and genetics (Ruzzante *et al*., 2006). It is reported that the most powerful method of stock discrimination is achieved through the use of multiple, complimentary stock identification methods that cover broad aspects of the biology of the species. This is particularly valid for species or populations with complex stock structures (Begg and Waldman, 1999). Herring populations in 6.a, 7.b and 7.c fall under this category and, coupled with the evidence of differences in biological profiles between herring caught in 6.a.N and 6.a.S, 7.b and 7.c and the results from the WESTHER project, it is clear further research is required in this area to ensure appropriate management can be put in place. Phenotypic variation is not under the sole control of genetics; the environment also has a significant influence on morphometric characters. This environmentally induced adaptation can occur significantly quicker than changes related to genetics (Turan, 1998). It is important to explore a holistic approach to discriminate between fish populations (Begg and Waldman, 1999) so an integrated approach with non-molecular stock identification methods and genetics will be explored for herring caught in ICES areas 6.a, 7.b and 7.c.

Morphometrics can generally be split into three different categories; traditional morphometrics is a series of length measurements, ratios or angles used to describe an object, landmark-based geometric morphometrics uses a collection of landmarks chosen strategically to represent the shape and form of the object, and outline-based geometric morphometrics can be used to describe the shape of open or closed curves and usually does not involve fixed landmarks. These three categories are linked by the fact that landmarks can be the starting point for each method (Webster and Sheets, 2010). Morphometric measurements can be taken from the body and from the otoliths.

Studies on body morphometrics have been applied to a variety of pelagic fish species including, sardine (Silva, 2003), horse mackerel (Murta *et al*., 2008; Turan, 2004), blue jack mackerel (Moireira, *et al*., 2019; Vasconcelos *et al*., 2018), thread herring (Pérez-Quiñonez *et al*., 2018) and Atlantic herring (Armstrong and Cadrin, 2001). During the

1980s and 1990s, different methods including genetics, tagging and parasite loadings were used to distinguish between different stocks, and of these methods, morphometrics and meristics were considered to be the most successful methods for stock identification (Armstrong and Cadrin, 2001). Concerns have been raised that the traditional distance measurements taken from body forms, including body depth, head height and length, could yield a biased coverage of the body (Strauss and Bookstein, 1982). As a result, landmark-based geometric methods, which make use of a configuration of points, generally based on anatomical features, are now more widely used in morphometric studies to describe the general form or shape of the body. (Ressel *et al*., 2020; Moreira *et al*., 2020; Vasconcelos *et al*., 2018). Biologically meaningful features can be measured through landmark placement, including fin position, mouth size and eye position (Cadrin, 2010). A truss network system using the x, y coordinates of the landmarks, calculates a network of measurements all over the body (Murta *et al*., 2008; Cadrin and Silva, 2005; Silva, 2003; Armstrong and Cadrin, 2001). The combination of traditional morphometric measurements and a landmark-based geometric method covers the entire body of the fish which increases the chance of detecting morphometric differences in body form (Turan, 1998).

Otoliths are a reliable, easily accessible tool for stock discrimination; their shape and appearance can vary among geographic stocks (Tuset *et al*., 2008), they are routinely collected by institutes across the world to gather age data for stock assessment models (Begg and Waldman, 1999; Turan, 2000), and they grow throughout the lifetime of the fish with re-absorption of material laid down in the otolith unlikely (Rodriguez-Mendoza, 2006). Otolith morphometry and shape have been successfully used to distinguish between different fish stocks in a number of marine fish species (Biolé *et al*., 2019; Duncan *et al*., 2018; Hüssy *et al*., 2016; Ihssen *et al*., 1981). The shape of an otolith can be defined by a combination of genetics and different environmental factors (Berg *et al*., 2018; Cardinale *et al*., 2004), including temperature, salinity and food availability (Mille *et al*., 2016; Feet *et al*., 2002; Fey, 2001). Otolith shape is also reported to be correlated with spawning period (Libungan *et al*., 2015a). Spring spawning herring hatch into ideal conditions and have been found to have small eggs and a small yolk sac due to food availability (Hempel and Blaxter, 1967), autumn and winter spawners hatch into less favourable conditions and the availability of food at the beginning of the life cycle can impact the shape of the otolith (Hüssy, 2008). When dealing with more regionalised population differences, the effect of growth can have a larger effect on otolith shape than the stock of origin (Campana and Casselman, 1993), and the effect of growth on otoliths manifests as a slower growing fish having larger otoliths than a faster growing fish of the same size (Hüssy *et al*., 2016). Otolith shape can be analysed using two different methods; landmark analysis (Cadrin, 2013) and outline analysis (Libungan *et al*., 2015a,b). Otolith morphology variables and shape descriptors are collected from the otolith to describe its form. Otolith morphology includes variables such as length, width, perimeter and area. Shape descriptors are calculated using transformation techniques, such as Fourier transform and Wavelet transform (Song *et al*., 2018). Several variations of Fourier transform techniques exist and have been applied to fish otoliths since the 1980s (Bird *et al*., 1986) but the more advanced technique of elliptic fourier analysis became more widely used in the 1990s (Murta *et al*., 1996). Elliptic fourier analysis does not require equally spaced points along the outline, which means it can more accurately map the outline of complex shapes when compared with other Fourier transform techniques (Tracey *et al*., 2006). The fundamental idea of Wavelet transform is to analyse data according to scale. Fourier analysis is based on sine and cosine functions, but these are non-local functions and do a poor job of estimating sharp edges. Approximating functions which are contained within finite domains are used in Wavelet analysis, making them well suited to approximating data with sharp discontinuities (Graps, 1995). The complex shape of herring otoliths makes it difficult to accurately describe the shape. Wavelet transform is reported to be more powerful than the commonly applied Fourier transform for shape analysis (Libungan and Pálsson, 2015), and it has been successfully applied to herring otoliths in the North Atlantic (Berg *et al*., 2018; Eggers *et al*., 2014; Libungan *et al*., 2015a, b).

Genetic assignment methods compare genetic data from individuals to genetic profiles of reference samples from potential source populations to determine population of origin, if

any, for a given individual (Manel *et al*., 2005). The methods can also be used to assess the amount of overlap or separation between the reference populations (McMillan and Fewster, 2017). Traditionally, genetic stock identification methods have promised to address the deficiencies in other methods, yet few have yielded results that have been integrated into effective management (Reiss *et al*., 2009; Waples *et al*., 2008). Many existing genetic studies have been hampered by high cost, few analysed individuals, inadequate sampling coverage, low numbers of suitable molecular markers, laborious genotyping and low power to detect genetic structure (see Mariani and Bekkevold, 2013). This scenario is particularly evident in studies of marine fish with large populations both in numbers and geographic spread, which may require a large number of molecular markers to provide sufficient power to detect significant population structure (see Hatfield *et al*., 2005). Incorporation of genetic assignment methods into regular fisheries data collection, assessment and management has been slow (Bernatchez *et al*., 2017; Reiss *et al*., 2009; Waples *et al*., 2008). Recently the advent of Next Generation Sequencing (NGS) technologies has fundamentally changed the way in which genetic sequence data are generated (see Hemmer-Hansen *et al*., 2014). It is now possible to generate large genomic data sets for non-model species, which facilitate the identification of genetic loci with high discriminatory power for specific population differentiation questions (Han *et al*., 2020; Martínez Barrio *et al*., 2016; Nielsen *et al*., 2012). NGS has also enabled high-throughput low cost genotyping that can be used for population genetics studies (Davey *et al*., 2011). This new approach is known as 'Genotyping By Sequencing' (GBS) and its primary advantage for population genetic studies is the generation of increased quantities of data with improved statistical power and higher genome representation (Narum *et al*., 2013). The ability to achieve genome-wide coverage has significantly improved our understanding of demographic and evolutionary processes in natural populations of well-studied species (Hemmer-Hansen *et al*., 2014).

Molecular markers such as selectively neutral microsatellites have been the workhorses of fisheries genetics for the past two decades and have played an important role in identifying stock delineations in numerous species (Hauser and Carvalho, 2008). However, before NGS based development methods (see Carlsson *et al*., 2013), microsatellites were costly and laborious to develop and most studies relied on small numbers of markers. Further problems associated with microsatellites include size-homoplasy (i.e. fragments of equal size in numbers of base pairs, but different in base pair composition leading to alleles falsely being treated as identical) and poor levels of inter-laboratory calibration with genotype based on fragment size rather than the underlying sequence information. Genotyping is laborious and often subjective (Mariani and Bekkevold, 2013). These microsatellite related problems have led to an increased focus on another type of molecular marker known as single nucleotide polymorphisms (SNPs), which comprise a change in a single base pair rather than a repeating unit (e.g. a change from an A to a T). There also remains unresolved problems with SNPs, such as ascertainment bias (i.e. markers developed on a particular population may show variability in the particular population but show less in other populations), transferability among SNP genotyping platforms (i.e. there are a number of different technical platforms for genotyping SNPs. Each of the platforms have their advantages and disadvantages, however a SNP marker working well on one may not work well on another and may need to be redeveloped), the requirement for high template DNA quality and the high cost associated with SNP-chip development (Helyar *et al*. 2011; Mariani and Bekkevold, 2013). Many of the issues associated with SNPs and microsatellite-based population studies could be mitigated using a genotyping by sequencing approach (Farrell *et al*., 2016; Helyar *et al*., 2012; Vartia *et al*., 2014, 2016) leading to less expensive and more robust marker panels for stock identification studies.

More recently there has also been a shift toward the analysis of sequence variation of functional, adaptive significance rather than just neutral DNA sequence variation (Mariani and Bekkevold, 2013). This approach focuses on identifying adaptive markers that are under diversifying selection and may reflect distinctive features of local populations (Nielsen *et al*., 2012). Small panels of such high-graded markers may provide efficient traceability tools for marine fisheries management (Han *et al*., 2020; Hemmer-Hansen *et al*., 2018; Martinez Barrio *et al*., 2016) although the temporal stability of genetic baselines

should be regularly monitored, particularly in situations of dynamic environmental conditions (Nielsen *et al*., 2012), and care must also be applied when deciding on appropriate assignment methods to avoid introducing 'high-grading bias' (see Anderson, 2010).

There is a long history of genetic studies on herring with a view to stock identification. The earliest studies in the northeast Atlantic (King, 1987) and Baltic Sea (Andersson, 1981) using allozymes to test for genetic population structure had limited power to detect structure. The move towards the use of randomly selected putatively neutral microsatellites (McPherson *et al*., 2001; O'Connell *et al*., 1998; Olsen *et al*., 2002) in the 21st century increased the resolution of stock identification however only the most differentiated populations could be discerned due to apparent low levels of population structure (Andre *et al*., 2010; Bekkevold *et al*., 2011; Mariani *et al*., 2005; Ruzzante *et al*., 2006). Recently the advent of Next Generation Sequencing (NGS) technologies has fundamentally changed the way in which genetic sequence data are generated (see Hemmer-Hansen *et al*., 2014) and consequently the way in which genetic markers are identified. The subsequent identification of both new microsatellites (Teacher *et al*., 2012) and SNPs (Helyar *et al*., 2012; Limborg *et al*., 2012a) heralded a new wave of attempts to refine the knowledge of population structure of Atlantic herring and also increased the focus on outlier loci (Bekkevold *et al*., 2016, 2016; Limborg *et al*., 2012b; Nielsen *et al*., 2012; Teacher *et al*., 2013). These studies illustrated that herring populations may in fact be highly differentiated, but this differentiation may only be detectable using particular genetic markers that may reflect local adaptation.

In 2015, the University College Dublin research group undertook an Irish industry funded pilot study to reanalyse three WESTHER spawning samples (3S04B Donegal n=86, 3S10B Cape Wrath n=84, and 4X01A Western Baltic n=22) from ICES Divisions 6.a.S, 6.a.N and 3.d, using NGS based approaches. Genetic markers were identified and developed through NGS following a modified protocol from Farrell *et al*. (2016). Locus specific primers were generated for forty-seven variable microsatellite loci. Additionally, forty-four transcriptome derived microsatellites from Teacher *et al*. (2012) were included as recent studies on Atlantic and Baltic Herring employing transcriptome derived microsatellites and SNPs have demonstrated the potential of employing adaptive markers that are under diversifying selection and may reflect distinctive features of local populations (Limborg *et al*., 2012b; Teacher *et al*., 2013). A novel method of microsatellite GBS using individual combinatorial barcoding, to allow pooling of individuals and samples, and custom bioinformatics scripts for data sorting (Farrell *et al*., 2016; Vartia *et al*., 2014; Vartia *et al*., 2016), was developed by the UCD research group. Analyses of twenty-nine of the ninety-one markers (nineteen transcriptome derived, ten novel) revealed significant genetic population structure between the three sample sites. The outlier Baltic sample was considered a positive control as it was assumed to be significantly different to the other two samples.

The WESTHER samples screened for population structure in the pilot study were collected in 2003/2004 and as such the results were temporally limited. Following discussion at the 10th September 2015 Pelagic Advisory Council 6.a Herring Meeting in Marine Scotland, Edinburgh it was deemed critical to collect and screen new baseline samples to verify the temporal stability of this structure. It was also deemed necessary to widen the spatial scale of the baseline samples to include other stocks which may be present in future mixed stock survey and fishery samples including the North Sea, Celtic Sea and Irish Sea samples. The resulting project was funded by the Northern Pelagic Working group of the European Association of Fish Producers Organisations (EAPO) and was undertaken by the UCD research group (see Farrell and Carlsson, 2018). The primary aims of the project, which commenced 10th March 2016, were to genetically screen baseline samples of 6.a herring and to establish a panel of high-graded temporally stable informative genetic markers (Farrell and Carlsson, 2018). In total 1,008 spawning herring were collected and screened with 100 microsatellite markers. The revised marker panel comprised both DNA derived (putatively neutral) and RNA derived (putatively adaptive) markers. The fifty-seven DNA derived markers included thirty-one novel microsatellites from the initial pilot study, seven from McPherson *et al*. (2001) that were also used in WESTHER, three from O'Connell *et al*.

(1998), two from Olsen *et al*. (2002) that were also used in WESTHER, and fourteen from Libungan *et al*. (2012). The forty-three RNA derived microsatellites were from Teacher *et al*. (2012). Preliminary results indicated a north-south division, where samples collected in 6.a.N and the North Sea clustered together and samples from 6.a.S, Celtic Sea and Irish Sea clustered together. Subsequent to the initiation of the expanded pilot study a draft herring genome was published in the scientific literature (Martinez Barrio *et al*., 2016) and a large number of potentially informative SNPs were also identified in herring (Bekkevold *et al*., 2016; Guo *et al*., 2016). In particular, seventy SNPs were identified which appeared to be related to spawning season and offered the potential to discriminate between herring stocks with temporally segregated spawning seasons (Martinez Barrio *et al*., 2016; Lamichhaney *et al*., 2012). Therefore, a subset of 142 of the 1,008 samples from the expanded baseline was genotyped at these seventy SNPs, of which fifty-five SNPS were successfully scored. Similar to the microsatellite analyses, results indicated a distinct north-south division.

Following presentation and discussion of preliminary results on 9th December 2016 at the Pelagic AC 6.a Herring Meeting in Dublin, it was decided to extend the project, to enable further baseline samples to be analysed and to investigate the use of single nucleotide polymorphism markers related to spawning period (Martinez Barrio *et al*., 2016) for the purposes of stock identification. Additional spawning samples were collected within the 6.a.N and 6.a.S spawning areas in the 2016/2017 spawning season. The full set of baseline samples (n=2,383) were genotyped with a further optimised panel of markers including the forty microsatellite markers and the fifty-five genotyped SNPs. An additional twenty novel polymorphic microsatellite markers were also identified by the UCD research group (Farrell and Carlsson, 2018). These microsatellites, which were located within informative areas of the herring genome, were identified by mapping the shotgun sequence data from the 2015 pilot study to the draft herring genome (Martinez Barrio *et al*., 2016). This enabled identification of regions of the genome which showed genetic variation between the putative populations. The genotyping approach followed again was NGS based GBS using the individual combinatorial barcoding method as followed in the pilot studies (see Farrell *et al*., 2016; Vartia *et al*., 2014, 2016). Both microsatellite and SNP markers indicated similar population structure, with significant population structure between the spawning fish collected from 6.a.N (Cape Wrath and NW of Cape Wrath) and 6.a.S (Donegal Bay). No significant population structure was detected between the 6.a.N and the North Sea spawning samples. However, significant structure was also detected between the Celtic Sea and Irish Sea samples and all other samples. It was concluded that the analyses could be strengthened by the collection and analysis of additional baseline samples. This work is continued within the EASME funded '*Herring in Divisions 6.a, 7.b and 7.c: Scientific Assessment of the Identity of the Southern and Northern Stocks through Genetic and Morphometric Analysis*' project.

The recent reduction in costs and increase in output of whole genome sequencing approaches have enabled the development of a fully annotated chromosome level genome for Atlantic herring (Pettersson *et al*., 2019), which was developed as part of a Norwegian/Swedish/Danish funded project entitled '*GENetic adaptations underlying population Structure IN herring*' (*GENSINC*). Through this work, Atlantic herring populations have been shown to be highly differentiated at only a small number of genetic markers (SNPs) associated with genes involved in ecological adaptation, whilst being largely indistinguishable with neutral markers (Han *et al*., 2020; Kerr *et al*., 2018; Lamichhaney *et al*., 2012; Martinez Barrio *et al*., 2016). Wide-scale whole genome sequencing of pooled samples collected over multiple years across the entire distribution range of the species has revealed, for the first time, the genetic population structure of the species (Han *et al*., 2020). Of particular relevance to the current study are the northeast Atlantic samples from around Ireland and Britain. The populations in this region are closely related and distinct from all other herring populations including those to the geographic north i.e. Norwegian Spring Spawning Herring. The populations can be further subdivided into three main groups: the spring spawning herring from the Minch and the Clyde, Cape Wrath and North Sea autumn spawning herring, and a southern group consisting 6.a.S, Celtic Sea, Irish Sea and Downs. Within this last group 6.a.S is more highly differentiated

from the other three groups. The Downs winter spawning herring are of particular interest as this is the first time, they have been shown to be highly differentiated from the North Sea autumn spawning herring. The Cape Wrath (6.a.N) sample was indistinguishable from the North Sea (Orkney) sample, thus supporting the conclusions of the review above that these stocks are most likely a single population. There was significant differentiation between the 6.a.N autumn spawning sample and the 6.a.S winter spawning sample. The toolbox of genetic markers from Han *et al*. (2020) can now be used to define genetic baselines for the individual populations by analysing multiple years of spawning samples from each population and confirming temporal stability of the results. If a sufficiently high level of assignment accuracy is achieved, then it will be possible to analyse potentially mixed samples and to assign the individuals back to their population of origin. This is the focus of the current study in Divisions 6.a, 7.b and 7.c.

2.9. Conclusions

The following conclusions can be drawn from the research reviewed here.

- There is an urgent need to reassess and revise the methods by which the herring stocks around Ireland and Britain are distinguished.
- The current approach of delimiting the boundaries between stocks by fixed geographical lines without a biological basis confounds the data used in the different assessments.
- A standardised and replicable method is required that can be used on commercial and survey catches.
- The 4°W boundary between the 6.a.N autumn spawning herring and North Sea autumn spawning herring has little biological support as a true population boundary.
- The term 'west of Scotland herring' originally referred to populations of spring spawning herring that spawned in the Minch. It now refers to autumn spawning herring that spawn west of the 4°W boundary and are likely to be the same population as North Sea autumn herring.
- The 6.a.S, 7.b and 7.c herring are a winter spawning population though there may be a later spawning component present in the area also.
- In summer the 6.a.S, 7.b and 7.c herring migrate offshore for feeding. In some years they migrate north of the 56°N line of latitude and feed west of the Hebrides during the time of the summer acoustic surveys.
- It is not known if 6.a.S, 7.b and 7.c herring also cross the 4°W boundary with the North Sea.
- There is no robust evidence that Irish Sea herring are found in large abundance west of the Hebrides during summer. Evidence does suggest that they may be found in the Clyde area at this time before returning to spawn in the Irish Sea in autumn.
- The Irish Sea and Celtic Sea are separate but closely related populations and there is exchange between them.
- The winter spawning Downs herring are a separate population to the North Sea autumn spawning herring.

Using stock identification methods, including genetics, body morphometrics and otolith shape analysis, this study aims to identify herring populations within the ICES areas 6.a., 7.b and 7.c and provide an estimate of stock proportions based on management areas where mixing occurs between different biological populations. The suitability of each stock identification method will be examined and combinations of the three methods will be explored to ensure the best fit model is used to distinguish between the herring populations in ICES areas 6.a., 7.b and 7.c.

3. Task 2 – Collection or gathering of samples

3.1. Objectives

- Collect herring samples over multiple years from summer acoustic survey (MSHAS)
- Collect herring samples over multiple years (at least 2014-2018) in the area at spawning time.
- Include other areas that may contribute to the putative stock mixtures.
- Collaborate with the fishing industry and fisheries research institutions.
- Determine number of required samples based on genetic analyses and power analyses.

3.2. Deliverables

- Sampling protocol for collection of baseline and mixed survey samples: **Detailed in Task 7**
- Archive of baseline samples from multiple years: **Collected**
- *Archive of mixed stock samples from tuning index surveys:* ***Collected***

3.3. Sampling

Extensive sampling was conducted between 2014 and 2020 in the core 6.a, 7.b and 7.c area and also on the surrounding stocks. Samples were collected from a range of sources including acoustic and groundfish surveys, industry based acoustic surveys, commercial catches and monitoring fishery catches (Table 3.1). The catch date, catch location, total length (to the 0.5cm below), weight (g), sex and maturity (Table 3.2) were recorded for each fish. Samples were collected and processed for morphometric and genetic samples according to the protocols detailed in in Task 7 (Section 7.3). Samples processed by MSS were maturity staged using the 9-point scale, those processed by the MI were maturity staged using the 8-point scale (excludes stage nine on the MSS scale) and samples processed by the Wageningen University and Research (WUR) on behalf of the Dutch Pelagic Freezer Trawlers Association (PFA) were maturity staged using the ICES 6-point scale. All maturity stages were converted to the ICES 6-point scale according to Table 3.2 so direct comparisons could made between samples.

Table 3.1. Fisheries surveys used as sampling platforms in the current study.

Survey	Years	Months	Area	Institute
6aN industry survey & monitoring fishery	2016-2020	Aug-Oct	6.a.N	Industry/Marine Scotland
6aS/7bc industry survey & monitoring fishery	2016-2020	Nov–Feb	6.a.S, 7.b-c	Industry/Marine Institute
Scottish West Coast groundfish survey (SWC-IBTS)	2018-2019	Feb-Mar	6.a	Marine Scotland
Malin Shelf Herring Acoustic Survey (MSHAS)	2010-2020	Jun-Jul	6.a, 7.b-c	Marine Institute/ Marine Scotland
Irish Groundfish Survey (IGFS)	2015-2020	Nov-Dec	Western shelf	Marine Institute

Table 3.2. Translation of Marine Scotland 9-point maturity scale to ICES 6-point scale. The Marine Institute 8-point scale is the same as the 9-point scale but excludes stage 9. From Mackinson *et al*. 2019.

9-point scale	Equivalent 6-point scale
1 – Immature Virgin	1 – Immature
2 – Immature	1 – Immature
3 – Early maturing	2 – Mature (not included in spawning category)
4 – Maturing	2 – Mature (not included in spawning category)
5 – Spawning prepared	3 – Mature (included in the spawning category)
6 – Spawning	3 – Mature (included in the spawning category)
7 – Spent	4 – Mature spent ((included in the spawning category)
8 – Recovering/resting	5 – Mature resting (not included in the spawning category)
9 – Abnormal	6 – Abnormal (not included in Mature or spawning categories)

3.4. Malin Shelf Herring Acoustic Survey (MSHAS)

The MSHAS has been carried out annually since 2008 and reports on the annual abundance of summer feeding aggregations of herring to the west of Scotland and to the north and west of Ireland for the Malin Shelf area (6.a, 7.b and 7.c combined). The Irish MSHAS is undertaken as part of the Marine Institute's Western European Shelf Pelagic Acoustic Survey (WESPAS) and covers the area from 54°N to 58°30'N. The remainder of Division 6.a to the north of 58°30'N and east to the 4°W line is surveyed by Marine Scotland Science as part of the broader International Herring Acoustic Survey (HERAS). This survey also covers areas to the east including the North Sea. Overall survey estimates of herring in the Malin Shelf area (6.a, 7.b and 7.c combined) are generated using data from these two surveys. Body and otolith morphometric samples have been collected following the SGHERWAY protocol (ICES, 2010) during the MSHAS since 2010. Since 2014 genetic samples have been collected together with the standard morphometric data from the same individual herring. The MSHAS index of abundance for herring is the primary tuning index available for use in the herring stock assessments in Divisions 6.a, 7.b and 7.c. As detailed in Section 2.4, any herring surveyed north of the 56°N line of latitude and west of the 4°W line of longitude (excluding the Clyde area) are currently considered to be part of the 6.a.N autumn spawning stock regardless of their population of origin, their biology or their spawning type. Herring surveyed to the south and west of the 56°N and 7°W lines are considered to be part of the 6.a.S stock in combination with herring in Divisions 7.b and 7.c. The primary aim of the current study was to develop a method to split the MSHAS samples into population of origin so that individual survey indices could be produced for the 6.a.N autumn spawning herring and the 6.a.S, 7.b and 7.c herring. Catch details of the MSHAS samples 2010 to 2020 are provided in Figures 3.1 and 3.2 and in Tables 3.3 and 3.4. Detailed length frequency and maturity data are provided in Annex 3 (Section 10.3) and survey maps in Annex 6 (Section 10.6).

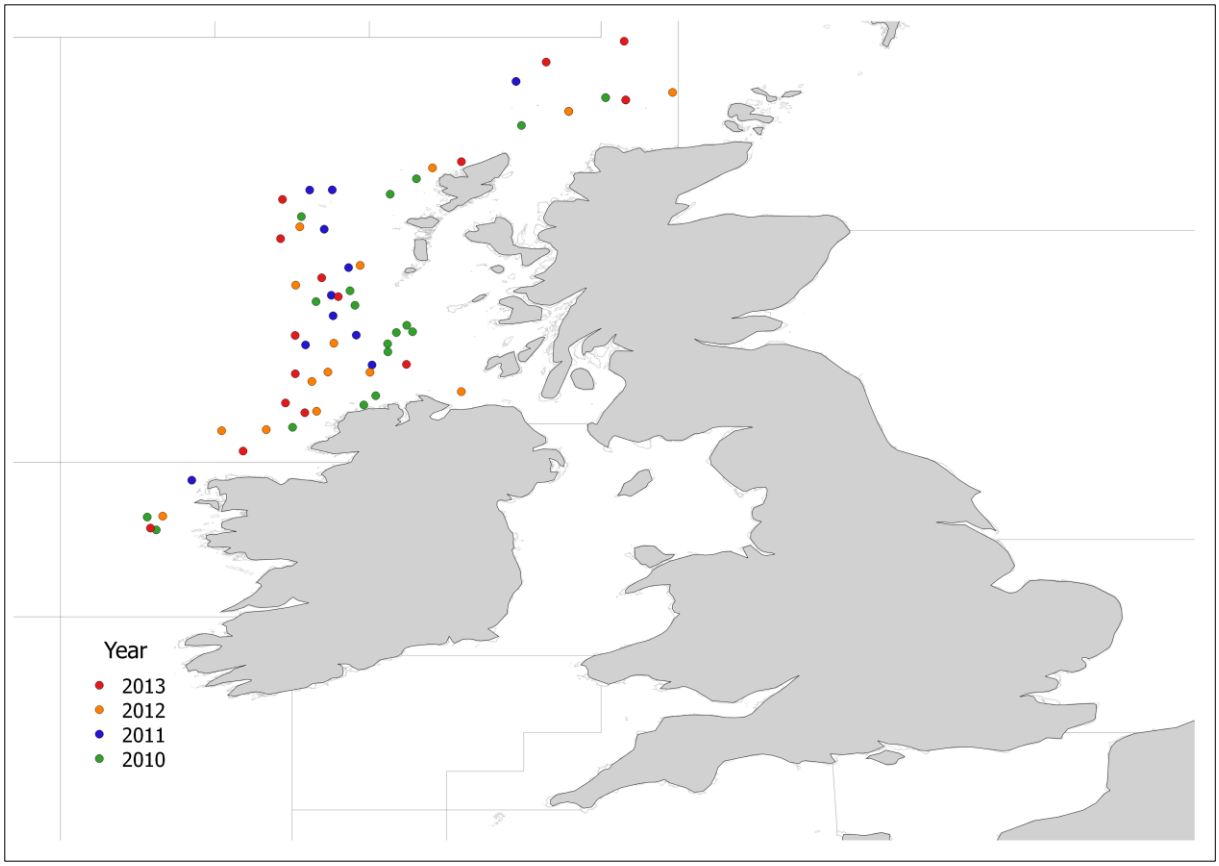


Figure 3.1. The distribution of MSHAS hauls sampled for body and otolith morphometrics during the MSHAS 2010-2013.

Table 3.3. Herring samples collected during the MSHAS 2010-2013 that have body and otolith morphometric data.

Date	Haul	Institute	# fish	Lat	Lon
20/06/2010	3	MI	74	53.63	-10.76
21/06/2010	5	MI	101	53.79	-10.88
26/06/2010	15	MI	122	54.95	-8.99
28/06/2010	18	MI	109	55.24	-8.07
29/06/2010	19	MI	105	55.36	-7.92
01/07/2010	21	MI	101	56.03	-7.76
02/07/2010	23	MI	41	56.27	-7.52
03/07/2010	24	MI	107	56.53	-8.19
30/06/2010	MSS_1	MSS	61	55.93	-7.76
02/07/2010	MSS_2	MSS	117	56.19	-7.44
02/07/2010	MSS_3	MSS	60	56.18	-7.65
05/07/2010	MSS_5	MSS	120	56.58	-8.69
05/07/2010	MSS_6	MSS	120	56.72	-8.25
09/07/2010	MSS_7	MSS	120	57.68	-8.88
09/07/2010	MSS_8	MSS	120	57.97	-7.73
10/07/2010	MSS_9	MSS	120	58.17	-7.39
13/07/2010	MSS_12	MSS	120	58.86	-6.03
15/07/2010	MSS_15	MSS	120	59.22	-4.94
23/06/2011	4	MI	110	58.02	-8.77
23/06/2011	5	MI	110	58.03	-8.48
24/06/2011	6	MI	115	57.52	-8.58
25/06/2011	8	MI	110	57.02	-8.27
26/06/2011	9	MI	110	56.66	-8.49
27/06/2011	10	MI	110	56.40	-8.47
28/06/2011	12	MI	57	56.15	-8.17
28/06/2011	14	MI	110	56.02	-8.83
30/06/2011	18	MI	115	55.76	-7.97
04/07/2011	23	MI	110	54.27	-10.30
22/07/2011	302	MSS	102	59.43	-6.10
24/06/2012	4	MI	120	58.31	-7.18
26/06/2012	5	MI	120	57.55	-8.90
27/06/2012	7	MI	120	57.05	-8.12
27/06/2012	8	MI	120	56.79	-8.95
30/06/2012	12	MI	78	56.04	-8.46
02/07/2012	16	MI	40	55.67	-7.99
02/07/2012	17	MI	120	55.67	-8.54
02/07/2012	18	MI	120	55.55	-8.74
03/07/2012	19	MI	60	55.41	-6.81
04/07/2012	22	MI	120	55.16	-8.68
05/07/2012	23	MI	120	54.92	-9.33
05/07/2012	24	MI	120	54.91	-9.91
07/07/2012	27	MI	74	53.80	-10.68
16/07/2012	313	MSS	120	59.29	-4.07
17/07/2012	314	MSS	29	59.04	-5.42
17/07/2012	315	MSS	120	59.04	-5.42
27/06/2013	3	MI	120	58.39	-6.81
28/06/2013	4	MI	120	57.90	-9.12
29/06/2013	6	MI	120	57.39	-9.15
30/06/2013	7	MI	120	56.89	-8.62
01/07/2013	8	MI	120	56.64	-8.40
03/07/2013	13	MI	120	56.14	-8.96
05/07/2013	14	MI	100	55.77	-7.52
06/07/2013	15	MI	120	55.65	-8.96
07/07/2013	17	MI	120	55.27	-9.09

08/07/2013	18	MI	120	55.14	-8.84
09/07/2013	21	MI	32	54.65	-9.63
11/07/2013	22	MI	39	53.65	-10.83
24/07/2013	184	MSS	123	59.95	-4.70
26/07/2013	188	MSS	134	59.68	-5.71
27/07/2013	190	MSS	136	59.19	-4.68

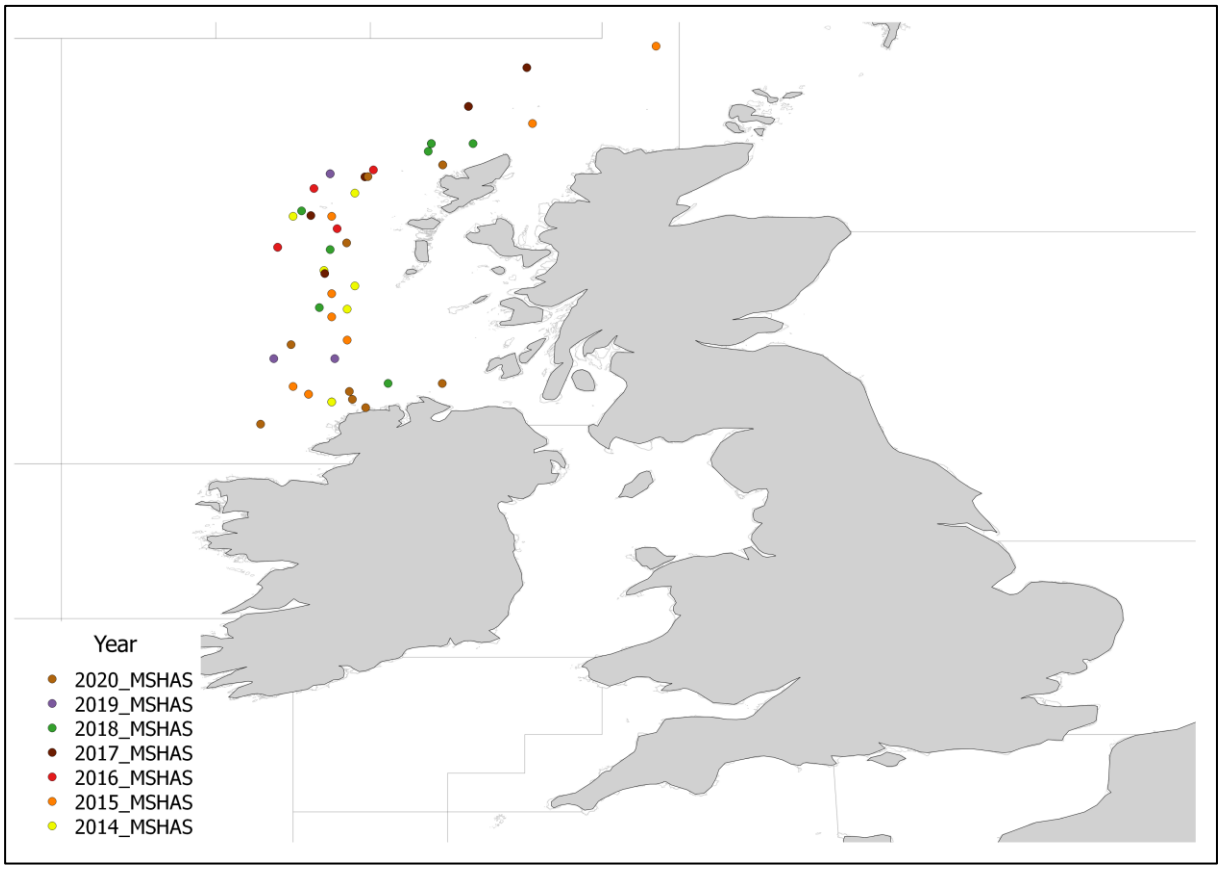


Figure 3.2. The distribution of MSHAS hauls sampled for body and otolith morphometrics and genetics during the MSHAS 2014-2020.

3.5. Division 6.a, 7.b and 7.c samples

In order to develop a baseline with which to compare the MSHAS samples it was necessary to sample the spawning populations in Divisions 6.a, 7.b and 7.c. Samples from both the 6.a.N autumn spawning herring and the 6.a.S spawning herring were readily accessible due to the spawning surveys (see Mackinson *et al*., 2016-2019) and monitoring fisheries in these areas (Figure 3.3 and Table 3.5). In both areas a wide range of maturity stages were identified in the samples (Annex 3). Sampling for 6.a.N spring spawning samples was opportunistic and relied on an industry survey conducted in February 2018 and also on the Scottish West Coast groundfish survey (SWC-IBTS). Only a small number of samples were collected from Division 7.b and none in Division 7.c, despite considerable efforts to obtain samples from these areas (Figure 3.3 and Table 3.5). No spawning fish were observed or collected in Divisions 7.b or 7.c throughout the duration of the project.

Table 3.4. Herring samples collected during the MSHAS 2014-2020 that have body and otolith morphometric data and genetic samples. The sample highlighted in red was collected in Division 4.a during the North Sea HERAS and is analysed in section 4.10.

Date	Haul	Institute	# fish	Lat	Lon
27/06/2014	6	MI	120	58.00	-8.20
28/06/2014	8	MI	120	57.70	-9.00
29/06/2014	9	MI	120	57.00	-8.60
30/06/2014	10	MI	120	56.80	-8.20
01/07/2014	13	MI	120	56.50	-8.30
06/07/2014	20	MI	120	55.30	-8.50
28/06/2015	2	MI	120	59.90	-4.30
30/06/2015	3	MI	120	58.90	-5.90
02/07/2015	5	MI	120	57.70	-8.50
04/07/2015	8	MI	120	56.70	-8.50
05/07/2015	10	MI	120	56.40	-8.50
06/07/2015	11	MI	120	56.10	-8.30
09/07/2015	16	MI	120	55.50	-9.00
09/07/2015	17	MI	120	55.40	-8.80
20/06/2016	5	MI	100	58.30	-7.96
21/06/2016	6	MI	100	58.06	-8.73
22/06/2016	7	MI	100	57.54	-8.43
22/06/2016	8	MI	100	57.30	-9.20
01/07/2016	206	MSS	120	59.59	-3.25
13/07/2017	36	MI	100	56.96	-8.59
15/07/2017	37	MI	100	57.71	-8.77
17/07/2017	39	MI	100	58.21	-8.07
13/07/2017	172	MSS	120	59.62	-5.97
14/07/2017	174	MSS	70	59.12	-6.73
12/07/2018	32	MI	100	55.54	-7.77
14/07/2018	35	MI	120	56.52	-8.66
16/07/2018	37	MI	119	57.27	-8.52
17/07/2018	39	MI	120	57.77	-8.89
19/07/2018	40	MI	120	58.54	-7.25
18/07/2018	181	MSS	120	58.64	-6.67
18/07/2018	182	MSS	120	58.64	-7.21
13/07/2019	35	MI	125	55.86	-8.46
13/07/2019	36	MI	29	55.86	-9.52
18/07/2019	42	MI	122	58.25	-8.52
29/06/2020	22	MI	13	55.01	-9.42
30/06/2020	23	MI	120	55.23	-8.06
01/07/2020	25	MI	120	55.33	-8.23
02/07/2020	26	MI	120	55.44	-8.27
02/07/2020	27	MI	100	55.54	-7.07
03/07/2020	28	MI	120	56.04	-9.03
06/07/2020	31	MI	120	57.36	-8.31
08/07/2020	32	MI	120	58.21	-8.03
08/07/2020	34	MI	100	58.36	-7.06

Table 3.5. Herring samples, sorted by date, collected in Divisions 6.a, 7.b and 7.c 2014-2019. Genetic samples were collected from all samples listed except those highlighted in green. Body and otolith morphometric samples were collected from those denoted with *. All samples except those highlighted in red were genetically analysed. Samples highlighted in blue were used for assignment validation in Section 4.9. Samples highlighted in yellow were used in Section 4.10.

Date	Sample	Stock	Location	#	Lat	Lon
16/12/2014	*6aS_14a**	6.a.S, 7.b-c	Inver Bay	120	54.65	-8.30
17/12/2014	*6aS_14b**	6.a.S, 7.b-c	Glen Head	120	54.66	-8.80
27/8/2014	*6aN_14a**	6.a.N_Aut	west of Hebrides	120	58.00	-7.20
01/9/2014	*6aN_14b**	6.a.N_Aut	Cape Wrath	85	58.60	-4.60
10/09/2015	*6aN_15*	6.a.N_Aut	Cape Wrath	96	58.61	-4.37
08/01/2016	*6aS_16a**	6.a.S, 7.b-c	Teelin Bay	142	54.63	-8.63
08/01/2016	*6aS_16b**	6.a.S, 7.b-c	Teelin Bay	100	54.63	-8.63
25/08/2016	*WoH_16a*	6.a.N_Aut	west of Hebrides	45	58.13	-7.25
28/08/2016	*WoH_16b*	6.a.N_Aut	west of Hebrides	51	58.17	-7.23
26/08/2016	*6aN_16d**	6.a.N_Aut	NW Cape wrath	120	58.77	-5.37
29/08/2016	*6aN_16e**	6.a.N_Aut	NW Cape wrath	116	58.72	-5.35
27/08/2016	*6aN_16f**	6.a.N_Aut	NW Cape wrath	120	58.78	-5.42
06/09/2016	*6aN_16b_H1*	6.a.N_Aut	N Cape Wrath	52	58.62	-4.37
06/09/2016	*6aN_16b_H2*	6.a.N_Aut	N Cape Wrath	44	58.62	-4.37
07/09/2016	*6aN_16c_H1**	6.a.N_Aut	N Cape Wrath	71	58.60	-4.25
09/09/2016	*6aN_16c_H2*	6.a.N_Aut	N Cape Wrath	30	58.60	-4.42
16/09/2016	*6aN_16a**	6.a.N_Aut	N Cape Wrath	120	58.77	-4.65
09/01/2017	*6aS_17a**	6.a.S, 7.b-c	Teelin Bay	120	54.63	-8.63
23/01/2017	*6aS_17b**	6.a.S, 7.b-c	Teelin Bay	120	54.63	-8.63
30/08/2017	*WoH_17*	6.a.N_Aut	west of Hebrides	96	58.14	-7.23
06/09/2017	*6aN_17d*	6.a.N_Aut	NW Cape Wrath	28	58.67	-5.30
06/09/2017	*6aN_17e*	6.a.N_Aut	NW Cape Wrath	42	58.67	-5.32
06/09/2017	*6aN_17f*	6.a.N_Aut	NW Cape Wrath	23	58.63	-5.33
08/09/2017	*6aN_17a*	6.a.N_Aut	NW Cape Wrath	20	58.65	-5.38
08/09/2017	*6aN_17b*	6.a.N_Aut	NW Cape Wrath	36	58.60	-5.48
09/09/2017	*6aN_17g**	6.a.N_Aut	NW Cape Wrath	99	58.63	-5.42
11/09/2017	*6aN_17c*	6.a.N_Aut	NW Cape Wrath	40	58.68	-5.53
09/11/2017	*6aS_17f*	6.a.S, 7.b-c	Lough Swilly	96	55.12	-7.49
29/11/2017	*6aS_17e*	6.a.S, 7.b-c	Bruckless Bay	92	54.61	-8.41
30/11/2017	*6aS_17c*	6.a.N_Aut	Lough Foyle	96	55.16	-7.04
20/11/2017	*6aS_17d*	6.a.S, 7.b-c	Inver Bay	96	54.62	-8.32
17/02/2018	*6aN_Sp_18a*	6.a.N_Sp	North Minch	96	57.82	-5.85
19/02/2018	*6aN_Sp_18c_H1*	6.a.N_Sp	W of Hebrides	10	58.47	-7.50
19/02/2018	*6aN_Sp_18c_H2*	6.a.N_Sp	W of Hebrides	10	58.58	-7.14
19/02/2018	*6aN_Sp_18c_H4*	6.a.N_Sp	W of Hebrides	17	58.58	-7.14
20/02/2018	*6aN_Sp_18c_H3*	6.a.N_Sp	W of Hebrides	7	58.21	-8.03
26/02/2018	*6aN_Sp_18b_H2*	6.a.N_Sp	Stanton	18	56.02	-7.13
23/02/2018	*6aS_18h*	6.a.S, 7.b-c	West of Donegal	42	54.95	-9.02
02/03/2018	*6aN_Sp_18b_H1*	6.a.N_Sp	Stanton	18	56.53	-7.40
04/03/2018	*6aN_Sp_18b_H3*	6.a.N_Sp	Stanton	16	57.01	-7.15
05/03/2018	*6aN_Sp_18d*	6.a.N_Sp	Minch	46	58.28	-5.49
27/03/2018	*6aS_18i*	6.a.S, 7.b-c	Galway Bay	48	53.18	-9.66
11/04/2018	*6aS_18j*	6.a.S, 7.b-c	Galway Bay	48	53.18	-9.66
16/09/2018	*6aN_18b*	6.a.N_Aut	Cape Wrath	48	58.82	-4.43
16/09/2018	*6aN_18c*	6.a.N_Aut	Cape Wrath	100	58.66	-4.40
17/09/2018	*6aN_18d*	6.a.N_Aut	Cape Wrath	100	58.68	-4.38
18/09/2018	*6aN_18e*	6.a.N_Aut	Cape Wrath	100	58.62	-4.42
18/09/2018	*6aN_18f*	6.a.N_Aut	Cape Wrath	100	58.65	-4.27
27/09/2018	*6aN_18a*	6.a.N_Aut	Cape Wrath	96	58.58	-4.28
05/11/2018	*6aS_18k*	6.a.S, 7.b-c	Tiree	96	56.24	-6.71

12/11/2018	*6aS_18e*	6.a.S, 7.b-c	Lough Swilly	96	55.12	-7.30
14/11/2018	*6aS_18d*	6.a.N_Aut	Lough Foyle	96	55.15	-6.90
20/11/2018	*6aS_18f*	6.a.S, 7.b-c	Bruckless Bay	48	54.61	-8.41
21/11/2018	*6aS_18g*	6.a.S, 7.b-c	Bruckless Bay	48	54.61	-8.41
02/12/2018	*6aS_18c*	6.a.S, 7.b-c	Inver Bay	96	54.65	-8.30
05/12/2018	*6aS_18a*	6.a.S, 7.b-c	St John's Point	96	54.61	-8.41
11/12/2018	*6aS_18b*	6.a.S, 7.b-c	Teelin Bay	96	54.63	-8.63
09/01/2019	*6aS_19b*	6.a.S, 7.b-c	Lough Swilly	66	55.12	-7.49
11/01/2019	*6aS_19a*	6.a.N_Aut	Lough Foyle	48	55.16	-7.04
19/02/2019	*6aN_Sp_19d*	6.a.N_Sp	N Cape Wrath	49	59.30	-4.79
19/02/2019	*6aN_Sp_19e*	6.a.N_Sp	N Cape Wrath	47	59.16	-4.16
21/02/2019	*6aN_Sp_19g*	6.a.N_Sp	NW of Hebrides	47	58.96	-6.65
21/02/2019	*6aN_Sp_19f*	6.a.N_Sp	NW of Hebrides	53	59.32	-6.13
22/02/2019	*6aN_Sp_19a*	6.a.N_Sp	W of Hebrides	50	58.55	-6.90
07/03/2019	*6aN_Sp_19b*	6.a.N_Sp	South Minch	17	56.74	-7.09
07/03/2019	*6aN_Sp_19c*	6.a.N_Sp	South Minch	22	56.88	-7.09
07/03/2019	*6aN_Sp_19i*	6.a.N_Sp	South Minch	66	57.13	-6.68
06/09/2019	*6aN_19e*	6.a.N_Aut	NW Cape Wrath	100	58.68	-5.55
07/09/2019	*6aS_19d*	6.a.S, 7.b-c	Galway Bay	35	53.23	-9.60
21/09/2019	*6aN_19a*	6.a.N_Aut	NW Cape Wrath	100	58.70	-5.35
21/09/2019	*6aN_19b*	6.a.N_Aut	NW Cape Wrath	100	58.72	-5.18
21/09/2019	*6aN_19d*	6.a.N_Aut	NW Cape Wrath	100	58.68	-5.25
30/09/2019	*6aN_19c*	6.a.N_Aut	NW Cape Wrath	100	58.53	-5.68
18/10/2019	*6aS_19e*	6.a.S, 7.b-c	Galway Bay	65	53.24	-9.04
03/11/2019	*6aS_19f*	6.a.S, 7.b-c	Lough Swilly	80	55.11	-7.49
03/11/2019	*6aS_19g*	6.a.S, 7.b-c	Inver Bay	98	54.62	-8.31
24/11/2019	*6aS_19c**	6.a.S, 7.b-c	Drumanoo Head	120	54.61	-8.49
31/12/2019	*6aS_19h*	6.a.S, 7.b-c	Clew Bay	80	53.83	-9.95

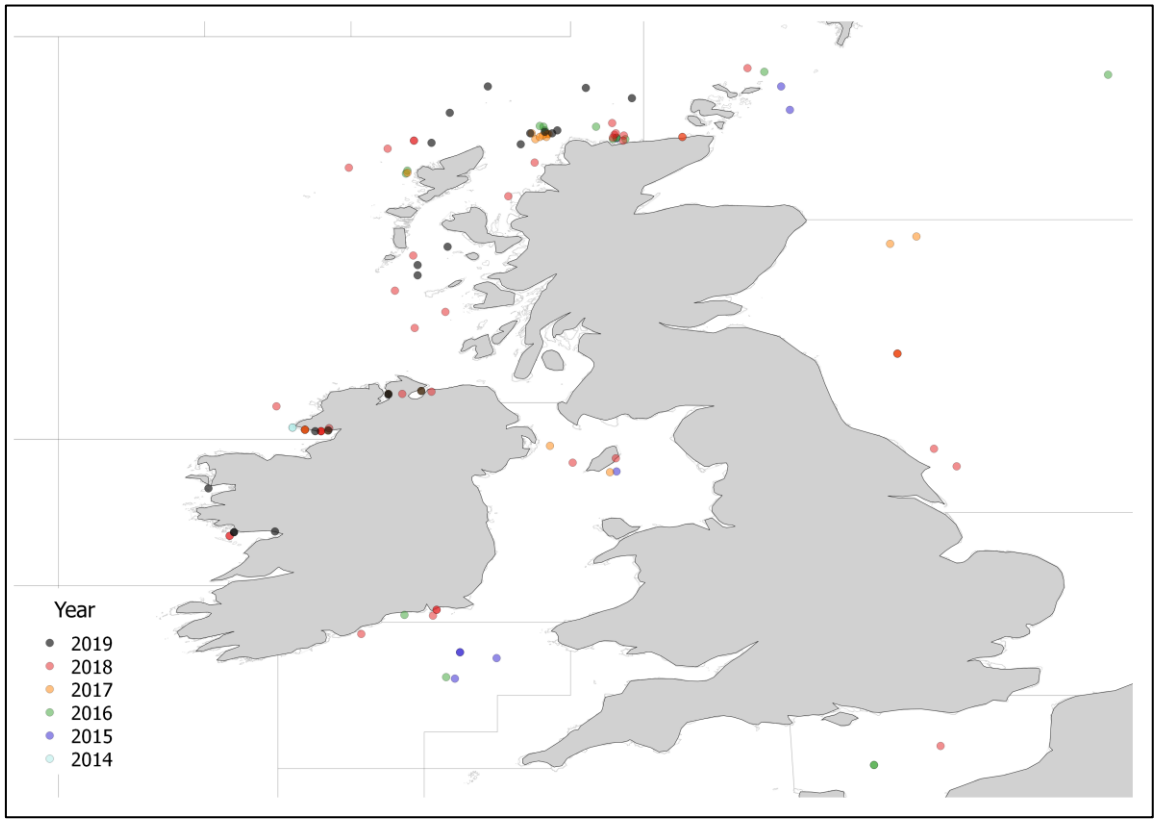


Figure 3.3. The distribution of contemporary herring samples from the current project 2014-2019 detailed in Tables 3.5 and 3.6.

3.6. Outgroup samples

Outgroup samples, defined as samples from the stocks surrounding ICES Divisions 6.a, 7.b and 7.c, were also collected from 2015 to 2018 from the autumn spawning Irish Sea herring, the winter spawning Celtic Sea herring, the autumn spawning North Sea herring and the winter spawning Downs herring for genetic analysis (Figure 3.3 and Table 3.6). Detailed length frequency and maturity data are provided in Annex 3 (Section 10.3).

Table 3.6. Herring genetic samples, sorted by date, collected in Division 6a 2014-2019.

Date	Sample	Stock	Division	Location	#	Lat	Lon
01/09/2015	*NS_15a*	NS	4.a	Orkney	96	59.00	-2.00
11/09/2015	*NS_15b*	NS	4.a	NE Orkney	48	59.32	-2.12
30/09/2015	*IS_15*	IS	7.a	Irish Sea	96	54.06	-4.37
11/10/2015	*CS_15a*	CS	7.g	Celtic Sea	48	51.51	-6.01
16/10/2015	*CS_15b*	CS	7.g	Celtic Sea	48	51.23	-6.58
01/12/2015	*CS_15c*	CS	7.g	Celtic Sea	96	51.59	-6.51
03/09/2016	*NS_16a*	NS	4.a	NE North Sea	95	59.48	2.35
03/09/2016	*NS_16b*	NS	4.a	North Sea	61	59.52	-2.35
11/10/2016	*CS_16a*	CS	7.g	Celtic Sea	46	52.10	-7.27
21/10/2016	*CS_16b*	CS	7.g	Celtic Sea	50	51.25	-6.70
13/12/2016	*DWN_16*	NS	7.d	Downs	34	50.05	-0.85
29/08/2017	*IS_17b*	IS	7.a	Rigg Bank	96	54.41	-5.28
07/09/2017	*NS_17a*	NS	4.a	Orkney	21	58.63	-3.47
09/09/2017	*NS_17b*	NS	4.b	Buchan	23	57.27	-0.27
09/09/2017	*NS_17c*	NS	4.b	Buchan	23	57.17	-0.63
11/09/2017	*IS_17a*	IS	7.a	Douglas Bank	90	54.05	-4.46
12/09/2017	*NS_17d*	NS	4.b	Banks	25	55.67	-0.53
08/02/2018	*CS_18b*	CS	7.g	Celtic Sea	96	52.09	-6.88
09/02/2018	*CS_18a*	CS	7.a.S	Celtic Sea	96	51.84	-7.86
31/08/2018	*NS_18a*	NS	4.a	North Orkney	48	59.57	-2.58
09/09/2018	*NS_18d*	NS	4.b	Banks	48	54.13	0.28
10/09/2018	*IS_18a*	IS	7.a.N	Laxey Bay	96	54.24	-4.38
11/09/2018	*IS_18b*	IS	7.a.N	N Chicken Bank	96	54.18	-4.97
19/09/2018	*NS_18e*	NS	4.b	Banks	48	54.37	-0.03
27/09/2018	*NS_18b*	NS	4.a	Orkney	48	58.63	-3.47
28/09/2018	*NS_18c*	NS	4.b	Banks	96	55.67	-0.53
09/12/2018	*DWNS_18*	NS	7.d	Downs	96	50.31	0.06
11/12/2018	*CS_18c*	CS	7.a.S	Baginbun	96	52.17	-6.83

3.7. WESTHER archive samples

The original spawning baseline genetic samples from the WESTHER project 2003-2004 were located in University College Cork, Ireland and the University of Liverpool, UK (Figure 3.4 and Table 3.7). The WESTHER genetic samples were collected using a variety of different microtube types of varying quality and had been stored, since the project ended, in a range of temperatures. Therefore, the quality of the genetic samples was highly variable, with some being very high and suitable for re-analysis and some being completely degraded. Detailed length frequency and maturity data are provided in Annex 3 (Section 10.3).

Table 3.7. Baseline spawning genetic samples from the 2003-2004 WESTHER project.

Year	Date	Sample	Division	Location	#	Lat	Lon
2003	14/03/2003	*Clyde_03 (3S05A)*	6.a.N	Clyde	44	55.14	-5.04
2003	01/09/2003	*6aN_03 (3S10B)*	6.a.N	Cape Wrath	84	58.62	-4.01
2003	02/10/2003	*IS_03 (3S06A)*	7.a	Douglas	48	54.12	-4.39
2003	21/10/2003	*6aS_03 (3S04B)*	6.a.S	Glen Head	85	54.68	-8.87
2003	17/12/2003	*CS_03 (3S01B)*	7.g	Hook Head	48	52.15	-6.80
2004	13/01/2004	*6aS_04 (4S04A)*	6.a.S	Aranmore	105	54.98	-8.59
2004	24/02/2004	*6aN_04 (4S10A)*	6.a.N	Skye	101	57.41	-6.15
2004	09/02/2004	*CS_04 (4S01A)*	7.g	Hook Head	105	52.03	-7.01
2004	22/08/2004	*6aN_04 (4S10B)*	6.a.N	Cape Wrath	100	58.60	-5.40
2004	23/09/2004	*IS_04 (4S06A)*	7.a	Douglas	40	54.13	-4.32
2004	19/10/2004	*7b_04 (4S03A)*	7.b	Achill	105	53.83	-10.11
2004	30/11/2004	*7j_04 (4S02A)*	7.j	SW Ireland	105	51.54	-9.83

Figure 3.4. The distribution of baseline spawning samples from the WESTHER project 2003-2004.

3.8. Conclusions

Extensive sampling was conducted between 2014 and 2020 in the core 6.a, 7.b and 7.c area and also on the surrounding stocks The objectives of Task 2 (Section 3.1) were completed, and all of the deliverables met. Further details on the analysis of the individual samples are provided in the relevant tasks.

4. Task 3 – Genetic analysis & Task 4 – Genetic data analysis and comparison with information from other stocks

4.1. Objectives

- Characterise the genetic variation from each of the spawning stocks in Task 2.
- Propose a state-of-the-art genetic method and justify why it is the best approach.
- If successful, propose development of high throughput collection and processing system.
- Analyse the genetic data using appropriate methods.
- Compare the outcome of the genetic analysis with existing genetic information currently available for these and other stocks in adjacent spawning areas.

4.2. Deliverables

- Genetic baseline database which to compare future mixed survey and fishery samples: **Completed**
- Confirmation of temporal stability of genetic structure: **Completed**
- Comparison of current baseline with WESTHER samples: **Completed**.
- Split of MSHAS samples into source stocks: **Completed**
- Panel of high-graded temporally stable informative genetic markers: **Completed**
- High-throughput methods for sample collection, processing and analysis: **Completed**
- Clear description of the methods and description of the results: **Completed**

4.3. Sample selection

Herring sampling between 2014 and 2020 was largely opportunistic and relied on the availability of existing surveys and commercial catches (Table 3.1). No ship-time was directly funded through the current project or in the previous industry funded studies. Therefore, samples were collected when available with the aim of collecting as many as possible, over as wide a temporal and spatial distribution as possible. As a result, the samples collected from Divisions 6.a, 7.b and 7.c and the outgroup populations contained a significant mix of length classes and maturity stages (Annex 3). For the purposes of developing robust baselines, it is critical to avoid including samples with uncertain origin. Therefore, only samples with a high proportion of maturity stage three individuals (6-point scale), caught in close proximity to known spawning grounds at spawning time were selected to be baseline samples. In order to further limit the potential for misclassification as a baseline spawning sample, only individuals classified as maturity stage three were included in the baseline analyses (Figure 4.1 and Table 4.1). It should be noted however that macroscopic maturity staging can be unreliable (ICES, 2011b) and as such there is a possibility of some contamination of baselines even with these strict criteria. This may be more of an issue for autumn spawning groups such as the 6.a.N autumn spawners, as during spawning there may be individuals from adjacent, winter and spring spawning populations mixing with the autumn spawners. These other individuals would most likely be maturity stage two on the 6-point scale, which equates to stage three and four on the 9-point scale. It can be difficult to distinguish the late pre-spawning stages from the early spawning stages, which leaves the potential for misclassification. Conversely any maturity stage two fish (6-point scale) observed on the 6.a.S, 7.b and 7.c spawning grounds during the winter spawning period are unlikely to be 6.a.N autumn spawning fish as these would be at maturity stage four and five on the 6-point scale at this time of year and these are readily distinguished from late stage two fish. Whilst initially excluded from the baseline dataset the pre-spawning samples and other samples of uncertain population of origin due to maturity stage (Table 3.5) will be compared to the baseline dataset to attempt to confirm their origin (Sections 4.9 and 4.10).

A large number of small samples of potential 6.a.N spring spawning samples were collected from the Scottish West Coast groundfish survey (SWC-IBTS) in quarter one 2018 and 2019 (Figure 3.3 and Tables 3.1 and 3.5). The 2018 samples were pre-screened prior to processing and only stage three fish were analysed. The 2019 samples comprised a range of maturity stages from pre-spawning, spawning and spent fish (Annex 3). A small number of stage three fish were caught west of the Hebrides and in the southern Minch area. These

are not recognised spring spawning grounds and as such were not included in the baseline dataset but will be compared to the baseline (Section 4.9). Only spring spawning herring caught in the northern Minch were included in the baseline dataset as this area is well documented as a spawning ground for this population (Baxter, 1958; 1963a). The 2019 samples had a high proportion of stage five fish and none of the samples were from the north Minch area. Therefore, they were all excluded from the baseline dataset.

A small number of spawning samples were caught in Lough Foyle in northwest Ireland. As described in Section 2.5, Lough Foyle is bisected by the 7°W line of longitude. Most of Lough Foyle is west of this line, however the mouth of Lough Foyle is east of this line and hence the herring in the whole lough are considered to be part of the 6.a.N autumn spawning stock despite having no obvious biologic or geographic proximity to this population. Due to this phenomenon the Lough Foyle samples were excluded from the baseline datasets and instead will be compared to the baseline dataset to attempt to confirm their origin (Section 4.10).

The 2019 spawning samples from the 6.a.S winter and the 6.a.N autumn spawning stocks (Figure 3.3 and Table 3.5) were also excluded from the baseline datasets and reserved to be used as 'known unknown' samples i.e. samples of known origin that will be used to test the assignments (Section 4.9). Similarly, the WESTHER baseline spawning samples (Figure 3.4 and Table 3.7) were reserved to be used as 'known unknown' samples to test both the accuracy of assignment and also the temporal stability of the genetic markers (Section 4.9). The MSHAS samples (Figure 3.2 and Table 3.4) were analysed with the SNP markers only and were compared with the SNP baseline in order to assign individuals back to population of origin (Section 4.11).

In total c.4,900 individuals from Divisions 6.a, 7.b and 7.c, 1,860 individuals from the outgroup populations, 650 individuals from the WESTHER samples and 3,665 individuals from the MSHAS samples were analysed as part of the genetic analysis tasks of the current project. The populations were defined based, on temporal and spatial separation of spawning, as: *6aS/7bc* (6.a.S, 7.b and 7.c), *6aN_Aut* (6.a.N autumn spawners), *6aN_Sp* (6.a.N spring spawners), *CS* (Celtic Sea winter spawners), *IS* (Irish Sea autumn spawners), *NS* (North Sea autumn spawners), *DWN* (Downs winter spawners) (Table 4.1).

Figure 4.1. The distribution of baseline spawning herring samples from the current project 2014-2018.

Table 4.1. Catch details and number of fish in the baseline spawning herring samples, sorted by population and date, included in the baseline analyses.

Date	Sample	Population	Location	# stage 3	Lat	Lon
10/09/2015	*6aN_15*	*6aN_Aut*	Cape Wrath	94	58.61	-4.37
26/08/2016	*6aN_16d*	*6aN_Aut*	NW Cape wrath	64	58.77	-5.37
27/08/2016	*6aN_16f*	*6aN_Aut*	NW Cape wrath	86	58.78	-5.42
29/08/2016	*6aN_16e*	*6aN_Aut*	NW Cape wrath	79	58.72	-5.35
06/09/2016	*6aN_16b_H1*	*6aN_Aut*	N Cape Wrath	34	58.62	-4.37
06/09/2016	*6aN_16b_H2*	*6aN_Aut*	N Cape Wrath	28	58.62	-4.37
07/09/2016	*6aN_16c_H1*	*6aN_Aut*	N Cape Wrath	50	58.6	-4.25
09/09/2016	*6aN_16c_H2*	*6aN_Aut*	N Cape Wrath	24	58.6	-4.42
16/09/2016	*6aN_16a*	*6aN_Aut*	N Cape Wrath	78	58.77	-4.65
06/09/2017	*6aN_17d*	*6aN_Aut*	NW Cape Wrath	27	58.67	-5.3
06/09/2017	*6aN_17e*	*6aN_Aut*	NW Cape Wrath	42	58.67	-5.32
06/09/2017	*6aN_17f*	*6aN_Aut*	NW Cape Wrath	21	58.63	-5.33
08/09/2017	*6aN_17a*	*6aN_Aut*	NW Cape Wrath	16	58.65	-5.38
08/09/2017	*6aN_17b*	*6aN_Aut*	NW Cape Wrath	34	58.6	-5.48
09/09/2017	*6aN_17g*	*6aN_Aut*	NW Cape Wrath	95	58.63	-5.42
11/09/2017	*6aN_17c*	*6aN_Aut*	NW Cape Wrath	36	58.68	-5.53
16/09/2018	*6aN_18b*	*6aN_Aut*	Cape Wrath	47	58.82	-4.43
27/09/2018	*6aN_18a*	*6aN_Aut*	Cape Wrath	93	58.58	-4.28
17/02/2018	*6aN_Sp_18a*	*6aN_Sp*	North Minch	73	57.82	-5.85
05/03/2018	*6aN_Sp_18d*	*6aN_Sp*	Minch	29	58.28	-5.49
17/12/2014	*6aS_14b*	*6aS/7bc*	Glen Head	41	54.66	-8.8
08/01/2016	*6aS_16a*	*6aS/7bc*	Teelin Bay	94	54.63	-8.63
08/01/2016	*6aS_16b*	*6aS/7bc*	Teelin Bay	20	54.63	-8.63
09/01/2017	*6aS_17a*	*6aS/7bc*	Teelin Bay	80	54.63	-8.63
23/01/2017	*6aS_17b*	*6aS/7bc*	Teelin Bay	87	54.63	-8.63
09/11/2017	*6aS_17f*	*6aS/7bc*	Lough Swilly	57	55.12	-7.49
20/11/2017	*6aS_17d*	*6aS/7bc*	Inver Bay	78	54.62	-8.32
29/11/2017	*6aS_17e*	*6aS/7bc*	Bruckless Bay	50	54.61	-8.41
23/02/2018	*6aS_18h*	*6aS/7bc*	West of Donegal	16	54.95	-9.02
05/12/2018	*6aS_18a*	*6aS/7bc*	St John's Point	92	54.61	-8.41
11/12/2018	*6aS_18b*	*6aS/7bc*	Teelin Bay	84	54.63	-8.63
08/02/2018	*CS_18b*	*CS*	Celtic Sea	83	52.09	-6.88
09/02/2018	*CS_18a*	*CS*	Celtic Sea	84	51.84	-7.86
30/09/2015	*IS_15*	*IS*	Irish Sea	80	54.06	-4.37
29/08/2017	*IS_17b*	*IS*	Rigg Bank	71	54.41	-5.28
11/09/2017	*IS_17a*	*IS*	Douglas Bank	67	54.05	-4.46
01/09/2015	*NS_15a*	*NS*	Orkney	74	59	-2
11/09/2015	*NS_15b*	*NS*	NE Orkney	47	59.32	-2.12
03/09/2016	*NS_16b*	*NS*	North Sea	49	59.52	-2.35
07/09/2017	*NS_17a*	*NS*	Orkney	22	58.63	-3.47
09/09/2017	*NS_17b*	*NS*	Buchan	19	57.27	-0.27
09/09/2017	*NS_17c*	*NS*	Buchan	24	57.17	-0.63
12/09/2017	*NS_17d*	*NS*	Banks	23	55.67	-0.53
31/08/2018	*NS_18a*	*NS*	North Orkney	40	59.57	-2.58
09/09/2018	*NS_18d*	*NS*	Banks	44	54.13	0.28
19/09/2018	*NS_18e*	*NS*	Banks	46	54.37	-0.03
27/09/2018	*NS_18b*	*NS*	Orkney	43	58.63	-3.47
28/09/2018	*NS_18c*	*NS*	Banks	85	55.67	-0.53
13/12/2016	*DWN_16*	*DWN*	Downs	32	50.05	-0.85
09/12/2018	*DWN_18*	*DWN*	Downs	95	50.31	0.06

4.4. Genetic marker development and primer design

The microsatellite marker panel used in the current study was developed as part of the industry funded *6a herring stock identification project 2015-2018* (see Farrell and Carlsson, 2018 and Section 2.8). Following publication of the chromosome level herring genome assembly (Pettersson *et al*., 2019), the microsatellites were mapped to the herring genome in order to identify the positions of the markers in the genome (Table 4.2). The microsatellites were spread across 19 chromosomes and a number of the markers were located in areas of the genome identified as being involved in the local adaption of herring populations (Han *et al*., 2020).

As described in the *1st Interim report*, a collaboration was developed with the Swedish/ Norwegian/Danish *GENSINC* project. The project is undertaking full genome sequencing of pooled samples from all herring populations across the species distribution in order to study the biological significance of the genetic variants underlying ecological adaptation in the Atlantic herring (Han *et al*., 2020; Lamichhaney *et al*., 2012; Martinez Barrio *et al*., 2016; Pettersson *et al*., 2019). In exchange for providing the *GENSINC* project with key samples from the populations around Ireland and Britain, access to the genome data and newly discovered markers was granted. The SNP markers used in the current study (Table 4.3) are derived from this collaboration. Twenty-three of the SNPs in the SNP marker panel were also used as part of the industry funded *6a herring stock identification project 2015-2018* (see Farrell and Carlsson, 2018 and Section 2.8) and were identified as being involved in the local adaptation of herring populations from the genome sequencing analyses detailed in Martinez Barrio *et al*. (2016). Seventeen additional SNPs, that are currently being used in DTU-Aqua, Silkeborg, Denmark as part of their *GENSINC* derived marker panel, were identified as being informative for the populations around Ireland and Britain. The final five SNPs were identified during the data analysis presented in Han *et al*. (2020).

Locus-specific forward and reverse primers were designed for polymorphic microsatellite and SNP loci with the *Primer3* application (Rozen and Skaletsky, 2000) in Geneious® 7.0 (Kearse *et al*., 2012) with optimal primer length set at 20bp, optimal T_m at 60°C and product size range at 120-180bp. Primers were designed to bind in conserved flanking regions to minimise the possibility of null alleles. Primers were cross-referenced with the original shot-gun sequence data set from the industry funded pilot study (Section 2.8) to identify primers that annealed to multiple regions, which if detected were excluded. The forward and reverse locus-specific primers were adapted, to facilitate combinatorial barcoding of amplicons, by adding either an M13-R (*5'-GGAAACAGCTATGACCAT-3'*) or CAG (*5'-CAGTCGGGCGTCATCA-3'*) universal tail to the 5' end as described in Vartia *et al*. (2016) and Farrell *et al*. (2016). The modified primers were tested for the formation of secondary structures (hairpins, primer dimers and hetero dimers) with the IDT OligoAnalyzer Tool 3.1 (http://eu.idtdna.com/calc/analyzer) and were ordered as 100µM stock solution (IDT, Leuven, Belgium). The microsatellite and SNP markers (Tables 4.2 and 4.3) were divided into multiplex panels in *MultiPLX 2.1* using the low grouping stringency setting and the maximum number of primers per group set at 20 (Kaplinski *et al*., 2005). Primers were diluted to 10µM working solution and combined according to the *MultiPLX 2.1* output to form five 0.25µM multiplexes.

Table 4.2. Microsatellite marker panel used in the current study. Linkage groups are indicated by colours in the 'Name' column.

Name	Chr #	Chr RefSeq #	Chr position	Source
Her133	3	NC_045154.1	25,248,408	Teacher *et al*., 2012
7_292	6	NC_045157.1	17,661,452	Farrell & Carlsson, 2018
Her117	6	NC_045157.1	19,571,197	Teacher *et al*., 2012
HerScaf46_B	6	NC_045157.1	22,481,476	Farrell & Carlsson, 2018
HerScaf46_A	6	NC_045157.1	22,508,472	Farrell & Carlsson, 2018
Her111	6	NC_045157.1	27,580,738	Teacher *et al*., 2012
Her36	7	NC_045158.1	11,455,701	Teacher *et al*., 2012
6_260	7	NC_045158.1	16,930,934	Farrell & Carlsson, 2018
5_260	7	NC_045158.1	16,931,448	Farrell & Carlsson, 2018
Her73	8	NC_045159.1	24,107,222	Teacher *et al*., 2012
Her100	8	NC_045159.1	29,147,695	Teacher *et al*., 2012
Her20	9	NC_045160.1	18,759,218	Teacher *et al*., 2012
10_553	10	NC_045161.1	12,535,476	Farrell & Carlsson, 2018
Her126	10	NC_045161.1	14,062,607	Teacher *et al*., 2012
Her84	11	NC_045162.1	6,299,147	Teacher *et al*., 2012
Her62	13	NC_045164.1	9,354,208	Teacher *et al*., 2012
Her12	14	NC_045165.1	25,102,849	Teacher *et al*., 2012
HerScaf1440_C	15	NC_045166.1	7,779,887	Farrell & Carlsson, 2018
Her63	15	NC_045166.1	8,470,641	Teacher *et al*., 2012
HerScaf1420_D	15	NC_045166.1	8,861,865	Farrell & Carlsson, 2018
Her40	15	NC_045166.1	24,107,339	Teacher *et al*., 2012
54_12444	16	NC_045167.1	8,895,473	Farrell & Carlsson, 2018
HerScaf211_A	16	NC_045167.1	9,016,332	Farrell & Carlsson, 2018
Her14	18	NC_045169.1	13,152,697	Teacher *et al*., 2012
Her97	18	NC_045169.1	19,622,734	Teacher *et al*., 2012
HerScaf481_A	19	NC_045170.1	20,421,904	Farrell & Carlsson, 2018
34_3848	20	NC_045171.1	12,369,303	Farrell & Carlsson, 2018
Her102	20	NC_045171.1	18,822,767	Farrell & Carlsson, 2018
8_365	21	NC_045172.1	20,264,572	Farrell & Carlsson, 2018
Her1	21	NC_045172.1	24,042,434	Teacher *et al*., 2012
HerScaf49_A	22	NC_045173.1	3,077,910	Farrell & Carlsson, 2018
Her64	22	NC_045173.1	3,735,724	Teacher *et al*., 2012
Her136	22	NC_045173.1	9,534,329	Teacher *et al*., 2012
Her101	22	NC_045173.1	11,565,699	Teacher *et al*., 2012
3_254	23	NC_045174.1	4,887,259	Farrell & Carlsson, 2018
Her43	25	NC_045176.1	13,940,119	Teacher *et al*., 2012
Her54	26	NC_045177.1	11,735,728	Teacher *et al*., 2012

Table 4.3. SNP marker panel used in the current study. Linkage groups are indicated by colours in the 'Name' column (Section 4.7).

Name	14_SNP panel	Chr #	Chr RefSeq #	Chr position	Source
Uher_309_092	Yes	6	NC_045157.1	6,922,915	DTU-Aqua SNP panel
HerSNP52		6	NC_045157.1	22,480,573	Martinez Barrio *et al*., 2016
HerSNP53		6	NC_045157.1	22,483,049	Martinez Barrio *et al*., 2016
HerSNP54	Yes	6	NC_045157.1	22,490,416	Martinez Barrio *et al*., 2016
HerSNP55		6	NC_045157.1	22,491,566	Martinez Barrio *et al*., 2016
HerSNP57b		6	NC_045157.1	22,498,055	Martinez Barrio *et al*., 2016
HerSNP60		6	NC_045157.1	22,522,166	Martinez Barrio *et al*., 2016
Uher_139_161		6	NC_045157.1	24,856,671	DTU-Aqua SNP panel
Uher_261_rd		8	NC_045159.1	27,790,385	DTU-Aqua SNP panel
Uher_115_045	Yes	8	NC_045159.1	30,463,168	DTU-Aqua SNP panel
Uher_356_099	Yes	14	NC_045165.1	12,123,905	DTU-Aqua SNP panel
Uher_276		15	NC_045166.1	7,750,607	DTU-Aqua SNP panel
HerSNP14	Yes	15	NC_045166.1	7,757,217	Martinez Barrio *et al*., 2016
Uher_1440_140		15	NC_045166.1	7,768,974	DTU-Aqua SNP panel
Uher_294		15	NC_045166.1	8,813,384	DTU-Aqua SNP panel
HerSNP9		15	NC_045166.1	8,864,451	Martinez Barrio *et al*., 2016
HerSNP7		15	NC_045166.1	8,873,811	Martinez Barrio *et al*., 2016
HerSNP4	Yes	15	NC_045166.1	8,896,838	Martinez Barrio *et al*., 2016
HerSNP3		15	NC_045166.1	8,900,089	Martinez Barrio *et al*., 2016
HerSNP2		15	NC_045166.1	8,900,455	Martinez Barrio *et al*., 2016
Uher_314		15	NC_045166.1	8,905,940	DTU-Aqua SNP panel
Uher_170		15	NC_045166.1	8,906,559	DTU-Aqua SNP panel
Uher_168		15	NC_045166.1	8,917,729	DTU-Aqua SNP panel
Uher_317		15	NC_045166.1	8,921,274	DTU-Aqua SNP panel
HerSNP22	Yes	15	NC_045166.1	9,045,589	Martinez Barrio *et al*., 2016
HerSNP24		15	NC_045166.1	9,049,286	Martinez Barrio *et al*., 2016
Uher_343_rd		15	NC_045166.1	10,884,020	DTU-Aqua SNP panel
Uher_312_093		15	NC_045166.1	10,905,964	DTU-Aqua SNP panel
Uher_348		15	NC_045166.1	10,910,459	DTU-Aqua SNP panel
HerSNP41		15	NC_045166.1	10,956,917	Martinez Barrio *et al*., 2016
HerSNP37	Yes	15	NC_045166.1	10,965,368	Martinez Barrio *et al*., 2016
HerSNP33		15	NC_045166.1	10,977,005	Martinez Barrio *et al*., 2016
chr17_1		17	NC_045168.1	26,007,645	Han *et al*., 2020
chr17_2		17	NC_045168.1	26,189,568	Han *et al*., 2020
chr17_5_rd	Yes	17	NC_045168.1	27,429,690	Han *et al*., 2020
Uher_161_061	Yes	18	NC_045169.1	9,244,125	DTU-Aqua SNP panel
Uher_148_057	Yes	19	NC_045170.1	717,401	DTU-Aqua SNP panel
HerSNP71		19	NC_045170.1	20,540,118	Martinez Barrio *et al*., 2016
HerSNP64		19	NC_045170.1	20,546,389	Martinez Barrio *et al*., 2016
HerSNP63		19	NC_045170.1	20,565,643	Martinez Barrio *et al*., 2016
HerSNP62	Yes	19	NC_045170.1	20,576,170	Martinez Barrio *et al*., 2016
Uher_246		19	NC_045170.1	20,608,343	Martinez Barrio *et al*., 2016
HerSNP1	Yes	19	NC_045170.1	23,666,857	Martinez Barrio *et al*., 2016
chr23_12		23	NC_045174.1	16,569,518	Han *et al*., 2020
chr23_13	Yes	23	NC_045174.1	17,263,595	Han *et al*., 2020

4.5. Sample processing

DNA extractions for the current project were either conducted inhouse using a modified Chelex with proteinase-K based extraction protocol (Section 7.4) or were outsourced to Weatherbys Scientific Ltd., Kildare, Ireland and were performed using a high-throughput magnetic bead-based method on the LGC oKtopure™ robotic platform. In both cases total genomic DNA (gDNA) was extracted from c.10mg of tissue from each fish. Extractions were performed in deep-well plates and extracted DNA was laid out on 0.2ml 96-well PCR plates. To avoid potential cross contamination between sampling areas, each area was separated into different PCR plates. Extracted DNA was quantified and quality checked on a NanoDrop™ spectrophotometer to ensure consistency both within and between plates. Plates were sealed with strip caps and stored at -20°C until further processing.

The genotyping approach followed was genotyping by sequencing using the individual combinatorial barcoding method as followed in the pilot studies (see Farrell *et al*., 2016; Vartia *et al*., 2016). The detailed protocol is provided in Section 7.5 and only key steps are summarised below. A set of ninety-six 11bp combinatorial barcodes suitable for amplicon sequencing on the Illumina MiSeq and HiSeq platforms were designed based on the 12-bp Golay-barcodes from Caporaso *et al*. (2012), following Vartia *et al*. (2016). The last base of the Caporaso *et al*. (2012) barcodes was removed and an M13-R universal tail was added to the 3' end of forty-eight of the barcodes and a CAG universal tail to the 3' end of the remaining forty-eight barcodes, yielding 2,304 possible combinations. The modified barcodes were tested for the formation of secondary structures (hairpins, primer dimers and hetero dimers) with the IDT OligoAnalyzer Tool 3.1 (http://eu.idtdna.com/calc/analyzer) and ordered as 100µM stock solution (IDT, Leuven, Belgium). Barcodes were diluted to 1µM working solution and laid out on 96-well PCR plates; M13-R tailed barcodes were arranged A to H and CAG tailed barcodes were ordered 1 to 12.

Microsatellite and SNP amplification and barcoding reactions were carried out using a two-step PCR as described in Farrell *et al*. (2016) and according to the protocol in Section 7.5. In short, the first PCR involves the amplification of the target genetic markers and the second PCR involves the incorporation of the combinatorial barcodes for individual identification (Figure 4.2). Following PCR amplification each plate of amplicons was pooled by pipetting 8µl from each well into a new 1.5ml Eppendorf tube. The concentration of the purified pooled amplicon samples was measured on a Qubit® Fluorometer (Invitrogen, ThermoFisher Scientific) using the Qubit® dsDNA HS Assay Kit (Invitrogen, ThermoFisher Scientific). Pooled amplicon samples were standardised and combined and sent for library preparation and amplicon sequencing by a third-party sequencing service provider. A number of different herring amplicon sequencing runs were conducted both before and during the current project using both the Illumina MiSeq and HiSeq platforms. The raw data from all of these runs was treated following the same protocols and was combined for the final analyses.

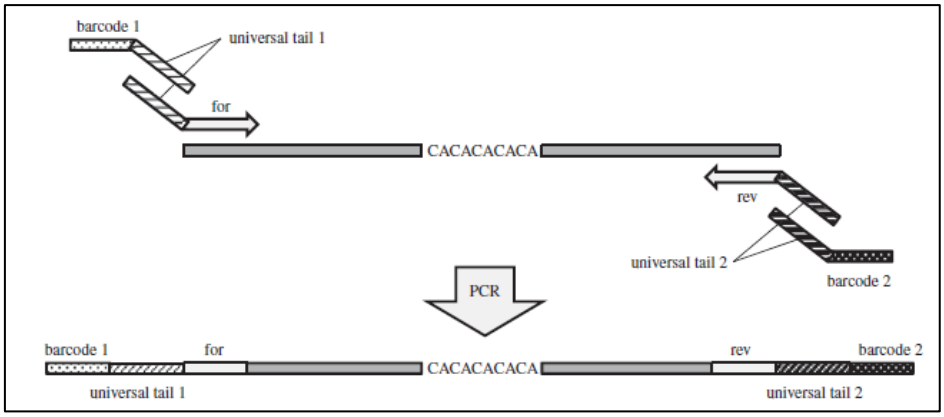


Figure 4.2. Diagram of the four-primer PCR and the structure of the resulting amplicon. From Vartia *et al*., 2016.

Following sequencing raw FASTQ sequence data were downloaded from Illumina BaseSpace and initial quality control was performed using FastQC (Babraham, 2016). Reads were sorted and grouped using a modified python script (Vartia *et al*, 2016) based on the Levenshtein distance metric, which measures the distance between two sequences of characters. In short, the raw sequence data was processed by identifying sequence reads containing the forward and reverse (combinatorial) barcodes and the locus-specific primers. The python script was set to allow for zero errors in either the combinatorial barcodes or primers. Reads were sorted hierarchically and grouped into five separate FASTA files as reads with: no barcode, one barcode, two barcodes and no primers, two barcodes and two non-matching primers, two barcodes and two matching primers. Only reads containing two barcodes and two matching primers were included in further analyses. These reads were grouped by locus and individual before removing the barcode from the sequences.

4.6. Genotyping

Microsatellite loci were manually genotyped using a custom '*classifier*' software to view all of the reads of a particular individual at a specific locus, as a read length histogram and verified by read alignment (Figure 4.3). This step can also be completed in Geneious® 7.0 (Kearse *et al*., 2012) as was done in Farrell *et al.* (2016) and Vartia *et al.* (2016). The '*classifier*' software was adapted to enable SNP genotyping by adding an option to change to single base calling (Figure 4.3). However, given the number of samples and the volume of data an alternate fully automated SNP genotyping option was subsequently used. Campbell *et al*. (2015) detailed a similar method of GBS of SNPs, termed 'Genotyping-in-Thousands by sequencing (GT-seq)'. The method used next-generation sequencing of multiplexed PCR products to generate genotypes from relatively small panels (50–500) of targeted SNPs for thousands of individuals in a single Illumina HiSeq lane. The method used a custom *Perl* script for genotyping, which counts amplicon-specific sequences for each allele, and used allele ratios to determine the genotypes. This *Perl* script was modified in the current project to use the output of the custom python scripts as its input. The default settings of the genotyping *Perl* script designate allele ratios >10.0 to be called as homozygous for allele 1, ratios <0.1 to be called as homozygous for allele 2, and ratios between 0.2 and 5.0 to be called as heterozygous (Campbell *et al*., 2015). These ratios were optimised for the data and markers in the current study by analysing each marker separately and visualising the genotyping calls (Figure 4.4) from which new ratios were calculated for each marker. The average designated allele ratios in the current study were >5.0 to be called as homozygous for allele 1, ratios <0.2 to be called as homozygous for allele 2, ratios between 0.3 and 3.33 to be called as heterozygous and ratios between 3.34-4.9 and 0.201-0.29 were called as NA (Figure 4.4).

When performing quality control of the genotype data only individuals with greater than 90% genotyping success were retained in the datasets. In this final analysis all genetic markers were successfully genotyped in greater than 90% of individuals.

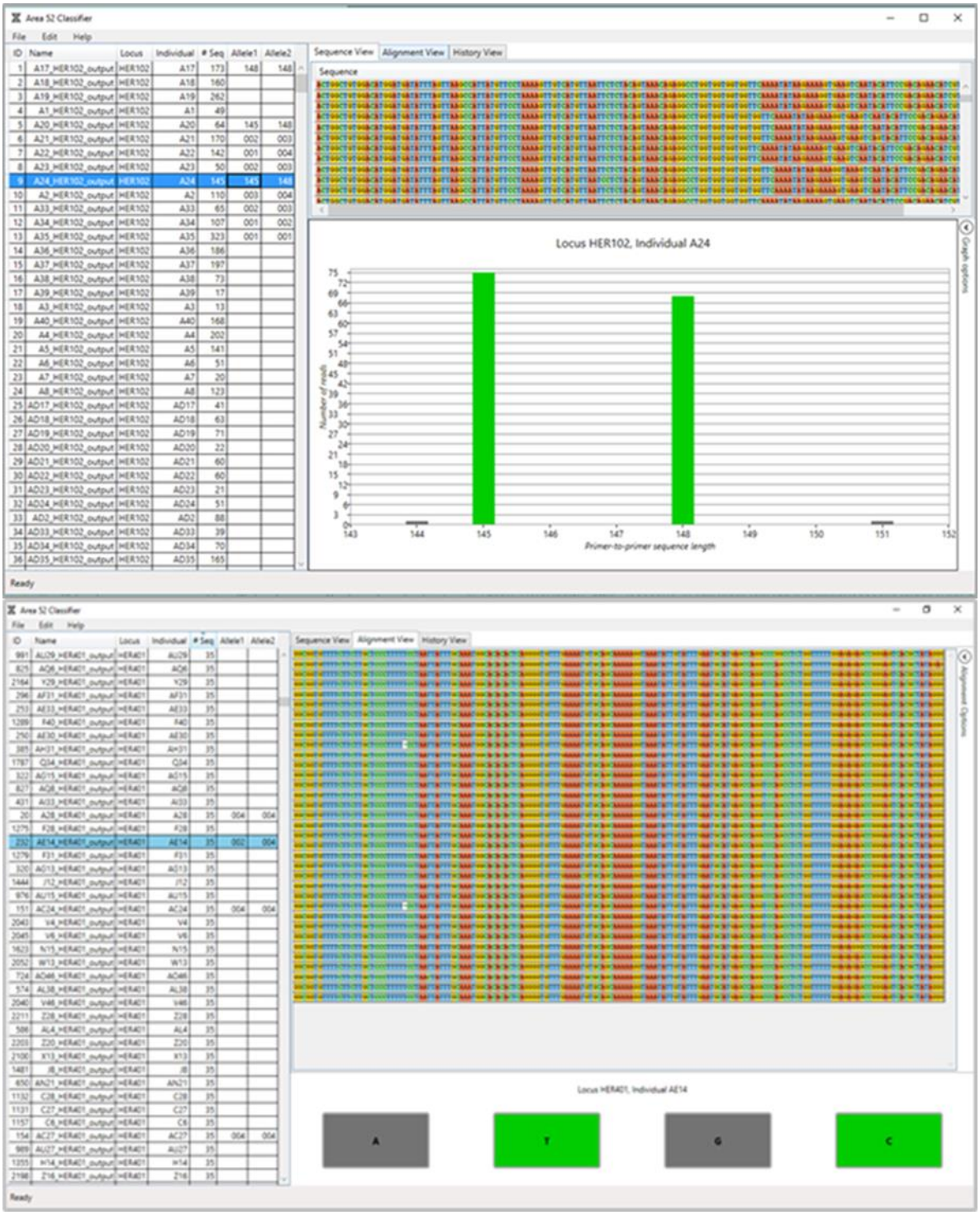


Figure 4.3. Screen shots of the *classifier* software illustrating microsatellite genotyping (top panel) and SNP genotyping (bottom panel).

Figure 4.4. An example scatter plot of read counts for a single locus, *chr17_1*. Each point represents an individual fish and shows the number of reads for allele 1 versus the number of reads for allele 2. The points in red are called as homozygous for allele 1, the points in blue as homozygous for allele 2, the points in purple as heterozygous and the points in yellow as NA. The full set of individuals is shown in the top panel and the bottom panel is zoomed to 200 reads on the x and y axes.

4.7. Baseline dataset analyses

The results of the baseline genetic analyses are detailed in this section. Only the samples listed in Table 4.1 were included in these analyses. It should be noted that the aim of the current study was not to undertake an exhaustive population genetics and demographic study of the herring populations around Ireland and Britain but was to develop a method to separate the herring caught in putatively mixed aggregations during the MSHAS into their population of origin. The analyses presented below are tailored to this specific task. Recently the wide-scale application of whole genome sequencing herring samples collected over multiple years across the entire distribution range of the species (Han *et al*., 2020), including around Ireland and Britain, has uncovered the genetic population structure of the

species (Section 2.8). The limited number of genetic markers used in the current study are high graded to maximise the power of discrimination between the core Division 6.a populations and may not be suitable for conventional population genetic analyses. Therefore, some of the analyses are presented as exploratory only.

Deviations from Hardy–Weinberg equilibrium (*HWE*), linkage disequilibrium (*LD*) and excess and deficiency of heterozygotes were tested with *Genepop 4.2* – default settings (Rousset, 2008). In all cases with multiple tests, significance levels were adjusted using the sequential Bonferroni technique (Rice, 1989). In the microsatellite dataset there were no significant patterns of deviation from *HWE* at the population level. At the locus level significant deviations from *HWE* were observed at locus *Her100* in twelve out of fifty samples and at locus *6_260* in nine out of fifty samples. Sample *6aN_16f* displayed indications of a significant heterozygote deficiency in six loci and there were no significant indications of a heterozygote excess in any of the samples. As expected, based on their close proximity in chromosome 6 (Table 4.2), microsatellite loci *HerScaf46_A* and *HerScaf46_B* displayed significant indications of *LD*, but only in three samples out of fifty. There were no other significant patterns of *LD*.

In the SNP dataset there were no significant patterns of deviation from *HWE* at the locus level. At the population level significant deviations from *HWE* were observed in samples *6aNSp_18d* and *6aS_17e* in six out of forty-five markers and in sample *6aS_17d* in nine out of forty-five markers. Samples *6aS_17d* and *6aS_17e* also displayed indications of a significant heterozygote deficiency in eleven and twelve loci, respectively. Samples *6aN_Sp_18d* and *DWN_18* displayed indications of significant heterozygote excess at eleven and seven loci, respectively. The significant indications of *LD* were in keeping with the linkage groups already identified (Table 4.3) from Han *et al*. (2020).

For the purposes of data exploration all samples and markers were retained in both datasets. *Microsatellite Analyzer* (MSA) 4.05 (Dieringer and Schlötterer, 2003) was used, under default settings, to assess multi-locus pairwise F_{ST} with 1000 bootstrap replications. Both the microsatellite and SNP datasets showed the same patterns of genetic differentiation (Annex 4: Figures 10.4.1 and 10.4.2) with significant differentiation between samples collected from the different putative populations except between the *6aN_Aut* and *NS*. There was no significant genetic differentiation between the two samples from the *CS* or between the temporal samples from the *6aN_Sp*, *IS* or *DWN*. There was little if any significant genetic differentiation among or between the temporal samples from the *6aN_Aut* and *NS* areas. There were some indications of differentiation between the temporal samples from *6aS*, with the samples collected in January and February (*6aS_17a*, *6aS_17b*, *6aS_18h*) showing a low level of differentiation from the other samples. In order to visualise the data and to clarify the relationships between the different samples *Principal Coordinate Analysis* (*PCoA*) using the covariance standardised method was conducted in *GenAlEx 6.51b2* (Peakall and Smouse, 2012). *PCoA* is a multivariate technique that allows one to find and plot the major patterns within a multivariate data set (e.g., multiple loci and multiple samples). *PCoA* does not assume a hierarchical genetic structure like tree building models such as neighbour joining trees. The *PCoA* of the microsatellite and SNP datasets indicated broadly similar clustering of samples. The temporal samples from each putative population clustered together apart from the *6aS* samples, which were relatively spread out (Figure 4.5). The *6aN_Aut* and *NS* samples all clustered together without any obvious pattern of separation.

In order to test the effect of the linked markers, the analyses above were conducted on two refined datasets. Locus *HerScaf46_A* (Table 4.2) was removed from the microsatellite baseline dataset, resulting in the *36_MSAT* baseline dataset. One high F_{ST} locus from each linkage group in the SNP dataset was selected, based on the F_{ST} per locus analyses of the *6aS* and *6aN_Aut* samples (Table 4.3), resulting in the *14_SNP* dataset. The *PCoA* of the reduced microsatellite and SNP datasets indicated little difference in the clustering of samples (Figure 4.6). For subsequent analyses only the *36_MSAT* and two SNP datasets were used, the *45_SNP* dataset with all the markers and the *14_SNP* dataset with a subset of the highest F_{ST} unlinked markers (Table 4.3).

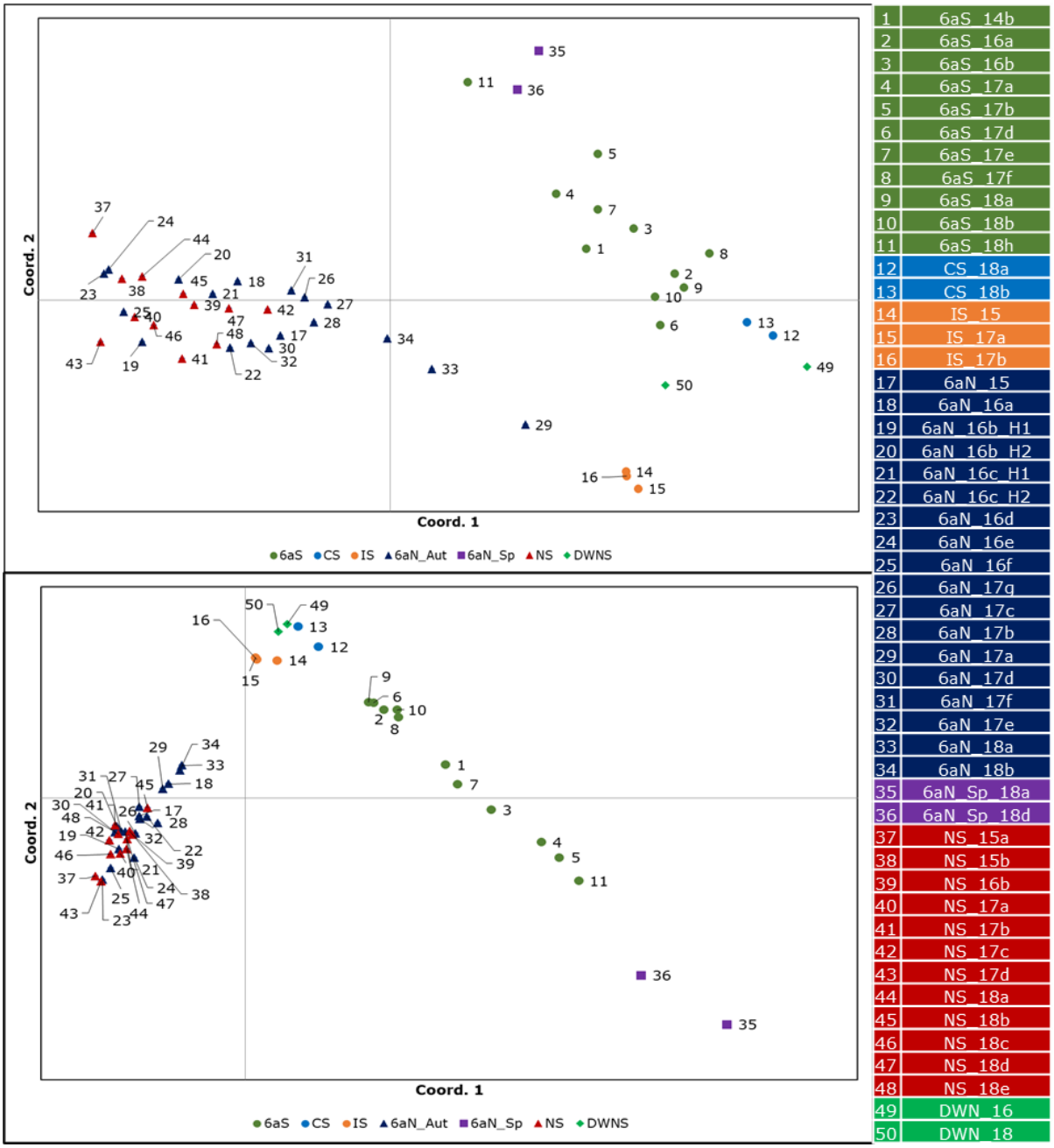


Figure 4.5. Principal Coordinate Analysis (PCoA) of the full microsatellite (top panel) and SNP (bottom panel) baseline datasets. Coordinate 1 explained 48% and 61% of the variation in the microsatellite and SNP analyses, respectively. Coordinate 2 explained 19% and 35% of the variation in the microsatellite and SNP analyses, respectively.

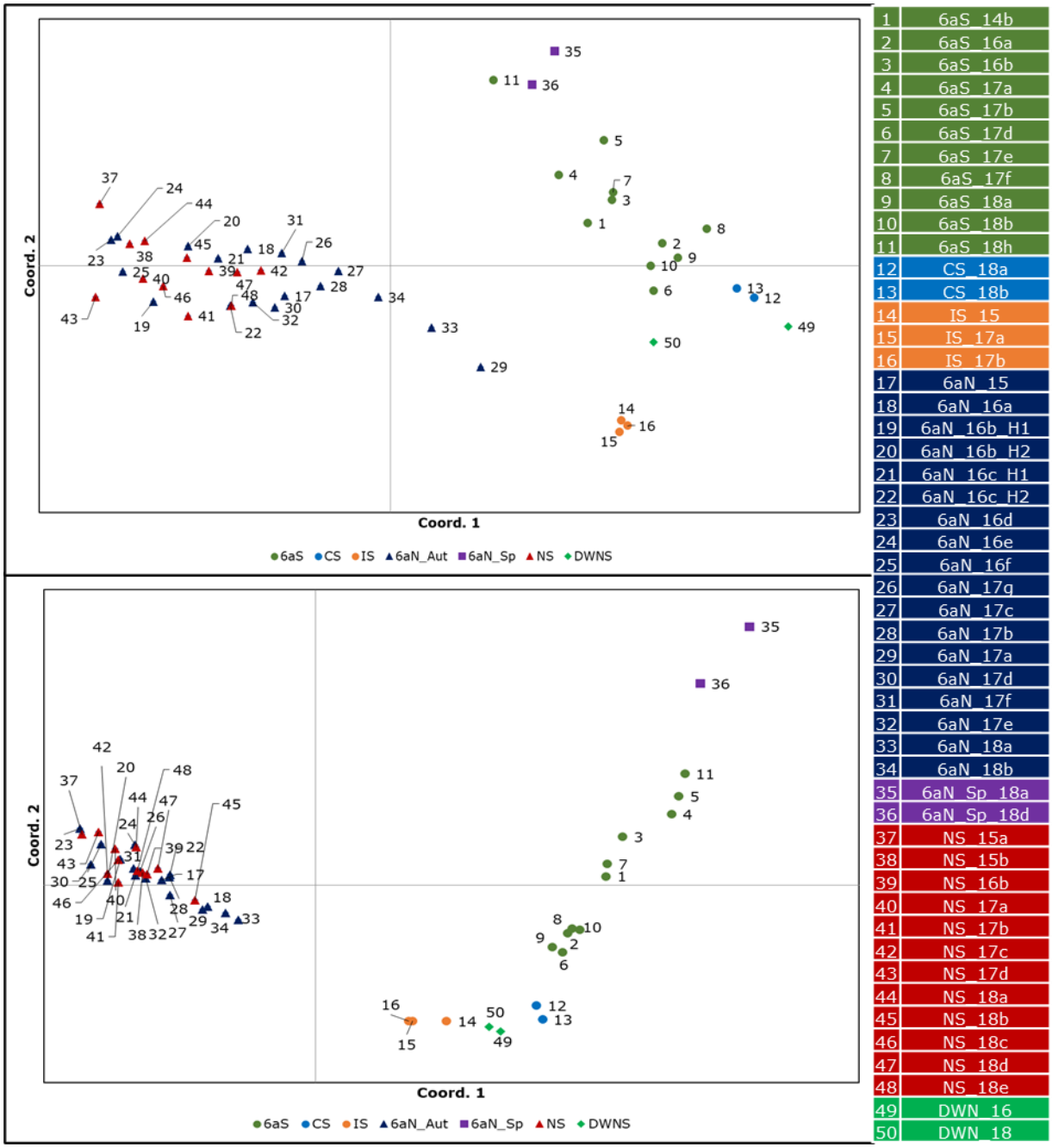


Figure 4.6. Principal Coordinate Analysis (PCoA) of the 36_MSAT (top panel) and 14_SNP (bottom panel) baseline datasets. Coordinate 1 explained 47% and 67% of the variation in the microsatellite and SNP analyses, respectively. Coordinate 2 explained 20% and 28% of the variation in the microsatellite and SNP analyses, respectively.

Clustering analyses of the three datasets (*36_MSAT, 45_SNP and 14_SNP*) were conducted using the *find.clusters* function of the a*degenet* package in *R* (Jombart, 2008). This function transforms the data using principal component analysis (*PCA*), then runs the *k-means* algorithm (function *kmeans* from the *stats* package) with increasing values of *k* and computes Bayesian Information Criterion (*BIC*) to assess the best supported model. The number of principal components (*PCs*) retained were 400, 45 and 14 for the *36_MSAT*, *45_SNP* and *14_SNP* datasets, respectively. The number of clusters indicated by the lowest *BIC* value was c.24 in the *36_MSAT* dataset and was indeterminate in the *45_SNP and 14_SNP* datasets (Annex 4: Figure 10.4.1). Therefore, a range of cluster numbers were used to test the data. The *36_MSAT* dataset did not provide a clear number of clusters that aligned with putative population structure (Annex 4: Figure 10.4.2). The *45_SNP* and *14_SNP* datasets both indicated a similar pattern of clustering for the temporal samples within each putative population (Annex 4: Figure 10.4.2).

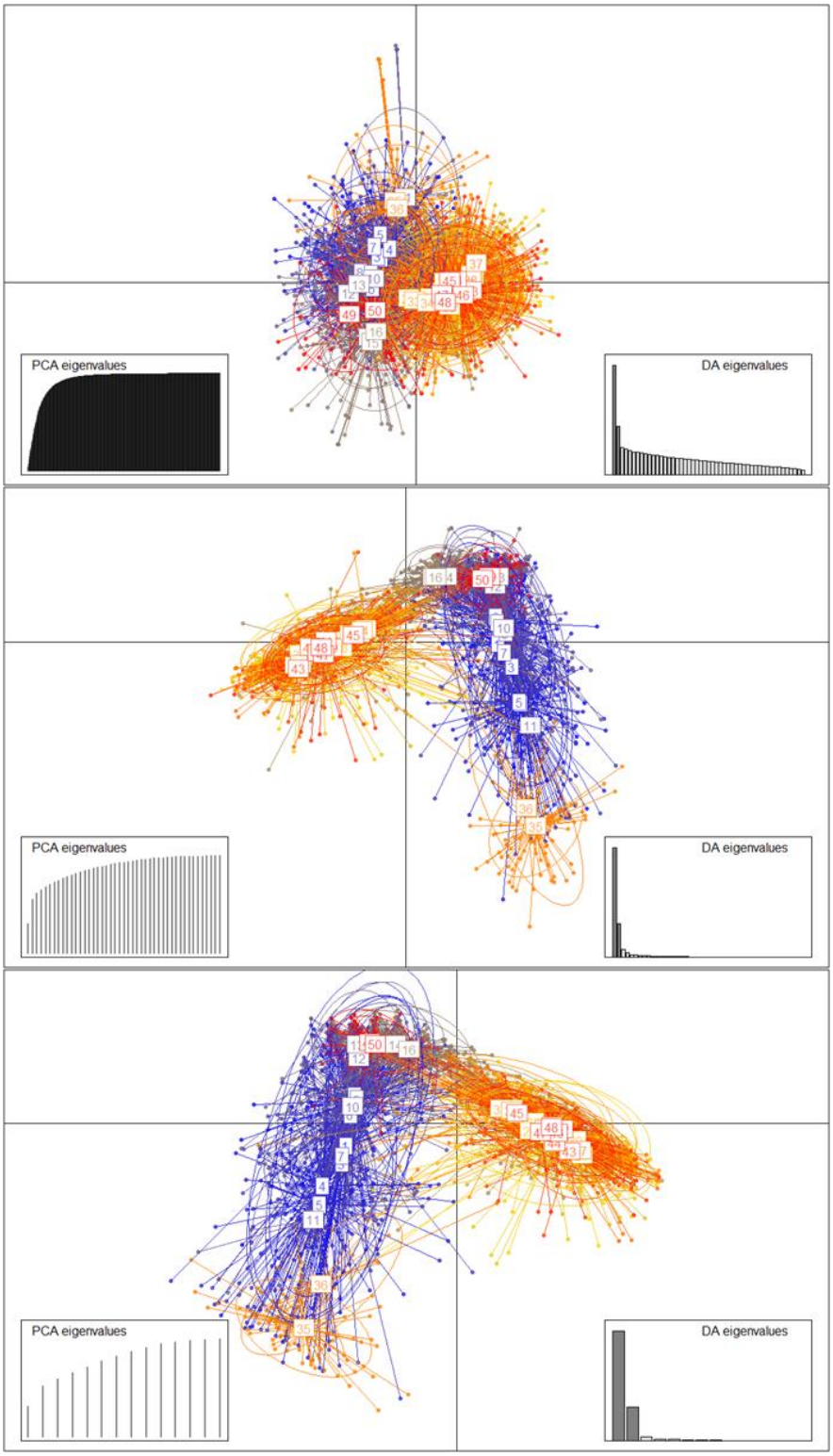


Figure 4.7. Discriminant Analysis of Principal Components (DAPC) of the (top) *36_MSAT*, (middle) *45_SNP* and (bottom) *14_SNP* baseline datasets performed with the a*degenet* package in *R*. The individuals are represented as dots and the samples as inertia ellipses. Sample numbers are the same as used in Figures 4.5 and 4.6. The blues are the *6aS* and *CS* samples, the oranges and yellows are the *6aN* and *NS* samples, the grey are the *IS* and the red are the *DWN* samples.

Discriminant Analysis of Principal Components (*DAPC*) was performed in *adegenet* (Jombart, 2008). *DAPC* is a multivariate approach that transforms multi-locus genotype data using *PCA* to derive a set of uncorrelated variables, which serve as input for discriminant analysis (*DA*). The *DA* aims to maximize among-group variation and minimize within-group variation. *DAPC* does not make assumptions of underlying population genetic

processes (e.g. neutrality, linkage equilibrium, Hardy–Weinberg equilibrium) common to other methods used to detect population structure. Therefore, it is appropriate to use this approach with the data in the current study, which are known to be derived from markers under selection that are in some cases linked. Most other genetic approaches, including F_{ST} based analyses, assume *HWE* and as such are only provided here for exploratory purposes. This is also true of commonly used Bayesian clustering analyses such as Structure (Pritchard *et al*., 2000), which are therefore not used in the current study.

The *DAPC* results supported the previous indications of temporal stability within each of the putative population areas with samples from the same putative populations clustering together (Figure 4.7). Therefore, the temporal samples were combined to form seven groups (*6aS*, *CS*, *IS*, *6aN_Aut*, *6aN_Sp*, *NS*, *DWN*), which represented the putative populations in the study area (Table 4.1). THE *DAPC* and *MSA* analyses were run again on the pooled samples (Table 4.4). In all three datasets there was significant differentiation between all of the pooled samples (Table 4.4). The lowest level of differentiation was between the *6aN_Aut* and *NS* autumn spawning groups in all three datasets. The level of differentiation between these pools was lower than the average differentiation between all of the samples within the *6aN_Aut* pool (Annex 4: Tables 10.4.1. and 10.4.2). The highest level of differentiation in all three datasets was between the *6aN_Sp* spring spawning groups and the other groups. The *6aN_Sp* group was most similar to the *6aS* group. There was also a very low level of differentiation between the *DWN* samples and the *CS* and *IS* samples, whilst the *DWN* samples had a high level of differentiation from the *NS* autumn spawners. The *DAPC* results indicated the same pattern of differentiation as the F_{ST} analyses (Figure 4.8) and also as those observed in Han *et al*. (2020) based on whole genome analyses. Therefore, the analyses presented here are considered robust.

Table 4.4. Pairwise multi-locus F_{ST} (above the diagonal) for the (top) *36_MSAT* (middle) *45_SNP* and (bottom) *14_SNP* baseline datasets and associated *P*-values (below the diagonal) with the temporal replicates condensed. *P*-values highlighted in bold were not significant after sequential Bonferroni correction.

	6aS	*CS*	*IS*	*6aN_Aut*	*6aN_SP*	*NS*	*DWN*
6aS		0.0027	0.0150	0.0141	0.0084	0.0189	0.0051
CS	0.0001		0.0085	0.0215	0.0206	0.0279	0.0013
IS	0.0001	0.0001		0.0203	0.0407	0.0262	0.0047
6aN_Aut	0.0001	0.0001	0.0001		0.0214	0.0007	0.0184
6aN_SP	0.0001	0.0001	0.0001	0.0001		0.0244	0.0267
NS	0.0001	0.0001	0.0001	0.0002	0.0001		0.0244
DWN	0.0001	**0.0568**	0.0001	0.0001	0.0001	0.0001	

	6aS	*CS*	*IS*	*6aN_Aut*	*6aN_SP*	*NS*	*DWN*
6aS		0.1078	0.1408	0.2220	0.3358	0.2476	0.1298
CS	0.0001		0.0957	0.2737	0.6568	0.3376	0.0112
IS	0.0001	0.0001		0.2279	0.6719	0.2987	0.0787
6aN_Aut	0.0001	0.0001	0.0001		0.5778	0.0076	0.2675
6aN_SP	0.0001	0.0001	0.0001	0.0001		0.5938	0.6818
NS	0.0001	0.0001	0.0001	0.0001	0.0001		0.3343
DWN	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	

	6aS	*CS*	*IS*	*6aN_Aut*	*6aN_SP*	*NS*	*DWN*
6aS		0.0821	0.1519	0.2665	0.2771	0.2860	0.1163
CS	0.0001		0.0912	0.3078	0.5531	0.3531	0.0216
IS	0.0001	0.0001		0.2437	0.6033	0.2942	0.0598
6aN_Aut	0.0001	0.0001	0.0001		0.5140	0.0049	0.2892
6aN_SP	0.0001	0.0001	0.0001	0.0001		0.5265	0.5914
NS	0.0001	0.0001	0.0001	0.0001	0.0001		0.3363
DWN	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	

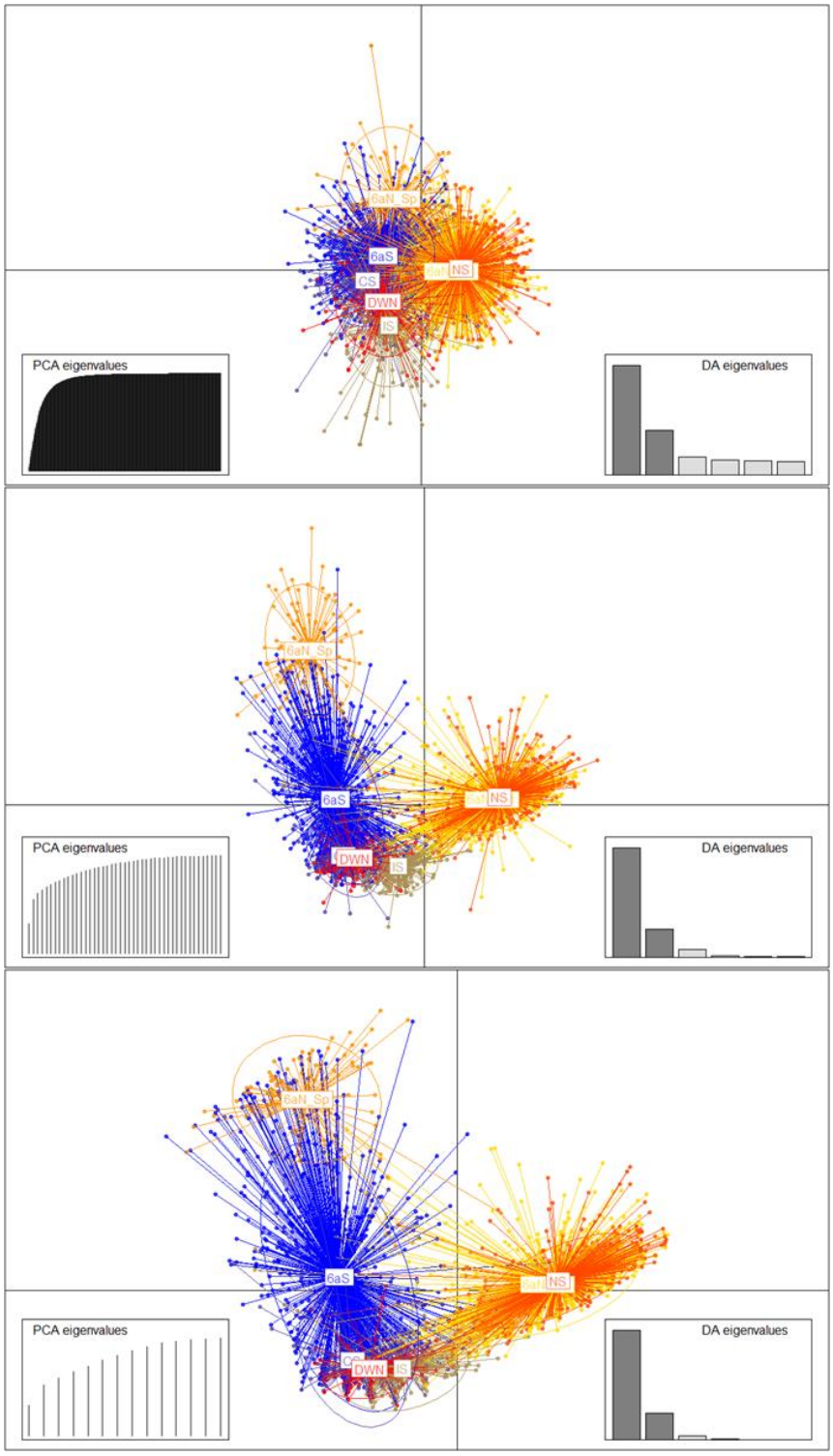


Figure 4.8. Discriminant Analysis of Principal Components (*DAPC*) of the (top) *36_MSAT*, (middle) *45_SNP* and (bottom) *14_SNP* baseline datasets performed with the *adegenet* package in *R*. The individuals are represented as dots and the samples as inertia ellipses. Sample numbers are the same as used in Figures 4.5 and 4.6. The blues are the *6aS* and *CS* samples, the oranges and yellows are the *6aN* and *NS* samples, the grey are the *IS* and the red are the *DWN* samples.

There was a significantly higher level of differentiation detected with the SNPs than with the microsatellites (Figure 4.8 and Table 4.4), illustrating the higher potential for discriminating between the different groups when using the SNPs from coding regions. The microsatellites did not provide a clear enough discrimination between the groups to enable individual assignment as is evident from the overlapping groups observed in the *DAPC* analyses (Figure 4.8). There was a complete overlap of *6aN_Aut* and *NS* groups in all three

datasets (Figure 4.8) and it is not possible to distinguish these groups with the current marker panel. There is currently no evidence to support the assertion that the North Sea autumn spawning herring comprise a different population to the 6.a.N autumn spawning herring (see also section 2.5) and based on the analyses presented in the current study the data from these groups could be pooled. However, this distinction was not the focus of the current study and pooling the data from these groups is likely to be a contentious undertaking, which would distract from the core focus of the current study. As such the North Sea samples were removed from further analyses. The Downs group was confirmed to be distinct from the North Sea autumn spawners though they cannot be reliably discriminated from the Celtic Sea and Irish Sea with the current panel of markers. Further analyses of the Downs group are beyond the scope of the current study and as such they too were removed from further analyses.

The highest level of discrimination observed in the *DAPC* analyses of the *45_SNP* and *14_SNP* datasets was along the primary axis and concerned the *6aS* and the *6aN_Aut* samples. Although there were some outliers and a small degree of overlap between these main groups, it should be possible to develop an assignment model based on this level of discrimination. The *6aS* and *6aN_Sp* groups were partially overlapped, indicating a lower potential to accurately discriminate between these groups. The *6aS*, *CS* and *IS* groups were overlapped, and the current marker panel cannot be used to distinguish these groups with a high level of accuracy. Therefore, the *CS* and *IS* groups were removed from the baseline data and excluded from further analyses. It should be noted that there is no evidence of significant numbers of herring from these groups being present in the feeding aggregations in Division 6.a when the MSHAS takes place. As described in section 2.7 the previous assertions made regarding the Irish Sea herring mixing in this area were unfounded. Addition of the *IS* samples to the current baseline would reduce the confidence in the assignments and be detrimental to the assignment of individuals from groups that are confirmed as being present in Division 6.a i.e. *6aS*, *6aN_Aut* and *6aN_Sp*.

There was a decrease in power to separate the *6aS* and *6aN_Sp* groups with the reduced *14_SNP* dataset when compared to the *45_SNP* dataset. This indicates that the fourteen SNPs in the reduced dataset, while near optimum for distinguishing the *6aS* and *6aN_Aut* groups, may not be the optimum markers for discriminating some of the other groups. Therefore, using the *45_SNP* dataset is preferable for future assignments, though as it contains linked SNPs the methods used must be appropriate and free of the assumptions of *HWE* and linkage equilibrium.

4.8. Assignment model

Assignment methods that attempt to solve classification problems rely on computing a discriminant function based on samples from potential source populations and then classify unknown individuals to the group with the highest discriminant score (Manel *et al*., 2005). In the case of genetic assignment methods, the discriminant function has traditionally been the genotypic frequency distribution under the assumption of *HWE* and linkage equilibrium in each source population (Manel *et al*., 2005). These genetic assignment methods can be broadly divided into Bayesian (Rannala and Mountain, 1997), frequency (Paetkau *et al*., 1995) and distance based (Cornuet *et al*, 1999) methods (Hauser *et al*., 2006). The underlying assumptions of the methods are quite similar however the distance-based methods may be less sensitive to violations of population genetic expectations such as *HWE* and linkage equilibrium (Cornuet *et al*., 1999). These methods are commonly implemented in the software *GeneClass2* (Piry *et al*., 2004). In the absence of baseline data to guide classification, Bayesian clustering methods may be used to delineate clusters of individuals based on their multi-locus genotypes and assign individuals to their individual clusters (Manel *et al*., 2005). However, these Bayesian clustering analyses such as that implemented in the software *Structure* (Pritchard *et al*., 2000) are also constrained by the underlying assumptions of *HWE* and linkage equilibrium.

Multivariate analysis has several advantages over other classical approaches used in population genetics, the foremost of which in the current study is that they do not require the assumptions of *HWE* or linkage equilibrium (Jombart *et al*., 2009). As shown in Section

4.7, *DAPC* is a powerful tool to identify and describe clusters of genetically related individuals (Jombart *et al*, 2010) and can be used both with prior definition of group membership or when group priors are lacking, sequential *K*-means and model selection can be used to infer genetic clusters. Multivariate approaches are particularly suited to solving classification problems when used in the form of supervised machine learning (*SML*) approaches. *SML* is concerned with predicting the value of a response label/category on the basis of the input variables/features (Schrider and Kern, 2018). When empirical data is available, *SML* trains an algorithm based on a training set of the labelled data, which can then be used to predict the category of unknown data. Support Vector Machines (*SVM*) are a set of supervised learning methods that can be used for classification problems. The objective of *SVM* algorithms is to find a hyperplane in an N-dimensional space (N - the number of features) that distinctly classifies the data point (see example in Figure 4.9 and also James *et al*., 2013). *SVM* models can also be used to classify non-linear data through use of non-linear kernels (James *et al*., 2013). The *SVM* model can be optimised by adjusting parameters, including cost and gamma, which control the stringency of the boundary and the influence of single training datapoints, respectively. A lower cost means a softer boundary between the classes and means more individual points on the wrong side of the division will be allowed. A low value for gamma means that each data point will have a wider influence than if the gamma was high. *SVM* models do not directly provide probability estimates, these are calculated using logistic regression on the *SVM* scores, fit by an additional cross-validation on the training data. The output probabilities can be converted to odds in order to make the values more understandable (Table 4.5). The threshold probability of 0.67 indicates a situation where one outcome is twice as likely as the alternate outcome.

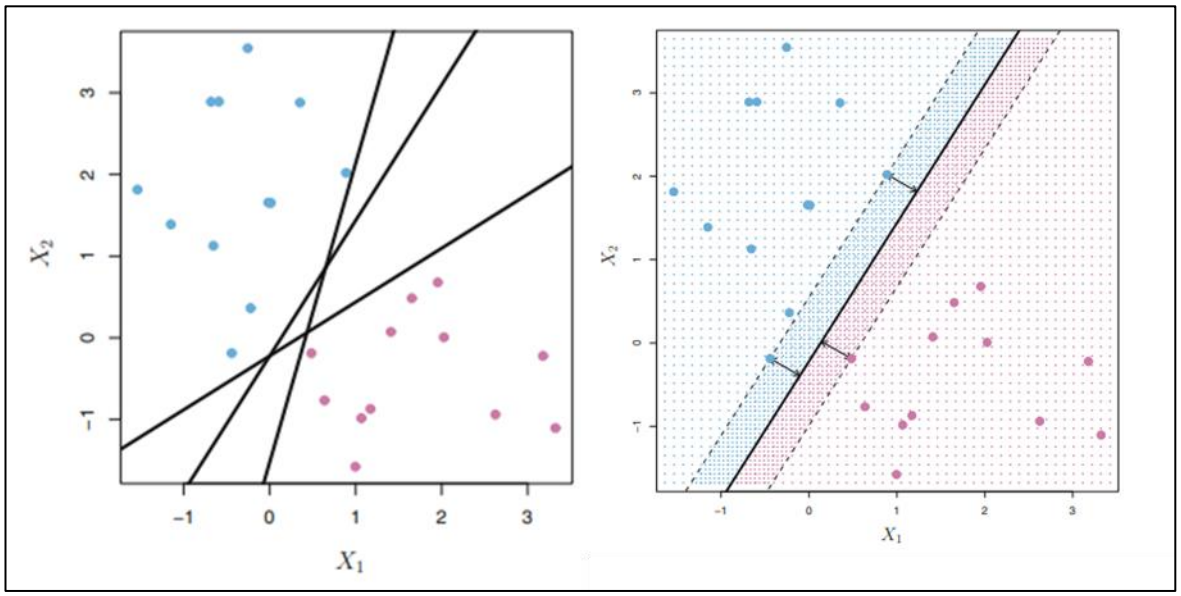


Figure 4.9. An example of support vector machine learning classification. Two classes of observations (blue and purple), each of which has measurements for two variables and three possible separating hyperplanes are shown on the left figure. The optimum hyperplane (right) is chosen based on training criteria and stringency. The two blue points and the purple point that lie on the dashed lines are the support vectors, and the distance from those points to the hyperplane is indicated by arrows. The purple and blue grid indicates the decision rule made by a classifier based on this separating hyperplane. (from James *et al*., 2013).

The *R* package, *assignPOP* (Chen *et al*., 2018), performs population assignment using a machine-learning framework. It employs supervised machine-learning methods to evaluate the discriminatory power of baseline data collected from source populations. The data from baseline samples are split into training and test data. The training data is used to train the model in *assignPOP* and then the remaining test data is compared with the training data to quantify the rate of correct self-assignment i.e. how many times an individual fish is assigned correctly to the baseline population which it came from. The

number of individuals used in the training set can be set as a proportion of the total samples per population or defined as a set number of individuals per population. Similarly, the number of loci (genetic markers) used in the tests can be set as a proportion of the total in the dataset or predefined numbers of loci can be used. The data can be tested for self-assignment using both Monte-Carlo cross-validation and also *K*-fold cross-validation to estimate membership probability. *assignPOP* has a number of classification model options including the *SVM* model from the *R* package *e1071* (Meyer *et al*., 2015). Based on the aforementioned analyses (Section 4.7) it was decided to develop the assignment model in *assignPOP* based on the *45_SNP* dataset as it provided the highest level of discrimination between the populations known to occur in Division 6.a, 7. And 7.c; *6aS*, *6aN_Aut* and *6aN_Sp*. In order to fully explore the power of the genetic data, the assignment was conducted using two different approaches, with each approach conducted at two hierarchical levels.

Table 4.5. Conversion of probabilities to odds. The proposed threshold of 0.67 is indicted with the red line.

Probability	Odds [p/(1-p)]
0.1	0.11
0.2	0.25
0.3	0.43
0.4	0.67
0.5	1
0.6	1.5
0.67	2.03
0.7	2.33
0.75	3
0.8	4
0.85	5.67
0.9	9
0.91	10.11
0.92	11.5
0.93	13.29
0.94	15.67
0.95	19
0.96	24
0.97	32.33
0.98	49
0.99	99
0.999	999

Approach 1 used the *45_SNP* dataset with the predefined *6aS*, *6aN_Aut* and *6aN_Sp* population groups. The assignment was conducted in two hierarchical levels based on the power to discriminate the different population groups in the *DAPC* analyses (Figure 4.10, top panel). In *level 1* the *6aS* and *6aN_Sp* groups were combined and tested against the *6aN_Aut* group. In *level 2* the combined *6aS/6aN_Sp* group was split into two and the individual population groups were tested against each other (Figure 4.10). The reason for this was that there was a higher power to discriminate between the groups in *level 1* than the groups in *level 2*. This was also the more important of the two assignment levels given the perceived abundance of the different groups in Division 6.a and the expected composition of the potentially mixed MSHAS samples. In order to avoid overfitting the model and to objectively determine the optimum number of *PCs* to be used in the *SVM* tuning in *assignPOP*, *DAPC* cross-validation was conducted with the *xvalDapc* function in *adegenet* (Annex 4: Figure 10.4.3). The optimum numbers of *PCs* were determined as the values with the lowest root mean squared error (*RMSE*) and were 20, 40 and 30 for complete dataset, the *level 1* dataset and *level 2* dataset, respectively. There was however little difference between the number of *PCs* retained in all cases suggesting that the assignment is not sensitive to this parameter (Annex 4: Figure 10.4.3).

It is evident from the *DAPC* in figure 4.10 that there were some outliers in the datasets i.e. individuals predefined as being from one population group but appearing to be clustered with another group in the *DAPC*. This appears to be more of an issue with the *6aN_Aut* dataset where a number of these individuals were clustered with the *6aS* and *6aN_Sp* group. This may be an indication of misidentification of individuals in the *6aN_Aut* spawning baseline or perhaps an indication of 'straying' between sympatric populations (Berg *et al*., 2020; Kerr *et al*., 2019).

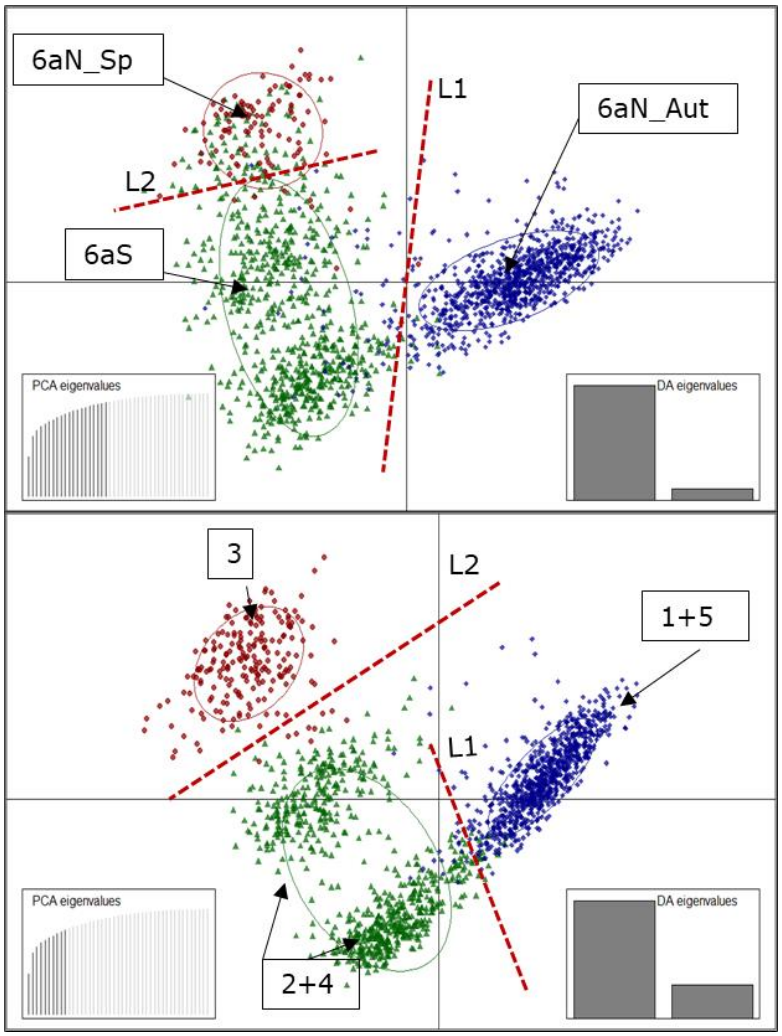


Figure 4.10. Discriminant Analysis of Principal Components (DAPC) performed with the *adegenet* package in *R* of the *45_SNP* baseline dataset (top) divided by predefined population groups i.e. Assignment *Approach 1* (bottom) groups based on *K*-means clustering analyses i.e. Assignment *Approach 2*. The dashed red lines indicate the hierarchical grouping levels.

In order to investigate the possibility of misidentification within the baselines and if present to mitigate for it, a second assignment approach was developed in which the baseline groups were not initially predefined based on their assumed population of origin. Instead clustering analyses of the *45_SNP* dataset with the individual samples from the three population groups (*6aS*, *6aN_Aut*, *6aN_Sp*) were conducted using the *find.clusters* function in *adegenet* (Tables 4.6 and 4.7). The optimum number of clusters was five clusters, which provided the most accurate division of the samples based on their assumed population of origin. *DAPC* of the clustered dataset indicated clear division between the clusters with minimal overlap (Figure 4.10, bottom panel), suggesting that an *SVM* model-based assignment using this approach would have a high accuracy at the cluster level. In the cases where multiple clusters represented a single assumed population of origin these clusters were pooled into the following cluster groups: *Group_1+5*, *Group_2+4*, *Group_3*.

90% of the *6aN_Aut* samples were represented by *Group_1+5* and 10% by *Group_2+4* (Table 4.7). The majority of the *6aN_Aut* individuals in *Group_2+4* were from the samples collected in 2018 (Tables 4.1 and 4.6). 84% of the *6aS* samples were represented by *Group_2+4* and 16% by *Group_3*. The majority of *6aS* individuals in *Group_3* were from the samples of late spawning herring collected at the end of the spawning season in January and February (Tables 4.1 and 4.6). 95% of the *6aN_Sp* samples were represented by *Group_3*, 3% by *Group_2+4* and 2% by *Group_1+5* (Table 4.7). In terms of cluster group composition, *Group_1+5* was comprised of 99% *6aN_Aut* samples (Table 4.7) and as such were considered, for the purposes of assignment, as a proxy for that population group. *Group_2+4* was comprised of 86% *6aS* and 14% *6aN_Aut*. There is evidence that the *6aN_Aut* individuals in these clusters were misidentified *6aS* herring (see Section 4.9). Therefore, *Group_2+4* was considered to represent *6aS* for the purposes of assignment. *Group_3* was comprised of 53% *6aS*, 46% *6aN_Sp* and 1% *6aN_Aut* and was considered, for the purposes of assignment, to represent a mix of *6aS* and *6aN_Sp* herring.

The *Approach 2* assignment was also performed in a hierarchical manner as per *approach 1*. In *level 1*, *Group_2+4* and *Group_3* were combined and tested against *Group_1+5*. In level 2 the combined *Group_2+3+4* was split back into *Group_2+4* and *Group_3* and these groups tested against each other (Figure 4.10, bottom panel). The optimum numbers of PCs , as determined through DAPC cross-validation, were 30, 35 and 10 for complete dataset, the level 1 dataset and level 2 dataset, respectively (Annex 4: Figure 10.4.4). There was however little difference between the number of PCs retained in all cases suggesting that the assignment is not sensitive to this parameter.

Exploratory analyses in *assignPOP* determined that the optimum model and kernel for the assignment model were the *SVM* model and the radial basis function (*RBF*) kernel. Exploratory analyses and the *tune*, *tune.control* and *best.svm* functions in *R* package *e1071* (Meyer *et al*., 2015) were used to perform a grid search for the optimum values for cost and gamma in *level 1* and *level 2* of *approach 1* and *approach 2* (Annex 4: Table 10.4.3). These parameters were used for testing the rate of self-assignment using both Monte-Carlo cross-validation (*assign.MC*) and also *K*-fold cross-validation (*assign.kfold*) to estimate membership probability. Monte-Carlo cross-validation is a method whereby the baseline dataset is divided into a training dataset and test dataset, based on user specified numbers of individuals or proportions (Chen *et al*., 2018). The assignment model is developed with the training dataset and subsequently tested with the independent test data, which avoids introducing 'high-grading bias' (see Anderson, 2010). In order to avoid unbalanced sample sizes among the baseline groups the number of individuals in the training sets were specified and were limited by the number of individuals in the smallest group (Annex 4: Table 10.4.3). As the Monte-Carlo procedure samples random individuals each time, it does not guarantee that every individual is sampled (Chen *et al*., 2018). Therefore, an additional method of *K*-fold cross-validation was also performed. *K*-fold cross-validation involves randomly dividing the individuals from each population into *K* groups and then using one group from each population as test individuals and the remaining *K*-1 groups as the training individuals to build the predictive model (Chen *et al*., 2018). Assignment tests are then performed until every group is tested, resulting in *K* tests. In this way, test individuals are independent from the training individuals, and every individual is guaranteed to be tested once. Both Monte-Carlo and *K*-fold cross-validation were performed using 25%, 50%, 75% of the highest F_{ST} loci.

Table 4.6. Clustering analyses, using the *find.clusters* function in *adegenet*, of the *45_SNP* dataset with the individual samples from the three population groups (*6aS*, *6aN_Aut*, *6aN_Sp*).

Sample	Cluster				
	1	2	3	4	5
6aS_14b	0	20	8	13	0
6aS_16a	0	63	5	25	1
6aS_16b	0	8	6	5	0
6aS_17a	0	15	24	41	0
6aS_17b	0	9	28	50	0
6aS_17d	2	56	9	11	0
6aS_17e	0	25	15	10	0
6aS_17f	0	38	7	12	0
6aS_18a	0	60	0	32	0
6aS_18b	1	50	4	29	0
6aS_18h	0	1	5	10	0
6aN_15	63	8	0	1	22
6aN_16a	51	11	0	2	14
6aN_16b_H1	21	0	0	0	13
6aN_16b_H2	17	2	0	0	9
6aN_16c_H1	31	2	0	1	16
6aN_16c_H2	18	0	0	1	5
6aN_16d	31	0	0	1	32
6aN_16e	43	3	1	1	31
6aN_16f	48	1	0	0	37
6aN_17g	59	2	1	5	28
6aN_17c	29	1	0	0	6
6aN_17b	18	2	0	3	11
6aN_17a	14	0	0	0	2
6aN_17d	19	0	0	0	8
6aN_17f	13	1	0	0	7
6aN_17e	29	1	0	2	10
6aN_18a	51	23	1	5	13
6aN_18b	29	11	0	3	4
6aN_Sp_18a	0	0	73	0	0
6aN_Sp_18d	0	0	24	3	2

Table 4.7. Clustering analyses, using the *find.clusters* function in *adegenet*, of the *45_SNP* dataset with the individual samples from the three population groups (*6aS*, *6aN_Aut*, *6aN_Sp*). The proportions of each population group split by cluster and the proportions of each cluster split by population are shown.

	Group_1+5	*Group_2+4*	*Group_3*
Population split by cluster group			
6aS	0.01	0.84	0.16
6aN_Aut	0.90	0.10	0.00
6aN_Sp	0.02	0.03	0.95
Cluster group split by population			
6aS	0.00	0.86	0.53
6aN_Aut	0.99	0.14	0.01
6aN_Sp	0.00	0.00	0.46

There was little difference between the self-assignment accuracy of *approach 1 level 1* (Figure 4.11 and Table 4.8) and assignment *approach 2 level 1* (Figure 4.12 and Table 4.9). Both approaches resulted in self-assignment rates greater than 90% and neither approach was observed to be particularly sensitive to the number of individuals in the training data, at the numbers tested. Similarly, neither approach was observed to be particularly sensitive to the proportion of highest F_{ST} loci used in the analyses. The main difference between the two approaches at *level 1* was the higher probabilities of assignment observed in the *K*-fold analyses in *approach 2* (Figures 4.11 and 4.12).

Conversely there were large differences between the two approaches in *level 2* assignment, where *approach 1* did not confidently assign *6aN_Sp* samples to their baseline (Figure 4.13 and Table 4.10), whereas *approach 2* achieved near perfect self-assignment (Figure 4.14 and Table 4.11). This is due to the overlap between the *6aS* late spawning herring and *6aN_Sp* samples, as previously discussed (Figure 4.10). *Approach 2* avoids this overlap by combining the overlapping individuals from these population groups into Group_3, which represents a mix of *6aS* and *6aN_Sp*. The benefit of this is that the majority of *6aS* individuals (Table 4.7) can be separated with a high level of accuracy and it is only the minority of *6aS* fish that are left in an unsorted mix with *6aN_Sp*. This is important when developing a method that can be implemented on the MSHAS samples. Both approaches will be used in subsequent analyses in order to investigate the differences between them and to ascertain which is more appropriate for splitting the MSHAS survey indices.

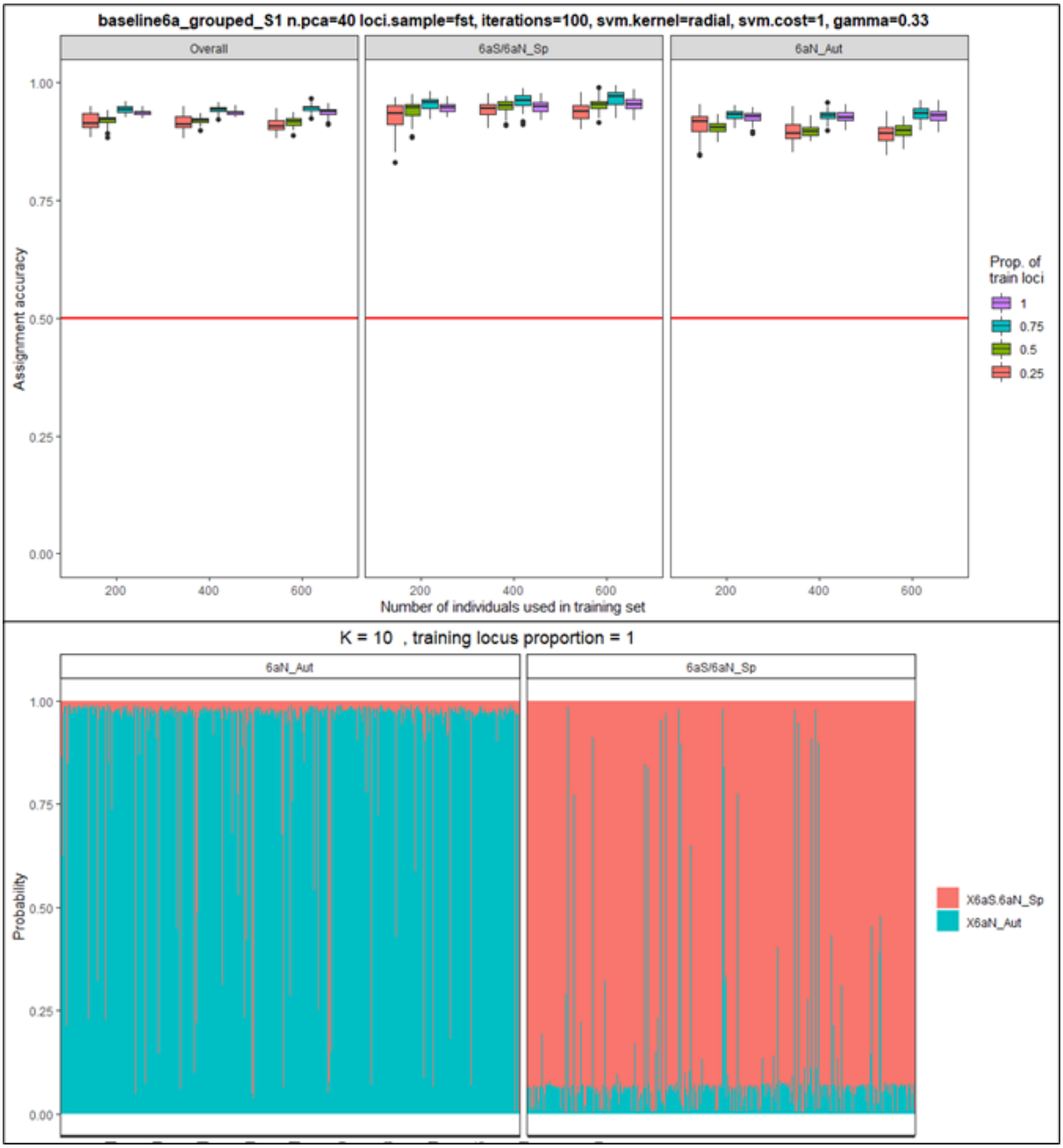


Figure 4.11. Monte-Carlo cross-validation (top) and *K*-fold cross-validation (bottom) of the *approach 1, level 1* assignment.

Table 4.8. Assignment matrix for the Monte-Carlo cross-validation (MC) and *K*-fold cross-validation of the *approach 1, level 1* assignment. SD = standard deviation.

Method	Origin	Assignment	
		6aS/6aN_Sp	*6aN_Aut*
MC	*6aS/6aN_Sp*	**0.95** ± 0.01 SD	0.05 ± 0.01 SD
	6aN_Aut	0.07 ± 0.01 SD	**0.93** ± 0.01 SD
K-fold	*6aS/6aN_Sp*	**0.95** ± 0.03 SD	0.05 ± 0.03 SD
	6aN_Aut	0.06 ± 0.02 SD	**0.94** ± 0.02 SD

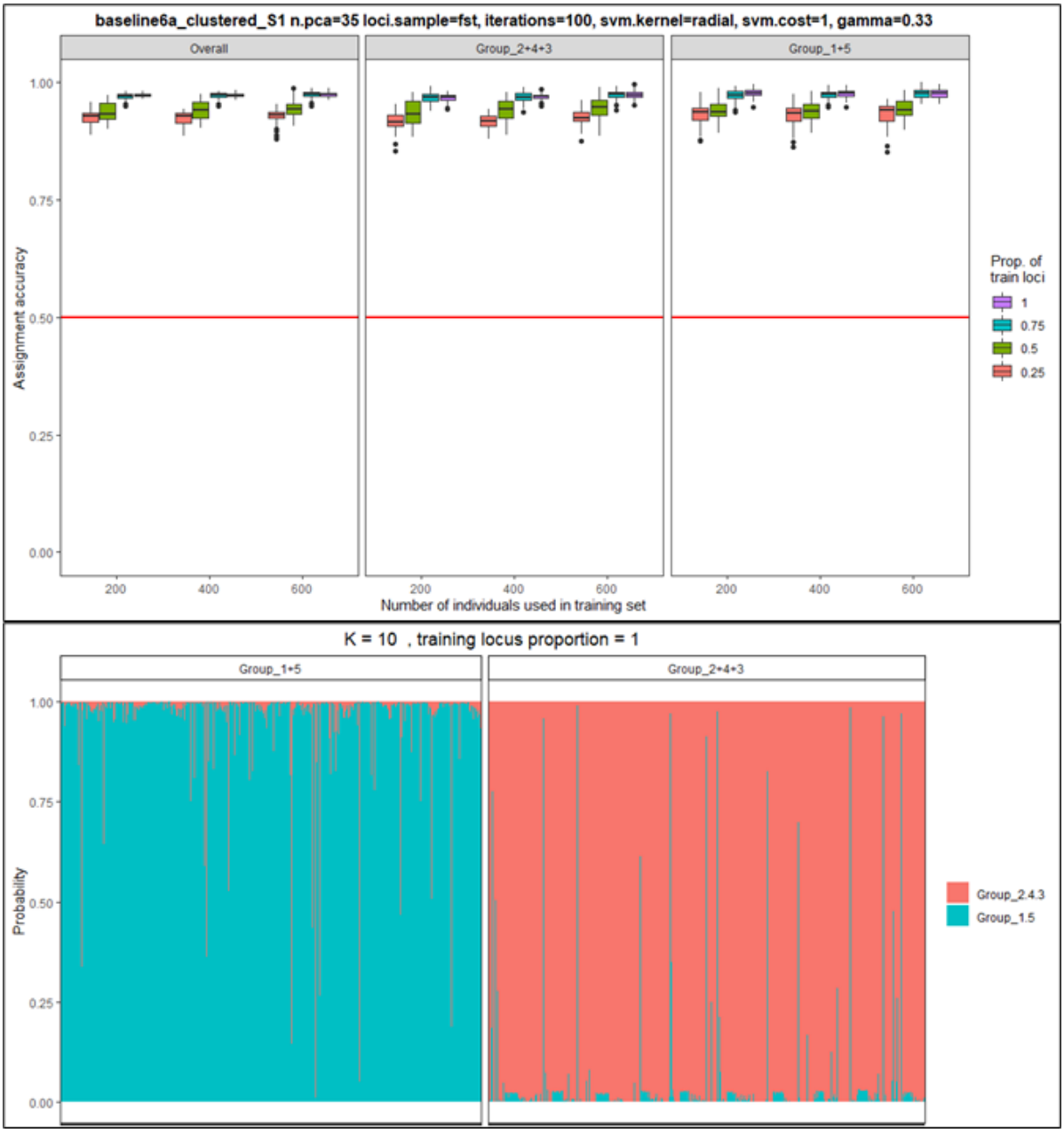


Figure 4.12. Monte-Carlo cross-validation (top) and *K*-fold cross-validation (bottom) of the *approach 2, level 1* assignment.

Table 4.9. Assignment matrix for the Monte-Carlo cross-validation (MC) and *K*-fold cross-validation of the *approach 2, level 1* assignment. SD = standard deviation.

Method	Origin	Assignment	
		Group_2+3+4	*Group_1+5*
MC	*Group_2+3+4*	**0.97** ± 0.01 SD	0.03 ± 0.01 SD
	Group_1+5	0.02 ± 0.01 SD	**0.98** ± 0.01 SD
K-fold	*Group_2+3+4*	**0.97** ± 0.03 SD	0.03 ± 0.03 SD
	Group_1+5	0.02 ± 0.02 SD	**0.98** ± 0.02 SD

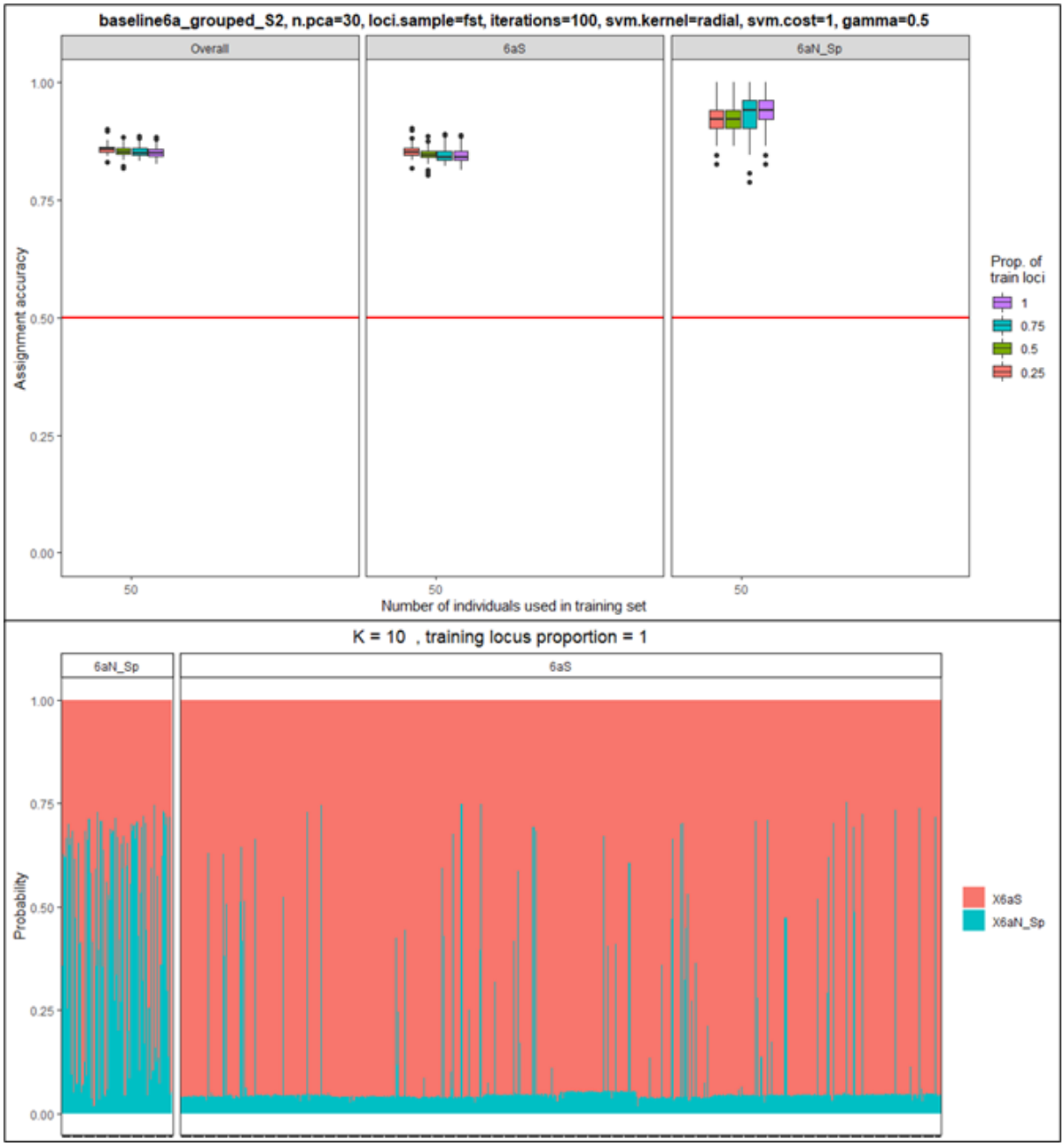


Figure 4.13. Monte-Carlo cross-validation (top) and *K*-fold cross-validation (bottom) of the *approach 1, level 2* assignment.

Table 4.10. Assignment matrix for the Monte-Carlo cross-validation (MC) and K-fold cross-validation of the *approach 1, level 2* assignment. SD = standard deviation.

Method	Origin	Assignment	
		6aS	*6aN_Sp*
MC	*6aS*	**0.84** ± 0.02 SD	0.16 ± 0.02 SD
	6aN_Sp	0.06 ± 0.02 SD	**0.94** ± 0.02 SD
K-fold	*6aS*	**0.95** ± 0.03 SD	0.05 ± 0.03 SD
	6aN_Sp	0.50 ± 0.18 SD	**0.50** ± 0.18 SD

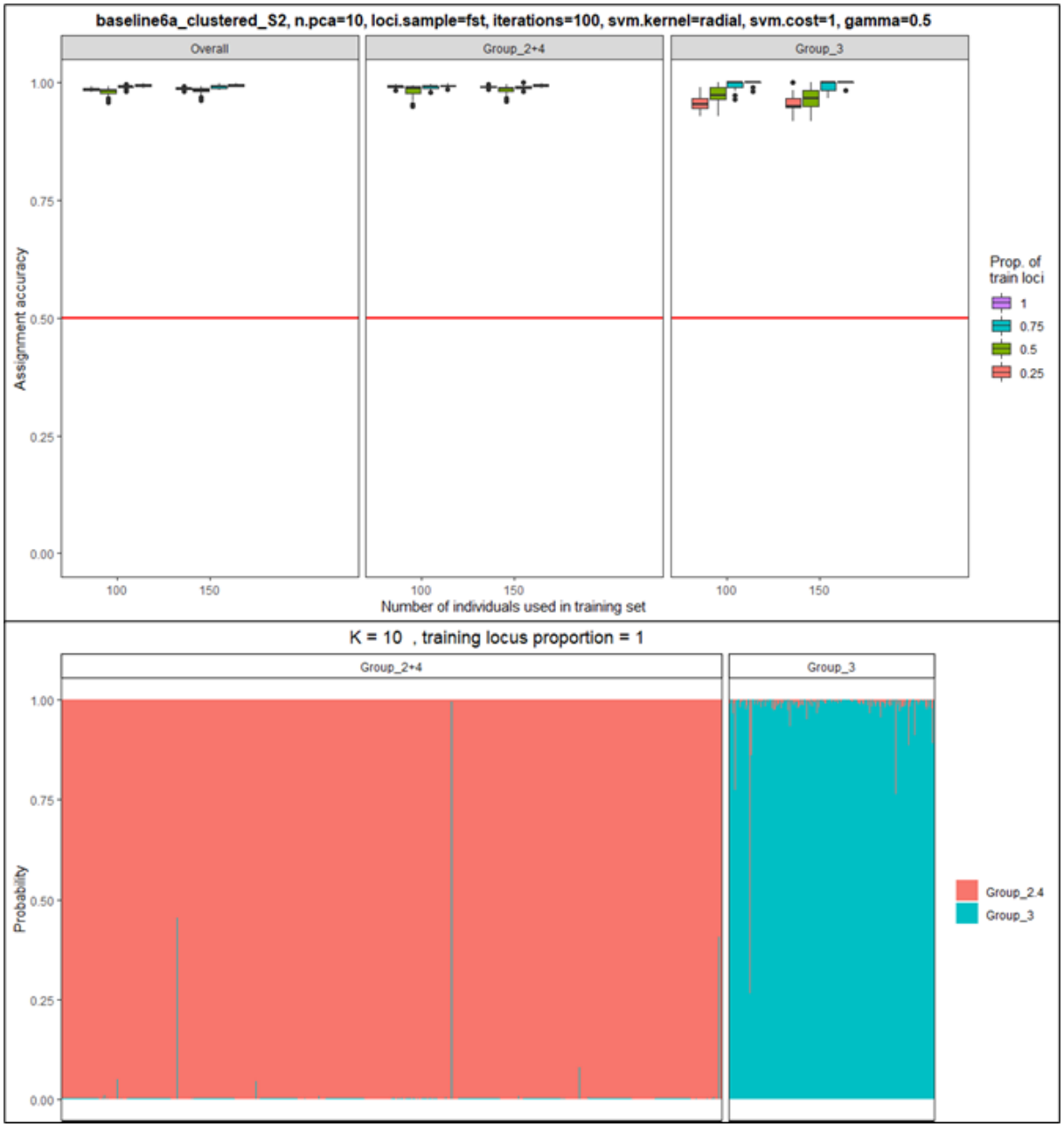


Figure 4.14. Monte-Carlo cross-validation (top) and *K*-fold cross-validation (bottom) of the *approach 2, level 2* assignment.

Table 4.11. Assignment matrix for the Monte-Carlo cross-validation (MC) and *K*-fold cross-validation of the *approach 2, level 2* assignment. SD = standard deviation.

Method	Origin	Assignment	
		Group_2+4	*Group_3*
MC	*Group_2+4*	**0.99** ± 0.00 SD	0.01 ± 0.00 SD
	Group_3	0.00 ± 0.00 SD	**1.00** ± 0.00 SD
K-fold	*Group_2+4*	**1.00** ± 0.00 SD	0.00 ± 0.00 SD
	Group_3	0.01 ± 0.03 SD	**0.99** ± 0.03 SD

One important consideration when developing the baseline is to determine how many genetic markers are required for accurate assignment using either of the approaches and at either of the assignment levels. This will enable the threshold for missing data of the unknown samples to be set with a robust basis without compromising the integrity of the assignments. In order to do this the Monte-Carlo cross validation analyses were run again with random sampling of loci (*loci.sample="random"*) rather than highest F_{ST} loci (*loci.sample="fst"*). In this instance 25%, 50% 75% and all the loci were randomly tested to determine the rate of accuracy of self-assignment of the different approaches and levels. The *approach 1 level 1* assignment was more sensitive to the number of loci than *approach 1 level 2* (Figure 4.15 and Table 4.12). This was particularly notable for the *6aS/6aN_Sp* group in *level 1*, where there was a significant drop in assignment accuracy and an increase in the number of outliers below 50% of loci. This indicates that at least twenty-three of the forty-five loci were required for accurate assignment at this level. Ideally over 75% (34 loci) should be genotyped at this level to ensure accuracy over 90%. The *approach 1 level 2* assignment was not very sensitive to the number of loci and there was little difference in the accuracy of assignment down to 25% of the loci.

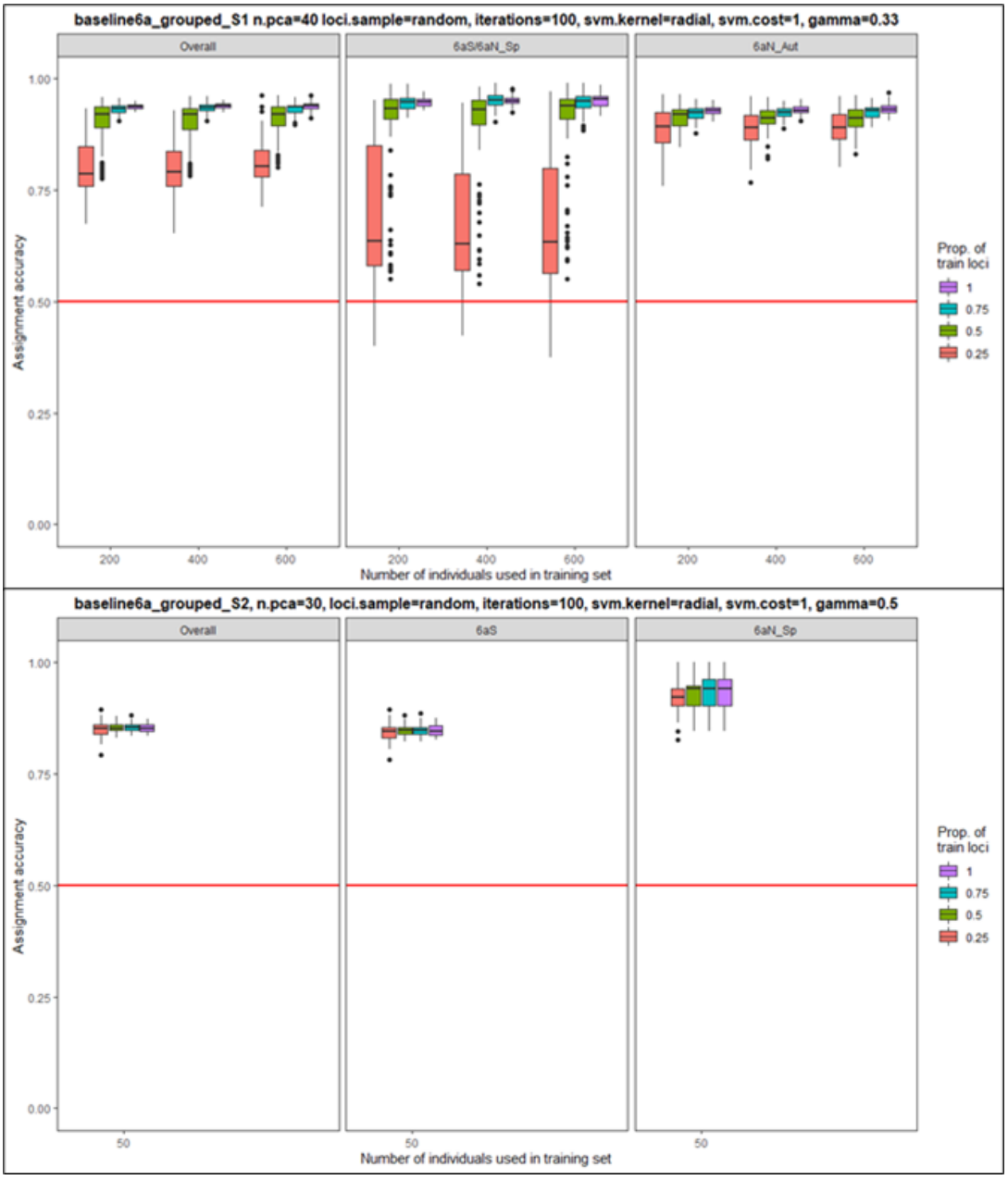


Figure 4.15. Monte-Carlo cross-validation of the *approach 1, level 1* (top) and *level 2* (bottom) assignments with random loci selection.

Table 4.12. Assignment matrix for the Monte-Carlo cross-validation of the *approach 1, level 1* and *level 2* assignments with random loci selection. SD = Standard deviation.

Level	%loci	Origin	Assignment	
			6aS/6aN_Sp	***6aN_Aut***
1	100	*6aS/6aN_Sp*	0.95 ± 0.01 SD	0.05 ± 0.01 SD
		6aN_Aut	0.07 ± 0.01 SD	0.93 ± 0.01 SD
1	75	*6aS/6aN_Sp*	0.95 ± 0.02 SD	0.05 ± 0.02 SD
		6aN_Aut	0.07 ± 0.01 SD	0.93 ± 0.01 SD
1	50	*6aS/6aN_Sp*	0.90 ± 0.11 SD	0.10 ± 0.11 SD
		6aN_Aut	0.09 ± 0.03 SD	0.91 ± 0.03 SD
1	25	*6aS/6aN_Sp*	0.67 ± 0.15 SD	0.33 ± 0.15 SD
		6aN_Aut	0.11 ± 0.04 SD	0.89 ± 0.04 SD
			6aS	***6aN_Sp***
2	100	*6aS*	0.85 ± 0.01 SD	0.15 ± 0.01 SD
		6aN_Sp	0.07 ± 0.01 SD	0.93 ± 0.01 SD
2	75	*6aS*	0.85 ± 0.01 SD	0.15 ± 0.01 SD
		6aN_Sp	0.07 ± 0.04 SD	0.93 ± 0.04 SD
2	50	*6aS*	0.85 ± 0.01 SD	0.15 ± 0.01 SD
		6aN_Sp	0.07 ± 0.04 SD	0.93 ± 0.04 SD
2	25	*6aS*	0.84 ± 0.02 SD	0.16 ± 0.02 SD
		6aN_Sp	0.08 ± 0.04 SD	0.92 ± 0.04 SD

The *approach 2* assignments had a similar pattern of sensitivity to the number of loci as the *approach 1* assignments (Figure 4.16 and Table 4.13). The *approach 2 level 1* assignment was more sensitive to the number of loci than *approach 2 level 2*. In particular the accuracy of the self-assignment of *Group_2+3+4* decreased rapidly below 50% of loci. This was also the case with the *Group_1+5* self-assignment, though to a lesser extent. Therefore, approximately 75% of the 45 loci are required for accurate assignment at this level. The *level 2* assignments were more accurate than the *approach 1 level 2* assignments however they were also slightly more sensitive to missing data. With only 25% of loci there was an increase in the number of outliers and a decrease in accuracy.

In each assignment approach at least 75% of the 45 loci were required to ensure accurate self-assignment. This indicates that there is a level of redundancy built into the panel of markers as was expected given that the markers are distributed among fourteen linkage groups (Table 4.3). This redundancy is an advantage when analysing unknown samples as it allows up to 25% missing data in the genotypes of individuals.

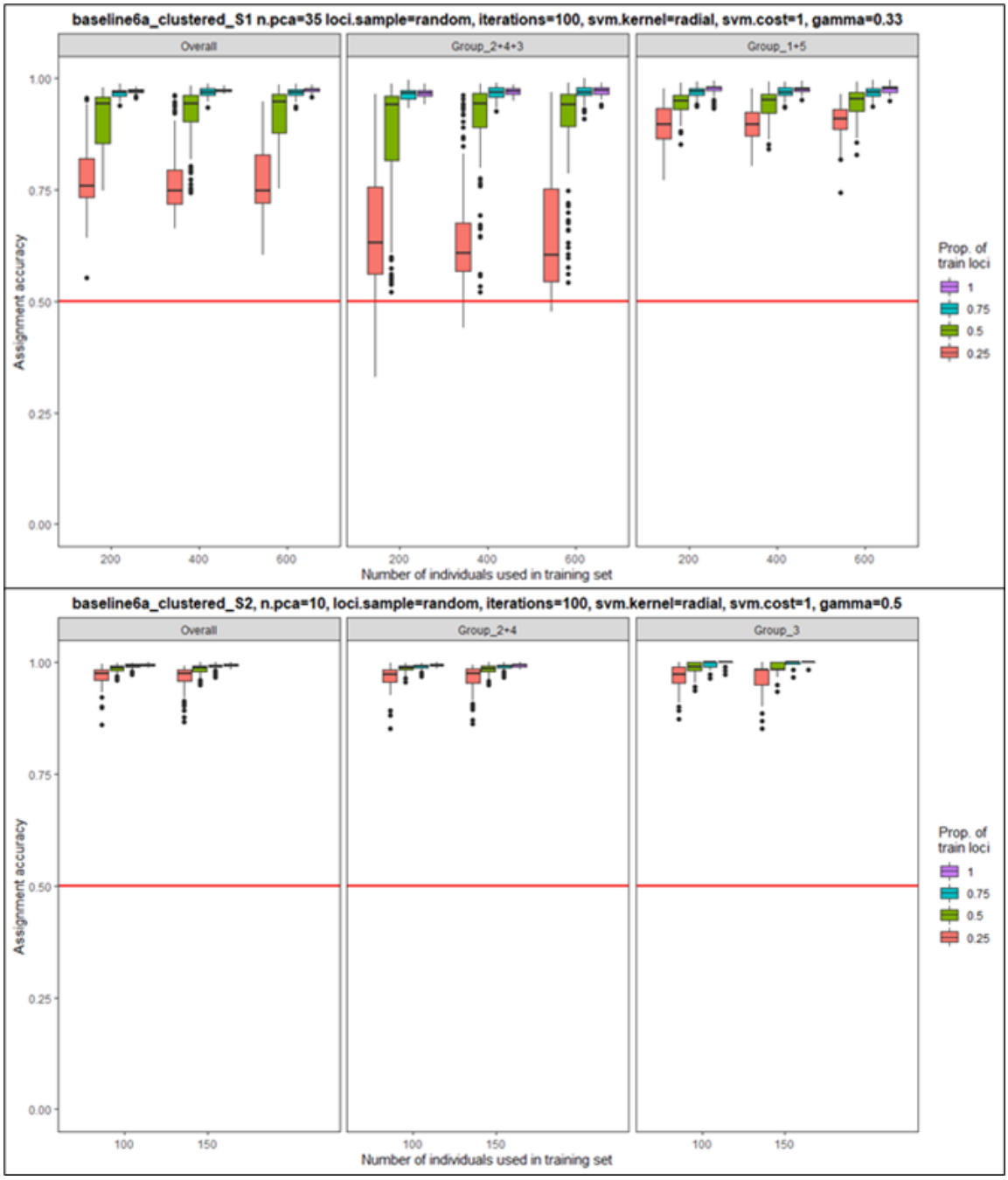


Figure 4.16. Monte-Carlo cross-validation of the *approach 2, level 1* (top) and *level 2* (bottom) assignments with random loci selection.

Table 4.13. Assignment matrix for the Monte-Carlo cross-validation of the *approach 2, level 1* and *level 2* assignments with random loci selection. SD = Standard deviation.

Level	%loci	Origin	Assignment	
			Group_2+3+4	***Group _1+5***
1	100	*Group_2+3+4*	0.96 ± 0.01 SD	0.03 ± 0.01 SD
		Group _1+5	0.02 ± 0.01 SD	0.98 ± 0.01 SD
1	75	*Group _2+3+4*	0.97 ± 0.02 SD	0.03 ± 0.02 SD
		Group _1+5	0.03 ± 0.01 SD	0.97 ± 0.01 SD
1	50	*Group _2+3+4*	0.89 ± 0.12 SD	0.11 ± 0.12 SD
		Group _1+5	0.06 ± 0.04 SD	0.94 ± 0.04 SD
1	25	*Group _2+3+4*	0.66 ± 0.15 SD	0.34 ± 0.15 SD
		Group _1+5	0.09 ± 0.04 SD	0.91 ± 0.04 SD
			Group _2+4	***Group _3***
2	100	*Group _2+4*	0.99 ± 0.01 SD	0.01 ± 0.01 SD
		Group _3	0.00 ± 0.00 SD	1.00 ± 0.00 SD
2	75	*Group _2+4*	0.99 ± 0.01 SD	0.01 ± 0.01 SD
		Group _3	0.01 ± 0.01 SD	0.99 ± 0.01 SD
2	50	*Group _2+4*	0.98 ± 0.01 SD	0.02 ± 0.01 SD
		Group _3	0.02 ± 0.02 SD	0.98 ± 0.02 SD
2	25	*Group _2+4*	0.96 ± 0.03 SD	0.04 ± 0.03 SD
		Group _3	0.03 ± 0.03 SD	0.97 ± 0.03 SD

4.9. Assignment validation with known-unknown samples

Though the assignments were tested comprehensively in section 4.8 and proven to be robust, it was decided to undertake additional validation steps. The first was to use the original WESTHER baseline samples from Division 6.a (Figure 3.4 and Table 3.7) as known-unknown samples to test the temporal stability of the assignment of the baseline. The WESTHER samples were processed and genotyped as per the methods for the baseline samples (Sections 4.5 and 4.6). The assignments were conducted using the *assign.X* function in *assignPOP* (Chen *et al*., 2018) using the two hierarchical approaches and with the same model parameters as described in Section 4.8. For each approach, the *level 1* assignment was conducted with all the individuals in the sample and the *level 2* assignment on a subset of individuals assigned to either *6aS/6aN_Sp* or *Group_2+3+4*, depending on the approach.

The 2003 and 2004 *6aN_Aut* WESTHER samples assigned near perfectly to the *6aN_Aut* population group (Figure 4.17) with an average probability of 0.97 and 0.98 for approaches 1 and 2, respectively. This means the individuals were 32 or 49 times more likely (see conversion in Table 4.5) to come from *6aN_Aut* or *Group_1+5* than from *6aS/6aN_Sp* or *Group_2+3+4*, respectively. Two individuals had an average probability of 0.93 and 0.99 of originating from *6aS/6aN_Sp* or *Group_2+3+4*. These two individuals were carried forward into the *level 2* assignments, where they assigned to *6aS* and *Group_2+4* with probabilities of 0.96 and 0.99, respectively.

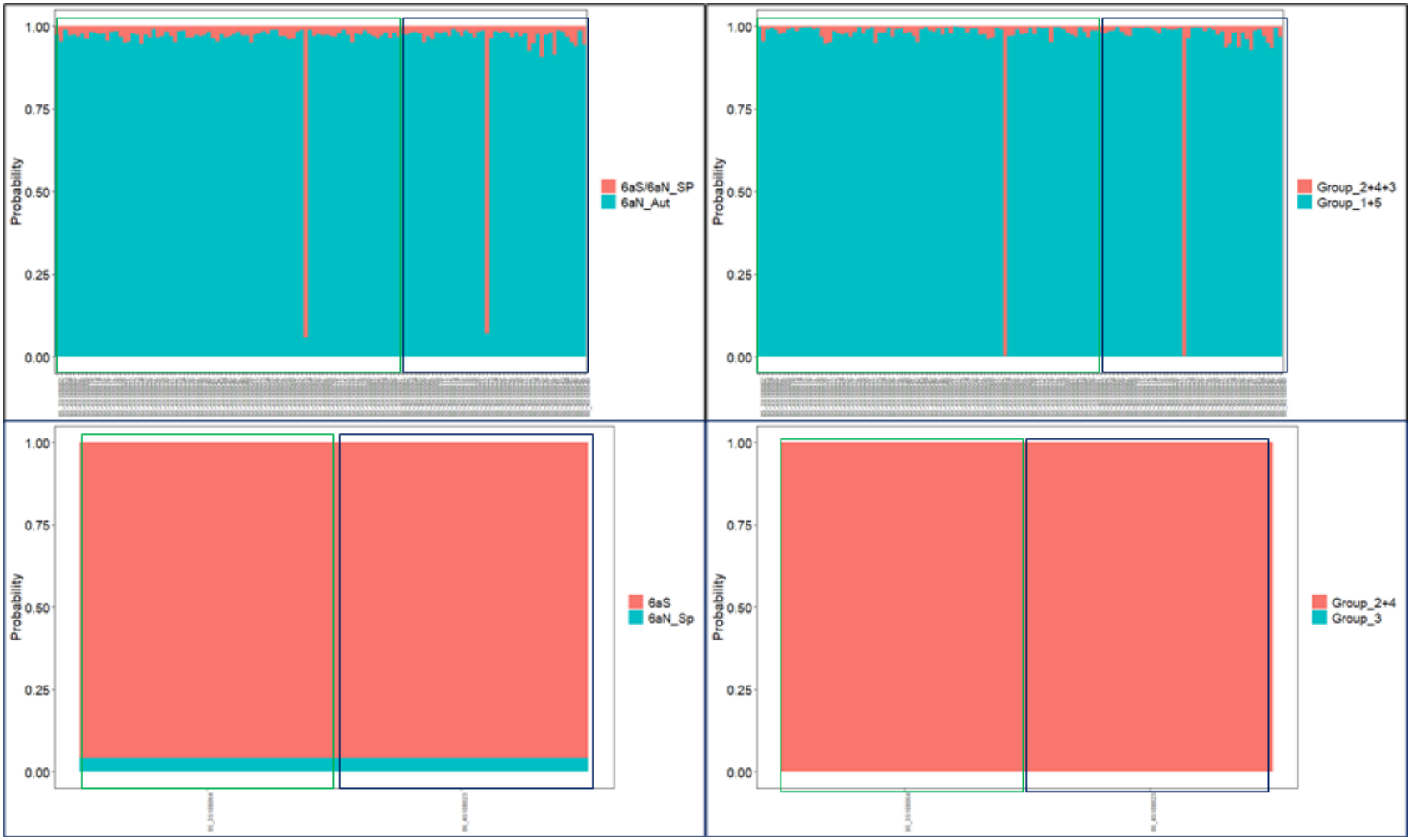


Figure 4.17. Assignment of the 2003 (green box) and 2004 (blue box) WESTHER *6aN_Aut* samples to the contemporary baseline with associated probabilities. (top left) *approach 1 level 1* (bottom left) *approach 1 level 2* (top right) *approach 2 level 1* (bottom right) *approach 2 level 2*.

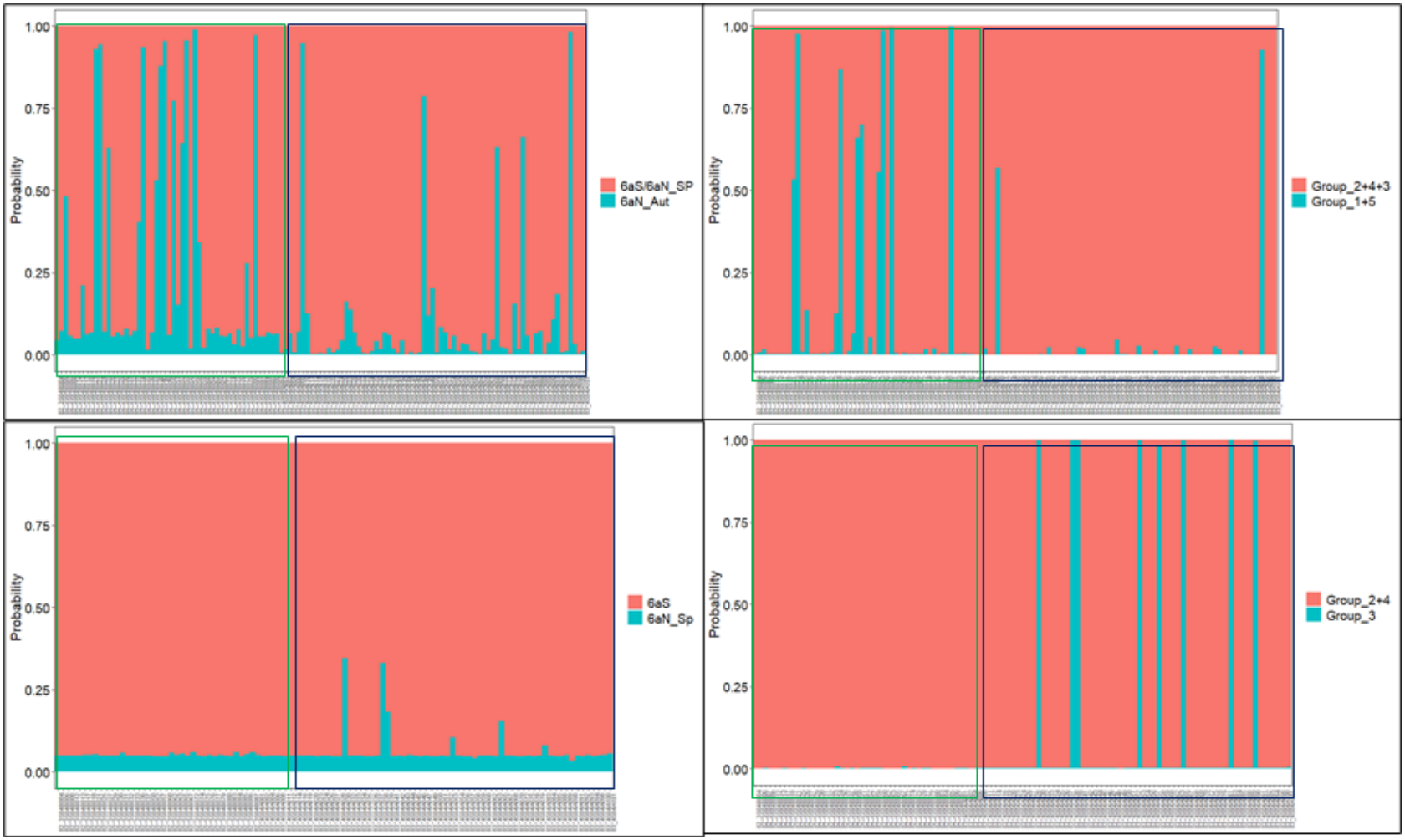


Figure 4.18. Assignment of the 2003 (green box) and 2004 (blue box) WESTHER *6aS* samples to the contemporary baseline with associated probabilities. (top left) *approach 1 level 1* (bottom left) *approach 1 level 2* (top right) *approach 2 level 1* (bottom right) *approach 2 level 2*.

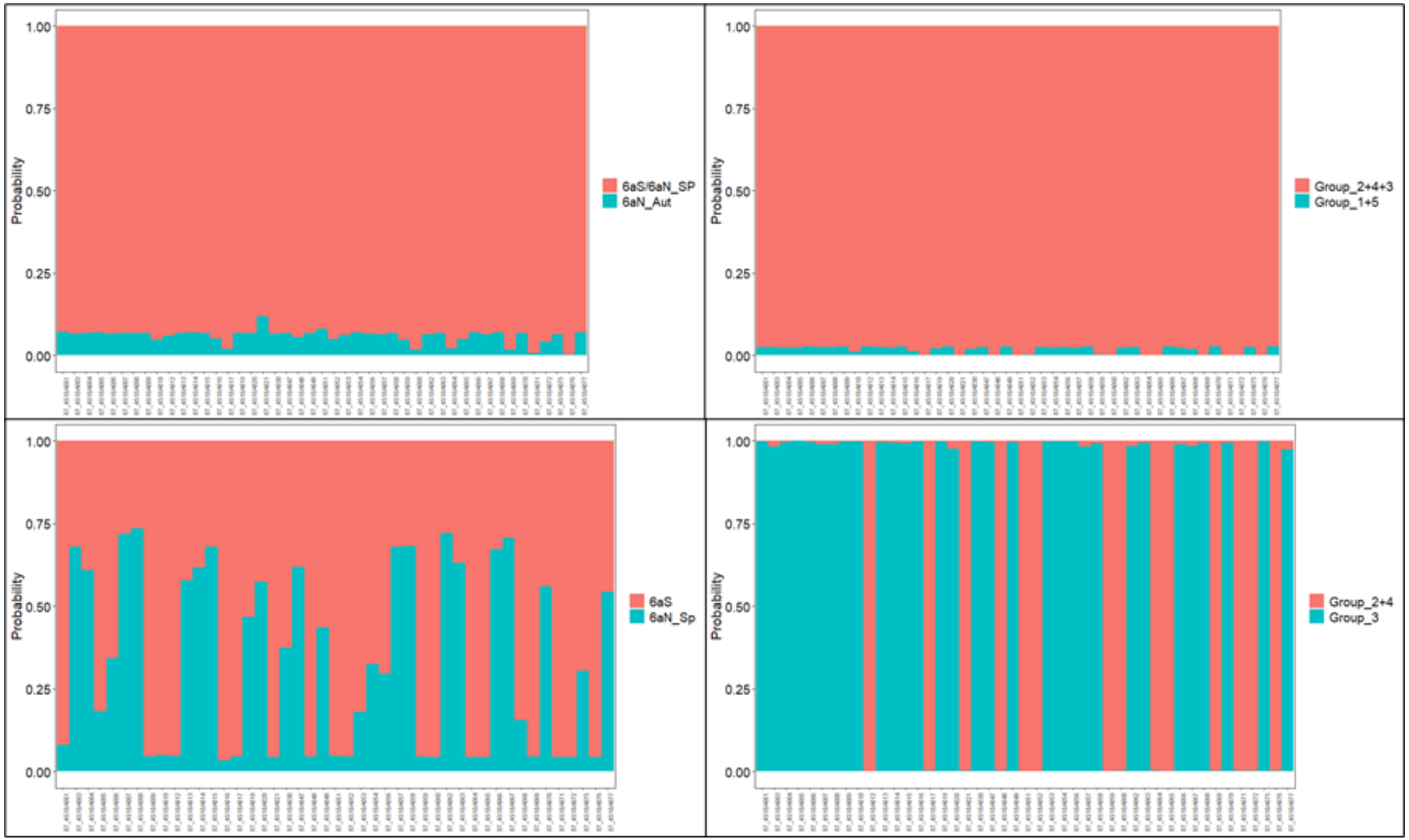


Figure 4.19. Assignment of the WESTHER *6aN_Sp* sample to the contemporary baseline with associated probabilities. (top left) *approach 1 level 1* (bottom left) *approach 1 level 2* (top right) *approach 2 level 1* (bottom right) *approach 2 level 2*.

The assignments of the 2003 and 2004 *6aS* WESTHER samples were not as confident as the *6aN_Aut* WESTHER samples (Figure 4.18). In the *approach 1 level 1* analysis of the 2003 samples, 12% of the samples did not meet the threshold assignment probability of 0.67, 17% misassigned to *6aN_Aut* and 71% assigned to *6aS/7bc*. In the *approach 2 level 1* analysis, 6% of the samples did not meet the threshold assignment probability of 0.67, 12% misassigned to *6aN_Aut* and 83% assigned to *6aS/7bc*. The *level 2* assignments of the 2003 samples indicated that all samples belonged to *6aS* or *Group_2+4*. The *approach 2 level 2* assignment of the 2004 sample indicated that 12% of the samples assigned to *Group_3*, which is a mixture of *6aS* and *6aN_SP,* and 82% to Group_2+4. The proportion of misassigned individuals in the *6aS* WESTHER samples was considered to be low and may be partly explained by the fact that the 2003 sample was collected in October (Table 3.7), which is earlier than any of the contemporary samples in the *6aS* baseline dataset. Autumn spawning has been previously recorded in Divisions 6.a.S. 7.b and 7.c (see section 2.6) though it was not observed during the course of the current study. Therefore, these groups are not represented in the baseline. Based on the analyses of the autumn spawning Irish Sea samples it is hypothesised that the 6.a.S, 7.b and 7.c autumn spawners may have a higher degree of overlap with the *6aN_Aut* group than the winter or late spawning 6.a.S. 7.b and 7.c herring (Figures 4.5 and 4.7). However, as these fish have not been observed in recent years despite extensive sampling this is not considered to be an issue for the assignment of contemporary MSHAS samples.

The assignment of the *6aN_Sp* WESTHER sample resulted in perfect assignment at *level 1* for both approaches (Figure 4.19). The average probability of assignment to *6aS/6aN_Sp* was 0.94 and to *Group_2+3+4* was 0.98. The *level two* assignments illustrated the issues related to the identification of the *6aN_Sp* spawners, as discussed in section 4.8. In *approach 1*, 27% of the individuals failed to meet the assignment probability threshold of 0.67, 53% were misassigned to 6aS and only 20% were correctly assigned to *6aN_Sp*. In *approach 2* all individuals confidently assigned with probabilities of greater than 0.97, with 31% being assigned to Group_2+4 and 69% to Group_3. Whilst neither approach is perfect for the assignment of *6aN_Sp*, it is clear that *approach 2* has a lower error rate.

Overall, the assignment of the WESTHER samples confirmed the temporal stability of the marker panel over a period of at least sixteen spawning seasons (2003/2004 – 2018/2019). This was considered sufficiently robust for the purposes of conducting further assignments.

The second additional validation step was to test the assignment model by attempting to assign additional known-unknown samples, collected in 2018 and 2019, that had had not been included in the development of the baselines. One sample was available for *6aS*, thirteen small samples from *6aN_Sp*, and three samples for *6aN_Aut* (*6aN_19a, 6aN_19b, 6aN_19c*) (Table 3.5).

The *level 1* assignments of the *6aS* sample (*6aS_19c*) indicated a high level of correct assignment, with 92% and 96% of individuals assigned to *6aS/6aN_Sp* and *Group_2+3+4*, respectively (Figure 4.20). In *approach 1 level 1*, 3% of individuals did not reach the assignment threshold and 5% of individuals were misassigned. In *approach 2 level 1*, 1% of individuals did not reach the assignment threshold and 3% of individuals were misassigned. In *approach 1 level 2*, all individuals assigned to *6aS* and in *approach 2 level 2*, 98% of individuals assigned to *Group_2+4* and 2% of individuals assigned to Group_3.

The thirteen *6aN_Sp* samples from 2018 and 2019 were run in the one assignment (Figure 4.21). As noted in Section 4.3, the 2018 samples were pre-screened prior to processing and only stage 3 fish were genetically analysed. The 2019 samples comprised a range of maturity stages from pre-spawning, spawning and spent fish (Annex 3) and only maturity stage 3 fish were included in the following analyses. One individual from the 2018 samples in *approach 1 level 1* did not meet the assignment threshold and the *level 1* assignments indicated near perfect assignment with only 1 individual, from the 2018 samples, being assigned to *6aN_Aut* or *Group_1+5*. The level 2 assignments were more uncertain in both the 2018 and 2019 samples with a similar pattern of misassignments in each. In the 2018 *approach 1 level 2* assignment, 31% of the individuals failed to meet the assignment

threshold, 43% of the individuals assigned to *6aS* and 26% assigned to *6aN_Sp*. In the 2019 *approach 1 level 2* assignment, 31% of the individuals failed to meet the assignment threshold, 45% of the individuals assigned to *6aS* and 24% assigned to *6aN_Sp*. The *approach 2 level 2* assignments had higher probabilities and no individuals failed to meet the assignment threshold. In the 2018 samples, 10% were assigned to *Group_2+4* and 90% were assigned to *Group_3*. In the 2019 samples 19% were assigned to *Group_2+4* and 81% to *Group_3*. These results support the conclusion that assignment *approach 2* is better for discriminating between the *6aS* and *6aN_Sp* population groups.

The three samples from *6aN_Aut* were run in three separate assignments and yielded surprising results. Based on maturity stage and collection location (Table 4.14), the samples *6aN_19a* and *6aN_19b* were both initially assumed to be potential baseline spawning samples. However, both the *level 1* assignments indicated that there was a significant number of *6aS/6aN_Sp* type fish in the samples (Figures 4.22 and 4.23). In sample *6aN_19a*, *approach 1* indicated 6% of the individuals failed to reach the assignment threshold, 22% of the individuals were *6aS/6aN_Sp*, and 72% of the individuals were *6aN_Aut*, whilst *approach 2* indicated that 5% were unassigned, 32% were assigned to *Group_2+3+4* and 63% were assigned to *Group_1+5*. In sample *6aN_19b*, *approach 1* indicated 12% of the individuals failed to reach the assignment threshold, 13% of the individuals were *6aS/6aN_Sp*, and 75% of the individuals were *6aN_Aut*, whilst *approach 2* indicated 5% were unassigned, 26% assigned to *Group_2+3+4* and 69% were assigned to *Group_1+5*. The level two assignments in both cases indicated near perfect assignment to *6aS* or *Group_2+4* (Figures 4.22 and 4.23). Both samples were caught on the same day, by the same vessel, within 5 nautical miles (nm) of each other and were processed in the same laboratory by the same technician. The two samples had very similar length-frequency distributions (Annex 3) and proportion of maturity stages (Table 4.14). As such they can be considered to be replicates and as the independent assignments both displayed the same patterns it lends support to their validity. The maturity staging of these samples (Table 4.14) indicated that there were some stage two herring present, though there was no pattern in the assignments related to maturity i.e. stage two and stage three fish were assigned to both *6aS/6aN_Sp* and *6aN_Aut*. There are three possible explanations for the high level of misassignments in these samples. The first is that the baseline is not temporally stable and that there has been a recent change in the genetic characteristics of the *6aN_Aut* group. Given the long-term temporal stability of the markers and the ability to accurately assign the WESTHER samples (Figure 4.17), this seems unlikely. The second is that there was a high rate of 'straying' between sympatric populations i.e. *6aS* herring had switched their spawning season to spawn with *6aN_Aut* herring (Berg *et al*., 2020; Kerr *et al*., 2019). Though this phenomenon is noted to occur in herring it is usually at lower rates than the misassignment rates observed here (Berg et al., 2020). Further if a high level of straying were common on an annual basis then it would be expected that this would be a common feature of all the *6aN_Aut* samples, which was not the case. If strayers were reproductively successful then the genetic differentiation between the populations would ultimately become insignificant, which was also not the case. The most plausible explanation is that there was misclassification of maturity stages during processing and the samples do actually contain a mix of the *6aS/6aN_Sp* and *6aN_Aut* population groups.

The third sample, *6aN_19c*, lends support to this explanation as it was caught nine days after the first two samples, 15nm to the south, by a different vessel but was processed in the same lab by the same technician as the *6aN_19a* and *6aN_19b* samples. One would expect that the sample would comprise the same mix of maturity stages or as the sample was collected later that it would be dominated by stage three fish. In fact, the converse is true. There were no maturity stage three fish in this sample and the majority of fish were stage two fish (Table 4.14), which is surprising given that it was caught in close proximity to the *6aN_Aut* spawning grounds at spawning time. The length-frequency distribution also indicated a wider distribution than the two other samples (Annex 3). The *level 1* assignments of this sample indicated that over 80% of the individuals were from *6aS/6aN_Sp* and *Group_2+3+4* and only 13% from *6aN_Aut* or *Group_1+5* (Figure 4.24). The *approach 1 level 2* assignment then indicated a mixture between *6aS* and *6aN_Sp* and the *approach 2 level 2* assignment similarly indicated a mix of *Group_2+4* and *Group_3*.

The significant difference between the maturity stages and assignments of this sample and those caught in close proximity only nine days prior lend support to the theory that the other two samples were confounded by mixing of populations and consequently by misclassification of maturity stages.

The three samples discussed above were all processed in the same laboratory by the same technician. Two alternate samples were identified that were collected in the same area at the same time by different vessels and processed in a different laboratory by a different technician. This enables a comparison to be made between the maturity staging of the samples, but it should be noted that the alternate samples have not been genotyped as they were not available at the time genetic processing was undertaken (Table 3.5). The *6aN_19e* sample was caught fifteen days prior to the two early samples and the *6aN_19d* sample was caught on the same day as the two earlier samples (Table 4.14). It might be expected that the *6aN_19e* sample would contain a majority of earlier maturity stage fish, whilst the *6aN_19d* sample should be comparable with the *6aN_19a* and *6aN_19b* samples. The *6aN_19e* was composed of 59% maturity stage two and 41% stage three fish, which fits with this theory. However, the *6aN_19d* sample comprised 43% stage two fish, 54% stage three fish and 1% each of stage one, four and five fish. This is in contrast to the *6aN_19a* sample that comprised 5% stage two and 95% stage three fish and the *6aN_19b* sample that contained 11% stage two, 87% stage three and 2% stage four fish. Both samples were caught on the same day only 3nm from the *6aN_19d* sample. The lack of agreement in the maturity staging between the samples supports the theory of misclassification of maturity stages.

Table 4.14. The catch details and number of fish at each maturity stage (6-point scale) for the samples with potential maturity staging issues.

Sample	Date	Lat	Lon	Maturity stage (6-point scale)					
				1	2	3	4	5	6
6aN_18b	16/09/2018	58.82	-4.43	0	23	75	2	0	0
6aN_18a	27/09/2018	58.58	-4.28	0	7	93	0	0	0
6aN_18c	16/09/2018	58.66	-4.40	0	24	75	2	0	0
6aN_18d	17/09/2018	58.68	-4.38	0	0	99	1	0	0
6aN_18e	18/09/2018	58.62	-4.42	0	70	31	0	0	1
6aN_18f	18/09/2018	58.65	-4.27	0	70	31	0	2	0
6aN_19a	21/09/2019	58.70	-5.35	0	5	95	0	0	0
6aN_19b	21/09/2019	58.72	-5.18	0	11	87	2	0	0
6aN_19c	30/09/2019	58.53	-5.68	8	81	0	11	0	0
6aN_19e	06/09/2019	58.68	-5.55	0	59	41	0	0	0
6aN_19d	21/09/2019	58.68	-5.25	1	43	54	1	1	0

As a result of the analyses above it was decided to also reanalyse the two 2018 *6aN_Aut* samples (*6a_18a* and *6aN_18b*) which had been included in the final baseline dataset. Previous clustering analyses indicated that a proportion of the fish in these two samples clustered in cluster groups two and four with the *6aS* samples (Table 4.6). These two samples were processed in the same laboratory and by the same technician as the three *6aN_Aut* samples with potential maturity classification issues discussed above. The *6aN_18a* sample comprised 7% maturity stage two and 93% stage three fish and the *6aN_18b* sample comprised 23% stage two, 75% stage three and 2% stage four fish (Table 4.14). Four additional samples were identified that were collected in the same area at the same time by different vessels and processed in a different laboratory by a different technician (Table 4.14). This enabled a comparison to be made between the maturity staging of the samples, but again it should be noted that the alternate samples have not been genotyped as they were not available at the time genetic processing was undertaken (Table 3.5). The four additional samples were caught over three days in close proximity to each other and contained a range of maturity stages (Table 4.14). Sample *6aN_18c* had the same proportions of stage two and stage three fish as samples *6aN_18b* but samples *6aN_18e* and *6aN_18f* were comprised of 70% stage two fish and sample *6aN_18d* was

comprised of 99% stage 3 fish. These results indicate either a high rate of misclassification of maturity stages, a large degree of mixing of autumn and later spawning populations close to the 6.a.N spawning area during the autumn spawning period or the most likely explanation, which is a combination of both.

The effect of including any misclassified samples in a baseline, which is composed of predefined population groups i.e. assignment *approach 1* baseline, is to blur the distinction between the groups. This could lead to a higher rate of misassignment of unknown samples. The *approach 2* baseline, which is based on groups defined by clustering analyses, is less susceptible to the effects of misclassification as the misclassified individuals will be clustered with a group according to their genetic characteristics and not a potentially subjective maturity stage classification. For this reason and for the assignments of the MSHAS samples the groups in the *approach 2* assignment are considered to represent the population groups as follows; *Group_1+5* represents *6aN_Aut*, *Group_2+4* represents *6aS*, *Group_3* represents an indistinguishable mix of *6aS* and *6aN_Sp*. The proportions of *6aS* and *6aN_Sp* in the *Group_3* mix cannot be estimated based on the sampling in the current project as neither population was exhaustively sampled due to the lack of specific survey and commercial catch cover in quarter one in both areas.

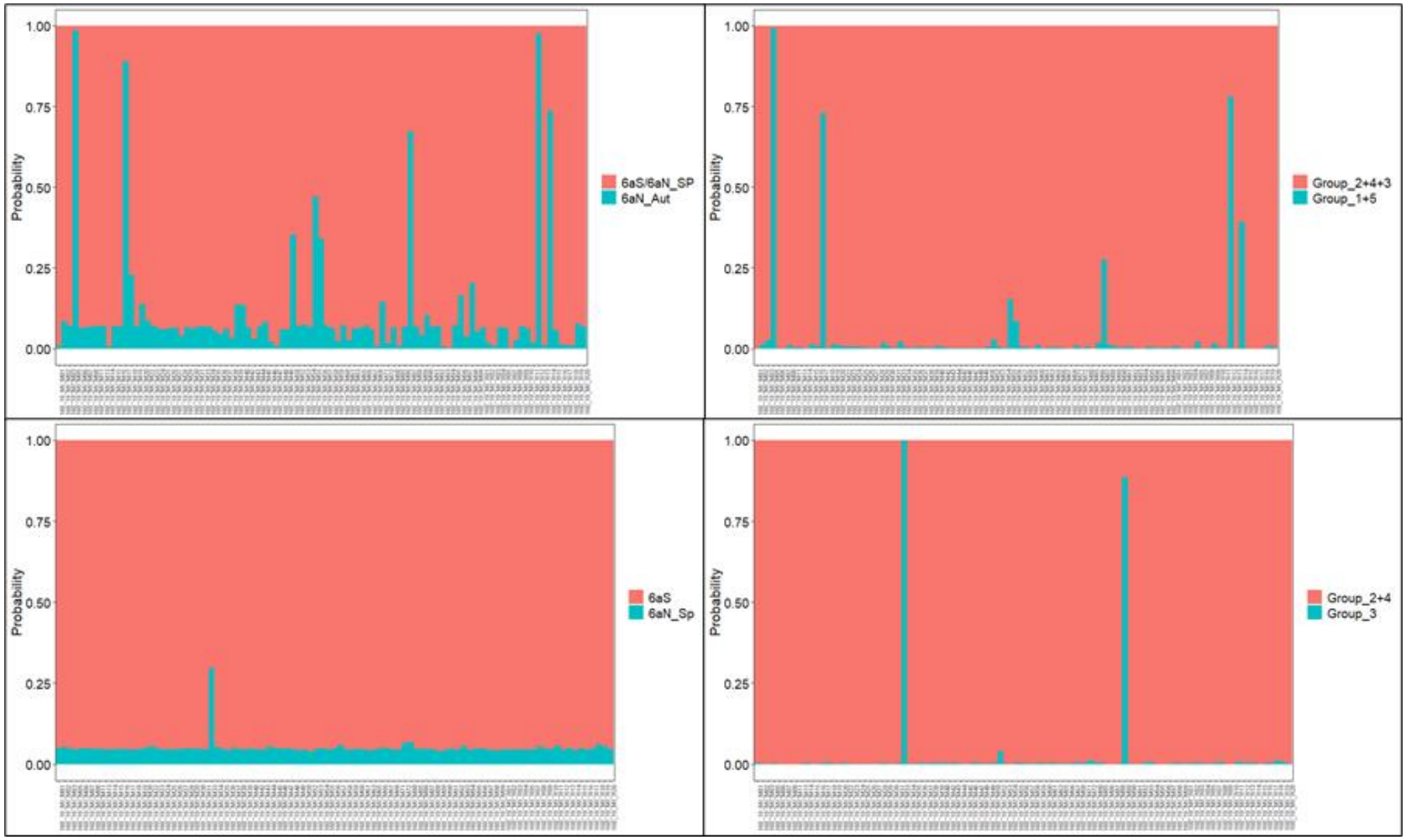


Figure 4.20. Assignment of the *6aS_19c* sample to the contemporary baseline with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

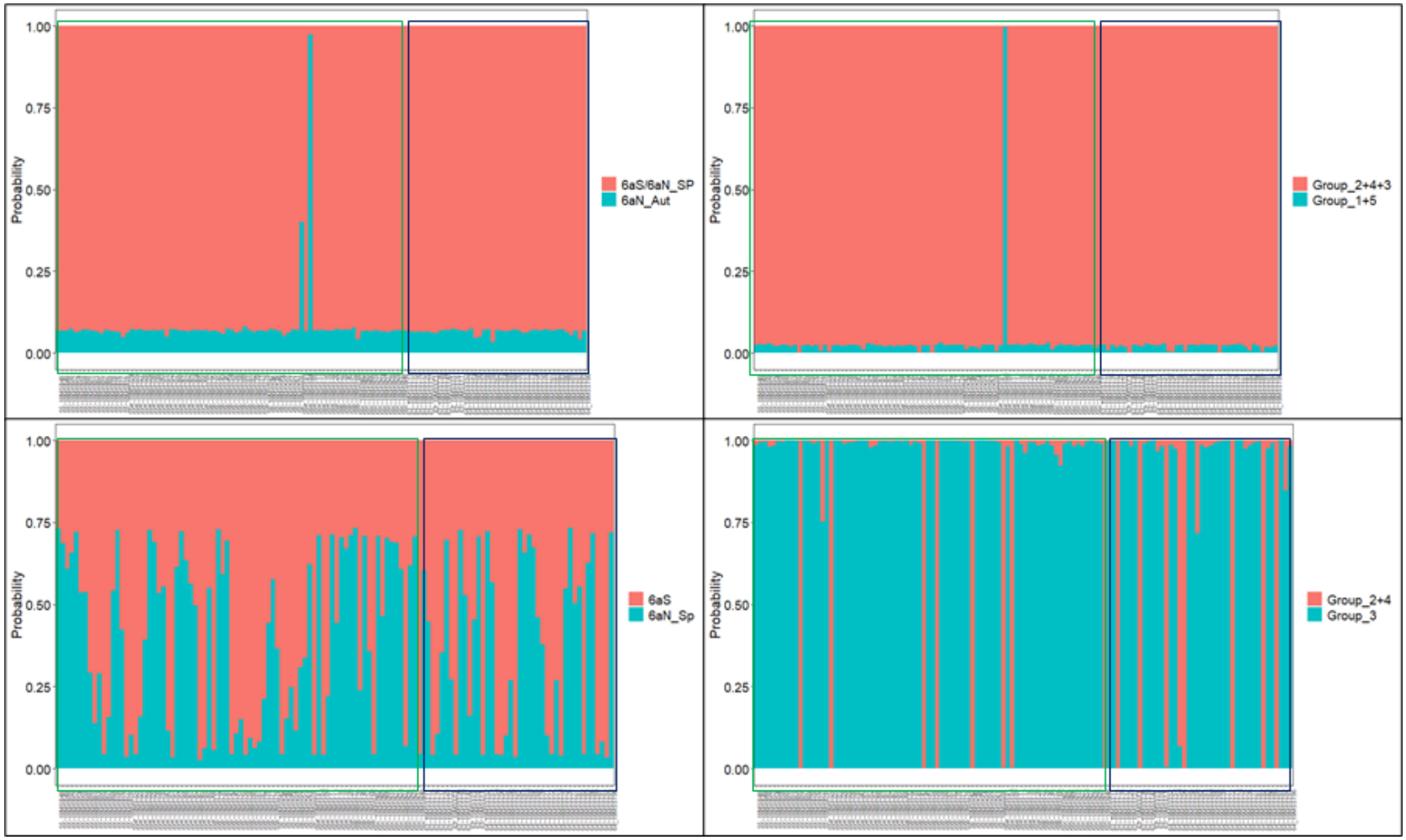


Figure 4.21. Assignment of the 2018 (green box) and 2019 (blue box) *6aN_Sp* samples to the contemporary baseline with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

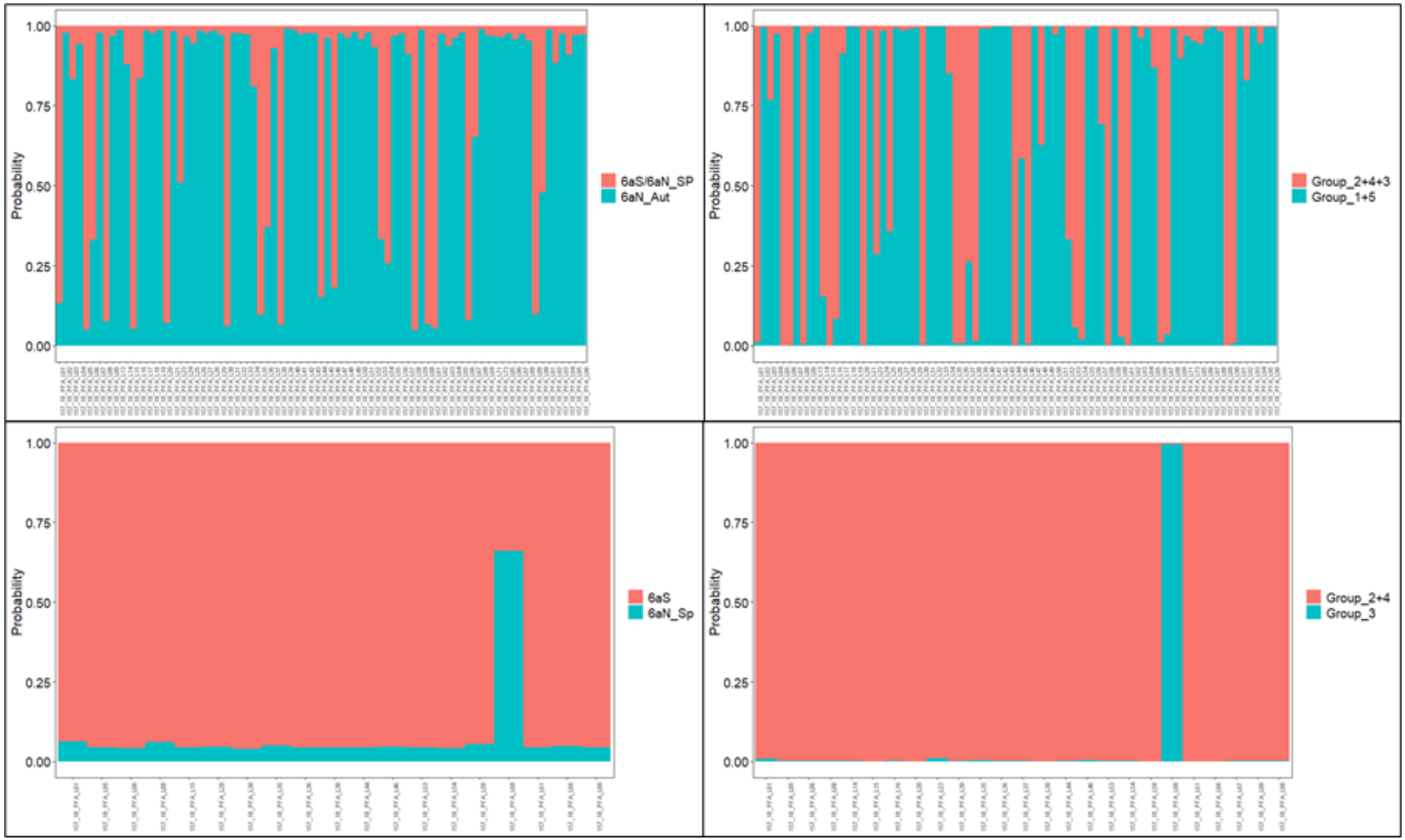


Figure 4.22. Assignment of the *6aN_19a* sample to the contemporary baseline with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

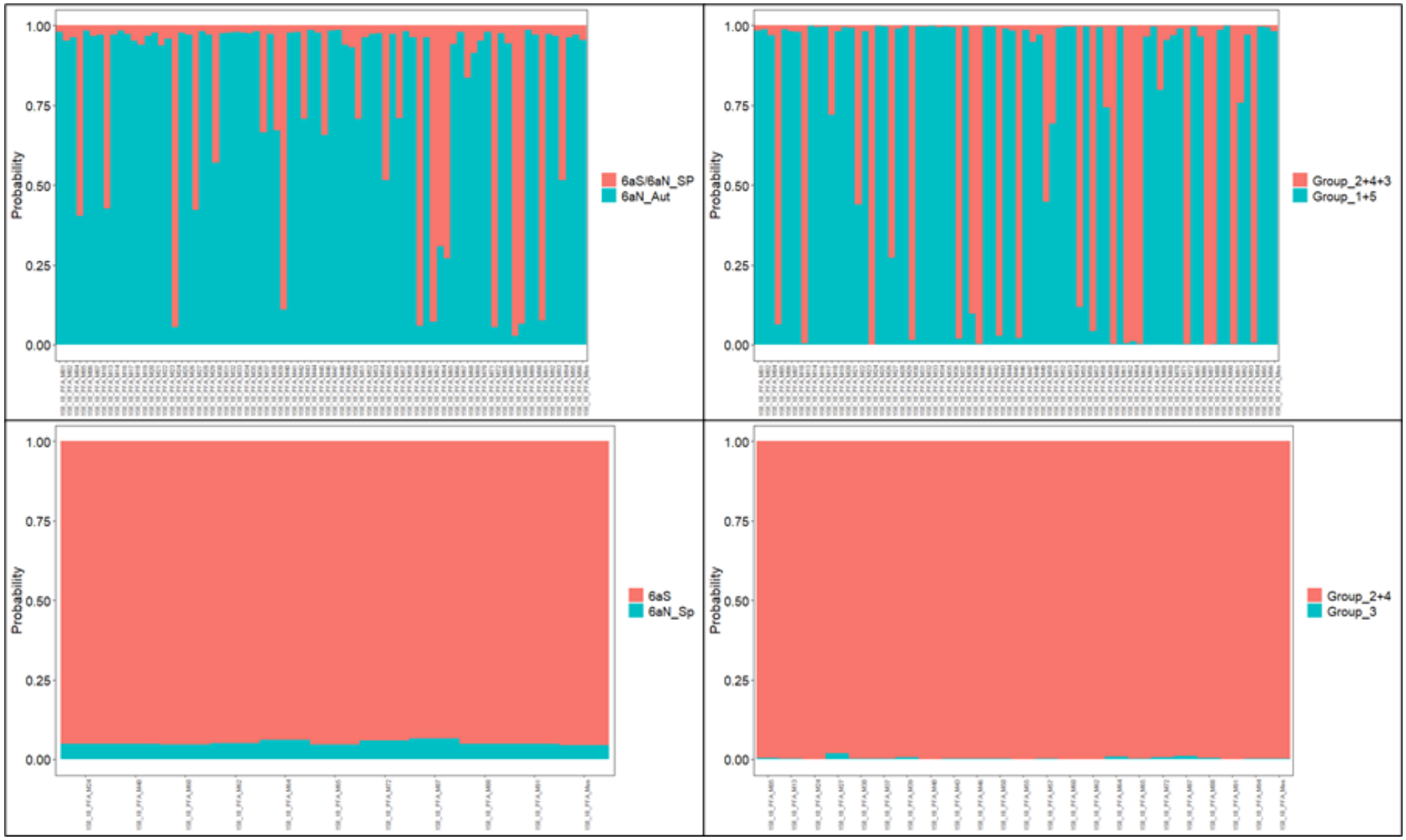


Figure 4.23. Assignment of the *6aN_19b* sample to the contemporary baseline with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

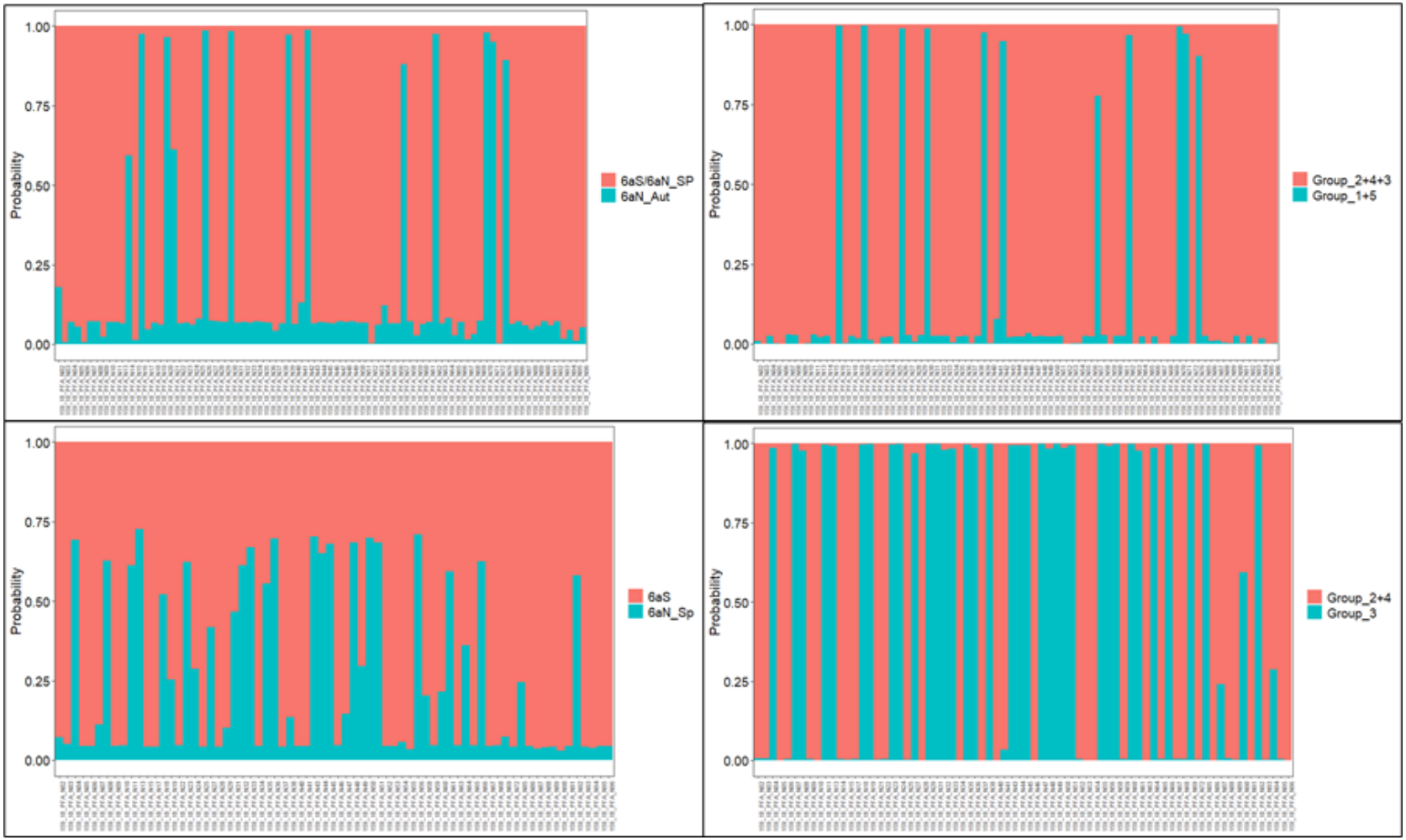


Figure 4.24. Assignment of the *6aN_19c* sample to the contemporary baseline with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

4.10. Additional samples of interest

There were a number of additional samples collected before and during the current study that were not included in the baseline datasets as they did not meet the criteria outlined in section 4.3. Some of these were used in the additional validation step outlined in section 4.9 and some others may be of further interest. Six samples were available from Division 6.a.S that were collected from November to January (Table 3.5) and comprised primarily of pre-spawning fish (Annex 3). These were considered likely to be 6.a.S, 7.b and 7.c herring as they were collected after the 6.a.N autumn spawning had concluded and were collected in areas that 6.a.S, 7.b and 7.c herring are known to aggregate prior to spawning including Lough Swilly, Inver Bay and Bruckless Bay. The *level 1* assignment of these samples indicated 89% and 94% of individuals assigned to *6aS/6aN_Sp* and *Group_2+3+4*, respectively (Figure 4.25). The *level 2* assignment of these individuals using *approach 1*, assigned 2% of the individuals to *6aN_Sp*, 5% were unassigned due to not meeting the assignment threshold and 94% were assigned to *6aS*. The *approach 2 level 2* assignment indicated 14% were from *Group_3* and 86% were from *Group_2+4*. These results were as expected and are a useful additional indicator of the assignment accuracy of the assignment model.

Three samples were collected in Lough Foyle, which as described in section 4.3 is officially part of the 6.a.N autumn spawning stock despite having no obvious biological or geographic connection to it. Two of the samples were collected in November 2017 (*6aS_17c*) and 2018 (*6aS_18d*) and the third sample was collected in January 2019 (*6aS_19a*) (Table 3.5). The *6aS_17c* sample was comprised of primarily stage three spawning fish, whereas *6aS_18d* and *6aS_19a* were mainly stage two fish. The *approach 1 level 1* assignment indicated 5% of individuals did not meet the assignment threshold, 6% were misassigned to *6aN_Aut,* and 88% of individuals assigned to *6aS/6aN_Sp* (Figure 4.26). The *approach 2 level 1* assignment indicated 1% of individuals did not meet the assignment threshold, 4% were misassigned to Group_1+5 and 95% of individuals assigned to *Group_2+3+4* (Figure 4.26). The *level 2* assignment of *6aS/6aN_Sp* individuals using *approach 1* assigned 7% of the individuals to *6aN_Sp*, 8% were unassigned due to not meeting the assignment threshold and 85% were assigned to *6aS*. The *approach 2 level 2* assignment indicated 26% were from *Group_3* and 73% were from *Group_2+4*. The results of the Lough Foyle assignments are in keeping with the biology of the fish in the area, in that they are not 6.a.N autumn spawning fish. Biologically and geographically they are part of the *6aS* population group.

Divisions 7.b and 7.c are also part of the 6.a.S, 7.b and 7.c stock area for assessment and management purposes (see section 2.5). However, no baseline spawning samples were collected in Divisions 7.b or 7.c during the study despite repeated attempts to locate spawning aggregations. The conclusion is that if spawning does still occur in this area then it is at a low level and it is not currently possible to sample it. Five samples were collected in Division 7.b during the current study. Two samples were collected in Galway Bay in 2018 in March (*6aS_18i*) and April (*6aS_18j*), two samples in Galway Bay in 2019 in September (*6aS_19d*) and October (*6aS_19e*) and one sample in Clew Bay in December 2019 (6aS_19h). The 2019 Galway Bay samples contained immature juvenile fish and were not genotyped (Annex 3). The 2018 Galway Bay samples comprised a mix of stage four and five fish, which would be expected at that time of year. It should be noted that as these are not spawning samples it cannot be concluded that their population of origin is in Division 7.b. The following assignments are conducted purely out of interest. The *approach 1 level 1* assignment indicated 2% of the individuals did not meet the assignment threshold, 7% were assigned to *6aN_Aut* and 93% were assigned to *6aS/6aN_Sp* (Figure 4.27). The *approach 2 level 1* assignment indicated 1% of the individuals did not meet the assignment threshold, 3% were assigned to *Group_1+5* and 96% were assigned to *Group_2+3+4* (Figure 4.27). The *level 2* assignment of *6aS/6aN_Sp* individuals using *approach 1* assigned 96% of the individuals to *6aS* and 4% were unassigned due to not meeting the assignment threshold. The *approach 2 level 2* assignment indicated 8% were from *Group_3* and 92% were from *Group_2+4*.

The 2019 Clew Bay sample (*6aS_19h*) comprised immature and juvenile stage one and two fish (Annex 3) but it was genotyped as it was the only sample collected in this area.

As with the Galway Bay samples, it should be noted that as these were not spawning individuals, it cannot be concluded that their population of origin is in Division 7.b. The following assignments are conducted purely out of interest. The *approach 1 level 1* assignment indicated 6% of the individuals did not meet the assignment threshold, 12% were assigned to *6aN_Aut* and 83% were assigned to *6aS/6aN_Sp* (Figure 4.28). The approach 2 level 1 assignment indicated 8% were assigned to *Group_1+5* and 92% were assigned to *Group_2+3+4*. In the level 2 assignments of *6aS/6aN_Sp* and *Group_2+3+4* individuals all individuals were assigned to *6aS* and *Group_2+4* (Figure 4.28).

Both the Galway Bay and the Clew Bay samples assigned with a high degree of accuracy to the *6aS* and *Group_2+4* baseline groups (Figures 4.27 and 4.28). Therefore, if fish from these areas are present in the potentially mixed aggregations encountered during the MSHAS it will be possible to distinguish them from the *6aN_Aut* fish despite a lack of baseline spawning samples from Divisions 7.b and 7.c.

The final sample of interest was collected in July 2016 during the North Sea Herring Acoustic Survey (HERAS) just east of the 4° line of longitude (Table 3.4). This sample, which was collected opportunistically, was not analysed as part of the MSHAS samples as it will not form part of the dataset that will be used to split the survey indices. The analysis here is purely exploratory. According to the current stock definitions the sample should be comprised of purely North Sea autumn spawning herring. As demonstrated in Section 4.7, the North Sea autumn spawning herring are indistinguishable from the 6.a.N autumn spawning herring with the current marker panels. Therefore, using the *45_SNP* dataset, it was assumed that the individuals in the sample would assign to the *6aN_Aut* population group, but this was not the case. In the *level 1* assignments, 52% and 54% of the individuals assigned to *6aS/6aN_Sp* and *Group_2+3+4*, respectively (Figure 4.29). The *approach 1 level 2* assignment of these individuals indicated 5% *6aN_Sp* and 86% *6aS*, whilst *approach 2 level 2* assignment indicated 17% *Group_3* and 81% *Group_2+4* (Figure 4.29). There is no suggestion that these assignments should be taken as completely accurate as there are a number of other population groups known to be in the North Sea that are not included in the baseline. However, what this exploratory assignment does suggest is that there was a mix of different populations in the sample that was analysed. This mix may potentially contain a proportion of *6aS* or *6aN_Sp* fish as there is no geographic boundary to limit the movement of herring from these populations across the 4° line of longitude. It is not possible to extrapolate the mixed nature of this single haul to the wider HERAS samples. It does suggest though that there may be a mixing issue in the North Sea area also and that future work should focus on developing an assignment model for this area also.

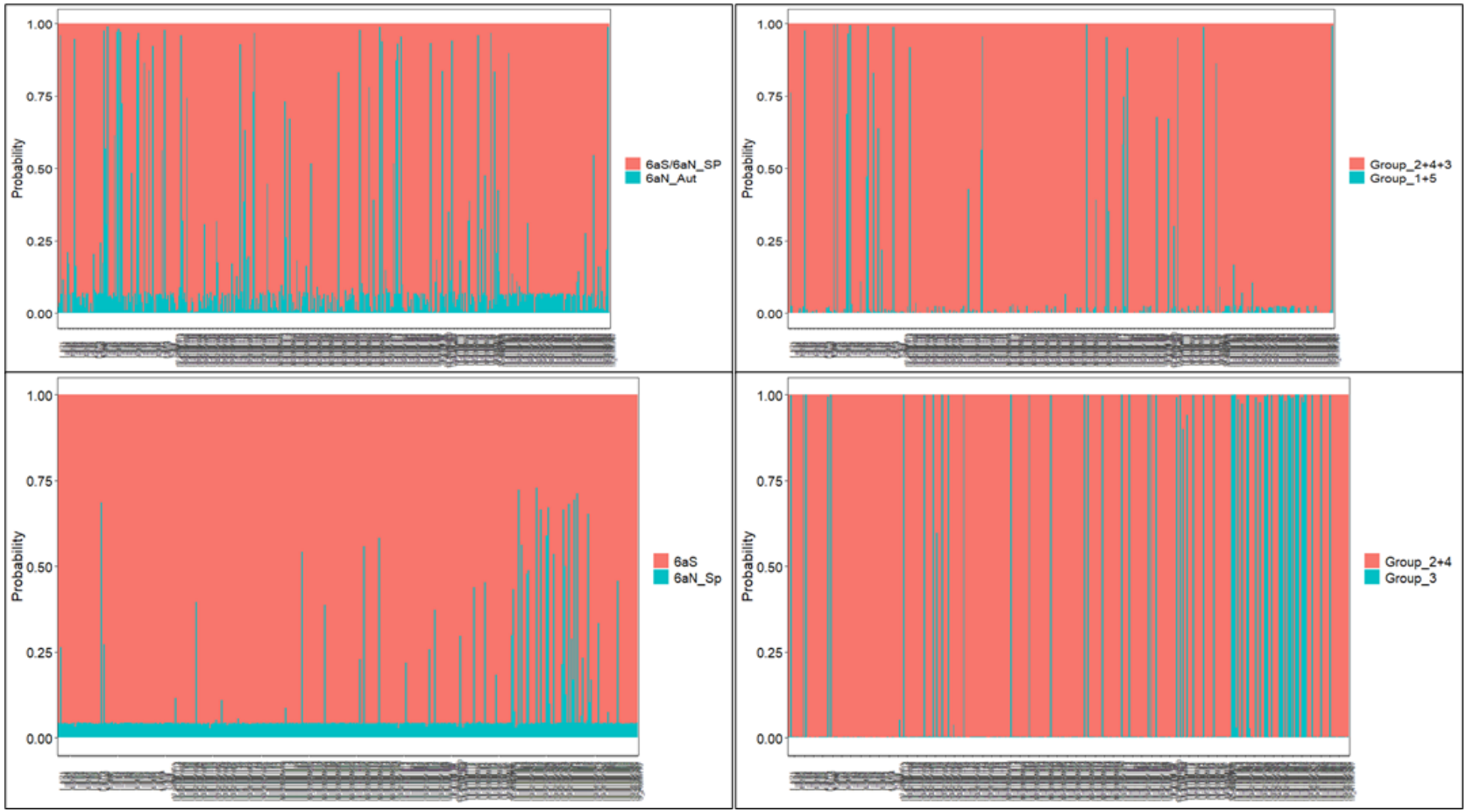


Figure 4.25. Assignment of the non-spawning 6.a.S samples collected at spawning time to the contemporary baseline with associated probabilities. (top left) *approach 1 level 1* (bottom left) *approach 1 level 2* (top right) *approach 2 level 1* (bottom right) *approach 2 level 2*.

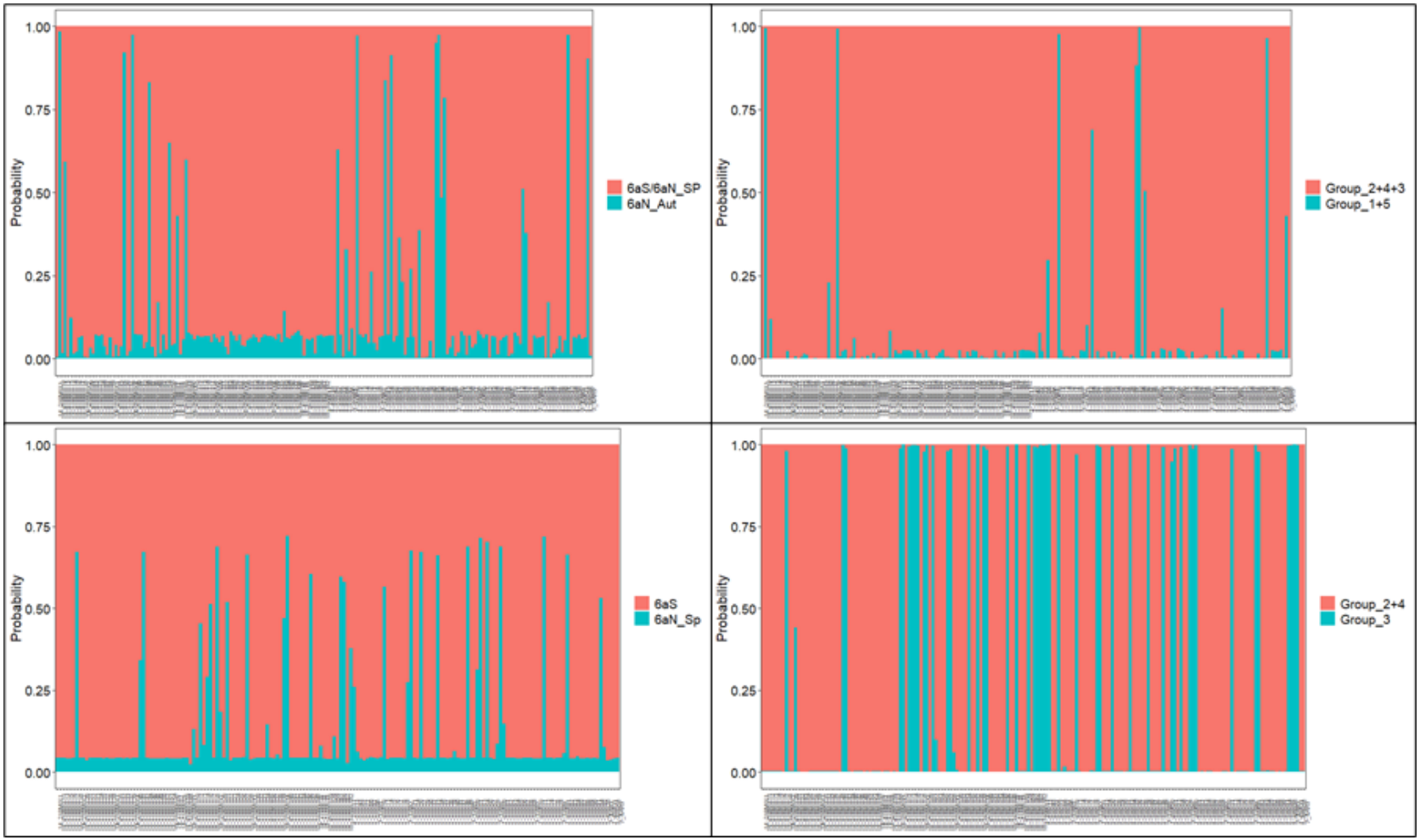


Figure 4.26. Assignment of the Lough Foyle samples to the contemporary baseline with associated probabilities. (top left) *approach 1 level 1* (bottom left) *approach 1 level 2* (top right) *approach 2 level 1* (bottom right) *approach 2 level 2*.

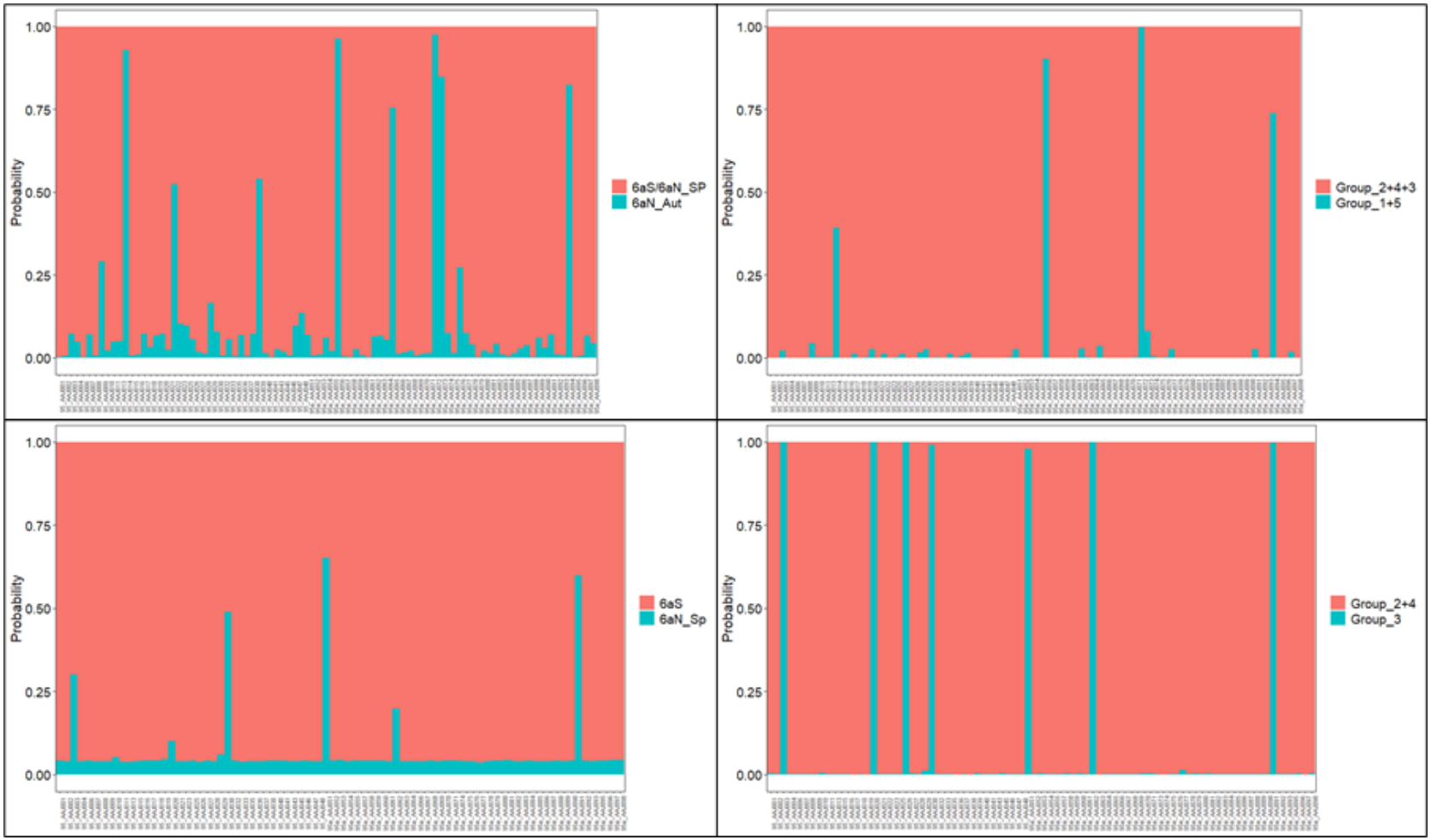


Figure 4.27. Assignment of the Galway samples to the contemporary baseline with associated probabilities. (top left) *approach 1 level 1* (bottom left) *approach 1 level 2* (top right) *approach 2 level 1* (bottom right) *approach 2 level 2*.

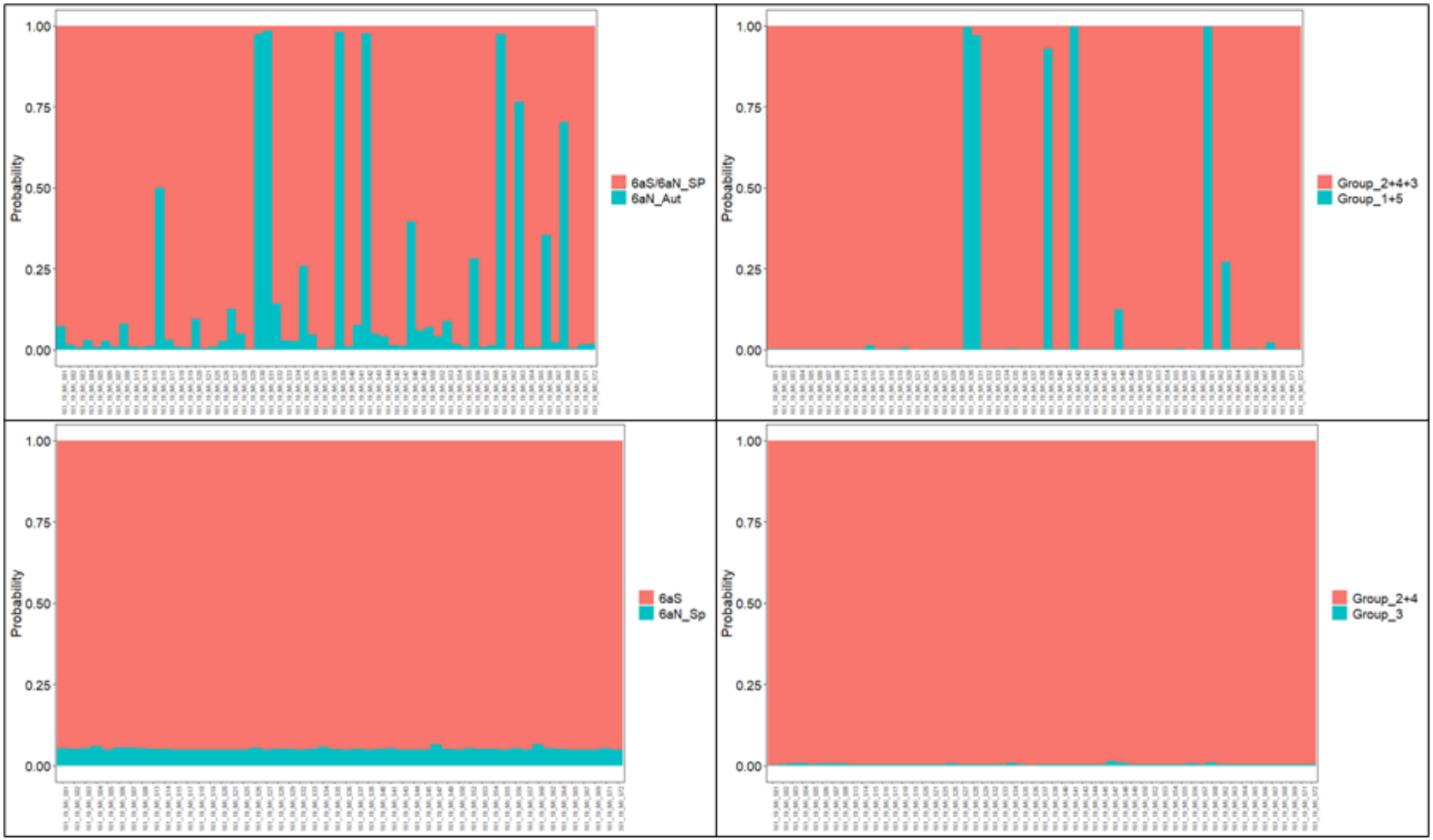


Figure 4.28. Assignment of the Clew Bay sample to the contemporary baseline with associated probabilities. (top left) *approach 1 level 1* (bottom left) *approach 1 level 2* (top right) *approach 2 level 1* (bottom right) *approach 2 level 2*.

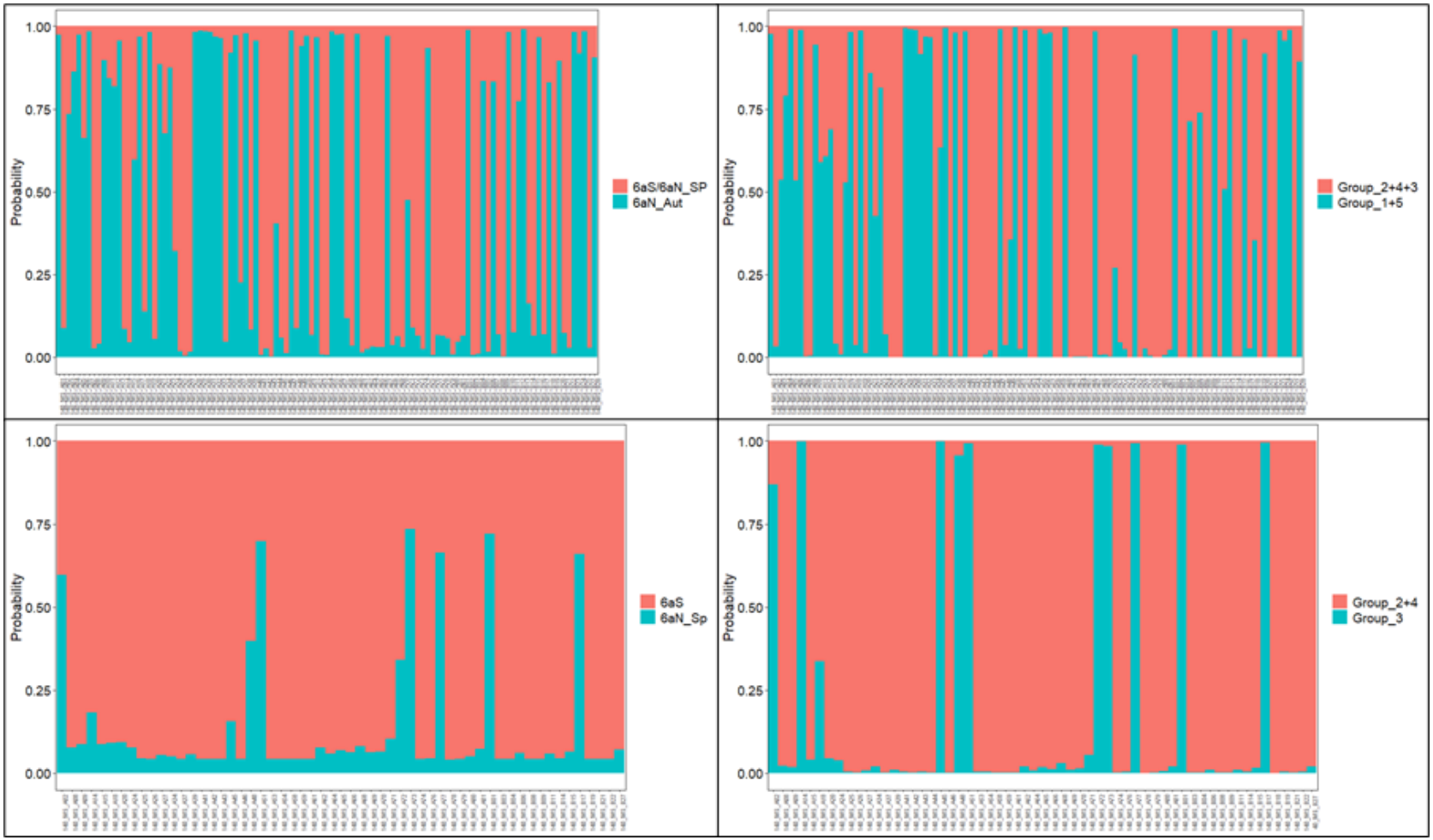


Figure 4.29. Assignment of the HERAS 2016 Haul 206 sample to the contemporary baseline with associated probabilities. (top left) *approach 1 level 1* (bottom left) *approach 1 level 2* (top right) *approach 2 level 1* (bottom right) *approach 2 level 2*.

4.11. MSHAS sample analyses

The reliability of the assignment model in distinguishing between the *6aN_Aut*, *6aS* and *6aN_Sp* fish has been demonstrated in sections 4.8-4.10. The 6.a.N autumn spawning and the 6.a.S, 7.b and 7.c stocks are the only stocks in Division 6.a that are assessed currently. The 6.a.N spring spawning herring are not assessed or managed and are believed to be at a significantly lower abundance than the other two stocks, having undergone a documented stock collapse in the late 1950s (see Section 2.6). There was higher power at the *level 1* assignment in both approaches and as this concerns the discrimination of 6.a.N autumn spawning herring from the two other groups, this will enable splitting of the MSHAS data in what is perceived to be the most significant split. The demonstrated potential misclassification of maturity stages in some of the *6aN_Aut* baseline samples adds a degree of uncertainty to the *approach 1 level 1 assignment*, though this can be mitigated for by using *approach 2 level 1*.

The *level 2* assignment is more uncertain, particularly in the case of the *6aN_Sp* population group where a variable proportion of these fish were be misassigned to *6aS*. Again, this can be mitigated for using *approach 2,* where the majority of *6aN_Sp* fish formed the mixed cluster *Group_3* with what is believed to be a small proportion of the *6aS* population group. In this way it is possible to separate the majority of *6aS* from *6aN_Sp*. It was decided to conduct the assignment of the MSHAS samples using both approaches so that a direct comparison would be possible. The MSHAS samples were processed and genotyped as per the methods for the baseline samples (Sections 4.5 and 4.6). In order to maximise the number of individuals genotyped, the quality control threshold was adjusted so that all individuals with greater than 75% of loci genotyped were retained. This threshold was set based on the analyses in section 4.8 (Figure 4.15 and 4.16). The assignments were conducted using the *assign.X* function in *assignPOP* (Chen *et al*., 2018) using the two hierarchical approaches and with the same model parameters as described in Section 4.8. For each approach, the *level 1* assignment was conducted with all the individuals in the sample and the *level 2* assignment on a subset of individuals assigned to either *6aS/6aN_Sp* or *Group_2+3+4*, depending on the approach. The final assignment call was based on a combination of *level 1* and *level 2* according to the assignment decision table (Table 4.15).

Table 4.15. Assignment decision table for the MSHAS samples.

Approach	level	Assigned	*P*	Action	Final assignment
1	1	*6aN_Aut*	≥ 0.67	Assigned	*6aN_Aut*
1	1	*6aS/6aN_Sp*	≥ 0.67	Move to level 2	-
1	1	*6aN_Aut*	<0.67	Not assigned	*NA*
1	1	*6aS/6aN_Sp*	<0.67	Not assigned	*NA*
1	2	*6aS*	≥ 0.67	Assigned	*6aS*
1	2	*6aN_Sp*	≥ 0.67	Assigned	*6aN_Sp*
1	2	*6aS*	<0.67	Not assigned	*NA*
1	2	*6aN_Sp*	<0.67	Not assigned	*NA*
2	1	*Group_1+5*	≥ 0.67	Assigned	*Group_1+5*
2	1	*Group_2+3+4*	≥ 0.67	Move to level 2	-
2	1	*Group_1+5*	<0.67	Not assigned	*NA*
2	1	*Group_2+3+4*	<0.67	Not assigned	*NA*
2	2	*Group_2+4*	≥ 0.67	Assigned	*Group_2+4*
2	2	*Group_3*	≥ 0.67	Assigned	*Group_3*
2	2	*Group_2+4*	<0.67	Not assigned	*NA*
2	2	*Group_3*	<0.67	Not assigned	*NA*

For ease of assessing the results from the large number of MSHAS samples, the samples from each year were summarised into a single figure with a pie chart representing the assignments from each haul sampled (Figures 4.30 – 4.35). Both the *approach 1* and *approach 2* assignments are presented in each figure. More detailed figures of the assignment levels, with the associated probabilities, are provided in Annex 5.

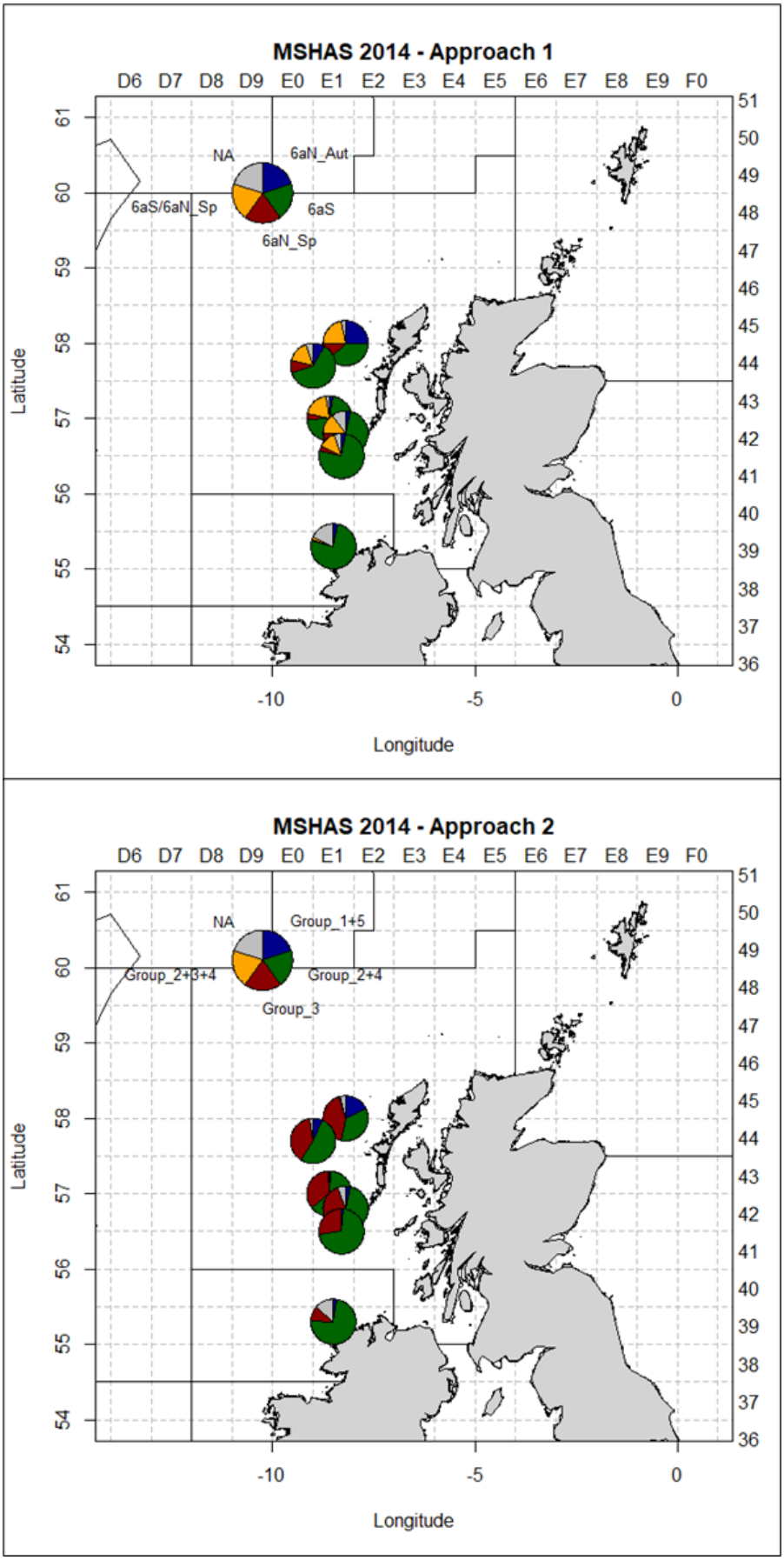


Figure 4.30. Summary of the *approach 1* (top) and *approach 2* (bottom) assignments of the 2014 MSHAS samples.

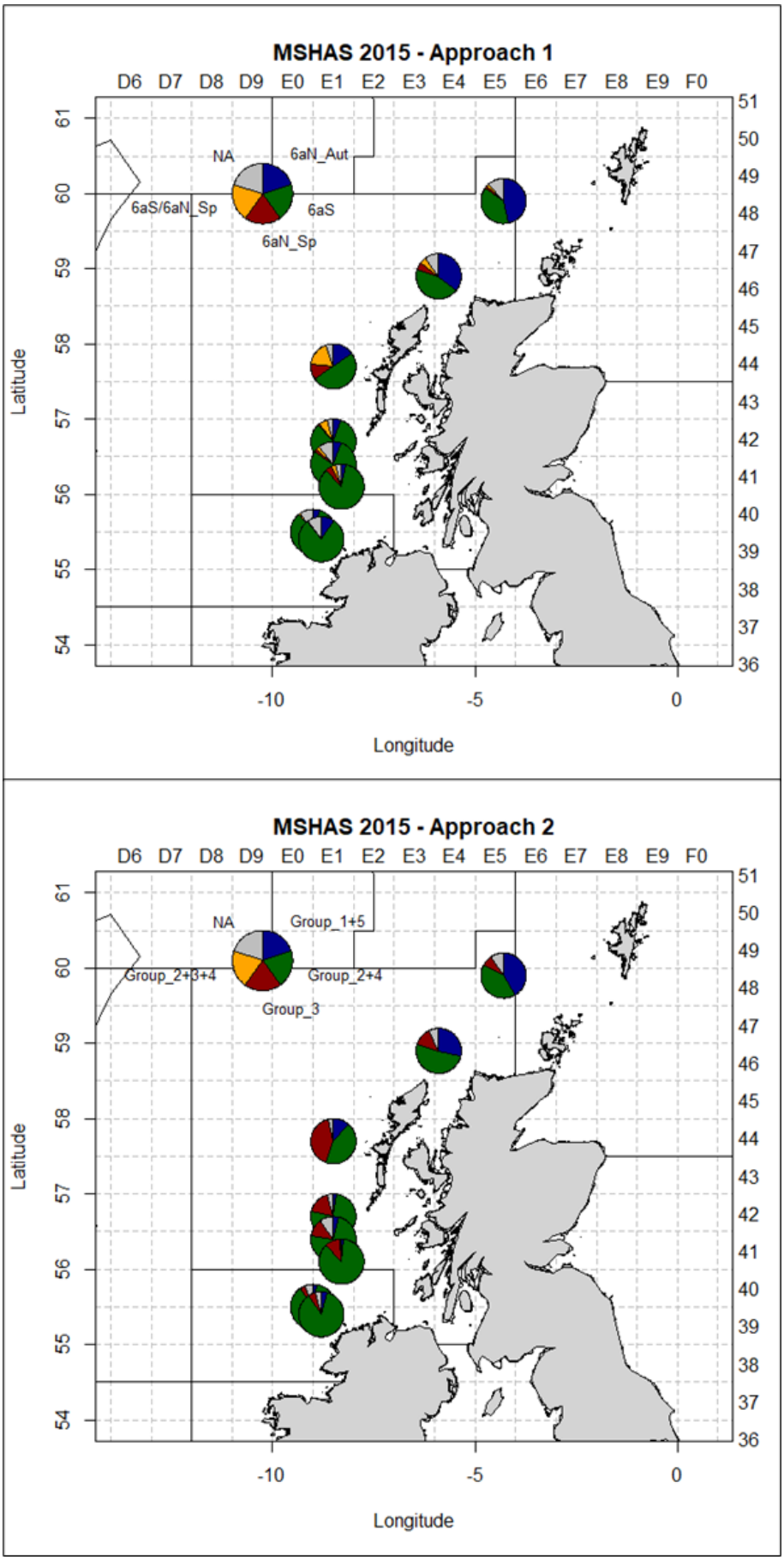


Figure 4.31. Summary of the *approach 1* (top) and *approach 2* (bottom) assignments of the 2015 MSHAS samples.

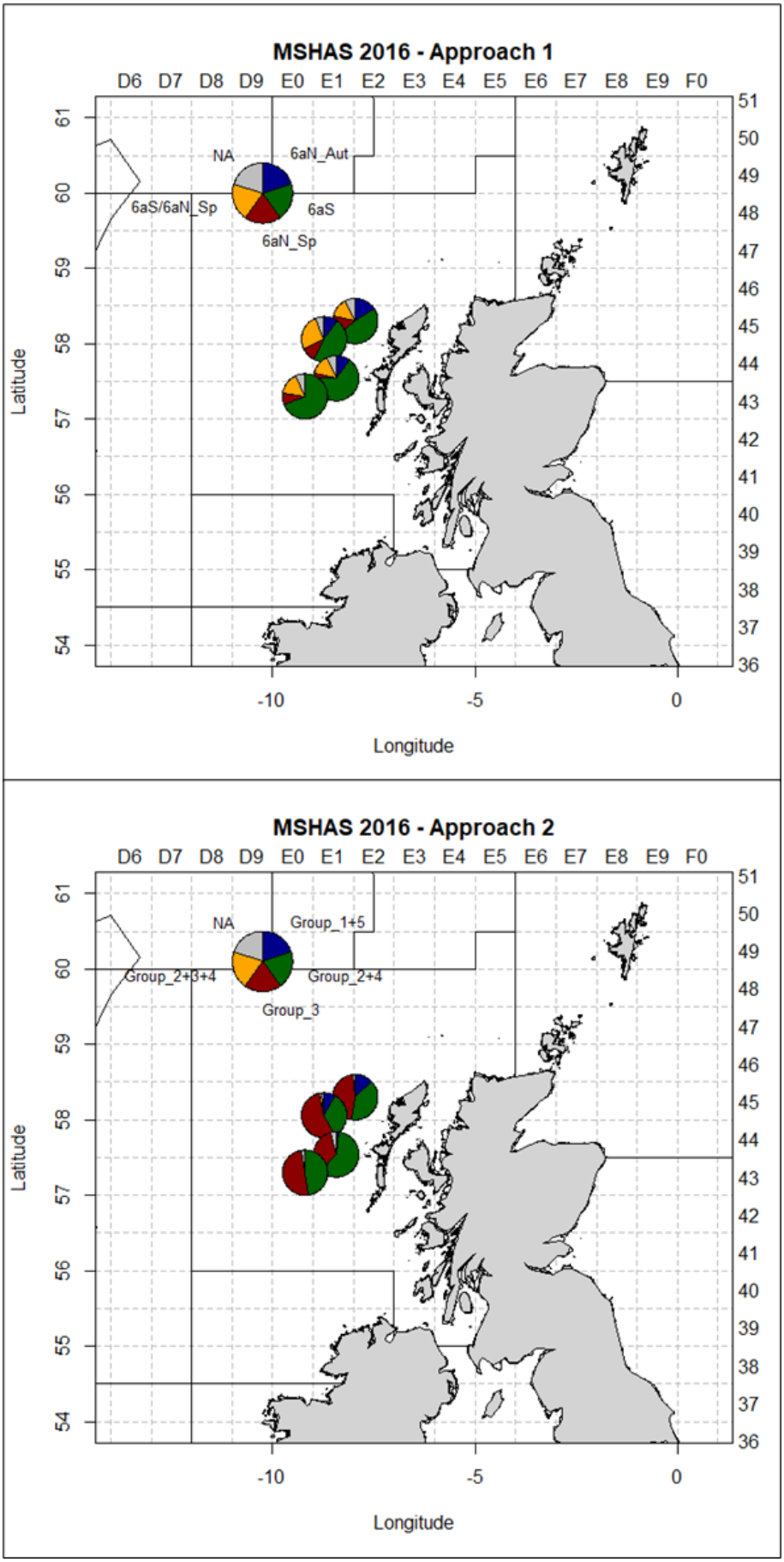


Figure 4.32. Summary of the *approach 1* (top) and *approach 2* (bottom) assignments of the 2016 MSHAS samples.

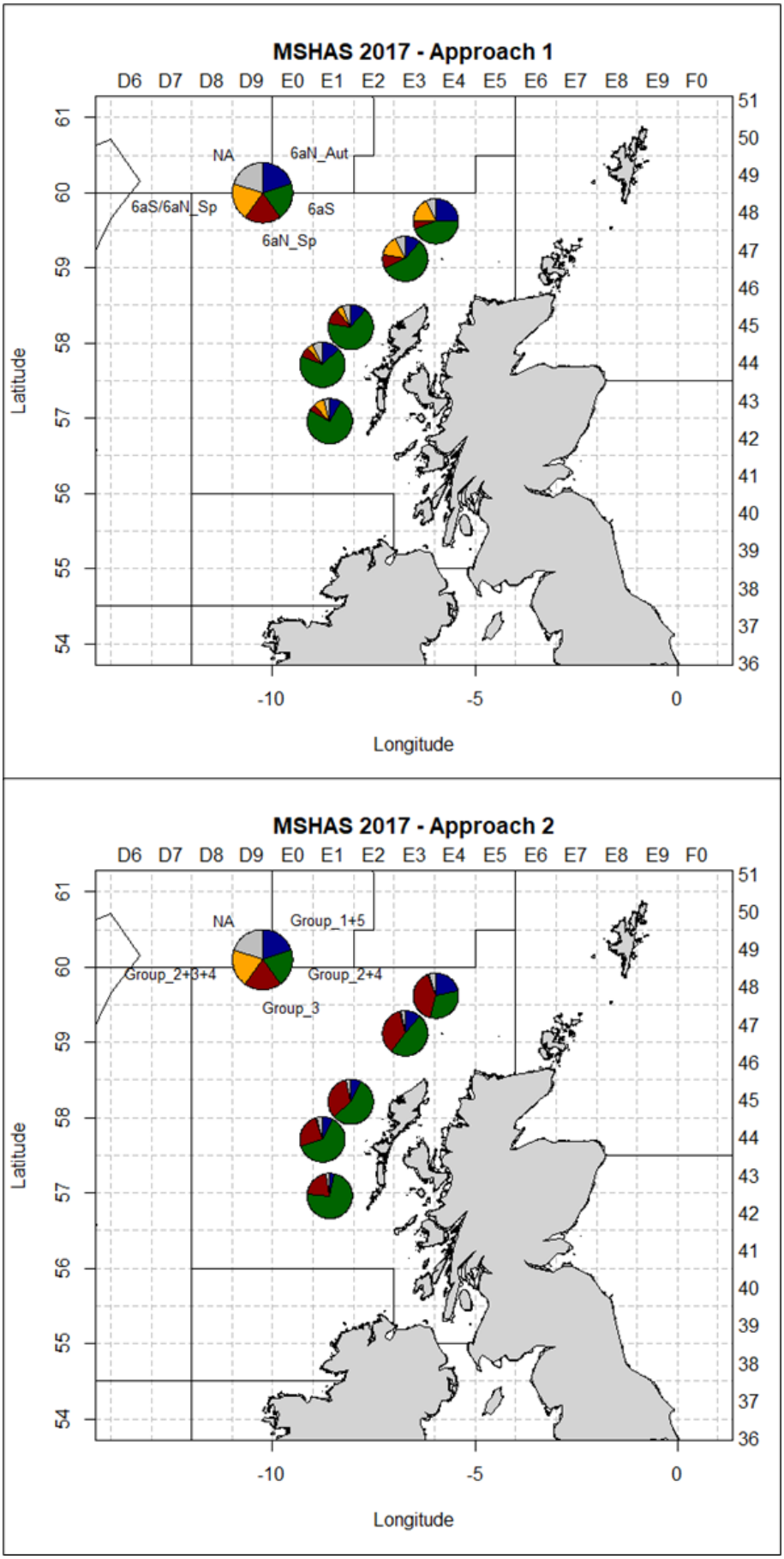


Figure 4.33. Summary of the *approach 1* (top) and *approach 2* (bottom) assignments of the 2017 MSHAS samples.

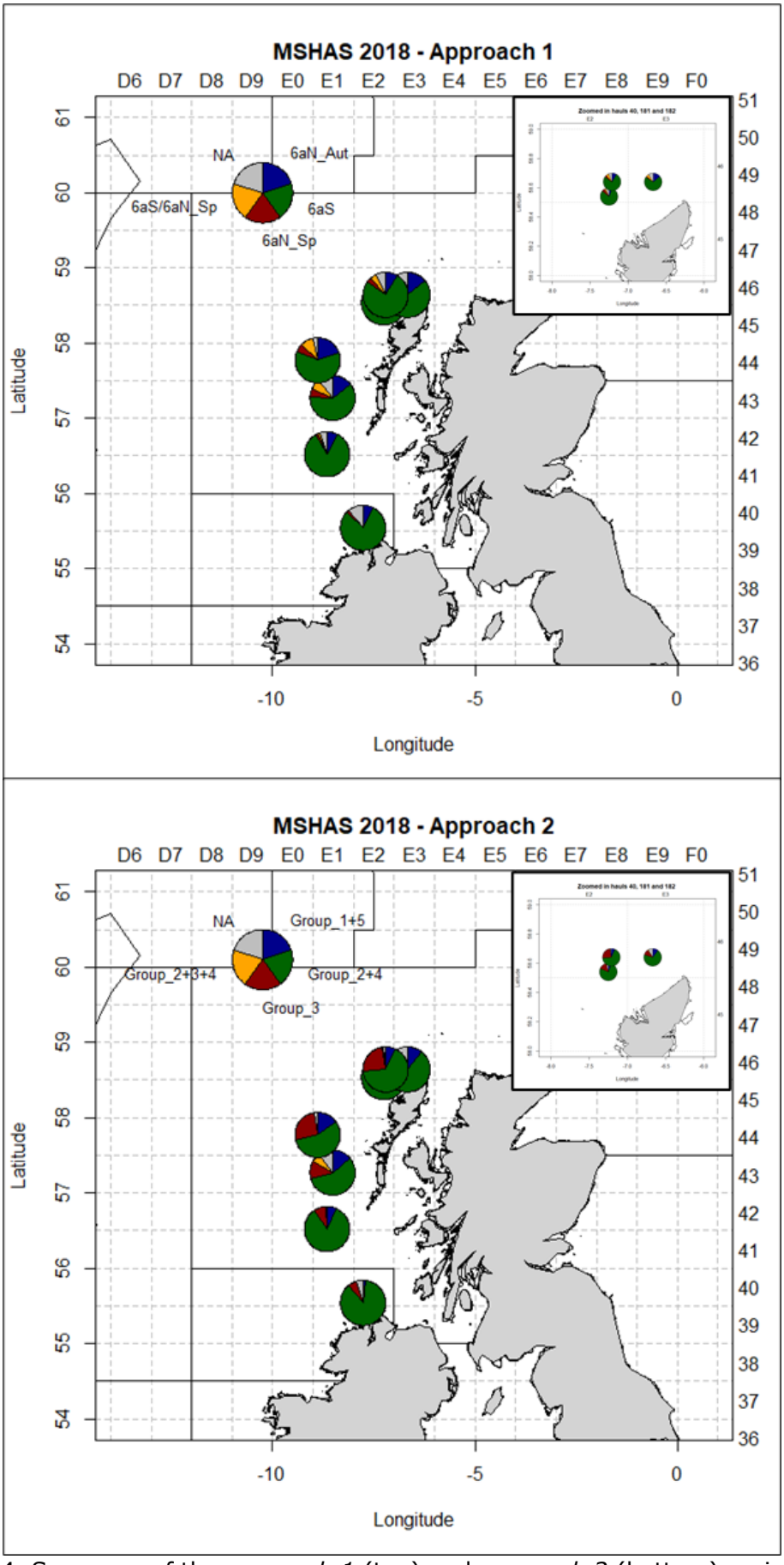


Figure 4.34. Summary of the *approach 1* (top) and *approach 2* (bottom) assignments of the 2018 MSHAS samples. The inset in both panels is zoomed in to the three northern hauls.

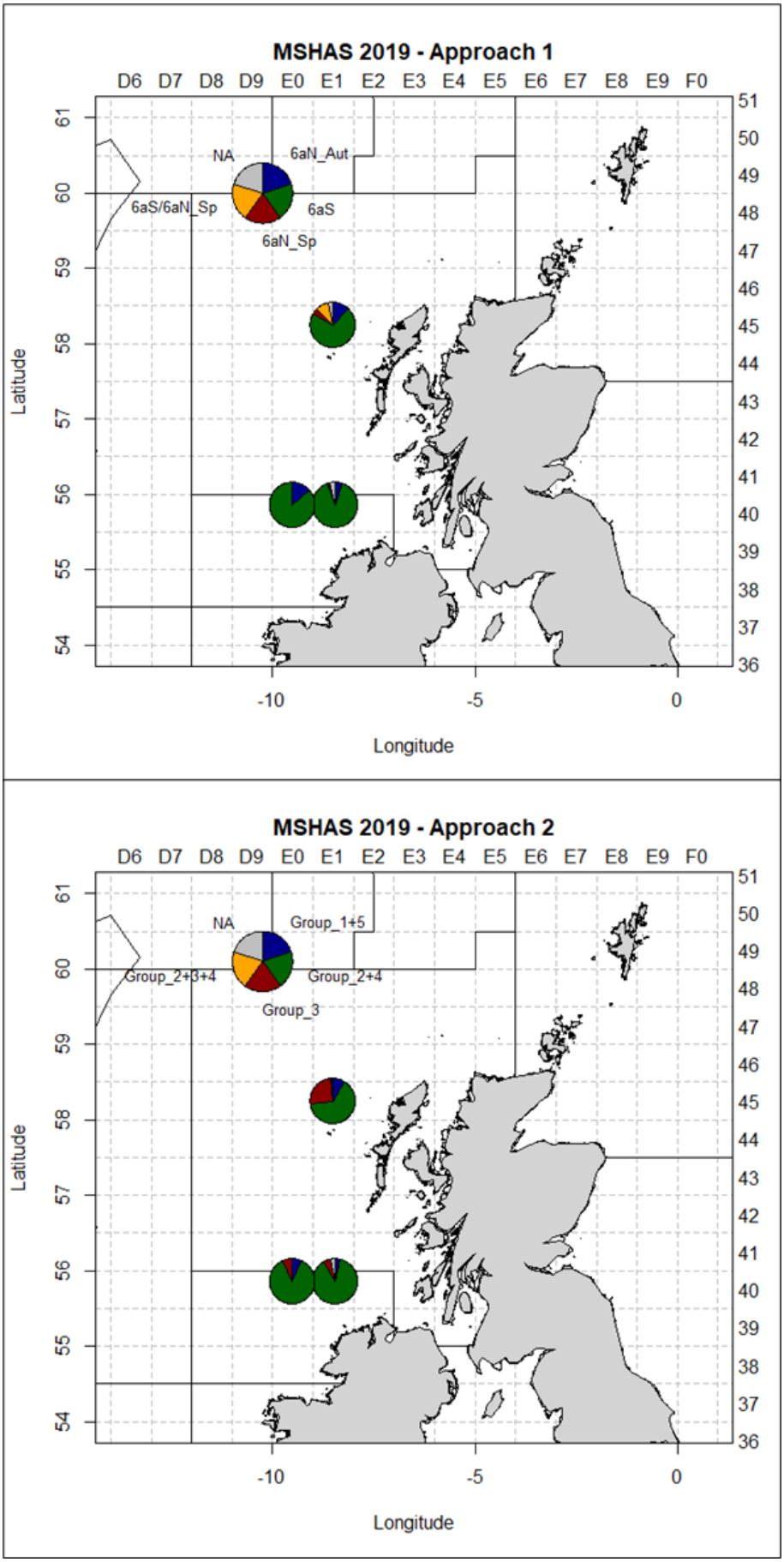


Figure 4.35. Summary of the *approach 1* (top) and *approach 2* (bottom) assignments of the 2019 MSHAS samples.

The distribution of MSHAS samples in the different years was quite variable and reflects the distribution of herring observed during the surveys (Annex 6: Figures 10.6.1 - 10.6.10). In some years (e.g. 2016) the majority of herring were distributed north of the 56° line of latitude and in other years there was a significant abundance of herring observed south of this line. Similarly, in the HERAS there were notable annual changes in the distribution of the herring. In 2013 the majority of herring were observed northwest of Orkney in close proximity to the 4°N line of longitude. In 2011 and 2014 the majority of herring were observed to be in the central part of Division 4.a and in 2018 and 2019 the majority of herring were observed to the east of Shetland. These temporal changes in the distribution of the herring shoals can have a significant impact on the survey abundance estimates when splitting of the estimates is based on geographically delineated areas and not on the biological composition of the observed shoals.

In Division 6.a the majority of herring sampled were in maturity stages one, two and five on the 6-point scale (stages two, three, four and eight on the 8-point scale, Annex 7 and Table 3.2). It was not possible to discriminate the origin of the herring based on maturity stage and as previously discussed macroscopic maturity staging is known to be imprecise (Sections 4.3 and 4.9).

Across the six years of MSHAS samples that were genetically assigned (2014-2019) there was a consistent pattern of a higher proportion of *6aS* or *Group 2+4* herring in the samples than *6aN_Aut* or *Group_1+5* herring, observed in the samples. The *6aS* or *Group 2+4* assigned fish were distributed across the survey area both south and north of the current stock delineation line of 56°N latitude, confirming that this geographic delineator for the collation of survey data is not appropriate. The highest proportions of *6aS* or *Group_2+4* fish were observed in the hauls closest to the Irish coast as seen in hauls 16 and 17 in 2015, where *6aS* fish comprised up to 93% of the hauls (Figure 4.31). The highest proportions of *6aN_Aut* fish were observed in the most northerly hauls adjacent to the 4°W stock delineator (Figure 4.31), where they represented up to 46% of fish in single haul in 2015. However, this haul was the exception and generally the proportion of *6aN_Aut* in the hauls was less than 20% (Figures 4.30 – 4.35). These patterns of distribution make geographic and biological sense, which supports the results of the assignment.

The *level 1* assignment probabilities for the two assignment approaches were generally greater than 90% for the majority of the samples (Annex 5: Figures 10.5.1 – 10.5.33), indicating a high level of assignment accuracy. This was not true of the *level 2* assignments following *approach 1*, where the same pattern of uncertainty between *6aS* and *6aN_Sp,* observed in the baseline assignment testing, was observed again. The *approach 2 level 2* assignments had probabilities of assignment to *Group_2+4* or *Group_3* of over 90% for the majority of samples. It must be stressed again though that *Group_3* represents an indistinguishable mix of *6aS* and *6aN_Sp* fish, the proportions of which are unknown. The approach two assignment also mitigates for the potential misidentification of baseline samples as discussed in section 4.9. Therefore, is it proposed as the preferred approach for the genetic assignments.

One notable finding were significant proportions of *6aN_Sp* and *Group_3* fish west of the Hebrides in almost all years. This consistent pattern was not expected though it is supported by the historical literature (Baxter, 1958), where it was noted that the spring spawning herring in the north Minch left the area in summer to feed west of the Hebrides before returning in the winter (see Section 2.6).

4.12. Conclusions

The following conclusions can be drawn from the genetic analyses in Section 4.

- The herring in ICES Divisions 6.a, 7.b and 7.c comprise at least three distinct populations; 6.a.S herring, 6.a.N autumn spawning herring and 6.a.N spring spawning herring.

- The 6.a.S herring are primarily a winter spawning population though there is a later spawning component present in the area also. These components are currently inseparable.

- No baseline spawning samples could be collected in Divisions 7.b or 7.c therefore the relationship between the herring that spawn in this area and those that spawn in 6.a.S is unknown. Non-spawning herring caught in Division 7.b assigned genetically to the 6.a.S population.

- The samples of herring from Lough Foyle, analysed in the current study, were shown to be genetically and biologically 6.a.S herring.

- There was no genetic differentiation between the 6.a.N autumn spawning herring samples and North Sea autumn spawning herring samples.

- The Downs samples were confirmed to be distinct from the North Sea autumn spawning samples though they cannot be reliably discriminated from the Celtic Sea and Irish Sea populations with the current panel of markers.

- The Irish Sea and Celtic Sea samples were genetically differentiated from each other and from the 6.a.S herring and 6.a.N autumn spawning herring. However, the current marker panel cannot be used to distinguish these groups with a high level of accuracy for the purposes of stock identification.

- The genetic stock identification method represents a standardised and replicable method to discriminate survey and commercial catches of the three population groups known to occur in ICES Division 6.a.

- Genetic assignment of the 2014-2019 MSHAS samples indicated that 6.a.S herring are distributed north and south of the 56°N line of latitude during the survey period. They were present as a significant proportion of the MSHAS hauls.

- Potential 6.a.N spring spawning herring comprised a significant proportion of the MSHAS hauls west of the Hebrides in the 2014-2019 MSHAS samples.

- 6.a.N autumn spawning herring comprised a small proportion of the MSHAS samples 2014-2019 except in the extreme north of the area in close proximity to the 4°W boundary with the North Sea.

- The current geographic delineation of the distribution of the stocks is not appropriate and should be revised according to the genetic assignment results.

5. Task 5 – Morphometric information data collection and analysis

5.1. Objectives

- Collect whole body and otolith morphometric data from each genetically sampled herring.
- Both spawning and acoustic surveys should be sampled.
- Analyse the data with suitable discriminant methods and present results clearly.
- Determine if morphometric sample collection should continue on the joint MSHAS.

5.2. Deliverables

- Comprehensive morphometric data from 2016 and 2017 spawning and survey samples: ***Completed***
- Updated morphometric baseline for spawning stocks: ***Completed***
- Split of MSHAS samples into source stocks back to 2010: ***Not possible***
- Clear description of the methods and description of the results: ***Completed***
- Comparison of current baseline with WESTHER samples: ***Not possible***

5.3. Background

The phenotype of an organism is the observable characteristics or traits displayed, which is influenced largely by genetics and the environment so populations from the same species can exhibit slightly different phenotypic characteristics depending on the environment they inhabit and their genetic make-up. The greater the genetic variation and the more distinct environments inhabited, the greater the difference between the phenotypic characters (Berg et al., 2018; Turan, 2000; Begg and Waldman, 1999). Atlantic herring found in ICES Divisions 6.a, 7.b and 7.c, consist of at least two reproductively isolated populations but these populations are believed to mix spatially on summer feeding grounds off the west coast of Scotland and north coast of Ireland. One method of distinguishing these populations is to use morphometrics. Morphometrics is a quantitative method of analysing the variability in size and shape of organisms and their organs (Elewa, 2010). The WESTHER project (Section 2.7) demonstrated that the combined power of body morphometrics and otolith shape analysis provided the best distinction between the two populations of herring in this area (Hatfield *et al.*, 2005). Since the WESTHER project was completed, new statistical approaches for morphometric data analyses have been developed and updated baseline data have been collected. Otolith shape analysis has also become more widespread in stock discrimination studies of Atlantic herring than body morphometrics (e.g. Libungan *et al.*, 2015; Eggers *et al.*, 2014). In the current study, otolith and body morphometric analyses were analysed separately and the results examined to test their suitability to this dataset, before being combined in a joint analysis.

5.4. Data collection and processing

Baseline samples were defined as samples collected in a known spawning area, during the spawning period for that area and the herring were in spawning condition – maturity stage 5 and 6 on the 8-point herring maturity scale or stage 3 on the 6-point scale (Table 3.2). Baseline samples for morphometric analysis were collected from 6.a.N autumn spawning herring and 6.a.S herring during the period 2014-2019 (Tables 3.5 and 5.1) and were processed according to Section 3.3 and Section 7. The list of baseline morphometric samples (Table 5.1) is significantly fewer than that of the genetics (Table 3.5). Genetic samples can be collected from frozen fish which allows more flexibility in the collection process, whereas the collection of baseline samples for morphometric analysis was more challenging, in particular, for body morphometrics.

Table 5.1. A summary of the baseline dataset collected from 6.a.N autumn and 6.a.S during the period 2014-2019.

ICES Division	Year	Month	Number	Length (cm)	Estimated Age
6.a.N	2014	August	120	29.6 (26.5-33.5)	5.33 (3 – 12)
6.a.N	2014	September	85	29.1 (26-32)	4.78 (2 – 11)
6.a.N	2016	August	357**	28 (23.5-32.5)	4.08 (2 – 14)
6.a.N	2016	September	216**	27.9 (22.5-33)	4.14 (2 – 9)
6.a.N	2017	September	120	28 (24.5-31.5)	3.86 (2 – 9)
6.a.S	2014	December	216**	27.4 (25.5-30.0)	4.03 (2 - 7)
6.a.S	2016	January	242**	27.2 (23.5-30.5)	5.24 (3 – 9)
6.a.S	2017	January	239**	27.5 (18.5-30)	5.62 (3 – 9)
6.a.S	2018	December	100*	26.4 (22-28.5)	4.47 (2 – 8)
6.a.S	2019	November	120	25.7 (21.5-29)	3.28 (1 – 8)

* No body morphometric data were collected from these fish

** More than one sample included in this total

Marine Scotland Science (MSS) collected 6.a.N autumn spawning herring during acoustic spawning surveys that took place on industry vessels in August and September. Scientists were onboard during these surveys and could sample the spawning fish immediately. No samples of 6.a.N spring spawning herring were available for morphometric analyses in the current study. Therefore, the term '6.a.N' in Section 5 refers to the 6.a.N autumn spawning herring only. The Marine Institute (MI) sampled 6.a.S spawning herring from landings from a scientific monitoring fishery (Table 3.1). Images for body morphometric analysis were taken while the fish were fresh to preserve the true shape of the body, so the MI relied upon good communication with the fishing industry so that spawning herring were reported when they were caught and sampled as soon as possible thereafter. Once spawning fish had been collected, the process required a minimum of two people, but an optimum of four, to prepare the fish for image capture. Staff resources were limited to 2 people onboard industry vessels during the acoustic surveys in 6.a.N. Rigor mortis can distort the shape of the body of the fish, so regardless whether samples were collected onboard a vessel or from landings, image capture of the body was completed within 24 hours of the herring being caught.

For the following analyses, there were 15 baseline samples used; eight samples from 6.a.N and seven samples from 6.a.S. Each sample consisted of a random selection of 120 individual fish (>20cm TL) that were pinned out on cardboard strips on the left side of the body and photographed using a standardised camera and camera stand setup. The target of 120 fish (ICES, 2010) was not always possible but samples containing less than 120 fish were still useful to the analysis. Twenty landmark points were identified on each herring photograph using the image analysis software *tpsDig 2.3.2* (Rohlf, 2006). Custom *R* scripts were used to convert the x, y coordinates of the body morphometry landmarks to twenty distance measurements and twenty-one truss measurements across the body (Figure 5.1 and Table 5.2).

Table 5.2. Description of the 41 morphometric distances.

Morphometric distances	Description	Morphometric distances	Description
LT	total length	*DPA*	pelvic fin to anal fin length
LF	fork length	*DDP*	dorsal fin to pectoral fin length
LS	standard length	*LPA*	anal fin to maxilla length
LD	dorsal fin length	*LA*	anal fin length
HOD	Orbital diameter	*DAC*	anal fin to peduncular length
LPO	eye to mouth length	*LSM*	standard length from maxilla
ML	mouth length	*HH*	head height
LH	head length	*HM*	mid-body height
LPD	mouth to dorsal fin length	*HDA*	dorsal fin to anal fin length
DPP	pectoral fin to pelvic fin	*HP*	peduncular height
LPV	maxilla to pelvic fin length		

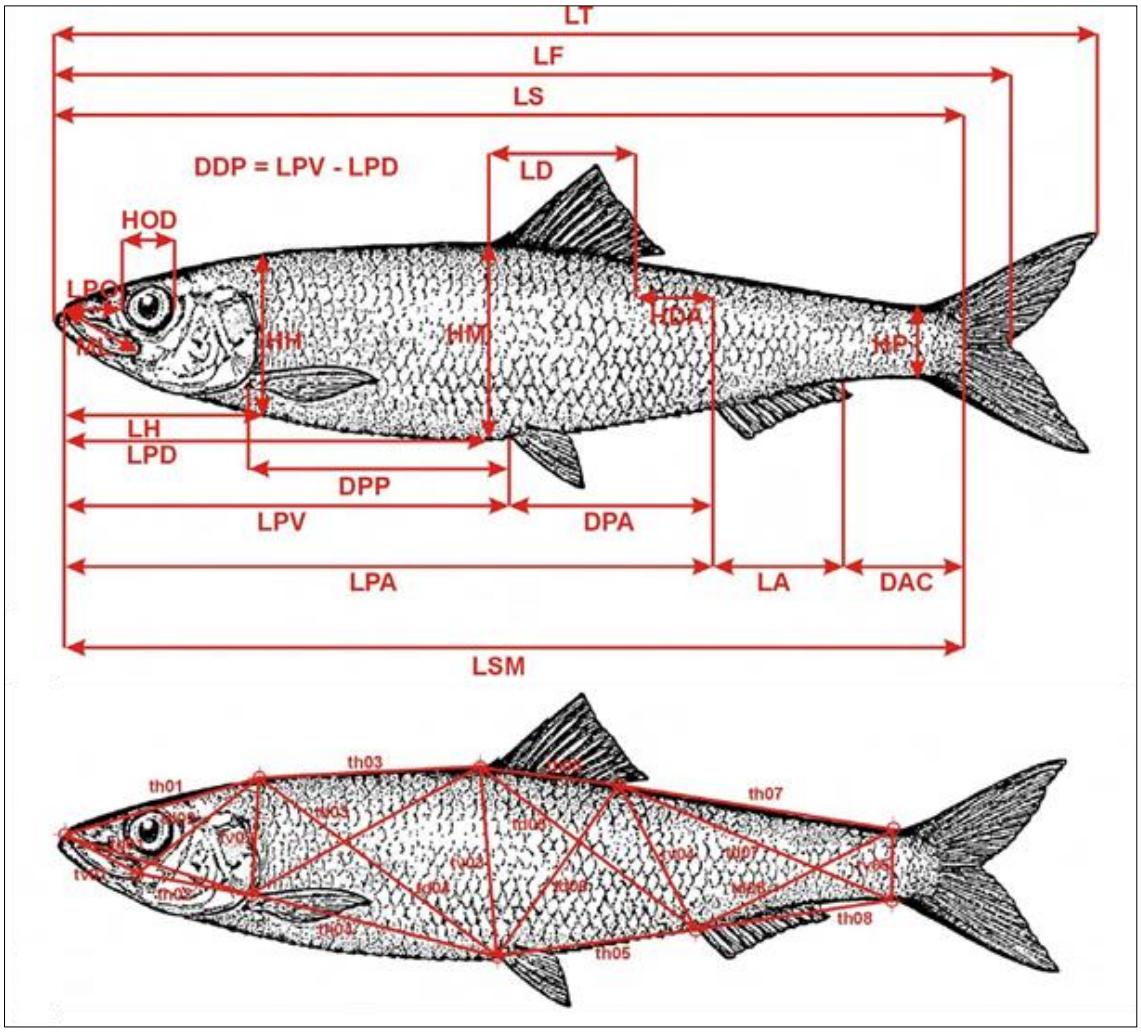


Figure 5.1. Body morphometry distance measurements (top) and truss measurements (bottom) available for use in the discriminant analysis.

The left otolith was mounted for age reading and the right otolith was stored clean and dry for photographs. The otoliths were placed sulcus side up and the rostrum facing the same direction under an *Olympus SZ12* dissecting microscope on a black background with adjustable lighting positioned above. Otolith images were captured using a mounted camera system and the image processing software *ToupView*. There was no significant difference found between the shape of the left and right otoliths in herring (Libungan *et al.*, 2015), so in cases where the right otolith was unavailable, the left otolith was photographed and the image was rotated to the required orientation. The scale of the image was confirmed at the start of each sample or if there was a change to the setup using a graticule. Otolith images were processed in *ShapeR* to trace the otolith outlines and calculate otolith shape descriptors using the x, y coordinates of the outline of each otolith. *ShapeR* can describe the shape of the otolith using Normalised Elliptic Fourier and Discrete Wavelet methods. Taking into consideration the ability of the two transformation techniques, the species being studied and the literature, the more powerful Wavelet transform was used to process the herring otoliths from ICES Division 6.a (Libungan and Pálsson, 2015). The package *ShapeR* was used to transform the otolith outline coordinates to discrete wavelet coefficients. The optimum Wavelet level, ranging from the finest scale (level 10) to the coarsest scale (level 0), was chosen based on the percentage deviation from the otolith outline. One wavelet coefficient is generated at level 0, and the number of coefficients doubles with every level. Level 5 was deemed the optimum level for the 6.a, 7.b and 7.c herring dataset, so by adding all of the coefficients generated from level 0 – level 5, the number of discrete wavelet coefficients that best described the herring otoliths was sixty-three wavelets (Song *et al.*, 2018; Libungan and Pálsson, 2015).

One of the primary objectives of the current study (Section 1) was to determine whether it is possible to use morphometric methods to distinguish between herring from different populations in ICES Divisions 6.a, 7.b and 7.c. In order to achieve this, factors influencing the body and otolith shape variables, other than population of origin, should be excluded where possible through transformation or removal of selected variables from further analyses. Forty-one body morphometric variables, five otolith morphometric variables and sixty-three otolith shape variables were collected for each fish. The suitability of each of the 109 measurements to be included and input to the discriminant analysis model were assessed using a series of *R* scripts that screen and test the raw morphometric data for outliers and missing data. Body and otolith shape variables are influenced by a number of factors that need to be considered prior to input to the discriminant analysis. For the 6.a, 7.b and 7.c herring dataset, these included the maturity stage of the herring, the effect of length on the shape variables and inter-annual variability across the six years of baseline data collection.

The baseline dataset included herring in spawning condition only, in order to give a high degree of confidence to the population of origin of the baseline samples. After filtering the baseline dataset for maturity stages 5 and 6 on the 8-point scale (Table 3.2), 302 individual herring collected between 2014 and 2019 were removed as a result. In order to eliminate any influence of the maturity stage on the shape of the body, two body shape variables were removed (Silva, 2004; Armstrong and Cadrin, 2001). *TV03* and *TD06* are truss measurements that describe the mid-body of the fish (Figure 5.2). Using the 2014 and 2016 6.a.S baseline samples, prior to filtering for maturity stages, both variables demonstrated a correlated relationship with maturity when plotted against length (LST) (Figure 5.3). Maturity stage seven (spent) on the 8-point scale showed a difference in the body truss measurement *TD06* and maturity stages eight and three (resting/recovering and early maturing) showed a difference in body truss measurement *TV03*.

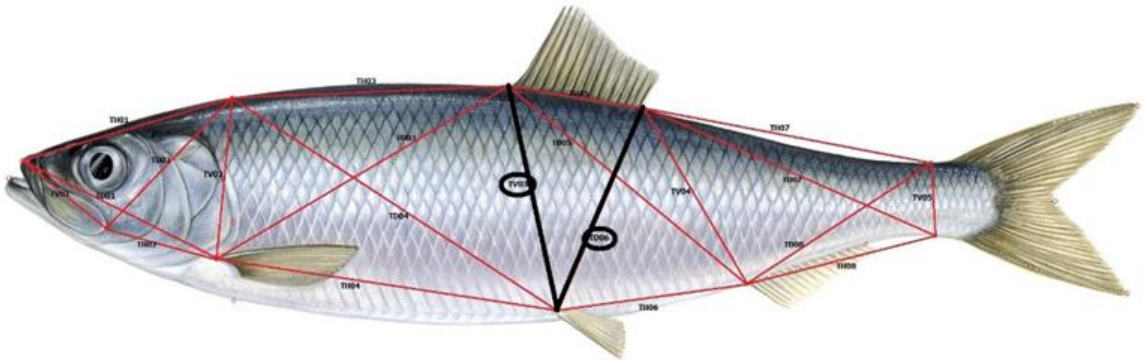


Figure 5.2. Bold black lines indicate the body morphometry truss variables that are correlated with maturity (*TV03* and *TD06*).

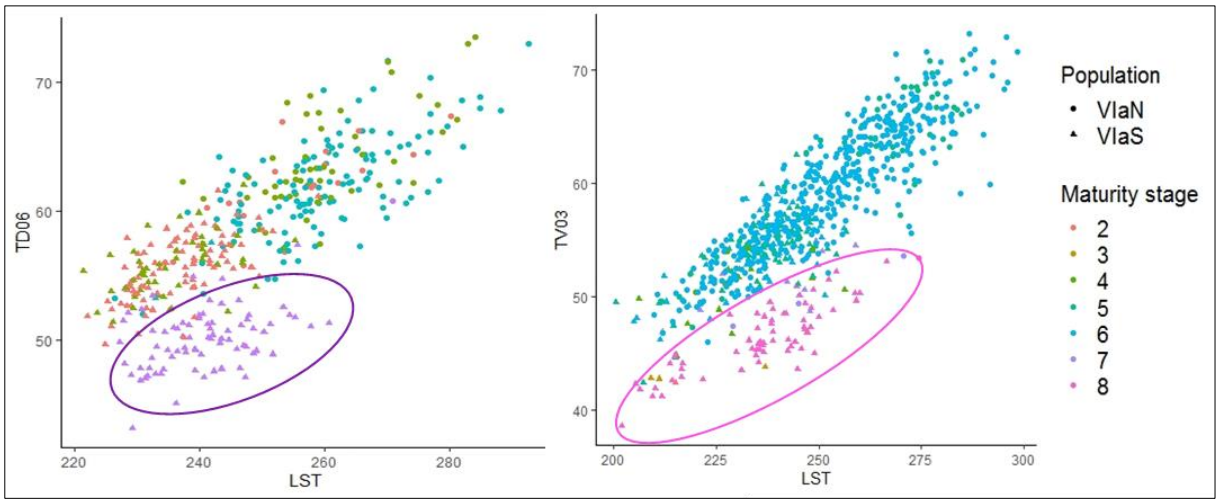


Figure 5.3. Relationship between body truss measurement (left) *TD06* and (right) *TV03* against length (LST) with respect to maturity stage.

The length effect on each body and otolith shape variable must be removed to allow comparison of the differences between shape (Campana and Casselman, 1993). All thirty-nine body shape variables demonstrated a correlated relationship with length (Pearson's correlation statistic < 0.05) the variables were transformed using the equation;

$$M_{adj} = M \times (L_T/L_O)^b, \text{ Elliott } et\ al., (1995)$$

where M is the morphometric measurement, M_{adj} is the size adjusted morphometric measurement, L_T is the total length of the fish, L_O is the total length overall mean and b was calculated for each variable as the slope of the regression of *Log M* on *Log* L_T using all individuals.

The otolith shape coefficients correlated with length (thirty variables, Pearson's correlation statistic < 0.05) were standardised using the common slope from an analysis of covariance (ANCOVA) with population of origin included as a factor and fish length used as a covariate. Variables with a significant population term only were included in the dataset. Those with a significant interaction term between length and population of origin showed an inconsistent relationship between the variable and total length across populations so they could not be standardised and were therefore removed from the analyses (Tracey *et al.*, 2006; Duncan *et al.*, 2018; Zhuang *et al.*, 2014). Out of sixty-eight otolith morphometric and shape variables, thirty-eight were not correlated with length and as a result no transformation was required, fifteen variables were transformed to eliminate the effect of length and fifteen variables were removed from the analysis.

When transforming both body and otolith shape variables to adjust for length, the parameters input to the transformation were calculated from the entire dataset and not based on the populations. Within population standardisation cannot be used on individuals from mixed samples because the population specific slope or mean length is unknown (Hussy *et al.*, 2016).

Baseline samples were collected over a six-year period (Tables 3.5 and 5.1) and MSHAS samples over a ten-year period (Tables 3.3 and 3.4). Inter-annual variability can have an effect on the statistical difference of body and otolith shape variables (Moreira *et al.*, 2020; Duncan *et al.*, 2018). This effect was removed as a source of variation in the baseline body and otolith shape by dividing the data into five groups:

- north: baseline data collected from all of the years in 6.a.N (2014-2017)
- south: baseline data collected from all of the years in 6.a.S (2014-2019)
- fourteen: the combined data from 6.a.N and 6.a.S for 2014
- sixteen: the combined data from 6.a.N and 6.a.S for 2016
- seventeen: the combined data from 6.a.N and 6.a.S for 2017

The first two groups were used to assess the year effect and the other three groups were used to assess the population effect for different years. In 2018 and 2019, baseline samples were only collected in 6.a.S so those years could not be used to look at the effect of population. For the groups north and south, the *P*-value from an analysis of variance (ANOVA) and the coefficient of determination (r^2) was calculated for each variable. A *P*-value < 0.05 indicated a significant difference between years. For the groups fourteen, sixteen and seventeen the *P*-values from an ANOVA and r^2 were calculated for each variable. A *P*-value < 0.05 indicated a significant difference between population. The coefficient of determination (r^2) is a measure of the proportion of variance in the dependent variable that can be explained by the independent variable (Fowler *et al.*, 1998) so this value for each variable is used to compare the two year groups (north and south) with each of the population groups (fourteen, sixteen, seventeen). If the value of r^2 is larger for the year groups, the variation for those variables is caused by year, if the r^2 is larger for the population groups, the variables are affected by population. The dataset contained multiple populations and data from multiple years. A variable was only used in the discriminant analysis if the effect across both populations and all years was a result of population (Table 5.3). Although only four of the body shape variables are influenced by a population effect across all comparisons, population was the strongest effect overall for both body shape and otolith shape variables.

Table 5.3. The r^2 was compared between year (S or N) and population (fourteen, sixteen or seventeen) groups for body shape variables. The cells highlighted in green demonstrate variables where the significant difference is caused by population. Only four variables were influenced solely by population; DDP, ML, TV01 and TD01.

Variable	EffectS_14	EffectS_16	EffectS_17	EffectN_14	EffectN_16	EffectN_17
LA	Pop	Year	Pop	Pop	Pop	Pop
DPP	Year	Pop	Pop	Pop	Pop	Pop
DDP	Pop	Pop	Pop	Pop	Pop	Pop
DPA	Pop	Year	Year	Pop	Pop	Year
DAC	Pop	Pop	Pop	Year	Year	Pop
HH	Year	Year	Year	Pop	Pop	Pop
HP	Year	Year	Pop	Year	Year	Pop
ML	Pop	Pop	Pop	Pop	Pop	Pop
OD	Pop	Pop	Pop	Pop	Pop	Year
TV01	Pop	Pop	Pop	Pop	Pop	Pop
TV02	Year	Year	Year	Year	Pop	Pop
TV04	Pop	Pop	Year	Pop	Pop	Year
TV05	Year	Year	Pop	Year	Year	Pop
TH01	Pop	Pop	Pop	Year	Year	Year
TH02	Pop	Pop	Pop	Year	Pop	Pop
TH03	Year	Pop	Year	Pop	Pop	Pop
TH04	Year	Pop	Pop	Pop	Pop	Pop
TH05	Pop	Pop	Pop	Year	Pop	Pop
TH06	Pop	Year	Year	Pop	Pop	Year
TH07	Pop	Pop	Year	Pop	Pop	Pop
TH08	Year	Pop	Pop	Year	Pop	Year
TD01	Pop	Pop	Pop	Pop	Pop	Pop
TD02	Pop	Year	Year	Pop	Pop	Pop

Efforts were made to select predictor variables that demonstrated the greatest difference in the response variable (population of origin) and as a result, some variables were transformed and some variables have been removed leaving four body shape variables and thirteen otolith shape variables. To ensure that both stock discrimination methods were performing optimally, the body morphometrics dataset and the otolith shape dataset were analysed separately, as well as in a combined dataset. This approach also enabled any technical artefacts to be detected prior to combining the datasets.

The body and otolith shape variables were measured in different units and on a different scale which means the distances, directions and covariances are not interpretable (Adams and Collyer, 2019). Therefore, in order to combine the body morphometric and otolith shape datasets, the variables were standardised (centred on mean and scaled by standard deviation).

Morphometric baseline data from the WESTHER project was not used in the current analysis. A new method for describing and analysing the outline of the otoliths was adopted according to Libungan and Pálsson (2015). An update of the otolith images was required for input to the new methodology but access to the otoliths was not obtained during this project. The body morphometric data from WESTHER was available for analysis but without the otolith shape data, the WESTHER dataset was incomplete.

5.5. *Discriminant analysis*

While Principal Component Analysis (*PCA*) provides weight to all of the body and otolith shape variables available in order to summarise the difference between individuals, Canonical Discriminant Analysis (*CDA*) uses the assigned groups, in this case population of origin, to calculate the variable weightings that show the greatest difference between the groups. *CDA* summarises between group variation, in the same way *PCA* summarises total variation (Dytham, 2003).

The *CDA* outputs a boxplot comparing the canonical variates from the two groups; 6.a.N and 6.a.S. The main body of the boxplot represents the interquartile ranges and the medians for 6.a.N and 6.a.S data. For body only dataset, otolith shape only dataset and the combined body and otolith dataset, the medians and the interquartile ranges did not overlap which indicated, with 95% confidence, that there was a difference between the populations (MANOVA $P < 0.002$). The whiskers represent data that falls outside of the interquartile range and outliers are plotted above and below the whiskers. Although a difference between the two populations is highly likely, there was some overlap with the boxplot whiskers for all three datasets (Figure 5.4). In order to clarify the extent of overlap, the distribution of the canonical variates, calculated from the *CDA*, were explored. The resulting histograms also illustrated a difference between the two populations, however there was also a large area of overlap between 6.a.N and 6.a.S for all three methods (Figure 5.5).

Exploratory classification of the baseline data using a discriminant analysis yielded self-assignment rates of >65% in all three datasets (Table 5.4). It should be noted that the accuracy of the model is an overall measure of how well the analysis performed, and as a result, may not be relied upon as a measure of the performance of individual groups. The self-assignments for all three datasets were lower for 6.a.S fish. This may be linked to a higher variability in the 6.a.S herring population and will be discussed in more detail.

The *body only* and *otolith only* discriminant analyses demonstrated a difference between the two populations and the self-assignment rate was highest for the *combined* dataset so this was used for further analyses.

Table 5.4. Output from the *CDA* for *body only*, *otolith only* and *combined* body and otolith datasets includes the percentage of individuals assigning back to the correct group for each population and the overall accuracy of the classification.

Dataset	Assigned to correct population		Accuracy
	6.a.N	6.a.S	
Body only	87.36%	66.16%	79.1%
Otolith only	81.38%	68.39%	75.9%
Combined	85.96%	70.16%	79.8%

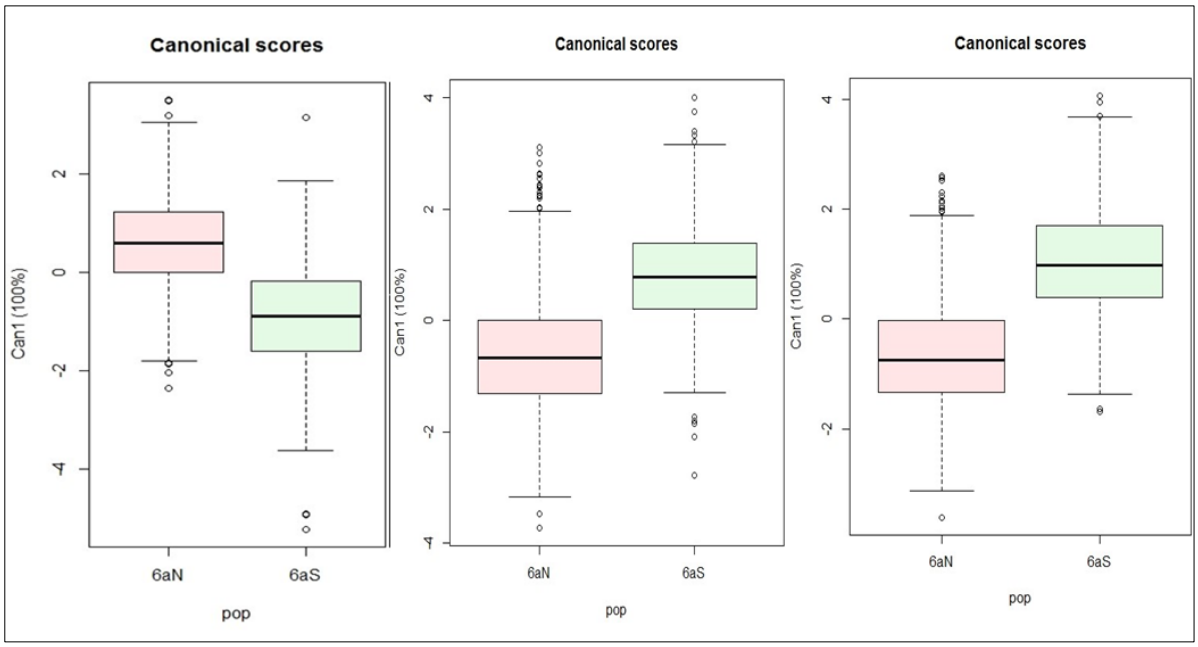


Figure 5.4. Boxplot output from the CDA for (left) *body only*, (middle) *otolith only* and (right) body and otolith *combined*.

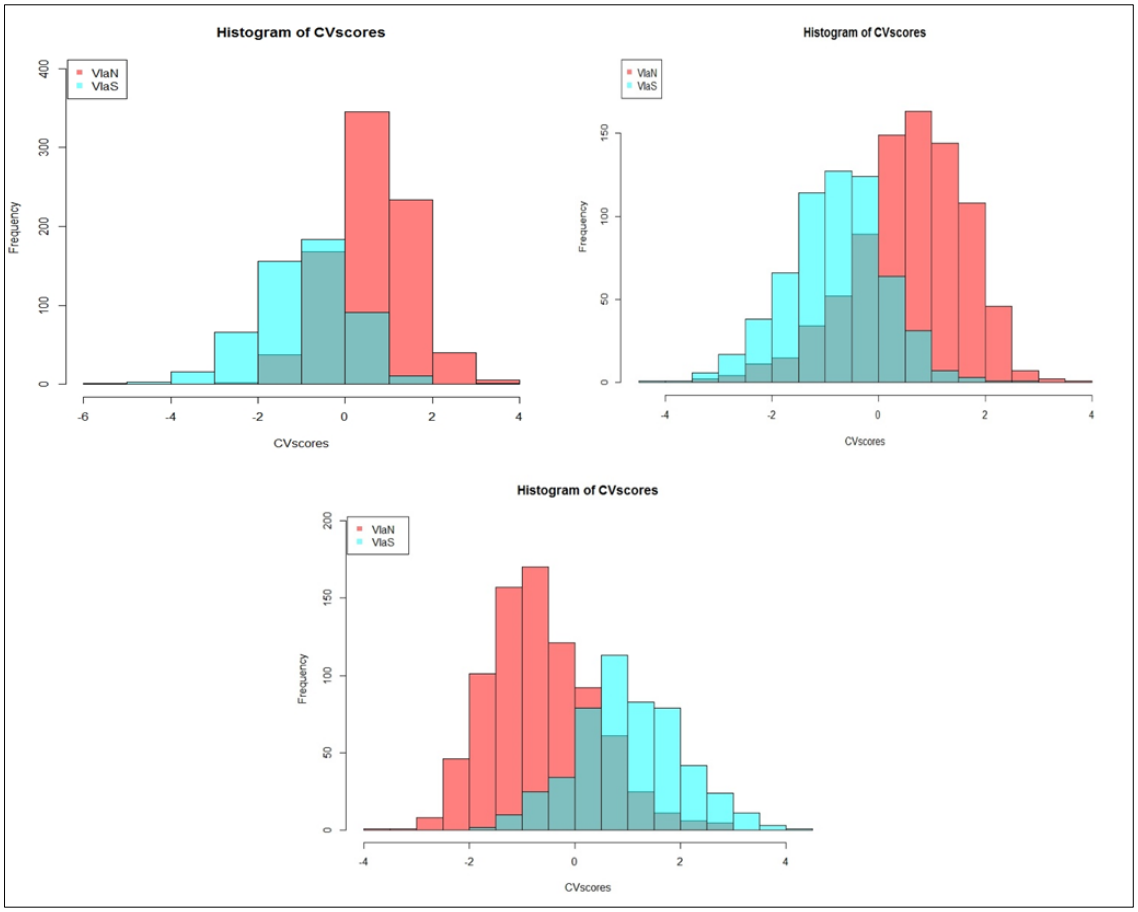


Figure 5.5. Histograms of the canonical variates for (top left) *body only*, (top right) *otolith only* and (bottom) body and otolith *combined*.

5.6. Mean otolith shape

Otolith shape analysis is used widely in a variety of species (Duncan *et al*., 2018; Hussy *et al*., 2016; Stransky, 2005). It has also been used successfully to discriminate between herring populations in the North Atlantic (Libungan *et al.*, 2015; Burke *et al.*, 2008). Using the mean otolith shape, Libungan *et al.* (2015) were able to highlight areas on the otolith that showed the greatest difference between herring from Canada, Faroe Islands, Iceland, Ireland, Norway and Scotland. The mean otolith shape of herring from 6.a.N and 6.a.S demonstrated low levels of differentiation across the whole otolith (Figure 5.6). Otolith shape is heavily influenced by growth (Campana and Cassellman, 1993) and growth is in turn influenced by environmental factors such as temperature and food availability (Mille *et al.*, 2016; Feet *et al.*, 2002; Fey, 2001). Herring populations from 6.a.N and 6.a.S are under similar environmental pressures due to their proximity, and at certain times of the year, overlap in spatial distribution. Another factor that influences the shape of otoliths is age.

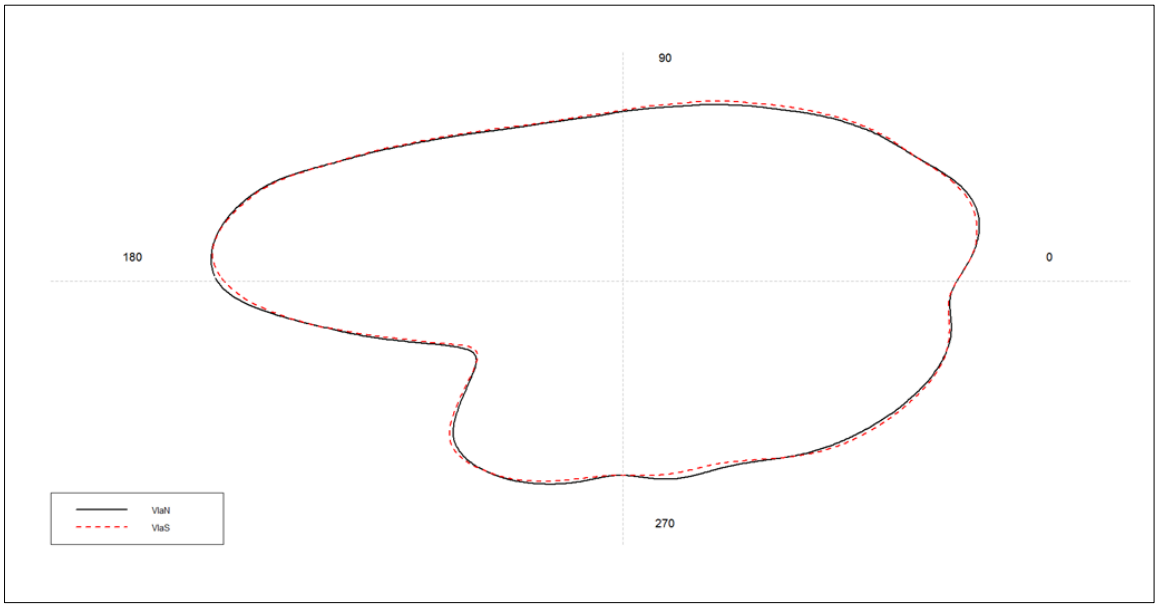


Figure 5.6. Overall mean otolith shape described by Wavelet coefficients using baseline data from 6.a.N (2014-2017) and 6.a.S (2014–2019).

The effect of age on the shape variability of herring otoliths has been recorded in previous studies and to address this, analysis has been carried out by age, age intervals, or specific ages have been selected for further analysis (Libungan *et al.*, 2015; Smolinski *et al.*, 2019). In the current study, herring of all ages may be collected in mixed survey samples so no ages can be eliminated from the analysis. In order to optimise the sample sizes, ensure that all ages were represented, and categorise the otolith ages to reflect change in shape with age, the baseline dataset was divided into three age intervals; *ages 1-3 years*, *ages 4-6 years* and *ages 7+years*.

Using the combined dataset, a separate *CDA* was run on the three age intervals and the results showed an increase in the accuracy of the self-assignments and also an increase in the percentage self-assignments for *6.a.S* (Table 5.5). The discriminant analysis showed there was a difference between 6.a.N and 6.a.S herring but there was still evidence of an overlap between the two populations that may affect the ability of the data to classify the mixed samples to their correct populations.

Table 5.5. Output from the *CDA* for the combined body and otolith datasets divided by age interval. Percentage of individuals assigning back to the correct group for each population and the overall accuracy of the classification.

Age interval	Assigned to correct population		Accuracy
	6.a.N	6.a.S	
1-3 years	92.71%	68.03%	86.2%
4-6 years	85.32%	84.64%	85.0%
7+ years	86.67%	74.72%	81.8%

5.7. *Classification models*

Some traditional classification models require assumptions that the current dataset does not meet, for example, a Linear Discriminant Analysis (*LDA*) assumes multivariate normality, although the model is generally robust to certain assumptions (Paukert and Wittig, 2002). As an alternative to traditional classification models, machine learning models have been explored (Doyle *et al.*, 2018). The use of machine learning models is still new to stock discrimination studies involving morphometric techniques (e.g. Zhang *et al.*, 2016; Mapp *et al.*, 2017) but there is evidence that machine learning algorithms can perform better than traditional classification models (Smoliński *et al.*, 2020).

A number of different classification models, both traditional and machine learning, have been explored through this work. An exploratory Linear Discriminant Analysis (*LDA*), also known as a Canonical Discriminant Analysis (*CDA*) (Dudzik, 2019), was conducted to investigate the performance of all three datasets used to describe the herring. A Quadratic Discriminant Analysis (*QDA*) model was successfully fitted to the data during WKWEST (2015) with classification success rates of 90% for 6.a.N and 94% for 6.a.S. The Support Vector Machine (*SVM*) model was found to be the optimum model when using data from four herring populations in the north Atlantic (Smoliński *et al.*, 2020). Therefore, it was explored as a possible option for the 6.a, 7.b and 7.c herring and, due to the data being measured in different units and at different scales (Section 5.5), a Random Forest model was suggested as a good fit for the data.

SVM is a popular machine learning model that aims to find a hyperplane to distinctly classify data points input to the model (see also Section 4.8 and Figure 4.9). *SVMs* are recognised for accuracy and the ability to deal with high dimensionality data (Ben-Hur *et al.*, 2008). *Random Forest* is also a well-known machine learning model developed by Breiman (2001), that uses a large number of decision trees which operate as an ensemble. Each decision tree provides a prediction of population of origin and the population with the most 'votes' becomes the models' prediction (Zhang *et al.*, 2016).

There are a number of packages in the statistical programme *R* that facilitate building different classification models, including *CARET* (Kuhn, 2008) and *assignPOP* (Chen *et al*., 2018). Classification and Regression Training (*CARET*) streamlines the process of creating predictive models. *assignPOP* uses a machine-learning framework to perform population assignments.

Using the *CARET* package, the performance of four classification models (*LDA, QDA, Random Forest* and *SVM*) were compared for the *all ages* dataset, *age interval 1-3 years* dataset, *age interval 4-6 years* dataset and *age interval 7+ years* dataset. Following Smoliński *et al*. (2020), the machine learning models were tuned to provide the best fit model. In order to replicate the *K*-fold cross validation performed in *assignPOP*, a 10-fold cross validation resampling procedure was run on each model for each dataset. The dataset was divided randomly into *K* groups, *K*=10 in this case, nine groups were used as the training dataset while the last group was classified by the training dataset in order to test the self-assignment rate. The model continues to run until all 10 groups have been used to test the training dataset. To ensure its validity, this process was repeated 100 times using a bootstrapping approach with independent resampling (Hastie *et al.*, 2009). A number of outputs were calculated for each dataset from each model, including classification accuracy and misclassification rates. Classification accuracy was calculated

using the proportion of herring correctly assigned to their population of origin. The models sampled show high accuracy for all of the datasets and there was little difference between them (Table 5.6). Although the difference between the three models is small, *SVM* demonstrated the highest accuracy across all datasets. The percentual average cell counts across resamples were calculated for the four datasets using *SVM*. The confusion matrix shows the percentage of individuals from the whole dataset assigned to the correct population and assigned to the wrong populations (Table 5.7). For example, the *age interval 4-6 years* dataset contains a total of 620 herring and according to the confusion matrix, 45.7% of 620 the individuals were assigned correctly to 6.a.N, 40.4% were assigned correctly to 6.a.S, 6.9% of the 620 individuals were misassigned as 6.a.S fish and 7.1% were mis-classified as 6.a.N herring. The highest misclassification occurred in 6.a.S fish for all datasets and the *age interval 7+* years dataset had the highest overall misclassification of 19% for 6.a.N and 6.a.S combined.

Table 5.6. Model accuracies for four datasets predicted using two machine learning classification models and two traditional classification models.

Model	Dataset			
	Full	*1-3 year*	*4-6 years*	*7+ years*
Random Forest	0.82	0.85	0.85	0.82
SVM	0.84	0.87	0.86	0.81
LDA	0.82	0.86	0.85	0.81
QDA	0.81	0.86	0.81	0.73

Table 5.7. Cross validated (10-fold, repeated 100 times) confusion matrix using the *SVM* output. Percentual average cell counts across resample and the average accuracy (sum of the diagonal cells).

Confusion matrices		
All ages		
	6.a.N	**6.a.S**
6.a.N	54.2	8.9
6.a.S	7.2	29.7
Average Accuracy	0.84	
Age Interval 1-3 years		
	6.a.N	**6.a.S**
6.a.N	69.1	7.9
6.a.S	4.6	18.3
Average Accuracy	0.87	
Age Interval 4-6 years		
	6.a.N	**6.a.S**
6.a.N	45.7	6.9
6.a.S	7.1	40.4
Average Accuracy	0.86	
Age Interval 7+ years		
	6.a.N	**6.a.S**
6.a.N	51.7	11.1
6.a.S	8.1	29.1
Average Accuracy	0.81	

The package *assignPOP* uses Monte-Carlo cross validation to estimate the assignment accuracy for each population and *K*-fold cross validation to calculate the average assignment to each response variable (in this case, population) across *K*-groups (Chen *et al.*, 2018). An assignment accuracy boxplot and a membership probability plot were created for all four datasets (Figure 5.7-5.10) and their corresponding mean and variation of assignment accuracies estimated across the assignment test (Table 5.8) using a tuned

radial *SVM* model. The self-assignment accuracies were >70% for all of the datasets, apart from *age interval 1-3 years*. The assignment accuracy was the lowest at 69% for 6.a.S and this was also reflected in the membership probability plot (Figure 5.8), demonstrating a lack of confidence in the assignments back to 6.a.S in this age category. One of the factors that may be influencing this was the imbalance in sample sizes, where 6.a.N (N=343) had double the number of fish as 6.a.S (N=122). The *age interval 4-6 years* had a balanced sample size (difference of 34 herring) and the assignment accuracies were highest for this dataset for both populations (Table 5.8).

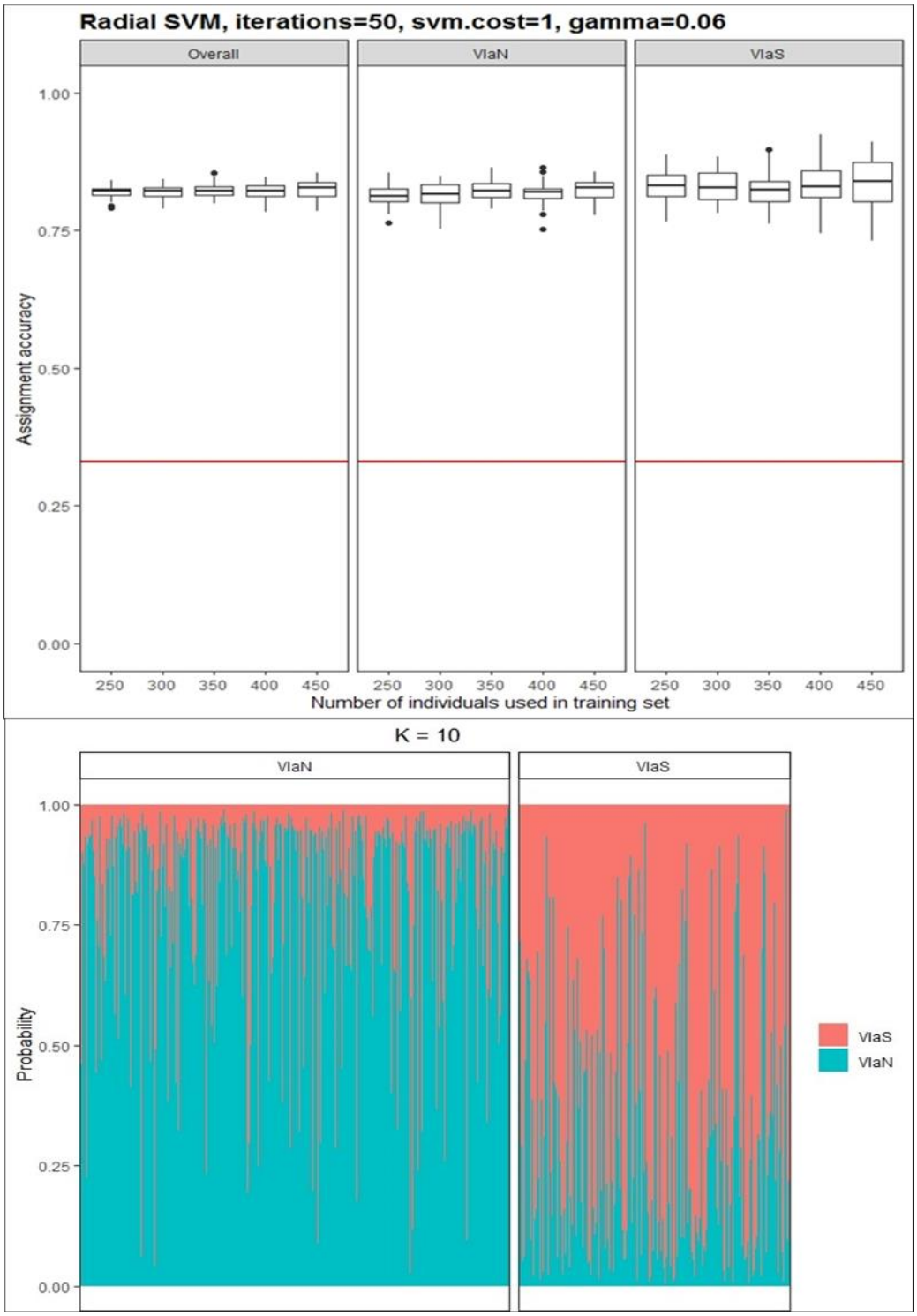


Figure 5.7. Assignment accuracy boxplot calculated using Monte-Carlo cross validation based on a range of training set sizes (top) and a membership probability plot for the *all ages* dataset based on 10-fold cross validation (bottom) using a tuned radial *SVM* classification model.

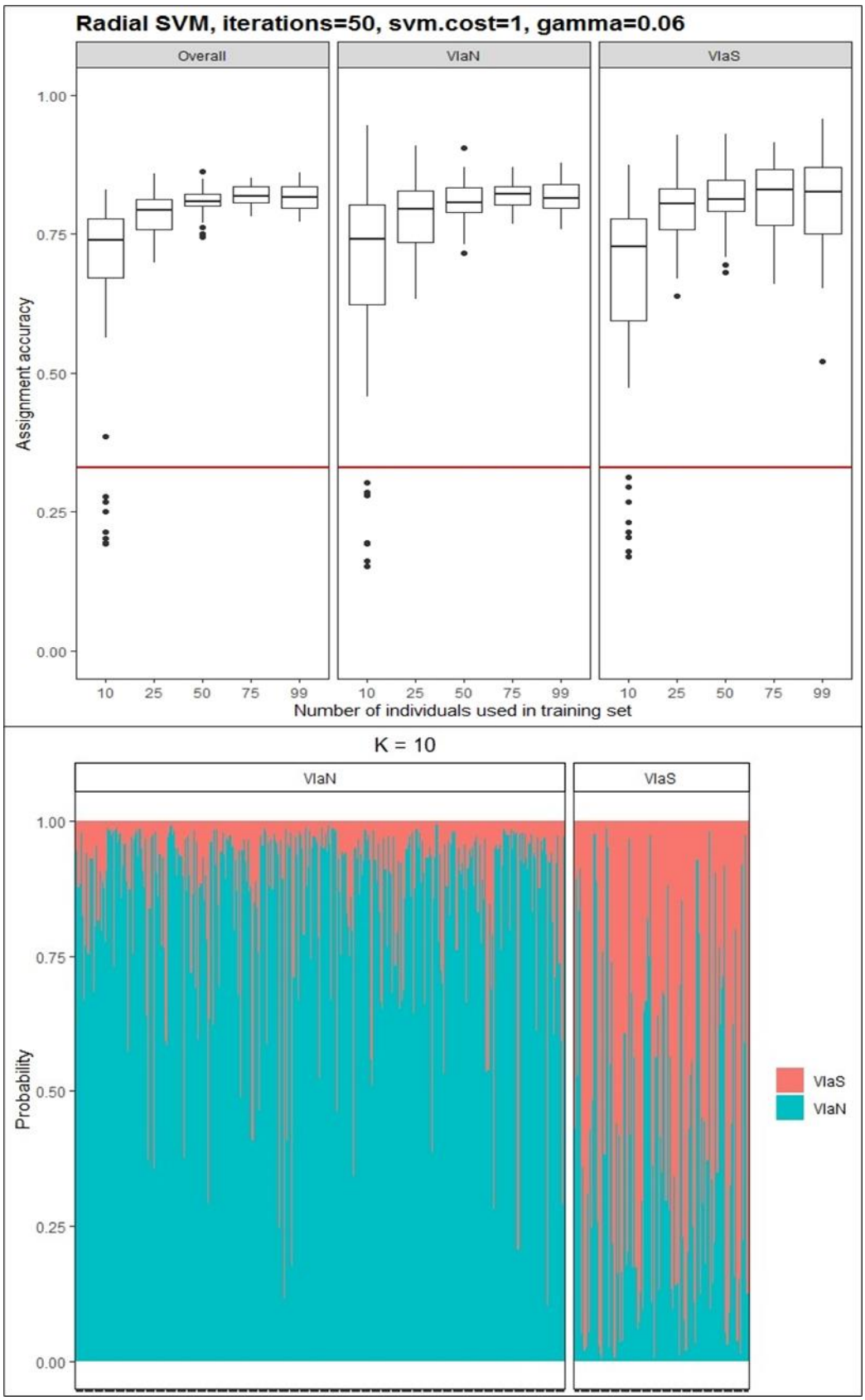


Figure 5.8. Assignment accuracy boxplot calculated using Monte-Carlo cross validation based on a range of training set sizes (top) and a membership probability plot for the *age interval 1-3 years* dataset based on 10-fold cross validation (bottom) using a tuned radial *SVM* classification model.

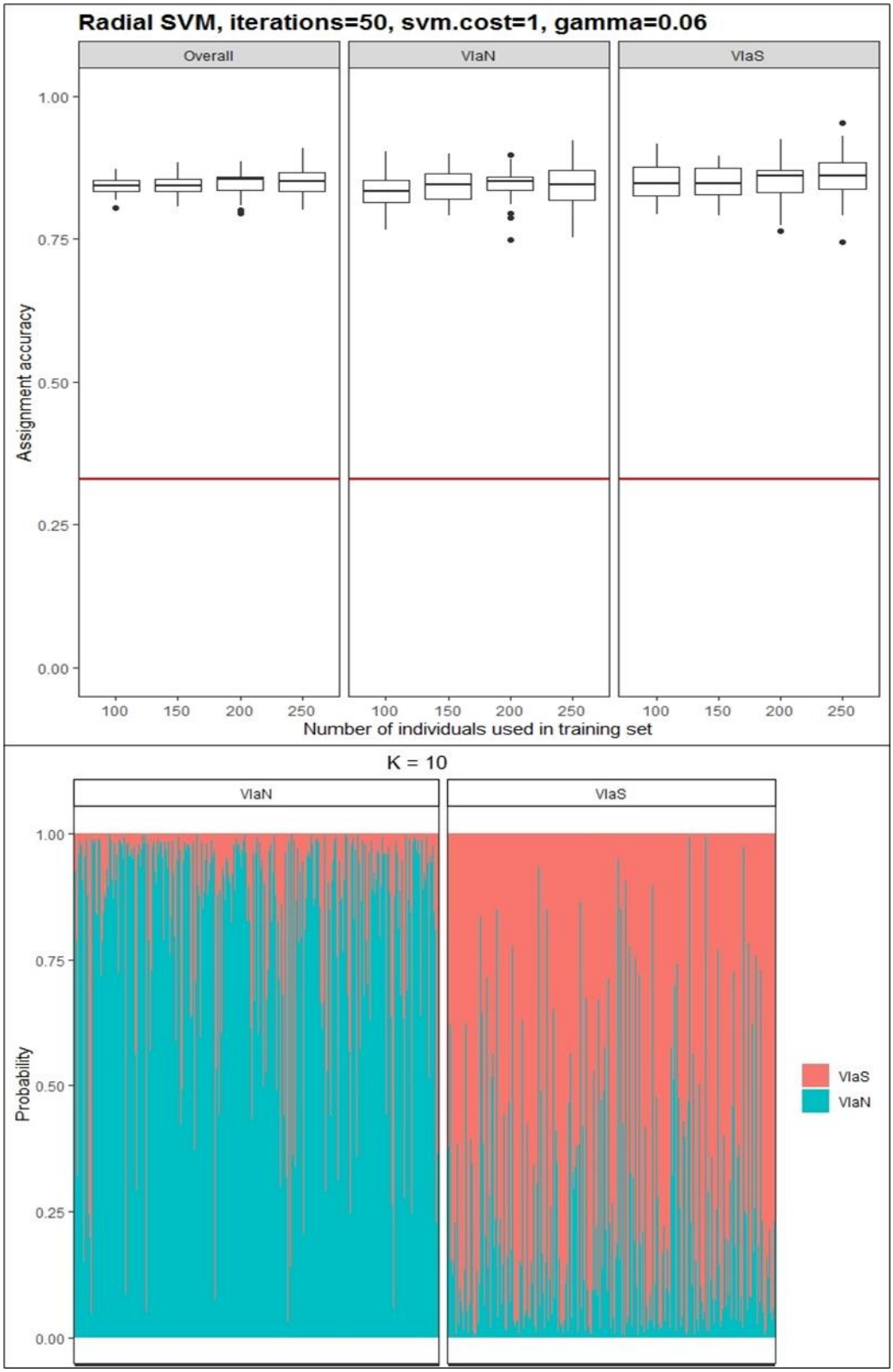


Figure 5.9. Assignment accuracy boxplot calculated using Monte-Carlo cross validation based on a range of training set sizes (top) and a membership probability plot for the *age interval 4-6 years* dataset based on 10-fold cross validation (bottom) using a tuned radial *SVM* classification model.

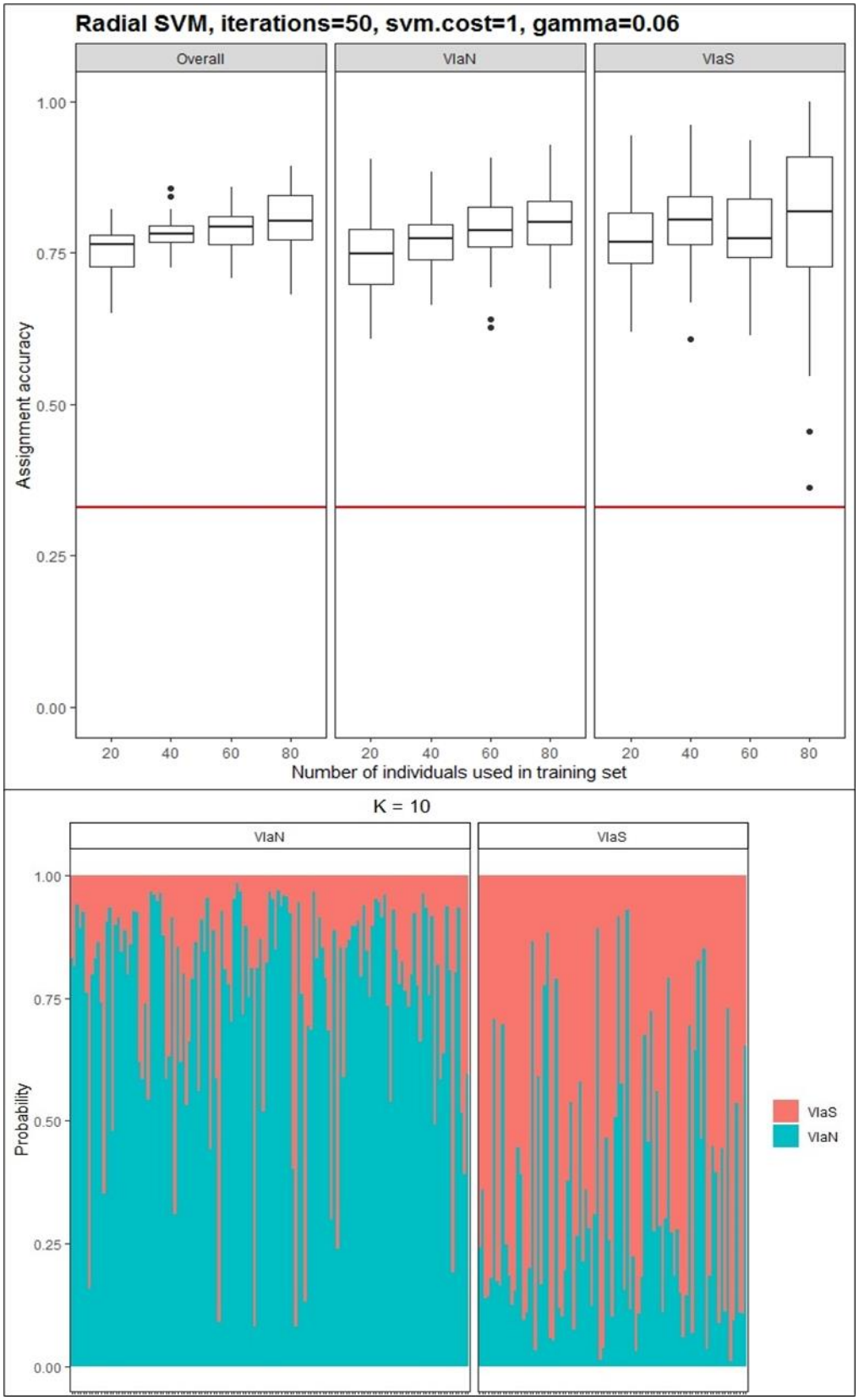


Figure 5.10. Assignment accuracy boxplot calculated using Monte-Carlo cross validation based on a range of training set sizes (top) and a membership probability plot for the *age interval 7+ years* dataset based on 10-fold cross validation (bottom) using a tuned radial *SVM* classification model.

Table 5.8. The mean assignment accuracy for Monte-Carlo (*MC*) and *K*-fold cross validation resampling procedures and the variation calculated using standard deviation (SD).

Method	Origin	Assignment	
		6.a.N	6.a.S
		All ages	
MC	6.a.N	**0.82** ± 0.02 SD	0.18 ± 0.02 SD
	6.a.S	0.16 ± 0.03 SD	**0.83** ± 0.03 SD
K-fold	6.a.N	**0.88** ± 0.04 SD	0.12 ± 0.04 SD
	6.a.S	0.24 ± 0.06 SD	**0.76** ± 0.06 SD
		Age interval 1-3 years	
MC	6.a.N	**0.81** ± 0.03 SD	0.19 ± 0.03 SD
	6.a.S	0.18 ± 0.05 SD	**0.82** ± 0.05 SD
K-fold	6.a.N	**0.95** ± 0.04	0.05 ± 0.04
	6.a.S	0.38 ± 0.16	**0.62** ± 0.16
		Age interval 4-6 years	
MC	6.a.N	**0.84** ± 0.03 SD	0.16 ± 0.03 SD
	6.a.S	0.15 ± 0.03 SD	**0.85** ± 0.03 SD
K-fold	6.a.N	**0.85** ± 0.09 SD	0.15 ± 0.09 SD
	6.a.S	0.16 ± 0.08 SD	**0.84** ± 0.08 SD
		Age interval 7+ years	
MC	6.a.N	0.78 ± 0.05 SD	0.22 ± 0.05 SD
	6.a.S	0.22 ± 0.07 SD	0.78 ± 0.07 SD
K-fold	6.a.N	**0.87** ± 0.11 SD	0.13 ± 0.11 SD
	6.a.S	0.28 ± 0.15 SD	**0.72** ± 0.15 SD

5.8. *Assignment validation with known-unknown samples*

An additional validation step used to validate the results of the classification model was to use 'known-unknown' samples to test the output. This involved removing a sample from the baseline dataset and re-running the model without this sample. The removed sample was treated as a mixed or unknown sample and assigned back to a population of origin using the adjusted baseline dataset. Three baseline samples were used to validate the results; one sample from 6.a.N and two samples from 6.a.S. The samples were removed one at a time to ensure the size of the baseline dataset was not significantly altered. The probability of assignment of each fish were calculated for 6.a.N and 6.a.S, and a threshold of 0.67 probability was set so that there is confidence in the assignment. For example, if a fish had assignment probabilities of 0.74 to 6.a.N and 0.36 to 6.a.S, that fish was assigned to 6.a.N but if the fish had probabilities of 0.55 to 6.a.N and 0.45 to 6.a.S, it was classed as unknown. A probability of >0.67 to a population indicates the fish is twice as likely to be from that population than the alternative population (Table 4.5).

Using the four datasets, membership probability plots and tables containing the percent of fish that assigned back to 6.a.N, 6.a.S or an Unknown category were produced for the known-unknown samples from 2016 caught in 6.a.N (Figure 5.11 and Table 5.9), a 2017 6.a.S sample (Figure 5.12 and Table 5.10) and the 2019 6.a.S sample (Figure 5.13 and Table 5.11). The 2016 6.a.N and 2017 6.a.S samples did show a majority of the herring assigning back to the correct stock, but the assignments were quite low for baseline samples. The highest correct assignment that was calculated was 87.5%. A significant proportion of fish also assigned back to the unknown category, which reduces the confidence of the model. The majority of the 6.a.S 2019 sample assigned back to 6.a.N.

The sample was scrutinised for errors, but no technical artefacts were found, therefore the high level of misassignment observed likely indicates a weakness in the assignment model.

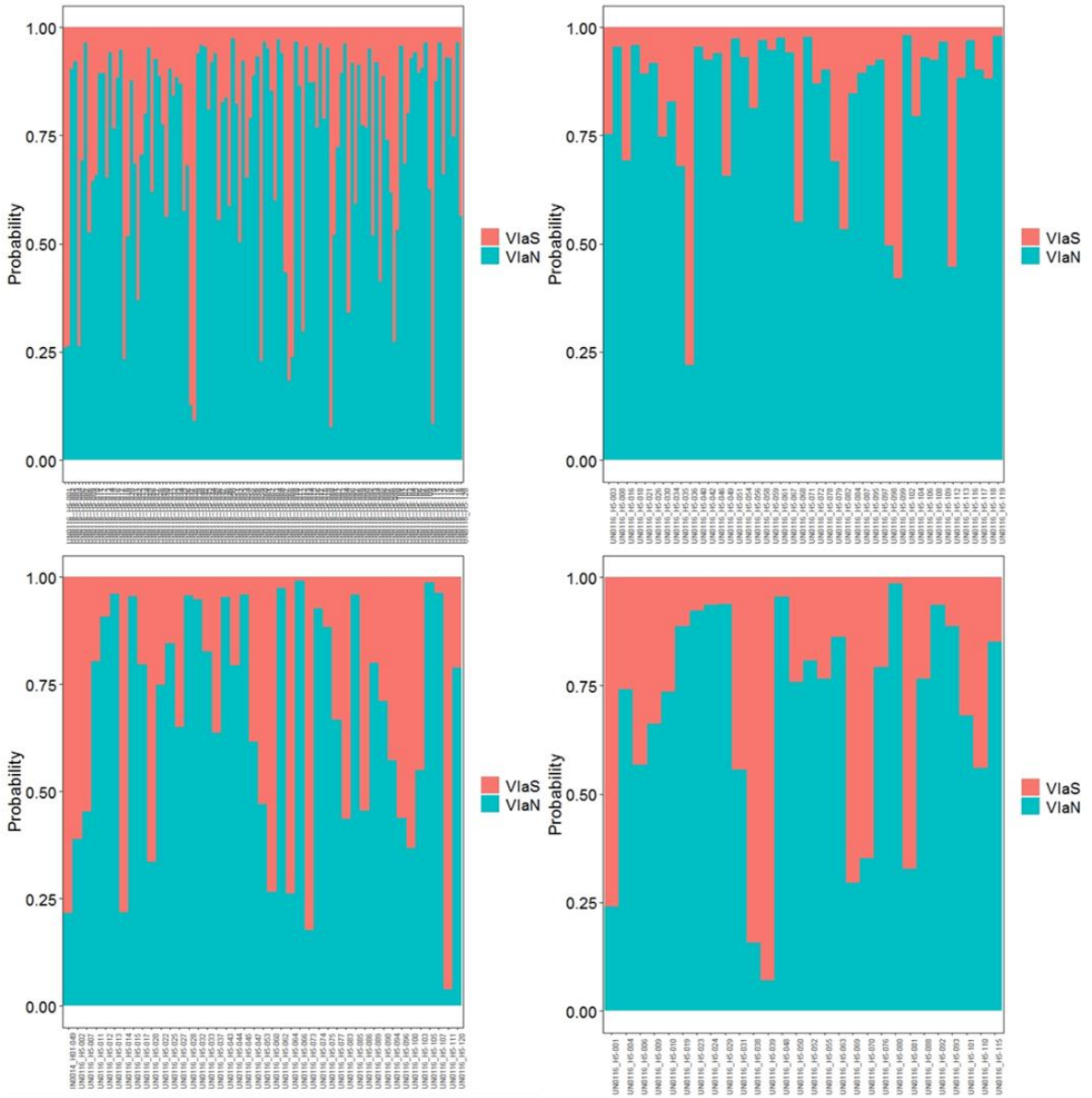


Figure 5.11. The assignment of the 2016 6.a.N known-unknown sample using *SVM* for (top left) *all ages*, (top right) *age interval 1-3 years*, (bottom left) *age interval 4-6 years*, and (bottom right) *age interval 7+ years*.

Table 5.9. The percent of individuals assigning back to 6.a.S, 6.a.N and Unknown using a 0.67 probability threshold for the four datasets from the known-unknown sample 2016 6.a.N. The column highlighted in grey is where the fish should be assigning back to.

	% 6.a.S	% 6.a.N	% Unknown
All ages	11.4	67.5	21.1
Age1-3	6.8	79.5	13.6
Age4-6	16.3	62.8	20.9
Age7+	10.7	64.3	25.0

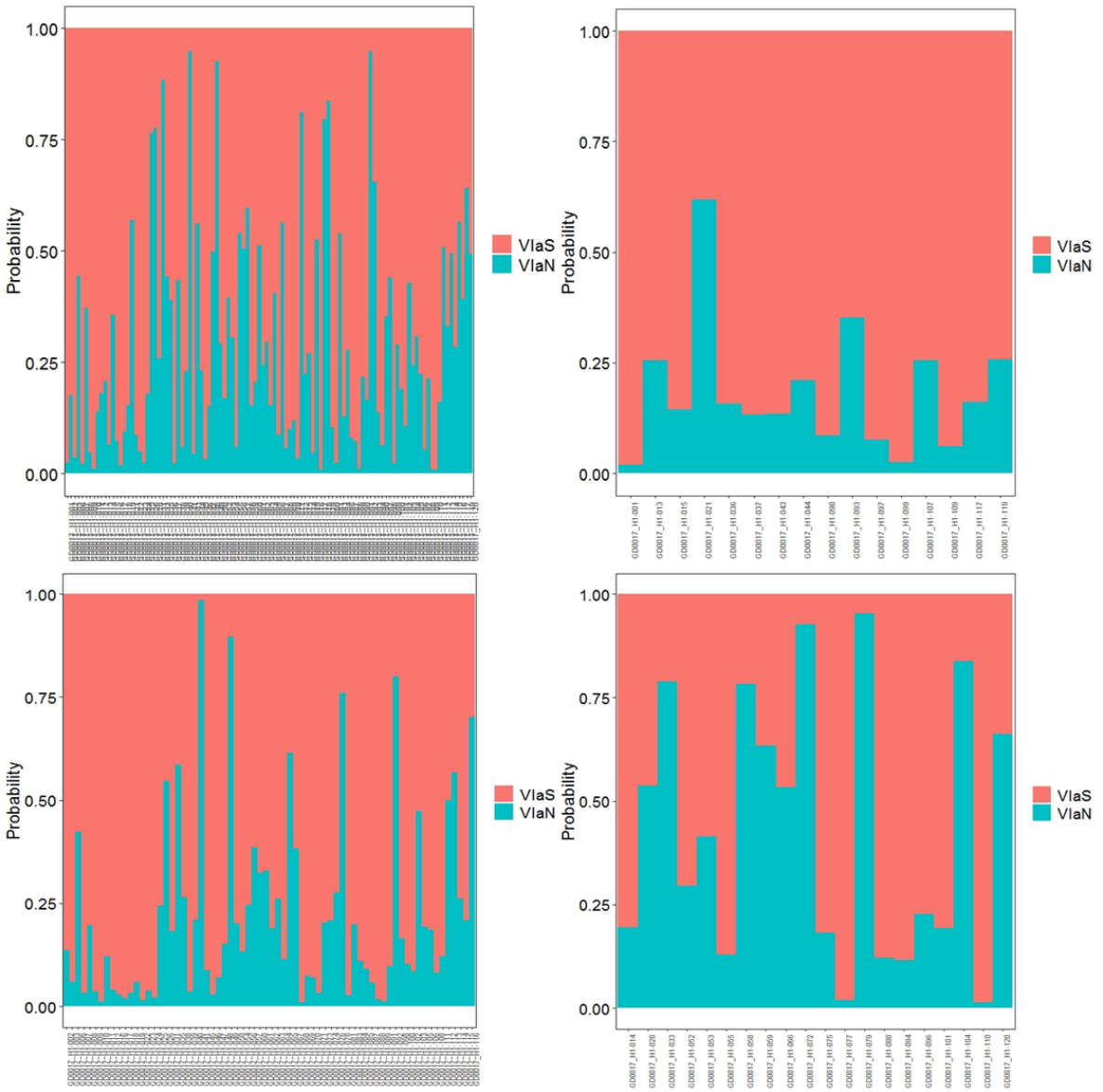


Figure 5.12. The assignment of the 2017 6.a.S known-unknown sample using *SVM* for (top left) *all ages*, (top right) *age interval 1-3 years*, (bottom left) *age interval 4-6 years*, and (bottom right) *age interval 7+ years*.

Table 5.10. The percent of individuals assigning back to 6.a.S, 6.a.N and Unknown using a 0.67 probability threshold for the four datasets from the known-unknown sample 6.a.S 2017. The column highlighted in grey is where the fish should be assigning back to.

	% 6.a.S	% 6.a.N	% Unknown
All ages	68.9	8.5	22.6
Age1-3	87.5	0.0	12.5
Age4-6	82.9	10.0	7.1
Age7+	50.0	25.0	25.0

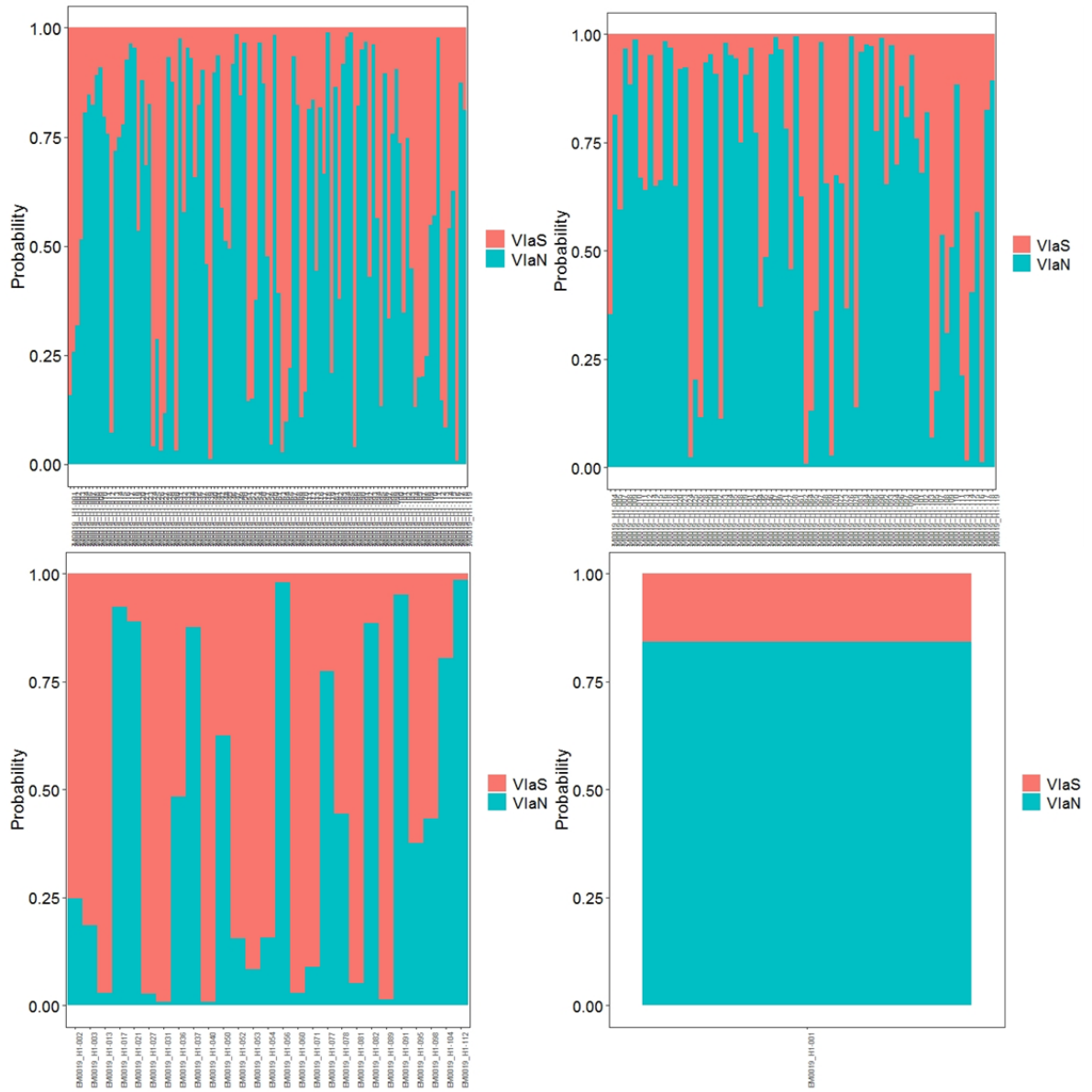


Figure 5.13. The assignment of the 2019 6.a.S known-unknown sample using *SVM* for (top left) *all ages*, (top right) *age interval 1-3 years*, (bottom left) *age interval 4-6 years*, and (bottom right) *age interval 7+ years*.

Table 5.11. The percent of individuals assigning back to 6.a.S, 6.a.N and Unknown using a 0.67 probability threshold for the four datasets from the known-unknown sample 6.a.S 2019. The column highlighted in grey is where the fish should be assigning back to.

	% 6.a.S	% 6.a.N	% Unknown
All ages	22.9	53.3	23.8
Age1-3	22.1	57.1	20.8
Age4-6	40.7	33.3	25.9
Age7+	0.0	100.0	0.0

5.9. MSHAS samples

The MSHAS samples were pre-processed using a series of *R* scripts to screen for errors and outliers. The transformation parameters that were used to correct for length in the body and otolith baseline data were common for both populations so these parameters were also used to correct the MSHAS data for length effect in order to ensure the data were treated in the same way as the baseline data (Hüssy *et al.*, 2016).

In order to test the assignment of the MSHAS data a single year was chosen that had a wide distribution of samples across the survey area, including south of the 56° line of latitude. The assumption being that the hauls in the geographic north would have a higher proportion of 6.a.N fish and those in the geographic south, a high proportion of 6.a.S fish. The MSHAS data from 2015 was assigned using the tuned *SVM* model for the four datasets. The probability threshold of 0.67 was used to ensure a high level of confidence in the assignments. The results for all datasets were very uncertain; the assignments contained a large proportion of unknown individuals and very low levels of 6.a.S fish were assigned to all of the hauls. Proportions of individuals classified as 6.a.N, 6.a.S and unknown were plotted on a map to show the distribution of hauls from the *all ages* dataset (Figure 5.14). The membership probability plots can be found in Annex 8 for the *all ages*, *age interval 1-3 years*, *age interval 4-6 years* and *age interval 7+ years* datasets for the eight hauls from the 2015 MSHAS.

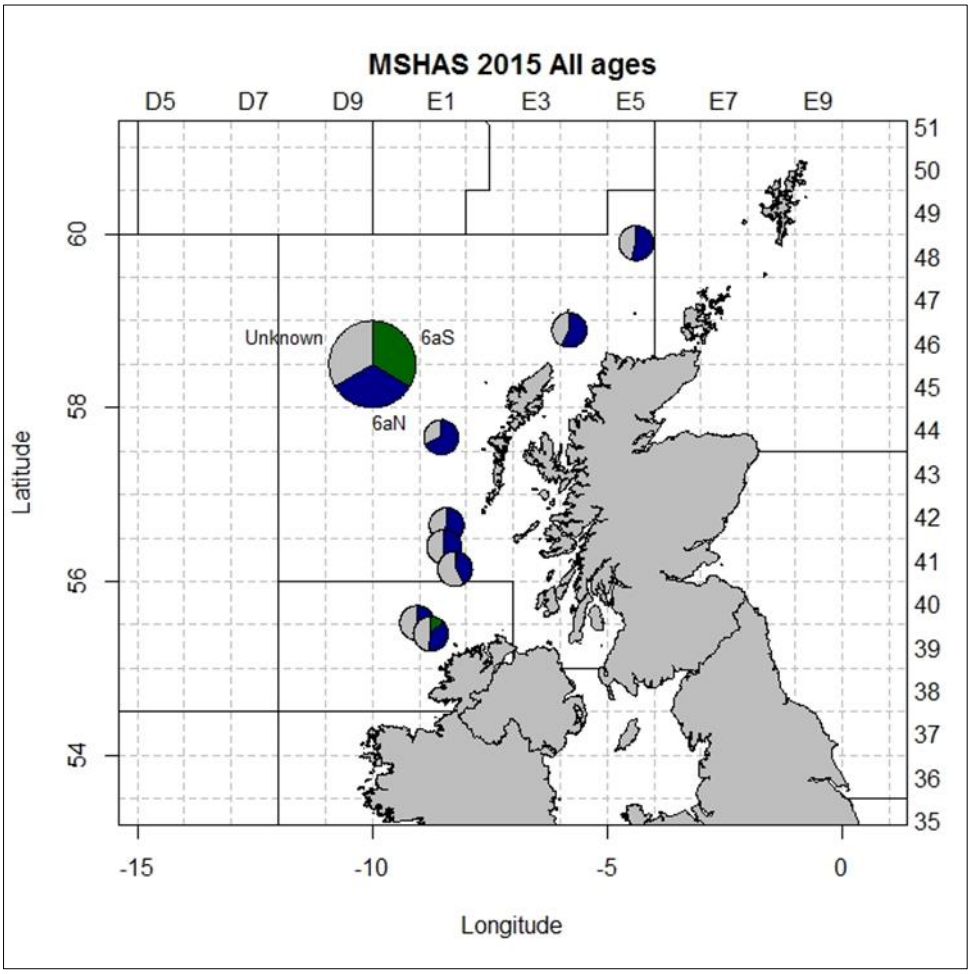


Figure 5.14. Summary of the morphometric assignments of the 2015 MSHAS samples for the all ages dataset.

5.10. *Morphometric limitations*

The morphometric baseline samples collected from 6.a.N and 6.a.S showed that the body and otolith shape differ between these two reproductively isolated stocks. Although the classification success rates compared well with the classification rates of peer reviewed stock discrimination studies for herring in other areas and other species (Libungan *et al.*, 2015; Duncan *et al.*, 2018; Armstrong and Cadrin 2001), the baseline dataset could not be used to assign herring from a mixed sample. There was very poor agreement between the population assignments of the MSHAS samples from genetic data and morphometric data. By running these analyses in parallel, limitations to the 6.a, 7.b and 7.c morphometric dataset were highlighted.

There was a difference in sample size between 6.a.N (N=805 herring) and 6.a.S (N=506 herring) baseline datasets. Imbalance between sample size is more of an issue when the sample sizes are small because this can cause bias in the results of statistical tests, such as ANOVA. In some cases, groups with a larger sample size can have a greater influence on the classification model but the *SVM* model chosen to analyse the 6.a herring data is resistant to sample size imbalances (Qiao *et al*., 2015).

The difficulty in obtaining a baseline sample for morphometrics, as discussed in section 5.4, limits the amount of samples that are feasible to collect. The herring must be pinned out and photographed when they are fresh and must not be in a state of rigor mortis, which depending on the conditions, can set in quite quickly (ICES, 2017a). Herring can spawn in an area within a very short space of time and the event could take place over one or two days. It requires enough fish in the catch to be in spawning condition to carry out the morphometric sampling, cooperation and coordination between the fishing industry and the project partners must good, and organisation of resources, including staff to be available last minute to ensure efficient processing of the sample. Being restricted in the number of spawning samples that can be collected reduces the ability of the data to represent all of the fish in the area. The genetic data noted a greater genetic diversity in 6.a.S herring, where these fish are known to spawn in winter (Molloy, 2005). Samples used in this dataset were collected from November through to January so collecting just one or two baseline samples for 6.a.S every year will not reflect this variation in the morphometrics. The classification success rates are generally lower for 6.a.S in all analysis carried out which maybe a result of the large variation in this population.

Genetic samples of spawning individuals were also obtained from a 6.a.N spring spawning component in the Minch. These herring are sampled annually during the MSHAS while they feed west of the Hebrides and mix with the 6.a.N autumn and 6.a.S herring. There is no baseline morphometric data available for 6.a.N spring spawning herring to include in the analysis and as a result the morphometric data will not be able to assign these fish correctly and they will end up being classed as one of the other two populations, or they will be classified as unknown which increases the uncertainty around the morphometric assignments of the MSHAS.

5.11. Conclusions

Baseline data is vital for the success of identifying phenotypic traits of a population and distinguishing between different populations (Hüssy et al., 2016; Cadrin et al., 2014). These samples build a profile of the herring that spawn in that area. Despite the success in classification of the 6.a.N and 6.a.S baseline samples, it appears that morphometric methods are not suitable to distinguish between the mixed samples of herring caught during the MSHAS. Although these populations are reproductively and genetically distinct, the overlap in their spatial ranges reduces the variation in environmental factors acting on the fish, which could result in very similar body and otolith shapes in the two populations. Issues with the morphometric baseline samples - such as vagrants, missing spawning populations and imbalanced sample sizes – could also have been a factor. This possibility is investigated in the next task by using genetically classified fish to form a morphometric baseline.

6. Task 6 – Genetic information vs. morphometric information

6.1. Objectives

- *Compare genetic identification to morphometric identification of same samples.*
- *Assess comparability of results of both methods.*
- *Attempt to retrospectively split acoustic time series to 2010.*
- *Compare samples from previous studies/projects to determine if these samples can also be divided.*
- *Determine if rapid genetic method can be used as sole method to split stocks in the summer acoustic surveys.*

6.2. Deliverables

- Retrospective split of survey time series data to 2010: ***Not possible***
- Test of applicability of the split to WESTHER samples: ***Not possible***
- If necessary, a test of new hybrid technique: ***Not possible***

6.3. Known-unknown comparison

Genetic and morphometric data were both collected from baseline and MSHAS samples since 2014 (Tables 3.5 and 3.7). The two datasets were analysed separately (Sections 4 and 5) to ensure the most suitable analysis has been applied to each method to optimise the datasets. The objective in the current section was to compare the MSHAS sample assignments of both methods and assess the comparability of the methods.

One of the indicators used, in the current study, as a measure of model success for the genetics and morphometric analyses was the ability of the baseline samples to assign known-unknown samples correctly. Baseline self-assignment figures were >90% for the genetic approaches and >65% for morphometric approaches. However, it was in the assignment of known-unknown samples that the issues with the morphometric methods became apparent. Known-unknown assignments were performed in both the genetic (Section 4.9) and morphometric (Section 5.9) analyses using a range of samples and two additional analyses are performed here in order for a direct comparison to be made between three of these samples: *6aS_19c, 6aS_17a, 6aN_16d* (Table 3.5).

The 2019 6.a.S sample (*6aS_19c*) was used as a known-unknown sample in both the genetic and morphometric analyses. The *level 1* genetic assignments of this sample indicated a high level of correct assignment, with 92% and 96% of the individuals assigned to *6aS/6aN_Sp* and *Group_2+3+4*, respectively (Figure 4.20 and Table 6.1). The morphometric analyses indicated the converse with the majority of the individuals misassigning to the 6.a.N autumn spawning group (Figure 5.14 and Tables 5.10 and 6.1).

One of the 2017 6.a.S samples (*6aS_17a)* was also used as a known-unknown by extracting the sample from the baseline datasets and then attempting to reassign it. The *level 1* genetic assignments of this sample indicated a high level of correct assignment, with 98% and 100% of the individuals assigned to *6aS/6aN_Sp* and *Group_2+3+4*, respectively (Figure 6.1 and Table 6.1). The morphometric assignment was more uncertain with only 68.9% of the individuals assigning to the correct population group and also had a higher level of unknown individuals, 22.6% (Figure 5.13 and Tables 5.9 and 6.1).

One sample from 6.a.N autumn spawning group (*6aN_16d*) was used as a known unknown by extracting the sample from the baseline datasets and then attempting to reassign it. The *level 1* genetic assignments of this sample indicated a high level of correct assignment, with 97% of the individuals assigned to *6aN_Aut* and *Group_1+5*, respectively (Figure 6.2 and Table 6.1). The morphometric assignment was again more uncertain and indicated that only 67.5% of the individuals assigned to the correct population group (Figure 5.12 and Tables 5.8 and 6.1).

In the analyses above, it is evident that there was a significant error rate associated with the morphometric assignments, which was not evident in the genetic assignments.

Table 6.1. Percentage assignment of known-unknown samples back to their correct population of origin.

Sample	Genetics		Morphometrics (all ages)
	Approach 1	*Approach 2*	
6aN_16d	97%	97%	67.5%
6aS_17a	98%	100%	68.9%
6aS_19c	92%	96%	22.9%

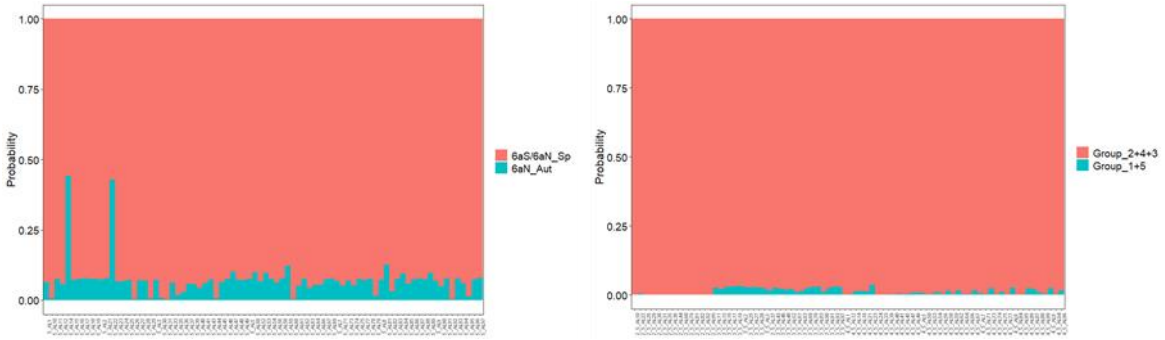


Figure 6.1. Genetic assignment of the *6aS_17a* sample to the genetic baseline with associated probabilities. (left) approach 1 level 1 (right) approach 2 level 1.

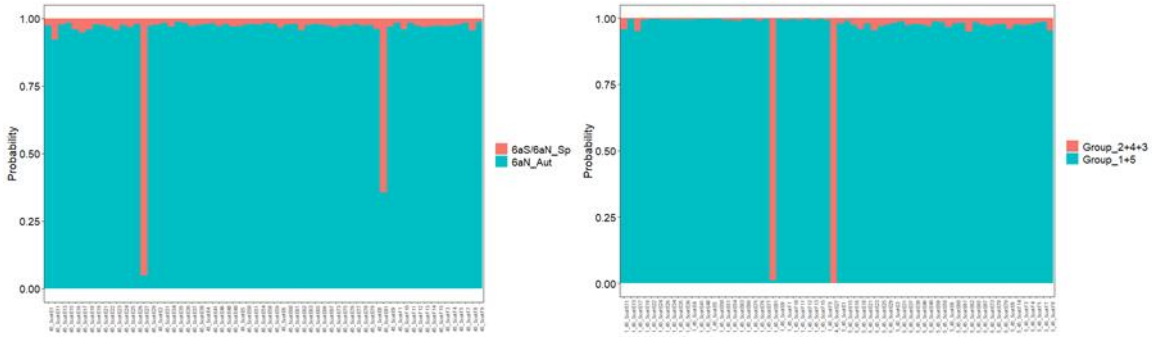


Figure 6.2. Genetic assignment of the *6aN_16d* sample to the genetic baseline with associated probabilities. (left) approach 1 level 1 (right) approach 2 level 1.

6.4. MSHAS samples

The genetic approach (Section 4) provided robust baseline assignments as confirmed through cross-validation and test assignments on known-unknown samples. Therefore, it was used for the further analyses of the 2014 – 2019 MSHAS samples (Section 4.11). Whilst the morphometric methods provided an acceptable level of self-assignment for the baseline dataset, they were not able to assign known-unknown samples robustly and exploratory assignment of the 2015 MSHAS samples indicated a high level of uncertainty in the assignments.

One additional significant limitation of the morphometric dataset that has been highlighted by the genetic dataset is the lack of samples from the 6.a.N spring spawning herring. It is evident from the genetic assignment of the MSHAS samples (Section 4.11), there was potentially a significant proportion of these fish in the MSHAS hauls (Figures 4.30 – 4.35). If the 6.a.N spring population is not represented in the morphometric baseline, it is not possible for them to be recognised or assigned in the mixed samples, thus adding further uncertainty to the morphometric assignments.

The overall conclusion is that the morphometric methods were not suitable for analysing the mixed MSHAS samples (Section 5.11). Therefore, there is little benefit in undertaking

further comparisons of the two methods as they will likely lead to erroneous conclusions. However, for completeness one further analysis was attempted.

6.5. Genetic MSHAS assignments to inform morphometrics

Due to the success of the genetic MSHAS assignments and the confidence in the results compared with the lack of confidence in the results of the morphometric MSHAS assignments, it was decided to conduct an exploratory assignment by using the genetic MSHAS assignments to inform the morphometrics.

Three hauls were selected from the 2015 and 2017 MSHAS samples and in this instance the genetic assignment was taken as the population of origin for each individual fish. These new groupings were used to create an exploratory '*new baseline*' dataset for the morphometric data. If the self-assignments of these selected samples were successful, this MSHAS baseline dataset would be used to provide a population assignment for individuals in the other MSHAS samples from 2014-2019 for comparison with the genetic assignments of the same samples. If consensus was found between the two sets of assignments, then it may be possible to use this new baseline to split the 2010-2013 MSHAS samples. After adding the population assignments from the genetic results to the morphometric data, the new baseline dataset was processed using the methods described in Section 5.8 for the actual baseline dataset. Maturity correlated variables were removed and length effect corrected, and variables influenced by inter-annual variability were removed. All of the body variables were temporally unstable and needed to be removed so this step of the process was not used for this exploration.

Using the tuned *SVM* model, self-assignment values were calculated for the '*new baseline*' dataset. The results showed a very poor assignment of individuals to their population, the data could not recognise any of the herring groups with any degree of confidence (Figure 6.3). A *DAPC* of the data showed a low level of clustering between the three populations (Figure 6.4). The *6aN_Sp* herring had the most distinct cluster but with no baseline sample, the morphometrics will always be limited in its ability to split these data. The clusters for *6aN_Aut* and *6aS* herring had a large area of overlap and added to the evidence that the morphometric data does not have the ability to assign the correct population of origin to the MSHAS samples. To ensure this dataset was explored thoroughly, variables influenced by inter-annual variability were removed, leaving 10 otolith variables only. The *DAPC* was run on this temporally stable data as well to see how it could improve the output but the clustering was largely overlapping between all populations so no improvement was made on the assignment (Figure 6.5).

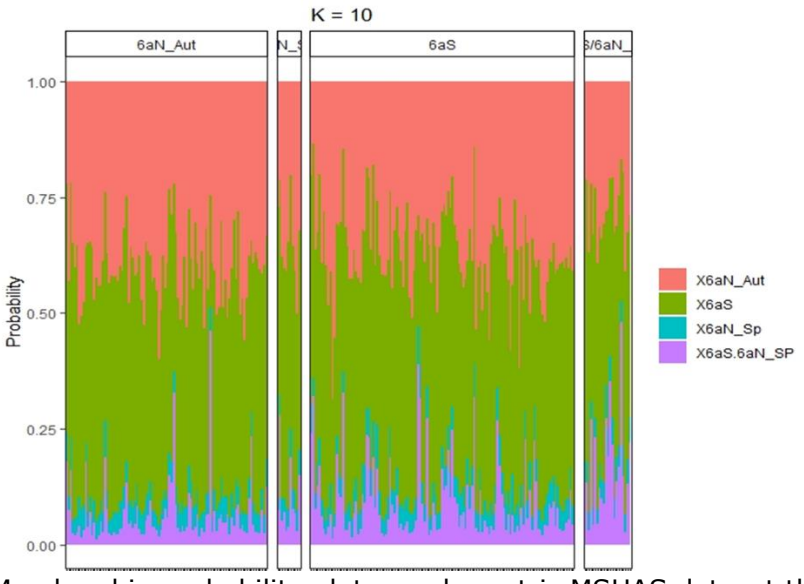


Figure 6.3. Membership probability plot morphometric MSHAS dataset that had been informed by the genetics.

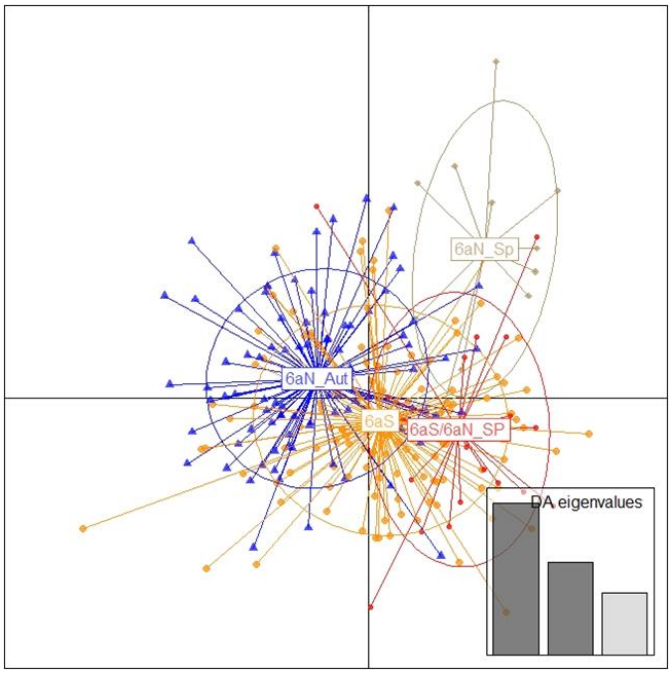


Figure 6.4. DAPC clustering plot of the four groups; *6aN_Aut*, *6aN_Sp*, *6aS* and a *6aS/6aN_Sp* mix.

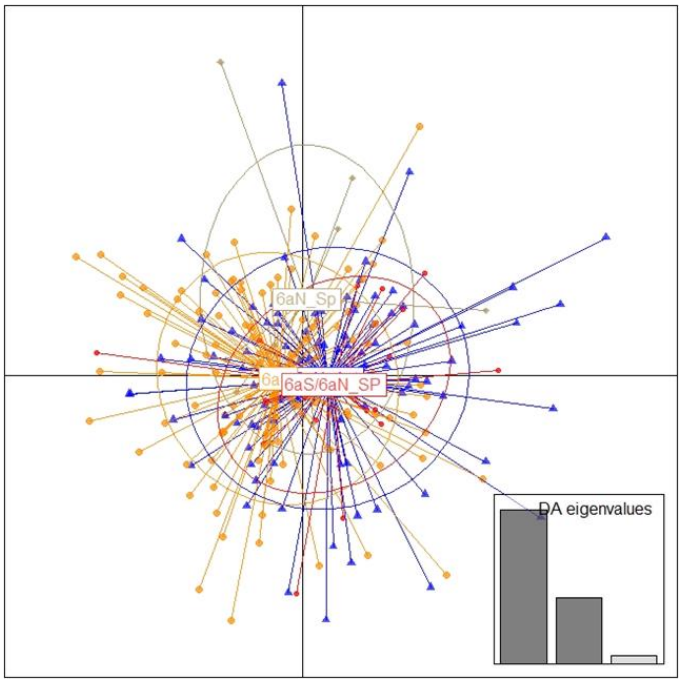


Figure 6.5. DAPC clustering plot of the four groups; *6aN_Aut*, *6aN_Sp*, *6aS* and a *6aS/6aN_Sp* mix using data corrected for interannual variability.

6.6. Conclusions

As described above the morphometric methods cannot be used to perform assignments of the MSHAS samples. Therefore, it was not possible to retrospectively split the time series data for period 2010-2013, for which genetic samples were not available. The only reliably split time series available for the herring stocks in Division 6.a, 7.b and 7.c commences in 2014. It is not possible to split the survey or commercial catch data prior to this period based on the analyses undertaken in this study.

7. Task 7 – Cost-effective protocol

7.1. Objectives

- Deliver a consistent and detailed protocol to divide samples into two different stocks.
- Protocol to be based on genetic method with minimum effort and cost.
- Protocol to include all stages from sampling to final differentiation of stocks.
- Provide a list of most suitable genetic markers.

7.2. Deliverable

- A consistent and detailed protocol to divide samples into two different stocks: ***Completed***

7.3. Genetic sample collection protocol

This is a detailed protocol for the collection of standardised tissue samples, for genetic analysis, from herring caught during scientific surveys or from commercial catches. The protocol is based on using the recently developed genetic tissue sample collection tool, which is produced and supplied by LVL Technologies GmbH & Co. KG, Crailsheim, Germany (http://www.lvl-technologies.com/). The novel system is based on LVL's range of SAFE® biobanking consumables and pairs a standard SBS format 96-tube barcoded rack and 2D barcoded tubes with a genetic sampling tool incorporated into the screwcap of the tubes. The unique sampling tool is designed specifically for use on fish and the pointed tip and rear facing cutting edge enables collection of a c.30mg tissue sample from beneath the skin of the fish being sampled, thus avoiding surface contamination. The tools, tubes and racks come pre-assembled and pre-sterilised. When used in conjunction with the 'Manual 1-Channel Capper/Decapper/Picker', it enables the user to select a sampling tool from a specific tube, to collect a genetic sample from the fish and return the tool, with the sample in it, to the collection tube without ever touching the tube or sample directly, thus reducing the possibility of cross contamination. It also removes the need for sterilising equipment (e.g. scissors, knives and forceps) between samples. The filled racks can be stored down to -196°C and, as they are SBS format, are compatible with all standard molecular laboratory equipment, enabling incorporation into automated workflows. The development of this tool represents a significant advance in the large-scale sampling of marine fish for genetics and it should be adopted as the standard system. The system also enables rapid and cost-efficient processing of samples post collection as detailed in Section 7.4 and long-term archiving of high-quality samples for future analysis.

All prices are list prices for the Irish marketplace. Equipment listed is the equipment available to and used in the current project. The prices for a single plate of 96 individual samples are detailed below.

Equipment and software

- Rainin Pipe-Lite™ XLS+ manual multi-channel pipette L8, 1000µL
- Grant UV/PCR cabinet
- Semi-automated 8-channel capper/decapper for SAFE® 96 IT tubes (LVL Technologies product code: CDC-8CH-IT)
- Manual 1-channel capper/decapper for SAFE® 96 IT tubes (LVL Technologies product code: CDC-1CH-UNI)
- SAFE® Multi standard rack scanner (LVL Technologies product code: DMTR-DF-MR-CP)
- Scanner software Ziath DataPaq™ version 3.18
- Ziath Samples database software

Table 7.1. Consumables required for tissue sample collection from herring and approximate costs excluding VAT for a single sample of 96 individuals.

Description	Cat #	Pk number	€ per unit	# for 1 x 96	€ per 1 x 96
LI 1000 96 Tube Rack PC with lid (slide lock), 1D barcode, 2D orientation code and stacked, blue capped tubes with tissue collection rack, with internal thread and 2D code	LVL Technologies DNC-I10-TC-NS-SLC-	50 racks per case	3,750	1	75
Gloves Nitrile	Anachem 95016910	1000	146.00	2	0.29
TerraRack™ Tips LTS 1000UL Fltr 768/8 TR-L1000F	Anachem	768 tips in 8 racks	97.00	0.2 rack	2.43
Ethanol Absolute 99.8+%, Certified AR for Analysis, meets analytical specification	Fisher Scientific 10437341	2.5L	20	0.04	0.80
				Total	78.52

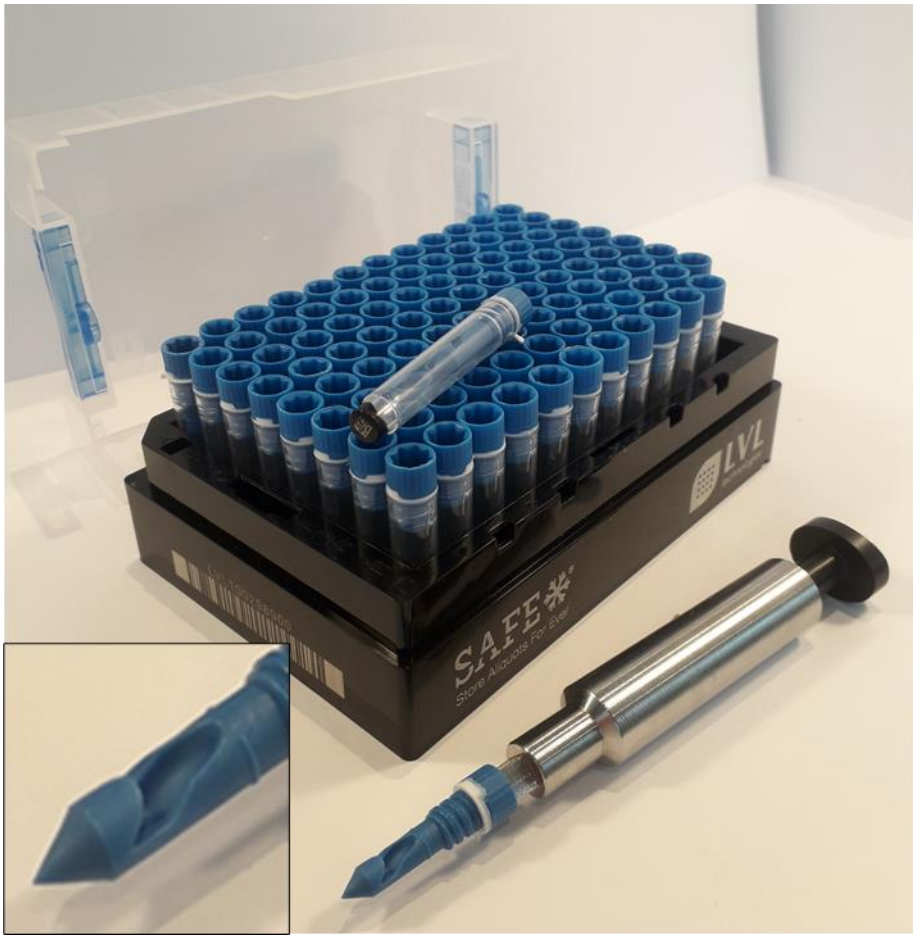


Figure 7.1. The LVL Technologies SAFE® genetic sampling system. A close up of the tissue collection window is shown in the inset.

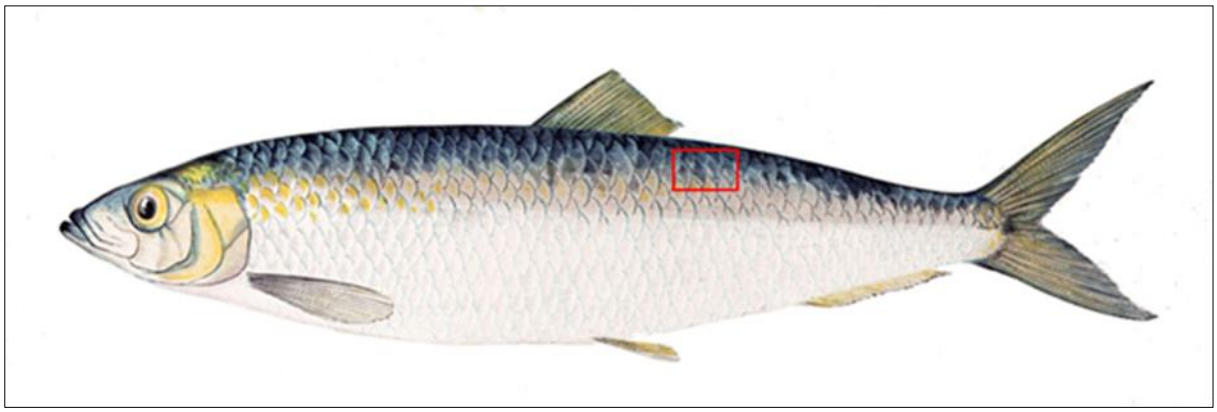


Figure 7.2. An Atlantic herring with the area for sample collection marked with a red box.

Pre-sampling preparation

1. Complete these steps in a 'clean' laboratory away from sources of potential contamination and observe aseptic conditions throughout.
2. Racks should be pre-scanned with *DataPaq*™ software, and rack and tube data saved in the relevant database, which was *Ziath Samples* software in the current study.
3. Sterilise the working area including the surfaces of the UV cabinet with Microsol 4 Decontaminant or a 10% bleach solution.
4. Sterilise all plastic consumables in the UV cabinet with 15 minutes of UV light.
5. Racks should be pre-filled with molecular grade absolute ethanol prior to starting to process samples. To achieve this, use the semi-automated 8 Channel Capper/Decapper to remove 8 sample tool caps at a time and add 875µl EtoH to each tube with the manual eight channel 1000ul pipette. Recap the tubes and repeat until all 96 tubes have been filled. Ensure aseptic conditions when preparing the tubes and racks.

Sampling Method

1. Take 96 randomly selected herring from a single haul if possible. When collecting baseline samples ensure that samples are in spawning condition and when collecting MSHAS follow the standard survey sampling protocols for collection of age samples. Ideally the genetic sampling stage should be included as stage 1 prior to extraction of the otoliths or to opening the body cavity for maturity stage classification. This will limit potential for cross contamination.
2. Lay out fish and wash down the surface to remove excess surface contamination e.g. blood, slime and loose scales.
3. 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.
4. Start sampling the first fish.
5. Using the manual 1-channel capper/decapper tool, select the first tube in the rack in the A1 position and unscrew the cap.
6. Push the sampling tool at an angle of c.45° into the dorsal musculature of the herring on the area indicated in Figure 7.2. When inserting the tool ensure that the tissue collection window is facing down. Once tool has been inserted up to the stop line indicated in the tool, rotate the handle 180° and withdraw from the fish. A c.30mg sample of white muscle tissue should be firmly contained within the tissue collection window (Figure 7.1).
7. Return the sampling tool to the relevant tube, screw closed and depress the plunger to eject the tube.
8. Measure the total length, weight, sex and maturity of the fish and record on data sheet beside the relevant sample no.
9. Move to the next fish and the next sample tube (A2) and repeat. Always work in the order A1-A12, B1-B12 etc.
10. Once sampling is competed store the box of sample tubes upright in a fridge (4°C) or freezer (-20°C) until further processing.

11. Sterilise all work surfaces and equipment with Microsol 4 Decontaminant or a 10% bleach solution and UV before proceeding to the next sample.

Costing
The approximate cost for sampling 96 individual fish as described above is €78.52 for materials or €0.81 per individual fish in materials. The labour required for the protocol described above is one person and approximately 1 hr. Labour cannot be costed here as this is wholly dependent on where the work is conducted and also by the level of the person undertaking it. Equipment is not costed for in this protocol as it is assumed that this is available in the laboratory undertaking the work.

Alternative methods
A number of different types of sample tubes and labelling systems have been tried during the current study, including hand labelling 2ml microtubes, manually applying solvent and freezer resistant labels to microtubes, prelabelled linear barcoded tubes and boxes etc. All require a significant amount of time to prepare tubes prior to sampling and none were compatible with downstream automated sample processing. None of these other methods had a built-in sample collection tool and all required additional sterilisation steps for the sampling implements used, thus adding significant time to the processing of samples. Further, despite a detailed protocol being provided there was significant variation in the size of piece of tissue collected by different samplers. In some instances, this led to poor preservation of the tissue when the ratio of tissue to preservative was too high. The original WESTHER samples were collected with a range of different tube types from simple 1.5ml eppendorf type tubes to 2ml microtubes with and without a rubber sealing gasket. As a result, there was significant variation in the state of preservation of the samples ranging from decomposed to well preserved.

The system described above is the best available approach for the collection of tissue samples for genetic analysis of marine fish such as herring. The system collects standard sized samples, which ensures proper preservation and removes the need for subsampling of samples for DNA extraction (Section 7.4). The system also removes the need for laborious labelling of tubes and ensures that the labels remain legible. The SBS format enables incorporation of the sample racks into automated workflows using standard molecular laboratory equipment. The SBS format is also smaller than existing sample collection tube boxes and reduces the space required for long term archiving of samples. Adoption of a single system for tissue sample collection method for genetic analyses across all surveys and commercial sampling programmes will enable easier transfer of samples between laboratories and will also enable easier establishment of standard protocols for processing the samples for genetic analyses. This is important given the international nature of fisheries surveys and data collection within the Northeast Atlantic area.

7.4. DNA extraction protocol

This is a detailed protocol for the high-throughput DNA extraction from herring muscle tissue samples collected with the LVL Technologies genetic tissue sample collection tool (Section 7.3). All prices are list prices for the Irish marketplace. Equipment listed is the equipment available to and used in the current project. The prices and work for a single sample of 96 individuals are detailed below.

Table 7.2. Consumables required for DNA extraction and approximate costs excluding VAT for a single sample of 96 individuals.

Description	Cat #	Pk number	€ per unit Ex VAT	# for 1 x 96	€ per 1 x 96
Tip Liq96 20 µL Filter 960/10 LQR-20F	Anachem 17011117	10 racks	190.00	1 rack	19.00
TerraRack™ Tips LTS 1000UL Fltr 768/8 TR-L1000F	Anachem 17014967	8 racks	97.00	0.2 rack	2.43
Encode syringe strl 0.5	Anachem 17001872	100	191.00	1	1.91
Gloves Nitrile	Anachem 95016910	1000	146.00	2	0.29
Low profile deep-well plate PP with round cavities 1.2ml	LVL Tech 225.DW.1.2.PP	50	105	1	2.10
4titude standard 96-well semi-skirted PCR plate clear polypropylene	Analab PCR1096	50	106.25	1	2.13
4titude 8-Strip flat caps optically clear polypropylene	Analab PCR1066	300	93.75	12	3.75
4titude foil heat seal plate seal pierceable sheets	Analab PCR0620	100	61.86	1	0.62
Proteinase K, recombinant, PCR grade 20 mg/µl	Biosciences EO0492	5 x 1 mL	151.19	0.5 mL	15.12
Chelex 100	Sigma C7901-100G	100g	275.00	0.14	7.70
				Total	55.05

Equipment

- Grant UV/PCR cabinet
- Simax Reagent Glass Bottle 250mL
- Magnetic Stirrer Bar PTFE with Pivot Ring 4.5x12mm
- AutoRep™ S manual repeater pipette
- Rainin Pipet-Lite™ XLS+ manual single-channel pipette, 100-1000 µL
- Semi-automated 8-channel capper/decapper for SAFE® 96 IT Tubes
- Stuart Stirrer US151 Stainless Steel Top
- Eppendorf Heatsealer S100 with low profile plate adaptor
- SLS Lab Basics Vortex Mixer
- Eppendorf Thermomixer C with Smartblock thermoblock for plates
- Rainin Benchsmart 96 semi-automated pipettor with 20 µl pipetting head
- Eppendorf Centrifuge 5804 with Eppendorf A-2-DWP deep-well plate swing out rotor

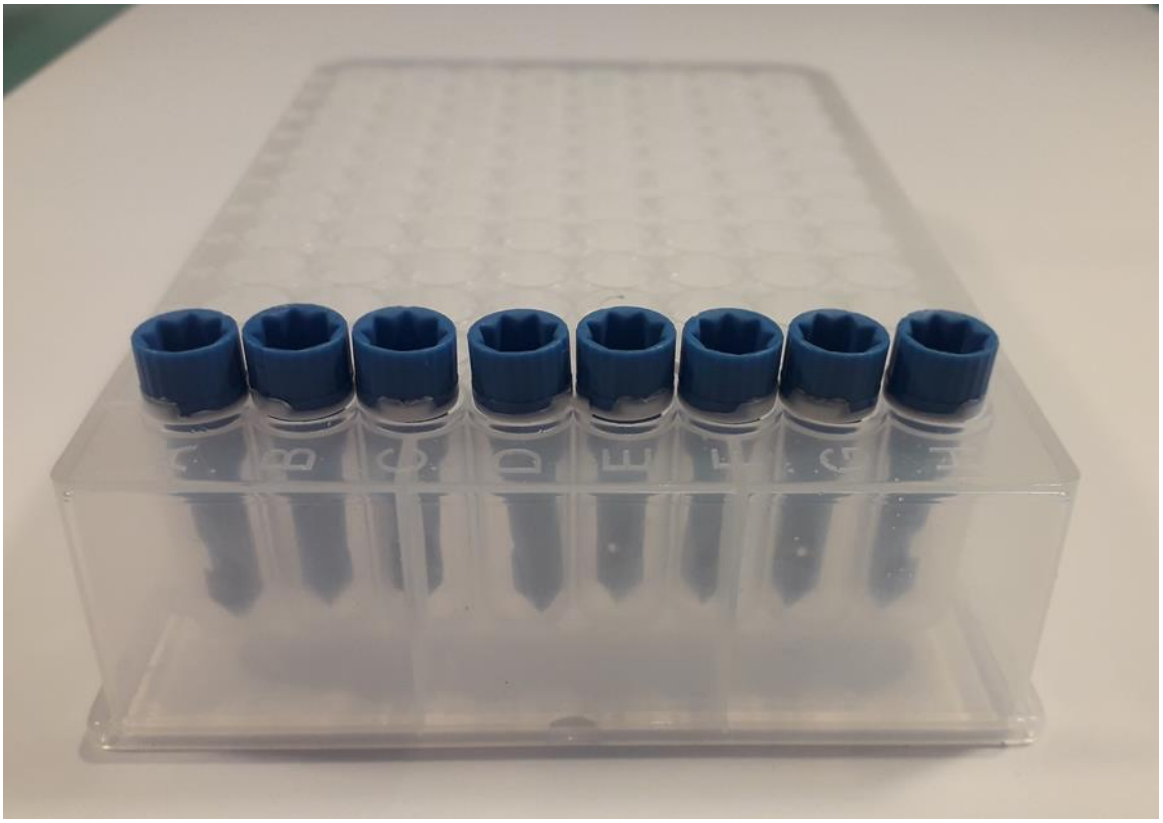


Figure 7.3. The LVL Technologies SAFE® genetic sampling tool in the LVL low profile deep-well plate for DNA extraction.

Digestion plate setup

1. Sterilise the working area including the surfaces of the UV cabinet with Microsol 4 Decontaminant or a 10% bleach solution.
2. Sterilise all plastic consumables and glassware in the UV cabinet with 15 minutes of UV light.
3. Ensuring aseptic conditions, prepare a 10% suspension of Chelex by adding 20g of Chelex resin to a glass reagent bottle and make up to 200ml with Ultrapure H_20.
4. Add the sterile magnetic stirrer bar to the bottle
5. Cut approximately 3mm off the end of twelve 1000µl pipette tips using a sterile scissors.
6. Using the AutoRep™ S manual repeater pipette with a 0.5mL Encode Syringe add 5µl of Proteinase K (20 mg/µl) to each of the 96 wells of the 1.2ml deep-well plate (digestion plate).
7. Put bottle of 10% Chelex suspension on the magnetic stirrer plate and mix to ensure all Chelex resin is in suspension. Keep mixing throughout step 8.
8. Using the Rainin Pipet-Lite™ XLS+ manual single-channel pipette, 100-1000µl, add 300µl of 10% Chelex suspension to each of the 96 wells of the digestion plate.
9. Digestion plates may be prepared in batches sealed and stored at -20°C until further use or they may be used immediately.

Extraction Method

1. Sterilise the working area including the surfaces of the UV cabinet with Microsol 4 Decontaminant or a 10% bleach solution.
2. Sterilise all plastic consumables in the UV cabinet with 15 minutes of UV light.
3. Use the semi-automated 8-channel capper/decapper to remove eight sample tool caps, with samples, from the sample tubes. Allow ethanol to drain through the drainage hole and rinse briefly with sterile water.
4. Eject the eight sample tools into their respective wells on the preprepared digestion plate (Figure 7.3).

5. Repeat step 2 with the next eight samples until all 96 samples have transferred to the digestion plate.
6. Transfer digestion plate to Eppendorf Thermomixer at 56°C for 60 mins.
7. After the 60 mins, use the semi-automated 8-channel capper/decapper to transfer the sample tools with the remaining samples in place back to their original storage rack. Save remaining undigested tissue for archiving.
8. Seal the digestion plate with a foil heat seal on the Eppendorf Heatsealer S100. Sealing takes approximately 6 seconds and ensure all wells are properly sealed.
9. Vortex the plate for 5 seconds to ensure all contents are thoroughly mixed.
10. Increase the Thermomixer temperature to 100°C and incubate the digestion plate for 15 mins.
11. Remove the digestion plate from the thermomixer, vortex for 5 seconds and place in a fridge at 4°C to cool for 15 mins.
12. Once cool, vortex the plate for 5 seconds and centrifuge for 1 minute at maximum speed on the Eppendorf Centrifuge 5804 with Eppendorf A-2-DWP deep-well plate swing out rotor
13. Remove the foil seal and transfer the digestion plate to the Rainin Benchsmart 96 semi-automated pipettor.
14. Load a new rack of sterile filter tips to the Benchsmart and transfer the required volume of supernatant from the digestion plate to a new sterile PCR plate. In the current project 60µl of supernatant were transferred. Special care must be taken to avoid aspirating the Chelex resin with the supernatant.
15. Seal the PCR plate with strip caps and freeze at -20°C if the samples will not be processed immediately.
16. Sterilise all work surfaces and equipment with Microsol 4 Decontaminant or a 10% bleach solution and UV before proceeding to the next box of samples.

Costing

The approximate cost per 96 individual fish run in a single plate as described above is €55.05 per 96 individuals for materials or €0.57 per individual fish in materials. The labour required for the protocol described above is one person and approximately 0.5 hrs excluding the incubation times. Labour cannot be costed here as this is wholly dependent on where the work is conducted and also by the level of the person undertaking it. Equipment is not costed for in this protocol as it is assumed that this is available in the laboratory undertaking the work. The use of the LVL genetic sampling system in the protocol above removes the need to subsample each individual sample prior to DNA extraction. This represents a saving of approximately 1.5 hrs per plate of 96 individuals.

Alternative methods

Using a proprietary DNA extraction kit such as the Qiagen DNeasy 96 Blood and Tissue Kit (Product code 69581) would cost approximately €3.00-3.80, excluding labour, per individual fish, depending on the number of samples to be extracted.

Outsourcing to a service provider for extraction of DNA and return of quantified DNA in 96-well PCR plates ranges from €2.00 per individual fish to €4+, including labour, depending on the method, the supplier used and the order volume.

7.5. Genotyping protocol

This is a detailed protocol for undertaking the PCR amplification, data processing and genotyping of herring genetic markers and preparing the pooled amplicons for sequencing.

Table 7.3. Consumables required for PCR amplification of genetic markers and approximate costs. Costs are based on the assumption of 24 plates of DNA amplified at 3 multiplexes comprising primers for 45 SNPs.

Description	Cat #	Pk number	€ per unit	# required	€
Molecular primers 100uM RxnReady Oligo	IDT	45	1197.00	1	1197.00
96 Barcode primers 100 uM LabReady Oligo	IDT	96	1056.00	11	1056.00
QIAGEN Multiplex PCR Kit	Qiagen 206145	25ml	2,004.00	3	6012
Nuclease-fee water	Qiagen 129115	1000ml	53.50	50ml	2.68
Gloves Nitrile	Anachem 95016910	1000	146.00	1	146.00
CLEARLock Microtube Polypropylene with Cap 1.5mL	Analab TUB1036	1000	32.50	200	6.50
4titude standard 96-well semi-skirted PCR plate clear polypropylene	Analab PCR1096	50	106.25	3.1	327.25
4titude 8-Strip flat caps optically clear polypropylene	Analab PCR1066	300	93.75	120	37.50
4titude foil heat seal	Analab PCR0620	100	61.86	1.44	89.00
Encode Syringe Strl 0.5 mL	Anachem 17001872	100	191.00	1	191.00
Tip Liq96 20 µL Filter 960/10 LQR-20F	Analab 17011117	10 racks	195.00	8	1,560
TerraRack™ Tips LTS 20UL Fltr 960/10 TR-L10F	Anachem 17014961	10 racks	126.00	3	378
TerraRack™ Tips LTS 200UL Fltr 960/10 TR-L200F	Anachem 17014963	10 racks	119.00	0.2	23.80
TerraRack™ Tips LTS 300UL Fltr 768/8 TR-L300F	Anachem 17014965	8 racks	119.00	0.125	14.88
TerraRack™ Tips LTS 1000UL Fltr 768/8 TR-L1000F	Anachem 17014967	8 racks	97.00	0.125	12.13
E-Gel agarose gels with SYBR Safe, 1.2%	Biosciences G521801	18	246.56	1	246.56
E-Gel 1 Kb Plus DNA Ladder with E-Gel Sample Loading Buffer (1X)	Biosciences 10488090	2x1000 µL	243.07	0.2	46.81
Qubit™ Assay Tubes	Biosciences Q32856	500	64.71	0.2	12.94
Qubit™ dsDNA BR Assay Kit	Biosciences Q32850	100 assays	74.92	1	74.92
Qubit® dsDNA HS Assay Kit	Biosciences Q32851	100 assays	88.75	1	88.75
Applied Biosystems™ ExoSAP-IT™ express PCR product cleanup reagent	Biosciences 75001.200.UL	100 rxn	104.67	1	104.67
Metafast library prep and MiSeq v3 2x250 bp sequencing	Fasteris	1 run	2,520.00	1	2520.00
				Total	14,148

Equipment

- Grant UV/PCR cabinet
- AutoRep™ S manual repeater pipette
- Rainin Pipet-Lite™ XLS+ manual single-channel pipette, 0.5-10µL
- Rainin Pipet-Lite™ XLS+ manual single-channel pipette, 2-20µL
- Rainin Pipet-Lite™ XLS+ manual single-channel pipette, 10-100µL
- Rainin Pipet-Lite™ XLS+ manual single-channel pipette, 20-300µL
- Rainin Pipet-Lite™ XLS+ manual single-channel pipette, 100-1000µL
- Rainin Pipet-Lite™ XLS+ manual 8-channel pipette, 0.5-10µL
- Eppendorf Heatsealer S100 with low profile plate adaptor
- SLS Lab Basics Vortex Mixer
- Rainin Benchsmart 96 semi-automated pipettor with 20 µl pipetting head
- Eppendorf Centrifuge 5804 with Eppendorf A-2-DWP deep-well plate swing out rotor
- Applied Biosciences SimpliAmp™ Thermal Cycler
- Invitrogen Qubit 4 Flurometer
- Invitrogen E-Gel powersnap electrophoresis system

Table 7.4. Forward and reverse primers for the 45 SNP used in the current study.

Name	Primer F	Primer R
Uher_309_092	TTTCCACGGCACACAGTCTT	TCTGACAGTGTGCTTGGTCC
HerSNP52	CGAGCTGCAGACCTTACAC	CATGAAAACTGCCTGCGATG
HerSNP53	GGGTGATTTCTTTGTGCAGGG	AGACACAGCACTGAATTCCTG
HerSNP54	ATAGCTCTGGTGGCAAAGGC	GCAGAATGCCATTTACCAGTG
HerSNP55	GCATACGGTGACAGGTGTAC	TGCCTTCAGATGCAGGTGAC
HerSNP57b	AGACATGGAAGCTGCAGTG	CATCTCAGGTGTGGCTCGAG
HerSNP60	TGCCAGAGCATTCGTGACC	TGCTGAGACCTCCACTTTGC
Uher_139_161	TGGAAACATGGCAAATCGCA	TTCCAACTAGGTGCTTGAAACC
Uher_261_rd	TACTCACGCTACACCCAGGT	TTCGGCTTCAGTTAGTGAGATGT
Uher_115_045	TTCTTGGGGCATCACTCCTG	TAGGTGACTTGCTTTGTAG
Uher_356_099	TGGTCTGTCCCTAATGGTGC	GAGGCAATGTGGAAACGTCG
Uher_276	GAATGCCAGAGCCTCCCTAC	CGGCTCACCTATAAGCATATCAG
HerSNP14	TGGCTGTAAATCTCGCACC	AATCTCTCCCACTGCGTGAC
Uher_1440_140	TGAGTAAAAGGGCCACTGGTC	CAGACATCCGCTAGAACCACA
Uher_294	TCCTGGGCAAGAAATGTGCT	TATCCTGGGTGCGGAAAAGG
HerSNP9	GATTGCTACTGAACTATGGGAAGC	TGTTGGTTATGAGTCAAACCTG
HerSNP7	TCTCTTACACACGCACGGAC	TCCAGCACTTTGTGACTGC
HerSNP4	GGCGGTGTTTTCTTCTTGCTC	GCACAGCCACTCTTGAAAGC
HerSNP3	GCCTGGAAATGTTCACCAGC	TGCTTGGAACATGAGCCCAT
HerSNP2	GAGACAGACACAACCCAGC	GCAGATGAGATGGGAGAGGC
Uher_314	ATGGAGCACAGAAGACAGGC	CAGACCTGACCTATCCCA
Uher_170	TTCAGCGTCTCACCGAATCC	ATAAGAGGTCTGTCGGGCCA
Uher_168	CCCATTGAGTTTTTGCCTTTGC	ACTAGAGACAGCACCTCCCA
Uher_317	AGTACAGCAGAACGCACAGG	ACTGTCAAACCAACAGAGGTGA
HerSNP22	GCCTCAGGTGAAAGAGTGAC	TCACAAAGACGGTGGACAG
HerSNP24	TGAGAGAGGGAGACACTGGTC	CCAATTGTCACACGAGCTTGG
Uher_343_rd	ACTGAGGATAATGCGACATTTACA	CTGCCATCCTTCTCACTGAA
Uher_312_093	AGCCCATTGCACACTGTCTAA	AGCCTAAATGAAATTATCACCCGT
Uher_348	TAGCACTCTACCCCAGTCG	TGAAGAAGATGGTGCCGGTG
HerSNP41	CATGGACCACCTGAGCCAG	CACAGACCAGGTGTGCGTAT
HerSNP37	ACCTGCCATTTCTGTCAGG	GCCAATGTCTGCAGCTCTCT
HerSNP33	CTGTGTGCCTGTAGCTCTGG	GGTCACTGAACAAAGGTGCAC
chr17_1	TAGGAGGATGGGACTGCACA	AGCGTCTGAGAACGTTTGGG
chr17_2	CTGTCTCTGTTGGCTGTGCA	TGTTCGGGTTTCTGAGGACG
chr17_5_rd	GTGGTGCGATAATTATACCTCCAT	TGTCAGCTGCCTCTGAGGA
Uher_161_061	CACCTCAGAGTGCCTCACT	CTTTGTTTGAAAGTGCTCC
Uher_148_057	CTGTTTTGAAGGCTCACCGC	AAGGAATGCACAGCTCTCTTC
HerSNP71	AGAGGTAGATCTAGCATGGGC	GCTCCAATTGAGTGAACCCAG
HerSNP64	ACACATTCAGGCAATCAAGAGC	AGCCTTCAGGTGAATATGCAC
HerSNP63	GCACAGAGACACATTTTCCTGC	CGGGCTAAACAGGGTTGTTG
HerSNP62	GCGCAGGTAGGAAGACTGAG	CCCCTTCAATTACGAGGCCA
Uher_246	ATGCAGTGTGGTGGTCTGAG	ACAAGCCAACAAACCACAGC
HerSNP1	CCACTTGGTAAAGGCAGAGC	TGCCAGCTTGCAAATCAGTT
chr23_12	AGGTCAAGGTTGTCGAAGGTT	CCGAGCCACCGTTAGAAATCT
chr23_13	TGCTCTTTCAATTGCATTCACCC	CAGCACTCTCGGCCTCTT

PCR reagent preparation

1. Sterilise the working area including the surfaces of the UV cabinet with Microsol 4 Decontaminant or a 10% bleach solution.
2. Sterilise all plastic consumables in the UV cabinet with 15 minutes of UV light.
3. Combine the SNP primers (Table 7.4) to make three multiplexes of 0.25 µM from 100 µM working solution, each comprising primers for fifteen SNP markers.
4. Dilute the barcodes with ddH_20 to 1 µM working solution and lay out on 96-well PCR plates; M13-R tailed barcodes should be arranged A to H and CAG tailed barcodes 1 to 12, yielding 4 plates for the CAG barcodes and 6 plates for the M13-R barcodes.
5. Prepare a PCR mastermix for each multiplex plate in a sterile 1.5ml eppendorf tube, with 500 µl Qiagen multiplex mastermix, 250µl ddH_2O, 100µl of primer multiplex.
6. Use an AutoRep™ S manual repeater pipette to add 8.5µl of the PCR mastermix (equates to 5µl Qiagen multiplex mastermix, 2.5µl H_2O, 1µl primer multiplex) to each well on a new sterile standard 96-well semi-skirted PCR plate.
7. Seal plate with 8-Strip flat caps and store at 4°C or -20°C if it is not to be used immediately or proceed to the next steps.

Step 1 PCR

1. Sterilise the working area including the surfaces of the UV cabinet with Microsol 4 Decontaminant or a 10% bleach solution.
2. Sterilise all plastic consumables in the UV cabinet with 15 minutes of UV light.
3. Carefully remove the strip caps from the DNA plate prepared in section 7.4.
4. If the multiplex plates have been prepared in advance, then vortex, centrifuge and unseal them.
5. Load a new rack of sterile filter tips to the Benchsmart 96 semi-automated pipettor.
6. Using the multi-dispense setting transfer 1.5µl of DNA into the multiplex plates.
7. Seal filled plates with a foil heat seal on the Eppendorf Heatsealer S100. Sealing takes approximately 6 seconds and ensure all wells are properly sealed.
8. Vortex the plate for 2 seconds to ensure all contents are thoroughly mixed and centrifuge for 10 seconds to remove bubbles.
9. Transfer plate to applied Biosciences SimpliAmp™ Thermal Cycler and run the Step 1 cycle (Table 7.5).
10. When cycle is completed remove plate and store at 4°C until further processing.

Step 2 PCR

1. The Step 2 PCR plate can be prepared when the Step 1 PCR plate is in the thermal cycler.
2. Use an AutoRep™ S manual repeater pipette to add 5µl of Qiagen multiplex mastermix to each well on a new sterile standard 96 well semi-skirted PCR plate.
3. Load a new rack of sterile tips to the Benchsmart 96 semi-automated pipettor.
4. Carefully remove the strip caps from the relevant CAG barcode plate.
5. Using the multi-dispense setting transfer 1µl of relevant CAG barcode into each well on the Step 2 plate.
6. Recap the CAG barcode plate and eject the used tips.
7. Load a new rack of sterile tips to the Benchsmart 96 semi-automated pipettor.
8. Carefully remove the strip caps from the relevant M13-R barcode plate.
9. Using the multi-dispense setting transfer 1µl of relevant M13-R barcode into each well on the Step 2 plate.
10. Recap the M13-R barcode plate and eject the used tips.
11. Move the Step 2 plate to the post-PCR area.
12. Vortex Step 1 PCR plate, centrifuge briefly and carefully remove the foil seal.
13. Using the manual 8-channel pipette, transfer 3µl of the Step 1 PCR product to the Step 2 plate.
14. Seal filled plates with a foil heat seal on the Eppendorf Heatsealer S100. Sealing takes approximately 6 seconds and ensure all wells are properly sealed.
15. Vortex the plate for 2 seconds to ensure all contents are thoroughly mixed and centrifuge for 10 seconds to remove bubbles.

16. Transfer plate to applied Biosciences SimpliAmp™ Thermal Cycler and run the Step 2 cycle (Table 7.5).
17. When cycle is completed remove plate and store at 4°C until further processing.

Amplicon Pooling

1. Vortex Step 2 PCR plate, centrifuge briefly and carefully remove the foil seal.
2. Take 8µl from each well and add to a new sterile 1.5 ml eppendorf tube.
3. Visualise on an E-Gel agarose gel, in order to confirm the amplicon length is as expected and the barcodes have been incorporated.
4. Purify the PCR products using ExoSAP-IT™ Express PCR Product Cleanup Reagent, following manufacturer protocols.
5. Measure the concentration of PCR product in each pooled sample with the Qubit™ dsDNA BR Assay Kit and if necessary, with the Qubit™ dsDNA HS Assay Kit.
6. Once all plates have been completed, combine the pooled plates to form a single 1.5ml of product for sequencing according to the specification of the sequence service provider.
7. Send for library preparation and sequencing. In the current project the best sequencing results have been achieved using FASTERIS SA, Switzerland. Library preparation was completed using their proprietary Metafast ligation-based method and sequencing was conducted on an Illumina MiSeq with a V3 2x250bp run.

Table 7.5. PCR cycling conditions for Step 1 and Step 2 PCR.

Cycle	Stage	Step	Temp °C	Time min:sec	
Step 1 PCR	Initial activation	1	95	15:00	
	Denaturation	2	94	00:30	Repeat 2-4 x30
	Annealing	3	60	01:30	
	Extension	4	72	01:00	
	Final extension	5	72	10:00	
	Hold	6	4	∞	
Step 2 PCR	Initial activation	1	95	15:00	
	Denaturation	2	94	00:30	Repeat 2-4 x8
	Annealing	3	53	01:30	
	Extension	4	72	01:00	
	Final extension	5	72	10:00	
	Hold	6	4	∞	

Data Processing and Genotyping

1. Download raw sequence data from Fasteris server as compressed FASTQ file.
2. Only the R1 file is used for further analyses.
3. Perform initial quality control of sequence data with FASTQC (Babraham, 2016).
4. Sort the raw data using the custom *python* scripts as detailed in Vartia *et al*., 2016 and Farrell *et al*., 2016. The *python* scripts are available from https://github.com/egenomics/micomba.
5. Sequence reads are sorted hierarchically and grouped into five separate FASTA files as reads with: no barcode, one barcode, two barcodes and no primers, two barcodes and two non-matching primers, two barcodes and two matching primers. Only reads containing two barcodes and two matching primers are included in further analyses.
6. Genotyping of this data is performed using the scripts from Campbell et al. (2015) available at https://github.com/GTseq/GTseq-Pipeline. The average designated allele ratios in the current study were >5.0 to be called as homozygous for allele 1, ratios <0.2 to be called as homozygous for allele 2, ratios between 0.3 and 3.33 to be called as heterozygous and ratios between 3.34-4.9 and 0.201-0.29 were called as NA.
7. Genotyped data is stored in *Genepop* format for further analyses in *assignPOP* (see section 4.8 for method and Annex 4: Table 10.4.5 for model parameters).

Costing
The approximate cost per sample of 96 individual fish genotyped at the 45 SNPs used for the assignments in the current study was €589.50 for consumables and materials or €6.14 per individual fish in materials. The labour required for the protocol described above is one person and approximately 1.5hrs excluding the PCR cycling times. Labour cannot be costed here as this is wholly dependent on where the work is conducted, the level of the person undertaking it and what equipment is available. Equipment is not costed for in this protocol as it is assumed that this is available in the laboratory undertaking the work.

Alternative methods
Whilst the protocol detailed above, which is based on genotyping by sequencing, was cost effective during the current study, it is not advised for regular monitoring of survey and commercial catches. The protocol, whilst straightforward, relies on significant user input from a trained technician and is also reliant on a single sequencing run. Therefore, in order to achieve cost effectiveness, it is prudent to wait until enough samples have been collected before conducting the sequencing run. This represents a potential time lag in the delivery of results. Further, any errors in the setup will potentially not become apparent until the final sequencing results are analysed. The benefit of the approach is that large numbers of markers can be tested during the development phase and both microsatellites and SNPs can be genotyped in the same sequencing run if required. It is also possible to combine up to twenty-four plates of DNA (2,303 individuals) analysed at up to c.100 markers in a single sequencing run, if required, which would further decrease the cost per individual. If a higher capacity sequencing platform and kit was used (e.g. Illumina HiSeq 2500 with RR 2x250 flow cell) then it is possible to combine even more individuals within a single run.

For the purposes of being able to genetically screen large numbers of survey or commercial catch samples rapidly, cost effectively and consistently with the 45 SNPs in the current study it is preferable to employ the services of a commercial genotyping service provider. Given the number of SNPs it would be most cost-effective to use a system that utilises fluorescently labelled allele-specific primers and probes for SNP genotyping, thus removing the need for sequencing. There would be initial assay design costs of c.€30 per SNP after which genotyping would be possible for c.€0.15 per SNP. Therefore, for the assignment approaches in the current study, that utilise a 45 SNP panel the cost per individual would be €6.75 including labour for genotyping only. This estimated cost does not include data analysis.

7.6. Conclusions
The protocols and information outlined in Section 7, for collecting and processing genetic samples for distinguishing between the herring populations known to inhabit ICES Division 6.a, are the best available at the current time. At the current sampling levels, using the genetic sampling tool system available from LVL Technologies (Section 7.3), and outsourcing DNA extraction and genotyping to a commercial service provider it is possible to generate genotype data for the 45 SNPs for c.€10 per individual fish.

8. Task 8 – Final Integration

8.1. Objectives

- Present advantages and disadvantages of the suggested protocol.
- Justify the accuracy of the method and potential uncertainties.
- Highlight practical difficulties in implementing the method and protocol.
- Propose measures to overcome the potential weaknesses of the protocol.
- Analyse in detail the implications of the results for herring fisheries management in the region.

8.2. Deliverables

- Report advising on all aspects of future implementation of methods**: *Completed***
- Advice to ICES HAWG on most appropriate data for stock assessment: ***Completed***

8.3. Review of the proposed genetic approach to assignment

The genetic markers and assignment methods presented in the current study constitute a tool that can be used for the assignment of herring caught in mixed survey and commercial catches in Division 6.a into their population of origin with a high level of accuracy (>90%). This will enable the splitting of the MSHAS indices into its constituent Division 6.a populations, which has not previously been possible. As a result, it will be possible to develop a separate stock assessment for the Division 6.a.S, 7.b and 7.c stock. Although, it should be noted that as there were no spawning herring observed or sampled in Divisions 7.b and 7.c, it was not possible to test the assumption that the herring that spawn in these Divisions are the same population as the 6.a.S herring. However, the non-spawning herring caught in Division 7.b, genetically assigned with a high probability to the *6aS* and *Group_2+4* baseline groups (Section 4.10). The lack of differentiation between the 6.a.N autumn spawning herring and the North Sea autumn spawning herring raises the question of whether it is appropriate to conduct a stand-alone assessment on the 6.a.N autumn spawning herring or whether it should be combined with the North Sea autumn spawning herring assessment. It is beyond the remit of the current project to make this recommendation.

To date there are few examples of genetic stock assignment being used for regular collation of survey or catch data of marine fish into population of origin for the purposes of stock assessment. These methods have primarily been used for one off studies, that at best have been used to inform management but few have been developed for regular monitoring and data collection (Reiss *et al.*, 2009; Waples *et al.*, 2008). Genetic stock identification methods have been most commonly used for salmonids, including sea trout, *Salmo trutta* (Prodöhl *et al.*, 2017), Atlantic Salmon, *Salmo salar* (Gilbey *et al.*, 2016) and species of pacific salmon including Coho Salmon, *Oncorhynchus kisutch* (Beacham *et al.*, 2020). In these studies, self-assignment accuracies of 70-80% were concluded to be acceptable levels of accuracy. The high level of self-assignment accuracy in the current study (>90%) exceeds this. Further, the assignment of known-unknown samples, with equally high probabilities, as an additional form of baseline validation adds an extra level of support for the results. Therefore, the levels of accuracy in the current study for the discrimination of the 6.a.S, 7.b and 7.c and 6.a.N autumn spawning herring stocks is considered sufficient for the prescribed purpose of splitting the MSHAS index.

The SNP panel in the current study was composed of adaptive markers that are known to be under diversifying selection and proven to be associated with local ecological adaptation (Han *et al.*, 2020; Martinez Barrio *et al.*, 2016). Genetic markers associated with loci under selection have been proven to provide better resolution to distinguish population structure in herring than random neutral genetic markers (Bekkevold *et al.*, 2016; Han *et al.*, 2020). However, such high-graded adaptive markers may undergo more rapid changes in the allele frequencies within populations than putatively neutral genetic markers, particularly in situations of dynamic environmental conditions (Jorde *et al.*, 2018; Nielsen *et al.*, 2012). In the current study the contemporary baseline spawning samples collected from 2014 to 2019 (five spawning seasons) indicated temporal stability of the genetic markers within the different populations (Section 4.7). The genetic assignment of the WESTHER samples

also confirmed longer term temporal stability of the SNP panel over a period of at least sixteen spawning seasons (Section 4.9), which is a temporally relevant time scale for the purposes of stock assessment. Thus, these SNPs are appropriate for the purposes of stock identification in the current study. However, it is advisable to continue to collect and analyse baseline spawning samples regularly to monitor any changes in allele frequencies within the populations in the assignment model in order to prevent erroneous assignments of mixed samples.

There remain some uncertainties in the assignments, the most notable of which is the difficulty in distinguishing the late spawning 6.a.S, 7.b and 7.c herring from the 6.a.N spring spawning herring. This is due in part to the genetic similarities of the two groups and in part to the small baseline sample sizes of each, particularly the 6.a.N spring spawning herring. There are inherent difficulties in sampling these groups for which no specific fishery currently exists and which spawn in areas that are subject to unfavourable weather conditions for sampling at the time of spawning. Of the two genetic assignment approaches presented, it is suggested that *approach 2* provided the most appropriate assignment for this discrimination. The majority of the 6.a.N spring herring were assigned to a mixed group (Group_3) with late spawning 6.a.S fish. It is not currently possible to split this group to a lower level and as such provision will have to be made in the processing of the MSHAS data for the existence of this mixed group.

One potential weakness of the genetic assignments in the current study is that they are solely based on the populations empirically proven to occur within Division 6.a. Genetic analyses have demonstrated that the Irish Sea herring and the Celtic Sea herring are distinct from each other and from the other populations in Division 6.a (Han *et al*., 2020; Section 4.7). However, they are genetically closely related to the herring in 6.a.S and as such it is difficult to distinguish them with a high degree of certainty. Inclusion of these populations in the baseline dataset would increase the overall uncertainty of the assignments. Despite the assertions of the WESTHER project (Section 2.7) there is no definitive evidence that a significant abundance of herring from either of these populations migrate to the Malin Shelf area at the time of the MSHAS. Therefore, their inclusion in the baseline datasets is not warranted at this time. The WESTHER project provided an illustration of the dangers of including multiple populations in a baseline when the power of discrimination between the populations is low. The inevitable outcome is that mixed samples will be weakly assigned and will have a high rate of misassignment which leads to the incorrect conclusion that mixed samples come from a larger number of source populations when the converse may be true.

Although there is the potential to misassign individuals from the Celtic Sea and Irish Sea populations, if they were present in the MSHAS area, the assignment in its current form is still a significant improvement on the existing method of splitting the stocks based on geographic delineation. It should also be noted that the set of markers may also be improved in the future as more genetic data and samples become available. The *GENSINC* project (Section 2.8) is currently completing an additional round of full genome sequencing, which includes temporal replicates from all of the samples, from populations around Ireland and Britain, included in the Han *et al.* (2020), including late spawning 6.a.S fish. The addition of extra samples will increase the power to identify population specific markers that may increase the discriminatory power between closely related populations. For this reason, the current marker panel should be considered the best available at the current time but continued efforts should be made to develop it further.

The ideal scenario may be to develop a universal marker panel that can discriminate all of the populations that could potentially be surveyed or caught in the Northeast Atlantic area (FAO Major Fishing Area 27). In theory this would solve some of the issues outlined above, it would, however, also create another in terms of cost and wasted resources. In order to differentiate a wider range of populations, including those in the Baltic Sea, the panel would certainly need to comprise a larger number of genetic markers. The markers that may be suitable for discriminating between some of the Baltic Sea populations would likely not be informative for the populations west of Ireland and Britain (see Han *et al.*, 2020).

Therefore, using a universal panel of markers on a sample caught to the west of Ireland and Britain, which is highly unlikely to contain any Baltic herring, would represent a degree of wasted resources as the Baltic markers would yield no useful information. If the universal panel was used on a sample caught in the eastern North Sea, then the presence of the Baltic markers may actually be beneficial as there is the potential for some Baltic Sea populations to be present in this area. The difficulty arises in defining the cut off points on where the use of the universal panel is justified and where it is wasteful. Such a definition is akin to delineating stocks based on geographic or statistical areas such as ICES Divisions and inevitably introduces an element of subjectivity that may bias the results. It also introduces issues concerning the temporal stability of such definitions in an era of changing environmental conditions and documented changes in species distributions. Therefore, the use of a universal or specific marker panel is a topic that requires very careful consideration and rigorous empirical testing, which is beyond the scope of the current project.

In terms of the practical application of the genetic assignment approaches to future MSHAS samples, there are no practical difficulties to immediately initiating a standard genetic screening protocol in the existing surveys. The recent development of a rapid genetic sample collection tool and archiving system (Section 7.3) will enable collection of standardised samples if it is adopted across all surveys as the preferred method of sample collection. This will significantly reduce the time required for sampling and processing of samples and will also ensure correct sample preservation and tracking, if the samples are to be archived. As stated above, the marker panel in the current study has been developed to resolve the specific stock identification issues in ICES Division 6.a. Therefore, further development is required before implementing these methods elsewhere. The SNP markers used in the current study can be genotyped on a number of different genotyping platforms and it is not necessary for the various Marine Institutes to undertake this themselves as it can be outsourced to commercial genotyping service providers (see Section 7.5). Therefore, there are no significant infrastructure costs to be considered in the short term. Longer term it is advisable to begin developing the capacity for the Marine Institutes to process and analyse the samples inhouse as genetic stock identification becomes more widespread and is adopted as part of routine sampling protocols.

The current study has also highlighted some of the potential stock identification issues that are apparent within the North Sea herring. The winter spawning Downs herring are a distinct and separate population to the North Sea autumn spawning herring and are relatively easily distinguished with the genetic markers in the current panel. The extent of distribution of the Downs herring in the North Sea area and their abundance in the HERAS or in the commercial catches in Divisions 4.a and 4.b are currently unknown. The current practice of managing the Downs stock by applying an arbitrary sub-TAC (Section 2.5) is likely not appropriate and may be improved through genetic stock identification. There are also known and demonstrated issues of mixing of the North Sea autumn spawning herring with Western Baltic herring to the east and with Norwegian Spring Spawning herring to the North. Both areas have been extensively sampled by partners (DTU-Aqua, Denmark, Institute of Marine Research, Norway, University of Bergen, Norway and Uppsala University, Sweden) in the GENSINC project and significant advances have been made in the genetic stock identification in these mixing zones. The current study has also demonstrated the uncertainty in the composition of HERAS hauls in close proximity east and west of the 4°N North Sea stock delineator (Sections 4.09 and 4.10). What is required now to address these issues is a cohesive project that brings together all of the research groups and institutes working on these different but overlapping issues. Such a project could capitalise on the recent advances in herring genetic stock identification and resolve the issues that have impeded the accurate assessment and consequently management of the northeast Atlantic herring stocks. At the very least standard genetic sampling of all hauls of herring should be immediately introduced on the HERAS, using the new sample collection tool, in order to start to build an archive of samples that can be used for future splitting of the survey index.

The immediate concerns of the current project are the implications of the results for the assessment and management of herring in ICES Division 6.a, 7.b and 7.c. It is important

that the results of the MSHAS assignments can be seamlessly incorporated in the stock assessment procedures for herring in this area. To this end the following sub-sections outline the steps for incorporation of the data, preparation of the assessment models and also preparing for the ICES benchmark assessment process. It should be noted however that this stage of the assessment process is beyond the scope of the current study and must be conducted following standard ICES procedures. The parties involved (the Marine Institute, Marine Scotland Science, ICES HAWG etc) will have to agree on the division of the MSHAS data prior to any new stock assessments being conducted.

8.4. Implications of the results for assessment and management

As detailed in Section 2 the herring stocks in ICES Divisions 6.a, 7.b and 7.c have historically been considered two separate stocks for the purposes of assessment and management. The 6.a.N autumn spawning stock is separated by the 4°W line of longitude from the North Sea autumn spawning stock and, within Division 6.a, it is separated from the 6.a.S, 7.b and 7.c herring by the 56°N line of latitude and 7°W line of longitude (ICES, 1982). Herring caught or surveyed to the north or east of this boundary in Division 6.a (excluding the Clyde area) are considered by ICES to be part of the 6.a.N autumn spawning stock regardless of their population of origin or their spawning type. Herring caught to the south and west of the 56°N and 7°W lines are considered to be part of the 6.a.S stock in combination with herring in Divisions 7.b and 7.c, regardless of their population of origin or their spawning type. Herring from the separate stocks are believed to form mixed aggregations on common feeding grounds on the Malin Shelf to the west of the Hebrides during summer (Hatfield et al., 2005). It is during this time that they are surveyed by the MSHAS, which is part of the HERAS. The inability to assign herring catches from the MSHAS into their population of origin prevents the development of separate indices of abundance for the two populations. At present ICES is unable to distinguish between the herring stocks in Divisions 6.a.N and 6.a.S, 7.b and 7.c in commercial catches or research surveys. This means that ICES has to conduct a combined assessment of these stocks and provide combined management advice, leading to a combined quota and management (ICES, 2015a).

It is evident from the genetic results of the current study (Section 4.7) and from recent genome sequencing studies (Han *et al.*, 2020) that the 6.a.N autumn spawning herring and the 6.a.S herring are distinct and separate biological populations from each other. The 6.a.S herring are a genetically diverse group which is reflected in the long duration of the spawning season in the area. There is currently no evidence that the 6.a.N autumn spawning herring are a distinct and separate population from the North Sea autumn spawning herring. It is also evident from the current study that the aforementioned geographic delineation of the stocks in Division 6.a is not appropriate. During, at least, the period of the MSHAS, the 6.a.S herring are distributed across the entire area of Division 6.a up to and potentially east the 4° line of longitude. Therefore, the genetic assignment method should be used as the basis for discriminating the survey samples for the purposes of developing separate survey indices and genetic sampling of commercial catches should also be developed as part of a statistically sound sampling regime to monitor levels of mixing. In the MSHAS 2014-2019 sample assignments the 6.a.N autumn spawning herring formed a minor component in almost all hauls in which they were present. They were more abundant in the extreme north of Division 6.a, in close proximity to the 4°N. The herring in Lough Foyle on the north coast of Ireland are genetically and biologically 6.a.S herring despite the current stock delineation considering them to be 6.a.N autumn spawning herring.

The presence of a third population inhabiting Division 6.a was confirmed, with the verification that the 6.a.N spring spawning herring from the north Minch are a distinct biological population. The extent of distribution of this population is at present unclear and the only recently confirmed spawning ground is in the vicinity of Gairloch on the west coast of Scotland. These 6.a.N spring spawning herring form a component of the mixed hauls on the MSHAS to the west of the Hebrides. As previously stated, there is no current assessment or management measures in place for this population.

8.5. Fishery independent data

There are three sources of fishery independent information for 6.a, 7.b and 7.c herring. Two international bottom trawl surveys that are conducted by Scotland annually in Quarter 1 and Quarter 4 and that provide one index of abundance each. Marine Scotland Science and the Irish Marine Institute also conduct an annual acoustic survey each in summer. Those two acoustic surveys are coordinated by the ICES Working Group of International Pelagic Surveys (WGIPS) that coordinates, implements, and reports on acoustic surveys for pelagic fish species in ICES Subareas 1-8 and Subdivisions 21–24. Based on the results of these two acoustic surveys, currently, one index of abundance is derived and used to tune the assessment.

The acoustic surveys in 6.a, 7.b and 7.c are carried out and analysed in accordance with the ICES survey manual for International Pelagic Surveys (ICES, 2015b). Currently vessels from Scotland and Ireland conduct the survey in 6.a, 7.b and 7.c and use Simrad EK60 echosounders with transducers mounted on the drop keel. Only data gathered at 38kHz is used for the analysis of acoustic data for herring. Data collected at other frequencies are used for target discrimination and identification of other species or echo types. Echo integration and further data analyses are carried out using Myriax Echoview software (currently Version 11). Parallel transects with random starting points are used throughout the survey area apart from the Minch strata area to the west of Scotland where zig-zag transects are used. Transect spacing is generally 15 nautical miles, but intensity is increased to 10 nmi spacing in some areas, according to abundance and variance results from previous surveys. Transect spacing within each stratum is consistent. The survey is designed to be analysed using *StoX* estimation software (StoX 2015; Johnsen *et al*., 2019) with an agreed strata system (Figure 8.1).

The global survey estimates of abundance and biomass are calculated using *StoX*, and raw acoustic and biological data are held on the ICES Acoustic Trawl Database (ICES DB). Estimation of abundance from acoustic surveys within *StoX* is carried out according to the stratified transect design model developed by Jolly and Hampton (1990). The *StoX* input files (acoustic and biotic .xml files) are generated as outputs from the ICES database. Stock splitting of abundance and biomass estimates from the survey can be performed within *StoX* provided the stock identification of sampled fish is given in the biotic input file (in *StoX*, the variable *BiologyStockCode* is used to allocate a stock ID; Johnsen *et al*., 2019). The output generated gives abundance and biomass for all of the stock ID codes used in the biotic input file. However, not all hauls in recent years on the HERAS/WESPAS survey have been fully sampled using stock identification procedures (e.g. genetics and/or morphometrics). For instance, when a haul contains fewer than 100 individual herring but more than 30 individuals, these fish may be used to work up the survey estimate in *StoX*, but may not have been sampled for stock identification genetics or morphometrics. Also, protocols developed during the WESTHER project for morphometric sampling included sampling only fish greater than 23cm; in these situations, stock identification sampling may not have always been completed. A haul that is used for age, length, weight, sex and maturity analysis may not always have a stock identification method applied to the sample. In such situations, currently within *StoX* the survey estimates can only be split to the level where there is information for the sampled fish. Therefore an "unknown" stock will be estimated when samples or hauls of unknown stock identification are used in the estimation process. In the current version of *StoX* (StoX 2.7) it is not possible to extrapolate a stock identification in situations where the sample was not analysed for stock identification by using other samples in the transect, strata or survey, similar to the way missing age data is imputed. For instance, in hauls where a fish is not aged, a missing age is generated using a random data imputation within the same length group at the station, strata or survey level. If no age information is available at any level for a specific length group, the abundance estimate is estimated with unknown age (Johnsen *et al.*, 2019). It may be possible to treat missing stock identification within *StoX* in a similar way to missing age, and *StoX* developers are currently looking into a solution for future versions of the software.

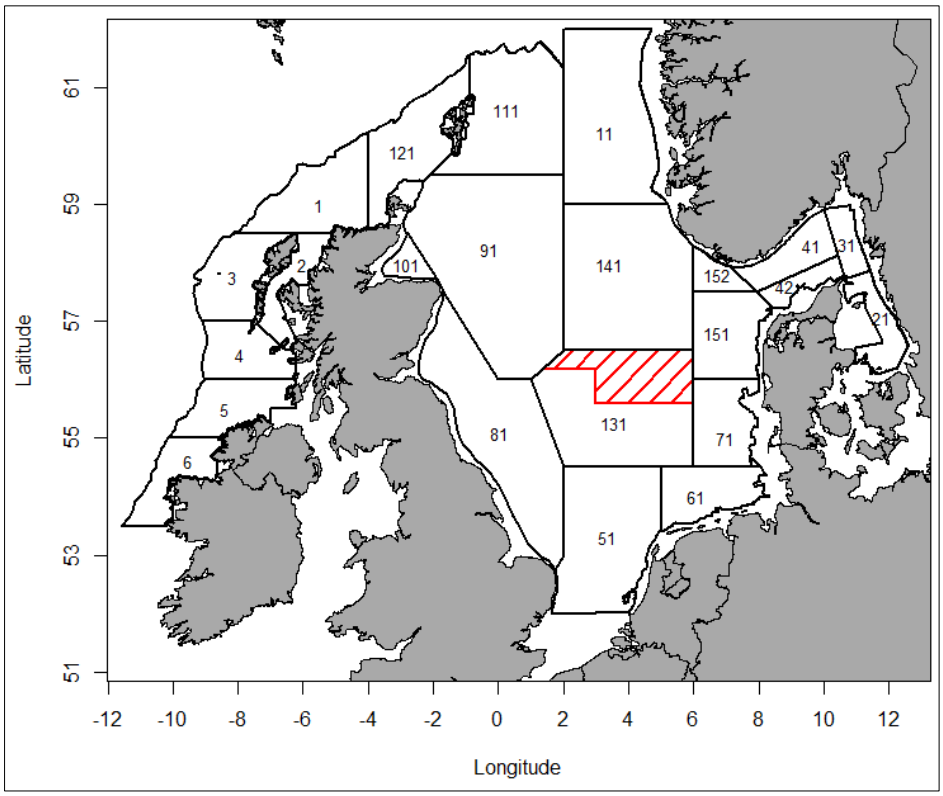


Figure 8.1. Strata used in the HERAS/WESPAS survey 2019. Strata numbers 1 to 6 are used to generate the Malin Shelf herring estimate (6.a., 7.b and 7.c). Reproduced from the 2020 WGIPS report (ICES, 2020a).

8.6. Fishery dependent data

Fishery dependent data are collected by member states involved in the fishery at landing ports or by scientific observers. In Divisions 6.a.S, 7.b and 7.c, herring are primarily fished by Ireland who receive 90% of the agreed TAC. The remaining 10% of the TAC is allocated to the Netherlands. The fishery takes place in Q1 and Q4 of each year when herring migrate inshore to spawn. A range of vessels participate in the fishery and the Marine Institute collect biological samples representative of the fishery catches in order to estimate catch numbers-at-age from this fishery. The 6.a.S, 7.b and 7.c stock was originally defined in 1982 (ICES, 1982) and was combined with 6.a.N in 2015 as a result of a benchmark process (ICES, 2015a).

In 6.a.N, the fishery starts in Q3 and takes place mainly on spawning aggregations. However, the genetic analyses have shown that mixed aggregations are also present at this time with 6.a.N autumn spawners as well as 6.a.N spring spawners and also 6.a.S, 7.b and 7.c fish present in this area. It is not possible to separate historical catches, but genetic techniques could be used to separate catches from this area in the future. The TAC is shared between the UK (60%), Ireland (15%), the Netherlands (11%), Germany (11%) and France (2%). The majority of the vessels involved in 6.a.N are offshore pelagic refrigerated seawater (RSW) and freezer trawlers. Landings are also sampled following national protocols and catch numbers at age are estimated.

Every year in Q1, ICES issue a data call detailing the data reporting requirements. Using information gathered during catch monitoring and national sampling programs, each nation submits data to ICES. For herring this includes quarterly estimates of total catch by ICES division. For sampled catches, estimates of catch numbers at age, mean weight and length at age are also provided along with an indication of the level of sampling undertaken. These data are then provided to the expert group HAWG and used to update the stock assessment.

8.7. Stock Assessment models and data preparation

The objective of this subsection was to identify the stock assessment models that could be considered as relevant candidates to perform an assessment of the individual stocks once a split of the MSHAS survey data is agreed. Input files have been created for a number of the models described below and will be updated when data are available. This will be the starting point to develop a new assessment.

The *State-Space Fish Stock Assessment Model* (*SAM*) is a statistical catch-at-age model developed in Template Model Builder (*TMB*; Kristensen et al., 2016) and described in Nielsen and Berg (2014).The SAM model uses the standard exponential decay equations to carry forward the N's (with appropriate treatment of the plus-group), and the Baranov catch equation to calculate catch-at-age based on the F's (ICES, 2013a). It is currently widely used in ICES to assess both demersal and pelagic stocks. It is the model currently used to assess the 6.a., 7.b and 7.c herring combined stock as well as many other stocks assessed by HAWG. SAM can be run via the web browser at www.stockassessment.org and within the *FLR* (*Fisheries Library in R*) system (www.flr-project.org). The configuration of this model will be further explored by the benchmark group.

The *Assessment For All initiative* (*a4a*) was initiated by the Joint Research Centre (Jardim et al., 2015). The stock assessment model framework is a non-linear catch-at-age model implemented in *R* that can be applied to a wide range of stocks with low parametrization requirements. The model structure is defined by five sub-models, which are the different parts that require structural assumptions. The sub-models in operation are: a model for F-at-age, a model for the initial age structure, a model for recruitment, a (list) of model(s) for abundance indices catchability-at-age, and a list of models for the observation variance of catch-at-age and abundance indices (Jardim et al., 2017). Different configurations of this framework will be tested by the benchmark group.

Age Structured Assessment Program (*ASAP*) is an age structured modelling program developed by Legault and Restrepo (1998). *ASAP* is a variant of a statistical catch-at-age model that can integrate annual catches and age compositions (by fleet), abundance indices and age compositions, annual maturity, fecundity, weight, and natural mortality at age. It is a forward projecting model that assumes separability of fishing mortality into year and age components but allows specification of various selectivity time blocks. It is also possible to include a Beverton-Holt stock-recruit relationship. *ASAP* is currently used to assess Celtic Sea Herring and was used in exploratory assessments for 6.a., 7.b and 7.c herring at WKWEST 2015 (ICES, 2015a). Further explorations using this model will be carried out when the split survey data are available.

Stock Synthesis (*SS*) is an age-structured population assessment tool used in several U.S. stock assessments and many other assessments around the world. It includes a population simulation model to calculate the abundance and mortality, an observation model to relate the population model to the observed data and a statistical model to adjust parameters of the population model and observation model to achieve the best fit to the data. *SS* can be applied to data from several fisheries and several surveys, each with its own pattern of selectivity. Although most population modelling is done within unit stocks, *SS* has the capability to model up to three geographic areas and to estimate the degree of migration between areas. Size based data can also be included and allows estimation of growth curves (Methot, 2000 and Methot et al., 2020). This is a more complex model to configure but may have the potential to address some of the issues with the 6.a., 7.b and 7.c herring assessment.

Stochastic Production In Continuous Time (*SPiCT*) is a surplus production model which in addition to stock dynamics also models the dynamics of the fisheries. This enables error in the catch to be reflected in the uncertainty of estimated model parameters and management quantities (Pedersen and Berg, 2017). An exploratory analysis for 6.a., 7.b and 7.c herring using *SPiCT* was carried out by the interbenchmark group in 2019 (ICES, 2019c). Issues with model convergence were highlighted but further exploration into model settings including adjusting uncertainties and including seasonality could be explored.

8.8. Benchmark assessment process

A benchmark assessment workshop is an established procedure conducted by ICES to review the assessment methodologies for fish stocks. In addition to this, the input data used in the assessment are reviewed as well as information on stock distribution, forecast methods and reference points. It is attended by scientists and stakeholders and the work is reviewed by external experts. The aim of the benchmark is to reach consensus agreement on the assessment methodology that is to be used in the future. Once the new method is agreed, it is detailed in the stock annex that will then be used to update the assessment at annual expert groups.

On an annual basis while the assessment is updated, stocks are also scored to determine the level of priority to undergo a new benchmark process. This evaluation is based on 5 criteria detailed in the headline of table 8.1 below. The 6.a., 7.b and 7.c herring scored in total 23/25 meaning that it is high priority to start a benchmark procedure. The scoring highlights that

1. The assessment is judged to be inadequate due to new information on stock identity and has the potential to be upgraded to a category 1 assessment which is a full analytical assessment and forecast. It is currently category 3 and is considered indicative of trends.
2. New information will be available when the results of the current study are published with the potential to use new survey indices and new assessment methods.
3. Herring in for 6.a., 7.b and 7.c is of high importance from the management perspective.
4. Herring in for 6.a., 7.b and 7.c is perceived to be significantly below possible reference points and at one of the lowest levels in the time series.
5. This stock has not been benchmarked for 7 years.

As a result of this scoring, the ICES Advisory Committee (ACOM) will make a decision regarding the timing of the benchmark. The benchmark will involve up to a year of work to agree between parties on the data to use and on the procedure to compile the data. A data compilation meeting will be organized in advance of the benchmark meeting where the assessment method will be agreed. The new assessment, integrating new methods developed during this study will then be applied by the Expert Group HAWG and the assessment updated on an annual basis.

Table 8.1: Stock prioritization procedure applied to the 6a/7bc herring stock. Scores range from 1 (lowest priority) to 5 (highest priority).

Criteria 1 – Quality of assessment to provide advice	Criteria 2 – Opportunity to improve the assessment	Criteria 3 – Management importance	Criteria 4 – Perceived stock status	Criteria 5 - Time since last benchmark
5	5	5	5	3
Assessment judged to be inadequate to provide advice (e.g., bias, stock id, unreliable catches, major change in biological processes/productivity), and high potential to be upgraded to Cat. 1 from Cat. 3 (ICES, 2019c)	New genetic and morphometric data will be available to address stock splitting issues and links with other stock components (e.g. North Sea) New acoustic indices will be available as trend indicators. New assessment models will be explored: SAM, ASAP, a4a, Stock Synthesis, SPICT biomass model	a) Advice on fishing opportunities is requested for the stock. b) Stock is the object of proposed rebuilding plan. c) Stock is the object of a directed fishery. d) Stock is the object of a pelagic fishery	Perceived to be below possible candidates for B_{lim}, at lowest ever biomass, and trend showing near continuous decline over last 20 years	7 years (by 2022) (ICES, 2015a)

8.9. Conclusions

This section lists the main conclusions from the analyses in the current study.

1. The genetic stock identification method represents a standardised and replicable method to discriminate survey and commercial catches of the three population groups known to occur in ICES Division 6.a.

2. The morphometric methods used in the current study did not show sufficient power to discriminate mixed survey catches in ICES Division 6.a.

3. The herring in ICES Divisions 6.a comprise at least three distinct populations; 6.a.S herring, 6.a.N autumn spawning herring and 6.a.N spring spawning herring.

4. The 6.a.S herring are primarily a winter spawning population though there is a later spawning component present in the area also. These components are currently inseparable and for the purposes of stock assessment should be combined as 6.a.S herring.

5. No baseline spawning samples could be collected in Divisions 7.b or 7.c therefore the relationship between the herring that spawn in this area and those that spawn in 6.a.S is unknown. Non-spawning herring caught in Division 7.b assigned genetically to the 6.a.S population.

6. Genetic assignment of the 2014-2019 MSHAS samples indicated that 6.a.S herring are distributed north and south of the 56°N line of latitude during the survey period. They were present as a significant proportion of the MSHAS hauls up to the 4°W boundary with the North Sea.

7. Potential 6.a.N spring spawning herring comprised a significant proportion of the MSHAS hauls west of the Hebrides in the 2014-2019 MSHAS samples.

8. 6.a.N autumn spawning herring comprised a small proportion of the MSHAS samples 2014-2019 except in the extreme north of the area in close proximity to the 4°W boundary with the North Sea.

9. The samples of herring from Lough Foyle, analysed in the current study, were shown to be genetically and biologically 6.a.S herring. They are currently defined as 6.a.N autumn spawning herring according to the ICES stock delineation.

10. There is no historical or contemporary evidence to support the differentiation of 6.a.N autumn spawning herring and North Sea autumn spawning herring.

11. The term ‘west of Scotland herring’ originally referred to populations of spring spawning herring that spawned in the Minch area. It now refers to autumn spawning herring that occur west of the 4°W boundary during the period of the MSHAS.

12. There is no robust evidence that Irish Sea herring are found in large abundance west of the Hebrides during summer. Historical evidence does suggest that they may be found in the Clyde area at this time before returning to spawn in the Irish Sea in autumn.

13. Exploratory assignment of a single haul from the 2016 HERAS immediately east of the 4°W boundary with the North Sea indicated a mixed sample with significant proportions of potential 6.a.S and 6.a.N spring spawning herring.

8.10. Recommendations

The following section lists the main recommendations arising from the analyses in the current study. Each recommendation is followed by suggested target groups in parentheses.

1. The 6.a.S, 7.b and 7.c stock and the 6.a.N autumn spawning stock should be assessed separately. (HAWG)

2. The genetic assignment approach detailed in the current study should be used for regular monitoring of MSHAS and commercial catches of herring in ICES Divisions 6.a, 7.b and 7.c. (HAWG, WGIPS, MI, MSS, WUR).

3. The continuation of morphometric sample collection on the MSHAS, for the purposes of splitting the mixed samples, is not recommended. (MI, MSS).

4. All herring sampled from hauls on the MSHAS should be sampled for genetics in future surveys. At a minimum, all aged fish should be genetically sampled but preferably up to 96 fish per haul. The exact protocol should be agreed between MI and MSS. (WGIPS, MI, MSS).

5. A programme for continuation of updating genetic baseline spawning samples of 6.a.N autumn spawning, 6.a.N spring spawning and 6.a.S herring should be agreed and targeted levels of sampling should be included in the program of work for these stocks. (MI, MSS).

6. Genetic sampling of commercial catches should be developed as part of a statistically sound sampling regime to monitor levels of mixing. (MI, MSS, IMARES).

7. All genetic sampling should be conducted with the genetic sampling tool available from LVL Technologies (see Section 7.3) in order to standardise the collection, preservation and archiving of samples using a common protocol. (MI, MSS, IMARES).

8. This report should undergo peer review by a relevant ICES expert group e.g. ICES Stock Identification Methods Working Group (SIMWG), prior to the results and conclusions being incorporated in the assessment and management of 6.a, 7.b and 7.c herring. (EASME, ICES).

9. Once the report has passed the peer review process the MSHAS genetic assignments should be used as the basis for splitting the survey data into indices for the different populations back to 2014. (ICES, HAWG, WGIPS).

10. The potential maturity staging issues of 6.a.N autumn spawning herring detailed in Section 4.9 should be resolved by genetic analysis of the additional samples. It should be a priority to complete this before the ICES benchmark. (MI, MSS, IMARES).

11. Work with *StoX* software developers to ensure that the results from stock splitting work can be routinely incorporated into the survey abundance and biomass results from the MSHAS. (IMR, WGIPS, MI, MSS).

12. Further study should be conducted to identify additional genetic markers that may help refine the discrimination of the 6.a.S, 7.b and 7.c stock from the 6.a.N spring spawning herring and also from adjacent stocks, including the Celtic Sea and Irish Sea.

13. Further research should also focus on the stock identification issues in the North Sea. In this light genetic sampling should be implemented on all herring surveys in

an effort to build an archive of samples that that may be used to split survey indices when new genetic markers become available. Genetic sampling should also be conducted, at the least, on commercial catches taken in areas of known mixing between populations. (WGIPS, MI, MSS, IMR, DTU-Aqua, WUR, TI).

9. References

Andersson, L., Ryman, N., Rosenberg, R. and Ståhl, G. 1981. Genetic variability in Atlantic herring (*Clupea harengus harengus*) description of protein loci and population data. Hereditas, 95: 69-78.

Anderson, E.C. 2010. Assessing the power of informative subsets of loci for population assignment: standard methods are upwardly biased. Molecular Ecology Resources, 10: 701–710.

André, C., Larsson, L.C., Laikre, L., Bekkevold, D., Brigham, J., Carvalho, G.R., *et al*. 2011. Detecting population structure in a high gene-flow species, Atlantic herring (*Clupea harengus*): direct, simultaneous evaluation of neutral vs. putatively selected loci. Heredity, 106: 1–11.

Andrews, W. 1866. On the herring fisheries of Ireland. Journal of the Royal Dublin Society, 5 (1865 - 1870): 12 - 27.

Anon. 2014. Proposal for a Council Regulation fixing for 2015 the fishing opportunities for certain fish stocks and groups of fish stocks, applicable in Union waters and, for Union vessels, in certain non-Union waters and repealing Council Regulation (EU) No 779/2014. European Commission, Brussels, 28.10.2014, COM.

Armstrong, M. P. and Cadrin, S. X. 2001. Morphometric Variation among Spawning Group of the Gulf of Maine–Georges Bank Herring Complex. Herring: Expectations for a New Millennium, Alaska Sea Grant College Program AK-SG-01-04.

Babraham Bioinformatics. 2016. FastQC. (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc)

Baxter, I.G. 1958. The composition of the Minch herring stocks. Rapports et procès-verbaux des réunions / Conseil permanent international pour l'exploration de la mer, 143: 814-94.

Baxter, I.G. 1963a. Features of the Minch herring fisheries in pre- and post-war years Rapports et procès-verbaux des réunions / Conseil permanent international pour l'exploration de la mer, 154: 227-35.

Baxter, I.G. 1963b. Herring tagging experiments in the Minch. Scottish Fisheries Bulletin, 20: 5-9.

Beacham, T.D, Wallace, C., Jonsen, K., McIntosh, B., Candy, J.R., Rondeau, E.B., Moore, J-S., Bernatchez, L. and Withler, R.E. 2020. Accurate estimation of conservation unit contribution to coho salmon mixed-stock fisheries in British Columbia, Canada, using direct DNA sequencing for single nucleotide polymorphisms. Canadian Journal of Fisheries and Aquatic Sciences, 77: 1302–1315

Begg, G. A. and Waldman, J. R. 1999. An holistic approach to fish identification. Fisheries Research, 43: 35–44.

Bekkevold, D., Clausen, L.A.W., Mariani, S., Andre, C., Hatfield, E.M.C., *et al*. 2011. Genetic mixed-stock analysis of Atlantic herring populations in a mixed feeding area. Marine Ecology-Progress Series, 442: 187–199.

Bekkevold, D., Helyar, S. J., Limborg, M. T., Nielsen, E. E., Hemmer-Hansen, J., Clausen, L. A. W., and Carvalho, G. R., FishPopTrace Consortium. 2015. Gene-associated markers can assign origin in a weakly structured fish, Atlantic herring. ICES Journal of Marine Science, 72: 1790–1801.

Bekkevold, D., Gross, R., Arula, T., Helyar, S.J., Ojaveer, H. 2016. Outlier loci detect intraspecific biodiversity amongst spring and autumn spawning herring across local scales. PLoS ONE11: e0148499.

Ben-Hur, A., Ong, C.S., Sonnenburg, S., Schölkopf, B. and Rätsch, G. 2008. Support Vector Machines and Kernels for Computational Biology. PLoS Computational Biology, 4: e1000173.

Berg, F., Almeland, O. W., Skadal, J., Slotte, A., Andersson, L. and Folkvord, A. 2018. Genetic factors have a major effect on growth, number of vertebrae and otolith shape in Atlantic herring (*Clupea harengus*). PLoS ONE 13(1): e0190995.

Berg, F., Østgaard, H.D, Slotte, A., Andersson, A. and Folkvord, A. 2020. A combination of genetic and phenotypic characterization of spring and autumn spawning herring suggests gene flow between populations. ICES Journal of Marine Science, doi:10.1093/icesjms/fsaa046.

Bernatchez, L. *et al*. 2017 Harnessing the Power of Genomics to Secure the Future of Seafood. Trends in Ecology and Evolution, 32: 665–680.

Biolé, F.G., Fortunato, R.C., Thompson, G.A. and Volpedo, A.V. 2019. Application of otolith morphometry for the study of ontogenetic variations of *Odontesthes argentinensis*. Environmental Biology of Fishes, 102: 1301 – 1310.

Bird, J.L., Eppler, D.T., Checkley, D.M.Jr. 1986. Comparisons of herring otoliths using Fourier series shape analysis. Canadian Journal of Fisheries and Aquatic Science, 43: 1228 - 1234.

Blaxter, J.H.S. and Hunter, J.R. 1982. The biology of the clupeoid fishes. Advances in Marine Biology, 20: 1–223.

Bracken, J. and Phillips, D.R. 1970. Donegal herring investigations 1967/68. The Scientific Proceedings of the Royal Dublin Society - Series B 2, 233 - 249.

Breiman, 2001. Random Forest. Machine Learning, 45: 15-32.

Brophy, D. and Danilowicz, B.S. 2002. Tracing populations of Atlantic herring (*Clupea harengus* L.) in the Irish and Celtic Seas using otolith microstructure. ICES Journal of Marine Science, 59: 1305 – 1313.

Cadrin, S.X. and Secor, D.H. 2009. Accounting for spatial population structure in stock assessment: past, present and future. In: Beamish R. J., Rothschild B. J., editors. The Future of Fishery Science in North America. Dordrecht: Springer. p. 405-425. 736 pp.

Cadrin, S.X. 2010. Stock Identification in Marine Populations in Elewa, A. M. T. Morphometrics for nonmorphometricians. Springer-Verlag Berlin Heidelberg, 219 – 232.

Cadrin, S.X. and Silva, V.M. 2005. Morphometric variation of yellowtail flounder. ICES Journal of Marine Science, 62: 683 - 694.

Cadrin, S.X., Kerr, L.A., and Mariani, S. 2013. Stock Identification Methods, 2nd edn. Applications in Fishery Science. Academic Press, London. 592 pp.

Campana, S.E. and Casselman J.M. 1993. Stock discrimination using otolith shape analysis. Canadian Journal of Fisheries and Aquatic Sciences, 50:1062-1083.

Campbell, N., Cross, M.A., Chubb, J.C., O'Cunningham, C., Hatfield, E., MacKenzie, K. 2007. Spatial and temporal variations in parasite prevalence and infracommunity structure in herring (*Clupea harengus* L.) caught to the west of the British Isles, in the Baltic and North Seas: implications for fisheries science. Journal of Helminthology, 81: 137–146.

Campbell. N.R., Harmon, S.A. and Narum, S.R. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources, 15: 855–867.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., et al. 2012 Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME Journal, 6: 1621-1624.

Cardinale, M., Doering-Arjes, P., Kastowsky, M. and Mosegaard, H. 2011. Effects of sex, stock and environment on the shape of known-age Atlantic Cod (*Gadus morhua*) otoliths. Canadian Journal of Fisheries and Aquatic Sciences, 61: 158-167.

Carlsson, J., Gauthier, D.T., Carlsson, J.E., Coughlan, J.P., Dillane, F., Fitzgerald, R. D., Keating, U., McGinnity, P., Mirimin, L. and Cross, T.F. 2013. Rapid, economical single-nucleotide polymorphism and microsatellite discovery based on de novo assembly of a reduced representation genome in a non-model organism: a case study of Atlantic cod *Gadus morhua*. Journal of Fish Biology, 82: 944–958.

Casey, J., Jardim, E. and Martinsohn, J.T.H. 2016. The role of genetics in fisheries management under the E.U. common fisheries policy. Journal of Fish Biology, 89: 2755–2767.

Chen, K., Marshall, E.A., Sovic, M.G., Fries, A.C., Gibbs, H.L., Ludsin, S.A. 2018. assignPOP: An r package for population assignment using genetic, non-genetic, or integrated data in a machine-learning framework. Methods in Ecology and Evolution, 9: 439-446.

Clarke, M.W., Egan, A., Mariani, S and Miller, D. 2011. Long term trends in the population dynamics of northwestern Ireland herring revealed by data archaeology. ICES CM 2011/D:04.

Cope, J.M. and Punt, A.E. 2011. Reconciling stock assessment and management scales under conditions of spatially varying catch histories. Fisheries Research, 107: 22–38.

Cornuet, J.-M., Piry, S., Luikart, G., Estoup, A. and Solignac, M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics, 15: 1989-2000.
Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M. and Blaxter, M.L. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics, 12: 499–510.
De Groot, S.J. 1980. The consequences of marine gravel extraction on the spawning of herring, Clupea harengus Linne. Journal of Fish Biology, 16: 605-611.
Dickey-Collas, M., Nash, R.D.M., Brunel, T., van Damme, C.J.G., Marshall, C.T., Payne, M.R., Corten, A., *et al*. 2010 Lessons learned from stock collapse and recovery of North Sea herring: a review. ICES Journal of Marine Science, 67: 1875-1886.
Dieringer, D. and Schlötterer, C. 2003 Microsatellite Analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Resources, 3: 167–169.
Doyle, D., Gammell, M. and Nash R. 2018. Morphometric methods for the analysis and classification of gastropods: A comparison using *Littorina littorea*. Journal of Molluscan Studies, 84: 190-197.
Dudzik, B. 2019. Landmark and semilandmark data collection using digitizers and data processing. In: Seguchi, N. and Dudzik, B. 3D Data acquisition for bioarchaeology, forensic anthropology and archaeology. Elsevier Inc. pp. 59-69.
Duncan, R., Brophy, D. and Arrizabalaga, H. 2018. Otolith shape analysis as a tool for stock separation of albacore tuna feeding in the Northeast Atlantic. Fisheries Research, 200: 68-74.
Dytham, C. 2003. Choosing and using statistics: a biologist's guide. 2nd Edition. Blackwell Publishing, Oxford.
Eggers, F., Slotte, A., Libungan, L. A., Johannessen, A., Kvamme, C., Moland, E., Olsen, E. M. and Nash, R. D. M. 2014. Seasonal dynamics of Atlantic herring (*Clupea harengus* L.) populations spawning in the vicinity of marginal habitats. PLoS ONE 9: e111985.
Elewa, A. M. T. 2010. Morphometrics for nonmorphometricians. Springer, Berlin.
Elliott, N. G., Haskard, K., and Koslow, J. A. 1995. Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. Journal of Fish Biology, 46: 202 - 220.
Ewart, J.D. 1884. Natural history of the herring. Annual report for the Fishery Board for Scotland 1884. Appendix F, IV.
Farran, G.P. 1930. Fluctuations in the stock of herrings on the north coast of Donegal. Rapports et procès-verbaux des réunions / Conseil permanent international pour l'exploration de la mer, 65(14).
Farran, G.P. 1937. The herring fisheries off the north coast of Donegal. Department of Agriculture Journal, 34(2).
Farran, G.P. 1944. The herring fishery in Eire 1921-1941. Department of Agriculture Journal, 41(2).
Farrell, E.D. and Carlsson, J. 2018. 6a/7bc Herring Genetic Stock Identification Baseline Project. Report for the European Association of Fish Producers Organisations (EAPO). 42pp.
Farrell, E.D., Carlsson, J.E.L. and Carlsson, J. 2016. Next Gen Pop Gen: implementing a high-throughput approach to population genetics in boarfish (*Capros aper*). Royal Society Open Science, 3:160651.
Feet, P.O., Ugland, K.I., and Moksness, E. 2002. Accuracy of age estimates in spring spawning herring (*Clupea harengus* L.) reared under different prey densities. Fisheries Research, 56: 59–67.
Fey, D.P. 2001. Differences in temperature conditions and somatic growth rate of larval and early juvenile spring-spawned herring from the Vistula Lagoon, Baltic Sea manifested in the otolith to fish size relationship. Journal of Fish Biology, 58: 1257–1273.
Fowler, J., Cohen, L. and Jarvis, P. P. 1998. Practical statistics for field biology. 2nd Edition. John Wiley & Sons Inc. New York.
Geffen, A. J. 2009. Advances in herring biology: from simple to complex, coping with plasticity and adaptability. ICES Journal of Marine Science, 66: 1688–1695.

Geffen, A.J., Nash, R.D.M. and Dickey-Collas, M. 2011. Characterization of herring populations west of the British Isles: an investigation of mixing based on otolith microchemistry. ICES Journal of Marine Science, 68: 1447–1458.
Gilbey, J., Cauweiler, E., Coulson, M.W., Stradmeyer, L., Sampayo, J.N., Armstrog, A., Verspoor, E., Corrigan, L. Shelley, J. and Middlemas, S. 2016. Accuracy of assignment of Atlantic Salmon (*Salmo salar* L.) to rivers and regions in Scotland and Northeast England based on single nucleotide polymorphism (SNP) markers. PLoS ONE 11(10): e0164327.
Grainger, R.J.R. 1978. Herring abundance off the west of Ireland in relation to oceanographic variation. ICES Journal of Marine Science, 38: 180-188.
Graps, A. 1995. An Introduction to Wavelets. IEEE Computational Science & Engineering 2: 50 – 61.
Green, H.J. 1884. The Herring Fisheries. International Fisheries Exhibition London, 1883. William Clowes and Sons, Limited, London
Guo, B., Li, Z. and Merilä, J. 2016. Population genomic evidence for adaptive differentiation in the Baltic Sea herring. Molecular Ecology, 25: 2833-2852.
Haegele, C.W., and J.F. Schweigert. 1985. Distribution and characteristics of herring spawning grounds and description of spawning behavior. Canadian Journal of Fisheries and Aquatic Science, 42: 39-55.
Han, F., Jamsandekar, M., Pettersson, M.E., Su, L., Fuentes-Pardo, A., Davis, B., Bekkevold, D., Berg, F., Cassini, M., Dahle, G., Farrell, E.D., Folkvord, A. and Andersson, L. (2020). Ecological adaptation in Atlantic herring is associated with large shifts in allele frequencies at hundreds of loci. eLife, 2020; 9:e61076 (DOI: 10.7554/eLife.61076)
Hatfield, E.M.C. *et al*., 2005. (WESTHER, Q5RS-2002-01056): A multidisciplinary approach to the identification of herring (*Clupea harengus* L.) stock components west of the British Isles using biological tags and genetic markers. Final Report. 320pp.
Hastie, T., Tibshirani, R. and Friedman, J. 2009. The elements of statistical learning: data mining, inference and prediction. 2nd edition. Springer.
Hauser, L., Seamons, T., Dauer, M., Naish, K., and Quinn, T. 2006. An empirical verification of population assignment methods by marking and parentage data: hatchery and wild steelhead (*Oncorhynchus mykiss*) in Forks Creek, Washington, USA. Molecular Ecology, 15: 3157-3173.
Hauser, L. and Carvalho, G.R. 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish and Fisheries, 9: 333-362.
Hay, D.E., Toresen, R., Stephenson, R., Thompson, M., Claytor, R., Funk, F., Ivshina, E., Jakobsson, J., Kobayashi, I., McQuinn, I., Melvin, G., Molloy, J., Naumenko, N., Oda, K. T., Parmanne, R., Power, M., Radchenko, V., Schweigert, J., Simmonds, J., Sjöstrand, B., Stevenson, D. K., Tanasichuk, R., Tang, Q., Watters, D. L. and Wheeler, J. 2001. Taking stock: an inventory and review of world herring stocks in 2000. In Herring: Expectations for a New Millennium? Funk, F., ed., pp. 385–392. Fairbanks, AK: University of Alaska Sea Grant College Program.
Heath, M.R. 1984. Report on the acoustic survey carried out in the northern part of division VIa on herring and mackerel. ICES Pelagic Fish Committee CM 1984/H:62
Heath, M. 1989. Transport of larval herring (*Clupea harengus* L.) by the Scottish Coastal Current. Rapports et procès-verbaux des réunions / Conseil permanent international pour l'exploration de la mer, 191: 85-91.
Heath, M. 1990. Segregation of herring larvae from inshore and offshore spawning grounds in the north-western North Sea – Implications for stock structure. Netherlands Journal of Sea Research, 25: 267-278.
Heath, M. and Copland, P. 1986. Report of an acoustic survey of Division VIa(N). ICES Pelagic Fish Committee CM 1986/H:6
Heath, M.R., MacLachlan, P.M. and Martin, J.H. A. 1987. Inshore circulation and transport of herring larvae off the north coast of Scotland. Marine Ecology Progress Series, 40: 11-23, 1987
Helyar, S.J., Hemmer-Hansen, J., Bekkevold, D., Taylor, M.I., Ogden, R., *et al*. 2011. Application of SNPs for population genetics of non-model organisms: new opportunities and challenges. Molecular Ecology Resources, 11: 123–136.

Helyar, S.J., Limborg, M.T., Bekkevold, D., Babbucci, M., van Houdt, J.K.J., Maes, G., *et al*. 2012. SNP discovery using next generation transcriptomic sequencing in Atlantic Herring (*Clupea harengus*). PLOS ONE. 2012; 7 (8): e42089.
Hempel, G and Blaxter, J.H.S. 1967. Egg Weight in Atlantic Herring (*Clupea harengus* L.). ICES Journal of Marine Science, 31: 170–195.
Hemmer-Hansen, J., Therkildsen, N.O. and Pujolar, J.M. 2014. Population genomics of marine fishes: Next-generation prospects and challenges. The Biological Bulletin, 227: 117-132.
Hintzen, N.T., Roel, B., Benden, D., Clarke, M., Egan, A., Nash, R.D.M., Rohlf, N. and Hatfield, E.M.C. 2015. Managing a complex population structure: exploring the importance of information from fisheries-independent sources. ICES Journal of Marine Science, 72: 528-542.
Hodgson, W. C. 1951. Fisheries of the northeast Atlantic Herring Atlas. International Council for the Exploration of the Sea, Copenhagen, Denmark.
Hüssy, K. 2008. Otolith shape in juvenile cod (*Gadus morhua*): Ontogenetic and environmental effects. Journal of Experimental Marine Biology and Ecology, 364: 35 –41.
Hüssy, K., Mosegaard, H., Albertsen, C.M., Eg Nielsen, E., Hansen, J H. and Eero, M. 2016. Evaluation of otolith shape as a tool for stock discrimination in marine fishes using Baltic Sea cod as a case study. Fisheries Research, 174: 210–218.
Huxley, T.H. 1881. The herring. Popular Science Monthly, 19.
ICES, 1970. Report on the state of the herring stocks around Ireland and north-west of Scotland. ICES Cooperative Research Report, Series A, No 21. 34 pp.
ICES, 1972. ICES Report of the North Sea Herring Assessment Working Group. ICES Pelagic fish (Northern) Committee, CM 1972/H:13.
ICES, 1975. Report of the ICES Working Group on the Bloden Tagging Experiment,1969/1970. ICES Cooperative Research Report No 47.
ICES, 1978. Report of the Herring Assessment Working Group for the Area South of 62ºN. ICES Pelagic Fish Committee CM 1978/H:67.
ICES, 1980. Report on the coordinate acoustic survey on herring stocks in 1980. ICES CM 1980/H:24.
ICES, 1981. Herring Assessment Working Group for the Area South of 62ºN. ICES Pelagic Fish Committee, CM 1981/H:8.
ICES, 1982. Report of the Herring Assessment Working Group for the Area South of 62ºN (HAWG). ICES CM 1982/Assess:7, 132 pp.
ICES, 1990. Report of the herring assessment working group for the area south of 62N. Copenhagen, 27 March-6 April 1990. ICES CM 1990/Assess:14.
ICES, 1991a. Herring assessment working group for the area south of 62N. Copenhagen, 2-12 April 1991. ICES CM 1991/Assess:15.
ICES, 1991b. Report of the planning group on acoustic surveys in sub-area IV and division IIIa. Aberdeen 9-10 January 1991. CM 1991/H:40.
ICES, 2010. Report of the Study Group on the evaluation of assessment and manage-ment strategies of the western herring stocks (SGHERWAY), 14-18 June 2010, Dublin, Ireland. ICES CM 2010/SSGSUE:08. 194 pp.
ICES, 2011a. Report of the Herring Assessment Working Group for the area South of 62 deg N (HAWG), 16 - 24 March 2011, ICES Headquarters, Copenhagen. ICES CM 2011/ACOM:06 .749 pp.
ICES, 2011b. Report of the Workshop on Sexual Maturity Staging of Herring and Sprat (WKMSHS), 20-23 June 2011, Charlottenlund, Denmark. ICES CM 2011/ACOM:46. 143pp.
ICES, 2012. Report of the Herring Assessment Working Group for the Area South of 62 N (HAWG), 13 - 22 March 2012, Copenhagen, Denmark. ICES CM 2012/ACOM:06. 835 pp.
ICES, 2013a. Report of the Benchmark Workshop on Pelagic Stocks (WKPELA 2013), 4–8 February 2013, Copenhagen, Denmark. ICES CM 2013/ACOM:46. 483 pp.
ICES, 2013b. Report of the Herring Assessment Working Group for the Area South of 62 N (HAWG), 12-21 March 2013, ICES Headquarters, Copenhagen. ICES CM 2013/ACOM:06. 1283pp.

ICES, 2014. Report of the Herring Assessment Working Group for the Area South of 62ºN (HAWG), 11-20 March 2014, ICES HQ, Copenhagen, Denmark. ICES CM 2014/ACOM:06. 1257 pp.
ICES, 2015a. Report of the Benchmark Workshop on West of Scotland herring (WKWEST 2015). 2–6 February 2015, Marine Institute, Dublin, Ireland.
ICES, 2015b. Manual for International Pelagic Surveys (IPS). Series of ICES Survey Protocols SISP 9 – IPS. 92 pp.
ICES, 2015c. Report of the Herring Assessment Working Group for the Area South of 62ºN (HAWG), 10-19 March 2015, ICES HQ, Copenhagen, Denmark. ICES CM 2015/ACOM:06. 850 pp.
ICES, 2016. Report of the Herring Assessment Working Group for the Area South of 62˚N (HAWG), 29 March-7 April 2016, ICES HQ, Copenhagen, Denmark. ICES CM 2016/ACOM:07. 867 pp.
ICES, 2017a. Workshop on Stock Identification and Allocation of Catches of Herring to Stocks (WKSIDAC), 20-24 November 2017, Galway, Ireland. ICES CM 2017/ACOM:37. 99 pp.
ICES, 2017b. Herring Assessment Working Group for the Area South of 62 deg N (HAWG), 14-22 March 2017, ICES HQ, Copenhagen, Denmark. ICES CM 2017/ACOM:07. 856 pp.
ICES, 2018. Report of the Herring Assessment Working Group for the Area South of 62°N (HAWG). 29-31 January 2018 and 12-20 March 2018. ICES HQ, Copenhagen, Denmark. ICES CM 2018/ACOM:07. 960 pp.
ICES, 2019a. Herring Assessment Working Group for the Area South of 62° N (HAWG). ICES Scientific Reports. 1:2. 971 pp.
ICES, 2019b. International Bottom Trawl Survey Working Group (IBTSWG). ICES Scientific Reports. 1:60. 159 pp.
ICES, 2019c. Interbenchmark Protocol for Herring in 6.a, 7.b-c 2019, (IBPher6a7bc). ICES Scientific Reports. 1:19. 74 pp. http://doi.org/10.17895/ices.pub.5261
ICES, 2020a. Working Group of International Pelagic Surveys (WGIPS). ICES Scientific Reports. 2:56. 473 pp.
ICES, 2020b. Herring Assessment Working Group for the Area South of 62° N (HAWG). ICES Scientific Reports. 2:60. 1054 pp.
Ihssen, P.E., Booke, H. E., Casselman, J.M., McGlade, J.M., Payne, N.R. and Utter, F.M. 1981. Stock Identification: materials and methods. Canadian Journal of Fisheries and Aquatic Science, 38: 1838–1855.
Iles, T.D. and Sinclair, M. 1982. Atlantic herring: stock discreteness and abundance. Science, 215: 627-633.
Jacobsen, V.H. and Hansen, A. 1976. Report on the international surveys of herring larvae in the North Sea and adjacent waters in 1975/76. C.M.1976/Ha26
James, G., Witten, D., Hastie, T. and Tibshirani, T. 2013. An introduction to statistical learning, volume 112. Springer, 2013.
Jardim, E., Millar, C.P., Mosqueira, I., Scott, F., Osio, G. C., Ferretti, M., Alzorriz, N., and Orio, A. What if stock assessment is as simple as a linear model? The a4a initiative. ICES Journal of Marine Science, 72: 232–236.
Johnsen, E., Totland, A., Skålevik, Å., Holmin, A.J., Dingsør, G.E., Fuglebakk, E.and Handegard N. O. 2019. StoX: an open source software for marine survey analyses. Methods in Ecology and Evolution, 10: 1523–1528.
Jolly, G.M., and Hampton, I. 1990. A stratified random transect design for acoustic surveys of fish stocks. Canadian Journal of Fisheries and Aquatic Sciences, 47: 1282-1291.
Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24: 1403-1405.
Jombart, T., Pontier, D and Dufour, A-B. 2009. Genetic markers in the playground of multivariate analysis. Heredity, 102: 330-341.
Jombart, T., Devillard, S. and Balloux, F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics, 11: 94.
Jorde, P.E., Synnes, A-E., Espeland, S.H., Sodeland, M. and Knutsen, H. 2018. Can we rely on selected genetic markers for population identification? Evidence from coastal Atlantic cod. Ecology and Evolution, 8:12547-12558.

Kaplinski, L., Andreson, R., Puurand, T., Remm, M. 2005 MultiPLX: automatic grouping and evaluation of PCR primers. Bioinformatics, 21: 1701-1702.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. and Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28: 1647-1649.
Kerr, L.A., Hintzen, N.T., Cadrin, S.X., Worsøe Clausen, L., Dickey-Collas, M., Goethel, D.R., Hatfield, E.M.C., Kritzer, J.P., and Nash, R.D.M. 2016. Lessons learned from practical approaches to reconcile mismatches between biological population structure and stock units of marine fish. ICES Journal of Marine Science, 74: 1708–1722.
Kerr, Q., Fuentes-Pardo, A.P., Kho, J., McDermid, J.L. and Ruzzante, D.E. 2019. Temporal stability and assignment power of adaptively divergent genomic regions between herring (*Clupea harengus*) seasonal spawning aggregations. Ecology and Evolution, 9: 500-510.
King, D.P.F., Ferguson, A. and Moffett, I.J.J. 1987. Aspects of the population genetics of herring, *Clupea harengus*, around the British Isles and in the Baltic Sea. Fisheries Research, 6: 35-52.
Kirkegaard, E., Aglen, A., Bailey, R.S., Forbes, S.T. and Hagstrom, O. 1989. Report on herring acoustic surveys in the North Sea, Skagerrak and Kattegat during summer 1988. ICES Pelagic fish Committee CM 1989/H:37.
Kirkegaard, E., Aglen, A., Degnbol, P., Hagstrom, O., Palmen, L-E. and Simmonds, E.J. 1990. Report on herring acoustic surveys in the North Sea, Skagerrak and Kattegat during summer 1989. ICES Pelagic fish Committee CM 1990/H:24.
Kristensen, K., Nielsen, A., Berg, C., Skaug, H. and Bell, B. 2016. TMB: automatic differentiation and Laplace approximation J. Stat. Softw., 70 (2016), pp. 1-21,
Kuhn, M. 2008. Building Predictive Models in R Using the caret Package. Journal of Statistical Software, 28(5).
Lamichhaney, S., Martinez Barrio, A., Rafati, N., Sundström, G., Rubin, C.-J., *et al*., 2012. Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. PNAS, 109: 19345–19350.
Legault, C. and Restrepo, V. 1999. A Flexible Forward Age-Structured Assessment Program. ICCAT Collective Volume of Scientific Papers, 49.
Lester, R.J.G. and MacKenzie, K. 2009. The use and abuse of parasites as stock markers for fish. Fisheries Research, 97: 1–2.
Libungan, L.A., Olafsdottir, G., Skirnisdottir, S., Palsson, S., Pampoulie, C., Bjornsdottir, S.H., Olafsson, K., Oskarsson, G.J. and Danielsdottir, A.K. 2012. Fourteen new microsatellite markers for Atlantic herring *Clupea harengus*. Journal of Fish Biology, 81:1422–1426.
Libungan, L.A. and Pálssom, S. 2015. ShapeR: An R package to study otolith shape variation among fish populations. PLoS ONE 10(3): e0121102.
Libungan, L.A., Óskarsson, G.J., Slotte, A., Jacobsen, J.A. and Pálsson, S. 2015a. Otolith shape: a population marker for Atlantic herring *Clupea harengus*. Journal of Fish Biology, 86: 1377 – 1395.
Libungan, L.A., Slotte, A., Husebø, A., Godiksen, J.A. and Pálsson, S. 2015b. Latitudinal gradient in otolith shape among local populations of Atlantic herring (*Clupea harengus* L.) in Norway. PLoS ONE 10(6): e0130847.
Limborg, M.T., Bekkevold, D. *et al*. 2012a. SNP discovery using next generation transcriptomic sequencing in Atlantic Herring (*Clupea harengus*). PLoS ONE, 7: e42089.
Limborg, M.T., Helyar, S.J., De Bruyn, M., Taylor, M.I., Nielsen, E.E., Ogden, R., Carvalho, G.R., Bekkevold, D. and FPT Consortium. 2012b. Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). Molecular Ecology, 21: 3686-3703.
MacKenzie, K. 1985 The use of parasites as biological tags in population studies of herring (*Clupea harengus* L.) in the North Sea and to the north and west of Scotland. ICES Journal of Marine Science, 42: 33-64.
MacKenzie, K. 1987. Long-term changes in the prevalence of two helminth parasites (Cestoda: Trypanorhyncha) infecting marine fish. Journal of Fish Biology, 31: 83–88

Mackinson, S., Pastoors, M., Lusseau, S., Armstrong, E., Fässler, S., Haan, D., Kleppe, R., O'Malley, M., Campbell, A., Clarke, M., Wiseman, A. 2017. Report on the 2016 industry-science survey of herring in the Western British Isles (ICES div 6a, 7bc). 82pp.

Mackinson, S., Pastoors, M., Lusseau, S., Armstrong, E., O'Connell, S, Haan, D., Burgraaf, D., Berges, B., McClean, A, Langlands, B., Scott, A., Wiseman, A., O'Malley, M., Clarke, M., Farrell, E.D. 2018. The 2017 industry-science survey of herring in the Western British Isles (ICES div 6a, 7bc). 89pp.

Mackinson, S., Pastoors, M., Lusseau, S., Armstrong, E., O'Connell, S, Sakinan, S., Berges, B., McClean, A, Langlands, B., Wiseman, A., O'Malley, M., Farrell, E.D. 2019. The 2018 industry-science survey of herring in the Western British Isles (ICES div 6a, 7bc). 99pp.

Mackinson, S., Pastoors,M., Lusseau, S., O'Connell, S, Forbes-Birnie, J., Sakinan, S., Berges, B., Brigden, K., O'Malley,M., Farrell, E.D. 2019. The 2019 industry-science survey of herring in the Western British Isles (ICES div 6a, 7bc). 96pp.

Mapp, J., Hunter, E., Van Der Kooij, J., Songer, S. and Fisher, M. 2013. Otolith shape and size: The importance of age when determining indices for fish-stock separation. Fisheries Research, 190: 43-52.

Manel, S., Gaggioti, O.E., Waples, R.S. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology and Evolution, 20: 136–142.

Mariani, S., and Bekkevold, D. 2013. The nuclear genome: neutral and adaptive markers in fisheries science. In Stock Identification Methods, 2nd edn, pp. 397–428. Ed. by S. X. Cadrin, L. A. Kerr, and S. Mariani. Elsevier, San Diego. 566 pp.

Mariani, S., Hutchinson, W.F., Hatfield, E.M.C., Ruzzante, D.E., Simmonds, E.J., Dahlgren, T.G., Andre, C. Brigham, J., Torstensen, E. and Carvalho, G.R. 2005. North Sea herring population structure as revealed by microsatellite analysis. Marine Ecology Progress Series, 303: 245–257.

Martinez Barrio, A., Lamichhaney, S., Fan, G., Rafati, N., Pettersson, M., Zhang, H., Dainat, J., Ekman, D., Höppner, M., Jern, P., Martin, M., Nystedt, B., Liu, X., Chen, W., Liang, X., Shi, C., Fu, Y., Ma, K., Zhan, X., Feng, C., Gustafson, U., Rubin, C.J., Sällman Almén, M., Blass, M., Casini, M., Folkvord, A., Laikre, L., Ryman, N., Ming-Yuen, Lee S., Xu, X., Andersson, L. 2016 The genetic basis for ecological adaptation of the Atlantic herring revealed by genome sequencing. eLife, 5:12081.

Matthews, J.D. 1885. Report as to the variety among the herrings on the Scottish coasts. Annual report for the Fishery Board for Scotland 1885, Appendix F No IV, 61-98.

McQuinn, I.H. 1997. Metapopulations and the Atlantic herring. Reviews in Fish Biology and Fisheries, 7: 297–329.

McMillan, L. and Fewster, R. 2017. Visualizations for genetic assignment analyses using the saddlepoint approximation method. Biometrics, 73: 1029-1041.

McPherson, A.A., O'Reilly, P.T., McParland, T.L., Jones, M.W. and Bentzen, P. 2001. Isolation of nine novel tetranucleotide microsatellites in Atlantic herring (*Clupea harengus*). Molecular Ecology Notes, 1: 31–32.

Methot, R.D., 2000. Technical description of the stock synthesis assessment program. NOAA Technical Memorandum NMFS-NWFSC-43. U.S. Department of Commerce National Oceanic and Atmospheric Administration National Marine Fisheries Service.

Methot, R.D., Wetzel, C.R., Taylor, I.G. and Doering. K. 2020. Stock Synthesis User Manual Version 3.30.15 Richard D. Methot, Jr.,1 Chantel R. Wetzel,2 Ian G. Taylor,2 and Kathryn Doering2 https://doi.org/10.25923/5wpn-qt71

Meyer, D., Dimitriadou, E., Hornik, K., Weingessel, A., Leisch, F., Chang, C.-C., and Lin, C.-C. 2015. e1071: Misc Functions of the Department of Statistics, Probability Theory Group (Formerly: E1071). TU Wien.

Mille, T., Mahé, K., Cachera, M., Villanueva, M. C., de Pontual, H. and Ernande, B. 2016. Diet is correlated with otolith shape in marine fish. Marine Ecology Progress Series, 555: 167–184.

Mitchell, J.M. 1864. The Herring: Its Natural History and National Importance. Edinburgh, Edmonston and Douglas, 370pp.

Molloy, J. 1983. The occurrence of winter and spring spawning herring off the Northwest coast of Ireland. ICES CM 1983/H:60.

Molloy, J., Barnwall, E. and Morrison, J. 1991. Herring tagging experiments around Ireland, 1991. Department of the Marine, Fishery Leaflet, 154.
Molloy, J. 2006. Herring fisheries of Ireland, 1900-2005. Biology, Research, development and assessment. Marine Institute, Galway, Ireland. 235pp.
Moreira, C., Froufe, E., Vaz-Pirez, P., Triay-Portella, R. and Correia, A.T. 2020. Landmark-based geometric morphometrics analysis of body shape variation in blue jack mackerel, *Trachurus picturatus*, from the North-east Atlantic. Journal of Sea Research 163.
Morrison, J.A. and Bruce, T. 1981. Scottish herring tagging experiments in the Firth of Clyde 1975-1979, and evidence of affinity between Clyde herring and those in adjacent areas. ICES CM 1981/H:53.
Munro, R.J The Herring Fisheries. International Fisheries Exhibition London, 1883. William Clowes and Sons, Limited, London.
Murta, A.G., Borges, M.F., Silveiro, M.L. 1996. Morphological variations in the sagitta otoliths of horse mackerel (*Trachurus trachurus*) in Portuguese waters (Div. IXa). ICES CM 1996/H: 27. 8p.
Murta, A.G., Pinto, A.L. and Abaunza, P. 2008. Stock identification of horse mackerel (*Trachurus trachurus*) through the analysis of body shape. Fisheries Research, 89: 152 – 158.
Narum, S.R., Buerkle, C.A., Davey, J.W., Miller, M.R. and Hohenlohe, P.A. 2013. Genotyping-by-sequencing in ecological and conservation genomics. Molecular Ecology, 22: 2841–2847.
Nielsen, E. E., A. Cariani, E. Aoidh, G. E. Maes, I. Milano, R. Ogden, M. Taylor *et al*. 2012. Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. Nature Communications, 3: 851.
Nielsen, M., Berg, C., 2014. Estimation of time-varying selectivity in stock assessments using state-space models. Fisheries Research, 158: 96–101.
Nolan, C., O'Donnell, C., Campbell, A., Sullivan, M. and Mullins, E. 2012. Northwest Herring Acoustic Survey Report, 21 June – 11 July, 2012. FEAS Survey Series: 2012/04. 48pp.
Nolan, C., O'Donnell, C., Campbell, A., Sullivan, M., Mullins, E. and Keogh, H. 2013. Northwest Herring Acoustic Survey Report, 22nd June – 12th July, 2013. FEAS Survey Series: 2013/04. 49pp.
Nolan, C., O'Donnell, C., Campbell, A., Sullivan, M. and Mullins, E. 2014. Northwest Herring Acoustic Survey Report, 22nd June – 12th July, 2014. FEAS Survey Series: 2014/04. 49pp.
Nolan, C., Campbell, A., O'Donnell, C., Marrinan, M. and Parker, M. 2015. Northwest Herring Acoustic Survey Report 24th June 14th July 2015. FEAS Survey Series: 2015/04. 51pp.
O'Connell, M., Dillon, M.C. and Wright, J.M. 1998. Development of primers for polymorphic microsatellite loci in the Pacific herring (*Clupea harengus pallasi*): Molecular Ecology, 7: 357-363.
O'Donnell, C., Mullins, E., Sullivan, M., Lyons, K., Kavanagh, L., Bunn, R., Beattie, S., McAuliffe, M., Griffin, N. and Boyd, J. 2010. Northwest Herring Acoustic Survey Report, 18 June – 07 July, 2010. FSS Survey Series: 2010/03. 32 pp.
O'Donnell, C., Nolan, C., Johnston, G., O'Malley, M., Mullins, E., Keogh, N., O'Callaghan, S., Keogh, H., Grassie, A. and Nicholas, S. 2016. Western European Shelf Pelagic Acoustic Survey (WESPAS),16 June – 30 July, 2016. FSS Survey Series: 2016/02. 55pp.
O'Donnell, C., O'Malley, M., Mullins, E., Lynch, D., Keogh, N. and O'Sullivan, C. 2017. Western European Shelf Pelagic Acoustic Survey (WESPAS),06 June – 21 July, 2017. FSS Survey Series: 2017/03. 61pp.
O'Donnell, C., O'Malley, M., Lynch, D., Mullins, E., Keogh, N., Power, J., Long, A. and Croot, P. 2018. Western European Shelf Pelagic Acoustic Survey (WESPAS), 10 June – 24 July, 2018. FSS Survey Series: 2018/03. 65pp.
O'Donnell, C., O'Malley, M., Lynch, D., Mullins, E., Connaughton, P., Power, J., Long, A. and Croot, P. 2019. Western European Shelf Pelagic Acoustic Survey (WESPAS), 13 June – 24 July, 2019. FSS Survey Series: 2019/03. 69pp.

Olsen, J.B., Lewis, C.J., Kretschmer, E.J., Wilson, S.L. and Seeb, J.E. 2002. Characterization of 14 tetranucleotide microsatellite loci derived from Pacific herring. Molecular Ecology Notes, 2: 101–103.
Paetkau D., Calvert, W., Stirling, I. and Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology, 4: 347-354.
Paukert, C.P. and Wittig, T.A. 2002. Applications of multivariate statistical Methods in Fisheries. Fisheries Magazine, 27: 16-22.
Peakall, R. and Smouse P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28, 2537-2539.
Pedersen, M.W. and Berg, C.W. 2017. A stochastic surplus production model in continuous time. Fish and Fisheries, 18: 226–243.
Pérez-Quiñonez, C. I., Quiñonez-Velázquez, C. and García-Rodríguez, F. J. 2018. Detecting *Opisthonema libertate* (Günther, 1867) phenotypic stocks in northwestern coast of Mexico using geometric morphometrics based on body and otolith shape. Latin American Journal of Aquatic Research, 46(4).
Pettersson, M.E., Rochus, C.M., Han, F., Chen, J., Hill, J., Wallerman, O., Fan, G., Hong, X., Xu, Q., Zhang, H., Liu, S., Liu, X., Haggerty, L., Hunt, T., Martin, F.J., Flicek, P., Bunikis, I., Folkvord, A. and Andersson L. 2019. A chromosome-level assembly of the Atlantic herring genome-detection of a supergene and other signals of selection. Genome Research, 29: 1919-1928.
Piry, S., Alapetite, A., Cornuet, J-M., Paetkau, D., Baudouin, L., Estoup, A. 2004. geneclass2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity, 95: 536– 539.
Pommeranz, T. 1975. Report on the international surveys of herring larvae in the North Sea and adjacent waters. C.M. 1975/H: 29
Pritchard, J.K, Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics. 155, 945–959.
Prodöhl, P. A., Antoniacomi, A., Bradley, C., Carlsson J., Carvalho, G. R., Coughlan, J., Coyne, J., Cross, M. C., Cross, M. E., Davies, C. A., Dillane, E., Gargan, P., Hynes, R., McGinnity, P. , Milner, N., Reed, T. , Roche, W., Taylor, M., Tyskind, N., Cross, T.F. 2017. Population genetics and Genetic Stock Identification of anadromous Salmo trutta from the Irish Sea and adjacent areas, using microsatellite DNA loci. In Sea Trout: from Science Management (Proceedings of the 2nd Sea trout Symposium, Dundalk, Ireland, 2017).
Qiao, X. and Zhang, L. 2015. Flexible high-dimensional machines and their asymptotic properties. Journal of Machine Learning Research, 16: 1547-1572.
Rankine, P.W. 1986. Herring spawning grounds around the Scottish coast. ICES Pelagic Fish Committee CM 1986/H:15
Rannala, B. and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. USA 94: 9197-9221.
Reiss, H., Hoarau G., Dickey-Collas M. and Wolff, W.J. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish and Fisheries, 10: 361–395.
Ressel, K.N., McNicholl, D.G. and Sutton T.M. 2020. Capelin, *Mallotus villosus*, population differentiation among and with regions using relative warps. Environmental Biology of Fishes, 103: 667 – 681.
Reyment, R. A. 2010. Morphometrics: An historical Essay in Elewa, A. M. T. Morphometrics for nonmorphometricians. Springer-Verlag Berlin Heidelberg, 9 – 24.
Rice WR. 1989 Analyzing tables of statistical tests. Evolution. 43, 223–225.
Rodriguez-Mendoza, R. P. 2006. Otolith and their application if fishery science. Ribarstvo, 64: 89–102.
Rohlf, F. J. 2006. tpsDig, Digitize Landmarks and Outlines, Version 2.05. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.
Rousset, F. 2008 Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources, 8: 103-106.
Rozen, S. and Skaletsky, H. 2000 Primer3 on the WWW for general users and for biologist programmers. Methods in Molecular Biology, 132: 365–386.

Ruzzante, D.E., Mariani, S., Bekkevold, D. *et al*. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Proceedings of the Royal Society of London Series B: Biological Sciences, 273: 1459–1464.
Saunders, R., O'Donnell, C., Campbell, A., Mullins, E., Sullivan, M., McKeogh, E. 2011. Northwest Herring Acoustic Survey Report, 18 June – 07 July, 2011. FSS Survey Series: 2011/03. 45pp.
Saville, A. 1961. A decline in the yield of Scottish estuarine spring spawning herring fisheries. Rapport et Proces-Verbaux des Reunions,154: 33-45
Saville, A. 1962. Herring tagging experiments in the Clyde. Scottish Fisheries Bulletin, 18:12-14.
Saville, A. 1970. The stock fished by the Scottish herring fishery to the west of Scotland. ICES Pelagic Fish (Northern) Committee, CM19/H: 29
Saville, A. 1971. The biology of young herring in the Moray Firth and their recruitment to the adult stocks. Rapp. P.-v. Reun cons perm. Int. Explor Mer, 160: 184-193.
Saville, A. and McKay, D. W. 1973. Report on the international surveys of herring larvae in the North Sea and adjacent waters in 1972/1973. ICES CM 1973/H: 13
Saville, A. and Morrison, J.A. 1973. A reassessment of the herring stock to the west of Scotland. ICES Pelagic Fish (Northern) Committee, CM 1973/H:24.
Saville, A., Parrish, B.B. and Baxter I.G. 1966. Review of Herring Fisheries and stocks off the west coast of Scotland. ICES CM 1966: H1.
Saville, A. and Bailey, R.S. 1980. The assessment and management of the herring stocks in the North Sea and to the west of Scotland. Rapport et Proces-Verbaux des Reunions, 177: 7-36.
Schnack, D. 1972. Report on the International Surveys of Herring Larvae in the North Sea and Adjacent Waters in 1971/72. ICES, C.M.1972/ H: 28
Schrider, D.R. and Kern, A.D. 2018. Supervised machine learning for population genetics: a new paradigm. Trends in Genetics, 34:301-312.
Silva, A. 2003. Morphometric variation among sardine (*Sardina pilchardus*) populations from the northeastern Atlantic and the western Mediterranean. ICES Journal of Marine Science, 60: 1352-1360.
Simmonds, E.J, Bailey, R. S. and McKay, D. W. 1986. Report on the 1985 ICES-coordinated herring acoustic survey in the northern North Sea. ICES Pelagic Fish Committee CM 1986/H:16.
Simmonds, E.J., Reid, D.G., Aglen, A., Skagen, and Corten, A. 1992. Report of the 1991 ICES coordinated acoustic survey of herring stocks in ICES divisions VIa, IVA and IVb. ICES Pelagic Fish Committee CM 1992/H:35.
Simmonds, E.J., Toresen, R., Corten, A., Pedersen, J., Reid, D.G. and Fernandes, P.G. 1995. 1994 ICES coordinated acoustic survey of ICES divisions IVa, IVb, VIa and VIIb. ICES Pelagic Fish Committee, CM 1995/H:15.
Simmonds, E.J., Bailey, M.C., Toresen, R., Couperous, B., Pedersen, J., Reid, D.G., and Hammer, C. 1998. 1997 ICES coordinated acoustic survey of ICES divisions IIIa, IVa, IVb and VIa. ICES Pelagic Fish Committee, CM 1998/OPEN:22.
Simmonds, E.J. 2009. Evaluation of the quality of the North Sea herring assessment. ICES Journal of Marine Science, 66: 1814–1822.
Stephenson, R.L. 2002. Stock structure management and structure: an ongoing challenge for ICES. ICES Marine Science Symposium, 215: 305-314.
Strauss, R. E. and Bookstein, F. L. 1982. The truss: body form reconstruction in morphometrics. Systematic Zoology, 31: 113-135.
Smith, P.J. and Jamieson, A. 1986. Stock discreteness in herrings: a conceptual revolution. Fisheries Research, 4: 223-34.
Smolinski, S., Schade, M. S. and Berg, F. 2020. Assessing the performance of statistical classifiers to discriminate fish stocks using Fourier analysis of otolith shape. Canadian Journal of Fisheries and Aquatic Sciences, 77: 674-683.
Song, J., Zhao, B., Liu, J., Cao, L., and Dou, S., 2018. Comparison of otolith shape descriptors and morphometrics for stock discrimination of yellow croaker along the Chinese coast. Journal of Oceanology and Limnology, 36: 1870-1879.
StoX (2015) StoX: An open source approach to acoustic and swept area survey calculations. Institute of Marine Research, Bergen, Norway. URL: http://www.imr.no/stox

Stransky, C. 2005. Geographic variation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the North Atlantic based on otolith shape analysis. ICES Journal of Marine Science, 62: 1691-1698.
Teacher, A.G.F., Kahkonen, K. and Merilä, J. 2012. Development of 61 new transcriptome-derived microsatellites for the Atlantic herring (*Clupea harengus*). Conservation Genetics Resources, 4: 71–74.
Teacher, A.G.F., André, C., Jonsson, P.R. and Merilä, J. 2013. Oceanographic connectivity and environmental correlates of genetic structuring in Atlantic herring in the Baltic Sea. Evolutionary Applications, 6: 549–567.
Tracey, S.R., Lyle, J.M. and Duhamel, G. 2006. Application of elliptical Fourier analysis of otolith form as a tool for stock identification. Fisheries Research, 77: 138 -147.
Turan, C. 1998. A Note on The Examination of Morphometric Differentiation Among Fish Populations: The Truss System. Turkish Journal of Zoology, 23: 259–263.
Turan, C. 2000. Otolith shape and meristic analysis of herring (*Clupea harengus*) in the North-East Atlantic. Archive or Fishery and Marine Research, 48: 283 – 295.
Turan, C. 2004. Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters. ICES Journal of Marine Science, 61: 774–781.
Tuset, V.M., Lombarte, A. and Assis, C.A. 2008. Otolith atlas for the western Mediterranean, north and central Eastern Atlantic. Scientia Marina, 72: 7-198.
Vartia, S., Collins, P.C., Cross, T.F., FitzGerald, R.D., Gauthier, D.T., McGinnity, P., Mirimin, L. and Carlsson J. 2014. Multiplexing with three-primer PCR for rapid and economical microsatellite validation. Hereditas, 151: 43–54.
Vartia, S., Villanueva, J.L., Finarelli, J., Farrell, E.D., Collins, P.C3:., Hughes, G., Carlsson, J.E.L., Gauthier, D.T., McGinnity, P., Cross, T.F., FitzGerald, R.D., Mirimin, L., Cotter, P. and Carlsson, J. 2016. A novel method of microsatellite genotyping-by-sequencing using individual combinatorial barcoding. Royal Society Open Science, 3:150565
Vasconcelos, J., Vieira, A. R., Sequeira, V., González, J. A., Kaufmann, M. and Gordo, L. S. 2018. Identifying populations of the blue jack mackerel (*Trachurus picturatus*) in the Northeast Atlantic by using geometric morphometrics and otolith shape analysis. Fishery Bulletin, 116: 81 – 92.
Waples, R.S., Punt, A.E. and Cope, J.M. 2008. Integrating genetic data into management of marine resources: how can we do better? Fish and Fisheries, 9: 423–449.
Wang, J. 2017. The computer program STRUCTURE for assigning individuals to populations: easy to use but easier to misuse. Molecular Ecology Resources, 17: 981–990.
Webster, M. and Sheets, H. D. 2010. A practical introduction to landmark-based geometric morphometrics in Alroy, J. and Hunt, G. Quatitative Methods in Paleobiology. The Paleontological Society Papers, Vol. 16.
Wood, H., 1960. The herring of the Clyde estuary. Scottish Fisheries Bulletin, 13: 23.
Wood, R.J. 1971. Autumn spawning grounds of herring to the west of Scotland. Rapports et Proees-Verbaux, 160: 65-73.
Wood, R.J. 1972. Herring larvae to the west of Scotland in the autumn of 1971. ICES Pelagic Fish (Northern) Committee, CM 1972/H:6.
Wood, R.J. 1974. Report on the international surveys of herring larvae in the North Sea and adjacent waters in 1973/1974. C.M. 1974/H: 13.
Wood, R.J. 1982. Report on the international surveys of herring larvae in the North Sea and adjacent waters in 1981/1982. ICES Pelagic Fish Committee CM 1982/H:2.
Zhang, C., Ye, Z., Li, Z., Wan, R., Ren, Y. and Dou, S. 2016. Population structure of Japanese Spanish mackerel *Scomberomorus niphonius* in the Bohai Sea, the Yellow Sea and the East China Sea: evidence from random forests based on otolith features. Fisheries Science, 82: 251-256.
Zijlstra, J.J. 1971. Report on the International Surveys of Herring Larvae in the North Sea and adjacent waters 1970/1971. ICES Pelagic Fish Committee, CM 1971/H: 15

10. Annexes

10.1. Annex 1 – List of acronyms

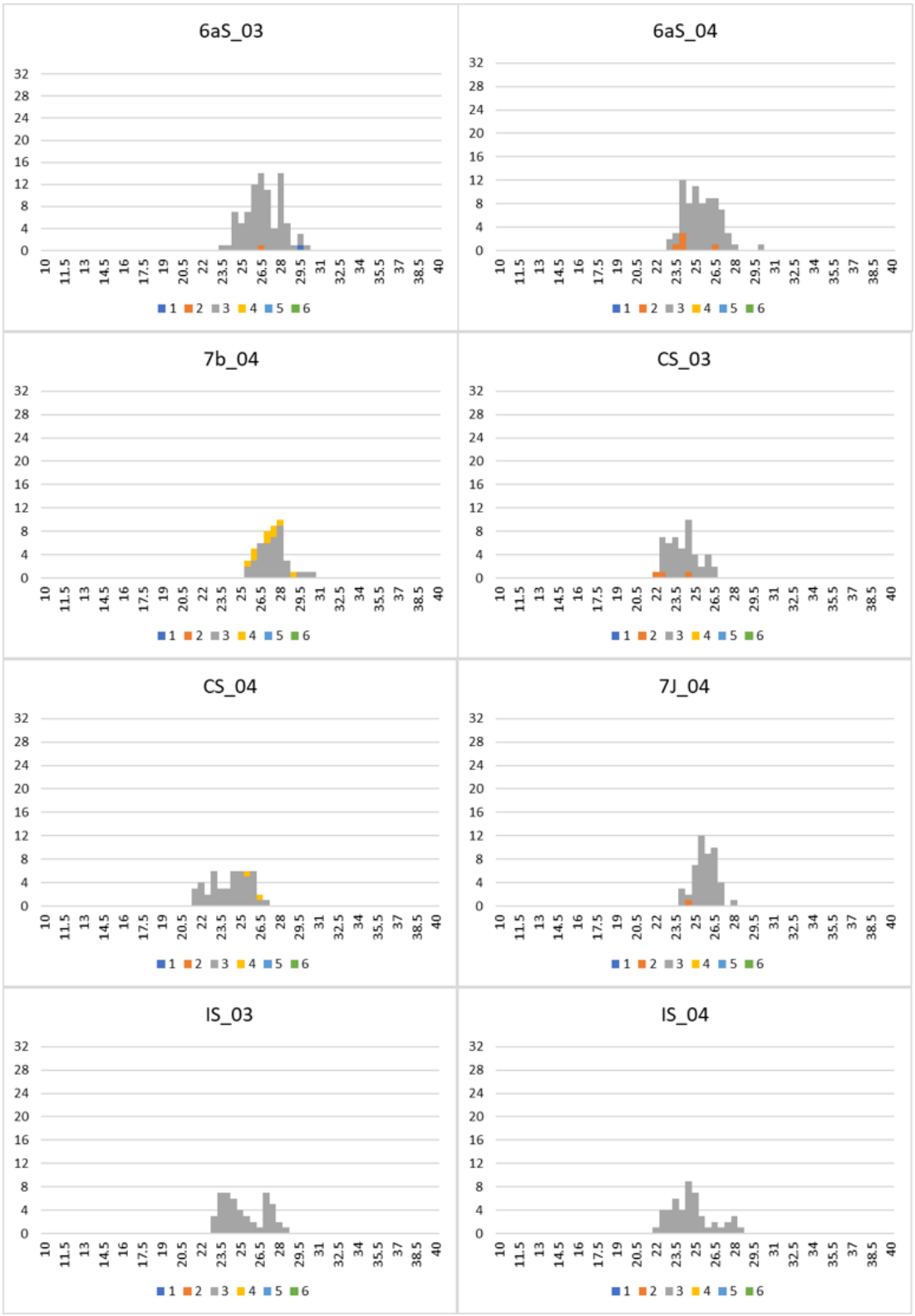
- ACFM – Advisory Committee on Fishery Management
- ACOM – ICES Advisory Committee
- BIC – Bayesian Information Criterion
- CDA – Canonical Discriminant Analysis
- DA – Discriminant Analysis
- DAPC – Discriminant Analysis of Principal Components
- DNA - Deoxyribonucleic acid
- EASME - Executive Agency for Small and Medium-sized Enterprises
- EMFF - European Maritime and Fisheries Fund
- EU – European Union
- GBS – Genotyping by Sequencing
- GENSINC - GENetic adaptations underlying population Structure IN herring
- GT-seq - Genotyping-in-Thousands by sequencing
- gDNA – Genomic Deoxyribonucleic acid
- HAWG – Herring Assessment Working Group
- HERAS - International Herring Acoustic Survey
- HWE - Hardy–Weinberg equilibrium
- ICES – International Council for Exploration of the Sea
- IMARES – Institute for Marine Resources and Ecosystem Studies
- IMR – Institute of Marine Research
- LDA – Linear Discriminant Analysis
- MI – Marine Institute
- MSHAS - Malin Shelf Herring Acoustic Survey
- MSS – Marine Scotland Science
- NASC – Nautical Area Scattering Coefficient
- NGS – Next Generation Sequencing
- PCoA – Principal Coordinate Analysis
- PCA – Principal Component Analysis

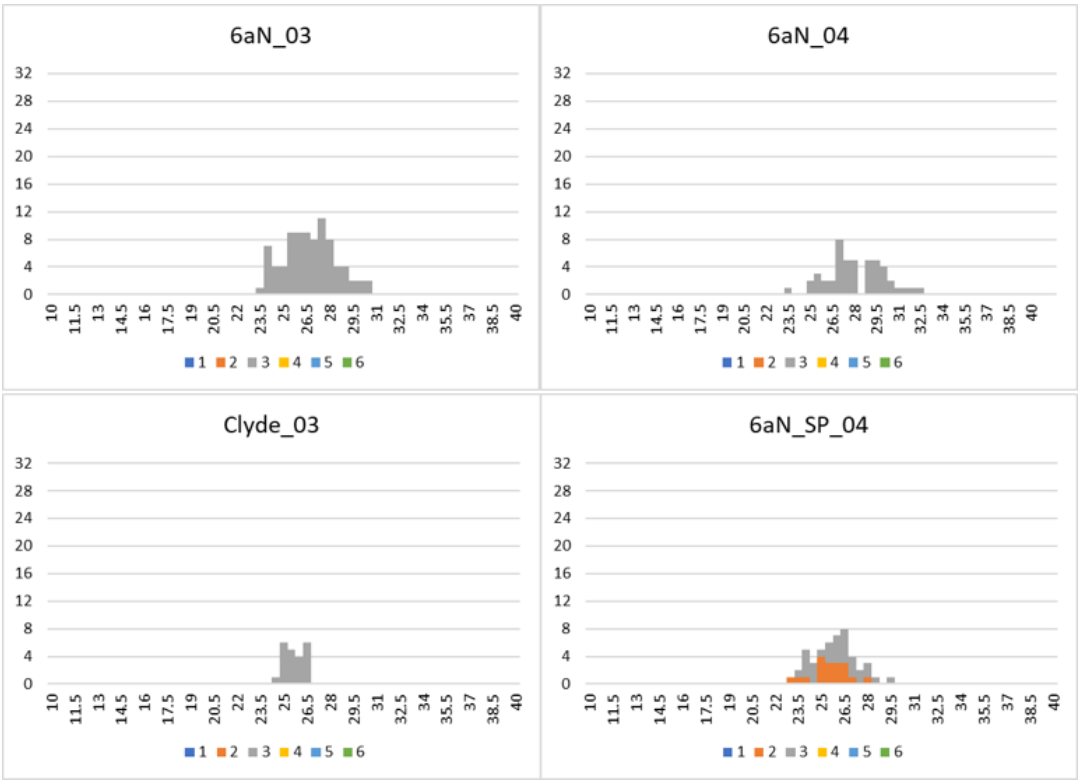
- PCR – Polymerase chain reaction
- PelAC – Pelagic Advisory Council
- QDA – quadratic discriminant analysis
- RBF – Radial Basis Function
- RMSE – Root Mean Square Error
- RNA - Ribonucleic acid
- SGHERWAY - ICES Study Group on the evaluation of assessment and management strategies of the western herring stocks
- SML – Supervised Machine Learning
- SNP – Single Nucleotide Polymorphism
- STO – Scientific and Technical Officer
- SVM – Support Vector Machine
- SWC-IBTS - Scottish West Coast International Bottom Trawl Survey
- TI – Thünen-Institut
- UCD – University College Dublin
- WESPAS - Western European Shelf Pelagic Acoustic Survey
- WGIPS – Working Group of International Pelagic Surveys
- WUR – Wageningen University and Research

10.2. Annex 2 - Labelled map of study area

10.3. Annex 3 – Length frequency and maturity stage plots of samples.

Total length (cm) is displayed on the x-axis and number of individuals on the y-axis. Maturtiy stage (6-point scale) is denoted by colour according to the legend below the x-axis.

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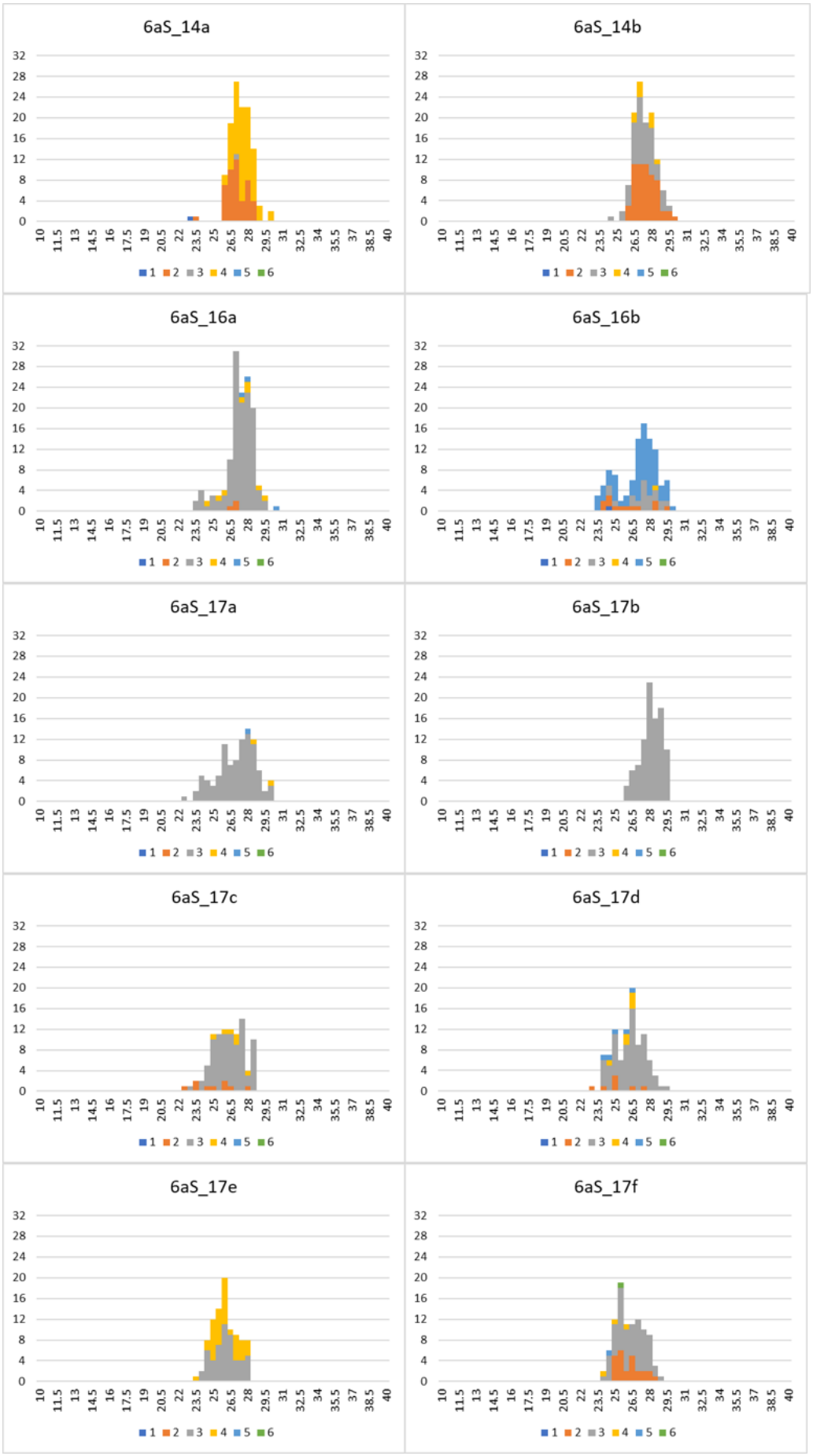
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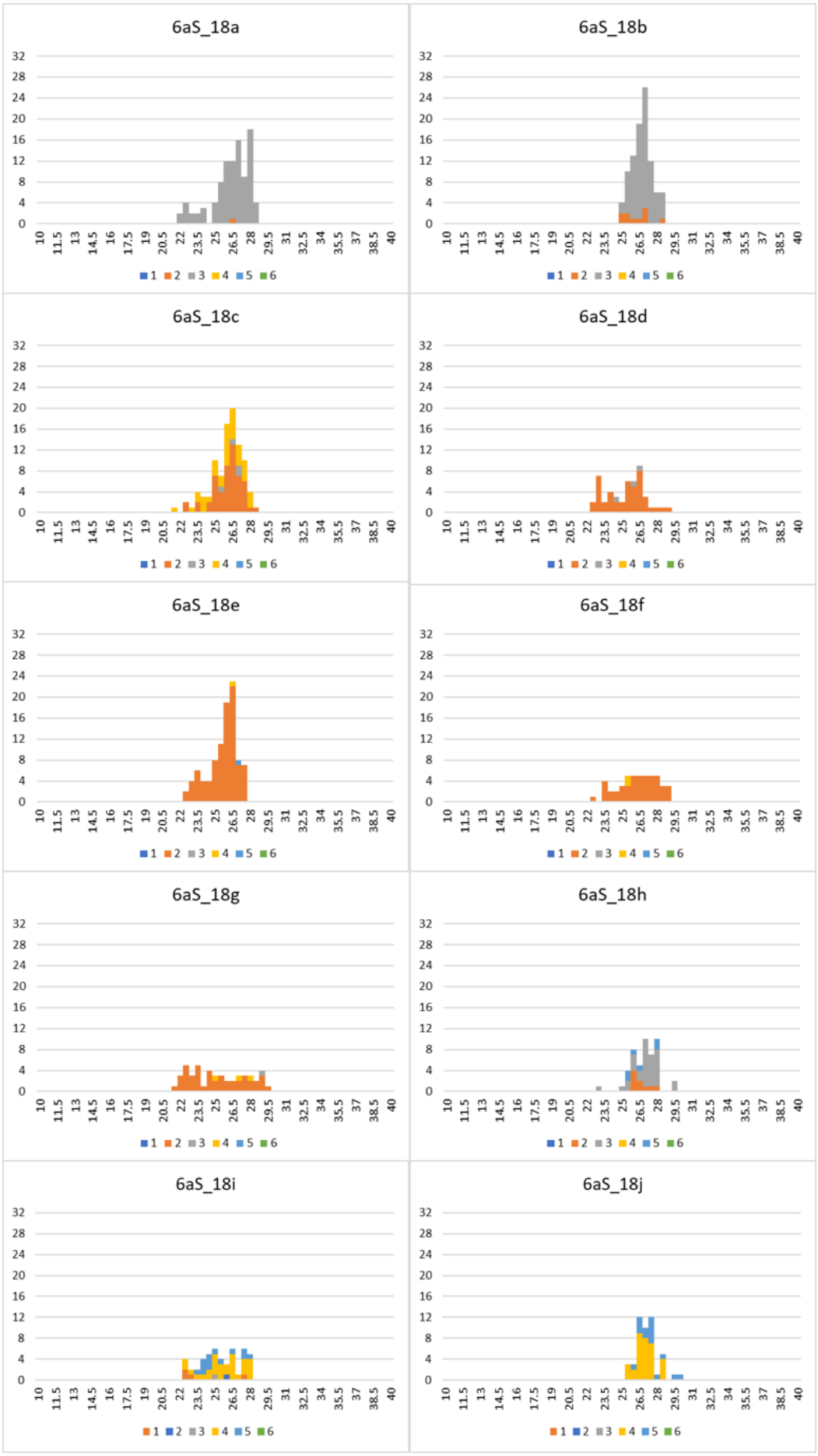
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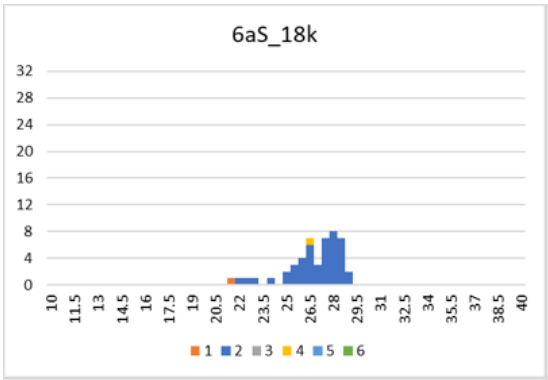
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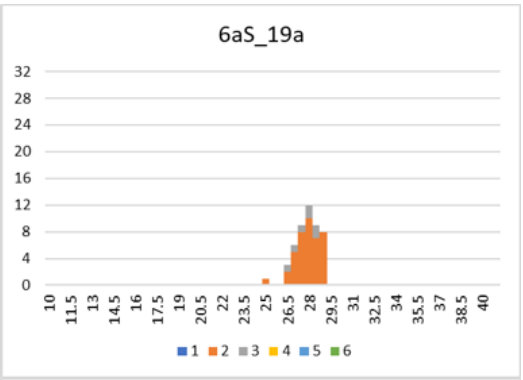
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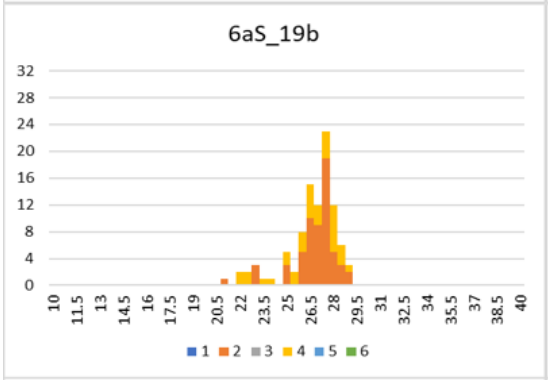
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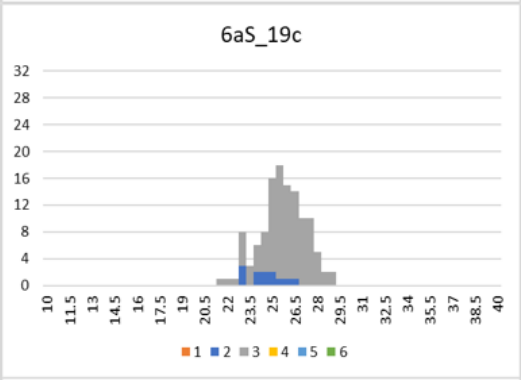
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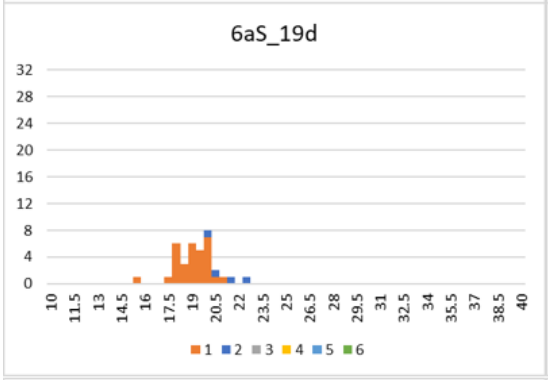
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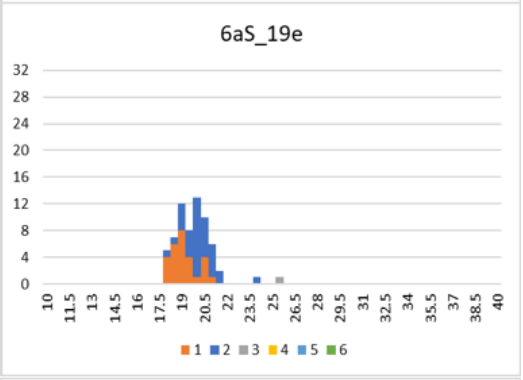
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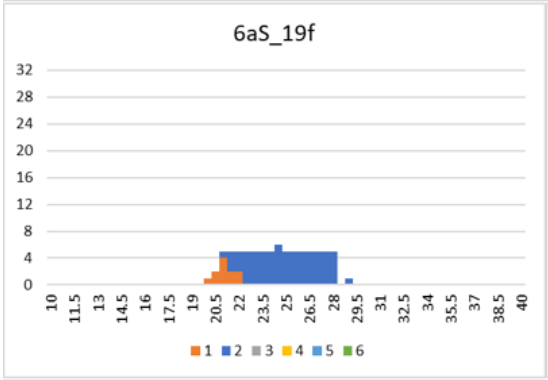
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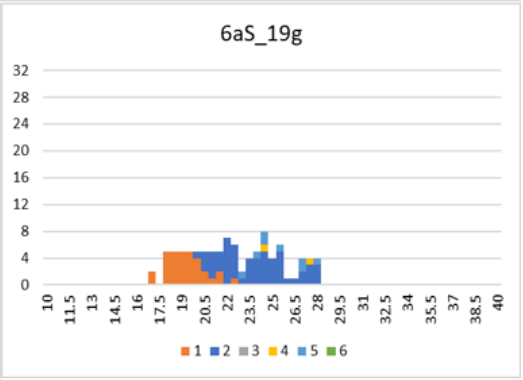
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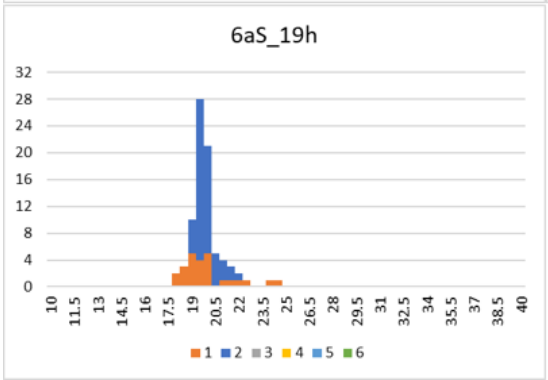
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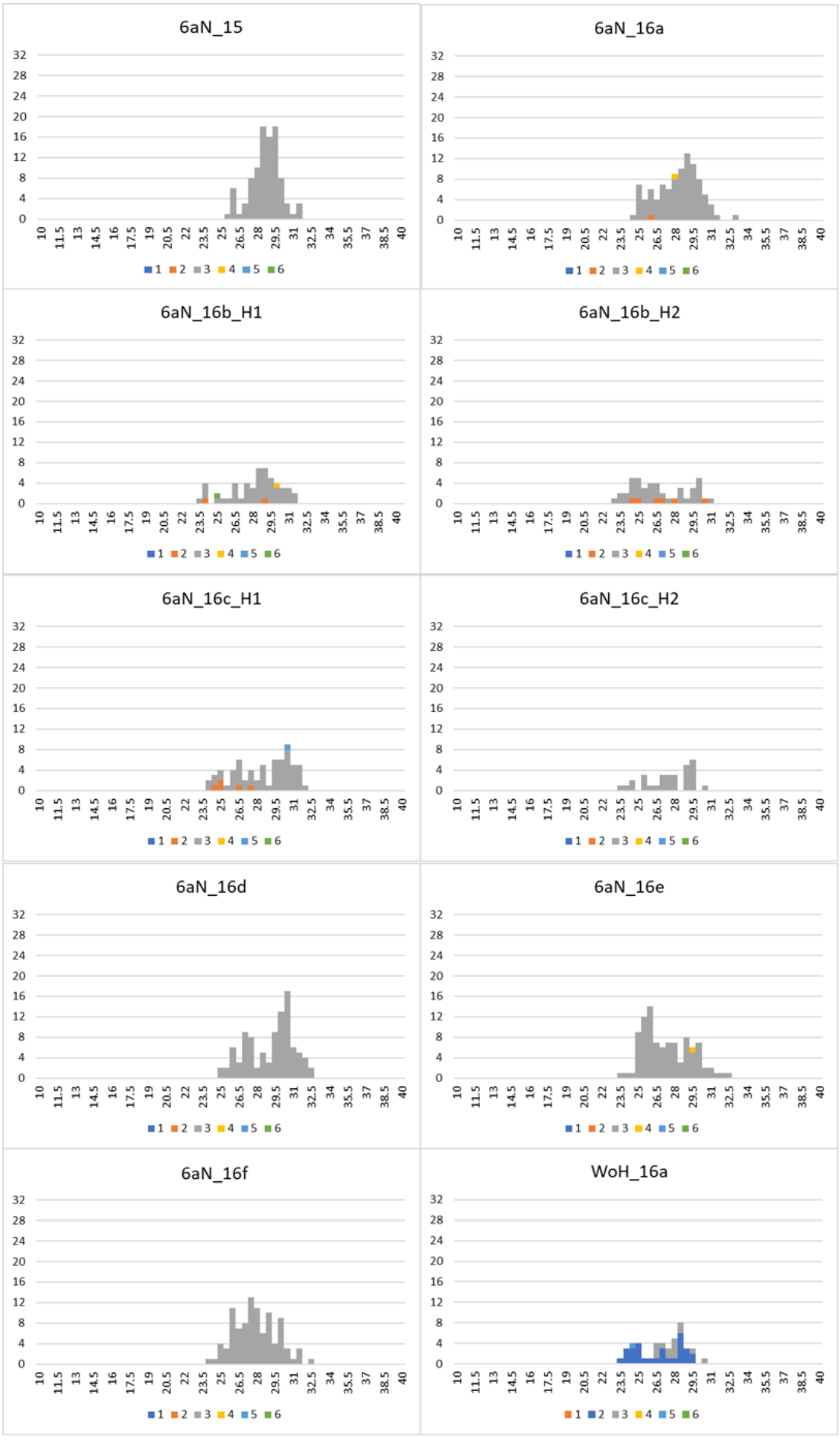
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6aN_16b_H1
6aN_16b_H2
6aN_16c_H1
6aN_16c_H2
6aN_16d
6aN_16e
6aN_16f
WoH_16a
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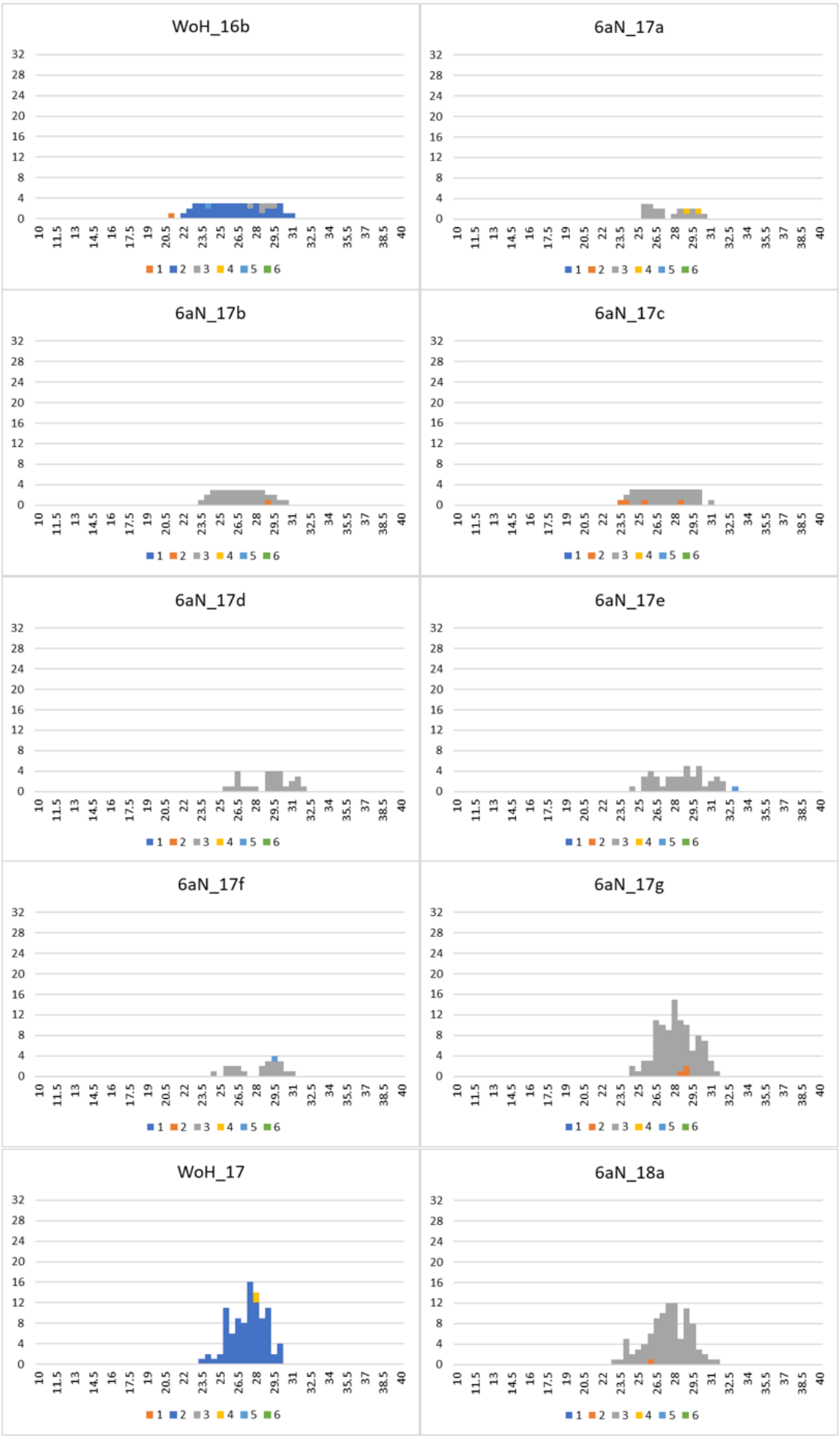
1 2 3 4 5 6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6aN_17b
6aN_17c
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6aN_17e
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6aN_17g
WoH_17
6aN_18a
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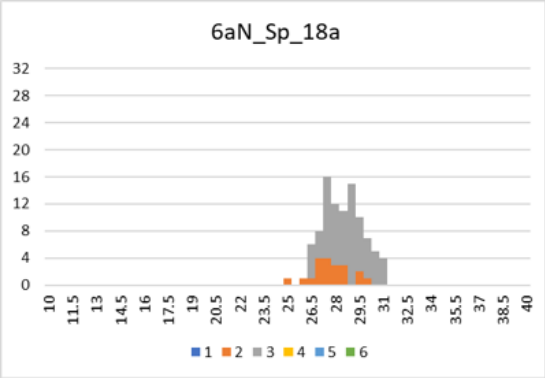
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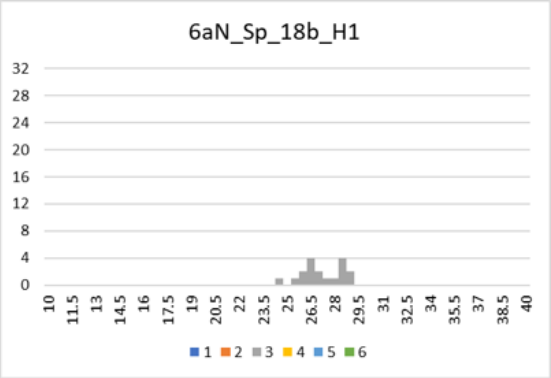
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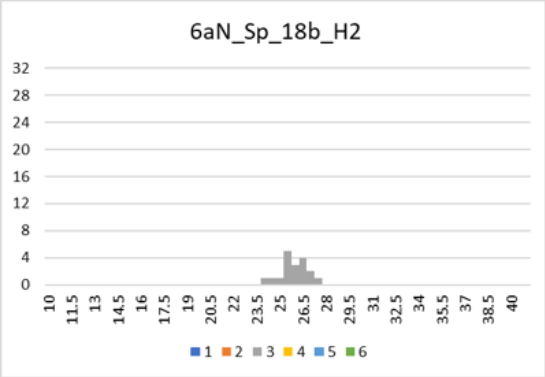
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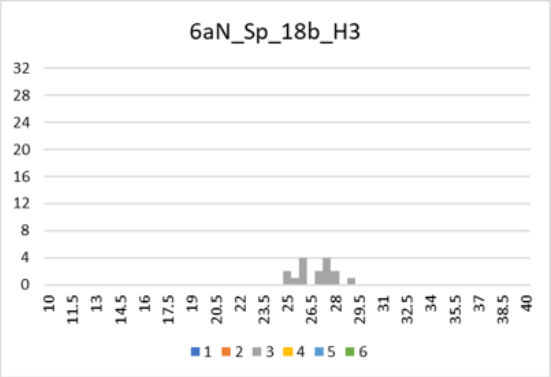
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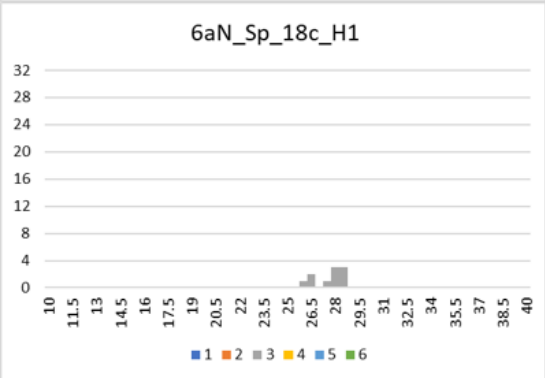
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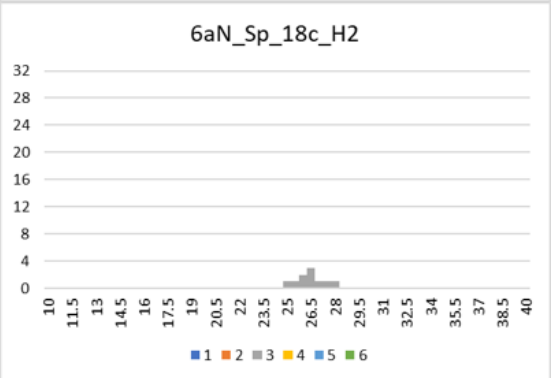
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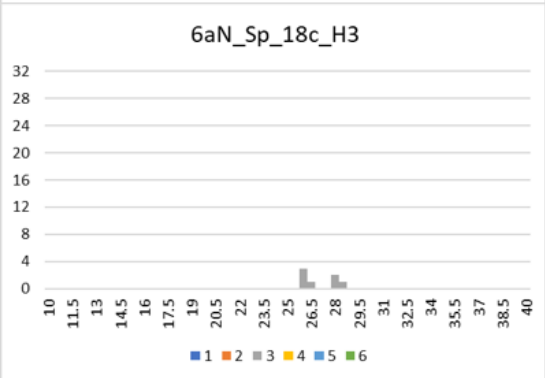
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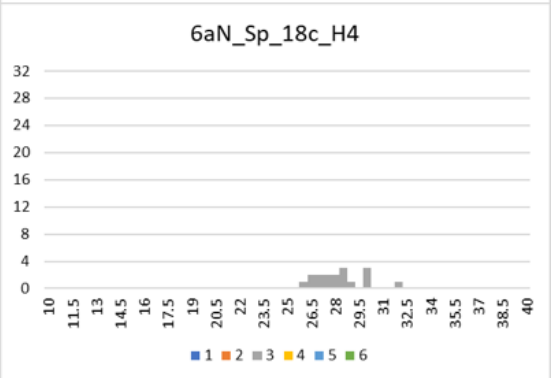
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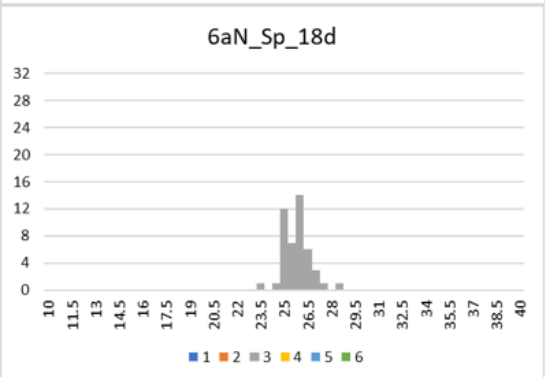
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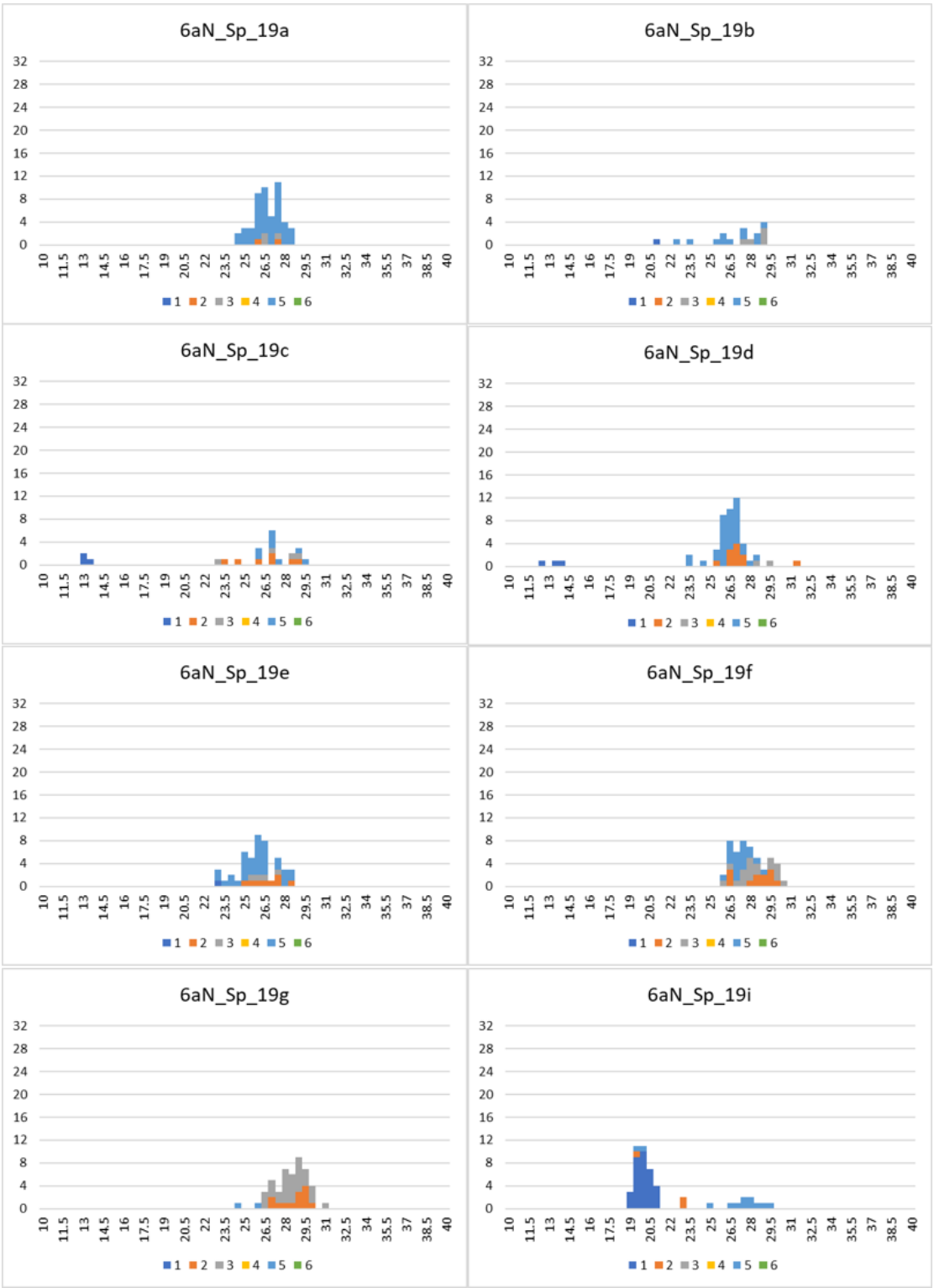
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6aN_Sp_19d
6aN_Sp_19e
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6aN_Sp_19i
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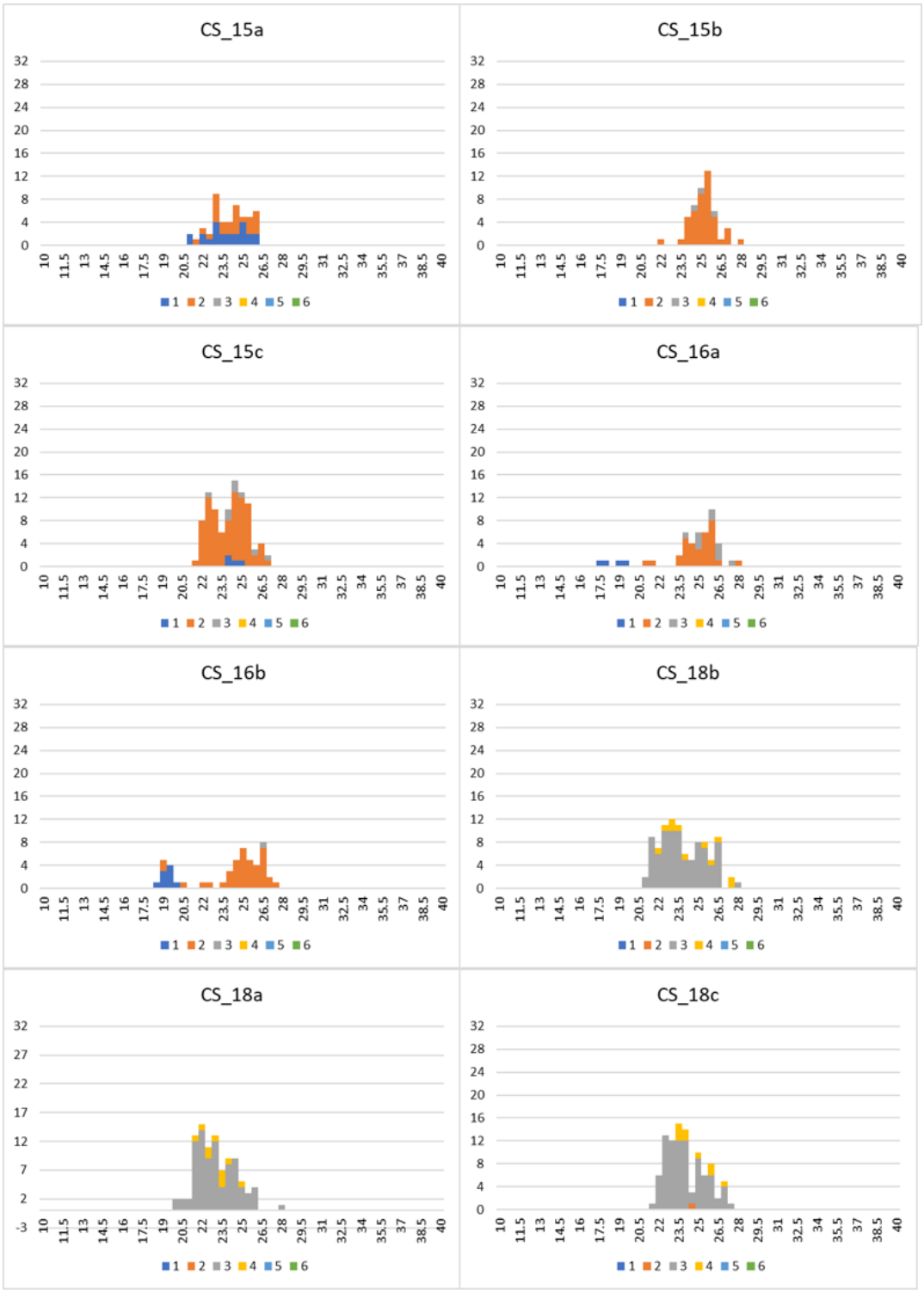
1 2 3 4 5 6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CS_15c
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CS_16b
CS_18b
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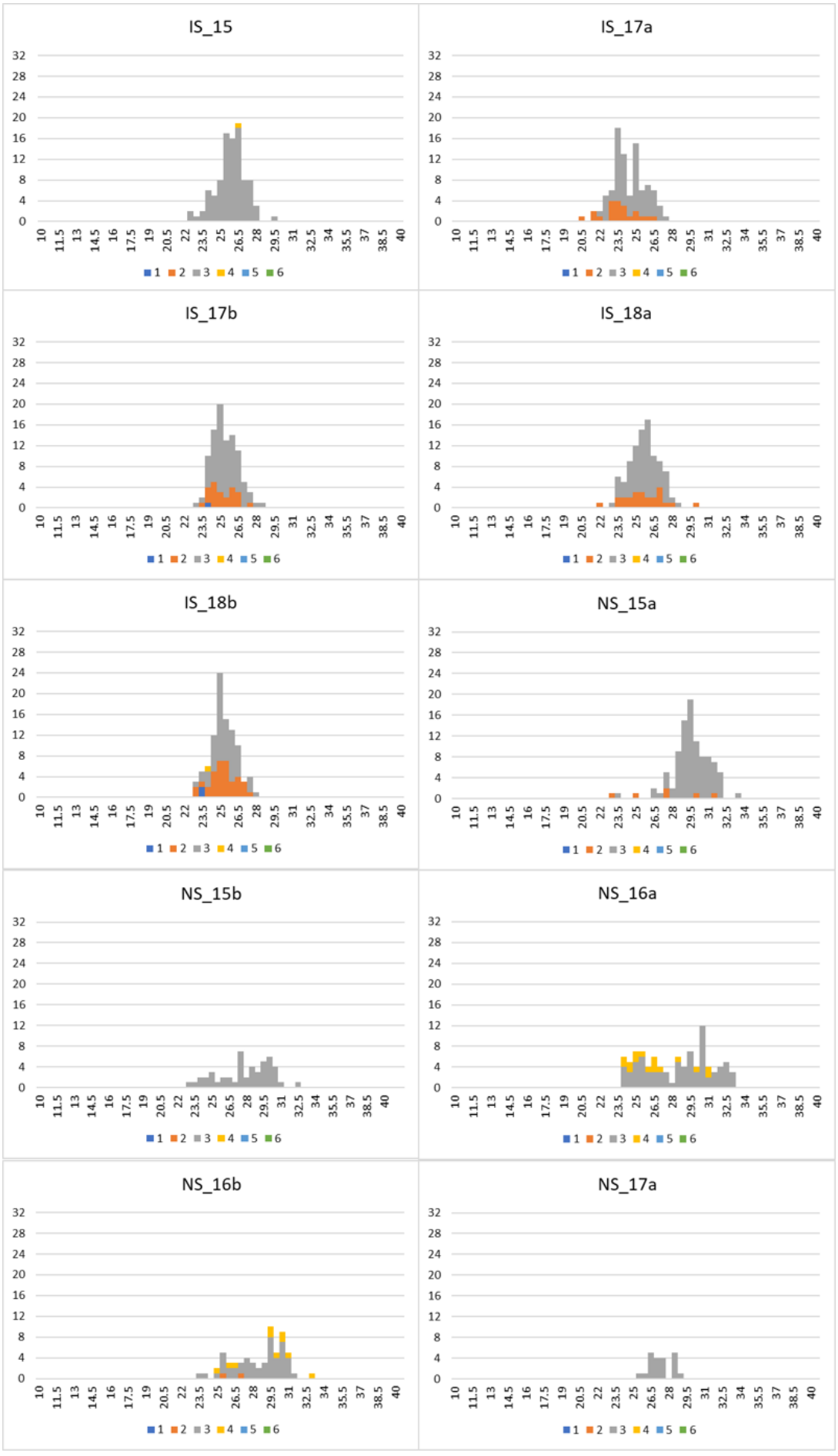
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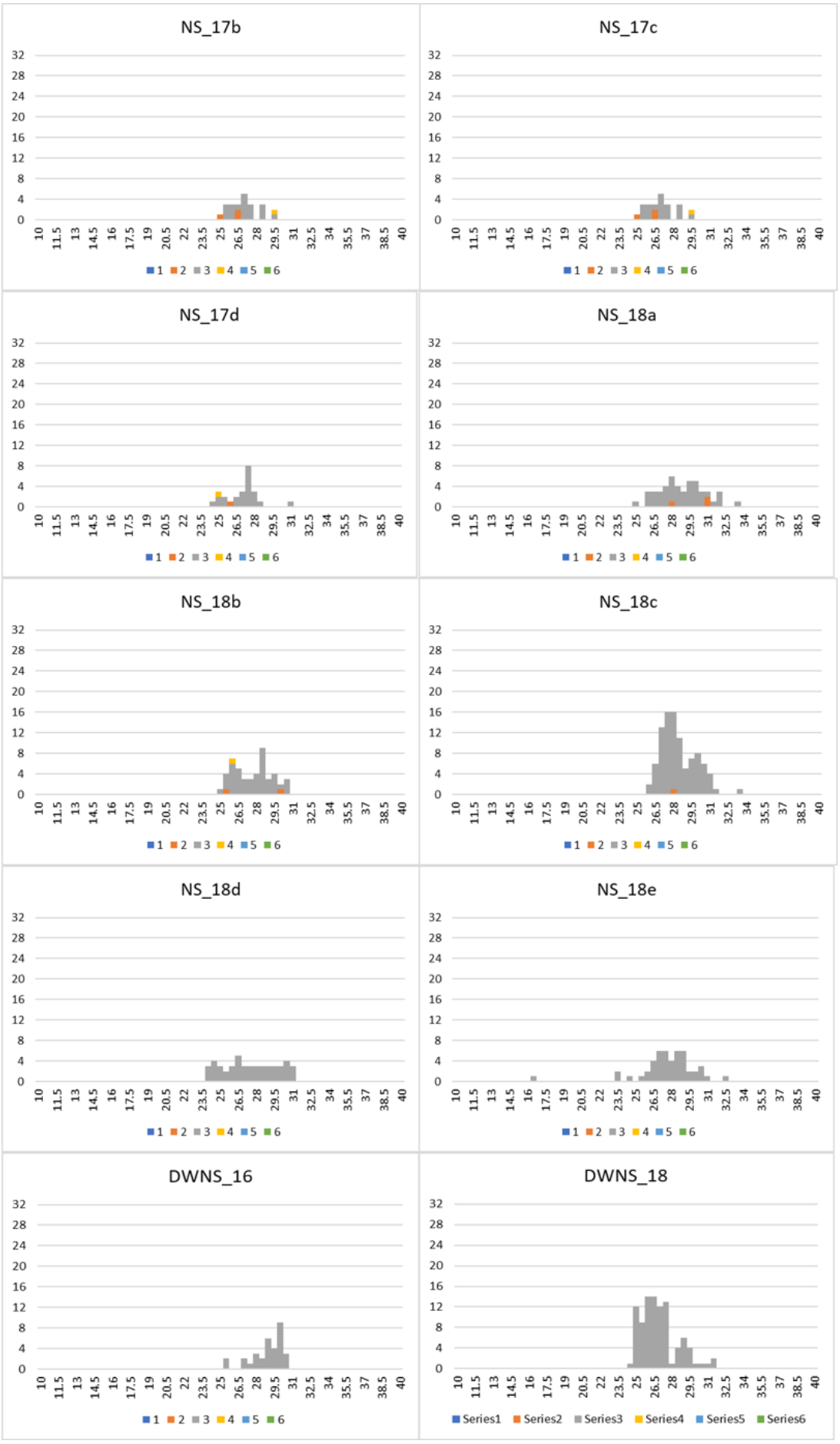
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1 2 3 4 5 6
Series1 Series2 Series3 Series4 Series5 Series6

10.4. Annex 4 – Supplementary genetic analyses figures and tables

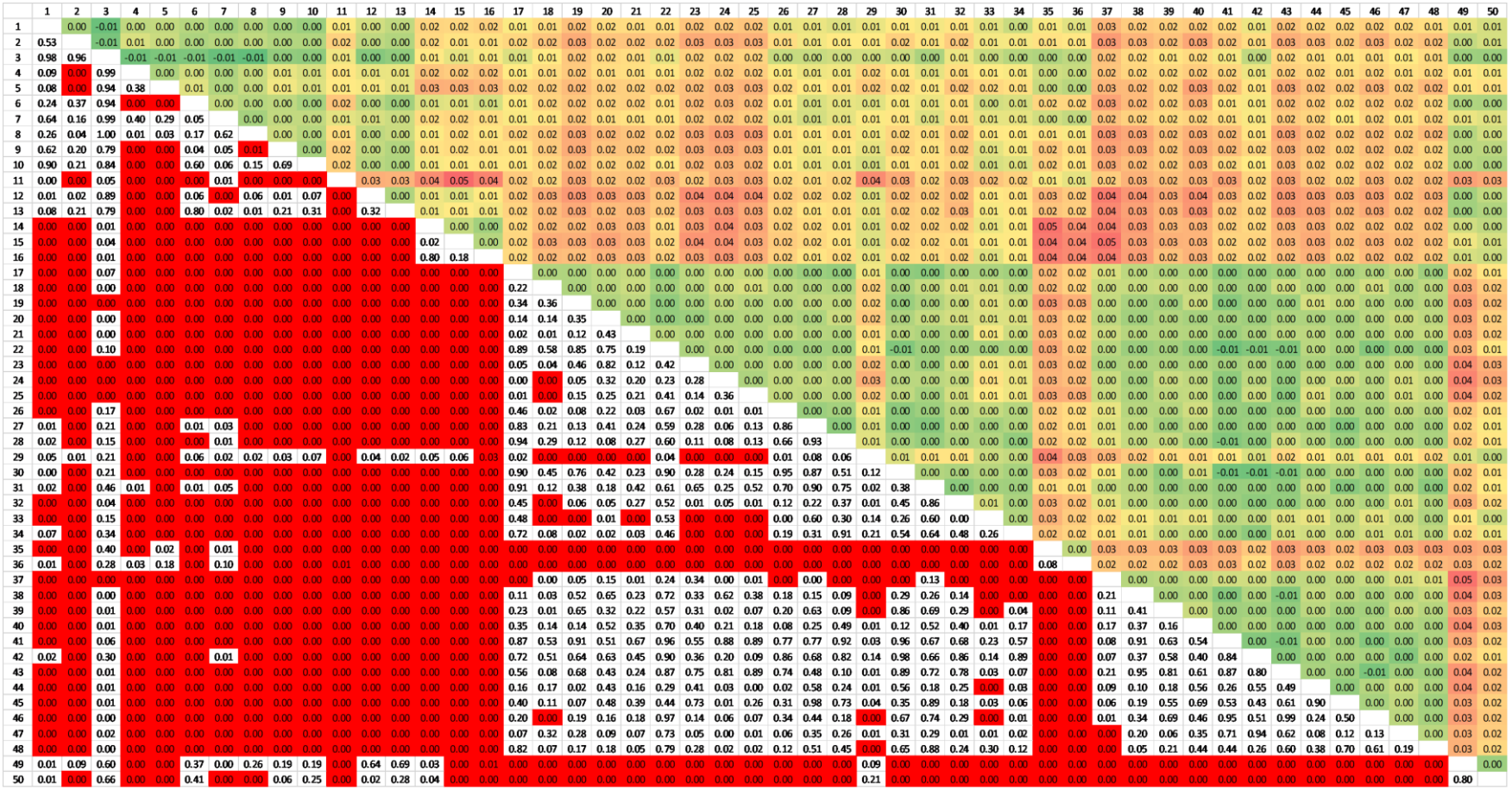


Table 10.4.1. Pairwise multi-locus F_{ST} (above the diagonal) for the 37-microsatellite baseline dataset and associated *P*-values (below the diagonal). *P*-values highlighted in red were still significant after sequential Bonferroni correction.

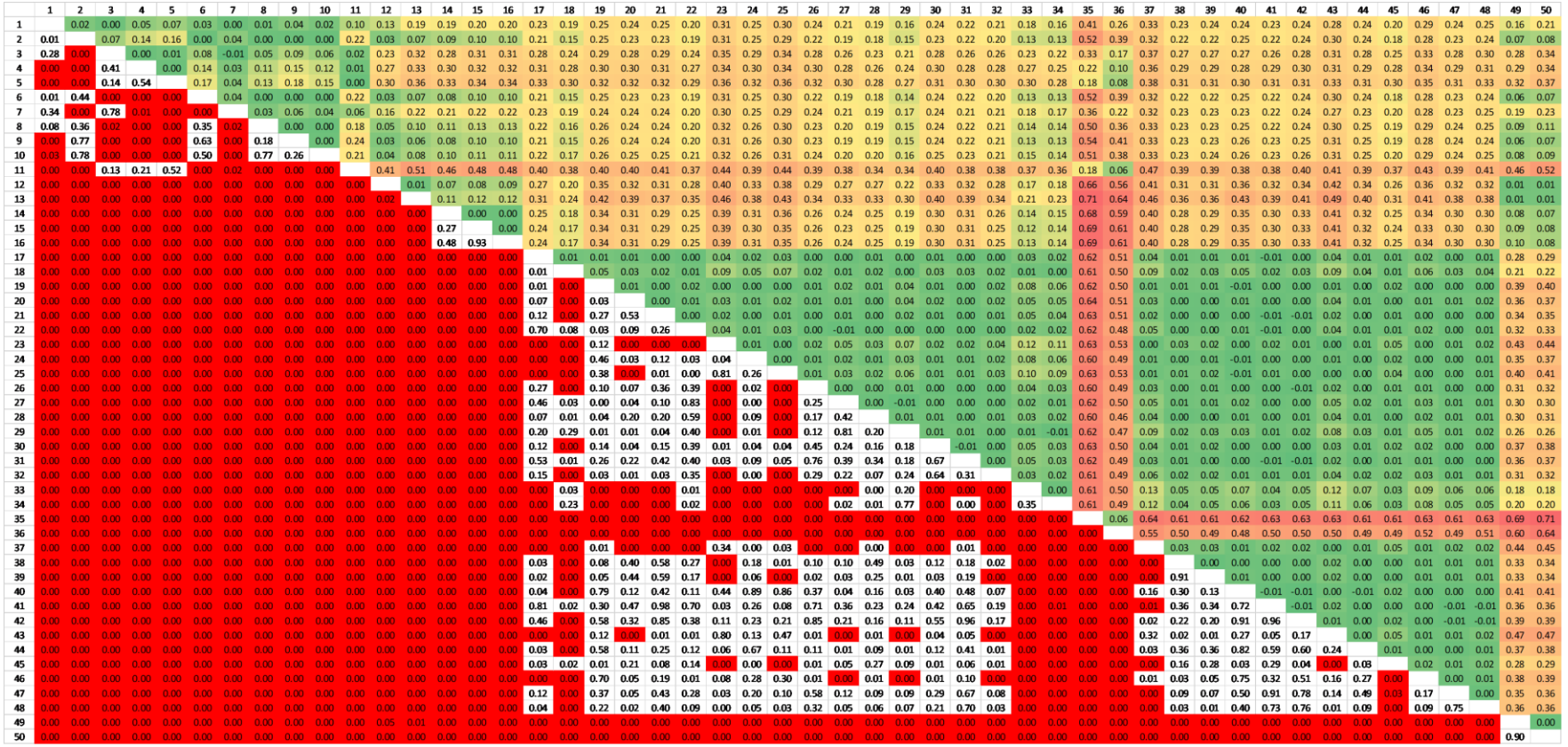


Table 10.4.2. Pairwise multi-locus F_{ST} (above the diagonal) for the 45-SNP baseline dataset and associated *P*-values (below the diagonal). *P*-values highlighted in red were still significant after sequential Bonferroni correction.

Table 10.4.3. The tuning parameters for the assignment models in assignPOP

approach	level	model	svm.kernel	n.pca	svm.cost	gamma	iterations	train.inds	train.loci
1	1	svm	radial	40	1	0.33	100	200,400,600	0.25, 0.5,0.75, 1
1	2	svm	radial	30	1	0.5	100	50	0.25, 0.5,0.75, 1
2	1	svm	radial	35	1	0.33	100	200,400,600	0.25, 0.5,0.75, 1
2	2	svm	radial	10	1	0.5	100	100,150	0.25, 0.5,0.75, 1

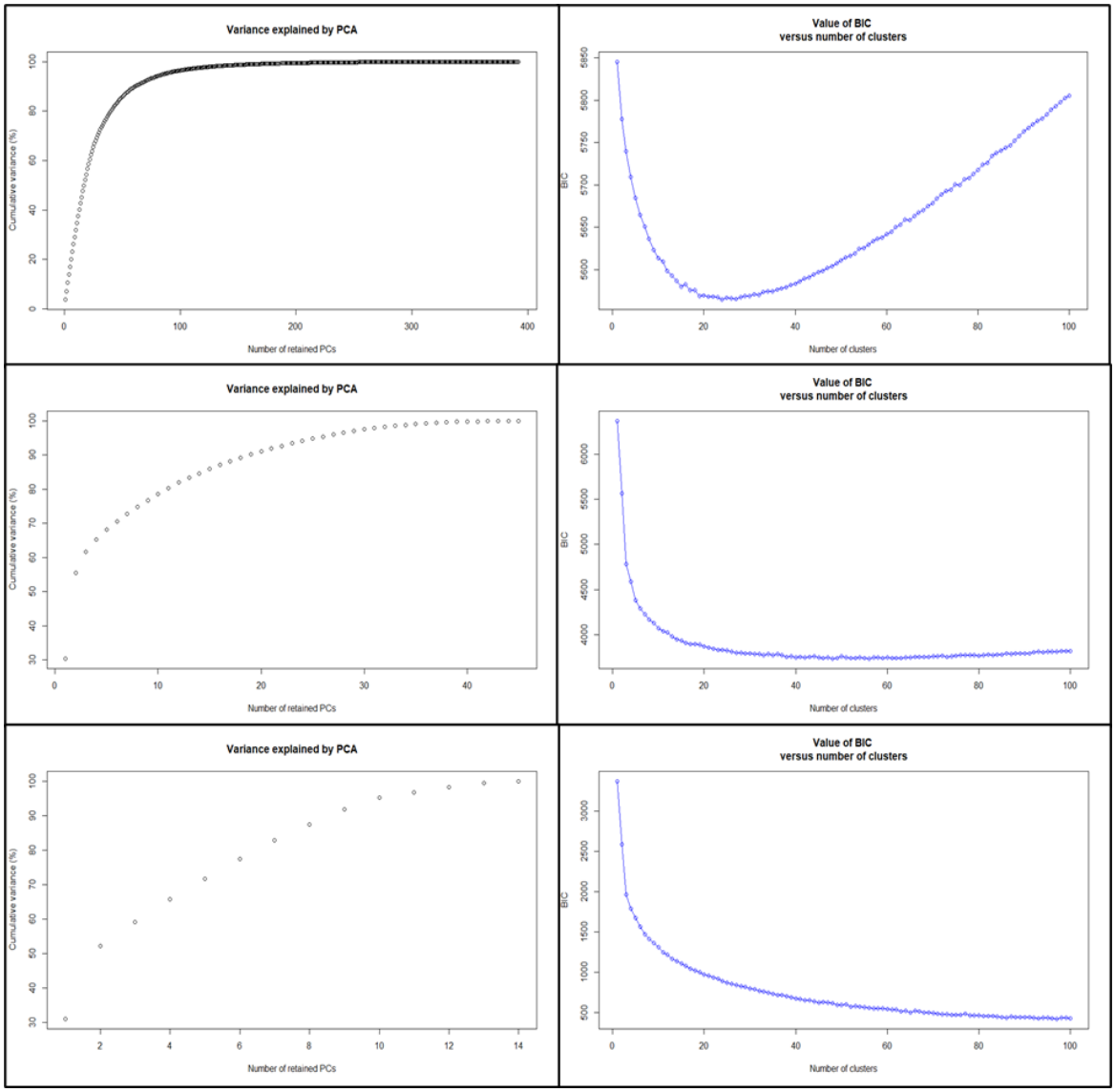


Figure 10.4.1. The figures of the left are graphs of the cumulated variance explained by the eigenvalues of the PCA for the (top) *36_MSAT*, (middle) *45_SNP* and (bottom) *14_SNP* baseline datasets. The figures on the right are BIC curves for the same data.

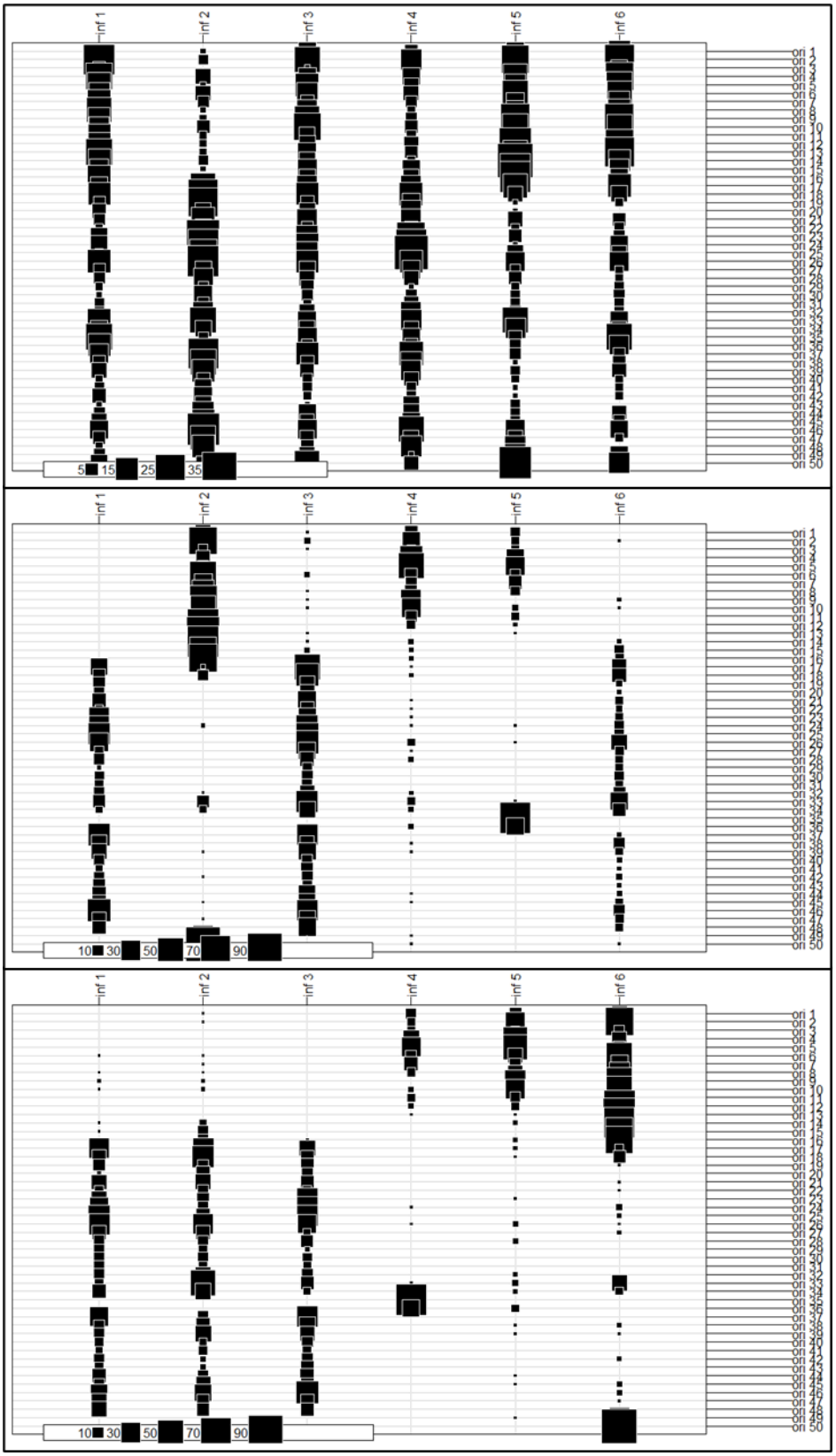


Figure 10.4.2. The output from the *find.clusters* function in *adegenet* in R for the (top) 36_MSAT, (middle) 45_SNP and (bottom) 14_SNP baseline datasets. The original samples are shown on the y-axis and the inferred clusters on the x-axis.

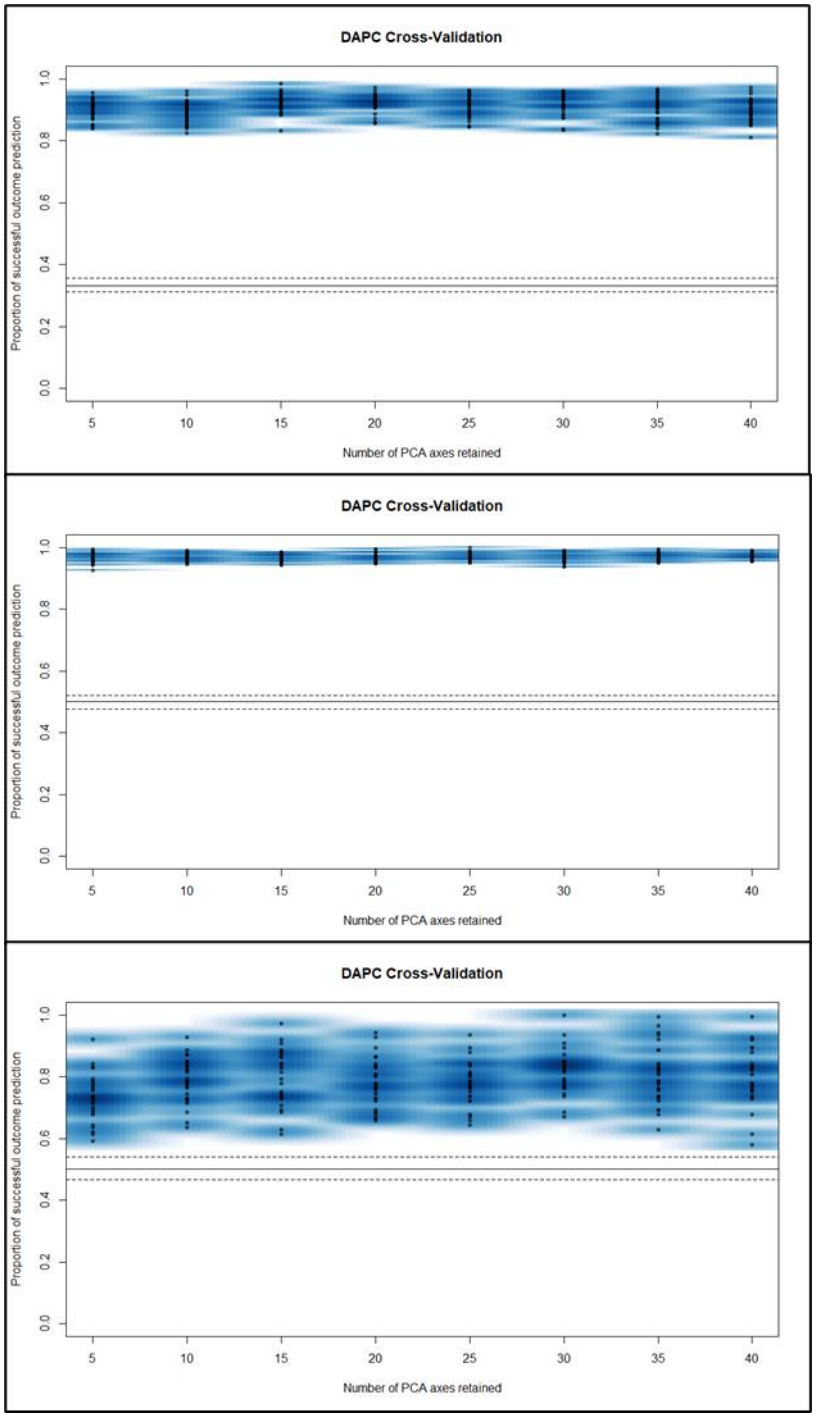


Figure 10.4.3. DAPC cross-validation of the *approach 1* assignment dataset conducted with the *xvalDapc* function in *adegenet*. (top panel) cross validation of the DAPC in Figure 4.10 (middle panel) cross validation of the assignment *approach 1-level 1* dataset (bottom panel) cross validation of the assignment *approach 1 – level 2* dataset.

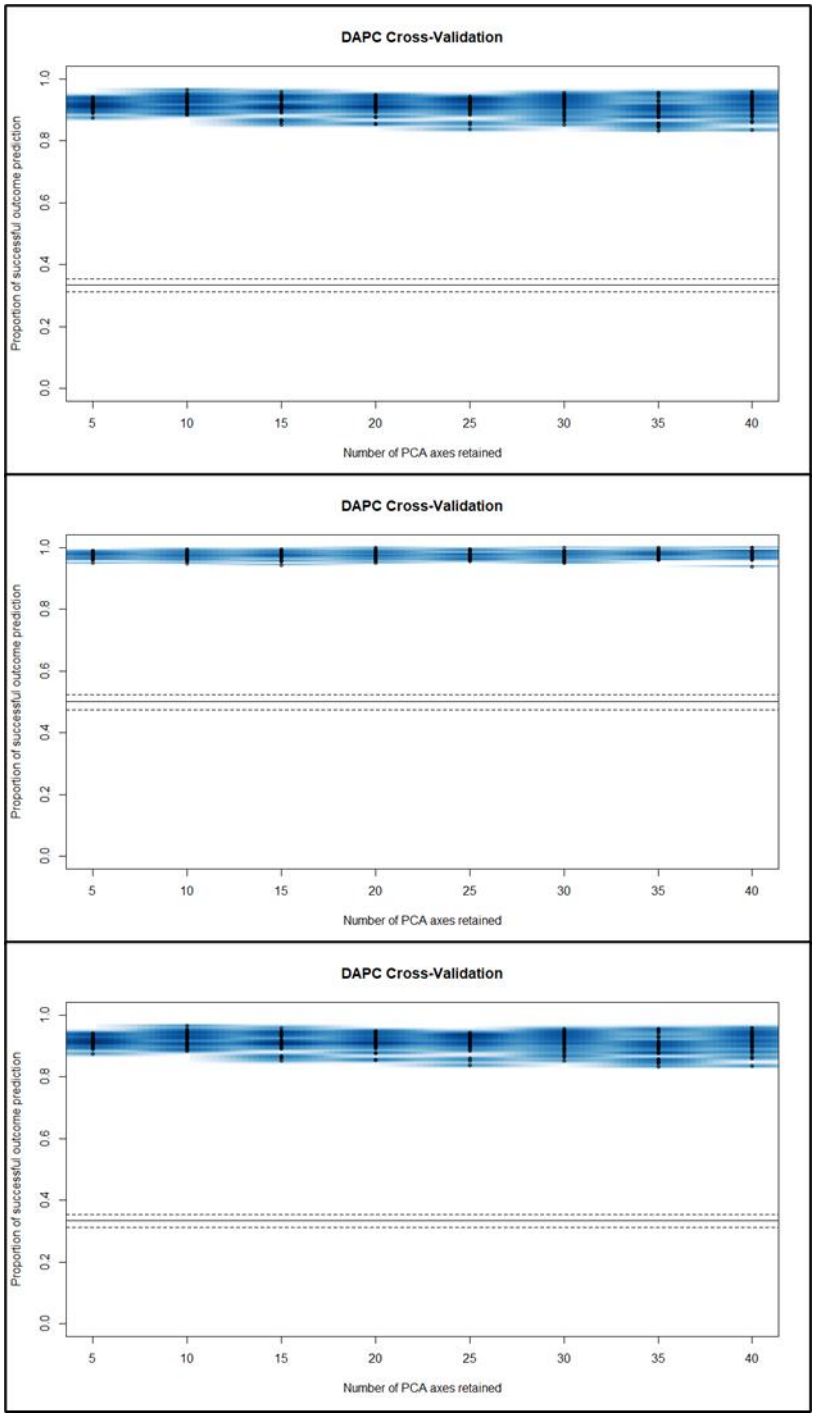


Figure 10.4.4. DAPC cross-validation of the approach 2 assignment dataset conducted with the *xvalDapc* function in *adegenet*. (top panel) cross validation of the DAPC in Figure 4.10 (middle panel) cross validation of the assignment approach 2 - level 1 dataset (bottom panel) cross validation of the assignment approach 2 – level 2 dataset.

10.5. Annex 5 – MSHAS genetic assignment probability plots

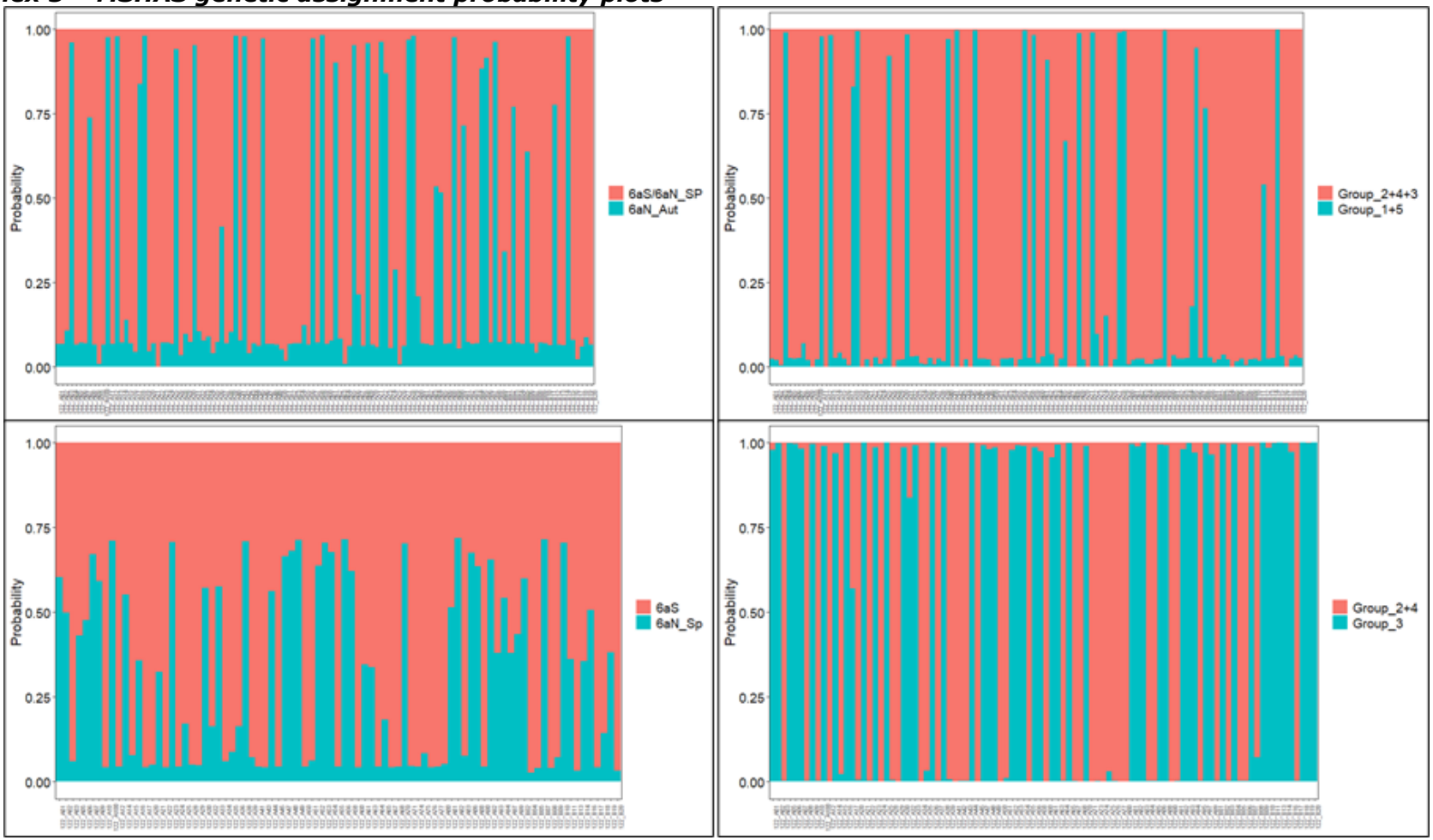


Figure 10.5.1. Assignment of the MSHAS 2014 Haul 6 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

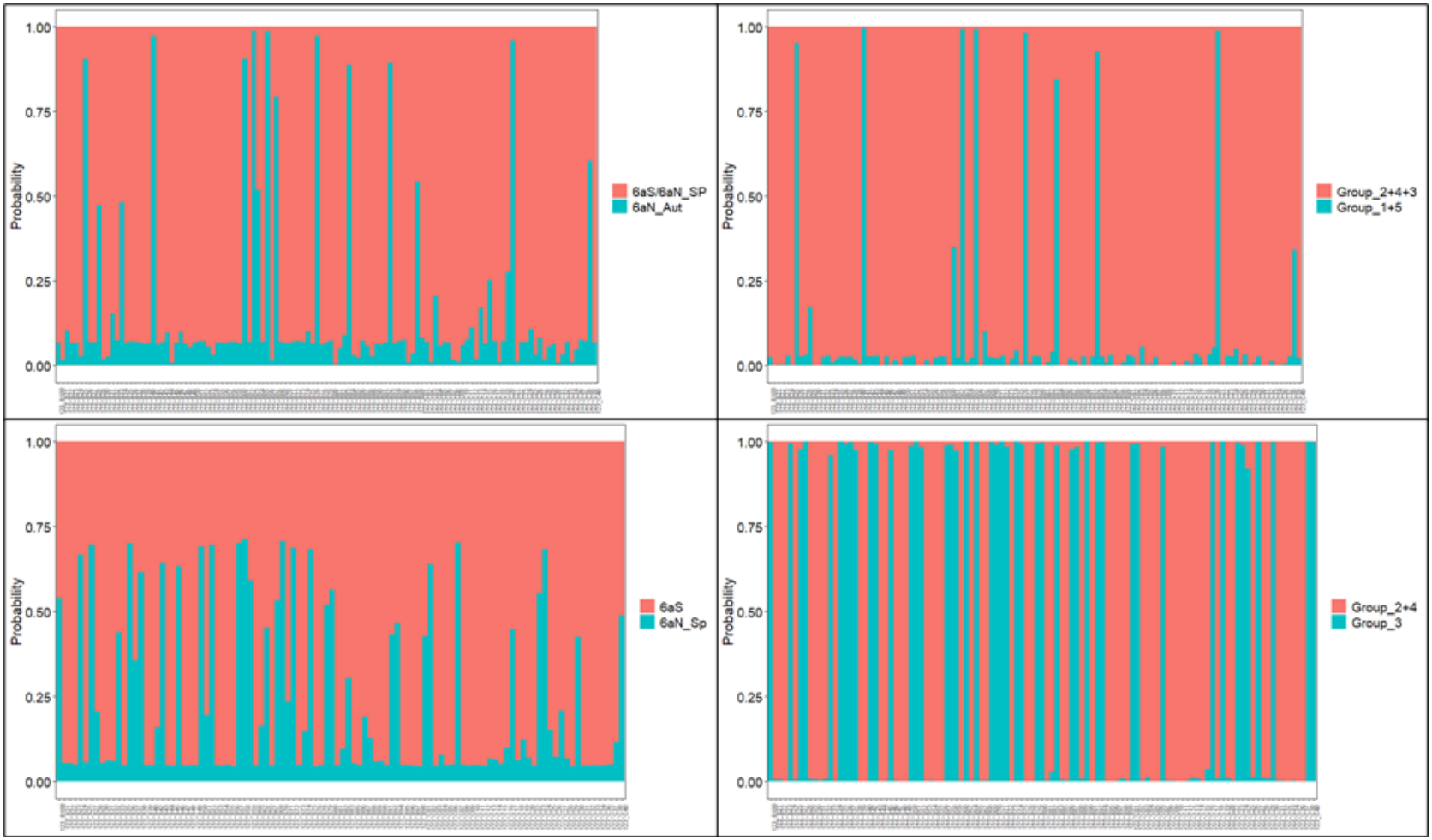


Figure 10.5.2. Assignment of the MSHAS 2014 Haul 8 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

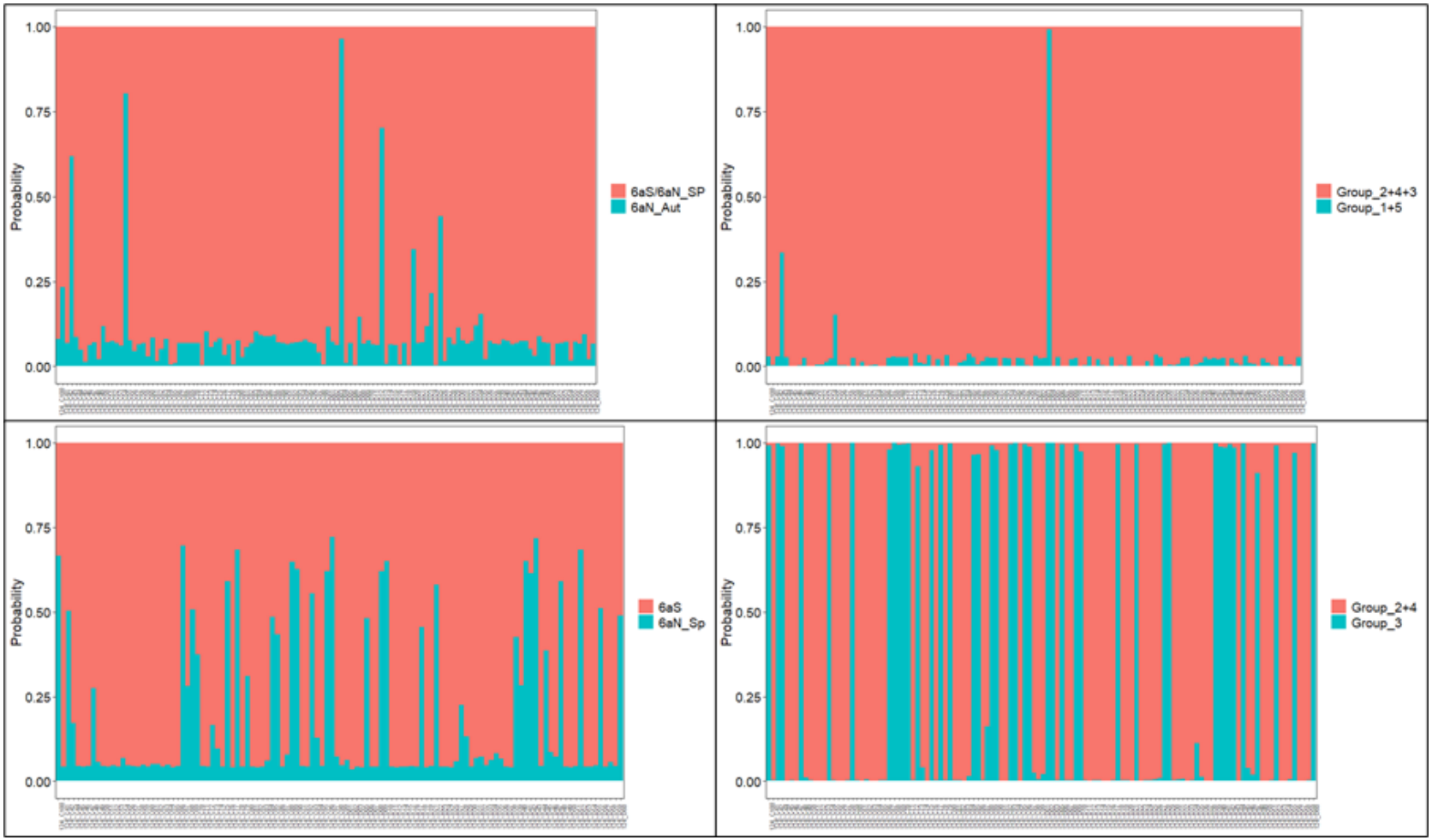


Figure 10.5.3. Assignment of the MSHAS 2014 Haul 9 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

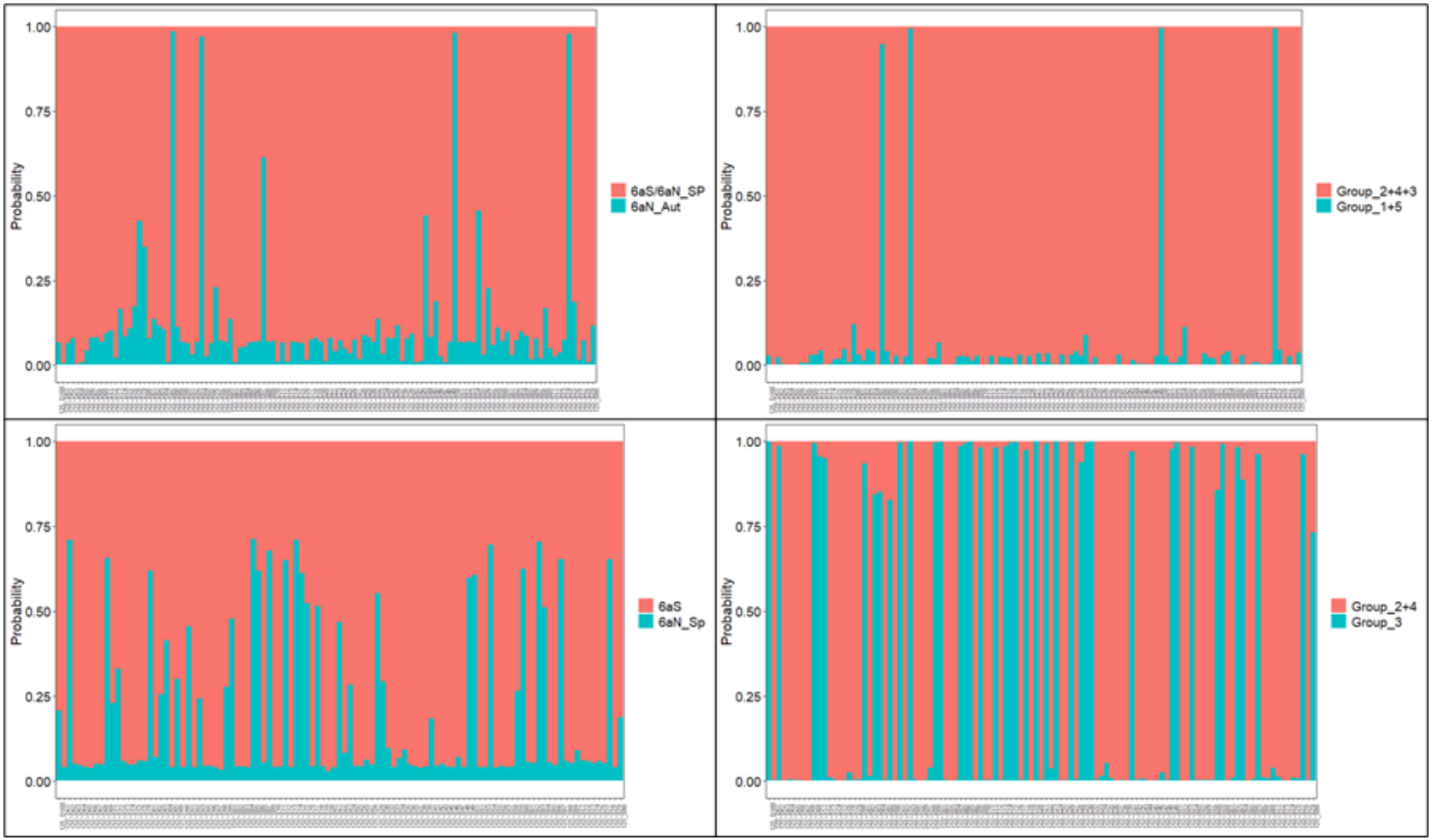


Figure 10.5.4. Assignment of the MSHAS 2014 Haul 10 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

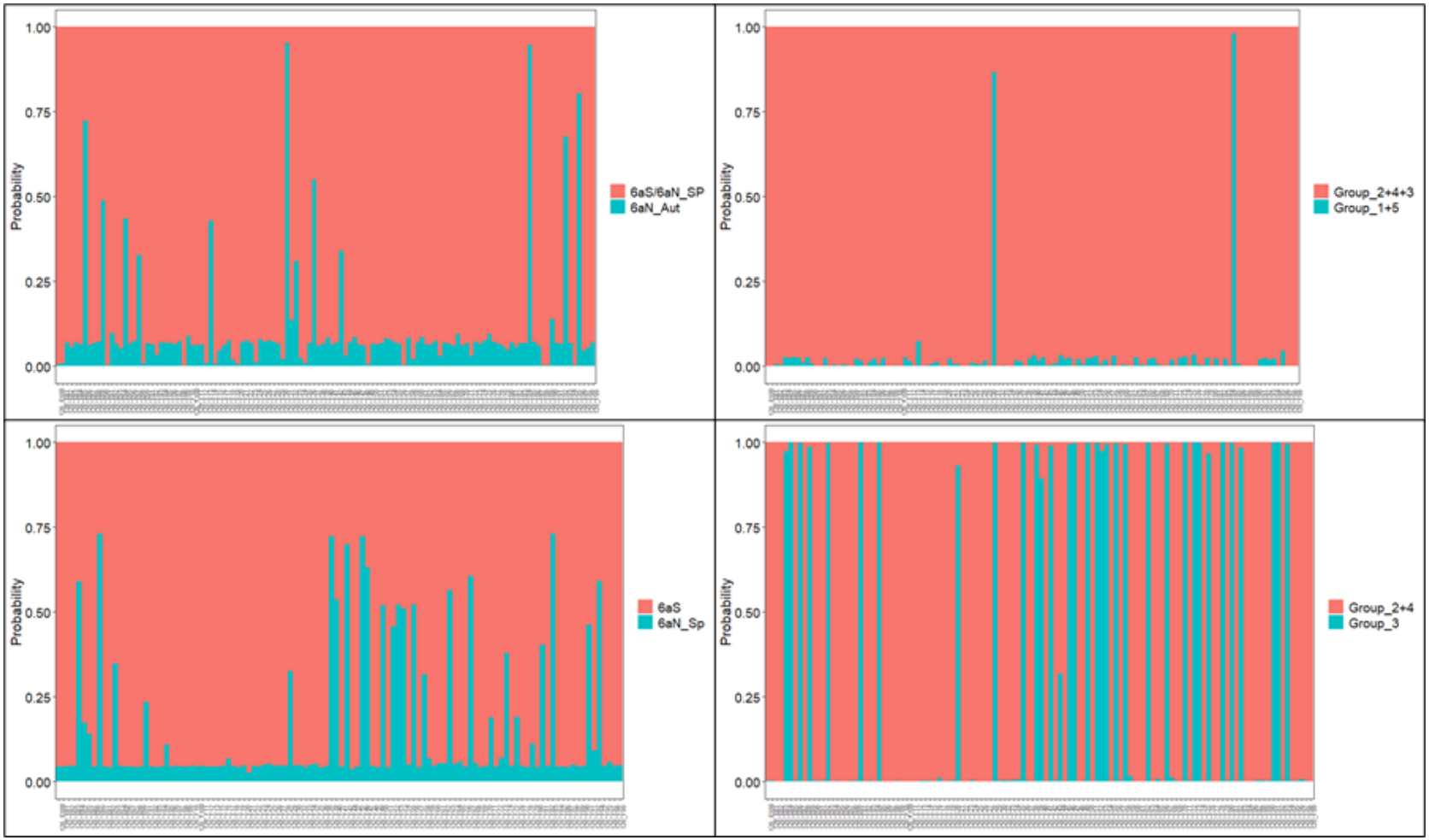


Figure 10.5.5. Assignment of the MSHAS 2014 Haul 13 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

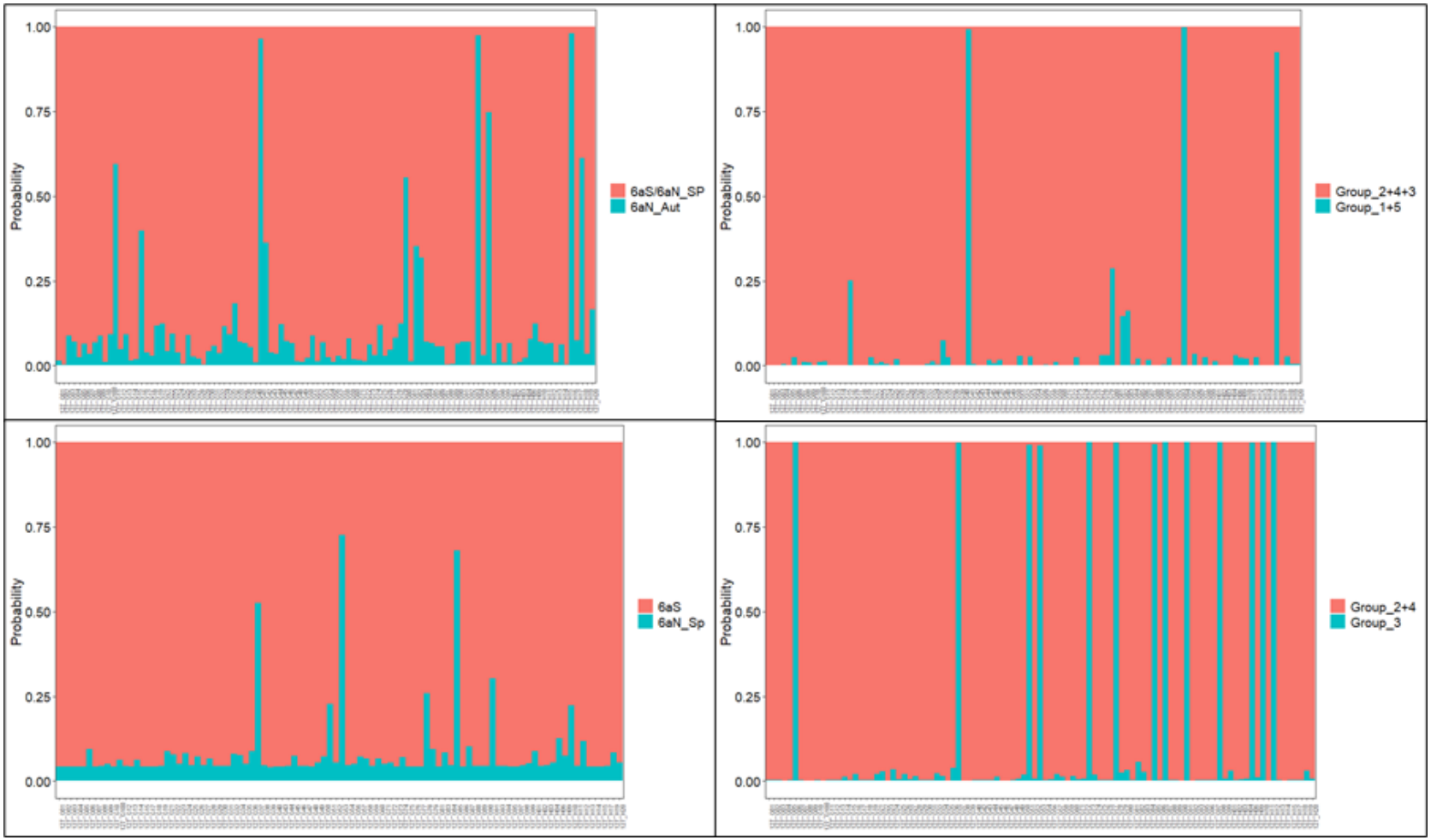


Figure 10.5.6. Assignment of the MSHAS 2014 Haul 20 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

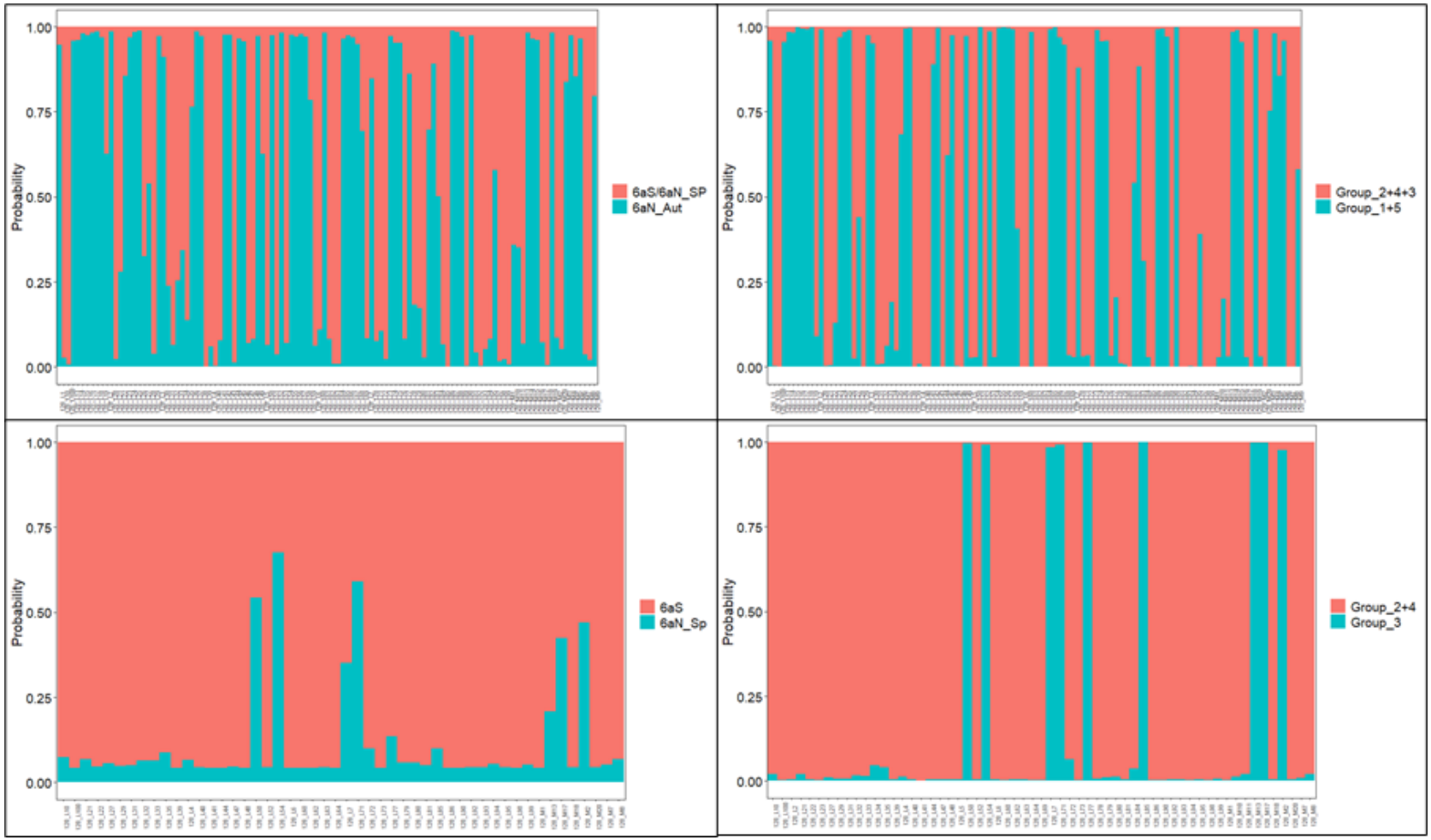


Figure 10.5.7. Assignment of the MSHAS 2015 Haul 2 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

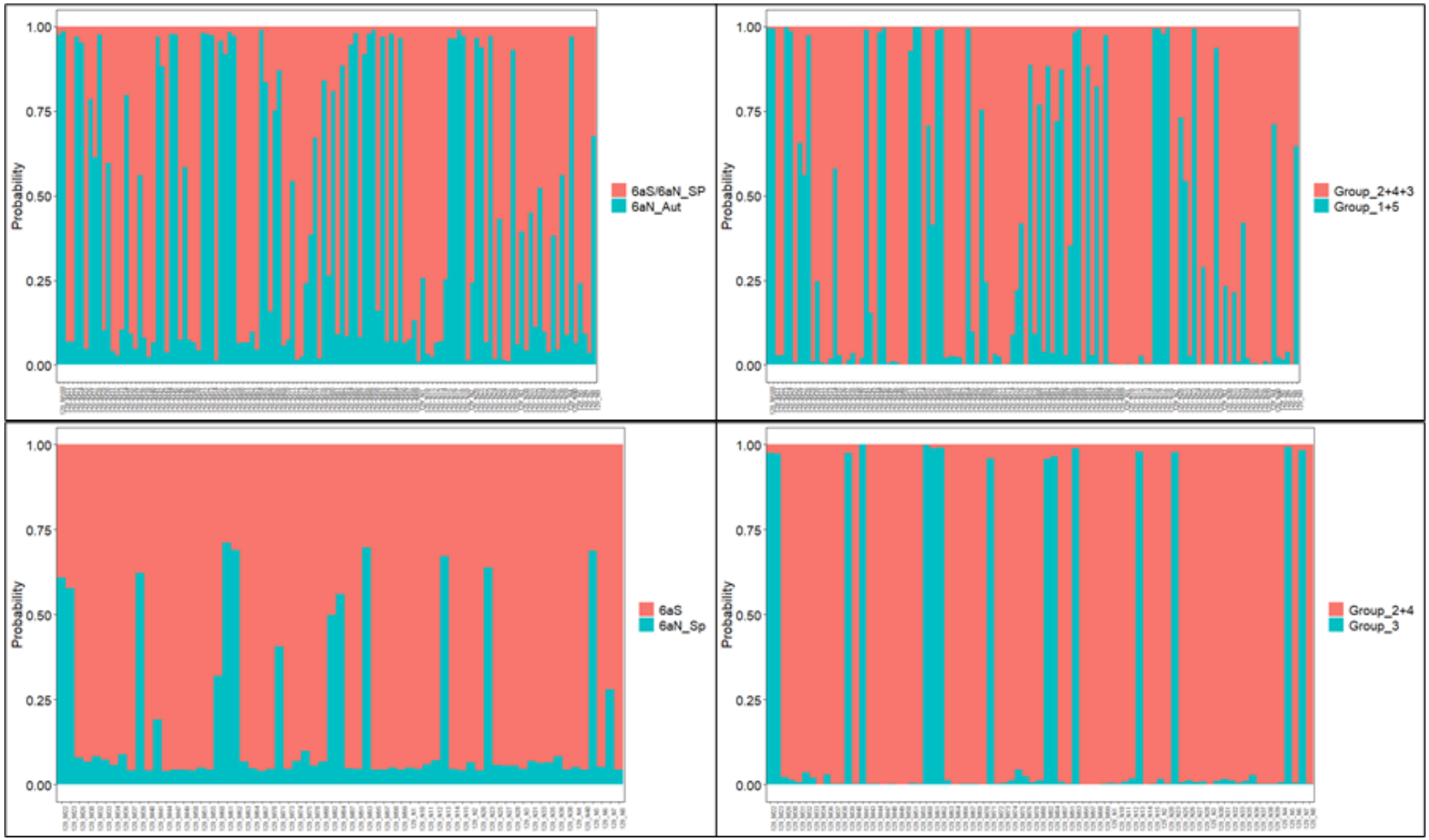


Figure 10.5.8. Assignment of the MSHAS 2015 Haul 3 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

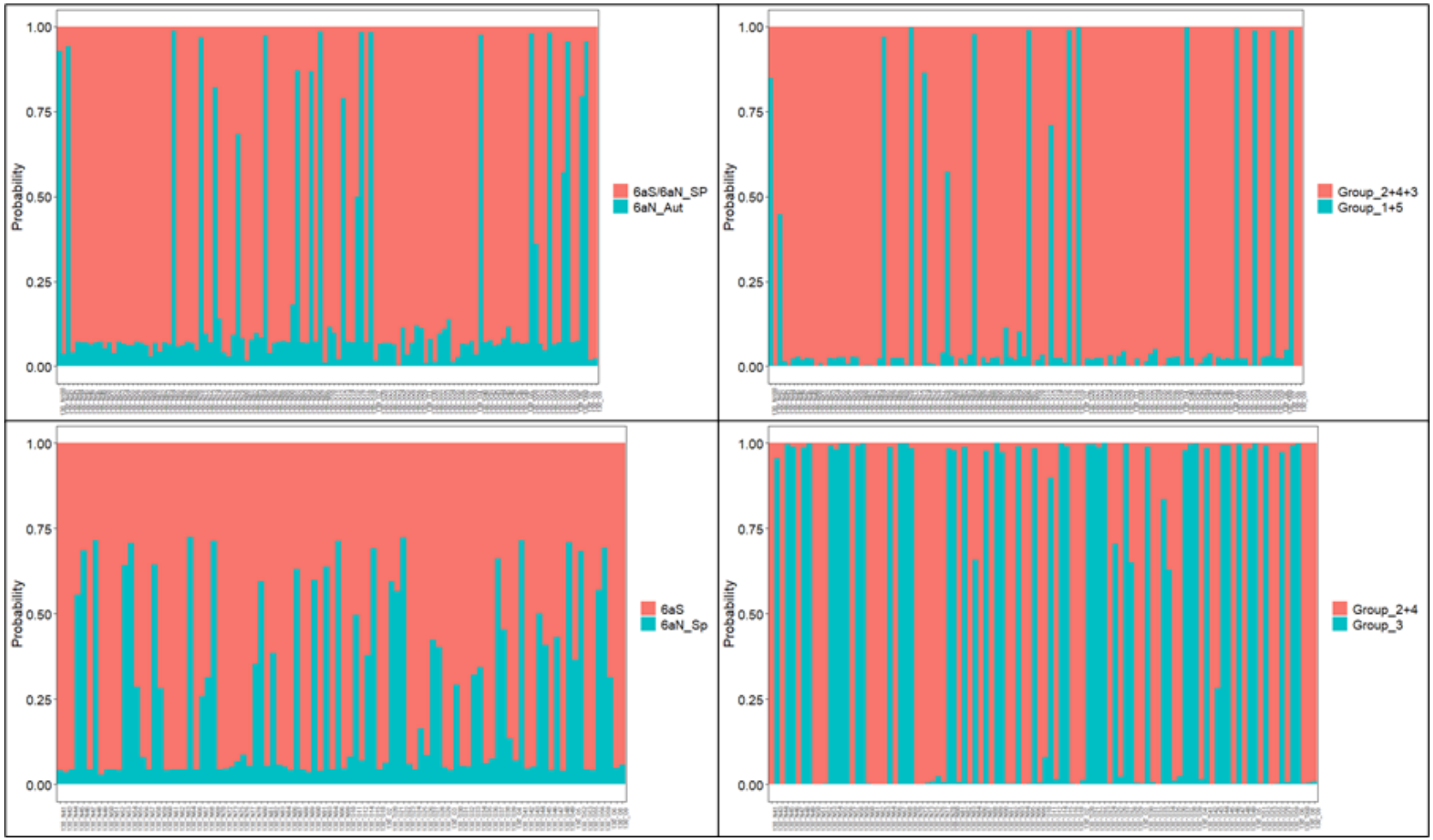


Figure 10.5.9. Assignment of the MSHAS 2015 Haul 5 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

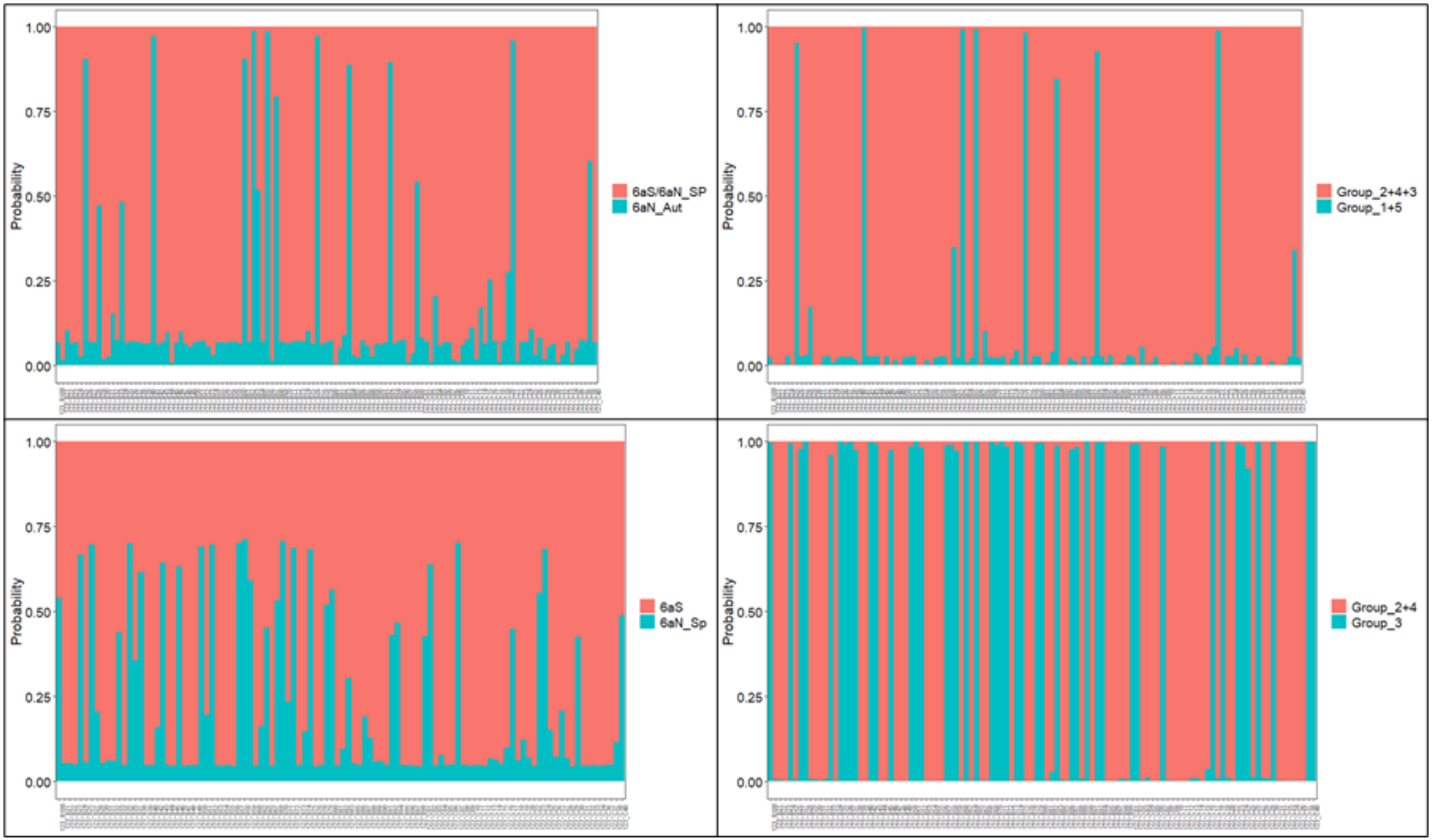


Figure 10.5.10. Assignment of the MSHAS 2015 Haul 8 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

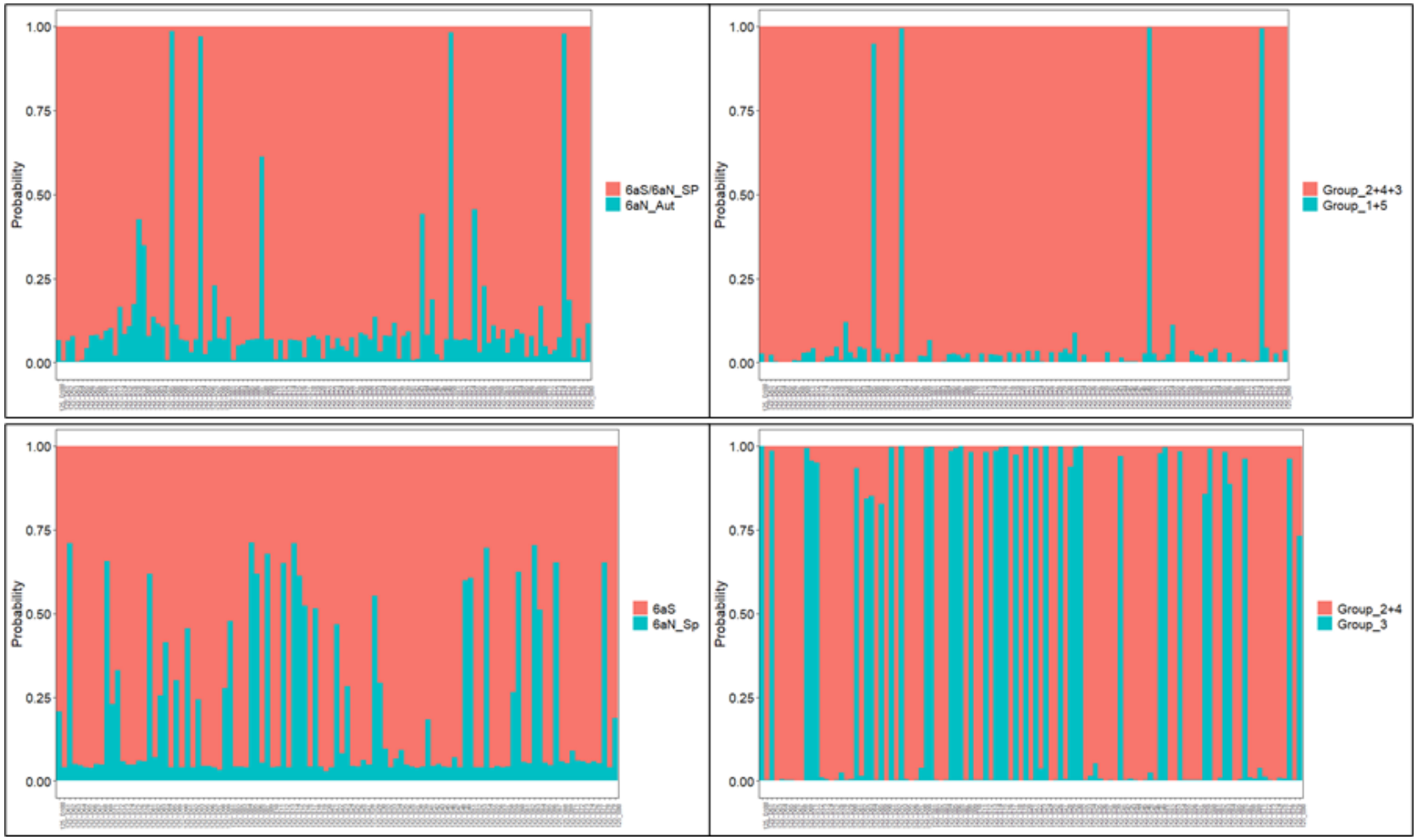


Figure 10.5.11. Assignment of the MSHAS 2015 Haul 10 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

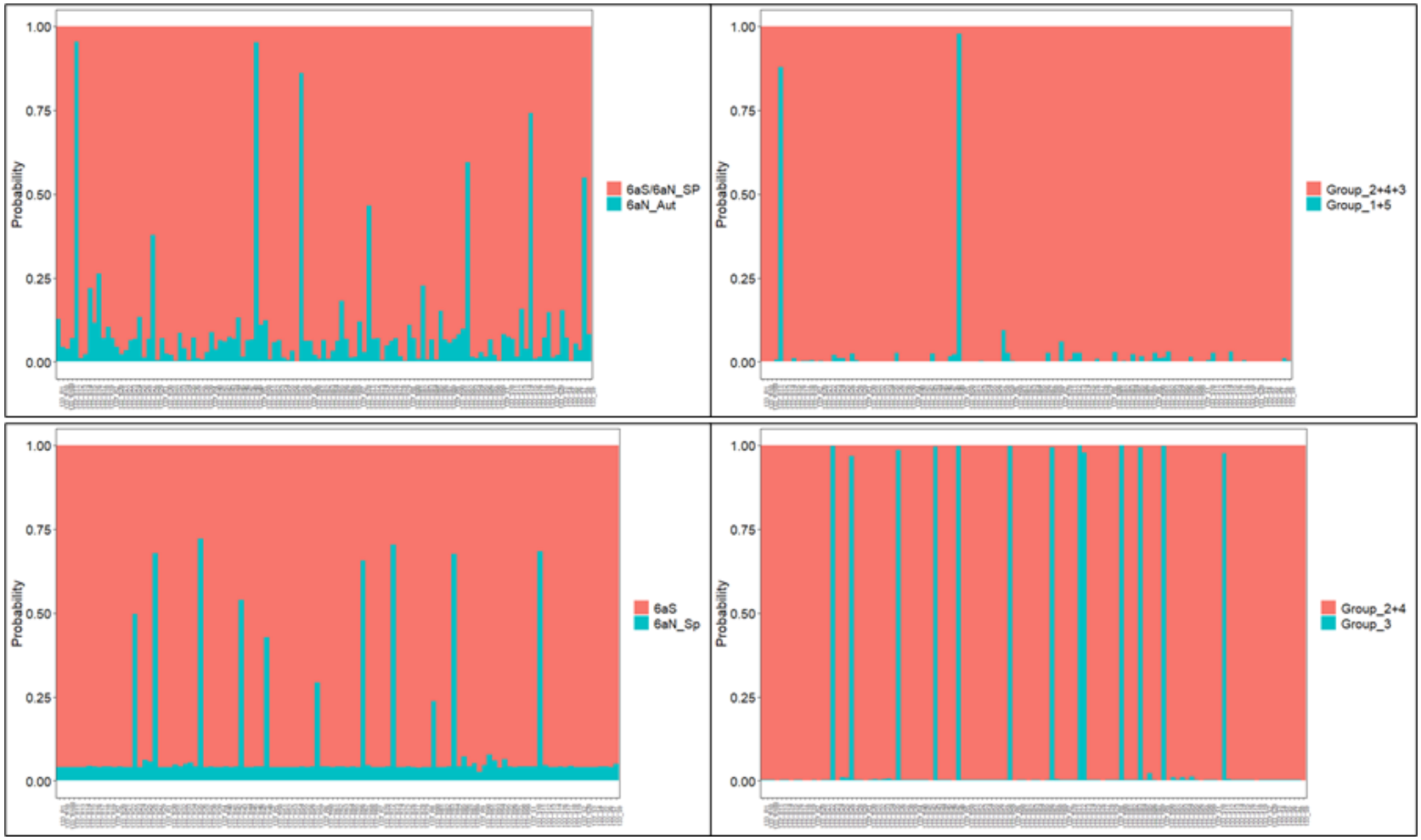


Figure 10.5.12. Assignment of the MSHAS 2015 Haul 12 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

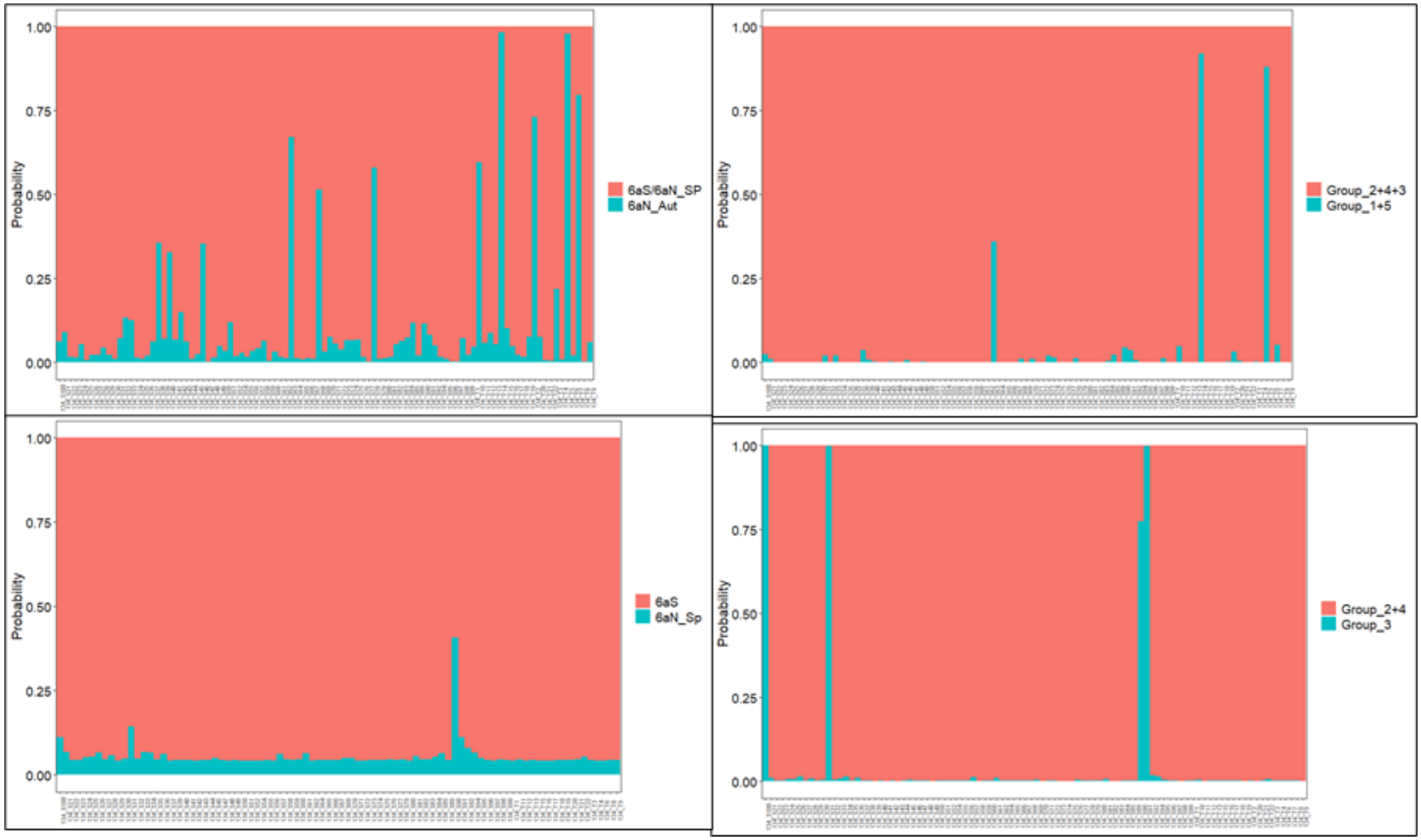


Figure 10.5.13. Assignment of the MSHAS 2015 Haul 16 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

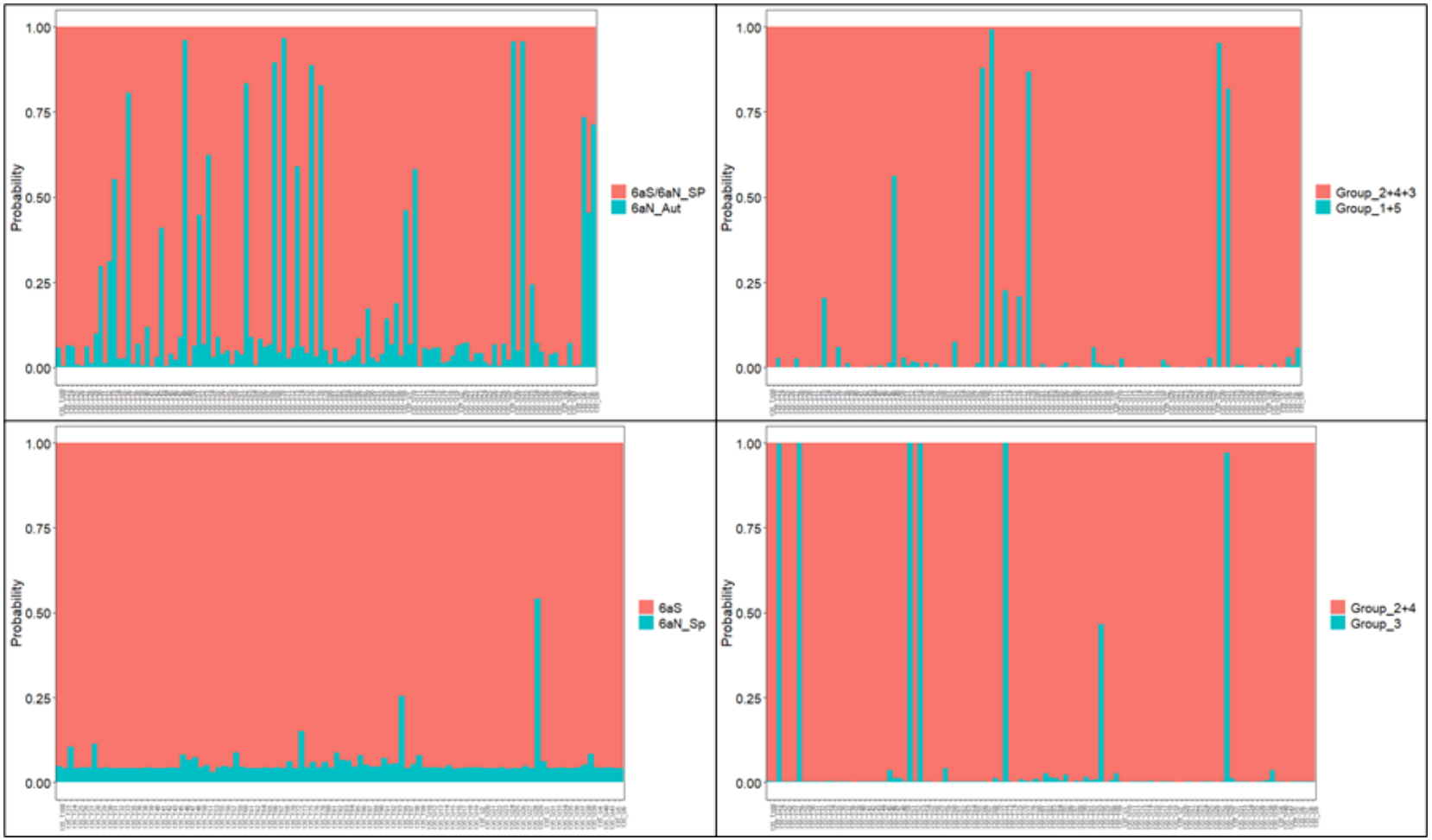


Figure 10.5.14. Assignment of the MSHAS 2015 Haul 17 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

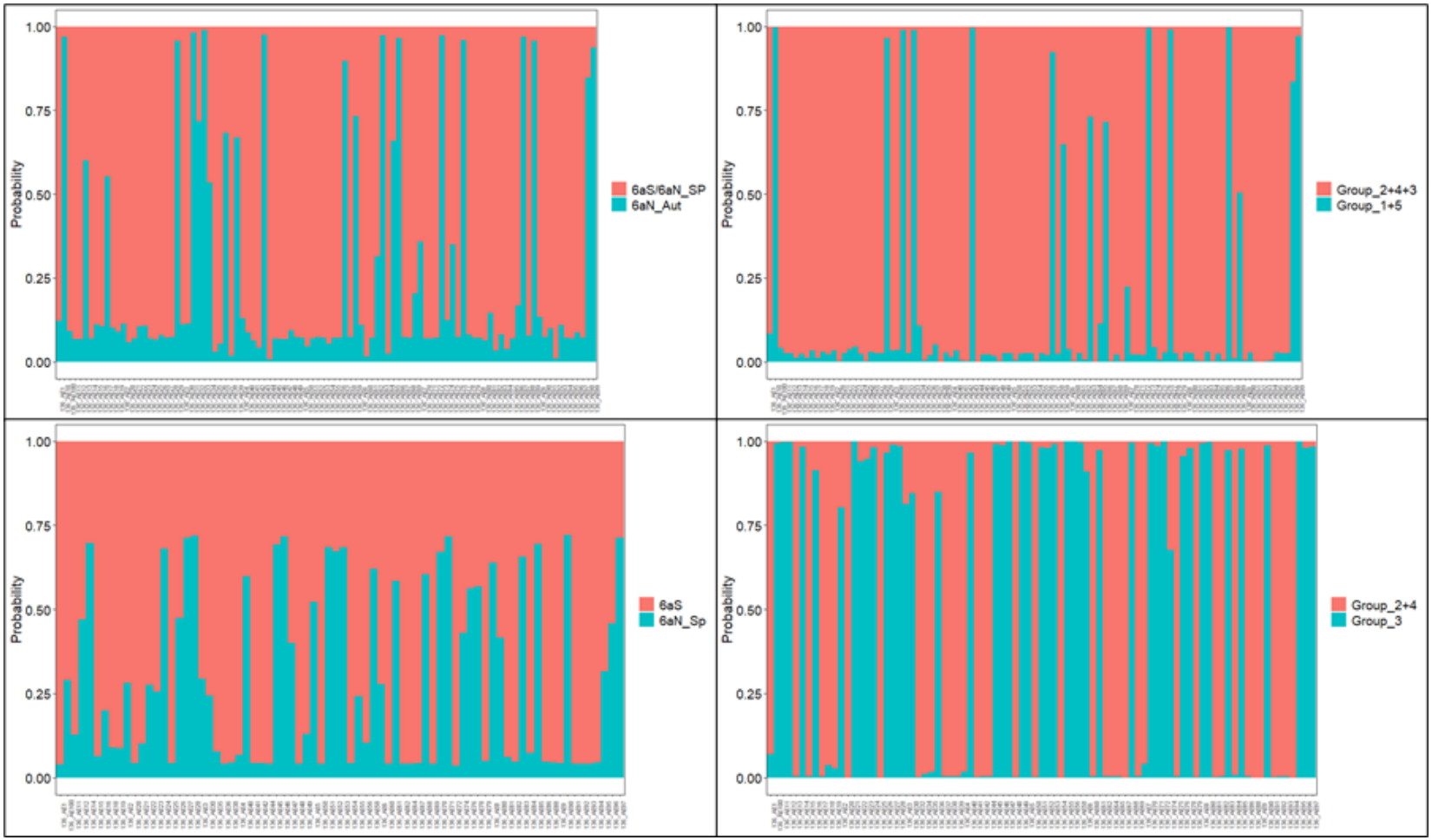


Figure 10.5.15. Assignment of the MSHAS 2016 Haul 5 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

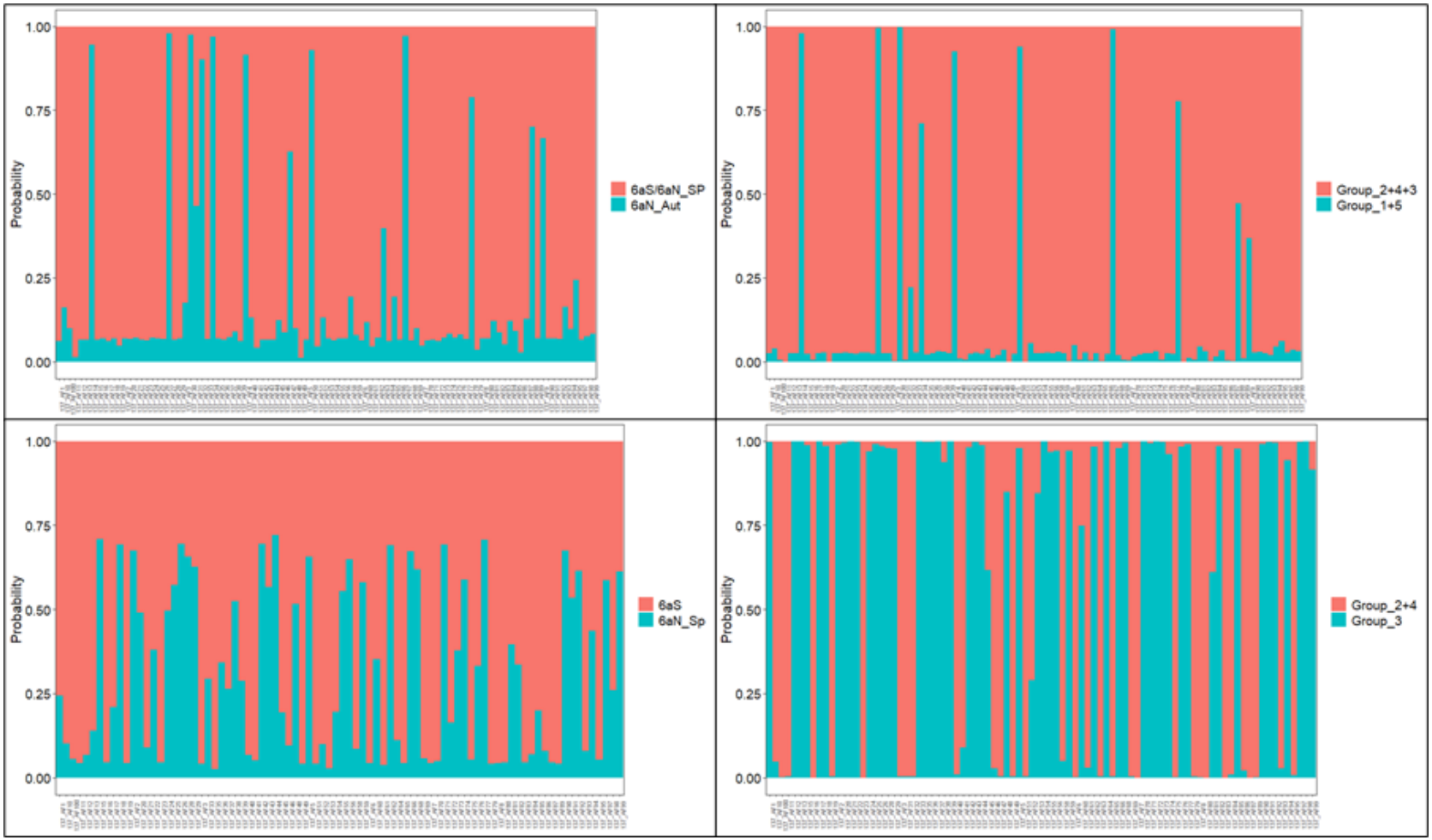


Figure 10.5.16. Assignment of the MSHAS 2016 Haul 6 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

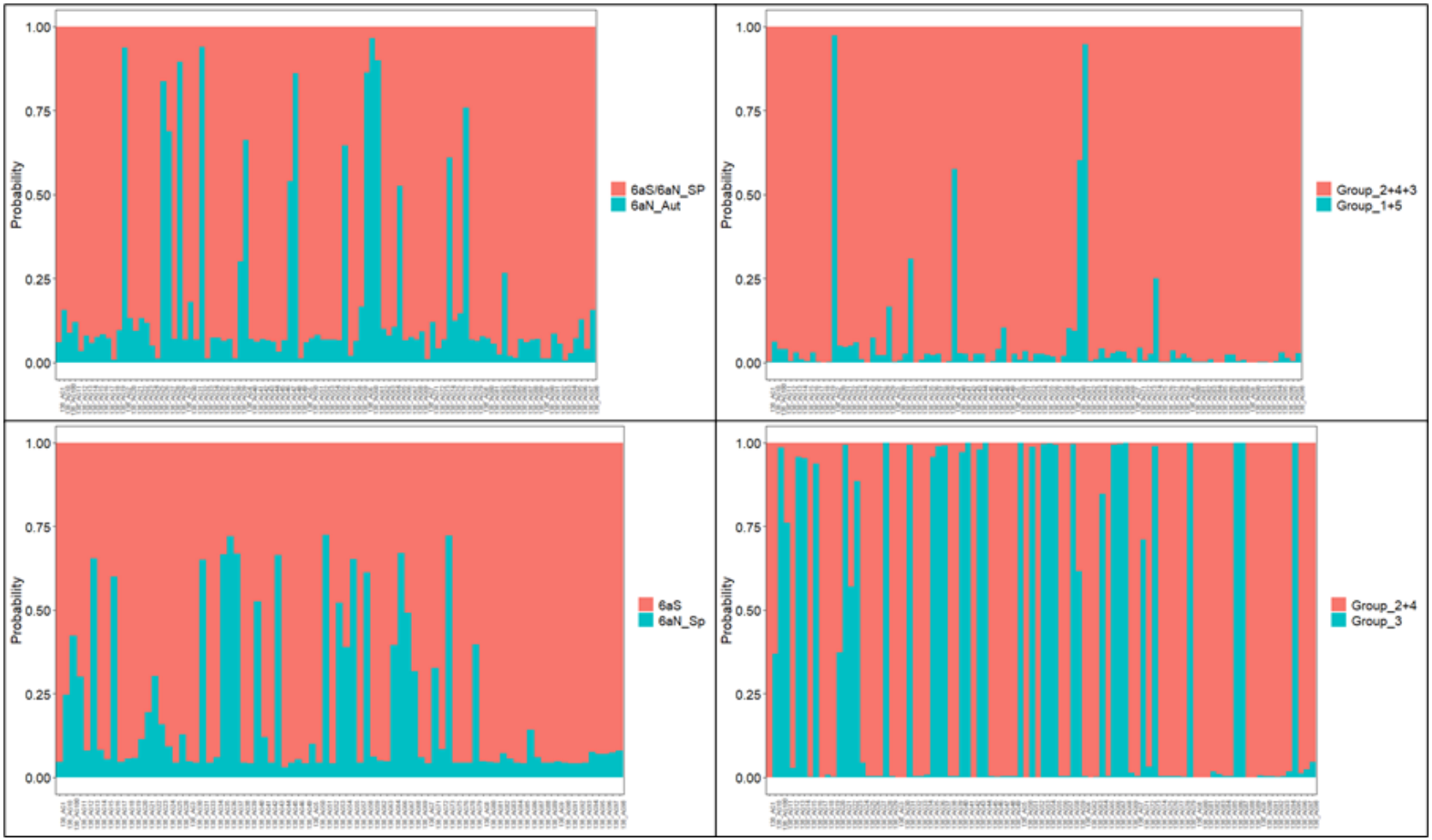


Figure 10.5.17. Assignment of the MSHAS 2016 Haul 7 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

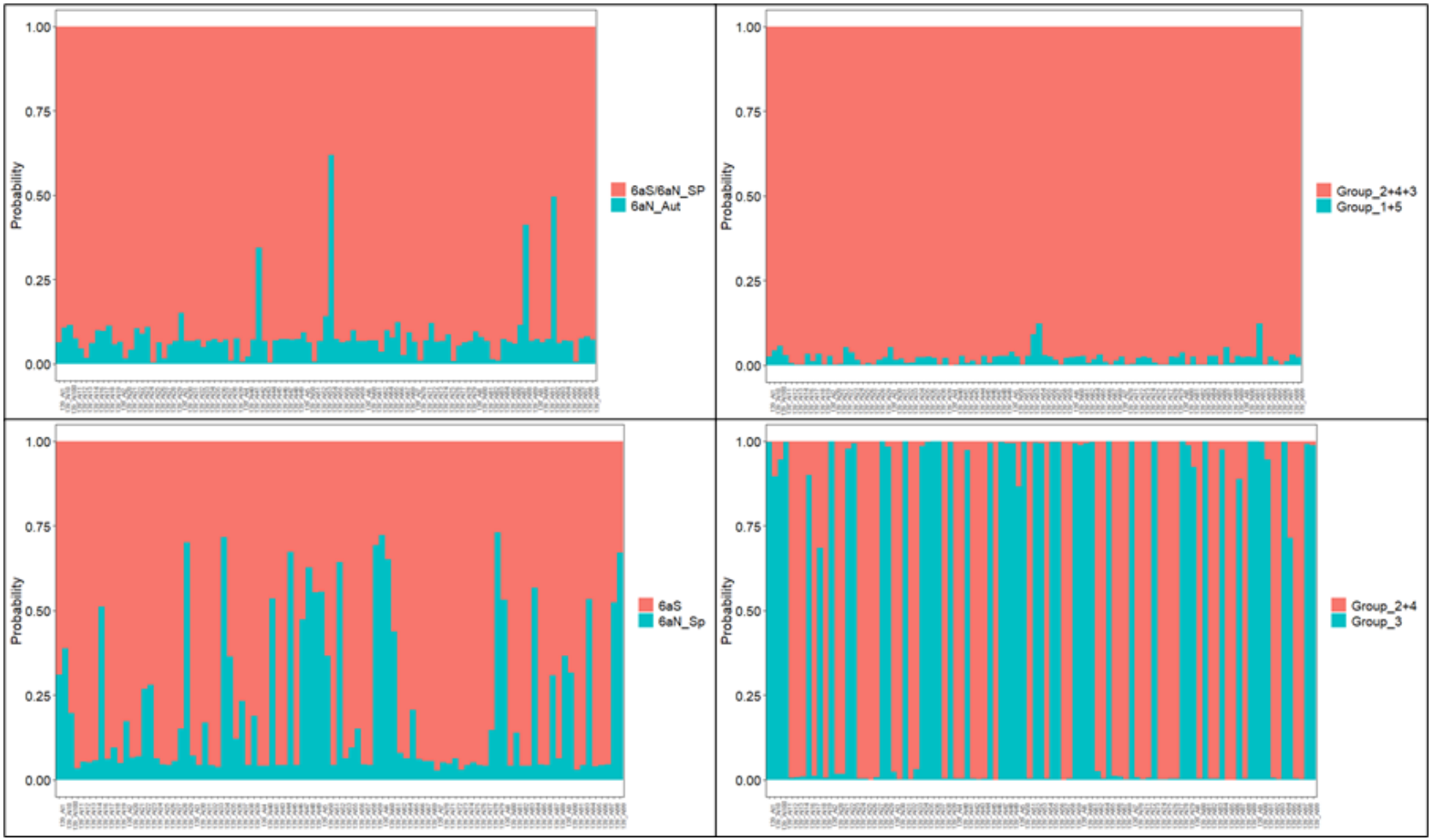


Figure 10.5.18. Assignment of the MSHAS 2016 Haul 8 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

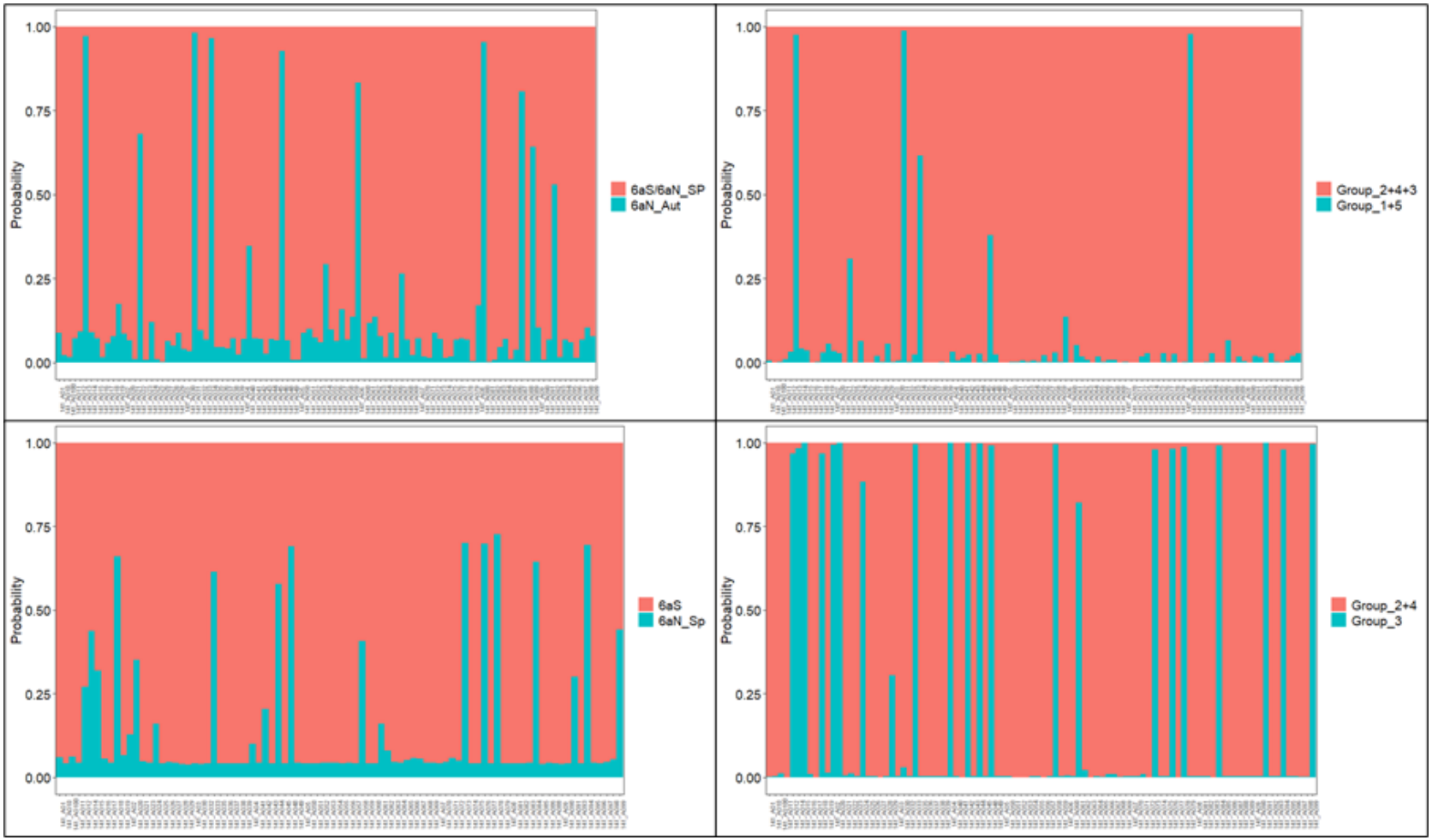


Figure 10.5.19. Assignment of the MSHAS 2017 Haul 36 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

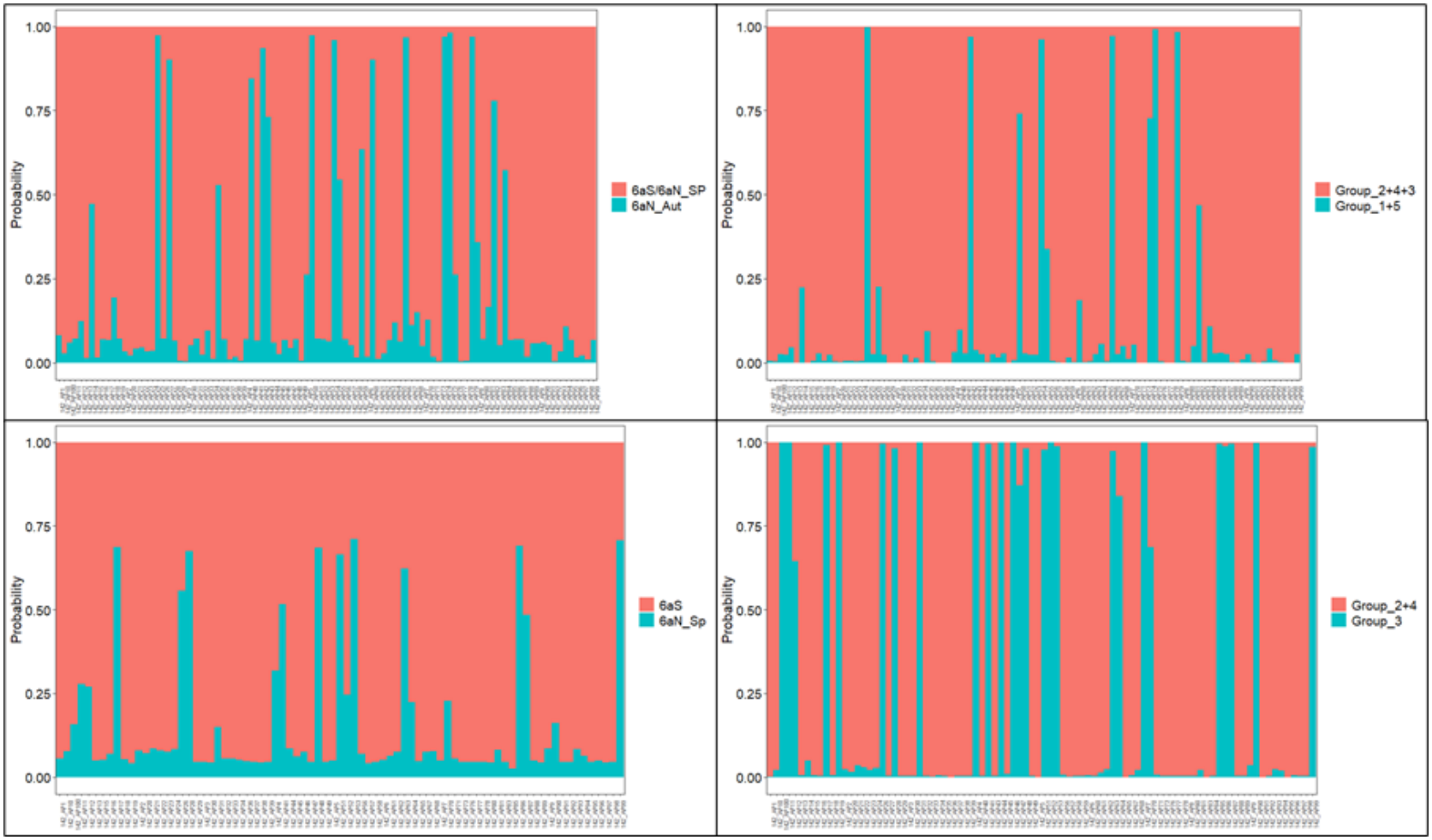


Figure 10.5.20. Assignment of the MSHAS 2017 Haul 37 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

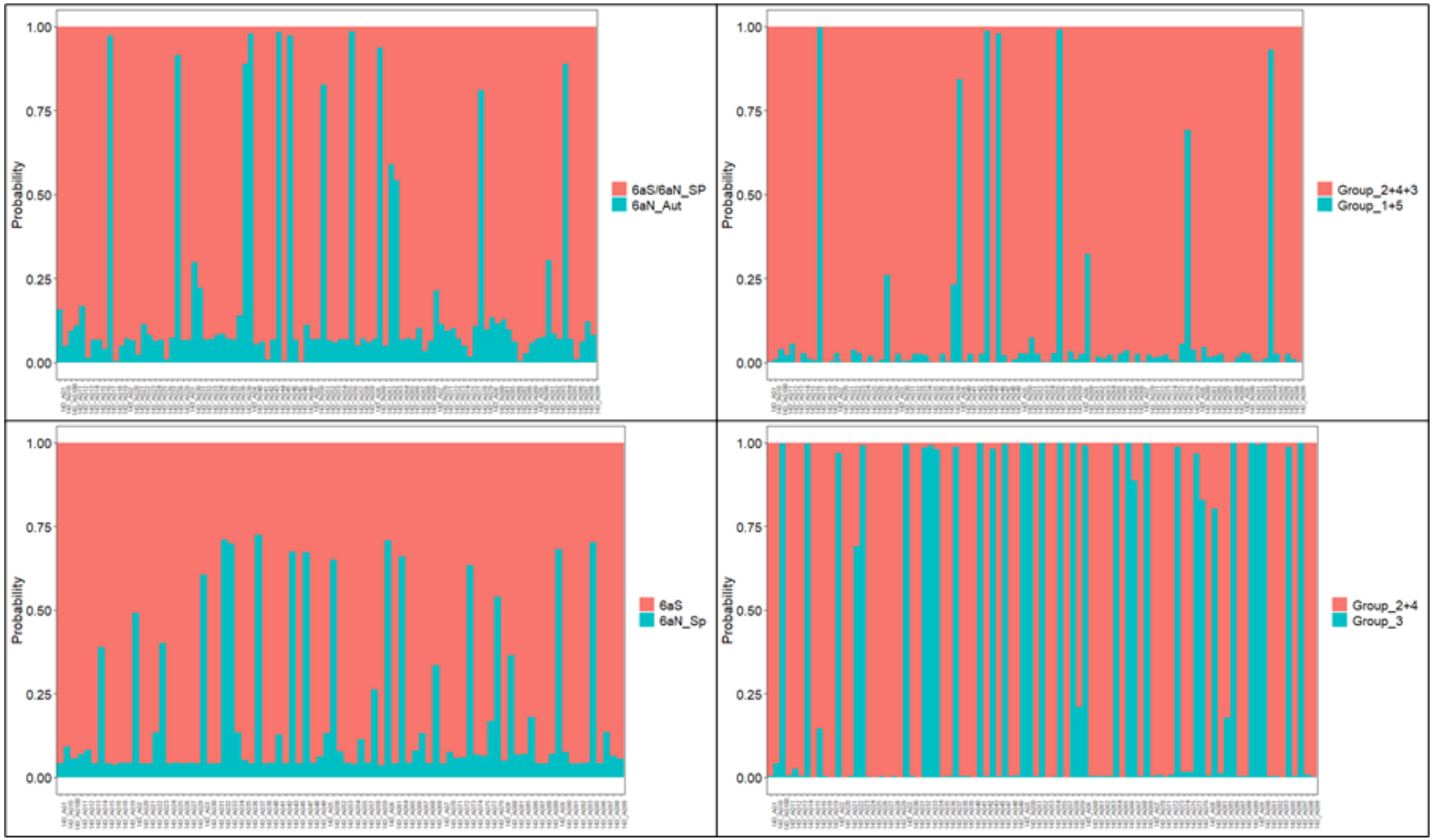


Figure 10.5.21. Assignment of the MSHAS 2017 Haul 39 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

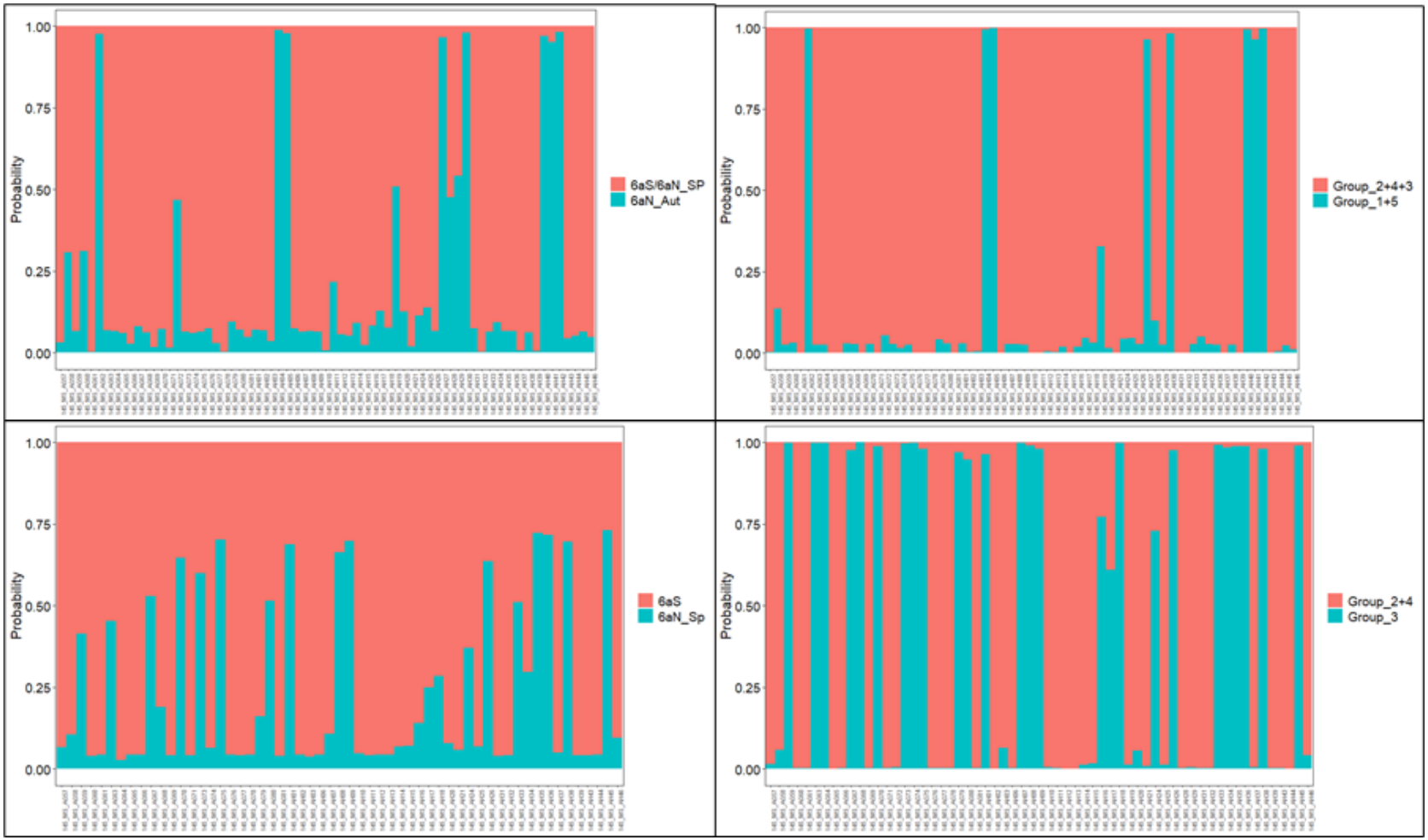


Figure 10.5.22. Assignment of the MSHAS 2017 Haul 174 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

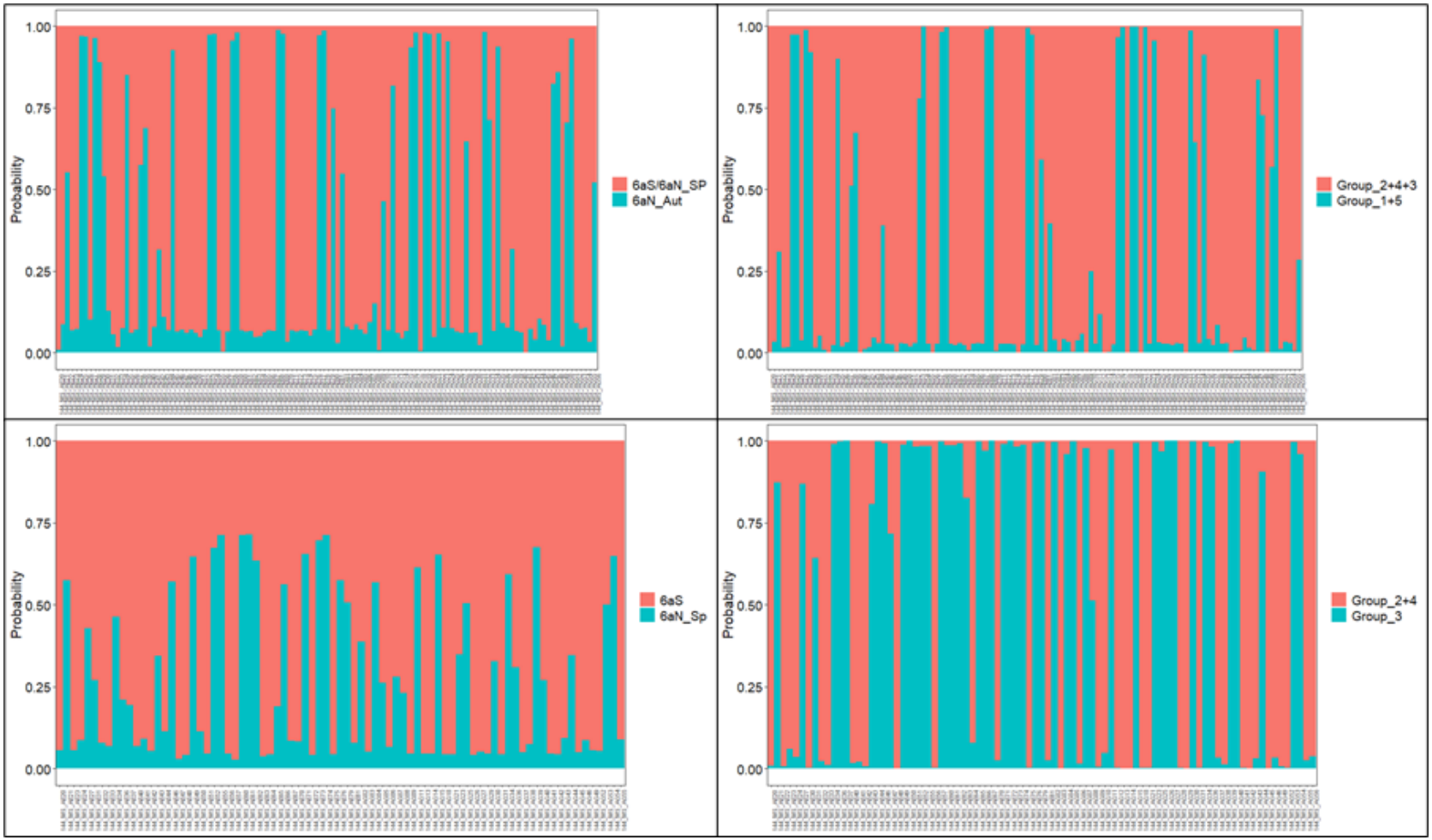


Figure 10.5.23. Assignment of the MSHAS 2017 Haul 172 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

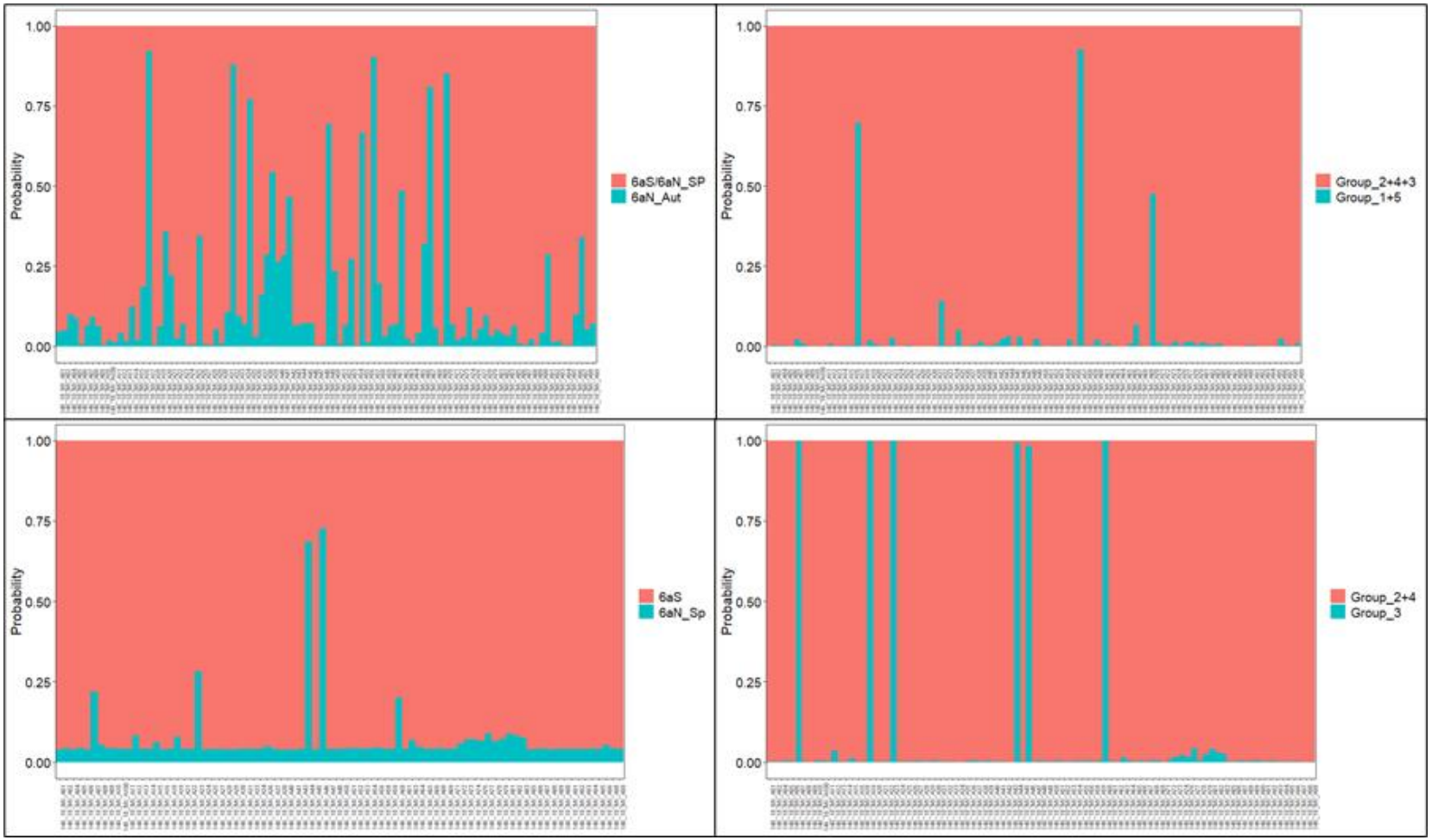


Figure 10.5.24. Assignment of the MSHAS 2018 Haul 32 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

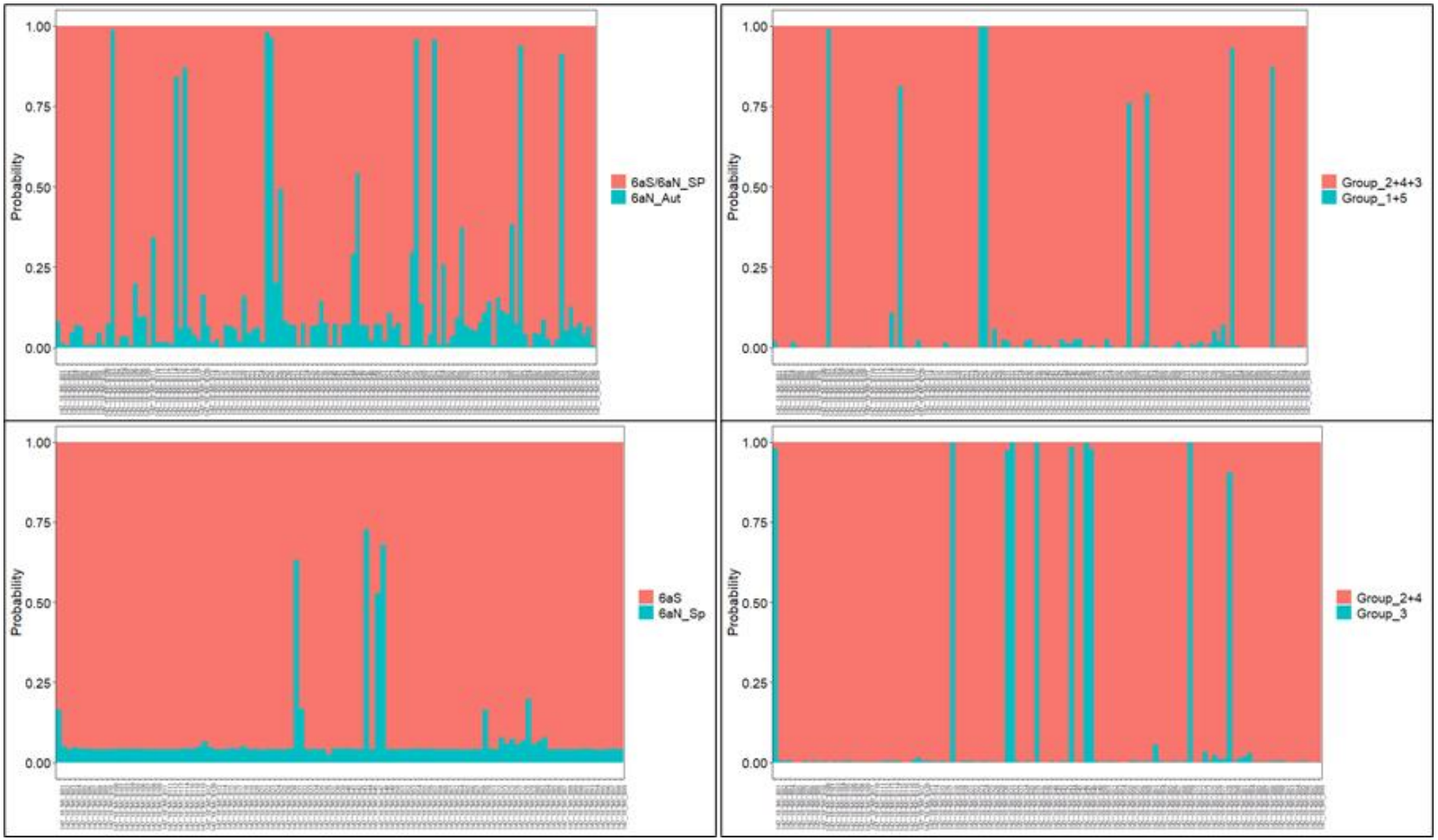


Figure 10.5.25. Assignment of the MSHAS 2018 Haul 35 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

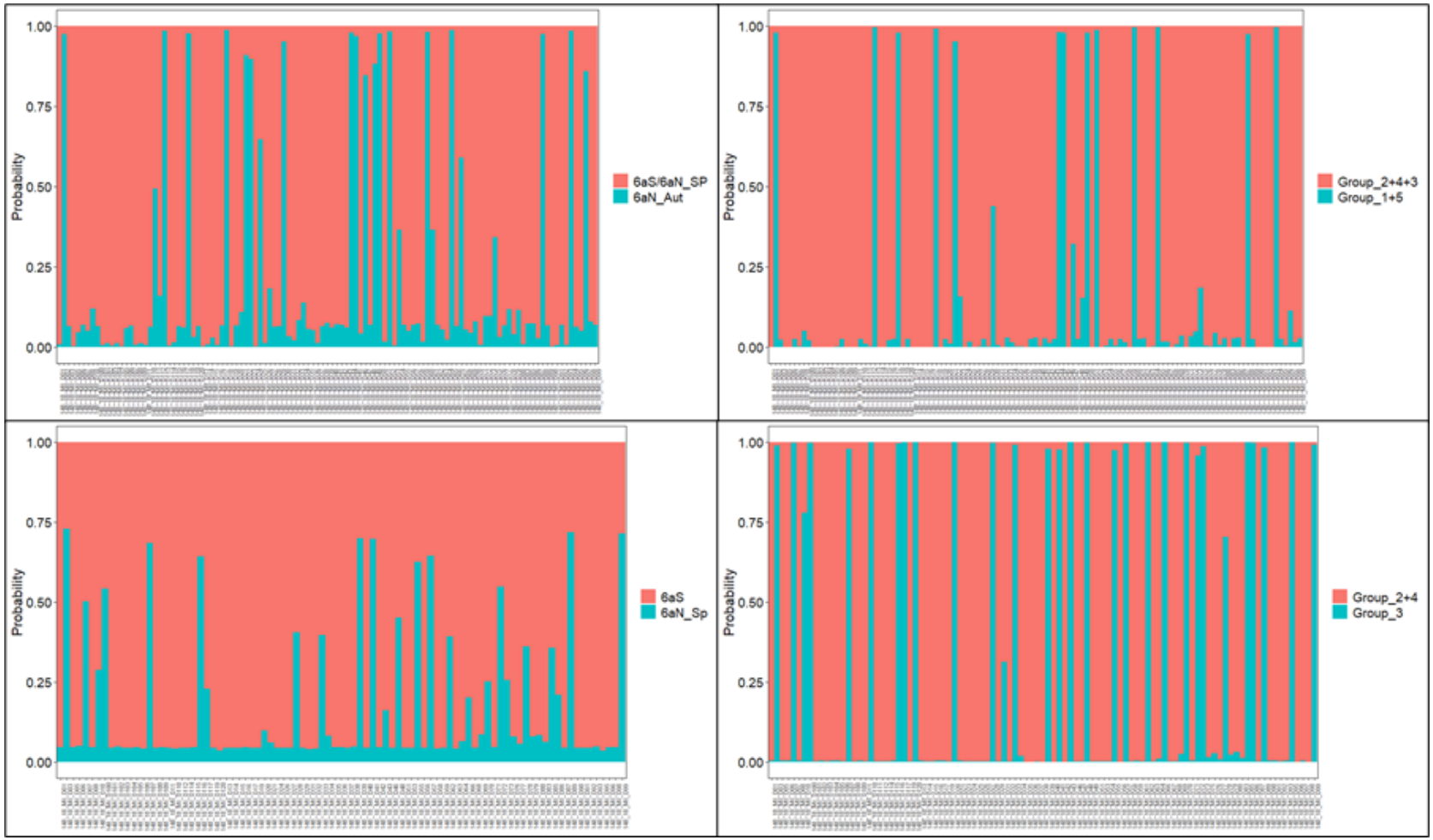


Figure 10.5.26. Assignment of the MSHAS 2018 Haul 37 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

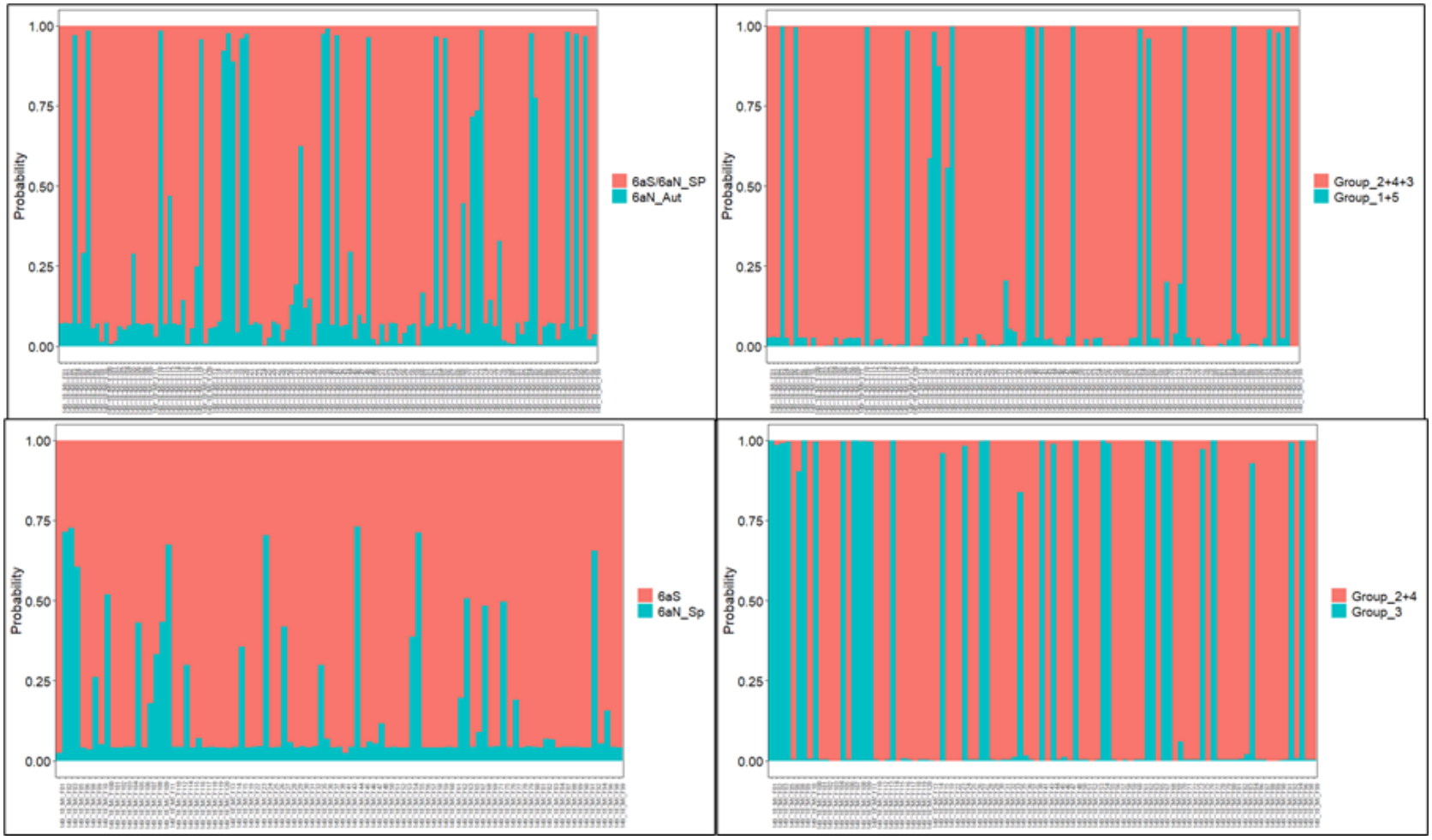


Figure 10.5.27. Assignment of the MSHAS 2018 Haul 39 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

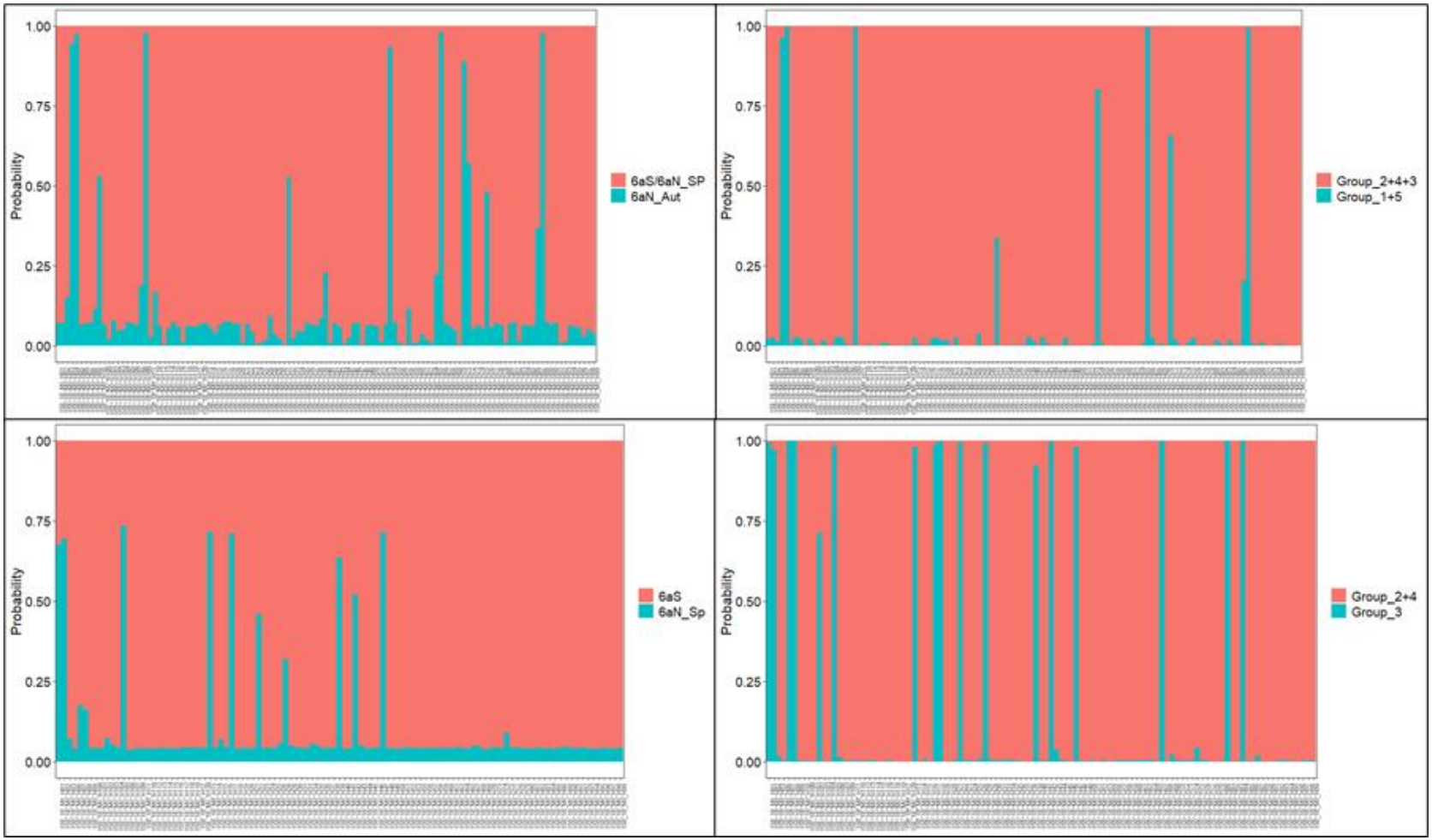


Figure 10.5.28. Assignment of the MSHAS 2018 Haul 40 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

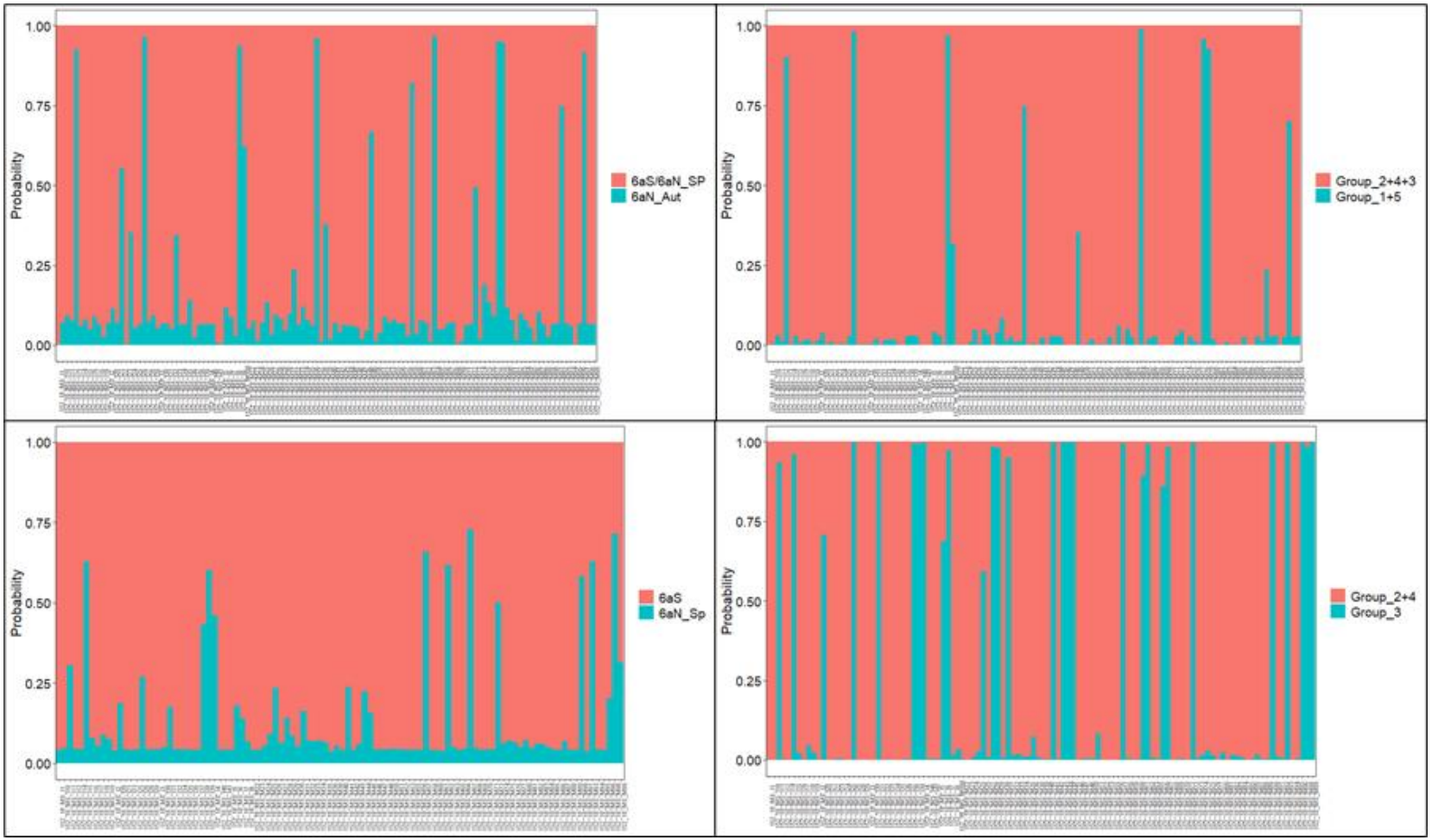


Figure 10.5.29. Assignment of the MSHAS 2018 Haul 182 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

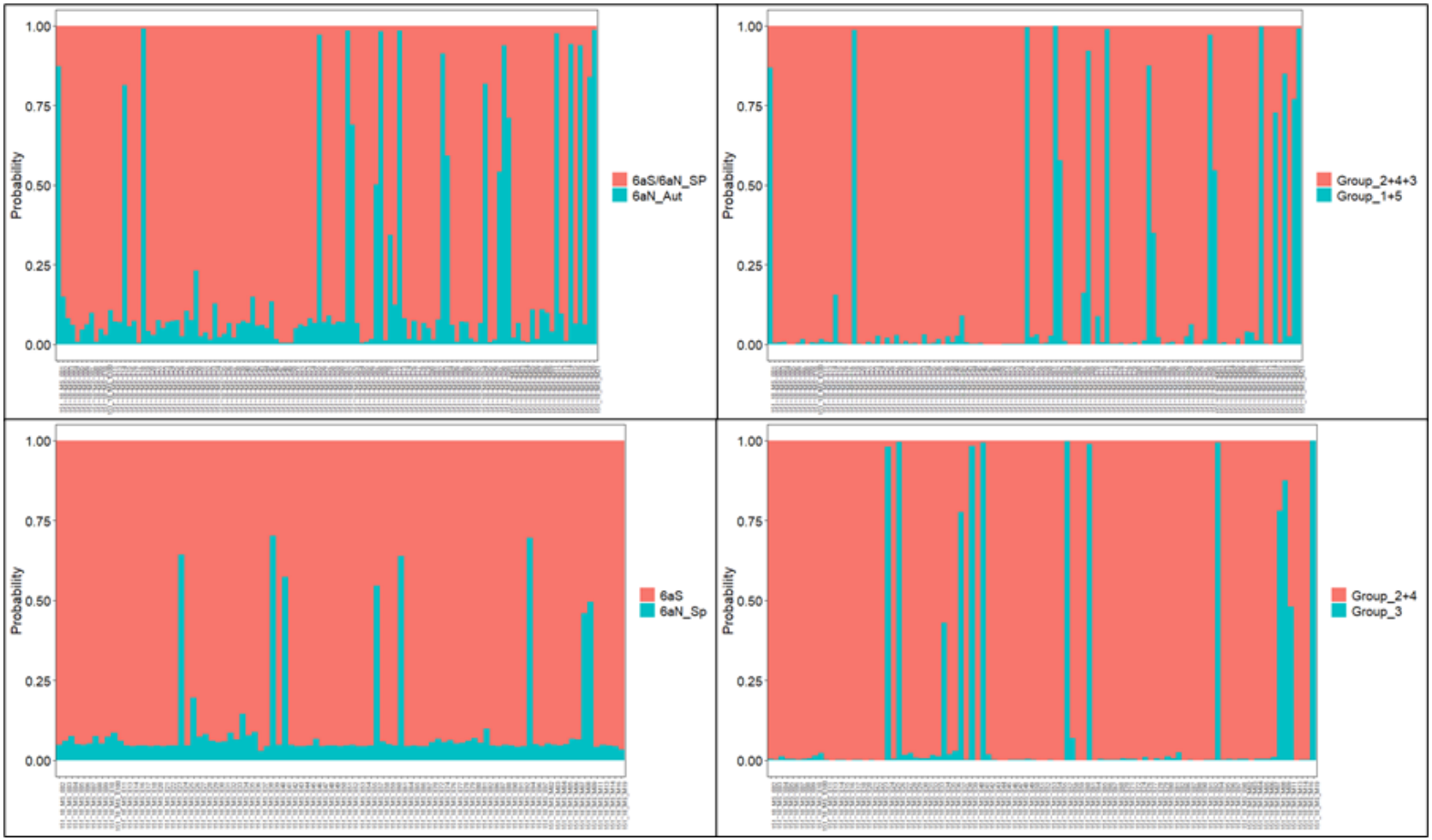


Figure 10.5.30. Assignment of the MSHAS 2018 Haul 181 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

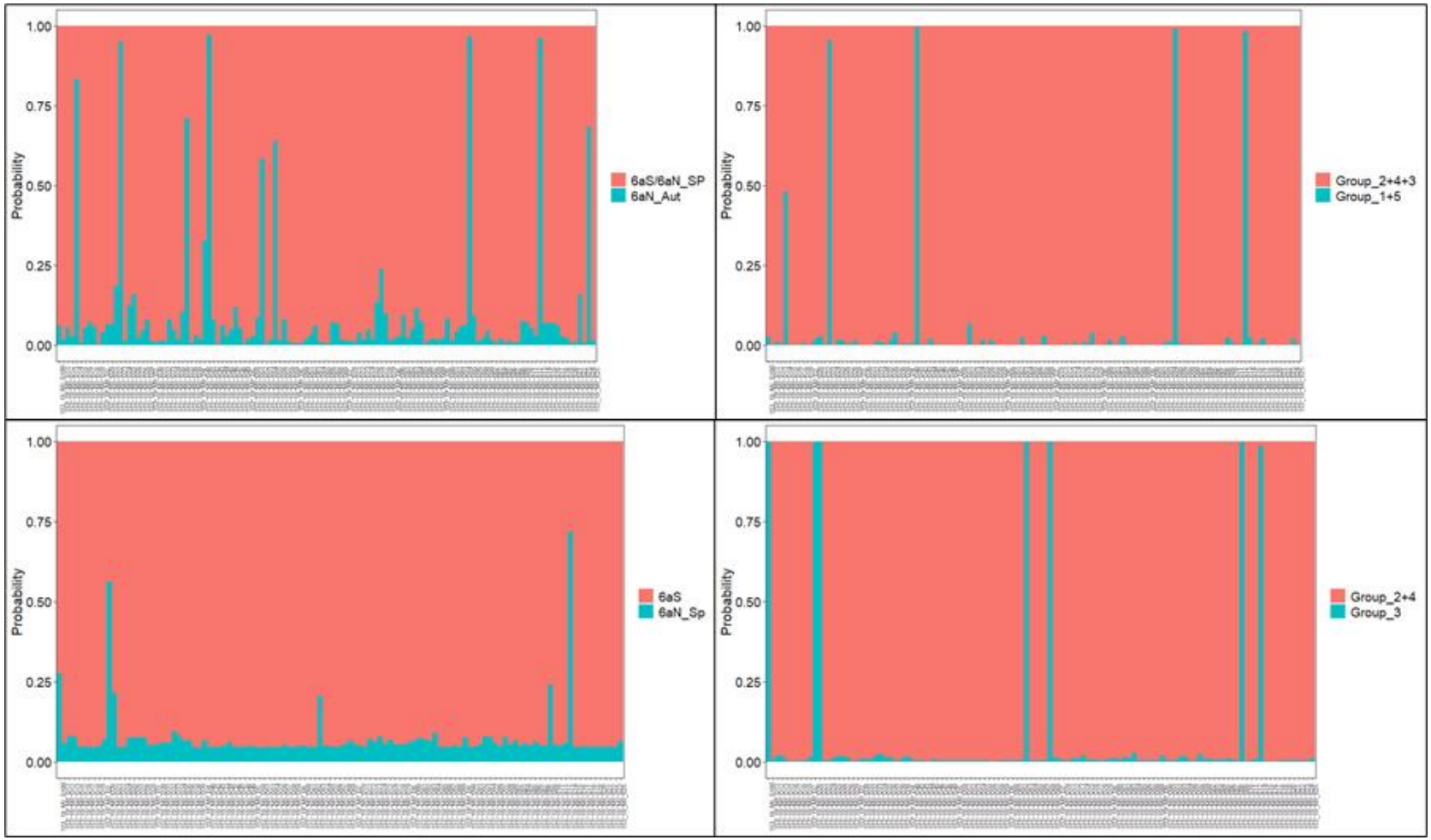


Figure 10.5.31. Assignment of the MSHAS 2019 Haul 35 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

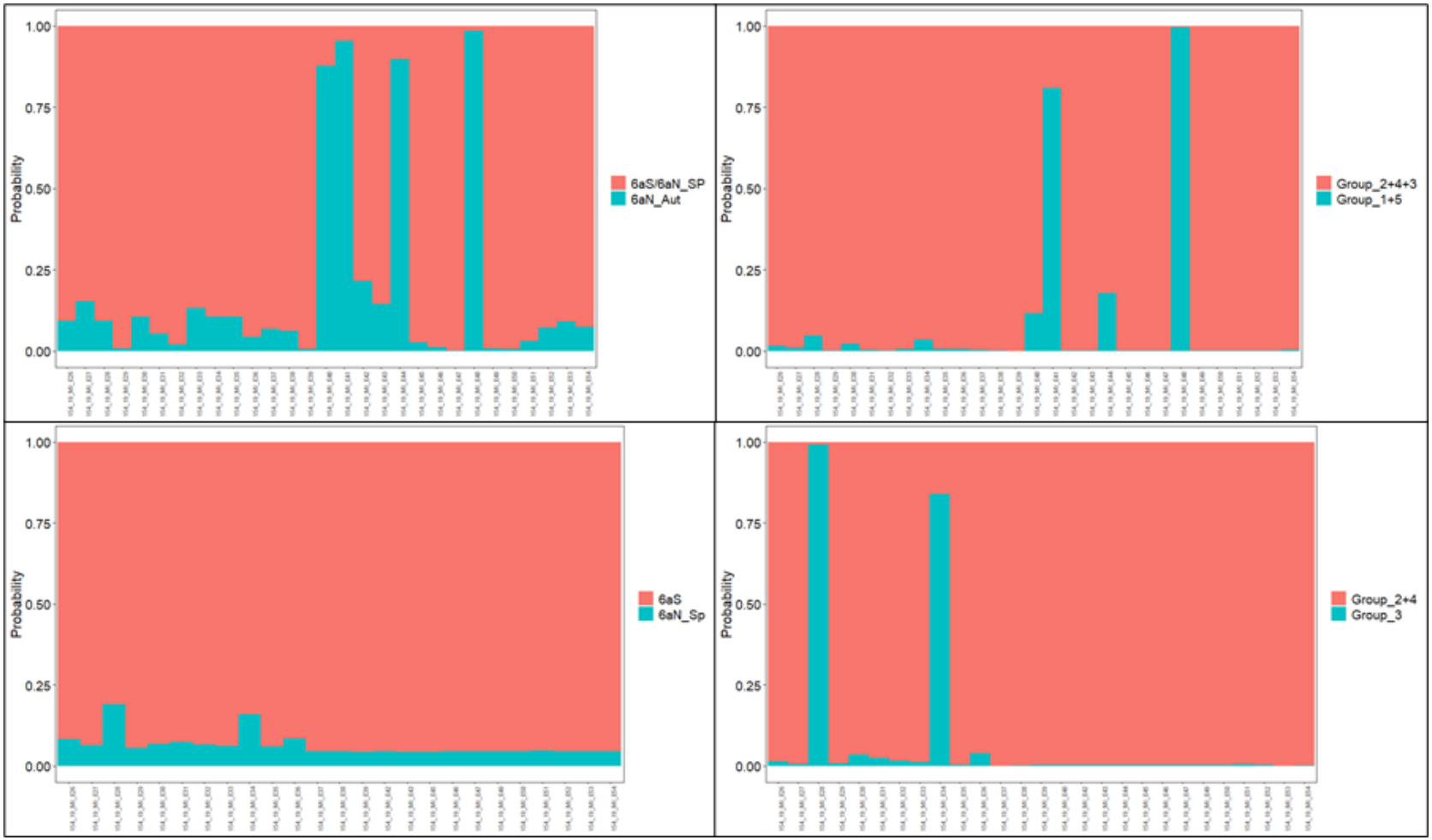


Figure 10.5.32. Assignment of the MSHAS 2019 Haul 36 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

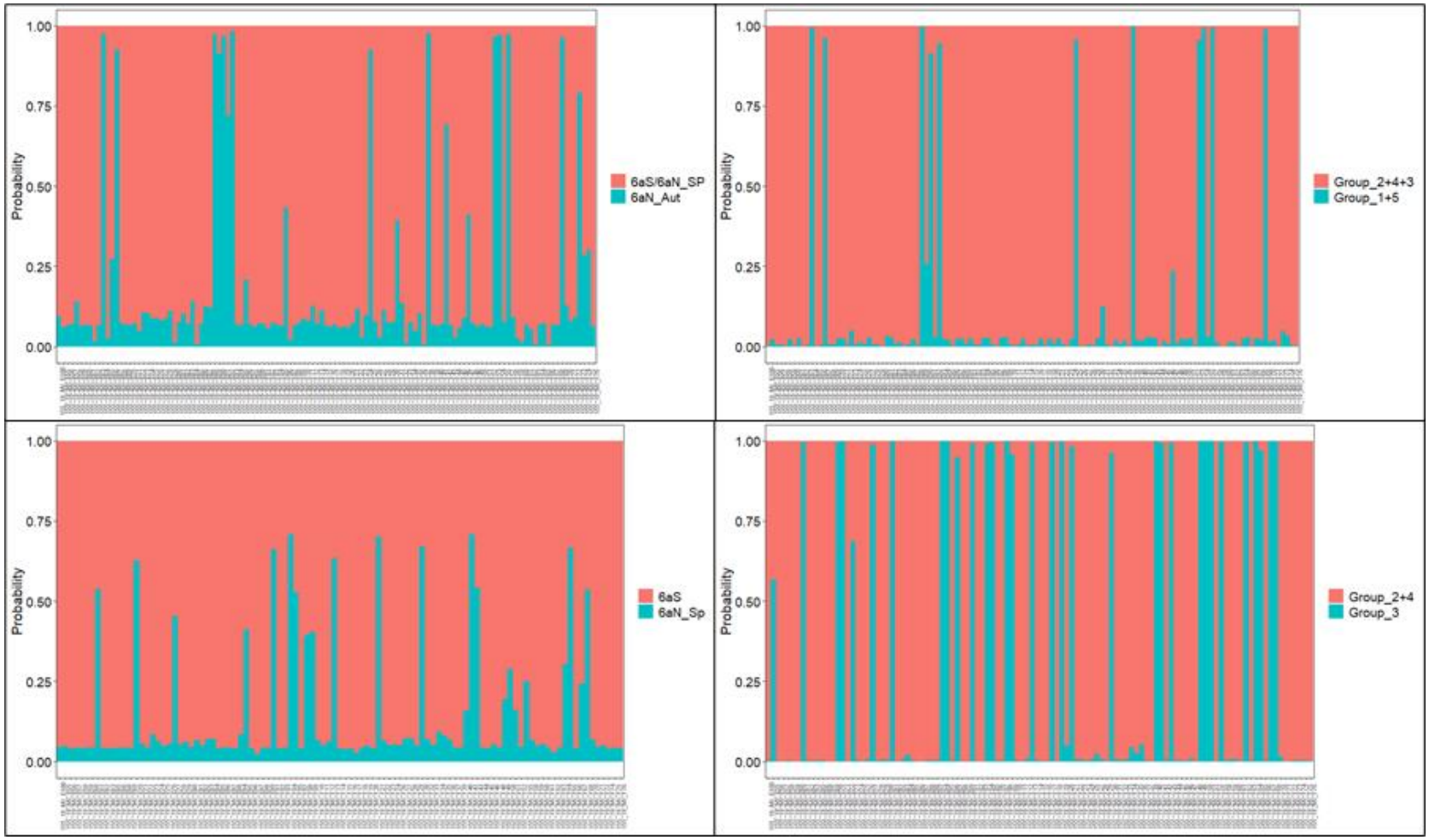


Figure 10.5.33. Assignment of the MSHAS 2019 Haul 42 sample with associated probabilities. (top left) approach 1 level 1 (bottom left) approach 1 level 2 (top right) approach 2 level 1 (bottom right) approach 2 level 2.

10.6. Annex 6 – HERAS and MSHAS survey maps

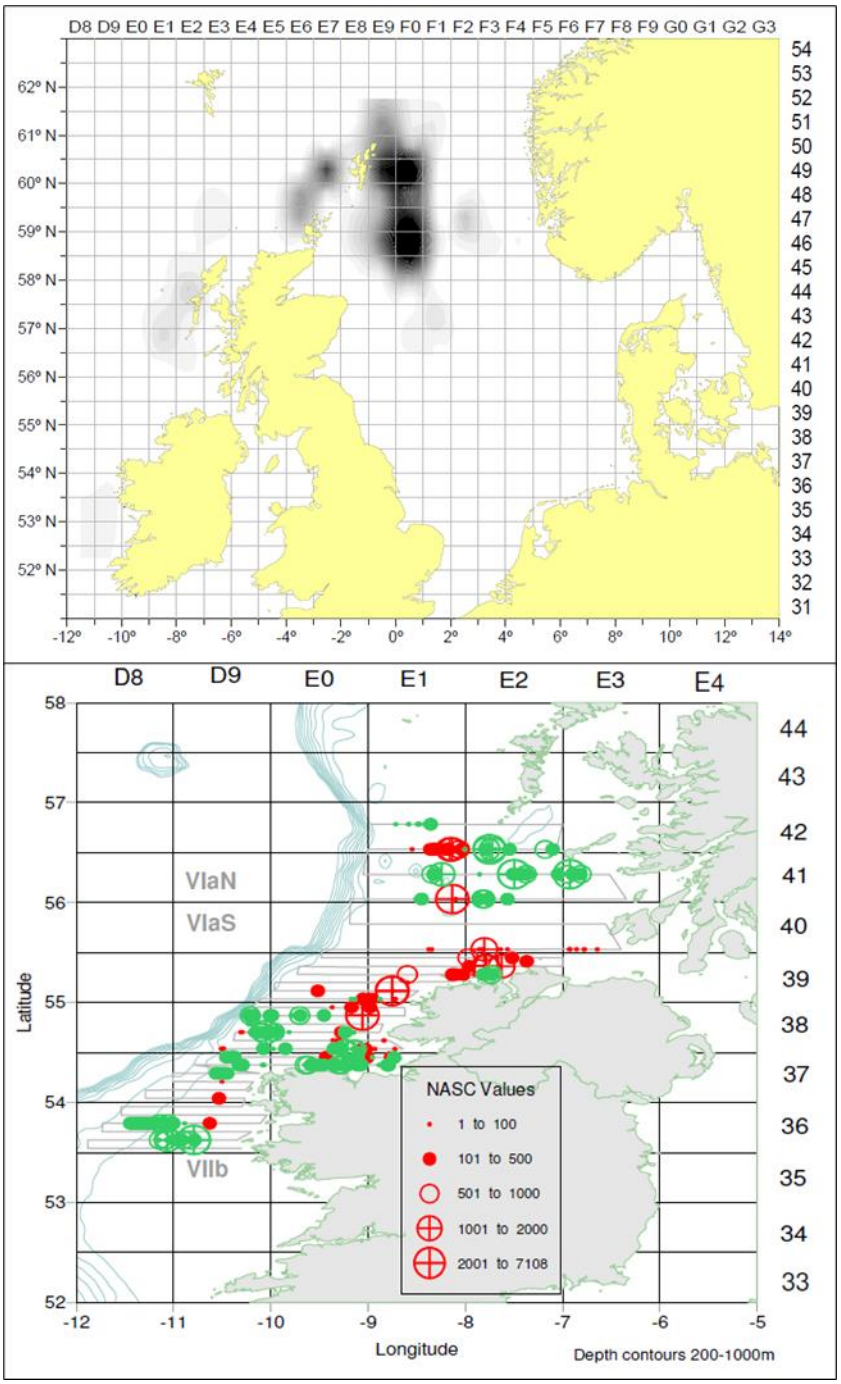


Figure 10.6.1. (top panel) Acoustic Surveys in the North Sea, West of Scotland VIa(N) and the Malin Shelf area in June-July 2010. Biomass of mature autumn spawning herring (maximum value = 220 000 t) from the HERAS combined acoustic survey 2010(from ICES, 2011a). (botom panel) Nautical Area Scattering Coefficient (NASC) plot of herring distribution from the 2010 Marine Institute northwest herring acoustic survey (NWHAS). Circle size proportional to NASC value. Red circles represent single herring schools, green circles represent herring occurring in mixed schools. (from O'Donnell *et al*., 2010).

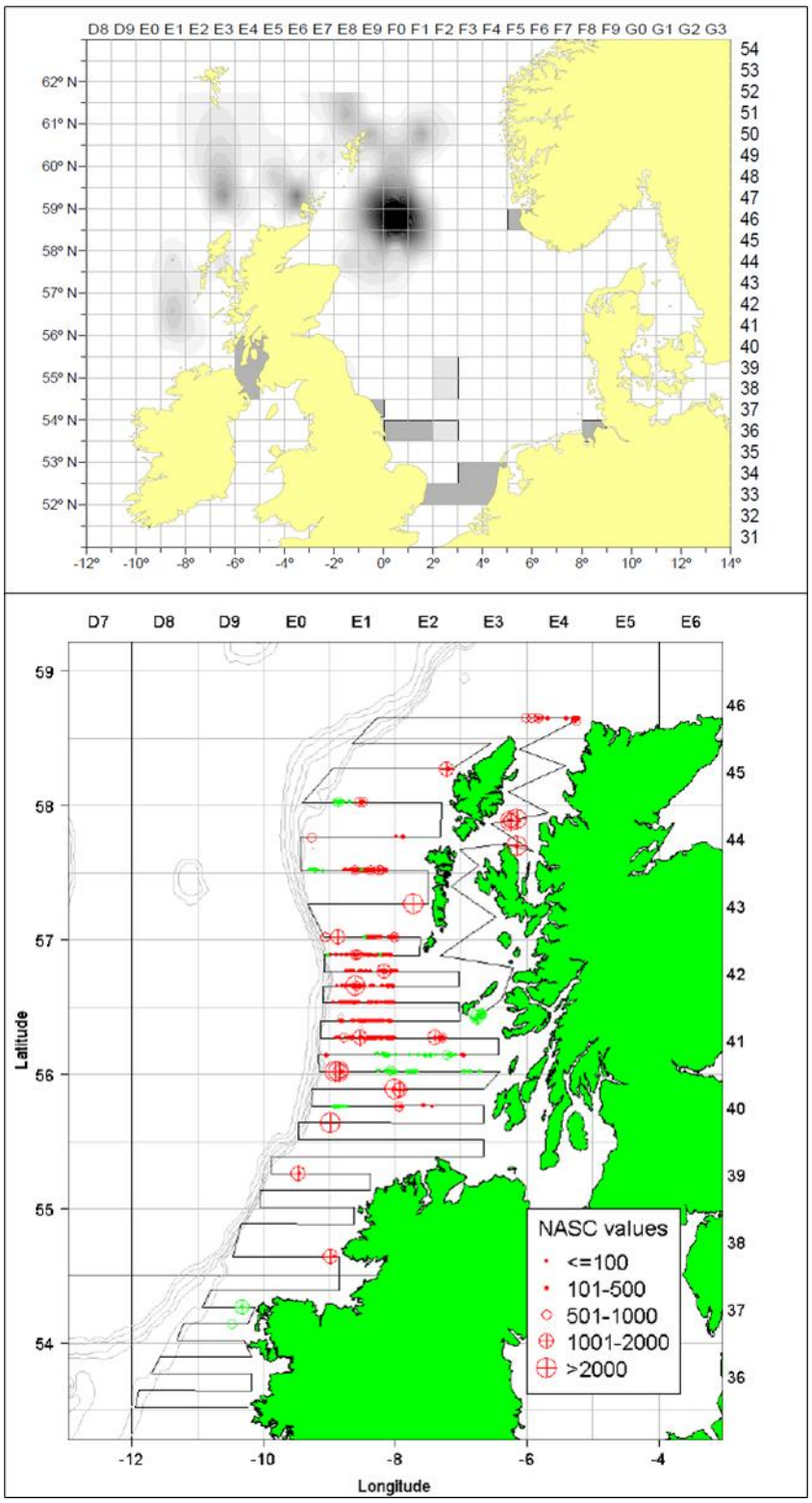


Figure 10.6.2. (top panel) Biomass of mature autumn spawning herring from the combined acoustic surveys in the North Sea, West of Scotland VIa(N) and the Malin Shelf area in June – July 2011 (maximum value = 220 000). Rectangles 36F2, 38F2 and 39F2 (light grey) were interpolated from surrounding ones. Rectangles in dark grey were left uncovered (from ICES, 2012). (botom panel) NASC plot of herring distribution during the NWHAS 2011 survey. Red circles represent single herring schools ("definitely and "probably" herring categories). Green circles represent herring occurring in mixed schools. (from Saunders *et al*., 2011).

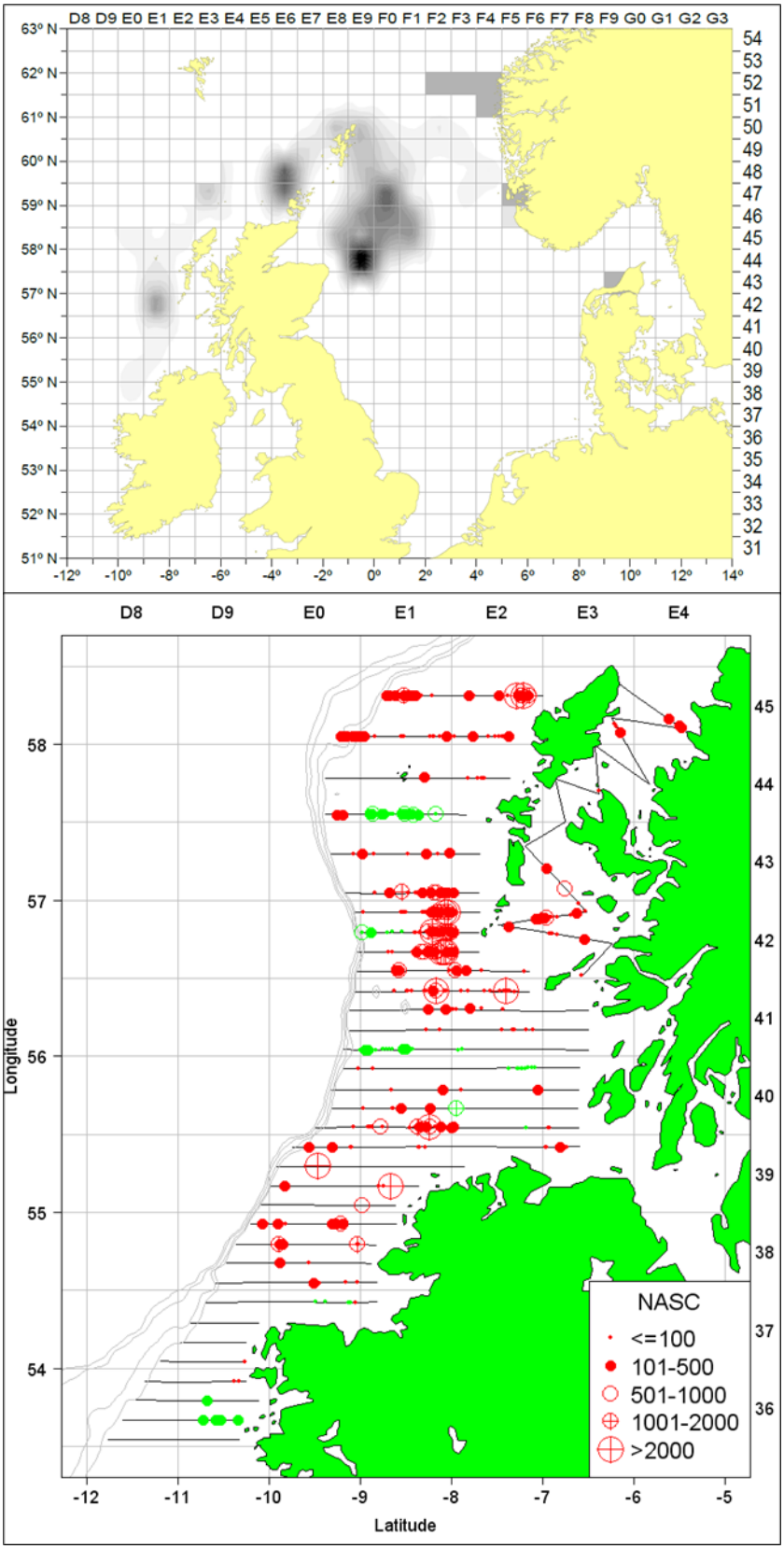


Figure 10.6.3. (top panel) Biomass of mature autumn spawning herring from the combined acoustic surveys in the North Sea, West of Scotland VIa(N) and the Malin Shelf area in June – July 2012 (maximum value = 220 000). Rectangles in light grey were interpolated from surrounding ones. Rectangles in dark grey were not covered. (from ICES, 2013b). (botom panel) NASC plot of herring distribution during the NWHAS 2012 survey. Red circles representsingle herring schools ("definitely" and "probably" herring categories). Green circles represent herring occurring in mixed schools. (from Nolan *et al*., 2012).

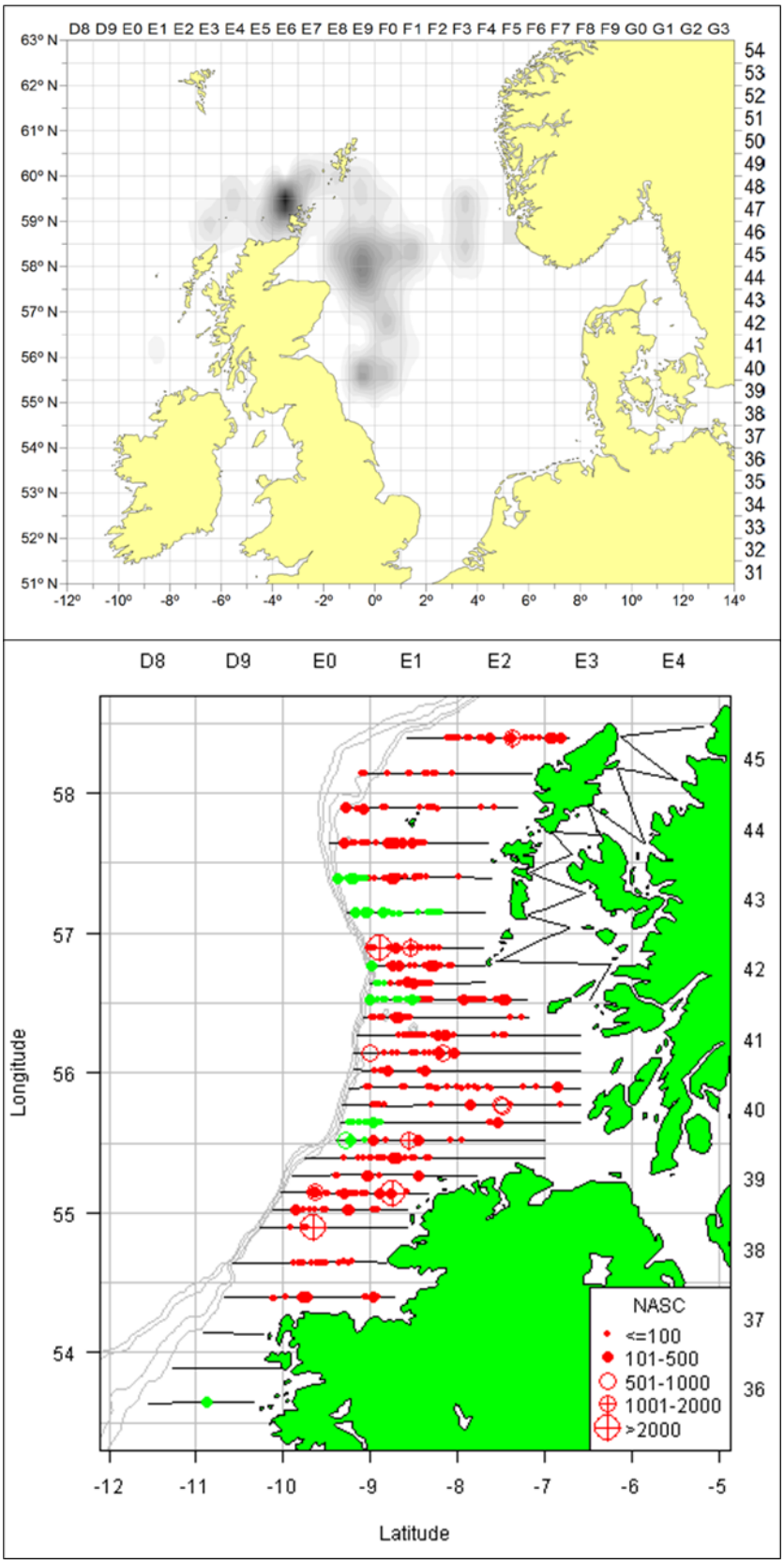


Figure 10.6.4. (top panel) Biomass of mature autumn spawning herring from the combined acoustic survey in June – July 2013 (maximum value = 237 546 t). Rectangles in grey was planned but not surveyed. (from ICES, 2014). (botom panel) NASC plot of herring distribution during the NWHAS 2013 survey. Red circles represent single herring schools ("definitely" and "probably" herring categories). Green circles represent herring occurring in mixed schools. (from Nolan *et al*., 2013).

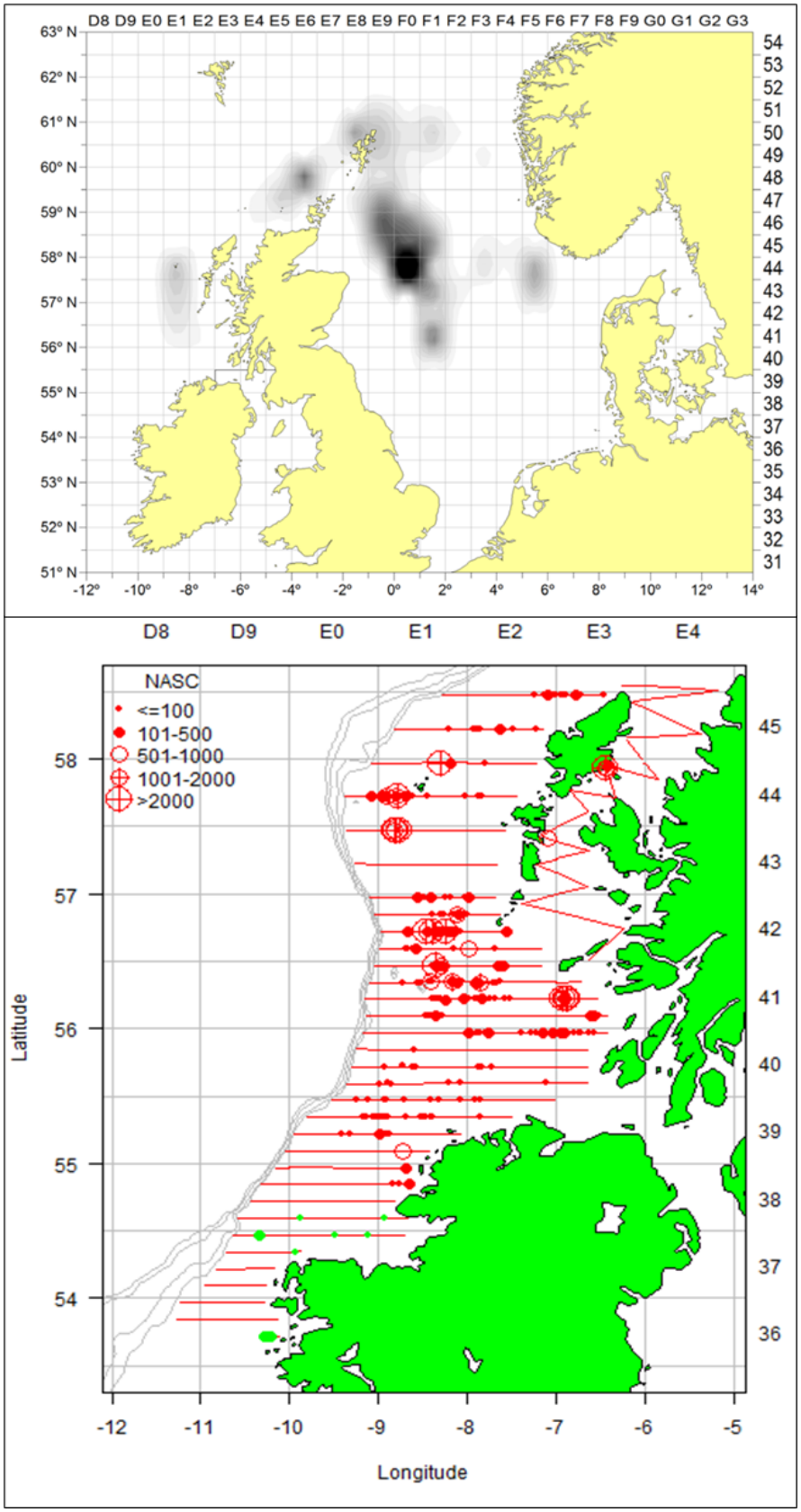


Figure 10.6.5. (top panel) Biomass of mature autumn spawning herring from the combined acoustic survey in June – July 2014 (maximum value = 443 537). (from ICES, 2015c). (botom panel) NASC plot of herring distribution during the NWHAS 2014 survey. Red circles represent single herring schools ("definitely" and "probably" herring categories). Green circles represent herring occurring in mixed schools. (from Nolan *et al.*, 2014).

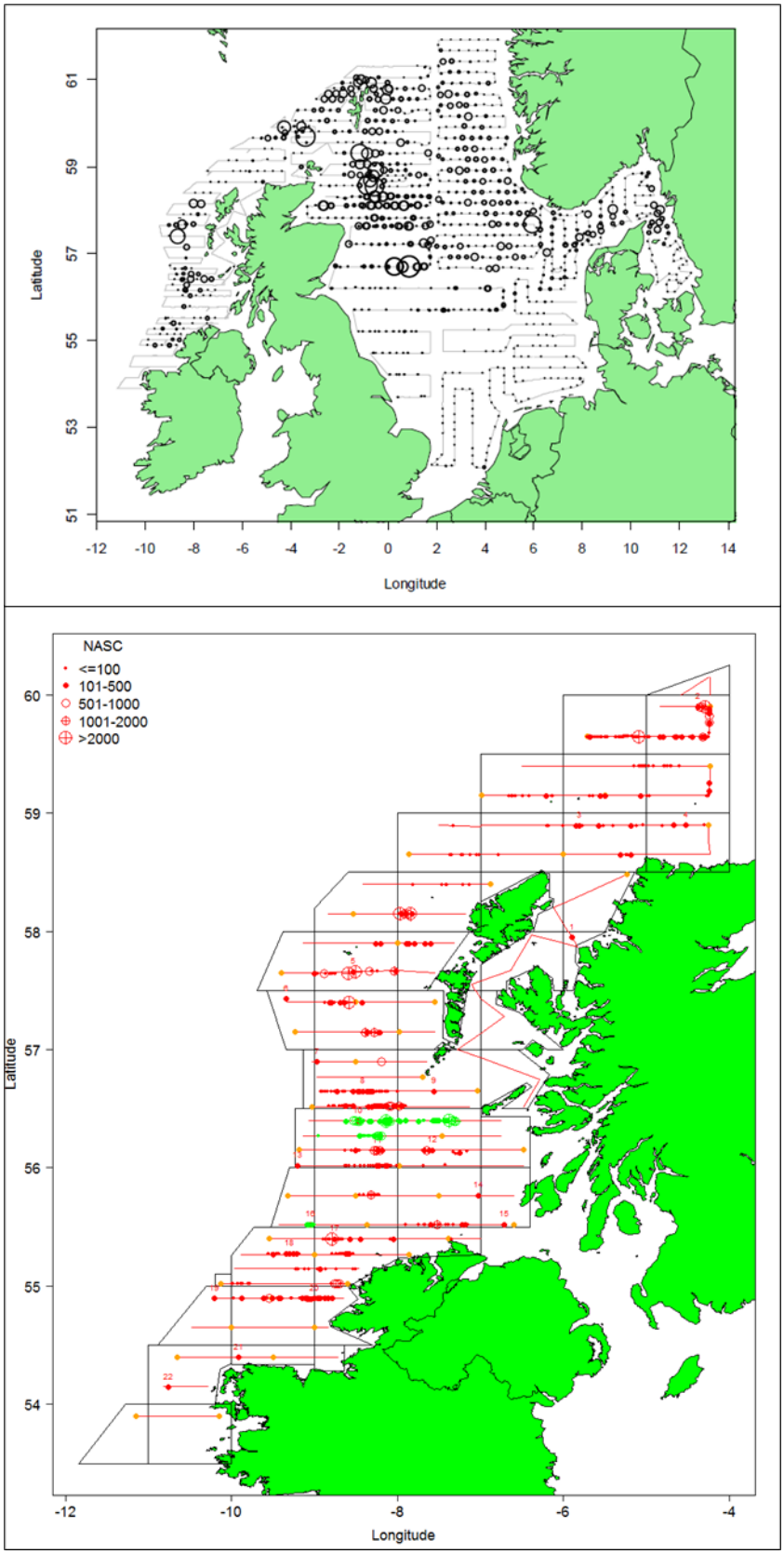


Figure 10.6.6. (top panel) Distribution of NASC attributed to herring in HERAS 2015. Cruise tracks are outlined in light grey with circles representing size and location of herring aggregations. NASC values are resampled at 15 nm intervals along the cruise track. Distribution displayed here is for all herring encountered in the HERAS survey regardless of stock identity. (from ICES, 2016). (botom panel) NASC plot of herring distribution during the NWHAS 2015 survey. Red circles represent single herring schools ("definitely" and "probably" herring categories). Green circles represent herring occurring in mixed schools. (from Nolan *et al*., 2015).

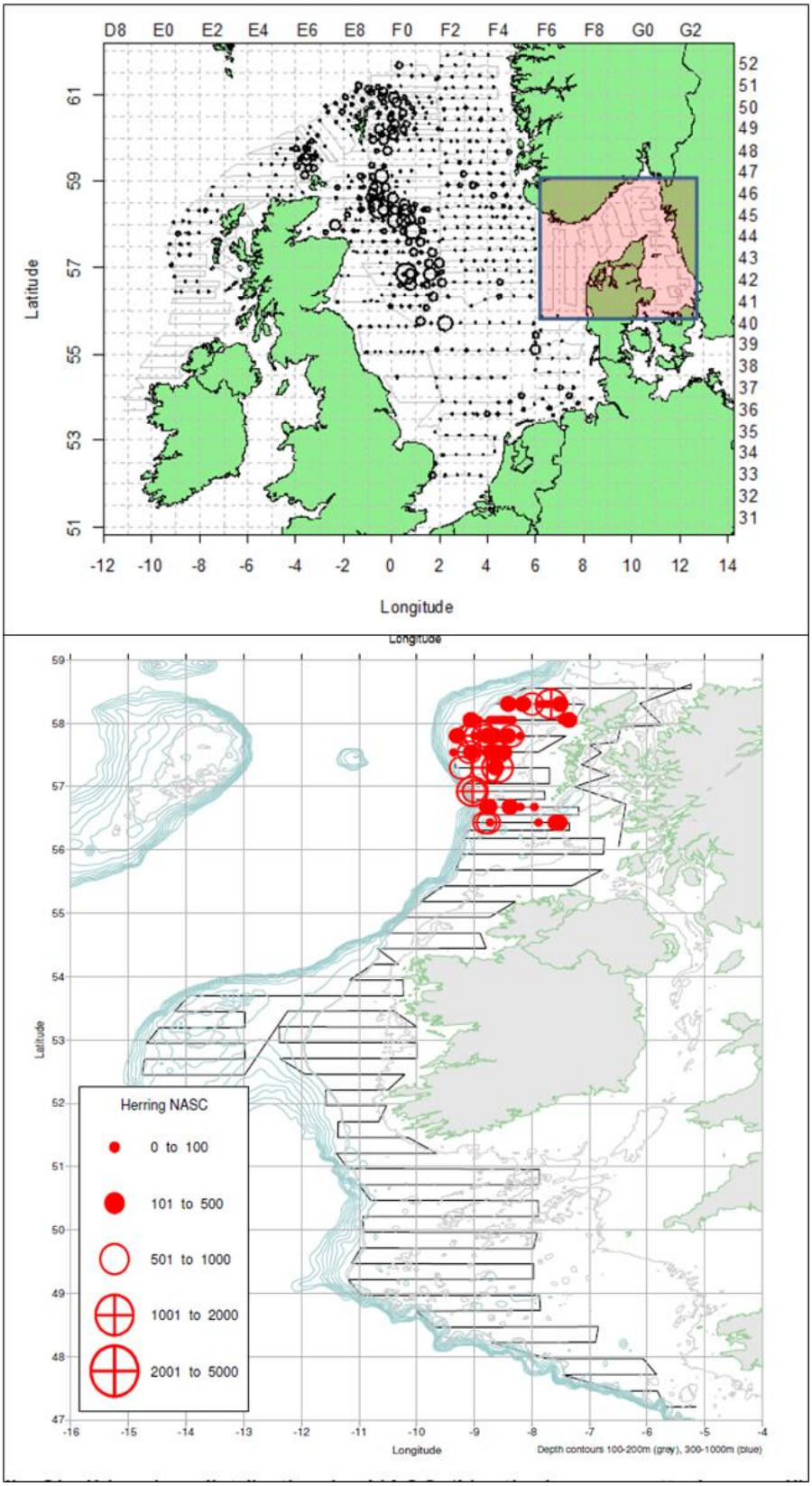


Figure 10.6.7. (top panel) Distribution of NASC attributed to herring in HERAS 2016. Cruise tracks are out-lined in light grey with circles representing size and location of herring aggregations. NASC values are resampled at 15 nm intervals along the cruise track. Distribution displayed here is for all herring encountered in the HERAS survey regardless of stock identity. (from ICES, 2017b). (botom panel) Malin Shelf herring distribution by NASC (Nautical area scattering coefficient) on the 2016 Western European Shelf Pelagic Acoustic Survey (WESPAS) (from O'Donnell *et al*., 2016).

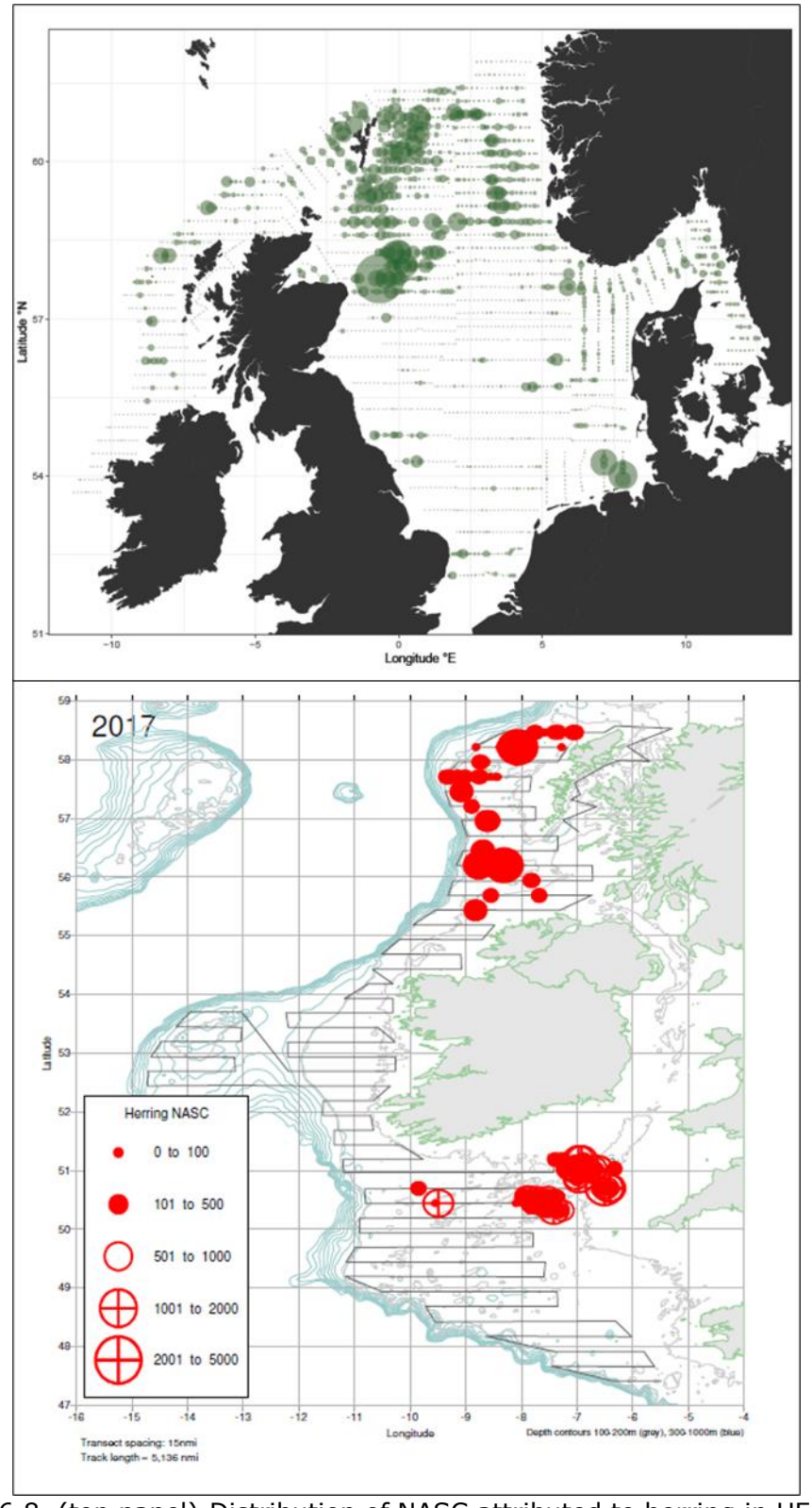


Figure 10.6.8. (top panel) Distribution of NASC attributed to herring in HERAS 2017. Cruise tracks are outlined in light grey with circles representing size and location of herring aggregations. NASC values are resampled at 15 nm intervals along the cruise track. Distribution displayed here is for all herring encountered in the HERAS survey regardless of stock identity. (from ICES, 2018). (botom panel) Malin Shelf herring distribution by NASC (Nautical area scattering coefficient) on the 2017 Western European Shelf Pelagic Acoustic Survey (WESPAS). Celtic Sea herring distribution also plotted. (from O'Donnell *et al*., 2017).

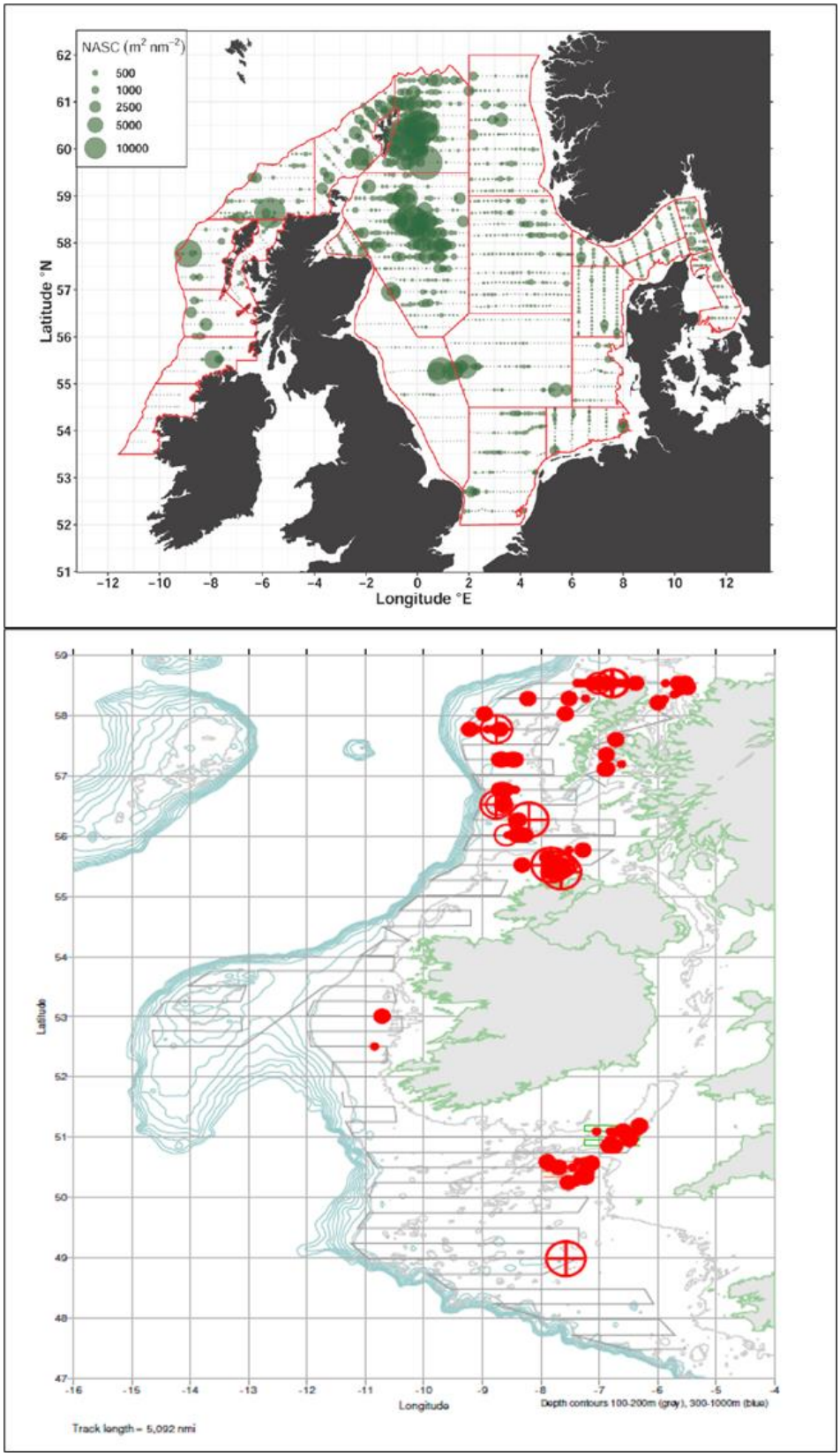


Figure 10.6.9. (top panel) Distribution of NASC attributed to herring in HERAS in 2018. Acoustic intervals represented by light grey dot with green circles representing size and location of herring aggregations. NASC values are resampled at 5 nmi intervals along the cruise track. The red lines show the strata system. (from ICES, 2019a). (botom panel) Malin Shelf herring distribution by NASC (Nautical area scattering coefficient) on the 2018 Western European Shelf Pelagic Acoustic Survey (WESPAS). Celtic Sea herring distribution also plotted. (from O'Donnell *et al*., 2018).

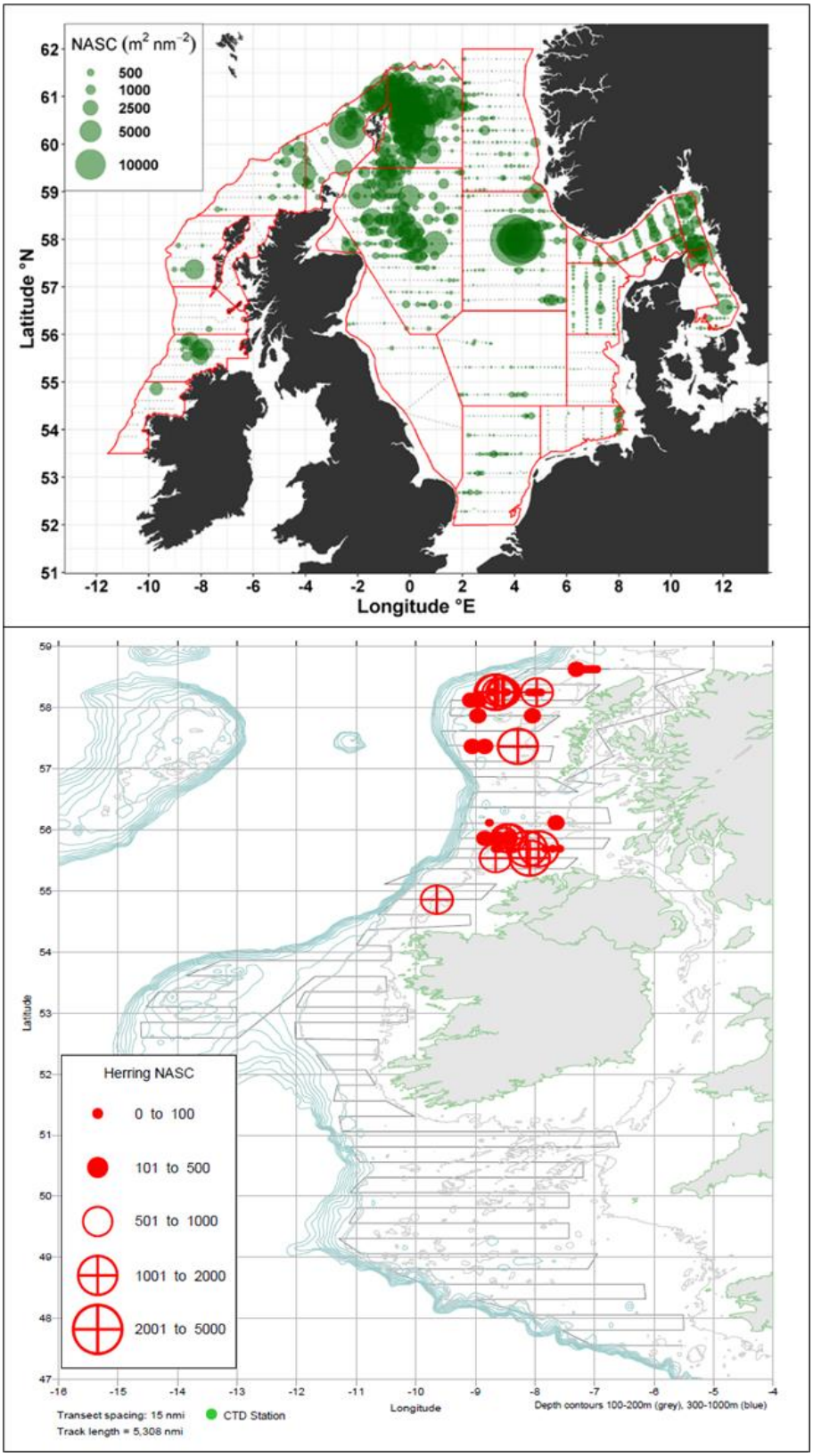
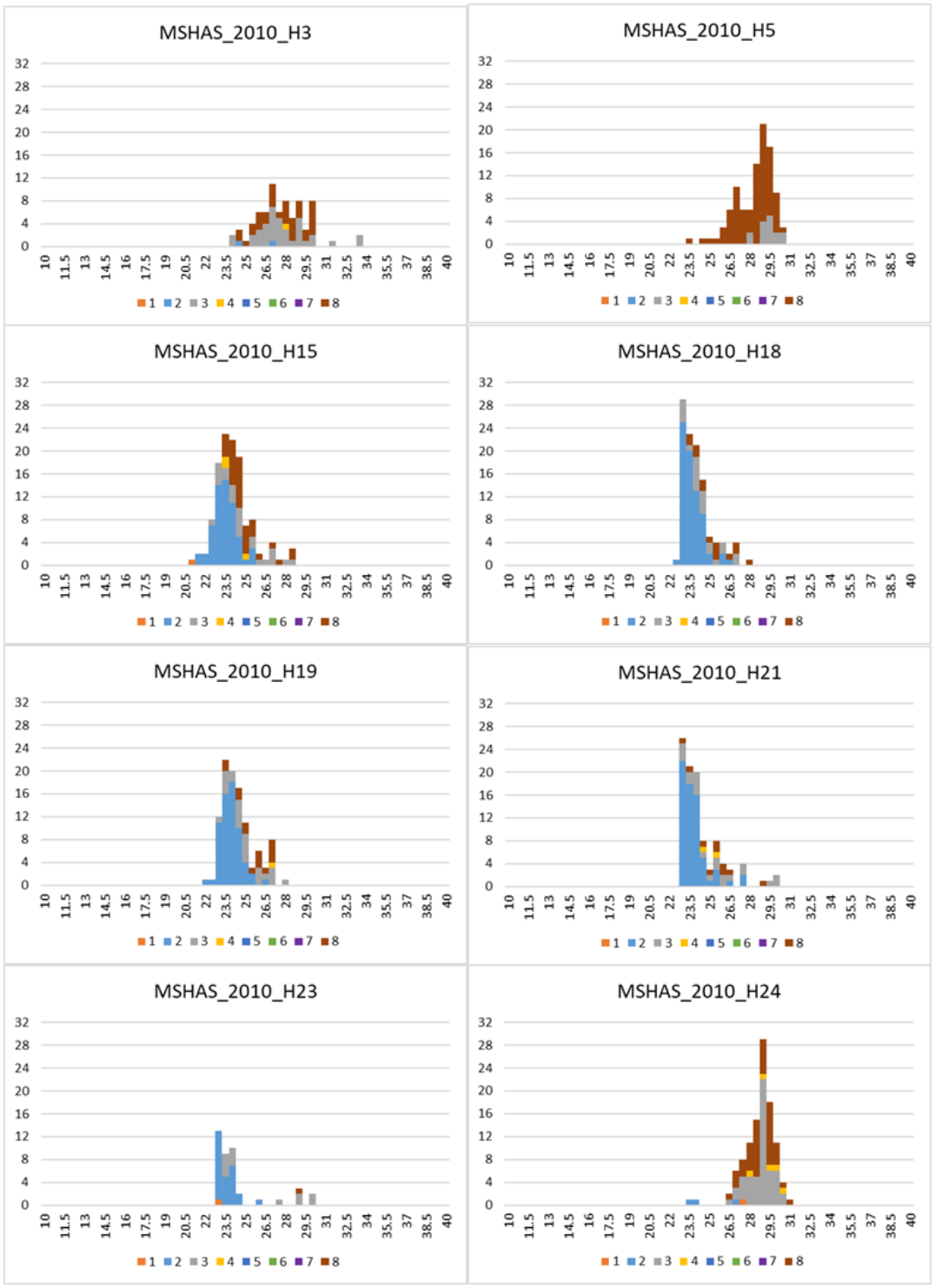
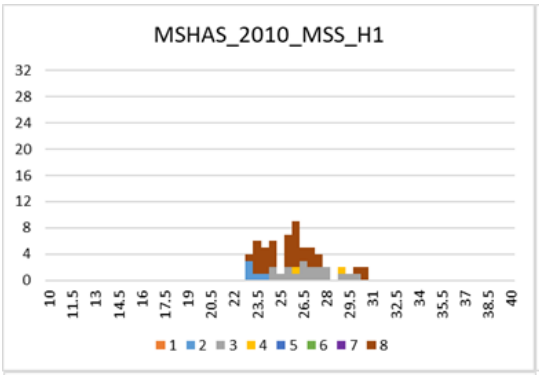


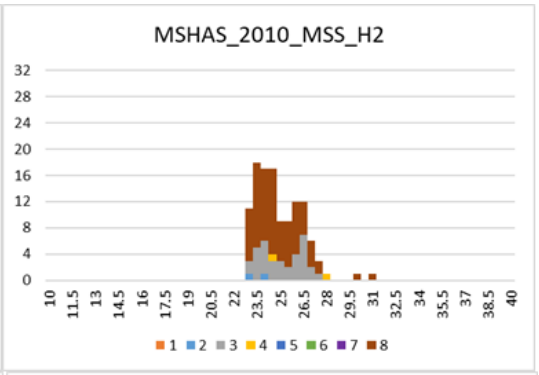
Figure 10.6.10. (top panel) Distribution of NASC attributed to herring in HERAS in 2019. Acoustic intervals represented by light grey dot with green circles representing size and location of herring aggregations. NASC values are resampled at 5 nmi inter-vals along the cruise track. The red lines show the strata system. (from ICES, 2020b). (botom panel) Malin Shelf herring distribution by NASC (Nautical area scattering coefficient) on the 2019 Western European Shelf Pelagic Acoustic Survey (WESPAS). (from O'Donnell *et al.*, 2019).

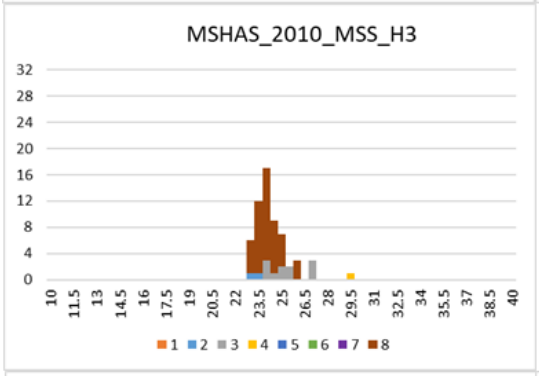
10.7. Annex 7 – Length frequency and maturity plots of MSHAS samples

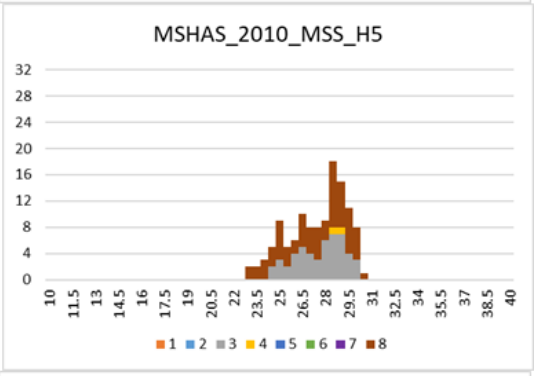
Total length (cm) is displayed on the x-axis and number of individuals on the y-axis. Maturtiy stage (6-point scale) is denoted by colour according to the legend below the x-axis.




MSHAS_2010_MSS_H1
32 28 24 20 16 12 8 4 0
10 11.5 13 14.5 16 17.5 19 20.5 22 23.5 25 26.5 28 29.5 31 32.5 34 35.5 37 38.5 40
1 2 3 4 5 6 7 8


MSHAS_2010_MSS_H2
32 28 24 20 16 12 8 4 0
10 11.5 13 14.5 16 17.5 19 20.5 22 23.5 25 26.5 28 29.5 31 32.5 34 35.5 37 38.5 40
1 2 3 4 5 6 7 8


MSHAS_2010_MSS_H3
32 28 24 20 16 12 8 4 0
10 11.5 13 14.5 16 17.5 19 20.5 22 23.5 25 26.5 28 29.5 31 32.5 34 35.5 37 38.5 40
1 2 3 4 5 6 7 8


MSHAS_2010_MSS_H5
32 28 24 20 16 12 8 4 0
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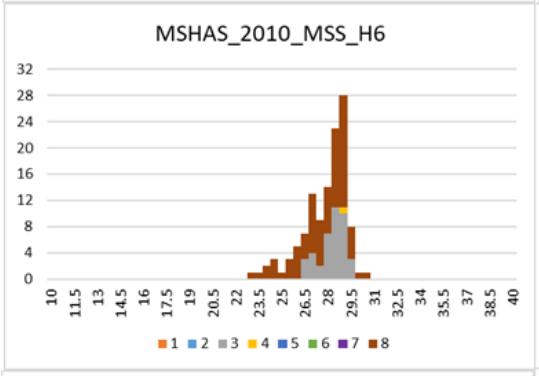
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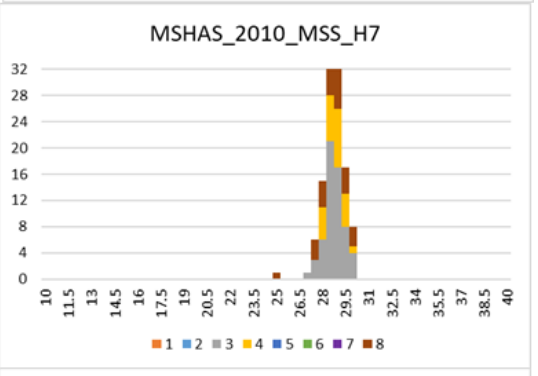
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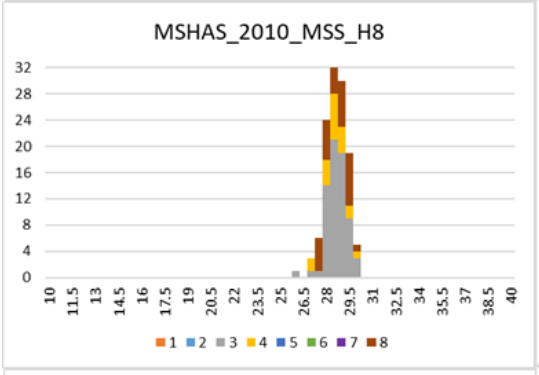
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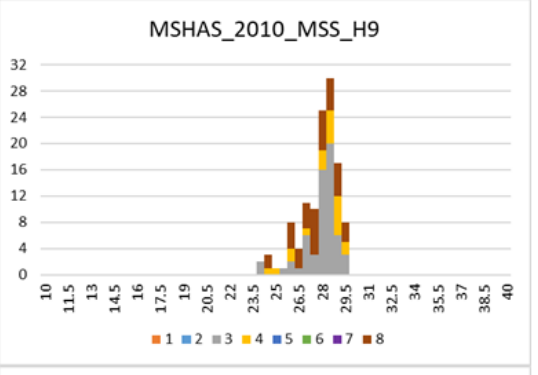
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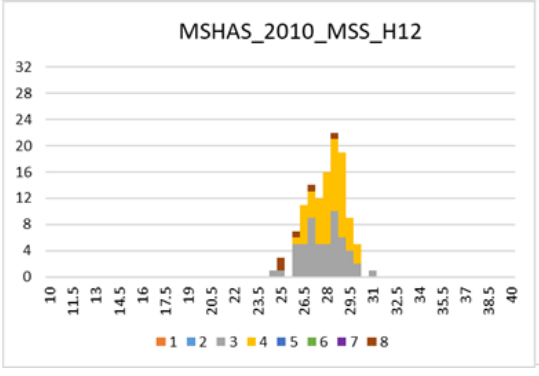
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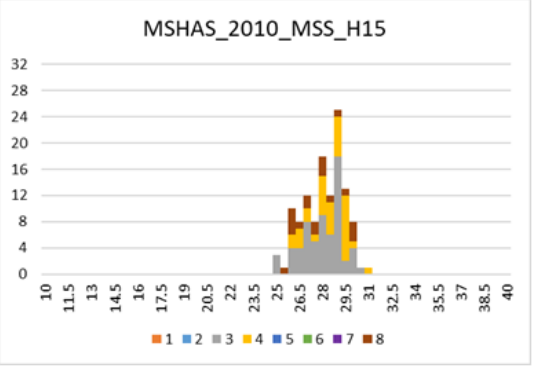
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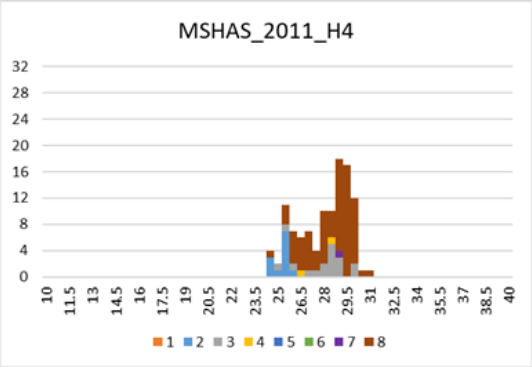
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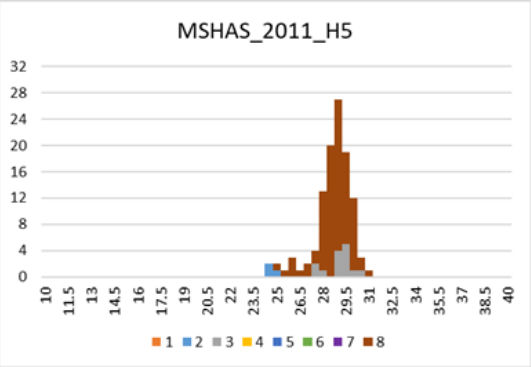
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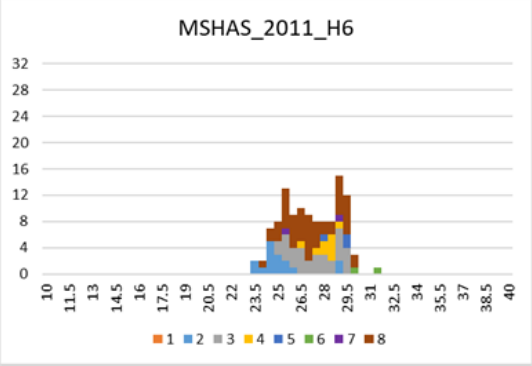
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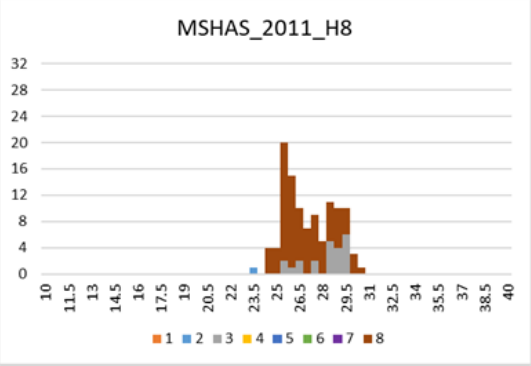
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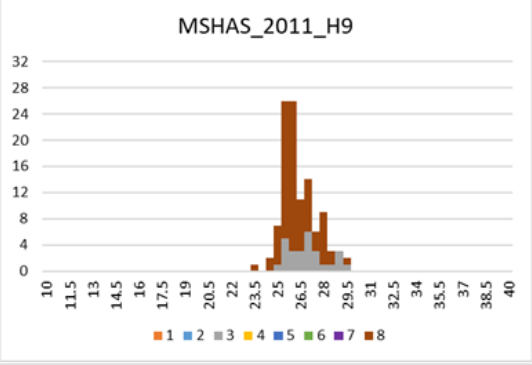
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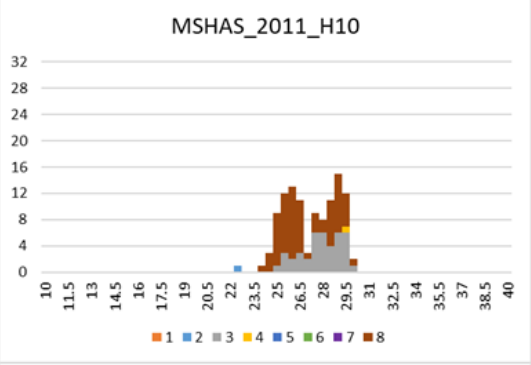
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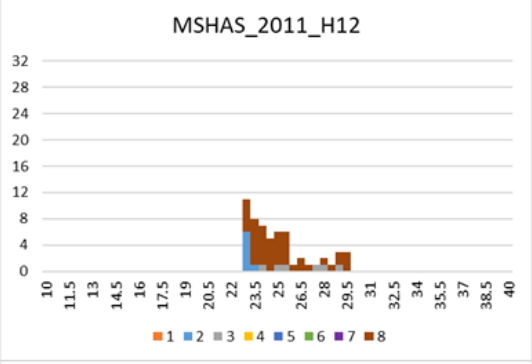
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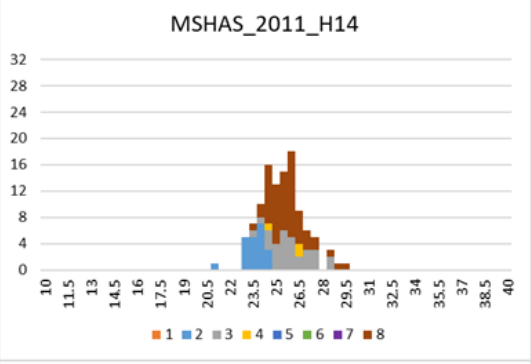
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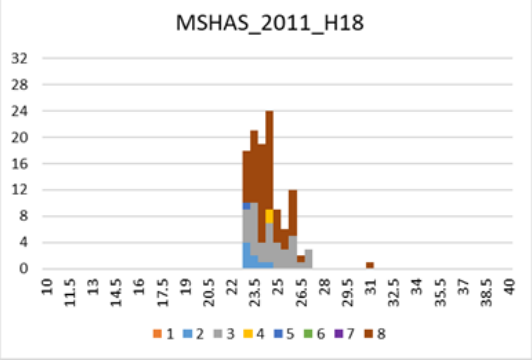
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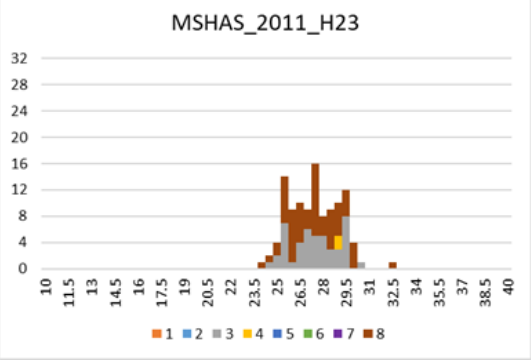
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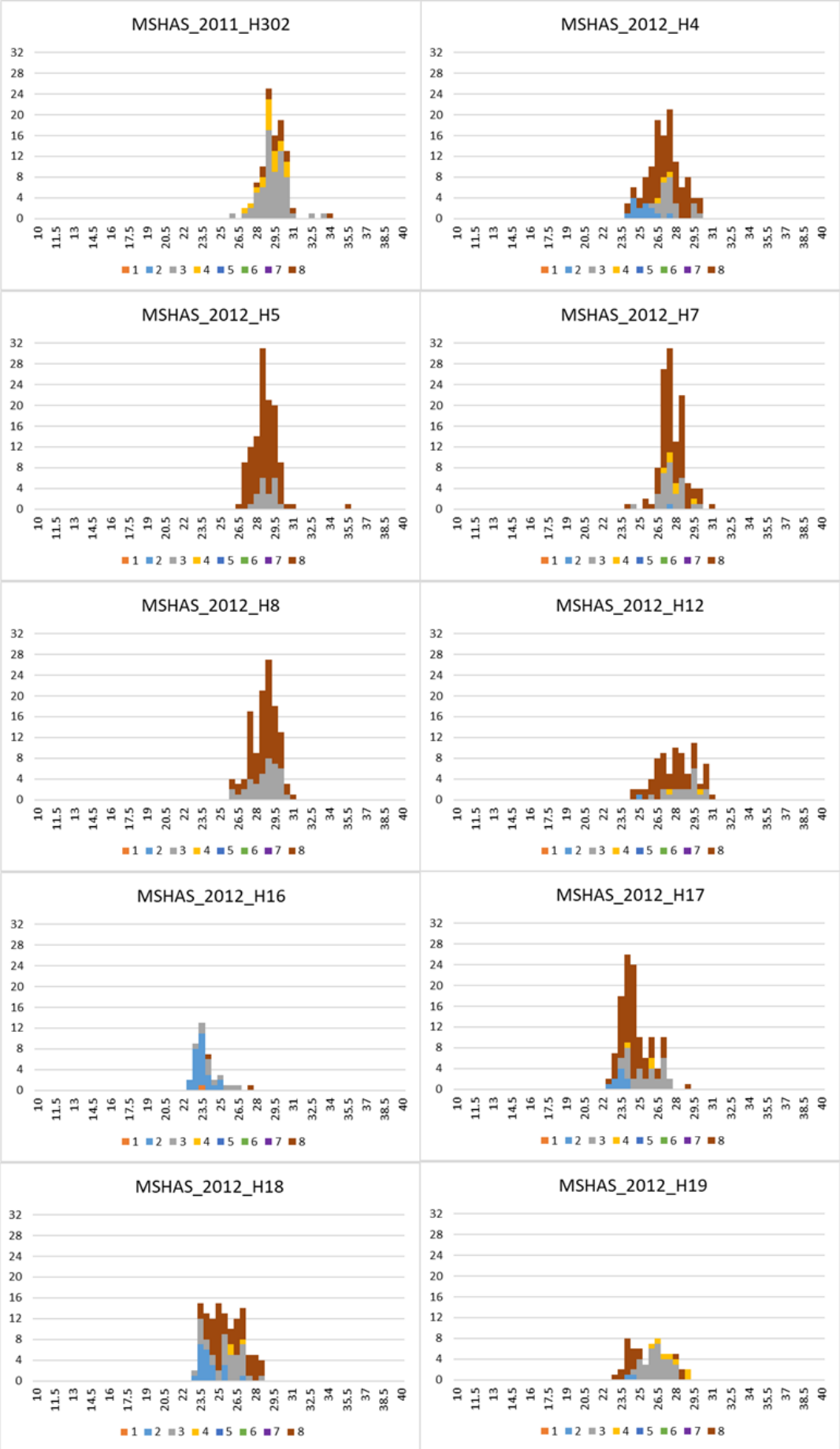
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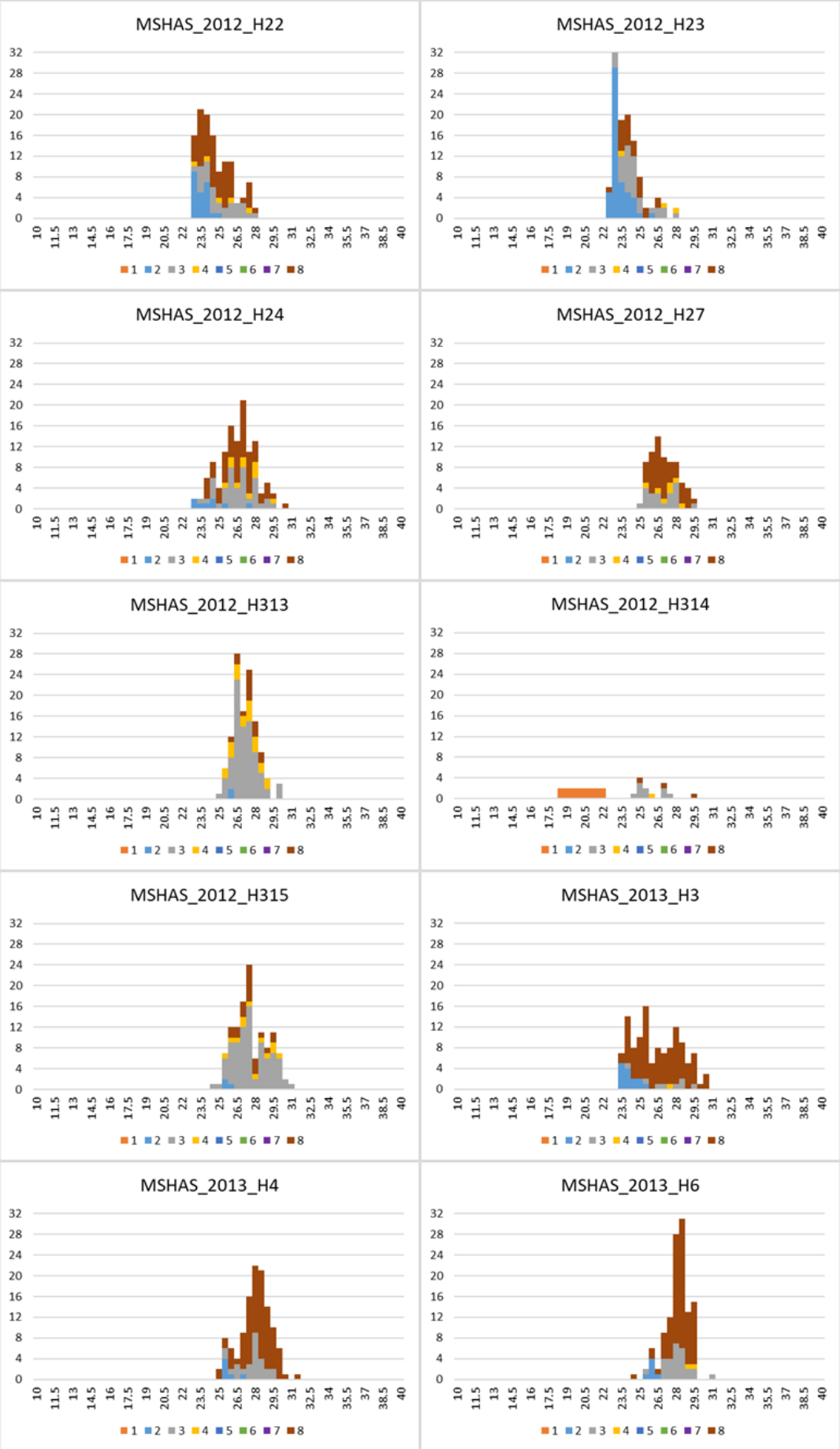
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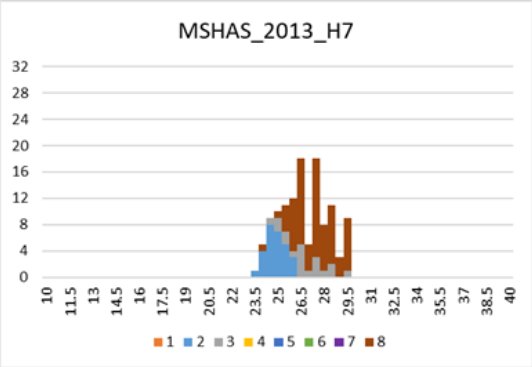
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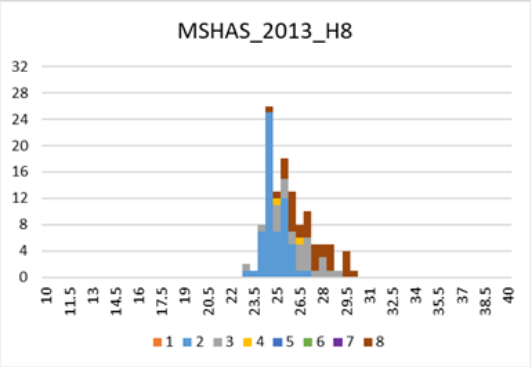
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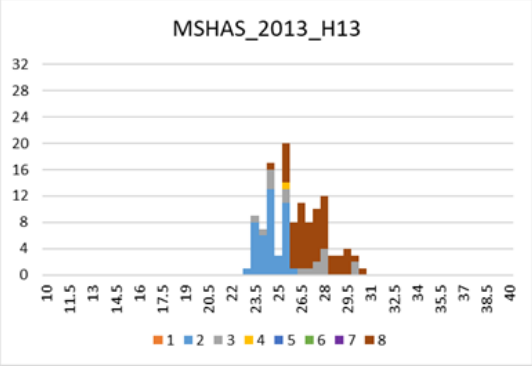
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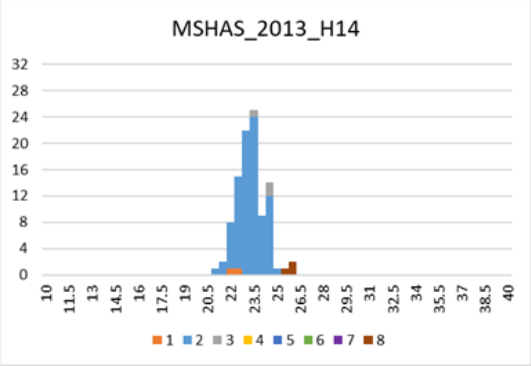
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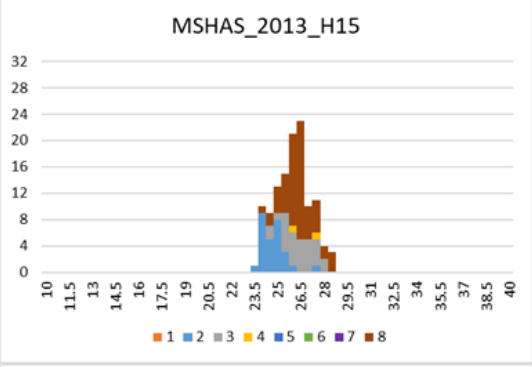
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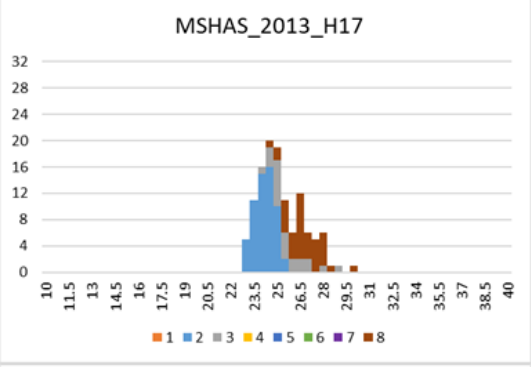
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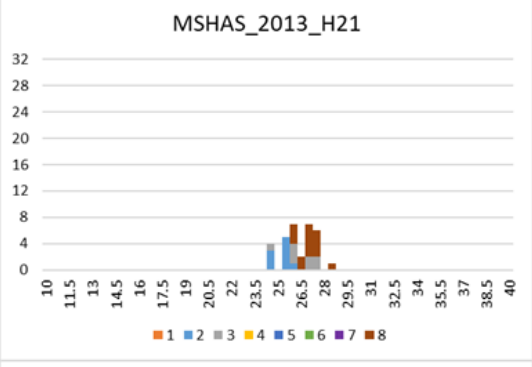
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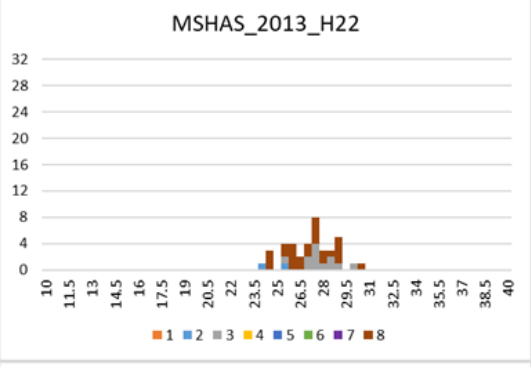
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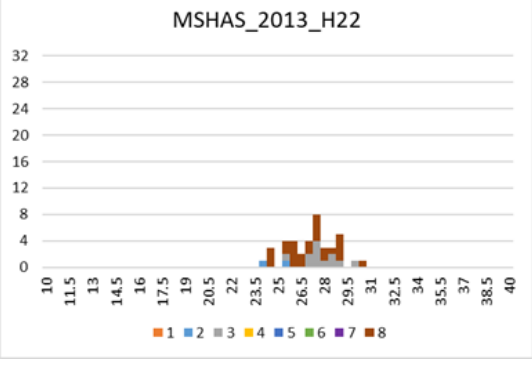
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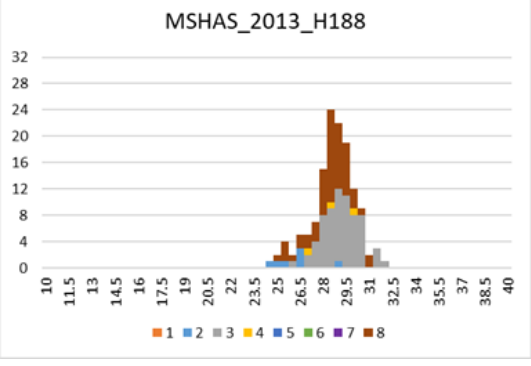
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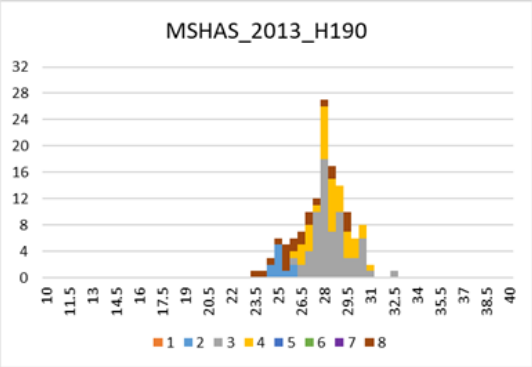
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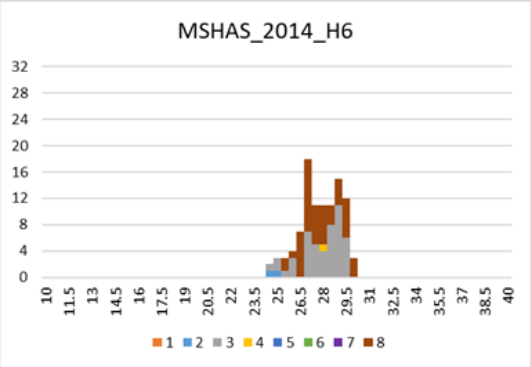
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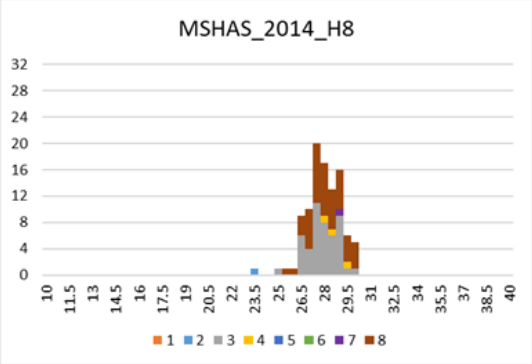
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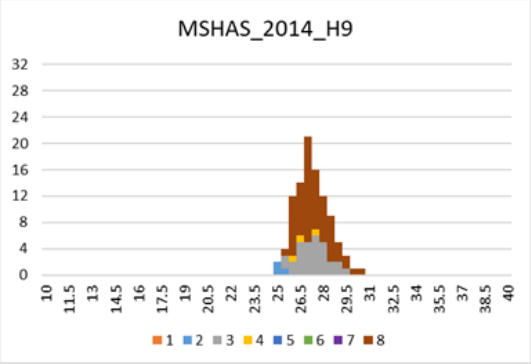
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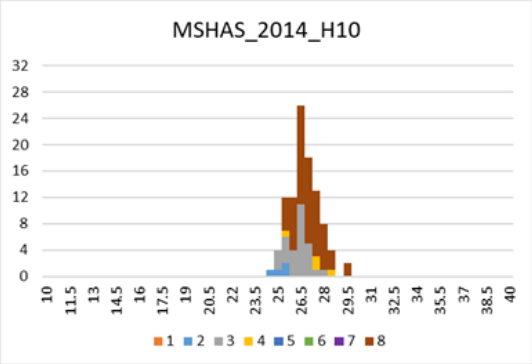
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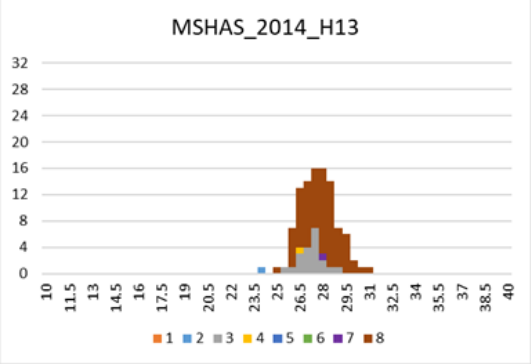
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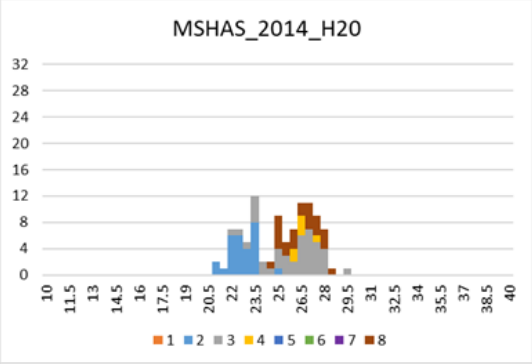
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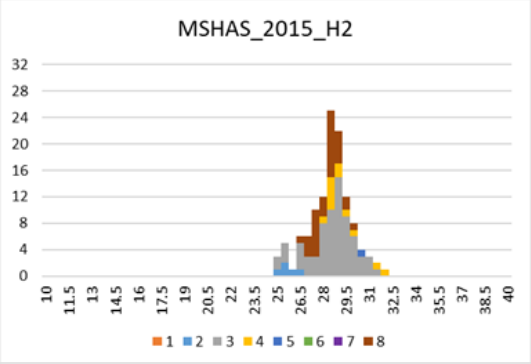
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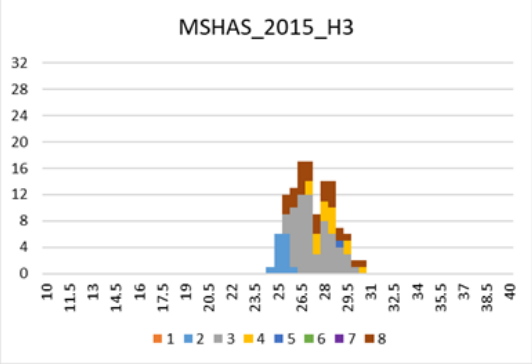
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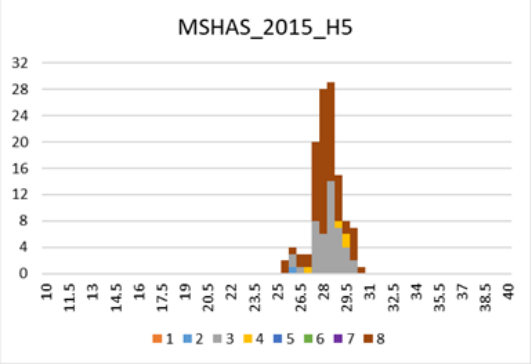
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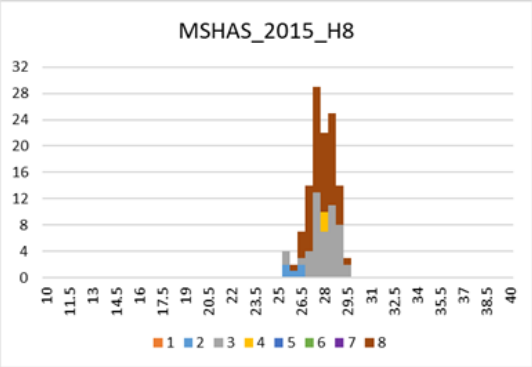
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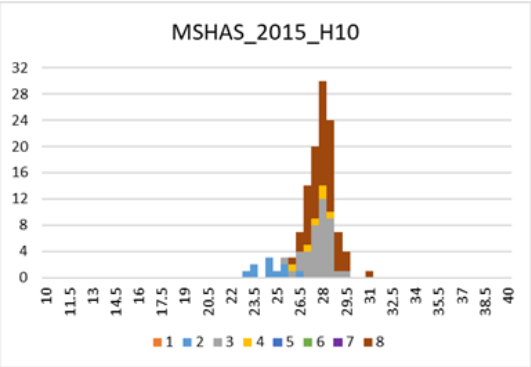
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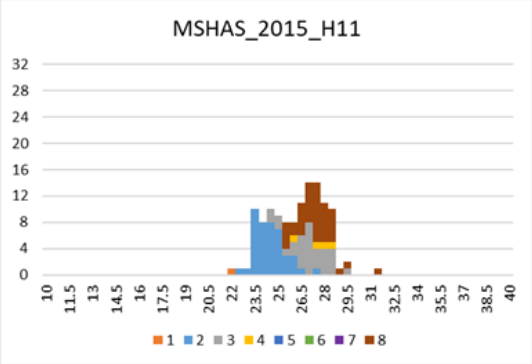
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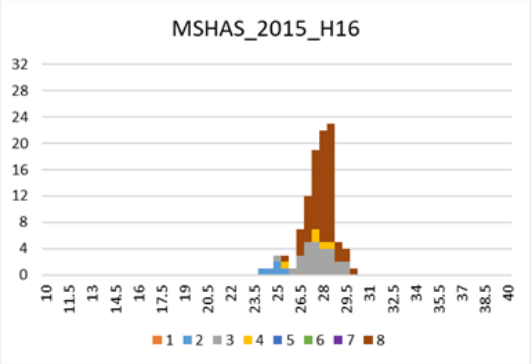
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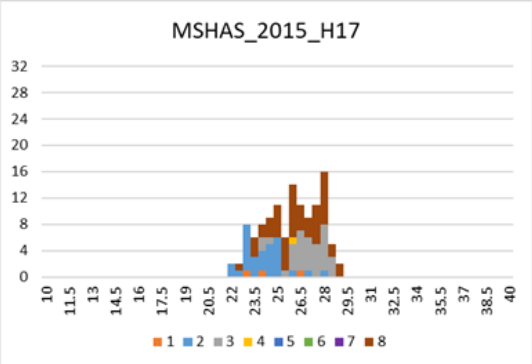
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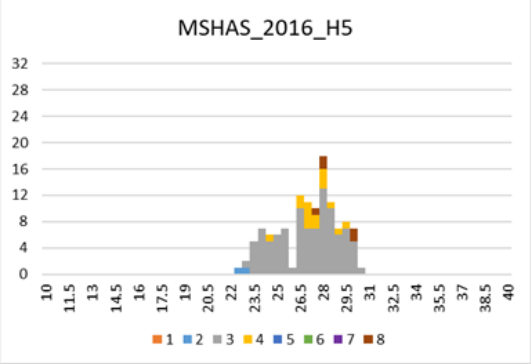
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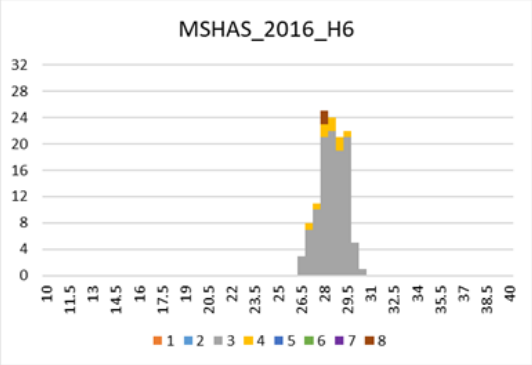
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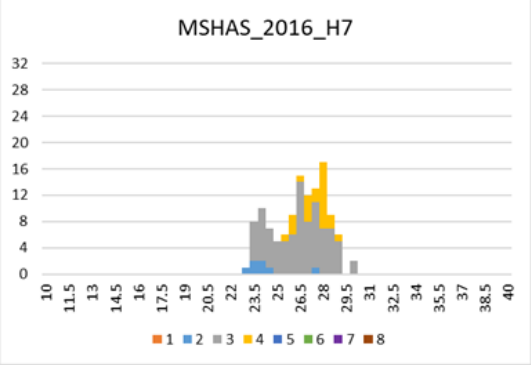
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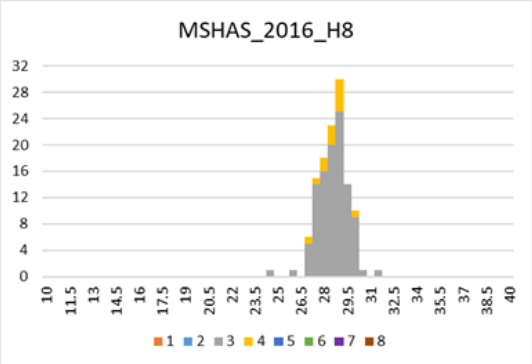
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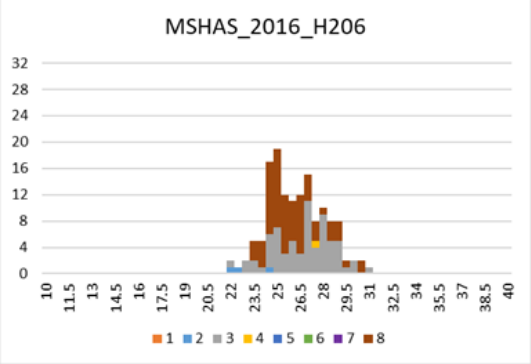
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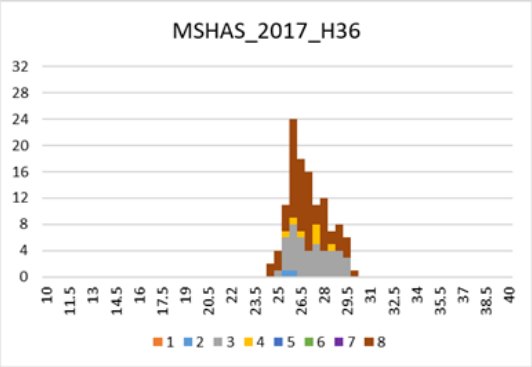
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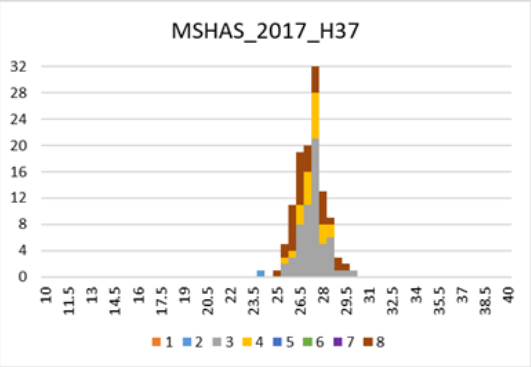
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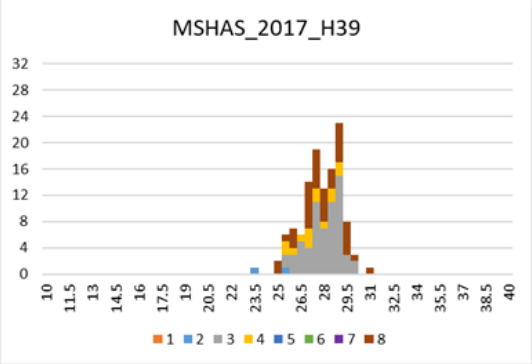
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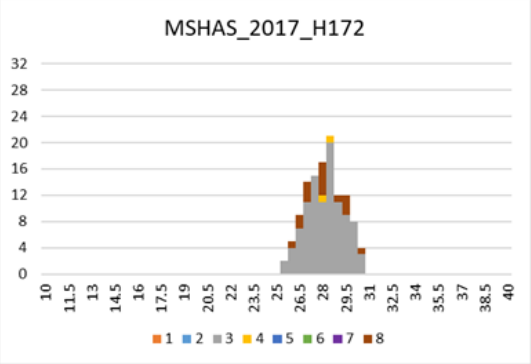
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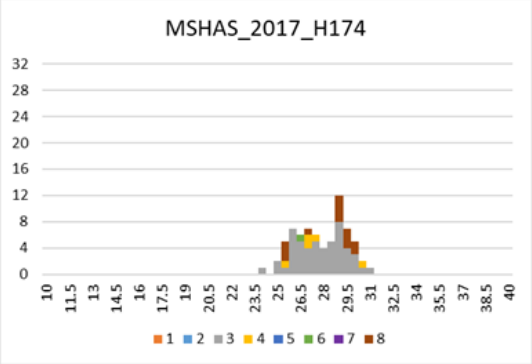
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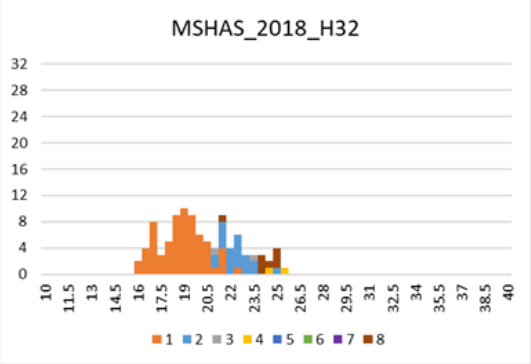
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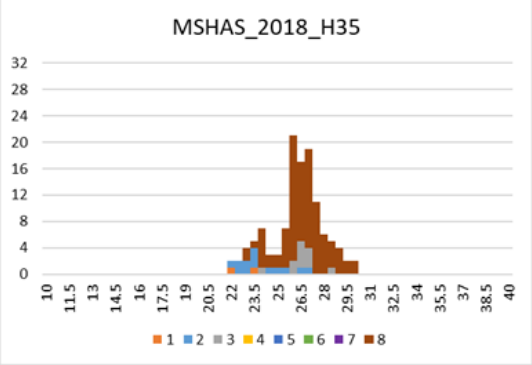
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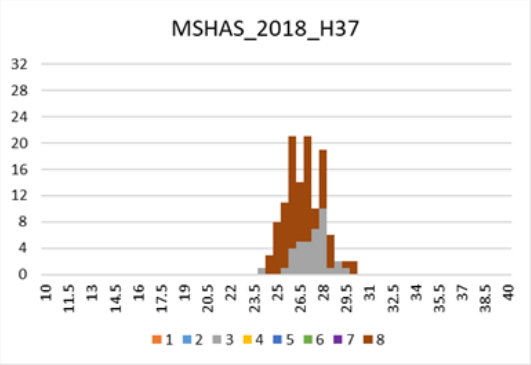
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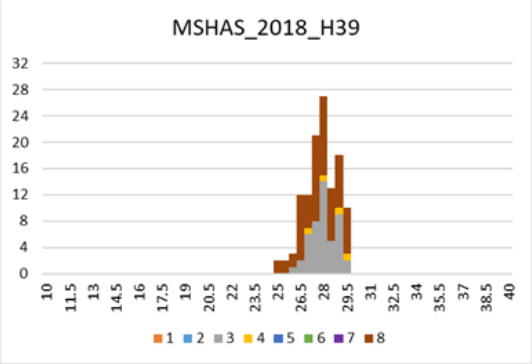
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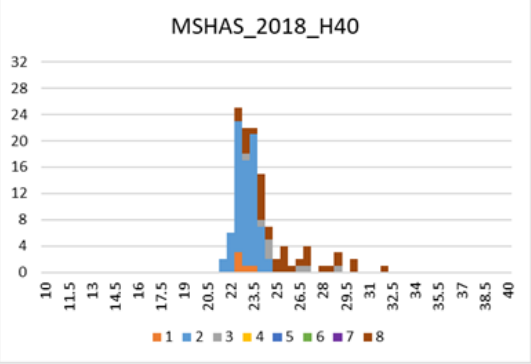
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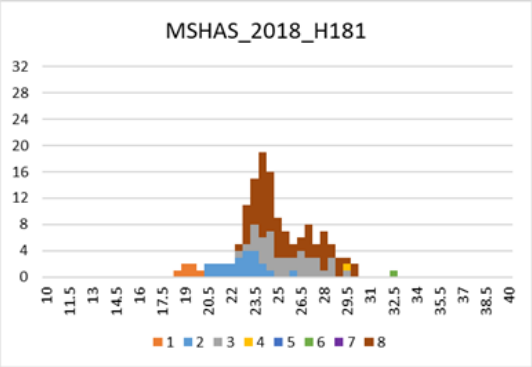
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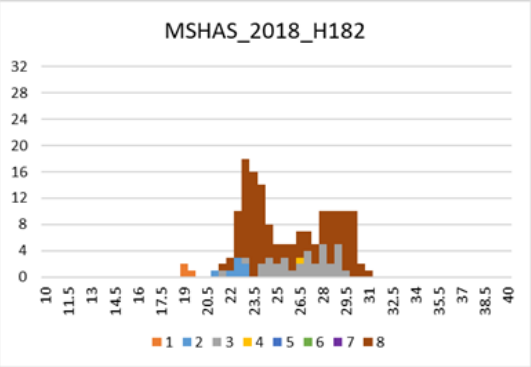
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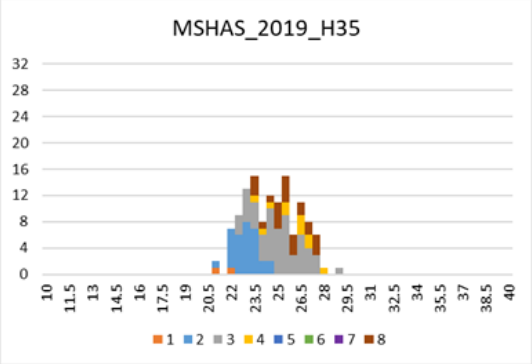
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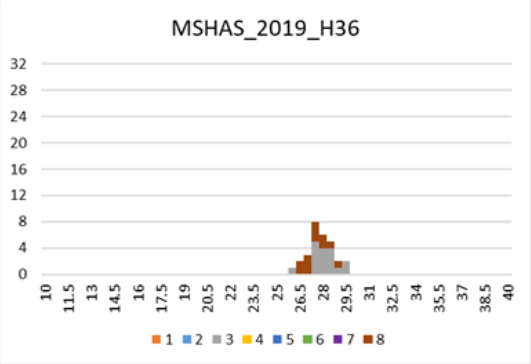
1 2 3 4 5 6 7 8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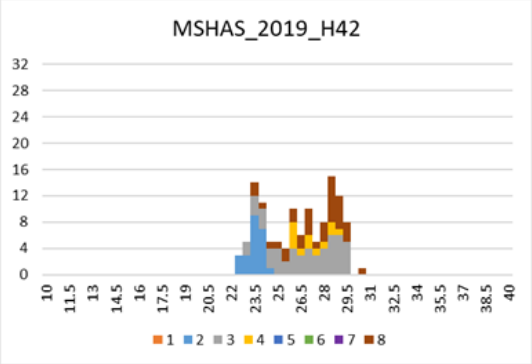
1 2 3 4 5 6 7 8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1 2 3 4 5 6 7 8

10.8. Annex 8 – MSHAS 2015 morphometric assignment probability plots

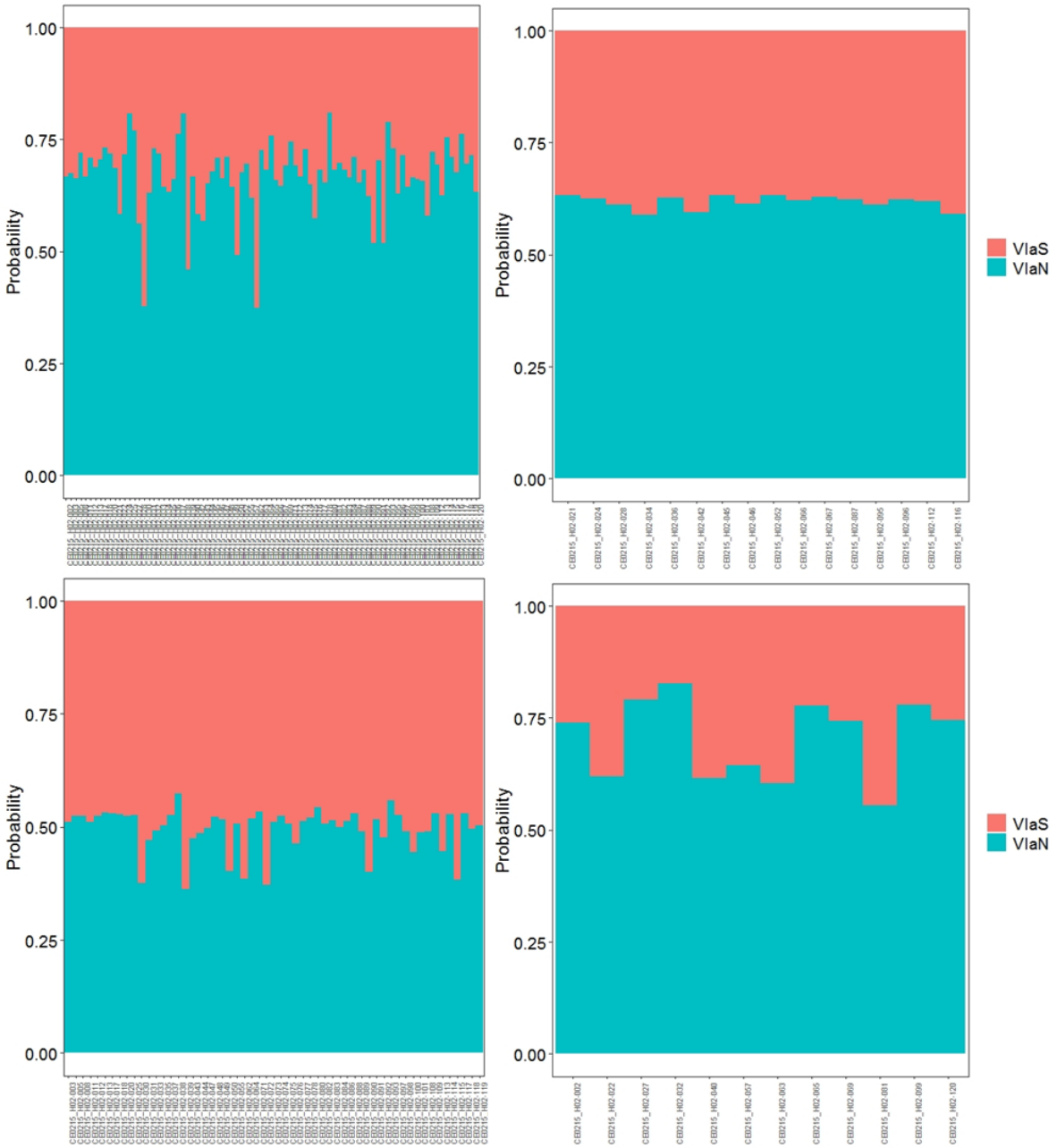


Figure 10.8.1. MSHAS 2015 Haul 2 membership probability plots for all ages (top left), age interval 1-3 years (top right), age interval 4-6 years (bottom left) and age interval 7+ years (bottom right).

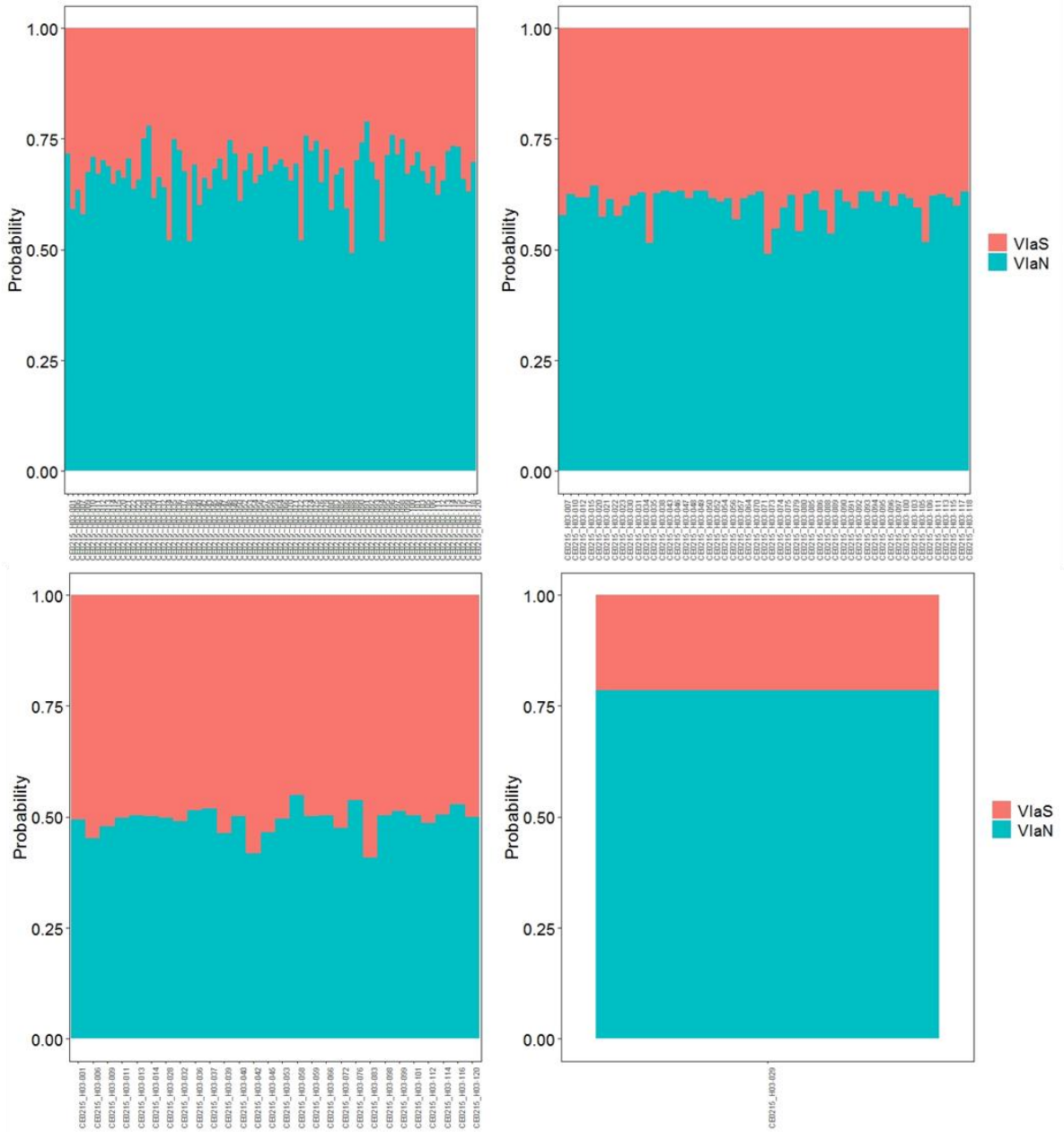


Figure 10.8.2. MSHAS 2015 Haul 3 membership probability plots for all ages (top left), age interval 1-3 years (top right), age interval 4-6 years (bottom left) and age interval 7+ years (bottom right).

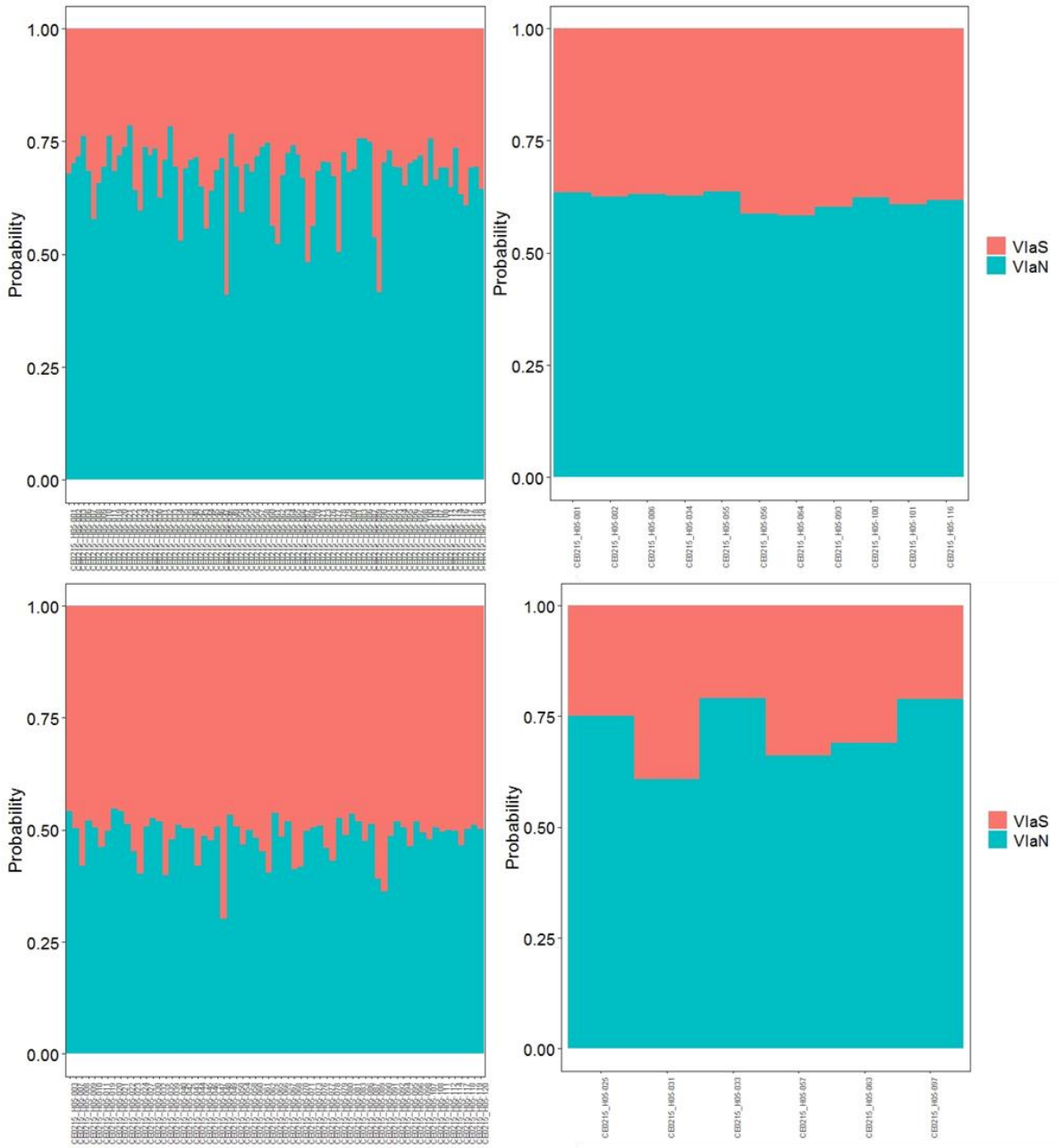


Figure 10.8.3. MSHAS 2015 Haul 5 membership probability plots for all ages (top left), age interval 1-3 years (top right), age interval 4-6 years (bottom left) and age interval 7+ years (bottom right).

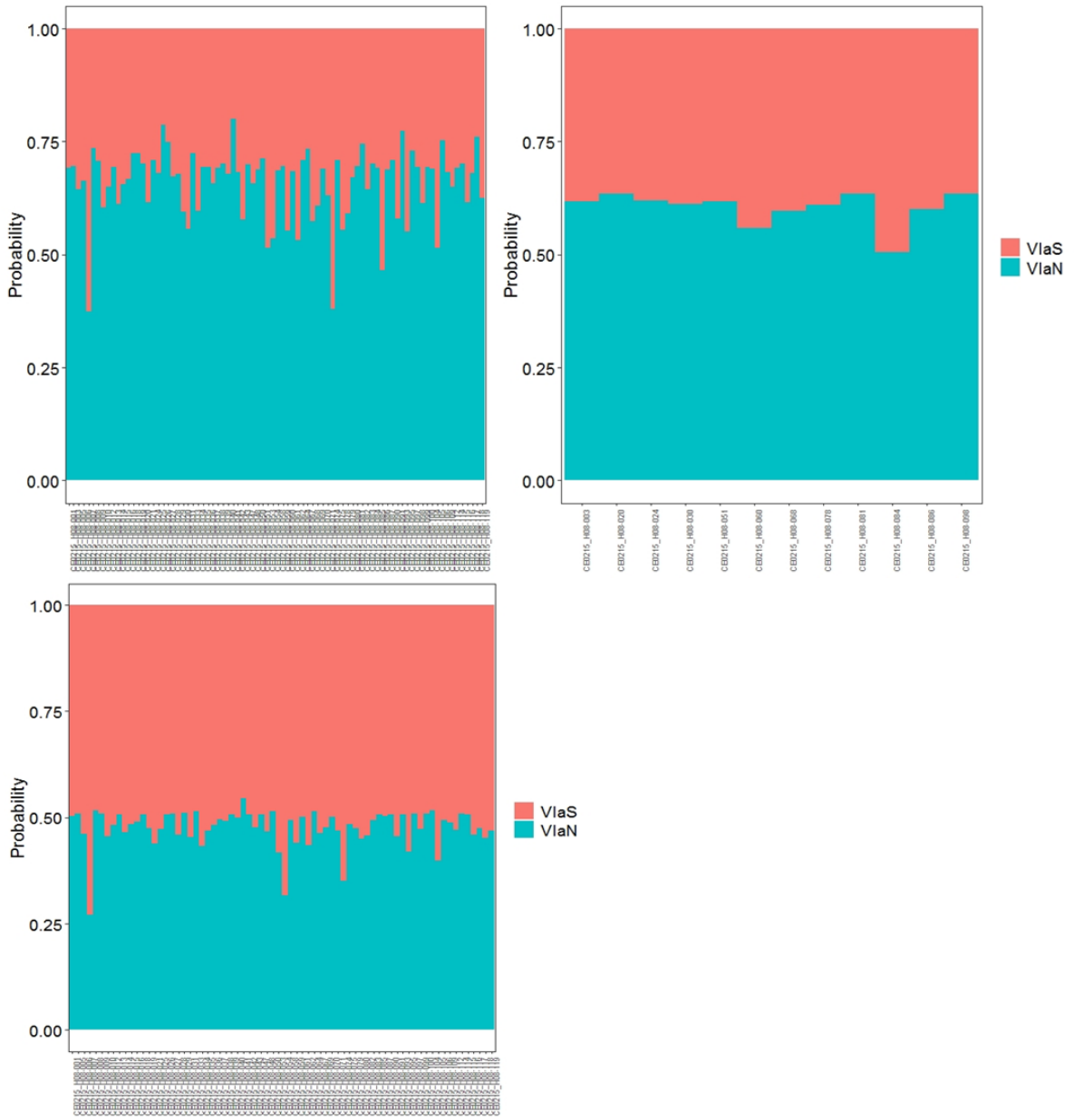


Figure 10.8.4. MSHAS 2015 Haul 8 membership probability plots for *all ages* (top left), *age interval 1-3 years* (top right) and *age interval 4-6 years* (bottom left). There were no *age interval 7+ years* in haul 8.

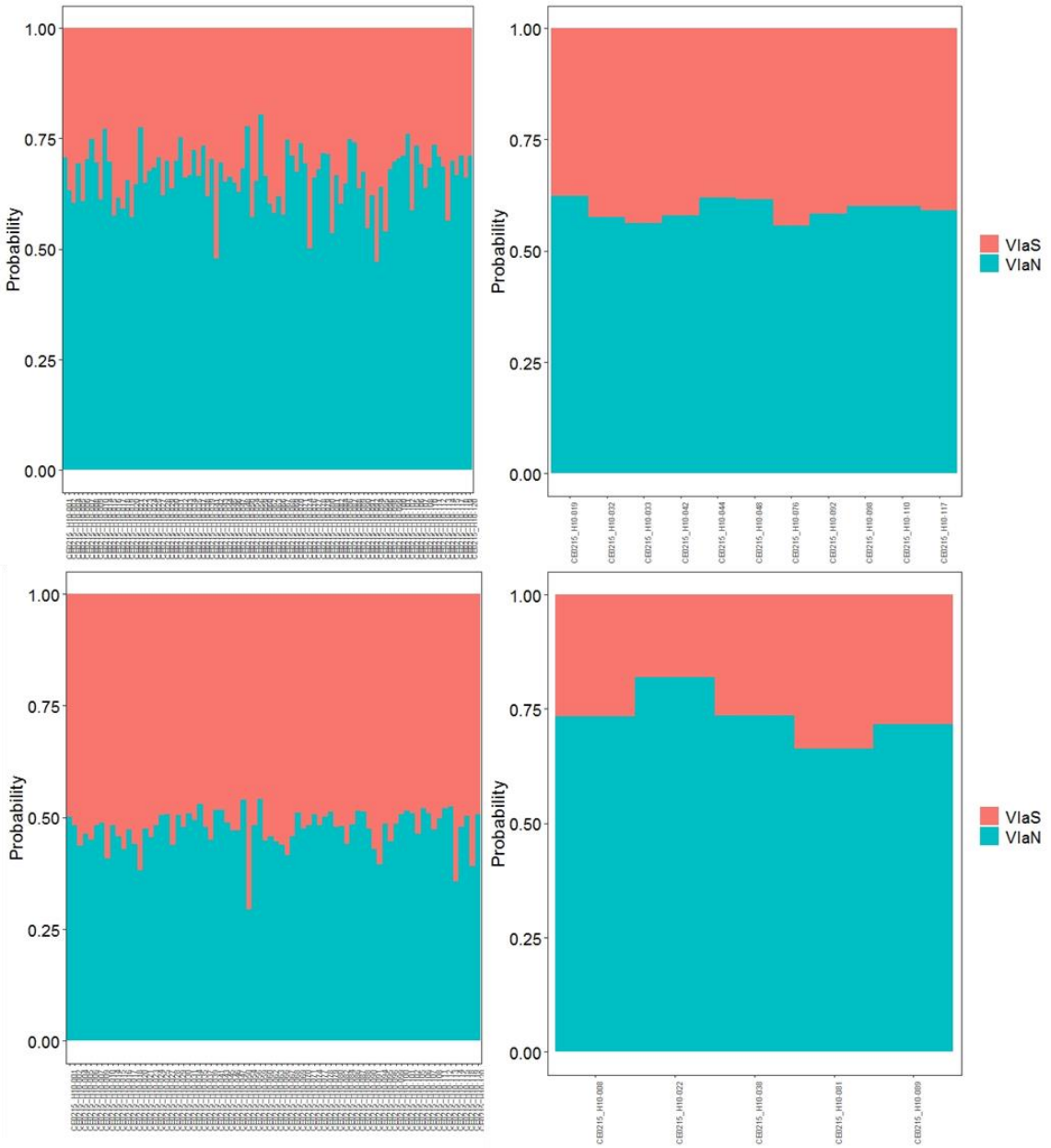


Figure 10.8.5. MSHAS 2015 Haul 10 membership probability plots for all ages (top left), age interval 1-3 years (top right), age interval 4-6 years (bottom left) and age interval 7+ years (bottom right).

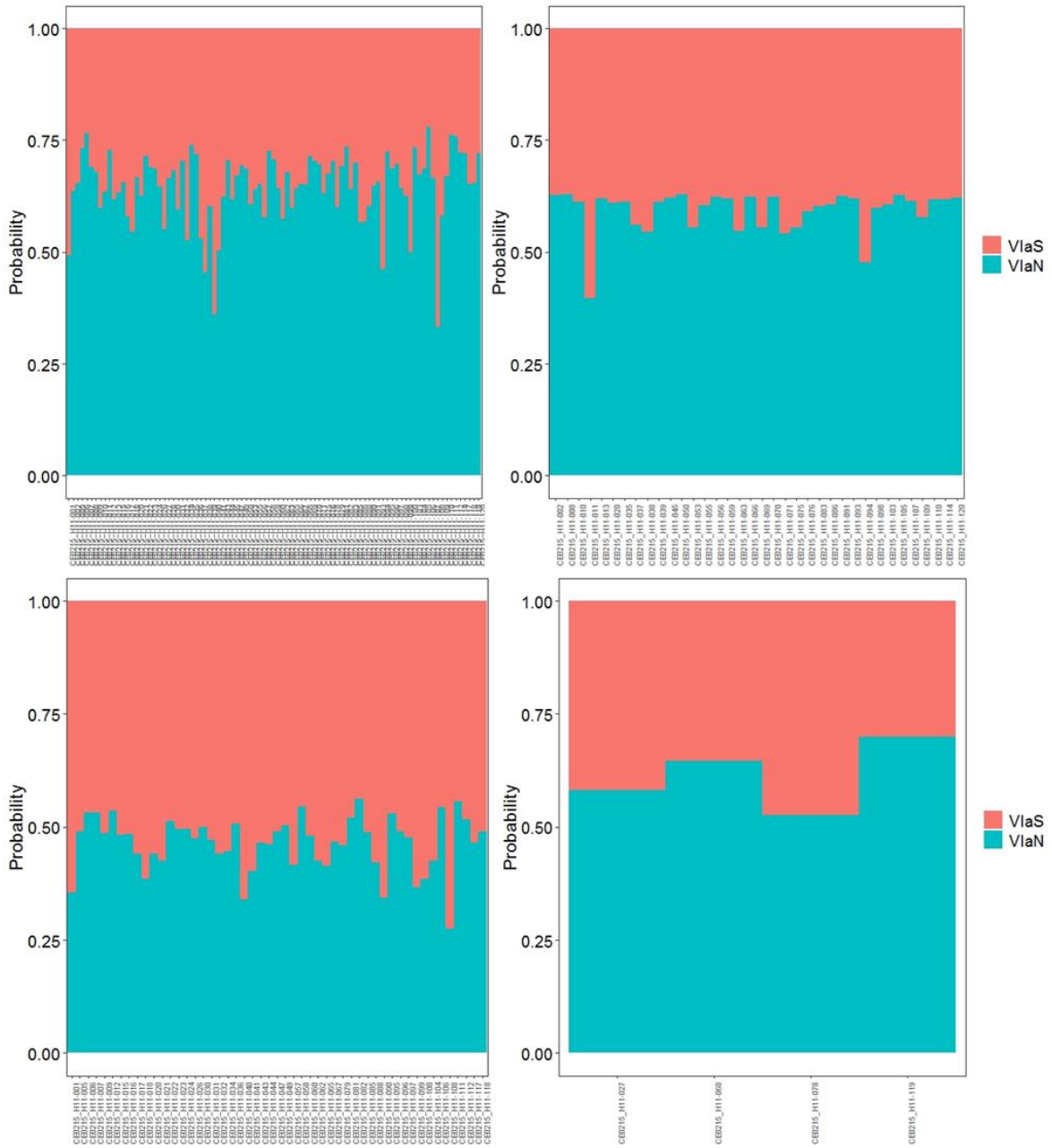


Figure 10.8.6. MSHAS 2015 Haul 11 membership probability plots for all ages (top left), age interval 1-3 years (top right), age interval 4-6 years (bottom left) and age interval 7+ years (bottom right).

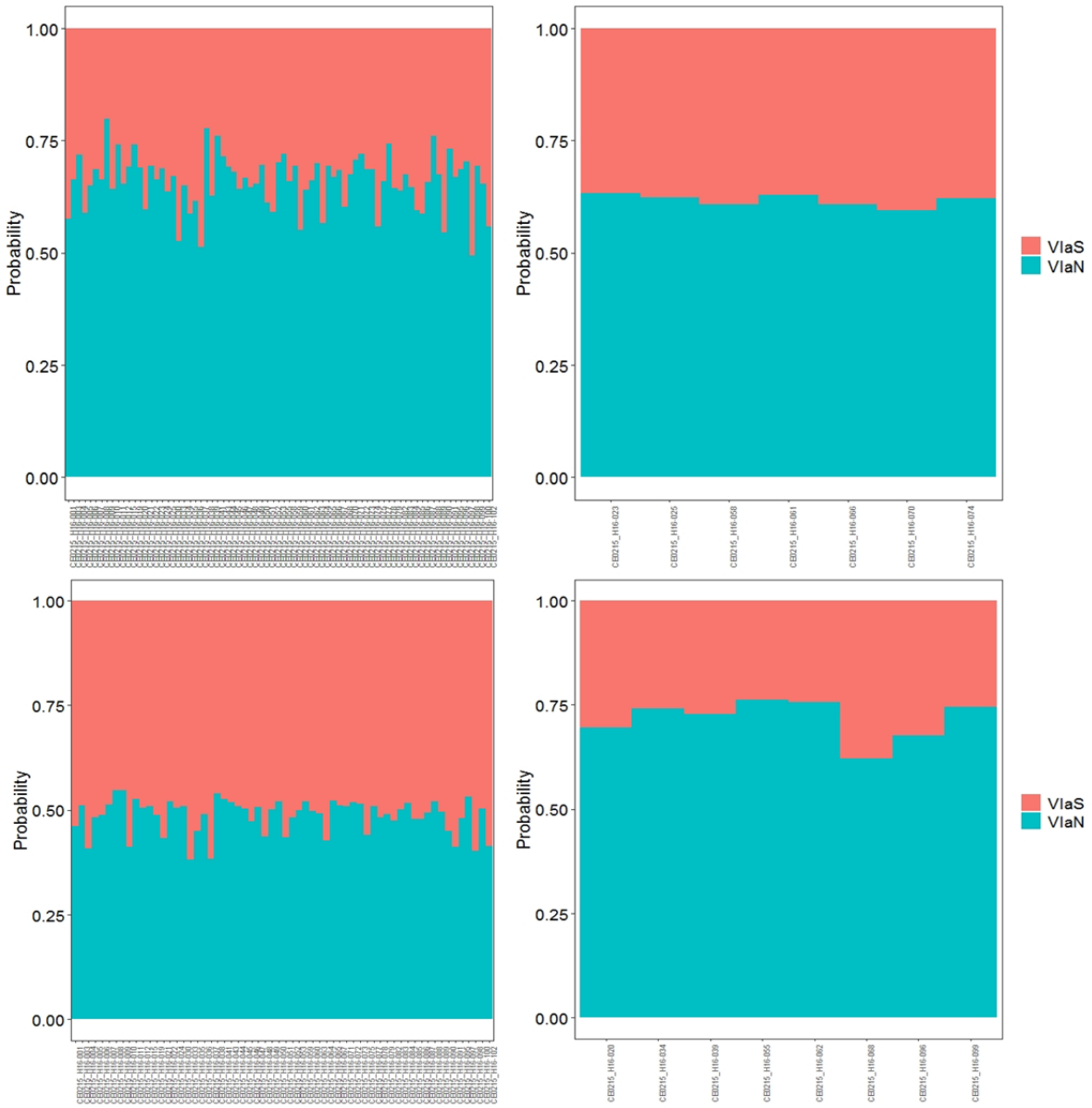


Figure 10.8.7. MSHAS 2015 Haul 16 membership probability plots for *all ages* (top left), *age interval 1-3 years* (top right), *age interval 4-6 years* (bottom left) and *age interval 7+ years* (bottom right).

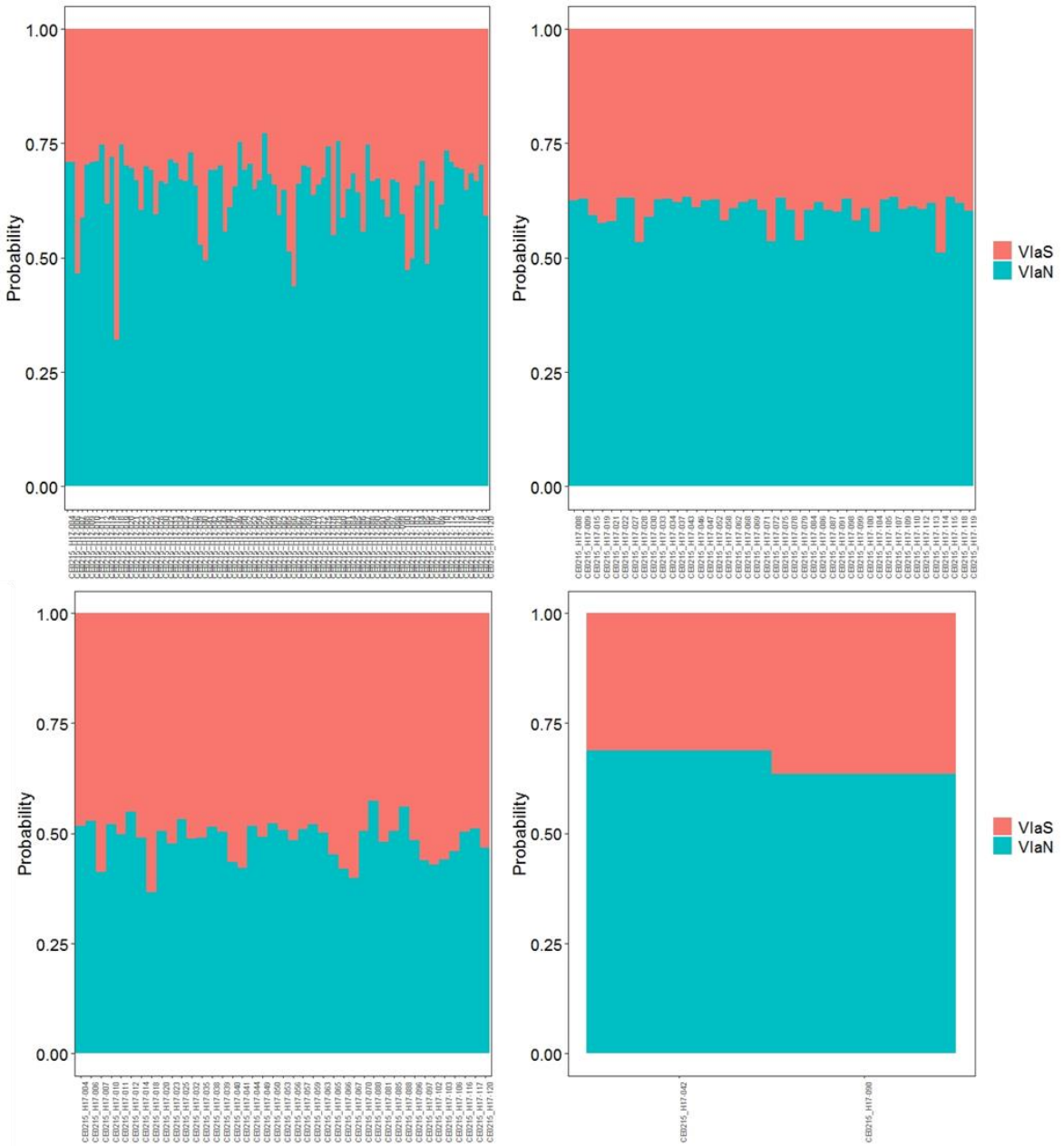


Figure 10.8.8. MSHAS 2015 Haul 17 membership probability plots for *all ages* (top left), *age interval 1-3 years* (top right), *age interval 4-6 years* (bottom left) and *age interval 7+ years* (bottom right).

10.9. Annex 9 – Body and otolith morphometric protocol

This is a detailed protocol for the capture of body morphometric images of herring used in the current project.

Body Morphometrics standard operating procedure (SOP)

Equipment used at the Marine Institute

- Kaiser Fototechnik Copylight System
- Nikon D7200 Digital SLR Camera
- Tamron lens
- Calibration mirror
- Nikon ViewNX-i software
- Nikon Camera Control Pro 2 software
- Cream coloured cardboard, approx. 20cm long
- Modelling pins with round plastic heads
- 30cm ruler
- Laminated gridded sheet

Once herring samples have been obtained from surveys or commercial samples, fish are sampled randomly from the haul. The ideal number of fish per sample is 100 so a sample of 120 fish is collected to provide a buffer of 20 fish in case any of the individuals need to be rejected for any reason (i.e. broken or crystalline otoliths, bowed body in photographs). The photographs need to be taken of fresh fish and this is done as quickly as possible before rigor mortis sets in. It is important to have the copylight and camera system set up and ready before any fish are caught.

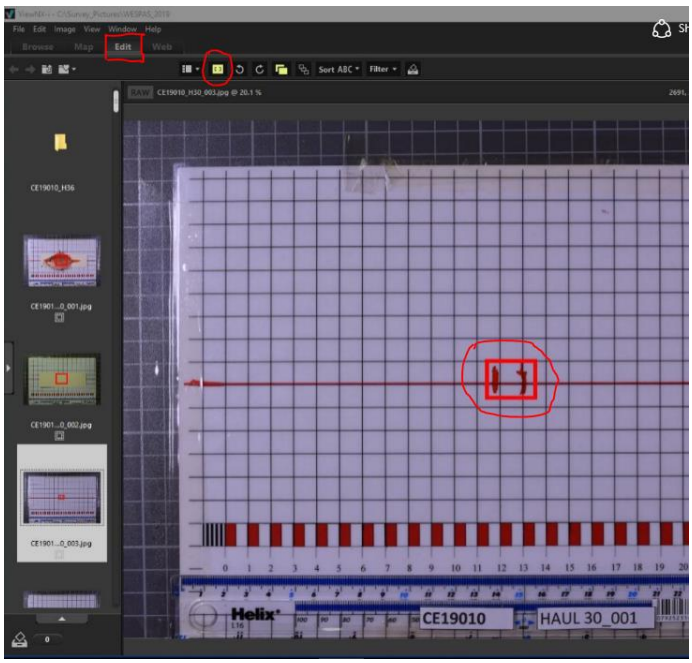
Camera and copylight setup

- Use bolts to secure the copylight system base to the countertop.
- Lights are attached to the base of the copylight system at the back and angled to avoid glare.

- The camera is attached to the movable part of the system which allows for height adjustments.
- Adjust the camera height to approximately 25inches/64.5cm.
- The height of the camera will determine the maximum size of fish that will fit within the frame.

- Adjust the height in order to fit a 30+cm fish within the photograph.
- Place a mirror underneath the camera and look through the camera viewer to line up the camera's focal point with the middle of the mirror.

- On occasion, it is required to place a folded piece of paper between the base of the camera and where it is fixed to the copylight system, this may help to tilt the camera slightly in the desired direction.
- Once centred in the mirror, place a laminated, gridded sheet on the base and line the sheet up with the centred camera.
- The *ViewNX-i* programme allows the user to display a focus point at the centre of the picture once it has been taken. Click the function  , in the edit tab, to display a focus point on the image and use this to help align with the centre the sheet.



- Using a red marker, draw a focal point on the laminated sheet that lines up with the focal point in the camera viewer and in *ViewNX-i* once the picture has been taken.
- When the sheet is correctly positioned, secure the sheet to the base with tape and draw a straight red line across the sheet, passing through the centre point; this will guide the placement of the fish on the sheet.

- Place a ruler at the end of the sheet furthest away from you and secure it with tape.
- The camera is secured in an inverted position on the copylight system, so the ruler is to be placed 'upside-down'.
- Print the survey code on a small piece of paper and secure it to the ruler, e.g. CE19010.
- On the *Tamron* lens switch off the VC to stop the lens from trying to stabilise the motion of the boat.
- Switch the lens to AF (Autofocus).
- Open *Camera Control Pro 2* and in the first tab 'Exposure 1'; adjust the Aperture setting to f/5.6.
- Turn off the flash on the camera.
- Adjust the dim switches of the lights to give the best illumination, without glare.
- Once the light is set at the right intensity, it is preferable to switch off at the socket when the system is not in use so no further adjustment will be required.
- In *Camera Control Pro 2*, go to 'Tools'; 'Transfer Options'.
- Browse for the 'Destination folder'; click Edit under 'File name to be used'; a 'File Naming' window will appear; under 'Prefix' input the Cruise Name_Haul Number; reset the 'Start numbering at:' box to 1; click OK and click OK.

Pinning Fish

- Herring are placed on a piece of cardboard, approx. 20cm long with the cream-colour side up.
- Pins are placed in the dorsal fin and pelvic fin to make sure they stand out.
- A third pin may be required to keep the mouth closed.
- Do not pierce the pin through to the other side of the card because the card will not lie flat and this will distort the photographs.

Photographing the fish

- The pinned fish have been placed in trays and are ready to be photographed, from now on the fish will be given a unique identification number.
- Place the haul number and fish number on the ruler so that it is included in the photograph, e.g. Haul 41_001.
- Place the pinned fish on the laminated sheet.
- Line the mouth and the middle of the tail up with the red line across the centre of the laminated sheet.
- Re-secure the pins on the dorsal fin and pelvic fin if necessary.
- Ensure no scales are obstructing the eye of the fish.
- Ensure the outline of the operculum can be seen.
- Pull the anal fin away from the body just before taking the picture to ensure the landmarks are clear, use a pin here if necessary.
- As time goes on, the mouths of the fish may begin to open, if this is the case, place a pin through the mouth to close it.
- When the position of the fish is correct, click 'AF and Start' in the *Camera Control Pro 2* programme.
- The picture will appear in *ViewNX-i* so you can check the quality.
- Once the photograph has been captured, remove the pins and the card from the fish, put the piece of paper with the haul number and fish number on the body of the fish and place the fish back in the tray for further processing.

Otolith Processing for Shape Analysis

Equipment used at the Marine Institute

- Microscope: Olympus SZ12, with Olympus 1 x DF Plapo lens and built in transmission light
- Camera: Canon mounted
- Microscope Camera Software: Toupview

Note: When extracting otoliths from herring, ensure the otoliths are cleaned as soon as possible. Residual tissue distorts the shape of the otolith and it is harder to remove once it is dried into the otolith.

Microscope settings

Magnification is set to 20x objective. Previously, transmission light was used and set to the maximum. This resulted in a dark otolith on a bright background. A new image analysis software specific to otoliths, *ShapeR*, is now being used to analyse the shape of the otoliths and it requires a white otolith on a black background. A microscope illumination unit is used to illuminate the otolith from above. Change the light settings accordingly so the otolith is not too bright.

ImageJ

Alternatively, the image analysis software, *ImageJ*, can be used to invert high quality otolith images to give a bright otolith on a dark background; open the image required for editing in *ImageJ*, select edit and invert. Alter the brightness of the image as required. Save image before closing. In order to invert a whole file of images; select Process -> Batch -> Macro. A 'Batch Process' pop-up box will appear, enter the location of the original images in the 'Input' box. The 'Output' box should contain the location of the file for storing the inverted images. Specify the 'Output format' to .jpeg from the drop-down list, select Invert from the 'Add Macro code' list and select 'Process' to batch invert your images.

Toupview settings

- Open the programme *Toupview*, navigate to Options -> Preferences, enter the file location and desired file name e.g. "IRL2018_Haul 1_", dictate the number format, e.g. "0000", and click OK.
- A photo of a graticule is taken at the start of each haul, after an adjustment has been made to the microscope or when the camera has been left unattended for a period of time. Once the graticule is in focus and use the save-as button, a pop-up box will appear, label the picture, e.g. IRL2018_Haul1_Graticule and save.
- Select the right otolith where possible. In the absence of the right otolith, the left otolith can be used and the orientation of the photograph changed using *ImageJ*. The otoliths are steeped in water to remove any residual tissue. If otoliths are particularly dirty, 70% alcohol can be used to loosen the dirt. Rub the otoliths between your fingers gently to remove any dirt and to dry the otolith before placing it on a slide with a black background. Orientate the otolith with the rostrum on top, facing to the left and in the centre of the frame (see figure below).

Right otolith orientated with the rostrum on top pointing to the left.

- Once the otolith's position is correct and the edges are clear save the image as above. Ensure the correct otolith sequence number, e.g. IRL2018_Haul5_0001, is entered in the pop-up box before saving.

Rotate image using ImageJ
- Open image with ImageJ, go to the tabs at the top and select Image -> Transform -> Flip Horizontally. Image should be orientated as shown in the figure above.

Resizing images
If the images are too large, *shapeR* will take a lot of time to outline all of the large images. Use the software *Gimp* to resize the images in a batch. An add-in to *Gimp* is required for batch processing

- Go to http://members.ozemail.com.au/~hodsond/dbp.html and click on the link for one of the versions.
- Download the dbp.exe file and copy it to Program Files/GIMP-2.0/lib/gimp/2.0/plug-ins.
- Open Gimp, go to the tabs at the top and click on Filters -> Batch Process…
- A pop-up box will appear called David's Batch Processor, use the input tab to add files for resizing.
- In the Resize tab tick the Enable box and move the tabs for the X and Y Scales to the desired setting – 0.6 was used for the current study.
- Use the Rename tab to select an output folder and the Output tab to choose .jpeg as the output type.
- In the Processing section of the pop-up box, click start.

10.10. Annex 10 - Presentations made to ICES and Pelagic Advisory Council

Copies of the presentations made to the Pelagic Advisory Council and detailed minutes of the resulting discussions are available through the PelAC website (https://www.pelagic-ac.org/).

- Farrell, E.D. and Carlsson, J. 2018. *Genetic stock identification of 6a Herring, Expanded Baseline Stage 2, Update*. 6a Herring Focus Group, Pelagic Advisory Council, WTC, Schiphol, 7th February 2018.

- Farrell, E.D. and Carlsson, J. 2018. *Genetic stock identification of 6a Herring, Update*. Pelagic Advisory Council, Working Group II, Den Haag, 2nd October 2018.

- Farrell, E.D. and Carlsson, J. 2019. *Genetic stock identification of 6a/7bc Herring, current status and future application*. 6a Herring Focus Group, Pelagic Advisory Council, 27th February 2019.

- Farrell, E.D. 2019. *Genetic stock identification of 6a/7bc Herring*. ICES HAWG, via Webex, 19th March 2019.

- Gras, M and White, E. 2019. *Herring stock Identification: Morphometrics*. ICES HAWG, 19th March 2019.

- Farrell, E.D. 2019. *Herring in Divisions 6.a, 7.b and 7.c: Scientific Assessment of the Identity of the Southern and Northern Stocks through Genetic and Morphometric Analysis*. Working Group II, Pelagic Advisory Council, Lisbon, Portugal, 10th July 2019.

- Farrell, E.D. 2019. *Genetic stock identifcation of 6a/7bc herring*. 6a Herring Focus Group, Pelagic Advisory Council, via webex, 28th September 2020.

- Farrell, E.D. 2019. *Herring in Divisions 6.a, 7.b and 7.c: Scientific Assessment of the Identity of the Southern and Northern Stocks through Genetic and Morphometric Analysis, Update*. Working Group II, Pelagic Advisory Council, via webex, 7th October 2020.

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EA-09-21-113-EN-N

doi:10.2826/208498
ISBN 978-92-9460-583-2

Publications Office
of the European Union