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Summary

There are several populations of herring (*Clupea harengus*) in the northeast Atlantic. Around the Shetland Islands, North Sea autumn-spawning herring are present, for which there is a valuable commercial fishery. Those herring undergo sexual maturation between March and August, ready to spawn in September. In recent years there have been observations in Shetland waters of herring undergoing maturation in December, which could potentially be spring-spawning herring (maturing between May and January, to spawn in February/March). The presence of these fish is of interest to both science and industry, with the possibility of a stock distribution which is currently unknown and unaccounted for, and which could have implications for stock management.

To provide information on the identity of herring in Shetland waters, this study worked with local fishers to collect specimens of herring for biological sampling and analyses. Between December 2020 and April 2021, and January 2022 and March 2022, 656 herring were collected by local fishers for sampling. Length, sex and maturity stage were recorded for all fish, with a tissue sample taken for genetic analysis, and a gonad sample taken for histological examination (providing microscopic maturity stage).

The genetic analysis showed a baseline sample of the Shetland collected herring to be distinct from other herring populations of 6.a.S; North Sea and 6.a.N autumn spawning; 6.a.N spring spawning; and Downs winter spawning herring. And while the Shetland baseline herring were shown to be different, there was indication from the genetic analysis that these fish were most similar to spring spawning 6.a.N fish. These results provide evidence of spring spawning herring in Shetland waters; with the potential for a previously unidentified Shetland spring spawning population component (in the context of this study and the available data). However, the identity and origin of this population is not known, and Norwegian or Western Baltic spring spawning herring cannot be discounted as the source. Across all the herring samples collected in Shetland, there was evidence of the presence and potential mixing of multiple herring population components (including autumn spawning North Sea and 6.a.N, and spring spawning 6.a.N and the potential Shetland component), with overlap likely occurring at various stages of spawning, feeding and over-wintering.

The application of maturity stage classification alongside genetic assignment further substantiated the genetic results. Using gonad samples to identify the maturity stage of fish caught between December and March, herring were classified as either autumn spawners (if immature, spent, recovering/resting) or spring spawners (if early maturing, maturing, spawning prepared and spawning). The majority of fish classified by maturity stage as spring spawners were genetically assigned as the potential Shetland component, while the majority of fish classified by maturity stage as autumn spawners were assigned as North Sea autumn spawners.

The study had some limitations. No genetic baseline data were available for Norwegian or Western Baltic spring spawning herring. Owing to the small sample sizes involved and limits to the genetic analyses, the findings are intended as exploratory indicators to genetic identification only. Nonetheless, this study has gathered valuable new information to provide insight into the identity of herring populations around Shetland, with clear evidence of autumn spawning and spring spawning herring. New connections with other academic institutions and staff working on similar research have been established, with further collaboration planned (e.g. genetic comparisons with other herring populations such as Norwegian and Western Baltic spring spawning), to provide more detailed information and to contribute information to a wider body of work.

1.0 Introduction

Herring is one of the most important pelagic species in the North Atlantic, with a history of exploitation which goes back centuries (ICES 2022). In the Northeast Atlantic, herring are widely distributed from the northern Bay of Biscay to Greenland, and east into the Barents Sea (ICES 2011; 2022). Herring comprise a number of different 'races' or populations (**Table 1, Figure 1**), varying in spawning characteristics e.g. autumn and spring spawners, and morphometric features e.g. number of vertebrae (ICES 2011; 2022). Herring undertake migrations between feeding, spawning and over-wintering areas, the differences between which characterise different populations (Cushing 1967) (**Figure 2**). Herring stocks with distinct patterns of growth, maturation and spawning come together to mix on common feeding grounds, making it difficult to separate stock components within a single catch, with implications for stock assessment (ICES 2011). Work has been carried out to distinguish different herring populations, using a number of different methods, including genetics, body morphometrics, otolith shape and chemistry analyses, tagging, parasite analysis and life-history analyses (see Farrell *et al*. 2021 for a review).

Table 1. Herring stocks in the Northeast Atlantic

Herring Stock	Information and characteristics
ICES Subarea 4, Divisions 3.a and 7.d. North Sea autumn spawning herring (NSAS)	One of the largest, most abundant, well-studied stocks (Simmonds 2009; ICES 2011). Historically recognised as a complex of multiple spawning components, differentiated by distinct spawning times and locations but largely managed as a single unit: Shetland/Orkney, spawning in August/September around those islands; Buchan, spawning in August/September east of Scotland; Banks, spawning in August/September off the English coast in the central North Sea; and Downs, somewhat a misnomer of NSAS herring, spawning in December in the English Channel (Cushing 1955; Dickey-Collas *et al.* 2010; ICES 2011; Iles and Sinclair 1982; Simmonds 2009). Recent genetic analysis (Farrell *et al*. 2021) has provided information on the identification of herring stocks: Downs winter spawning herring have been shown to be highly differentiated from the North Sea autumn spawning herring, while a lack of genetic differentiation between North Sea autumn spawning herring and 6.a.N autumn spawning herring suggests a single population.
Division 6.a.N 6.a.N autumn spawning herring and 6.a.N spring spawning herring	Part of the Malin Shelf Stock Complex (ICES 2011). Historically recognised as an autumn spawning stock (late August to early October), with mixing of other populations e.g. components of neighbouring herring stocks to the south known to be present seasonally in Division 6.a.N (Farrell *et al.* 2021; ICES 2011). Recent genetic analysis (Farrell *et al*. 2021) indicated herring in 6.a comprise at least three distinct populations: 6.a.N autumn spawning, 6.a.N spring spawning, and 6.a.S.
Divisions 6.a.S, 7.b and 7.c	Part of the Malin Shelf Stock Complex (ICES 2011). 6.a.S herring are primarily a winter spawning population (November-January), though there is also a later spawning component (Farrell *et al.* 2021).

Divisions 7.a North of 52°30'N (Irish Sea)	Part of the Malin Shelf Stock Complex (ICES 2011). Spawns in Autumn (September/October) mainly on the Douglas Bank east of the Isle of Man. No evidence that these are part of the Malin shelf complex; previous 'evidence' shown to be incorrect (Farrell *et al.* 2021).
Divisions 7.a South of 52°30'N, 7.g, 7.h, 7.j and 7.k (Irish Sea, Celtic Sea, and South of Ireland)	Spawns in winter (November-January) off the south coast of Ireland (Farrell *et al.* 2021; Hay 2001). Celtic Sea and Irish Sea herring are distinct from each other and from 6.a (Farrell *et al.* 2021).
Norwegian spring-spawning herring (NSSH) Barents Sea (Divisions 2.a, 2.b, 5.a, 5.b and 14.a) and Norwegian Sea	Largest herring stock in the world, widely distributed and highly migratory throughout NE Atlantic (ICES 2011). Spawns in spring (February-April) along the Norwegian west coast (ICES 2011).

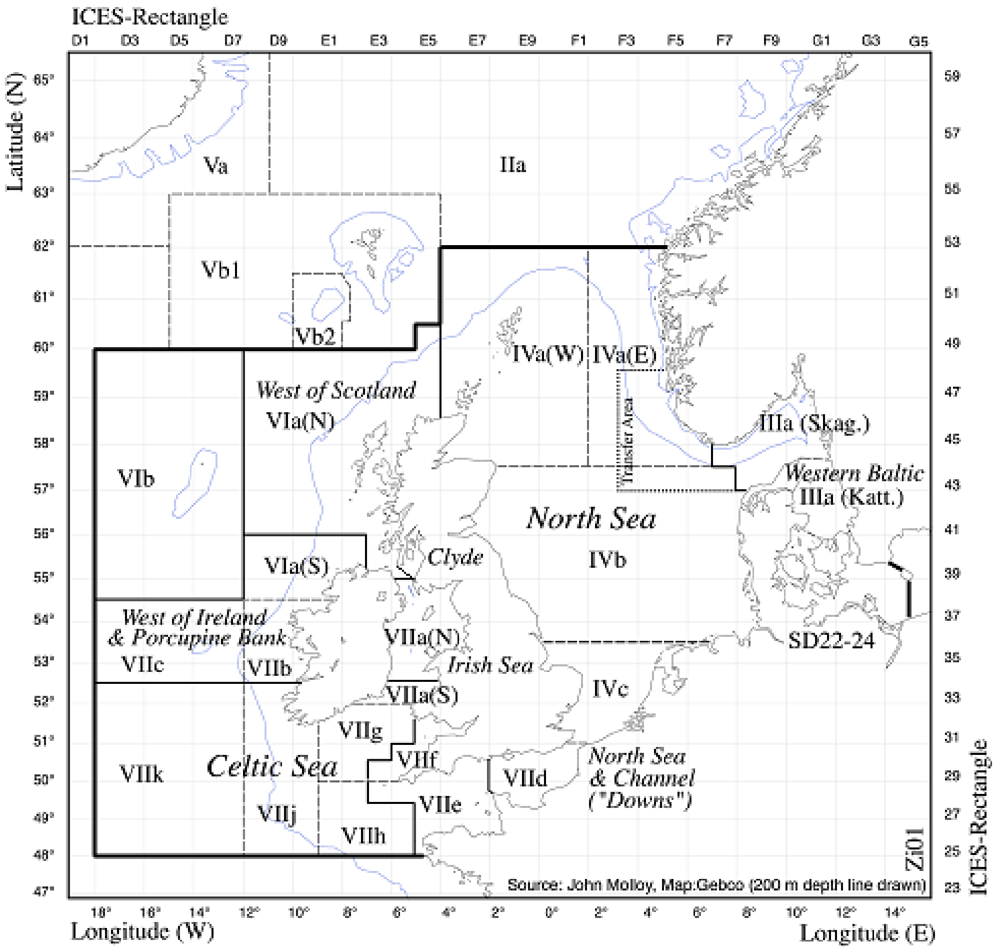

Figure 1. ICES areas used for the assessment of herring stocks south of 62°N (ICES HAWG). Area names in italics indicate the area separation applied to the commercial catch and sampling data kept in long term storage. "Transfer area" refers to assessment of the migratory western Baltic spring spawning herring (Rügen herring), where information about catches etc. in the North Sea are transferred to this stock. (Source: ICES 2011, p13).

Around the Shetland Islands, North Sea autumn-spawning (NSAS) herring are present, for which there is a valuable commercial fishery. Those herring undergo sexual maturation between March and August, ready to spawn in September (McPherson and Kjesbu 2012; dos Santos Schmidt *et al.* 2017). NSAS herring spawn in the north-western North Sea (Baxter 1959; Corton 2001; Hay *et al.* 2001), after which they move eastward to overwinter in deeper waters in the region of the Norwegian Trench off the southwest coast of Norway (Corton 2001; Hay *et al.* 2001). In spring (March-April), migration to the feeding grounds begins, with fish moving north along the Norwegian Trench, then west towards Shetland (Corton 2001). An intense period of feeding takes place into the summer (April-July) in the central and northern North Sea before fish return to spawning grounds (Corton 2001; Cushing and Burd 1957; Hay *et al.* 2001; dos Santos Schmidt *et al.* 2017; Zijlstra 1969). During summer feeding and overwintering, different spawning components of North Sea herring are known to mix (Hay *et al.* 2001). Herring of the southern Downs component will depart the feeding grounds to spawn in the eastern English Channel in December/January, before overwintering in the southern North Sea (Corton 2001).

While NSAS herring spawn before overwintering, Norwegian spring spawning (NSS) herring spawn afterwards. NSS herring mature between May and January, to spawn in February-April along the Norwegian coast, in an area between Lista (58°N) and Lofoten (68 °N) (Hay *et al.* 2001; Kurita *et al.* 2003; McPherson and Kjesbu 2012; dos Santos Schmidt *et al.* 2017; Slotte 1999a; b). Following spawning, fish feed in the Norwegian sea during the summer (May-August), before migrating to overwintering grounds off the northern coast of Norway (67-68 °N) in September (Kurita *et al.* 2003; Slotte 1999a). They remain in this area (without feeding) until January when they migrate back to the spawning grounds (Kurita *et al.* 2003).

Herring population structure is highly complex and it has been shown that different populations with distinct spatial and temporal patterns of spawning come together and mix during feeding and overwintering (Husebo *et al.* 2005; Iles and Sinclair 1982; Ruzzante *et al.* 2006). In the North Sea and Skagerrak (north-eastern parts of Division 4 and Division 3.a) mixing of NSAS herring and spring-spawning herring (NSS and Western Baltic) is known to occur (Clauson *et al.* 2015; Hay *et al.* 2001; ICES 2011). Around Shetland, there have been historic reports of a 'winter herring' fishery, and more recent fisher observations of maturing herring in December, sparking questions from the fishing industry over the potential for these fish to be spring spawners, rather than the typical NSAS herring known to be present. The identification of herring in Shetland is yet to be fully explored, and the potential presence of an unknown and unaccounted for spring spawning component requires further investigation, with correct population identification and discrimination fundamental to fisheries management and stock assessments (Begg *et al.* 1999; ICES 2011).

This aim of this study was to gather more information to determine the identity of spawning herring found in Shetland waters in winter. The research intended to a) consult with local fishers to document their observations of December mature herring in past and recent years, and b) work with local fishers in collecting specimens of these herring in order to carry out biological sampling and analyses, including i) identification through genetics; ii) determination of sex and maturity; and iii) documentation of spatial and temporal distribution. The work provides an initial assessment of the spatial and temporal distribution of Shetland winter herring and their biology, including breeding patterns. This is a key first step in addressing currently unknown and unassessed activity and will provide valuable knowledge and understanding of the life history patterns of a species of international commercial importance.

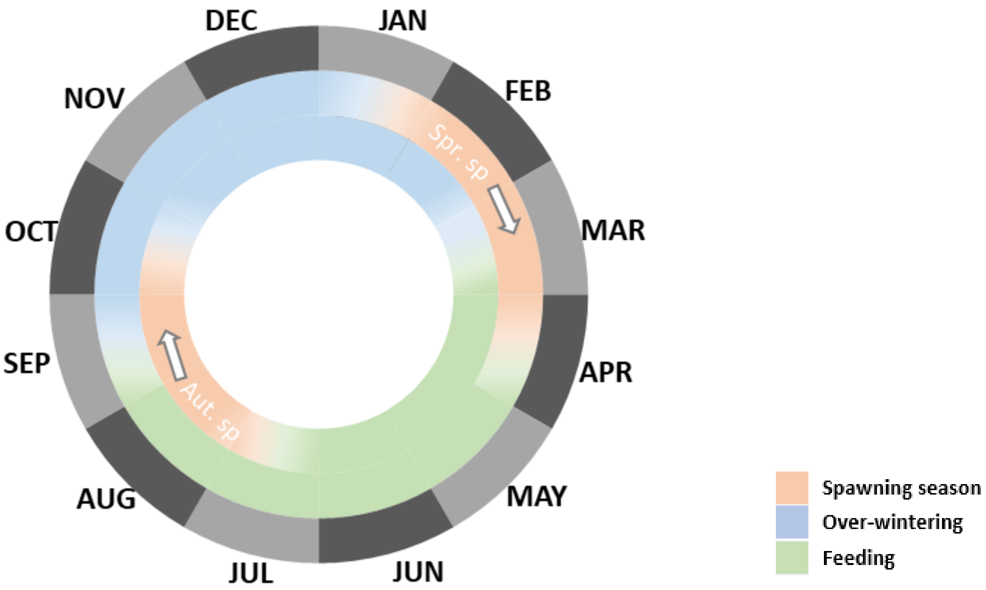

Figure 2. Schematic of timing of spawning season, over-wintering and feeding of North Sea autumn spawning herring and Norwegian spring spawning herring. Inside ring=North Sea autumn spawning herring ('Aut. sp'); middle ring=Norwegian spring spawning herring ('Spr. sp'); outside ring=month.

2.0 Methods

2.1 Herring samples

Local Shetland fishers were asked to retain any herring caught between December 2020 and April 2021, as well as between January 2022 and March 2022. Information was distributed via email (from the Shetland Fishermen's Association (SFA) and Shetland Fish Producers Organisation (SFPO)); social media posts (by SUHI, SFA and SFPO); notices at fish markets and other locations frequented by fishers; a local press release and a SUHI project webpage (**Appendix 1, 2**).

All herring retained were either caught by demersal or pelagic trawl in the waters around Shetland (**Figure 3**). A total of 719 herring were collected by fishers, from which 656 herring had associated metadata and were of sufficient quality to be fully utilised in analyses. The 656 herring were collected across twenty-eight samples, varying between 1 and 105 fish per sample (**Table 2, Figure 3**).

All herring were frozen (for between 10 days and 3 months) and thawed for sample processing. Total length (to the nearest lowest half cm), sex and macroscopic maturity stage were recorded for all 656 fish. Macroscopic maturity stage was determined by visual inspection of the gonads using the MSS 9-point scale (**Table 3**). For all herring collected in 2020/21 (456 fish), a gonad sample (approximately 1-2cm^3) was taken and preserved in formalin for histological examination. Tissue was taken from all 656 fish (approximately a pea sized piece, clean and without scales) from in front of the dorsal fin, and stored in ethanol for genetic analysis. Otoliths were removed from all fish and retained but no ageing, microstructure or microchemistry was carried out during the timeframe of the project owing to limited resources. (Since project completion, ageing is being investigated, which would provide a valuable addition to the dataset).

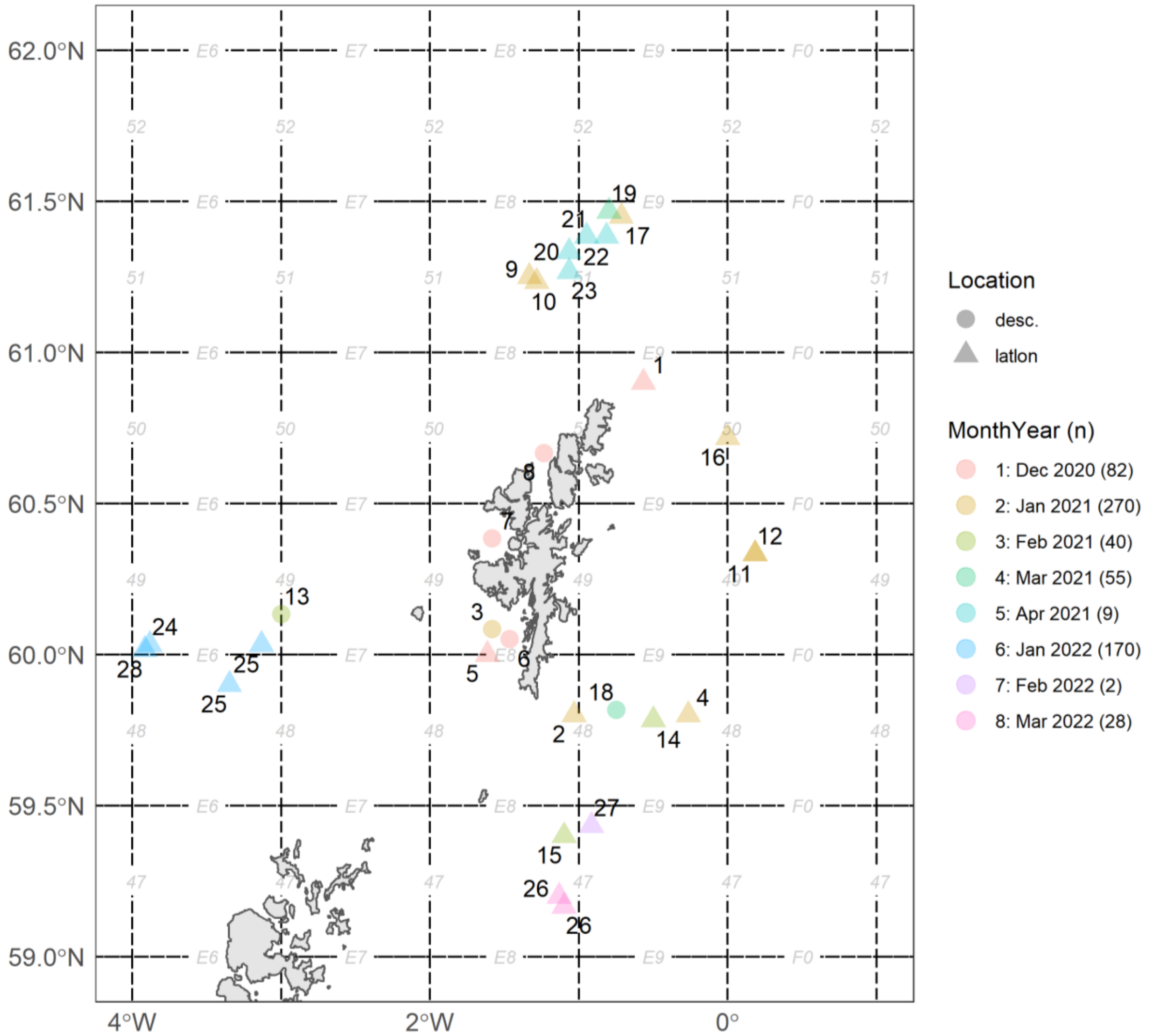

Figure 3. Location of herring samples. Where Location=desc, only a descriptive location was provided for samples e.g. 'approximately 10 miles W of Burra'. Where Location=latlon, latitude and longitude were provided for samples.

Table 2. Herring sample information (see notes below table for further details).

Sample	Haul date	Trawl	No. fish in sample†	Data
1 (A)	14 Dec 2020	Demersal	3 (3)	Length, sex, macroscopic maturity, gonad, otolith, tissue
2 (B)	24 Jan 2021	Demersal	5 (5)	Length, sex, macroscopic maturity, gonad, otolith, tissue
3 (C)*	20 Jan 2021	Demersal	11 (11)	Length, sex, macroscopic maturity, gonad, otolith, tissue
4 (D)	26 Jan 2021	Demersal	54 (54)	Length, sex, macroscopic maturity, gonad, otolith, tissue
5 (E)	08 & 09 Dec 2020	Demersal	24 (23)	Length, sex, macroscopic maturity, gonad, otolith, tissue
6 (F)*	01 Dec 2020	Demersal	15 (14)	Length, sex, macroscopic maturity, gonad, otolith, tissue
7 (Ga)*	10 Dec 2020	Demersal	7 (7)	Length, sex, macroscopic maturity, gonad, otolith, tissue
8 (H)*	04 Dec 2020	Demersal	33 (31)	Length, sex, macroscopic maturity, gonad, otolith, tissue
9 (I)	31 Jan 2021	Demersal	15 (15)	Length, sex, macroscopic maturity, gonad, otolith, tissue

10 (J)	26 Jan 2021	Demersal	16 (14)	Length, sex, macroscopic maturity, gonad, otolith, tissue
11 (K)	19 & 20 Jan 2021	Demersal	4 (4)	Length, sex, macroscopic maturity, gonad, otolith, tissue
12 (L)	23 Jan 2021	Demersal	45 (42)	Length, sex, macroscopic maturity, gonad, otolith, tissue
13 (N)*	08 Feb 2021	Demersal	37 (31)	Length, sex, macroscopic maturity, gonad, otolith, tissue
14 (Pa)	28 Feb 2021	Demersal	1 (1)	Length, sex, macroscopic maturity, gonad, otolith, tissue
15 (Pb)	28 Feb 2021	Demersal	2 (2)	Length, sex, macroscopic maturity, gonad, otolith, tissue
16 (Q, T)	26 Jan 2021	Demersal	105 (99)	Length, sex, macroscopic maturity, gonad, otolith, tissue
17 (R)	28 Jan 2021	Demersal	15 (15)	Length, sex, macroscopic maturity, gonad, otolith, tissue
18 (S)*	20 Mar 2021	Demersal	33 (33)	Length, sex, macroscopic maturity, gonad, otolith, tissue
19 (U)	31 Mar 2021	Demersal	22 (22)	Length, sex, macroscopic maturity, gonad, otolith, tissue
20 (V)	16 Apr 2021	Demersal	3 (-)	Length, sex, macroscopic maturity, gonad, otolith, tissue
21 (V)	15 Apr 2021	Demersal	3 (-)	Length, sex, macroscopic maturity, gonad, otolith, tissue
22 (V)	17 Apr 2021	Demersal	1 (-)	Length, sex, macroscopic maturity, gonad, otolith, tissue
23 (V)	15 Apr 2021	Demersal	2 (-)	Length, sex, macroscopic maturity, gonad, otolith, tissue
24 (W)	24 Jan 2022	Demersal	5 (5)	Length, sex, macroscopic maturity, otolith, tissue
25 (Xa)	21 Jan 2022	Pelagic	45 (45)	Length, sex, macroscopic maturity, otolith, tissue
25 (Xb)	21 Jan 2022	Pelagic	35 (23)	Length, sex, macroscopic maturity, otolith, tissue
26 (Y)	01 & 02 Mar 2022	Demersal	28 (28)	Length, sex, macroscopic maturity, otolith, tissue
27 (Y)	02 Feb 2022	Demersal	2 (2)	Length, sex, macroscopic maturity, otolith, tissue
28 (Z)	24 Jan 2022	Pelagic	85 (85)	Length, sex, macroscopic maturity, otolith, tissue

Sample letter is the original assigned sample label during sample processing.
ƚ No. in brackets is no. of fish to pass genetics QC; for V samples (20-23), 7 out of total 9 fish passed QC.
*No lat/lon provided, descriptive location only.
Sample 16 (T) maturity stages 5 and 6 used for genetic baseline (64 fish).
Sample 25 (Xa, Xb): Factory landed sample, 2 haul locations.
Sample 26 (Y): 2 labels for one sample with 2 dates and 2 haul locations.
Sample 27 (Y): Landed with 26 (Y) but separate label with different date and location.
For pelagic samples, herring caught as bycatch in mackerel fishery.

2.1.1 Histological analysis

For histological preparation, the gonad sections were removed from formalin and a small portion of tissue cut from the middle of the sample and placed in a labelled cassette. The cassettes were placed in a tissue basket (in batches of 55 cassettes) and loaded into a Shandon Citadel tissue processor for automated processing through reagents and wax in order to dehydrate and embed the tissue (see **Appendix 3** for specified time periods at each stage). For the final stage of wax embedding, the tissue was placed into a mould, wax poured over the tissue and a labelled cassette placed on top. The wax blocks were left to cool and stored in the fridge. Prior to sectioning, the wax blocks were placed on a cool plate. Using a Leica rotary microtome, 4 µm sections were taken and then placed on the surface of a hot water bath (set at 40^{o}C) in order to remove any folds and aid the slide mounting. The slides were left to dry prior to staining with haematoxylin and eosin (see **Appendix 4** for details). After staining, a coverslip was fixed on each slide with Neo-Mount. The sections were then examined using a compound microscope.

For each slide examined, maturity stage was identified using standard histological criteria (West 1990; McMillan 2007; Bucholtz *et al.* 2008; Brown-Peterson *et al.* 2011). Histological staging was classified by the most advanced type of oocyte present (West 1990; Brown-Peterson *et al.* 2011). The diameter of ooctyes was also measured to the nearest millimetre using an ocular micrometer, to aid in the classification of maturity stage. Histological stages were matched with the corresponding macroscopic stages provided in the MSS maturity scale for herring (**Table 3**) and a histologically derived macroscopic stage was assigned in order to offer a direct comparison to the original macroscopic staging.

Table 3. Herring macroscopic maturity stages. Marine Scotland Science (MSS) 9-point maturity scale (with equivalent ICES 6-point scale). From Mackinson *et al.* 2019.

MSS 9-point scale	Equivalent ICES 6-point scale
1: Immature Virgin	1 (Immature)
2: Immature	1 (Immature)
3: Early maturing	2 (Mature – but not included in spawning category)
4: Maturing	2 (Mature – but not included in spawning category)
5: Spawning prepared	3 (Mature – included in spawning category)
6: Spawning	3 (Mature – included in spawning category)
7: Spent*	4 (Mature [Spent] – included in spawning category)
8: Recovering/resting*	5 (Mature [Resting] - not included in spawning category)
9: Abnormal	6 (Abnormal – not included in spawning categories)

*Note, macroscopic stages 7 and 8 largely classified as 7, as difficult to differentiate between spent and recovering/resting.

2.1.2 Genetic analysis

Tissue samples were sent to EDF Scientific in Ireland for genetic analysis. Full methodological details of the sample processing, genotyping and baseline dataset analyses are documented in Farrell *et al*. 2021.

For the genetic analysis, a baseline sample was needed, requiring a sample with a high proportion of mature spawning fish. Following a review of the samples, Sample 16 (T) was selected as the Shetland herring baseline, having the highest proportion of mature spawning fish out of all the samples. Only maturity stages 5 and 6 (MSS 9-point scale, **Table 3**) from Sample 16 (T) were used as the baseline (64 fish out of 85). Fish of the remaining maturity stages from Sample 16 (T) were utilised in analysis for all samples (see below). Note that baseline selection was carried out prior to histological analysis, therefore maturity stage used was macroscopic. Following histological analysis, there was no change to maturity stage for 63 of the 64 stages 5 and 6, with one stage 5 downgraded to stage 4.

The Shetland baseline was compared to other existing herring baselines for 6.a.S, 6.a.N autumn spawning, 6.a.N spring spawning, North Sea autumn spawning, and Downs. Multi-locus pairwise F_{ST} analyses were conducted to assess genetic differentiation between baseline samples, and Principal Coordinates Analysis (PCoA) of pairwise F_{ST} values provided an indicator of where Shetland baseline fish lie in comparison to adjacent baseline populations.

Discriminant Analysis of Principal Components (DAPC) and Principal Component Analysis (PCA) were then performed for each of the non-baseline samples as a whole, and then broken down by maturity stage, with the aim to identify clusters of genetically related individuals amongst the Shetland samples, with comparison to baseline datasets.

Note that owing to the small sample sizes involved here, such analyses are intended as exploratory indicators to genetic identification only.

2.1.3 Spawner classification

Fish sampled were classified as either autumn or spring spawners based firstly on maturity stage at month of capture, then on genetic assignment; with the outcomes of the two classifications compared. Stage 1 fish were excluded from the classification, and classification was restricted to samples taken during the main sampling period December – March (excluding the small sample of 9 fish in April 2021).

Using microscopic maturity stage, herring of maturity stages 2, 7 and 8 caught between December and March were classified as autumn spawners (having spawned in September, with subsequent maturation between March and August). Stages 3, 4, 5 and 6 herring caught in the same period were classified as spring spawners (with spawning occurring in February-April, following maturation between May and January).

A genetic assignment model was used to classify each fish to either North Sea Autumn, Shetland Spring or Downs winter herring, using a probability score derived from baseline datasets which provided input training to the model. Similar to the earlier genetic analysis carried out (see 2.1.2 above), Sample 16 (T *and* Q) was selected as the Shetland herring baseline, and the existing North Sea autumn spawning and Downs winter baselines were again used. (Following the outcomes of the genetic analysis (see 3.1.2 below), the output model classification from the Shetland baseline was termed 'Shetland Spring').

Note that the assignment has limitations: while the North Sea autumn and Downs winter baseline datasets are from multiple samples from different dates/years, the Shetland baseline only used a single sample. The assignment can test the samples and classify each as a spring spawner or not, separating them from North Sea autumn and Downs winter. However, other populations are not considered by the model (e.g. 6.a.N spring spawning; 6.a.S spring spawning; Norwegian spring spawning; and Western Baltic spring spawning herring). The assignment is therefore viewed as exploratory only. Genetic assignment was carried out by EDF Scientific; details of a similar genetic assignment model are available in Farrell *et al*. 2021.

2.2 Local observations

In addition to the sampling information distributed to local fishers (see 2.1 above), a link to an online questionnaire was also provided in order to document observations of December mature herring in past and recent years (**Appendix 5**). Responses were limited (receiving only 1), and interviews which had been planned were unable to take place due to concerns over covid. Given these difficulties and, by contrast, the success of herring samples collected for the project, the focus of analysis and discussion became directed to the samples and associated histological and genetic examinations.

3.0 Results

3.1 Herring samples

The opportunistic approach to sampling achieved a broad range of samples, spatially distributed around Shetland throughout the winter sampling period (**Figure 3**). While samples were obtained in all months requested (December 2020 - April 2021, and January 2022 - March 2022), the majority were caught in December 2020 (82 fish), January 2021 (270 fish) and January 2022 (170 fish) (**Table 2, 4**). All fish caught were between 20 and 33.5 cm (**Table 5, Figure 4**), typical of the adult fish found and commercially exploited in the North Sea. The majority were either maturity stage 4 (152 fish), 5 (170 fish) or 7 (258 fish), with fewer than 25 fish in each of the stages 1, 2, 3, 6 and 8 (**Table 4**).

Table 4. Number of sampled fish at each maturity stage. For 2021, histologically derived maturity stage is used and number in brackets is macroscopic maturity stage when no stage was able to be derived histologically (e.g. poor slide, unable to be read microscopically). For 2022, only macroscopic maturity available to be used (no gonad sample taken for histology).

	1 Immature virgin	**2** Immature	**3** Early maturing	**4** Maturing	**5** Spawning prepared	**6** Spawning	**7** Spent	**8** Recovering / resting	
Dec 2020	1	0	4	27	8	0	41	1	*82*
Jan 2021	1	1	6	37 (3)	76 (4)	15	124 (2)	1	*270*
Feb 2021	0	0	0	0	1	0	38	1	*40*
Mar 2021	8	0 (7)	1 (1)	4	0 (1)	0	30 (3)	0	*55*
Apr 2021	0	0	0	0	0	0	9	0	*9*
Jan 2022	0	0	2	77	80	8	3	0	*170*
Feb 2022	0	0	0	2	0	0	0	0	*2*
Mar 2022	0	15	3	2	0	0	8	0	*28*
	10	*23*	*17*	*152*	*170*	*23*	*258*	*3*	

Table 5. Length of fish sampled

Sample	No. fish	Mean length, cm (min-max)	Sample	No. fish	Mean length, cm (min-max)
1 (A)	3	29.7 (29 - 30.5)	15 (Pb)	2	27.2 (23 - 31.5)
2 (B)	5	28.0 (26 - 30)	16 (Q, T)	105	30.3 (23.5 - 33.5)
3 (C)*	11	26.5 (23 - 30)	17 (R)	15	30.9 (27.5 - 32.5)
4 (D)	54	27.0 (23.5 - 30)	18 (S)*	33	24.5 (21.5 - 31)
5 (E)	24	27.7 (25 - 32)	19 (U)	22	30.0 (28 - 31)
6 (F)*	15	25.1 (20 - 30)	20 (V)	3	29.8 (29 - 31)
7 (Ga)*	7	27.6 (25 - 29)	21 (V)	3	29.7 (28.5 - 31)
8 (H)*	33	29.7 (25.5 - 32)	22 (V)	1	29.5 (29.5 - 29.5)
9 (I)	15	31.4 (30 - 33)	23 (V)	2	29.0 (29 - 29)
10 (J)	16	30.7 (29 - 33)	24 (W)	5	28.2 (24 - 31)
11 (K)	4	28.8 (26 - 31)	25 (Xa)	45	28.9 (25 - 32.5)
12 (L)	45	29.5 (25.5 - 33)	25 (Xb)	35	29.7 (27.5 - 32.5)
13 (N)*	37	30.0 (27 - 33)	26, 27 (Y)	30	25.9 (22.5 - 29.5)
14 (Pa)	1	30.0 (30 - 30)	28 (Z)	85	29.2 (25 - 32.5)

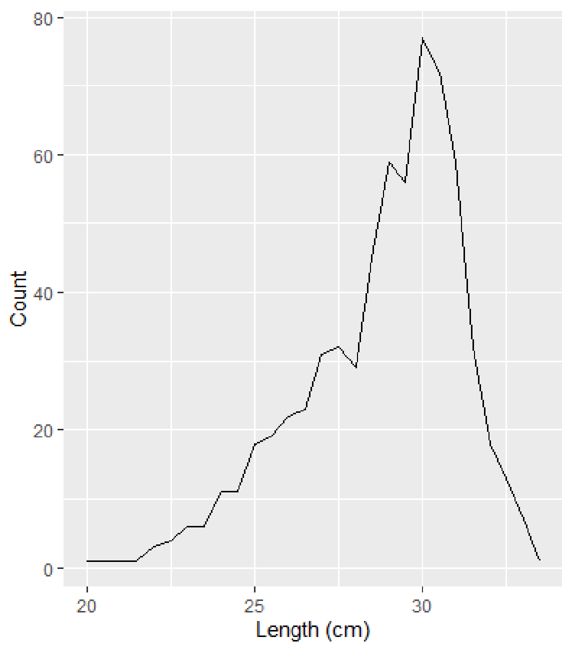

Figure 4. Length distribution of fish sampled

3.1.1. Histological analysis

For the majority of samples (259 of 456), the original macroscopic and histologically derived maturity stages matched (**Table 6**). Where mismatch did occur, it was most commonly (119 samples out of 459) in identifying stage 7 fish as stage 2 – two stages which are known to be difficult to separate macroscopically (Bucholtz *et al.* 2008). Other cases of mismatch assigned a stage above (nine stage 1 fish were assigned as stage 2) or a stage below (20 stage 4 fish were staged as 3, and fifteen stage 5 fish were staged as 4).

For samples collected in 2020/21, the histologically derived macroscopic stage was used in onward analysis (note, when no stage was able to be derived histologically, the original macroscopic maturity stage was used e.g. instances when a poor quality slide was unable to be read microscopically).

Table 6. Match/mis-match between original macroscopic maturity staging and histologically derived maturity staging.

		HISTOLOGICALLY DERIVED MATURITY								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	***Too poor to stage***
MACROSCOPIC MATURITY	**1**	**1**						2		
	2	9	**1**					119		*7*
	3			**10**	20			1		*1*
	4				**46**	15				*3*
	5				2	**67**				*5*
	6					3	**15**			
	7			1				**119**	3	*5*
	8							1*		

* Note made that the gonad appeared unusual

3.1.2 Genetic analysis

The F_{ST} analyses showed the Shetland baseline sample to be significantly different to the baselines from the adjacent populations of 6.a.S, 6.a.N autumn spawning, 6.a.N spring spawning, North Sea autumn spawning, and Downs (**Table 7**). The *PCoA* of F_{ST} of the baseline datasets further indicated the Shetland baseline to be distinct from the others examined (**Figure 5**). While the Shetland baseline was shown to be different, the results of the F_{ST} and *PCoA* indicated that the Shetland baseline was more similar to the baseline of spring spawning 6.a.N fish than the other baseline datasets. This was also further reflected when the baseline datasets were plotted with each Shetland sample for the DAPC and PCA, with the Shetland baseline dataset heavily clustered with that of the 6.a.N spring spawning baseline (note the yellow and red data points, **Appendix 6a-6z**).

When comparing each sample to baseline datasets, the DAPC results showed Shetland fish clustering with different baselines across – and within – samples (note the pink data points, **Appendix 6a-6z**), indicating varied genetic differentiation throughout the samples.

Samples 1 (A), 13 (N), 14 (Pa), 17 (R) all clustered with autumn spawning North Sea and 6.a.N fish (**Appendix 6a, 6n, 6Pa, 6r**). Other samples had majority clustering with autumn spawning North Sea and 6.a.N fish, with a few fish in the sample (between 1 and 5) conversely clustering with the Shetland baseline (2 (B), 4 (D), 6 (F), 7 (Ga), 9 (I), 10 (J), 11 (K), 19 (U), 20-23 (V), **Appendix 6b, 6d, 6f, 6g, 6i, 6j, 6k, 6u, 6v**).

Four samples (including two collected from by pelagic trawl) demonstrated clustering with the Shetland baseline (15 (Pb), 24 (W), 25 (Xb), 28 (Z), **Appendix 6Pb, 6w, 6Xb, 6z**), with three other samples (containing the remaining pelagic trawl sample) showing majority clustering with the Shetland baseline and some clustering (between 2 and 5 fish) with the North Sea and 6.a.N autumn spawners (3 (C), 8 (H), 25 (Xa), **Appendix 6c, 6h, 6Xa**).

Sample 16 (Q) which was collected along with Sample 16 (T) but was not used as part of the baseline dataset shows close clustering with the Shetland baseline (**Appendix 6q**). Most of the non-spawning fish from Sample 16 (T) also cluster with the Shetland baseline, except for two fish which cluster with autumn spawning North Sea and 6.a.N baselines (**Appendix 6t**).

Samples 5 (E), 12 (L), 18 (S) and 26, 27 (Y) show more of an even split in clustering between the Shetland baseline and the autumn spawning North Sea and 6.a.N baselines (**Appendix 6e, 6l, 6s, 6y**).

Note that some samples that clustered with the Shetland baseline also shared clustering with the 6.a.N spring spawning baseline, with similarity between these two baseline datasets.

Generally, fish from the Shetland samples tended to show genetic clustering with autumn spawning North Sea and 6.a.N fish, and spring spawning Shetland and 6.a.N baselines. The differences in sample clustering (within and across samples) were substantiated by the addition of maturity data (**Appendix 6a-6z**; discussed in more detail below, see 3.1.3), whereby stages 3,4 and 5 fish largely grouped with the Shetland T sample and stage 2 and 7 fish largely grouped with the North Sea autumn spawners.

When looking at the spatio-temporal distribution of autumn and spring spawning fish, no clear overall patterns were apparent, though there was some grouping of fish at locations offshore (**Figure 3, Table 2**). For example, to the north of Shetland, there was a collection of eight samples which predominatly demonstrated clustering with autumn spawning North Sea and 6.a.N fish (9 (I), 10 (J), 17 (R), 19 (U), 20-23 (V)). To the west of Shetland and to the south, there were two groups of samples which generally demonstrated clustering with the spring spawning Shetland and 6.a.N baselines (24 (W), 25 (Xa, Xb) and 28 (Z) to the west, all of which were caught in January; and 15 (Pb) and 26, 27 (Y) to the south, caught at the end of February/beginning of March). In general, fish of both spawning strategies were present in samples throughout Shetand waters, across the sampling period December-April.

Table 7. Pairwise multi-locus F_{ST} (above the diagonal) for the baseline datasets and associated *P*-values (below the diagonal): Datasets: 6.a.S ('6aS'), 6.a.N autumn spawning ('6aN_Aut'), 6.a.N spring spawning ('6aN_Sp'), North Sea autumn spawning ('NS'), winter spawning Downs ('DWN'), Shetland Sample 16 (T) ('Shet_T').

	6aS	6aN_Aut	6aN_Sp	NS	DWNS	Shet_T
6aS		0.2020	0.3480	0.2431	0.1198	0.4758
6aN_Aut	0.0001		0.5699	0.0161	0.2376	0.6279
6aN_Sp	0.0001	0.0001		0.5946	0.6818	0.1897
NS	0.0001	0.0001	0.0001		0.3299	0.6451
DWNS	0.0001	0.0001	0.0001	0.0001		0.8079
Shet_T	0.0001	0.0001	0.0001	0.0001	0.0001	

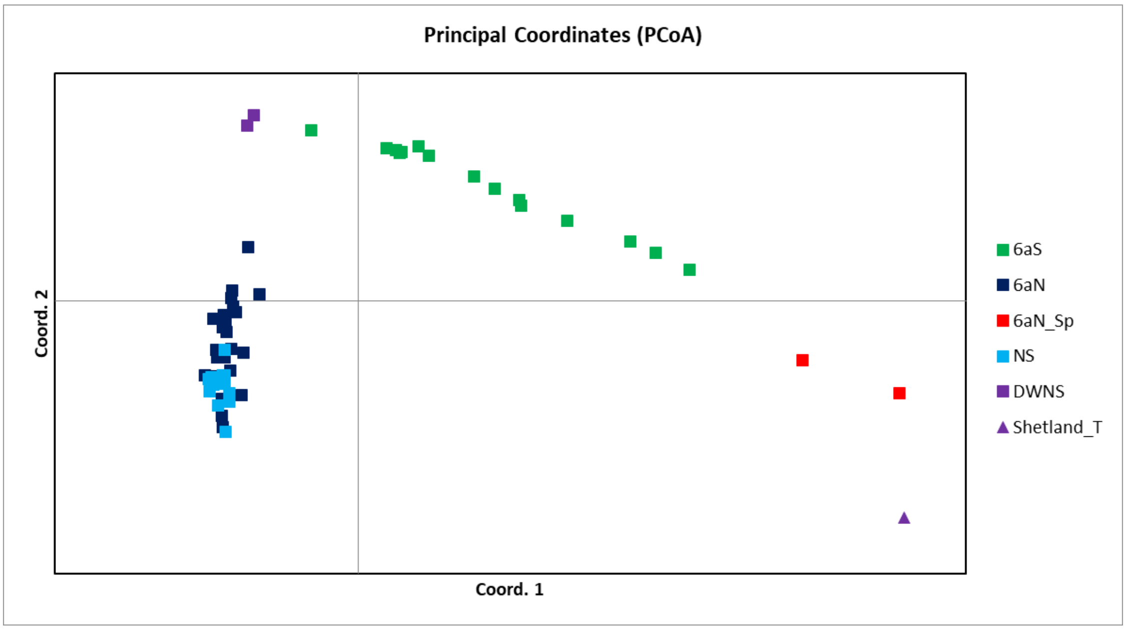

Figure 5. Principal Coordinate Analysis (PcoA) of F_{ST} of the baseline datasets. Datasets: 6.a.S ('6aS'), 6.a.N autumn spawning ('6aN_Aut'), 6.a.N spring spawning ('6aN_Sp'), North Sea autumn spawning ('NS'), Downs ('DWN'), Shetland Sample 16 (T) ('Shetland_T').

3.1.3 Spawner classification

For the genetic assignment, 530 fish were assigned with the model (excluding the baseline dataset, those samples which did not pass genetics QC, stage 1 fish, and April samples). The majority of fish classified by maturity stage as spring spawners were genetically assigned as Shetland spring spawners with the model (189 fish, 71.0%), and, similarly, the majority of fish classified by maturity stage as autumn spawners were assigned as North Sea autumn spawners with the model (248 fish, 93.9%), **Table 8**. There were 76 fish maturity classified as spring spawners which were then genetically assigned as North Sea autumn spawners (28.6%). These were mainly stage 4 (38) and 5 (25) fish, of which 37 came from pelagic samples 25 (Xa, Xb) and 28 (Z). Very few fish (6 individuals, 2.3%) classified as autumn spawners were genetically classified as Shetland spring spawners; and overall, only 11 were genetically assigned as Downs winter spawners.

Across the samples, four contained only fish which were genetically assigned as North Sea autumn spawners (1 (A), 13 (N), 14 (Pa), 17 (R), **Table 9**). A further 11 samples comprised mainly genetically assigned North Sea autumn spawners (2 (B), 4 (D), 5 (E), 6 (F), 7 (Ga), 9 (I), 10 (J), 11 (K), 18 (S), 19 (U), 26 (Y)). Only one of the samples were made up solely of Shetland spring spawners (27 (Y)); while five contained majority Shetland spawners (3 (C), 8 (H), 16 (Q/T), 25 (Xb), 28 (Z)). The four remining samples (12 (L), 15 (Pb), 24 (W) and (25) Xa) contained more of an equal split of assigned North Sea autumn and Shetland spring spawners.

Table 8. Breakdown of spawner classification demonstrating match/mis-match between classification by maturity stage and genetic assignment. Number of fish (percentage of fish from total spring or autumn maturity classification)

		Genetic assignment		
		Shetland spring spawner	North Sea autumn spawner	Downs winter spawner
Maturity classification	Spring spawning	189 (71.0%)	76 (28.6%)	1 (0.4%)
	Autumn spawning	6 (2.3%)	248 (93.9%)	10 (3.8%)

Table 9. Genetic assignment of samples

	Genetic assignment			
Sample	Shetland spring spawner	North Sea autumn spawner	Downs winter spawner	(No. fish)
1 (A)	0	3	0	(3)
2 (B)	1	4	0	(5)
3 (C)*	8	2	0	(10)
4 (D)	1	53	0	(54)
5 (E)	4	17	2	(23)
6 (F)*	0	12	1	(13)
7 (Ga)*	1	6	0	(7)
8 (H)*	20	11	0	(31)
9 (I)	1	14	0	(15)
10 (J)	2	12	0	(14)
11 (K)	1	3	0	(4)
12 (L)	18	24	0	(42)
13 (N)*	0	31	0	(31)
14 (Pa)	0	1	0	(1)
15 (Pb)	1	1	0	(2)
16 (Q, T)	22	2	1	(6, 19)
17 (R)	0	15	0	(15)
18 (S)*	3	19	3	(25)
19 (U)	2	20	0	(22)
20 (V)	0	0	0	(0)
21 (V)	0	0	0	(0)
22 (V)	0	0	0	(0)
23 (V)	0	0	0	(0)
24 (W)	3	2	0	(5)
25 (Xa)	27	18	0	(45)
25 (Xb)	19	4	0	(23)
26 (Y)	2	23	3	(28)
27 (Y)	2	0	0	(2)
28 (Z)	57	27	1	(85)

4.0 Discussion

This study has gathered valuable new information to provide insight into the identity of herring populations around Shetland, with clear evidence of the presence autumn and spring spawning herring, as well as Downs winter spawning herring between the months of December and March.

Genetic analysis showed the Shetland baseline to be significantly different and distinct from adjacent populations (6.a.S; North Sea and 6.a.N autumn spawning; 6.a.N spring spawning; and Downs winter spawning herring), with most similarity to spring spawning 6.a.N fish; indicative of spring spawning herring in Shetland waters, with the potential for a previously unidentified Shetland spring spawning population component in the context of this study and the available baseline datasets (see 3.1.2).

Comparison of the Shetland non-baseline samples against baseline data indicated genetic clustering with the Shetland baseline, as well as with other baseline datasets, demonstrating the presence of multiple herring population components, including autumn spawning North Sea and 6.a.N, and spring spawning Shetland and 6.a.N (see 3.1.2). Throughout the non-baseline samples, stages 3, 4 and 5 fish largely grouped with the spring spawning Shetland baseline, while stage 2 and 7 fish largely grouped with the autumn spawning North Sea baseline, providing a corroboration of the genetic results.

There was further indication of herring of differing populations within single samples from both genetic assignment and maturity stage at month of capture (see 3.1.3). Such results suggest the potential for mixing of populations in Shetland waters, though owing to the opportunistic nature of sampling and limited sample information available, it is not known if those fish caught were single shoals or scattered fish. If mixing is taking place, there is likely overlap occurring at various stages of spawning, feeding and over-wintering. That herring (mainly showing genetic clustering with the Shetland baseline) were caught alongside mackerel in samples from the pelagic fishery also demonstrates potential wider species interactions.

There was good agreement of spawner classification between maturity stage at month of capture and genetic assignment (see 3.1.3), with results demonstrating the two main herring populations present in Shetland waters to be North Sea autumn spawning and Shetland spring spawning. North Sea autumn spawning fish are known to be present, with a valuable commercial fishery in place here since the 1970s. Less is known about the presence of spring spawning herring in Shetland waters, though there have been historical reports of such 'winter fish' and a 'winter fishery'. The agreement between the two methods of spawner classification provides confidence in using maturity stage at time of capture as an indicator of population identity. This is useful to know, as genetic analysis is not always practical, timely or affordable to carry out, so maturity stage may be utilised as a rapid method of early indication to population identity. It remains however preferable to carry out genetic analysis, which provides the most robust method for discriminating populations, at least until a deeper understanding of patterns of mixing is obtained. That the histological analysis showed macroscopic staging to be largely correct (see 3.1.1) is also useful information in this regard. The most common error was in identifying stage 7 fish as stage 2, a known difficulty in herring maturity staging.

While the results here have provided evidence of spring spawning fish in Shetland waters, the origin of this population is not known. No baseline datasets were available for Norwegian or Western Baltic spring spawning herring (NSSH or WBSSH), which cannot be discounted as the source of spring spawning fish found in Shetland waters. Since the project completion, NSSH and WBSSH baseline data have been obtained, and a universal genetic assignment model is being developed which can provide more insight into the Shetland samples (EDF, *pers. comm.*).

No clear spatio-temporal relationship was observed in the presence and distribution of autumn and spring spawning fish. It is noted that the timing of the Shetland spring spawning seemed earlier than Norwegian and Western Baltic spring spawning fish. Spawning of NSSH is typically between February-April and WBSSH is generally April-June (Hay *et al.* 2001; Kurita *et al.* 2003; McPherson and Kjesbu 2012; dos Santos Schmidt *et al.* 2017; Slotte 1999a, b). Spring spawning in the Shetland herring samples in this study was generally observed mid-late January, with some in December, February and March. Owing to the limited scope of this study, the information provides a snapshot in time, with further sampling necessary to better understand the presence of spring spawning herring in Shetland waters.

The opportunistic nature of the sampling carried out provided a sound sample base from which to examine the morphometrics, biology and genetics of herring found in Shetland waters during the winter. The histological analysis carried out provided improved accuracy to the maturity staging of the herring, which was of value when assessing spawning strategies as an indicator of population identity. Being reliant on opportunistic samples from commercial vessels had the potential to impact on the outcomes of the study (for example, not knowing if, or how many, samples could be obtained to address the aims and objectives). However, there are benefits to such ways of sampling: dedicated ship time was not constrained by limited funds or poor weather; a wider spatio-temporal distribution of sampling could be covered; and relationships with industry developed.

It was unfortunate not to have obtained observations from questionnaires and interviews. The initial concept for the project originated from questions from the fishing industry, sparked by recent observations of maturing herring in December, suggesting that there is knowledge of 'winter herring' within the community and an inclination to engage with local researchers. The reason for the low response to questionnaires can only be speculated upon, and the inability to carry out interviews was an unexpected circumstance beyond control. Local fisher knowledge remains a valuable source of information, with further engagement worthwhile should the opportunity arise. It is possible that dissemination of the project findings may stimulate wider discussions which could be taken forward. In contrast to the questionnaire, local fishers were highly responsive to the request for herring samples, and the number of samples received from fishers exceeded expectations, providing a good number of contemporary observations for a valuable dataset.

Utilising skills and expertise from external organisations had the advantage of establishing connections with other academic institutions and staff working on similar research. Preliminary results were presented to the ICES Working Group of International Pelagic Surveys (made up of staff from a number of research and academic institutes), whose work includes reviewing the use of genetic stock separation methods. Additional collaboration is planned, with further genetic assessment of the samples obtained in this study, to provide more detailed information and to contribute information to a wider body of work. Such work can contribute to addressing questions of stock discrimination which can inform science and management.

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Appendix 1: Information distributed to fishers via local press release

Fri 4th December 2020

Shetland Herring Wanted for New Scientific Study

The NAFC Marine Centre UHI is asking Shetland fishermen for help with a new project to determine the identity of herring found in Shetland waters.

The fishermen are being asked to collect samples of herring caught around Shetland during the winter and spring so that their identity can be investigated.

There are several populations of herring in the north-east Atlantic. Around Shetland, North Sea autumn-spawning herring are present and support a valuable commercial fishery. These herring are observed to undergo sexual maturation between March and August, ready to spawn in September. Yet there have also been observations in Shetland waters of other herring which instead undergo maturation in the winter months, to spawn early in the year.

NAFC Fisheries Scientist Dr Katie Brigden, who is leading the project, explained "while we are aware of previous fishing for 'winter herring' there is little documented information available, and the presence of these fish is of interest to science and industry – raising the question of a stock distribution which is potentially unknown and unaccounted for. There is a need to gather more information to confirm which population these 'winter herring' belong to."

The new project is asking all fishermen in Shetland waters to look out for and retain any herring that are caught between December 2020 and April 2021 so that they can be biologically sampled and genetically identified. Any herring caught should be kept on ice or frozen, with details recorded of the date and location of capture. Fishermen are asked to contact the NAFC Marine Centre for the samples to be collected or to arrange drop-off (herring@uhi.ac.uk, 01595 772000).

In addition to collecting samples, fishermen are also being asked to complete a short questionnaire (available at Questionnaire: Identification of herring in Shetland waters - https://bit.ly/2JqShf2) to provide information on historical observations of winter herring around Shetland in order to better understand the spatial and temporal distribution of these fish.

Further Information

Full details of the project are available on the NAFC website at: www.nafc.uhi.ac.uk/research/pelagic/herring/.

For further information, please contact Dr Katie Brigden or Dr Shaun Fraser at the NAFC Marine Centre (herring@uhi.ac.uk, 01595 772000).

Appendix 2: Information distributed to fishers via email, social media posts, notices at fish markets and other locations frequented by fishers

Appendix 3: Specified solutions and time periods for gonad samples during histological sample processing (dehydrating and embedding)

Step	Solution	Time Hrs:Mins	Time (Mins)
1	70% Ethanol	00:45	(45)
2	96% Ethanol	01:30	(90)
3	96% Ethanol	01:30	(90)
4	100 % Ethanol	01:30	(90)
5	100 % Ethanol	01:30	(90)
6	Ethanol/Histo-clear 50%	02:15	(135)
7	Histo-clear	01:30	(90)
8	Histo-clear	01:30	(90)
9	Histo-clear/Paraffin wax 50%	02:15	(135)
10	Paraffin wax	03:00	(180)
11	Paraffin wax*	Indefinite	Indefinite

*Remove cassettes from wax bath and fill with wax from wax dispenser

Appendix 4: Specified solutions and time periods for gonad samples during histological sample processing (staining)

Step	Reagent	Time Mins:Secs	Time (Secs)
1	Neo-clear	10:00	(600)
2	100% Ethanol	04:00	(240)
3	80% Ethanol	03:00	(180)
4	Water	02:00	(120)
5	Harris' hematoxylin	00:24	(24)
6	Water	02:00	(120)
7	Acid alcohol	00:10	(10)
8	Water	03:00	(180)
9	Lithium carbonate	00:10	(10)
10	Water	01:00	(60)
11	70% Ethanol	01:00	(60)
12	Eosin/Phloxin b solution*	02:00	(120)
13	96% Ethanol	02:00	(120)
14	100% Ethanol	02:00	(120)
15	Neo-clear	05:00	(300)
16	Neo-clear	03:00	(180)

Appendix 5: Online questionnaire

Questionnaire: Identification of herring in Shetland waters

The NAFC Marine Centre has recently started a new project to determine the identity of herring found in Shetland waters in winter (between November and April).

In addition to collecting samples of herring, we are asking for historical observations of these winter herring in order to document the spatial and temporal distribution. This information is useful for informing on stock distribution, and any shifts in such distribution which may be unknown or unaccounted for. Questionnaire responses will contribute to this information.

Questionnaire responses are anonymous unless you provide your email at the end of the questionnaire (this is optional). More information on participation and use of data is available in the Participant Information Sheet.

For further information on the project or any questions, please contact Dr Katie Brigden (katie.brigden@uhi.ac.uk (mailto:katie.brigden@uhi.ac.uk)) or Dr Shaun Fraser (shaun.fraser@uhi.ac.uk (mailto:shaun.fraser@uhi.ac.uk)).

By clicking the 'Submit' button below, you are consenting to the information being used in this project.

1

Have you ever caught full/ripe/running herring in Shetland waters between November and April?

If not sure, please select other and give more information.

○ Yes

○ No

○ [ ]

Other

2

What was the method of fishing?

3

Which years have you caught full/ripe/running herring between November and April?

Select all that apply. If "other" please provide details.

- ☐ 1960s
- ☐ 1970s
- ☐ 1980s
- ☐ 1990s
- ☐ 2000s
- ☐ 2010s
- ☐ 2020s
- ☐ Other

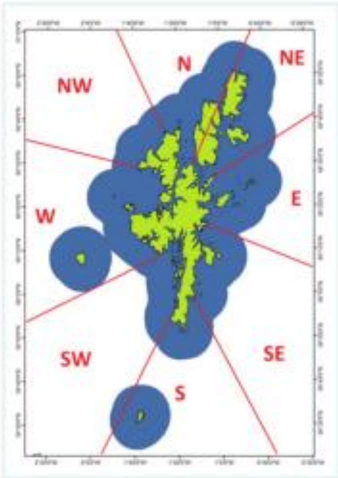
4

Between November and April, which months have you most commonly caught full/ripe/running herring?

Select all that apply. If "other" please provide details.

- ☐ November
- ☐ December
- ☐ January
- ☐ February
- ☐ March
- ☐ April
- ☐ Other

5



Typically, where have you caught full/ripe/running herring between November and April?

Please refer to map, select all options that apply. If you can be more specific, please also enter details in "other".

- ☐ Inshore (blue areas)
- ☐ Offshore (white areas)
- ☐ N
- ☐ NE
- ☐ E
- ☐ SE
- ☐ S
- ☐ SW
- ☐ W
- ☐ NW
- ☐ [ ]

Other

6

Do you have any other information on the presence of full/ripe/running herring in Shetland waters between November and April?

7

Are you happy to be contacted to discuss your observations?

A member of the NAFC Fisheries Team may be in touch. Your contact details will not be shared outside of this project.

- Yes
- No

8

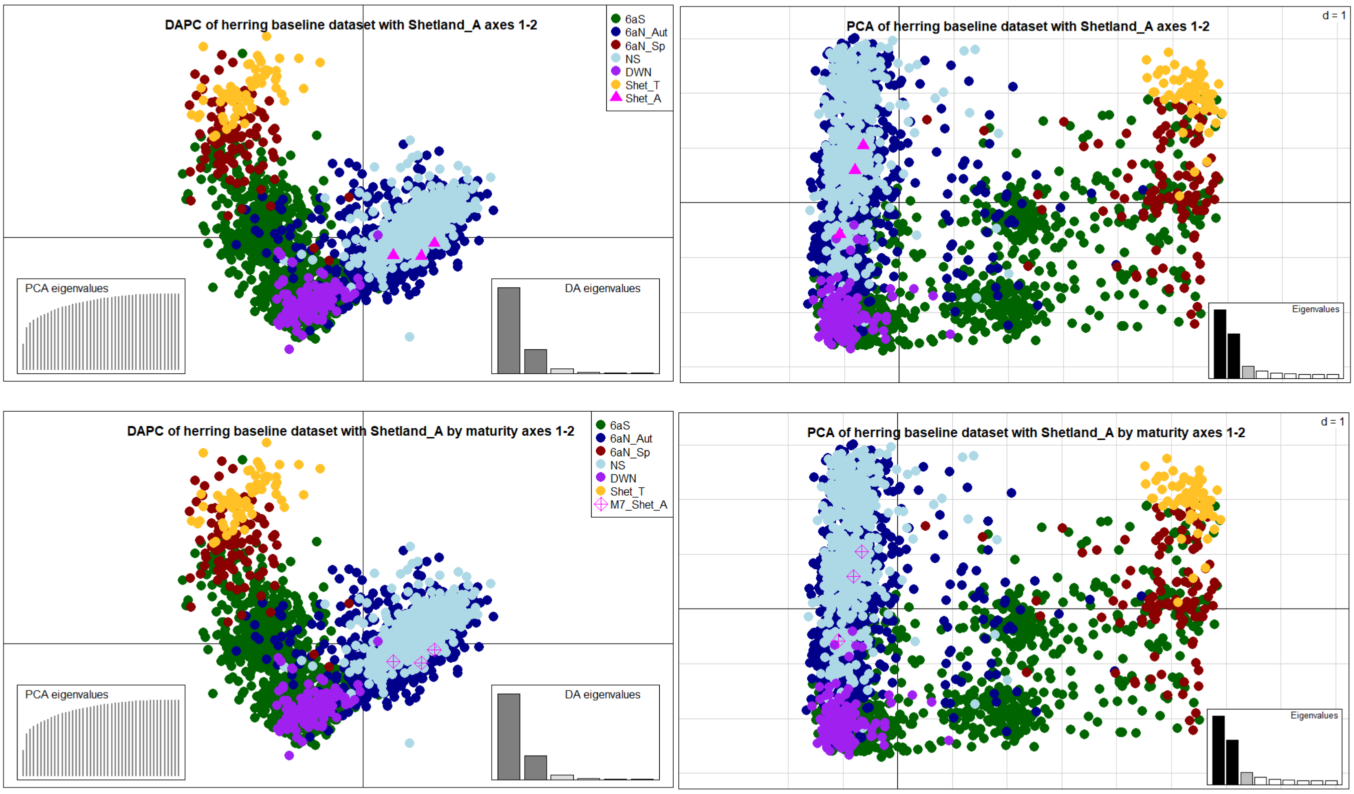
Please enter your contact details below.

Name, contact number, email address.

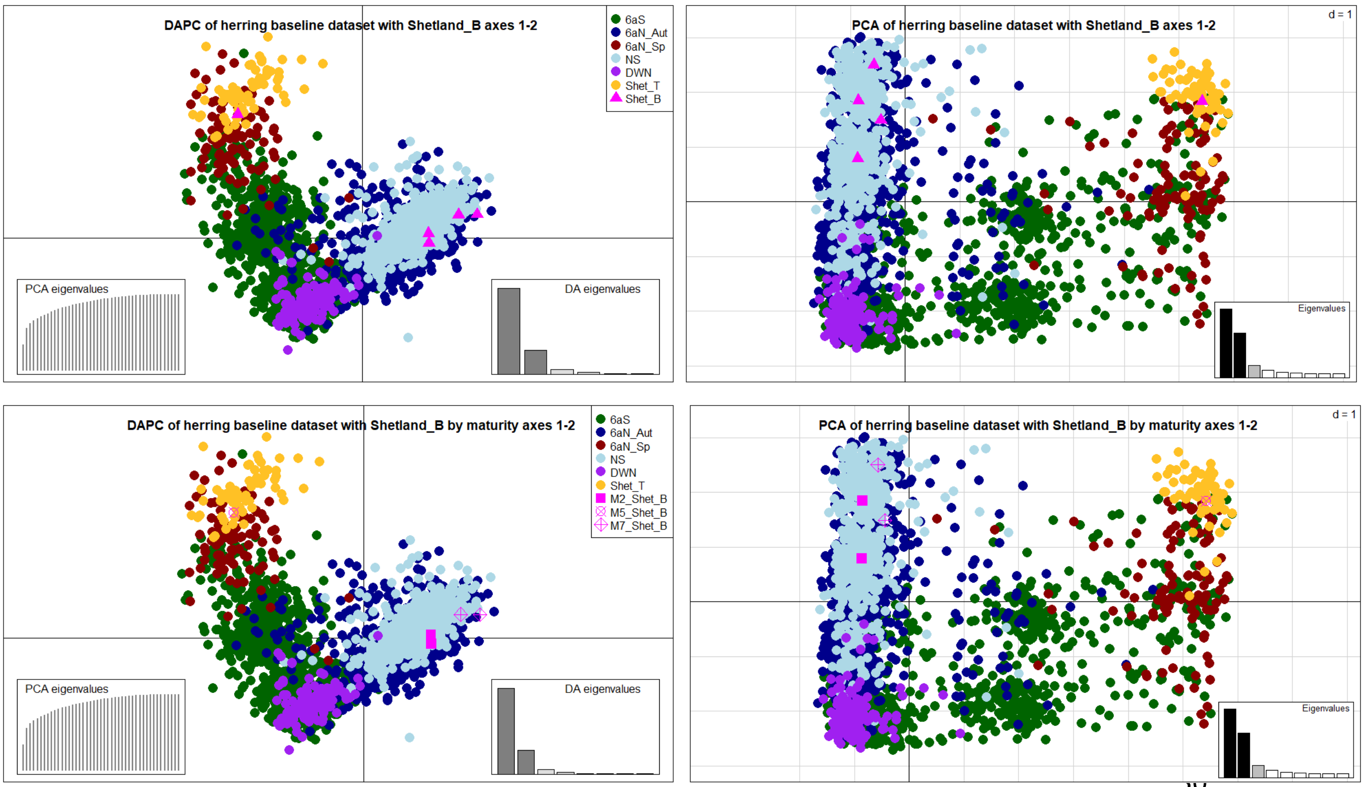
(The NAFC Marine Centre is GDPR compliant and any personal data will be processed in accordance with the provisions of the Data Protection Act 1998).

Appendix 6: Discriminant Analysis of Principal Components (DAPC) and Principal Component Analysis (PCA) for each of the Shetland samples as a whole, and broken down by maturity stage

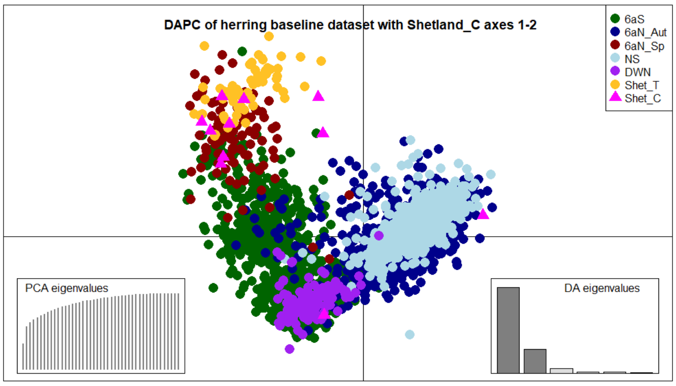
Appendix 6a: Sample A

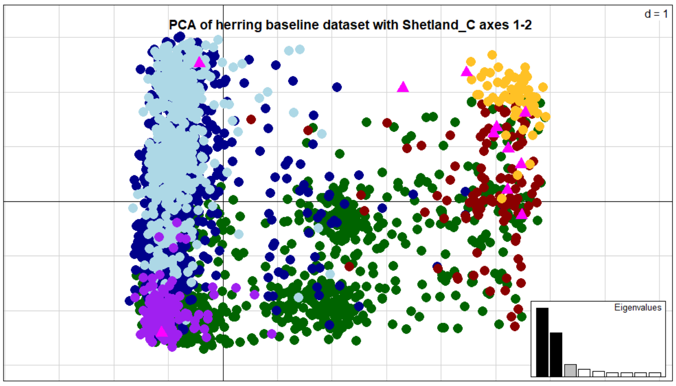


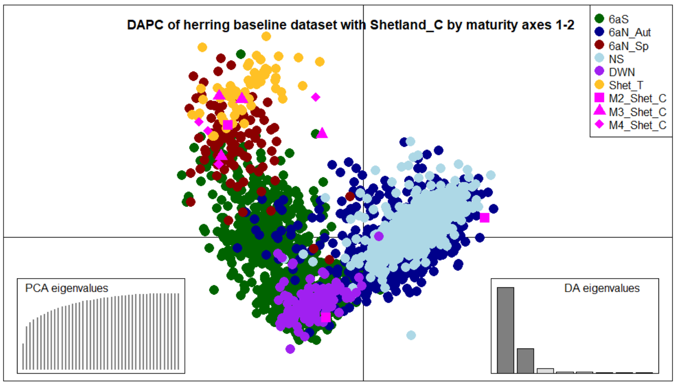
Appendix 6b: Sample B

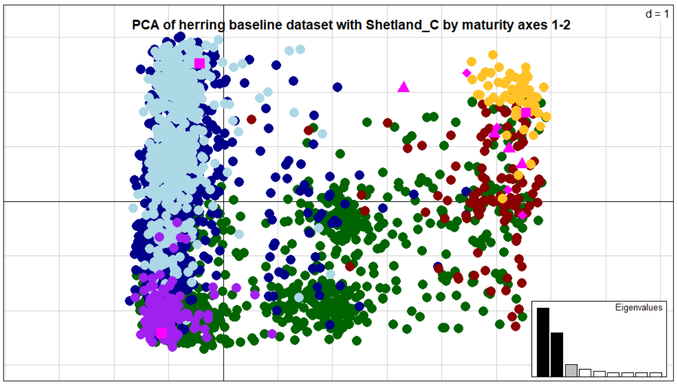


Appendix 6c: Sample C

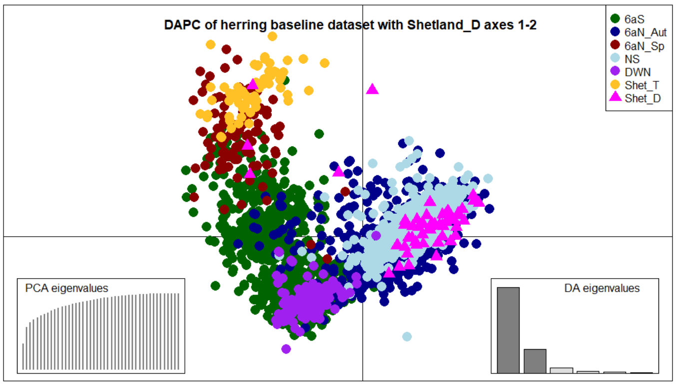


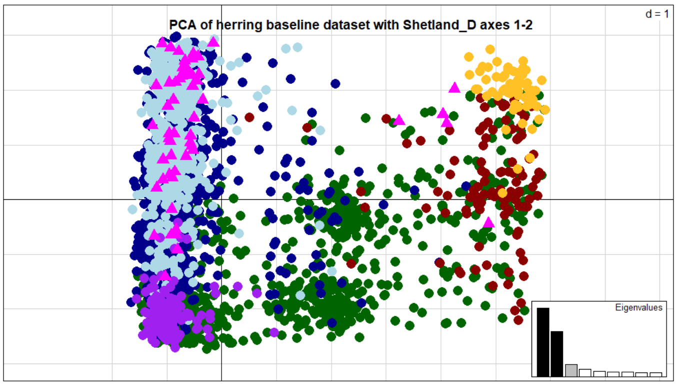


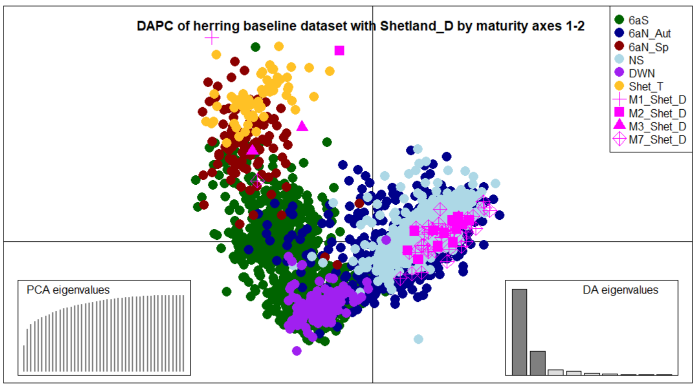


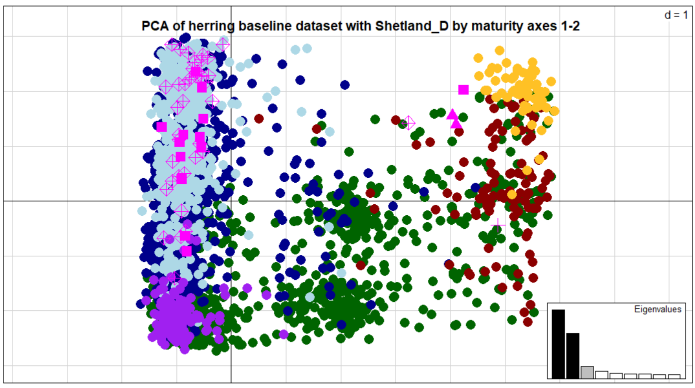


Appendix 6d: Sample D

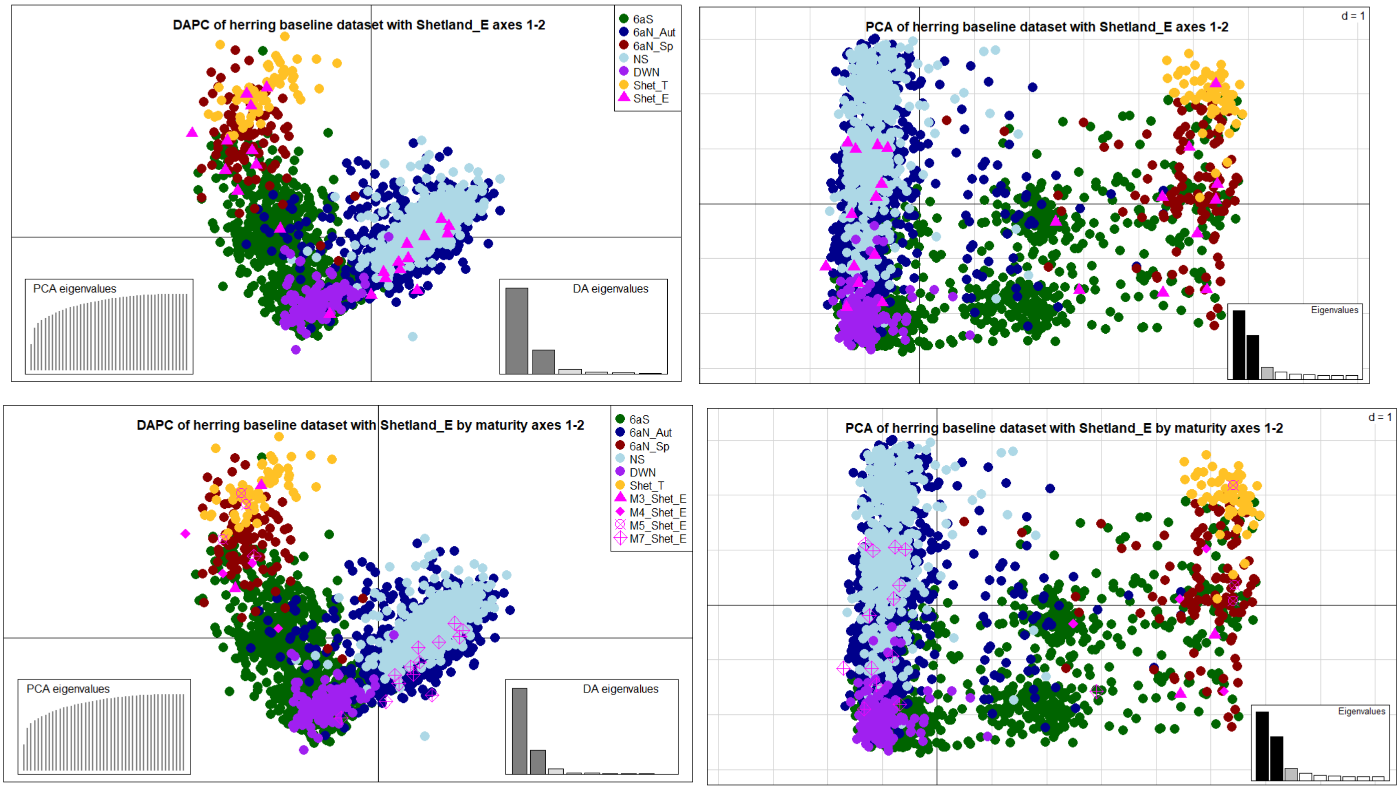




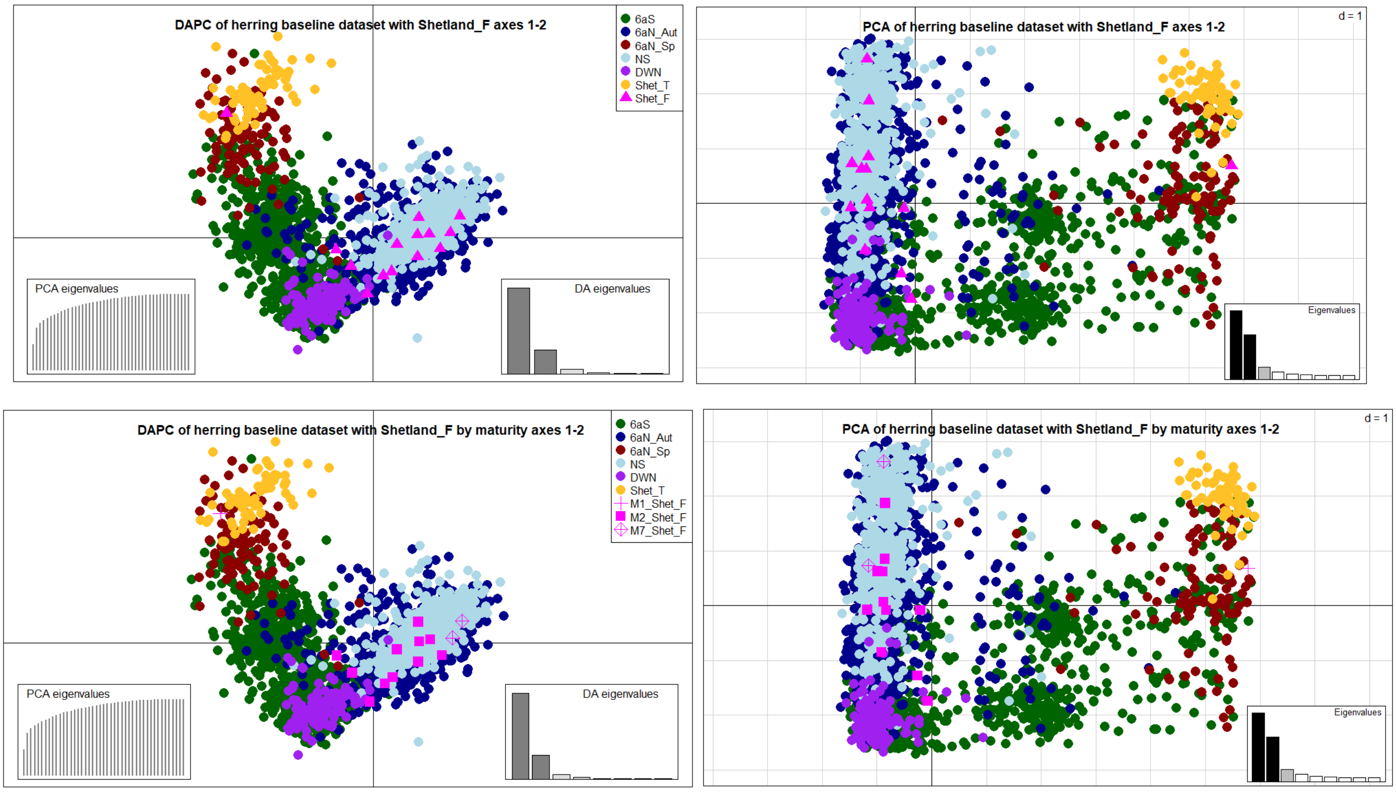




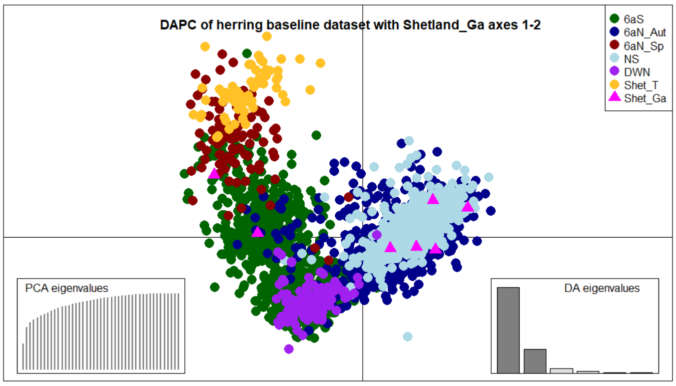
Appendix 6e: Sample E

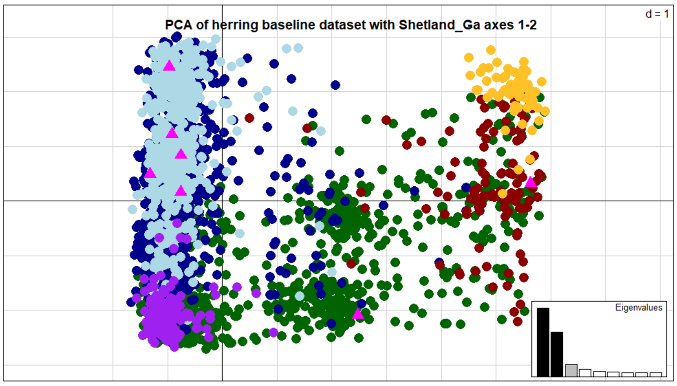


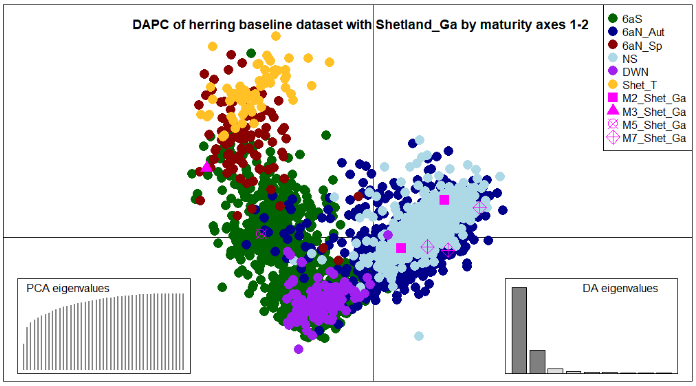
Appendix 6f: Sample F

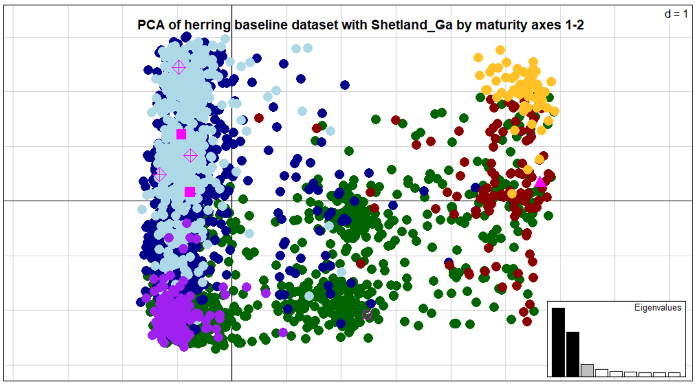


Appendix 6g: Sample Ga

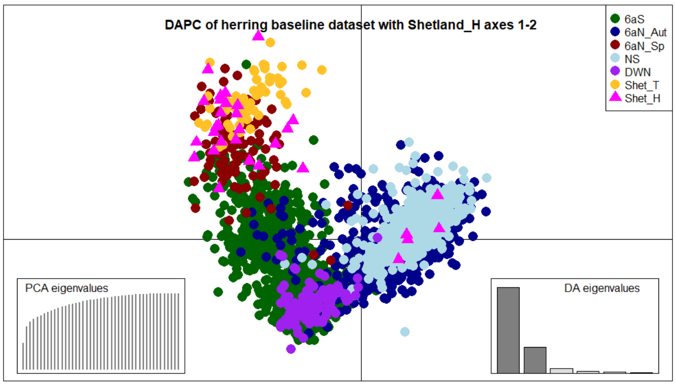


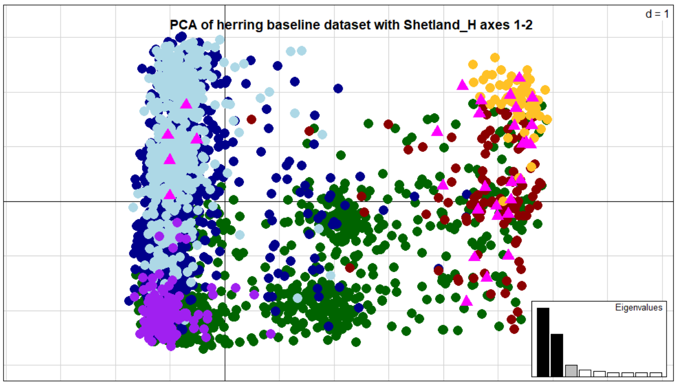


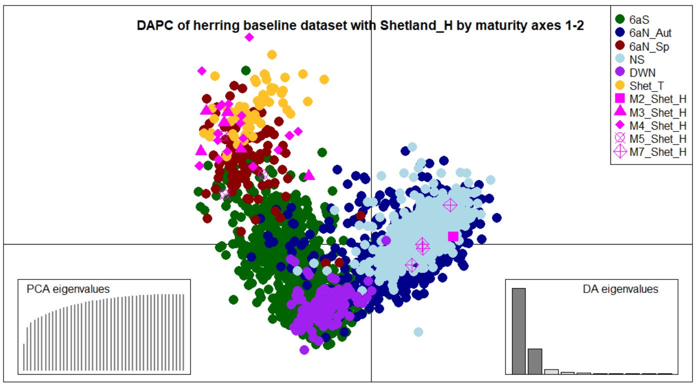


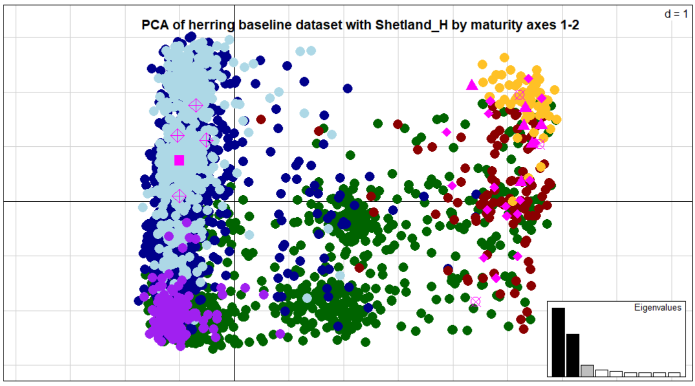


Appendix 6h: Sample H

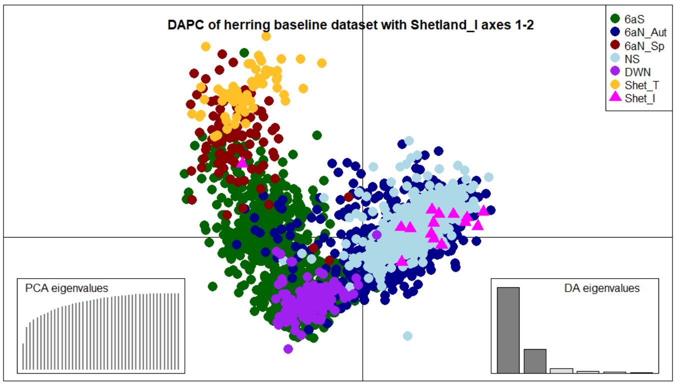


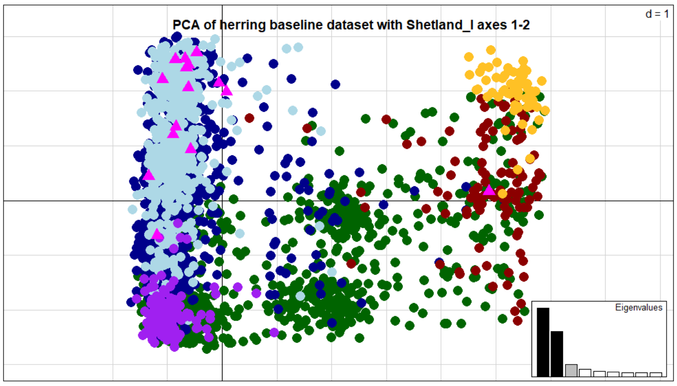


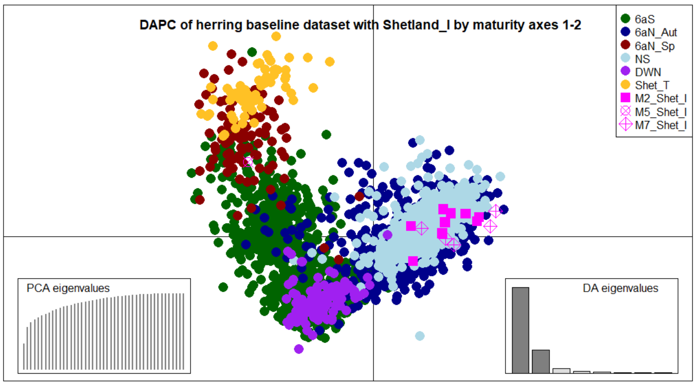


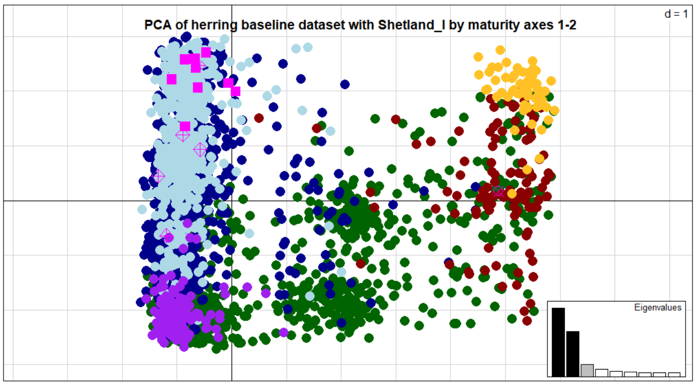


Appendix 6i: Sample I

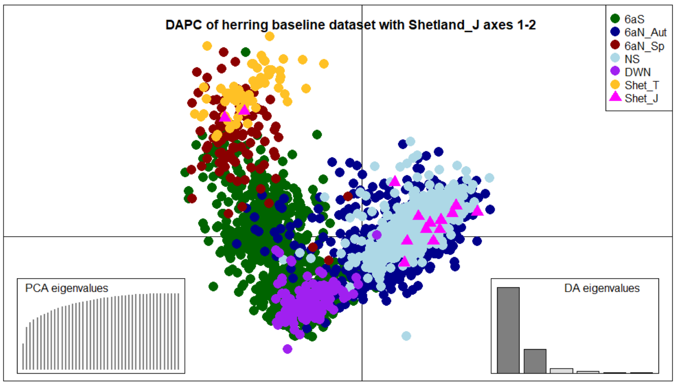


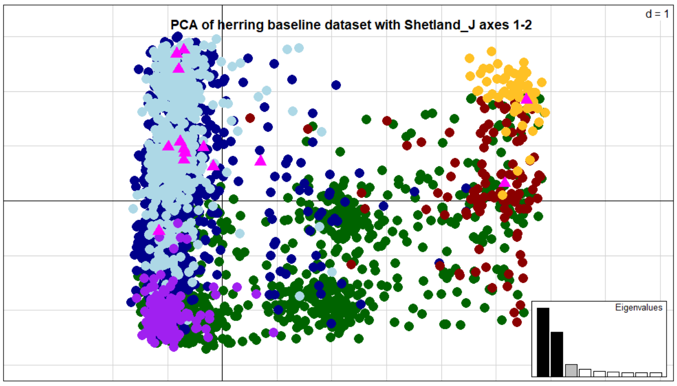


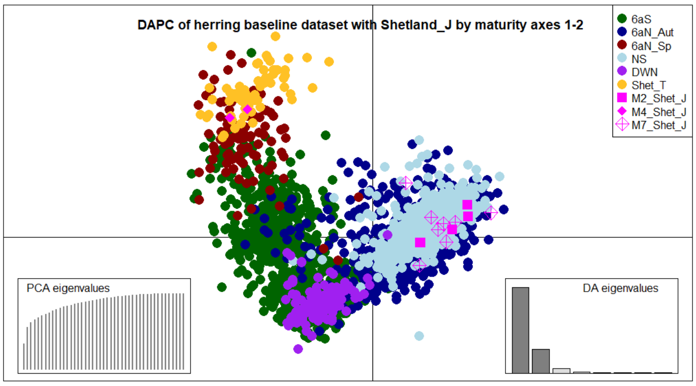


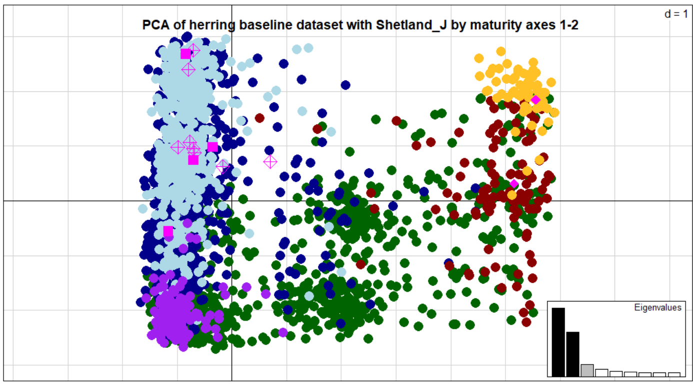


Appendix 6j Sample J

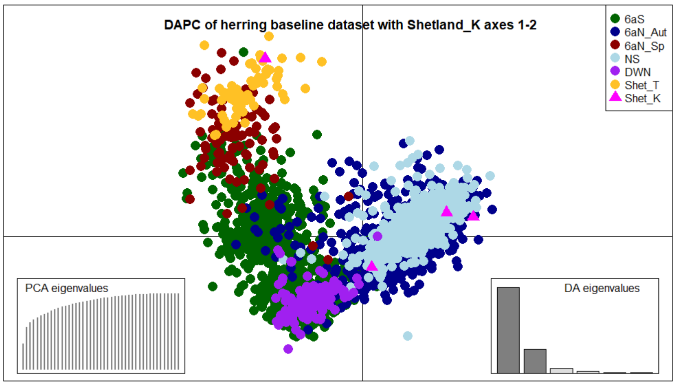


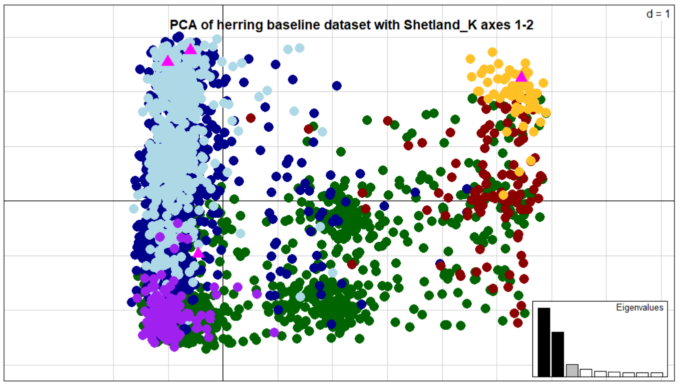


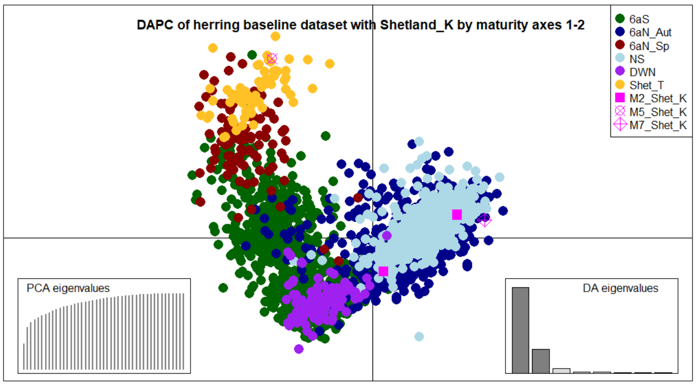


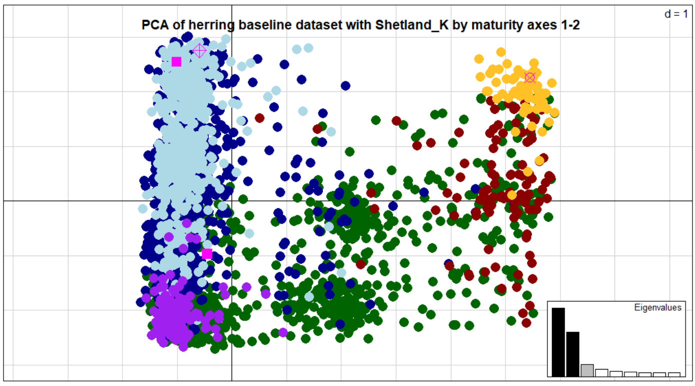


Appendix 6k: Sample K

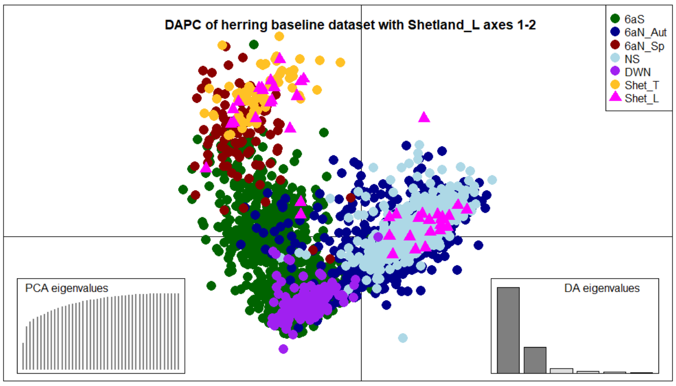


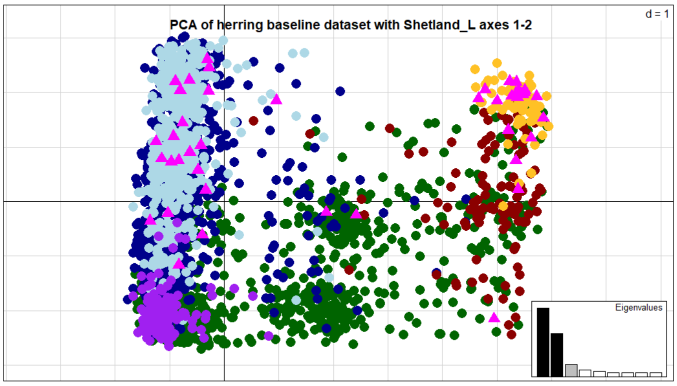


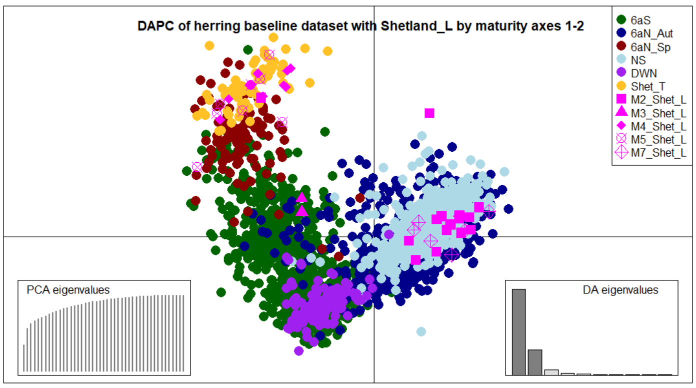


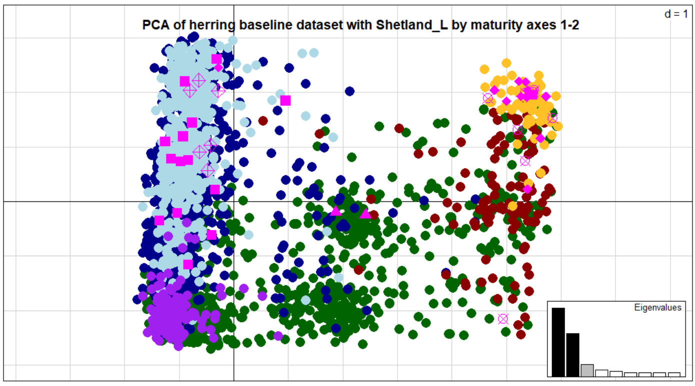


Appendix 6l: Sample L

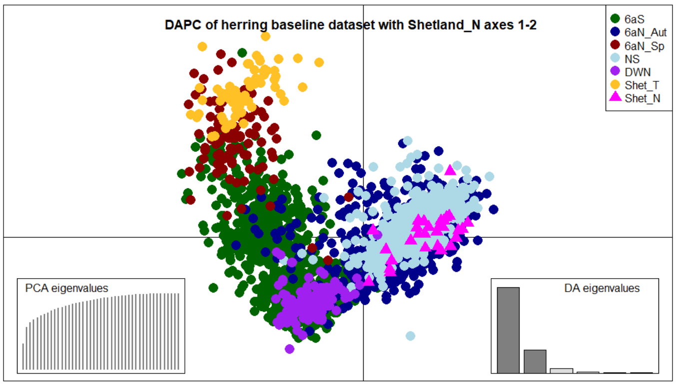


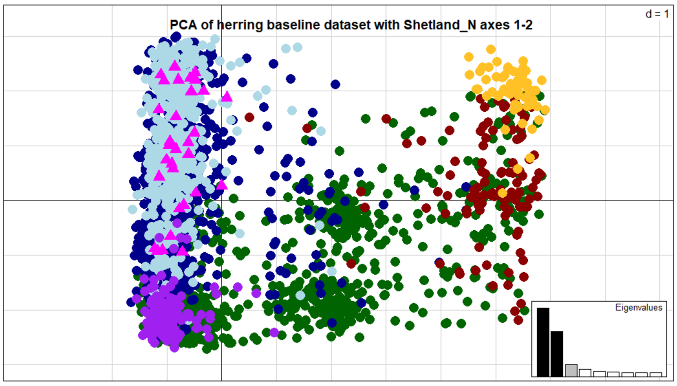


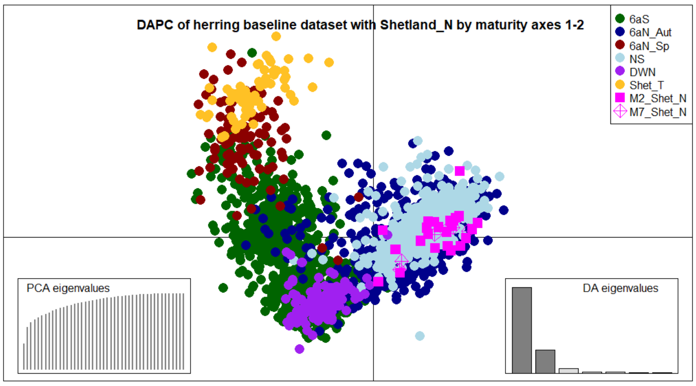


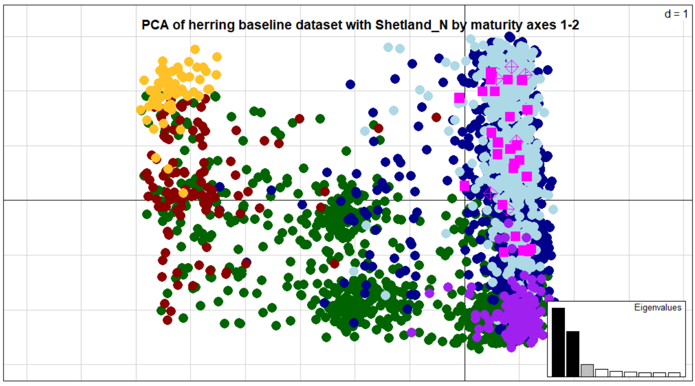


Appendix 6n: Sample N

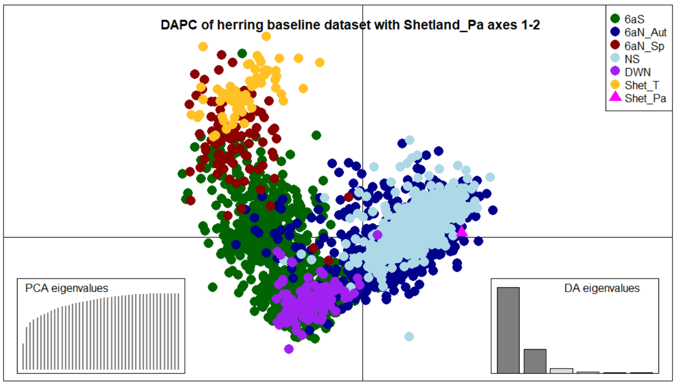


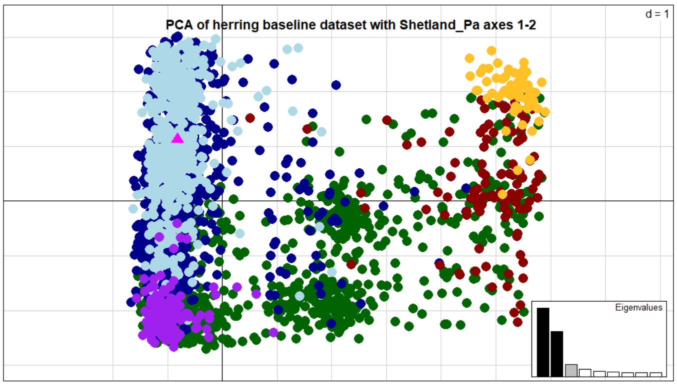


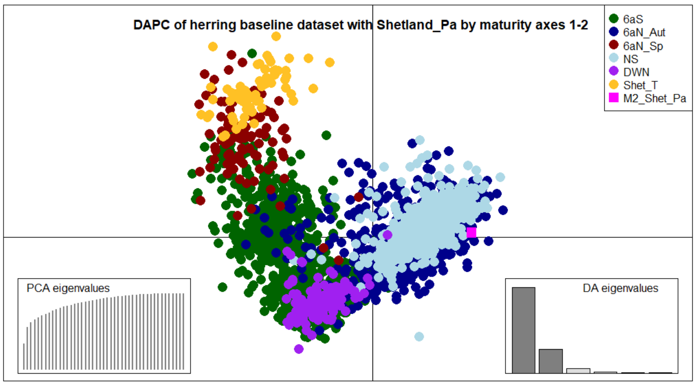


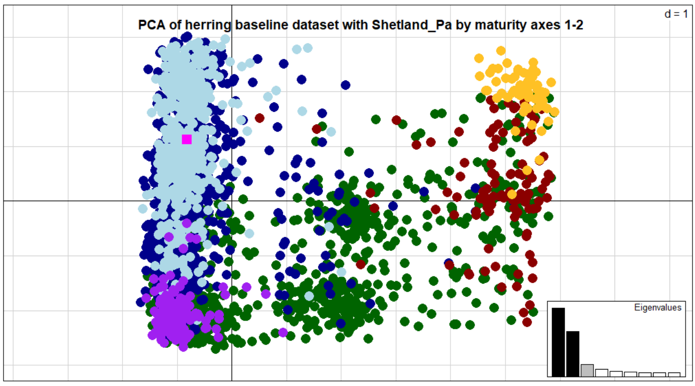


Appendix 6Pa: Sample Pa

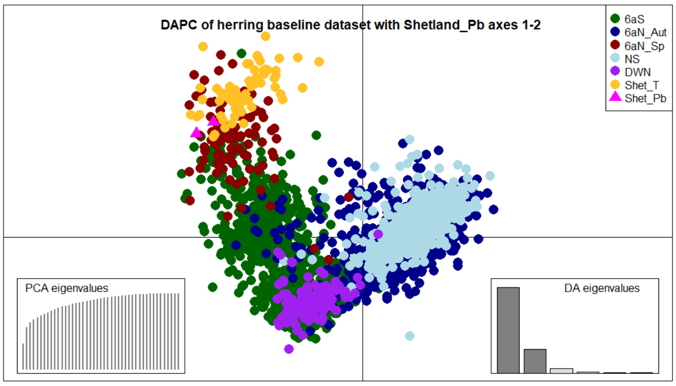


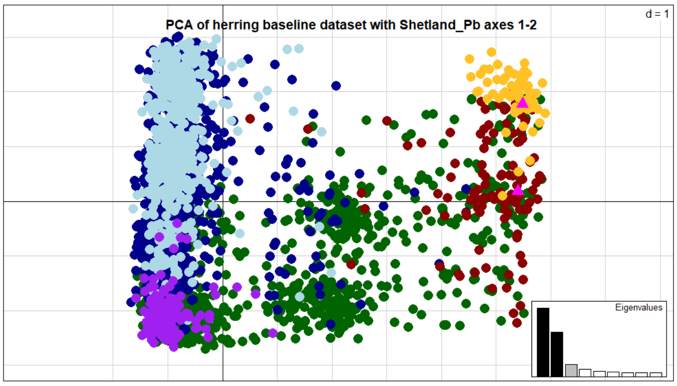


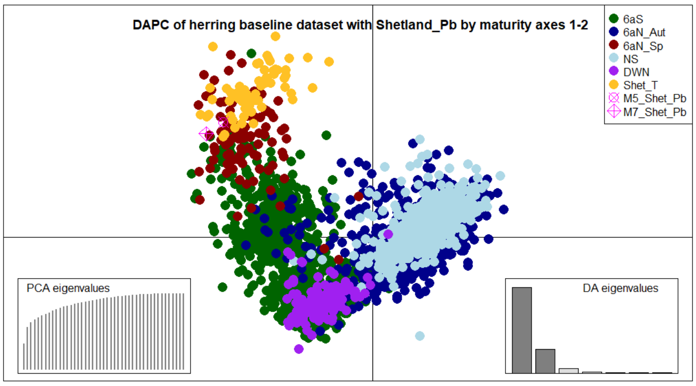


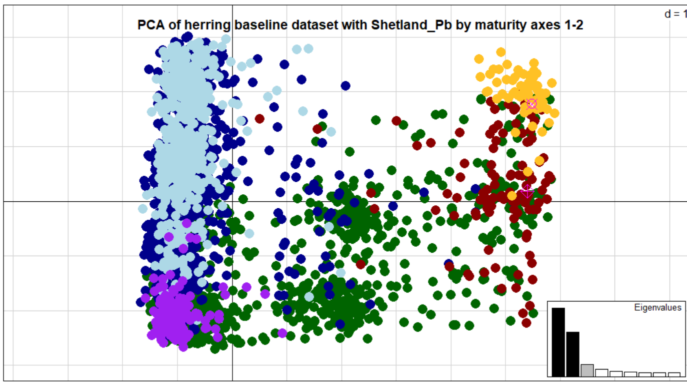


Appendix 6Pb: Sample Pb

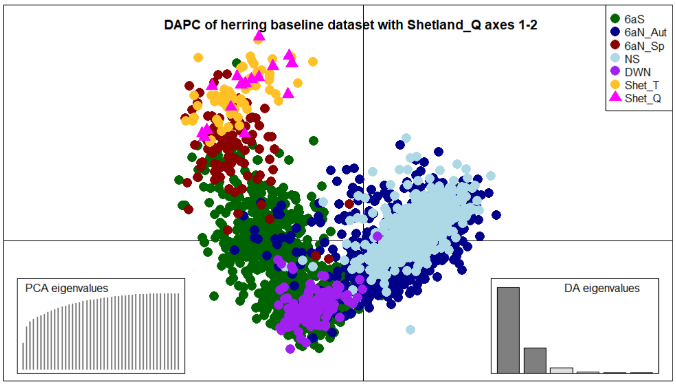


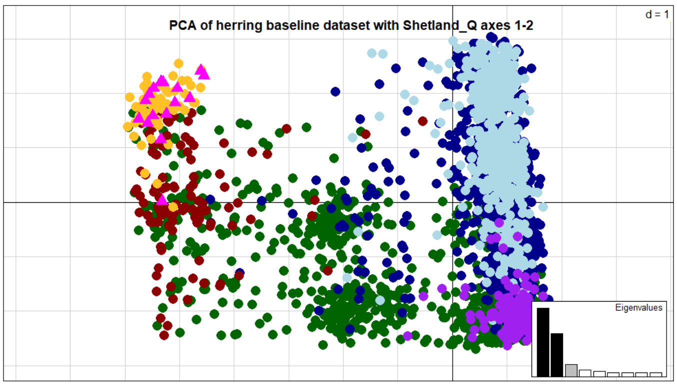


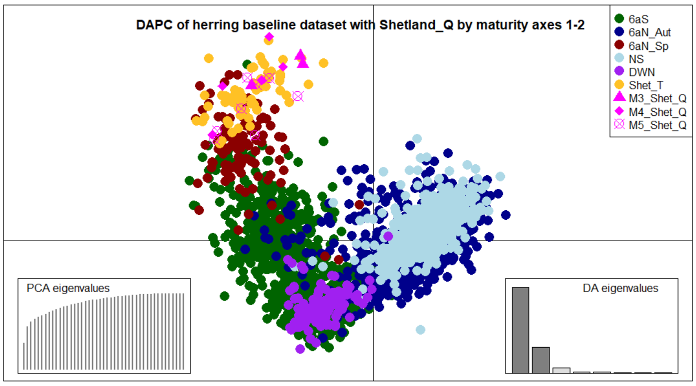


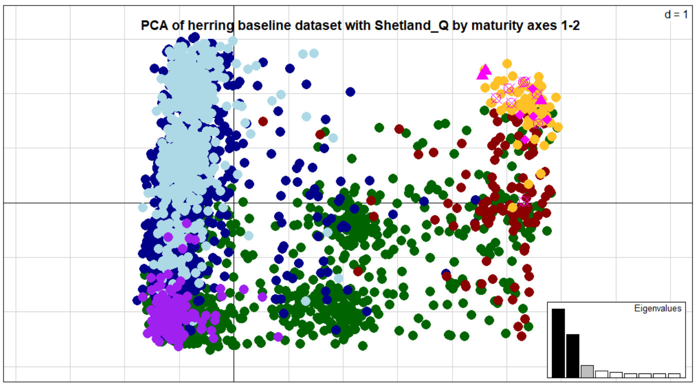


Appendix 6q: Sample Q

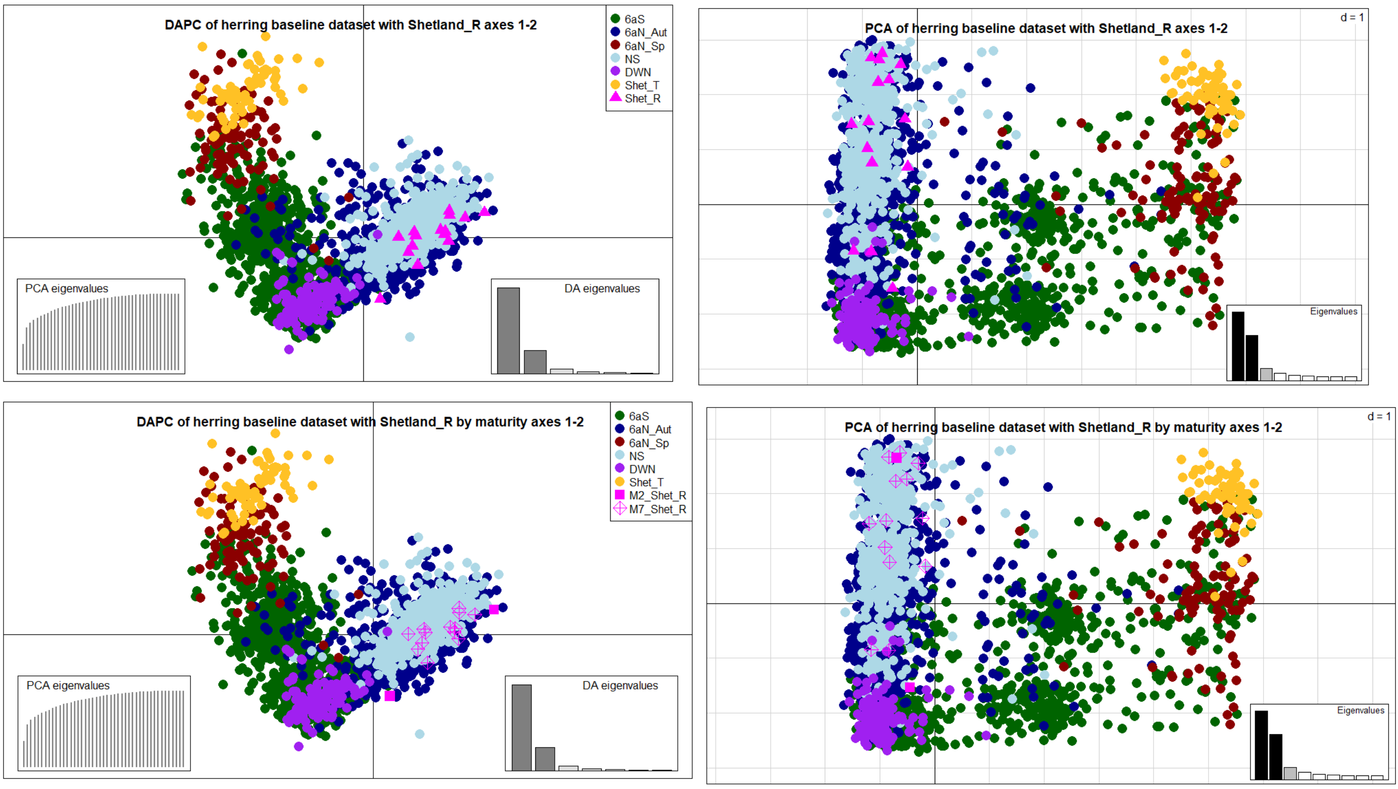




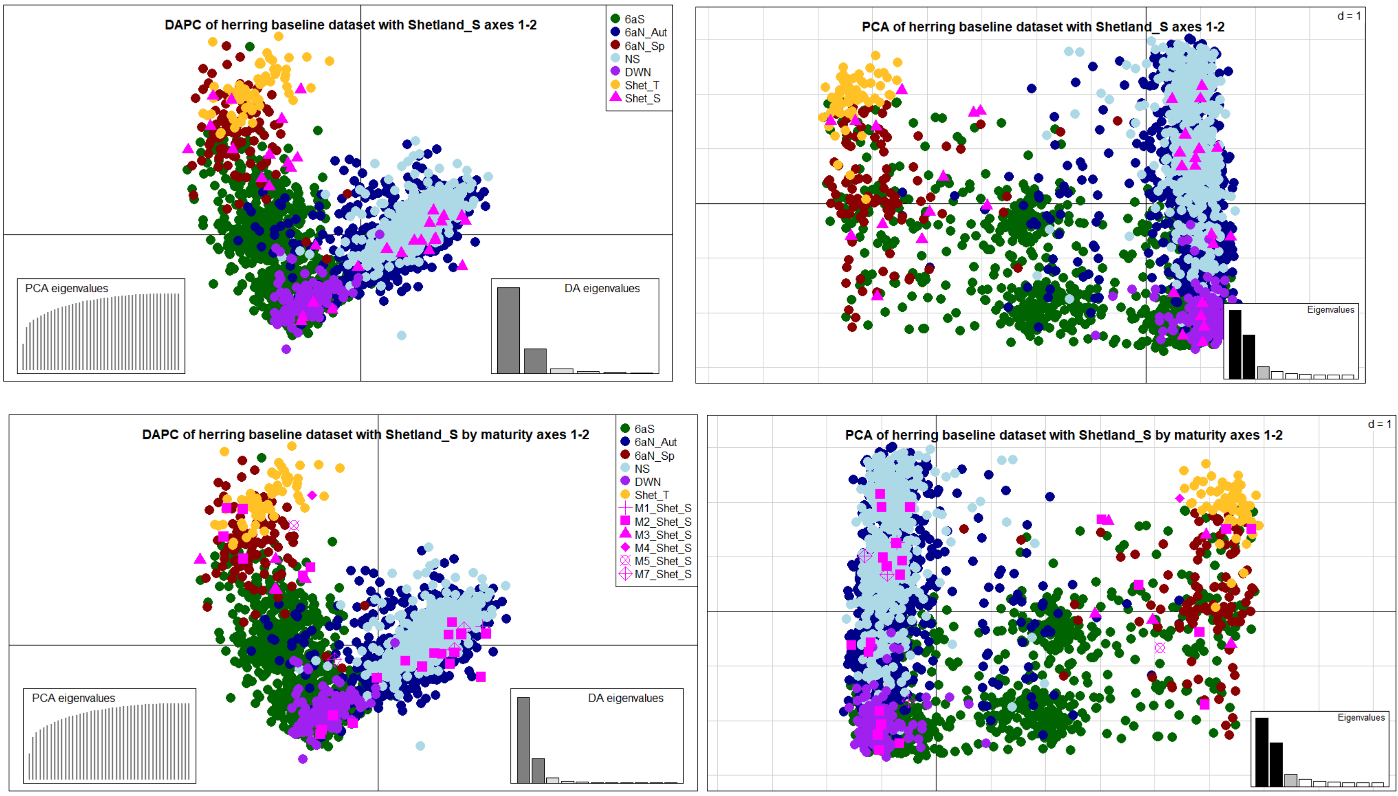




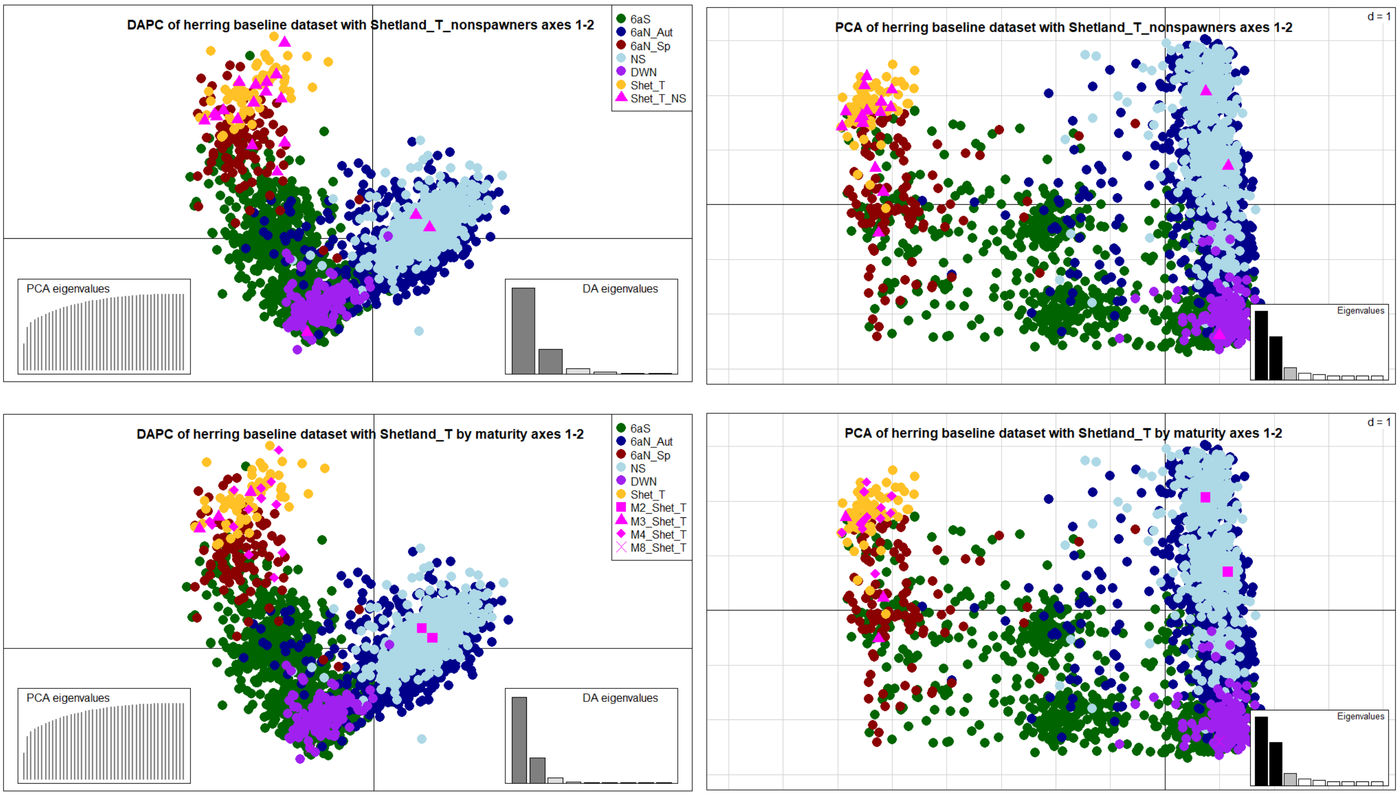
Appendix 6r: Sample R



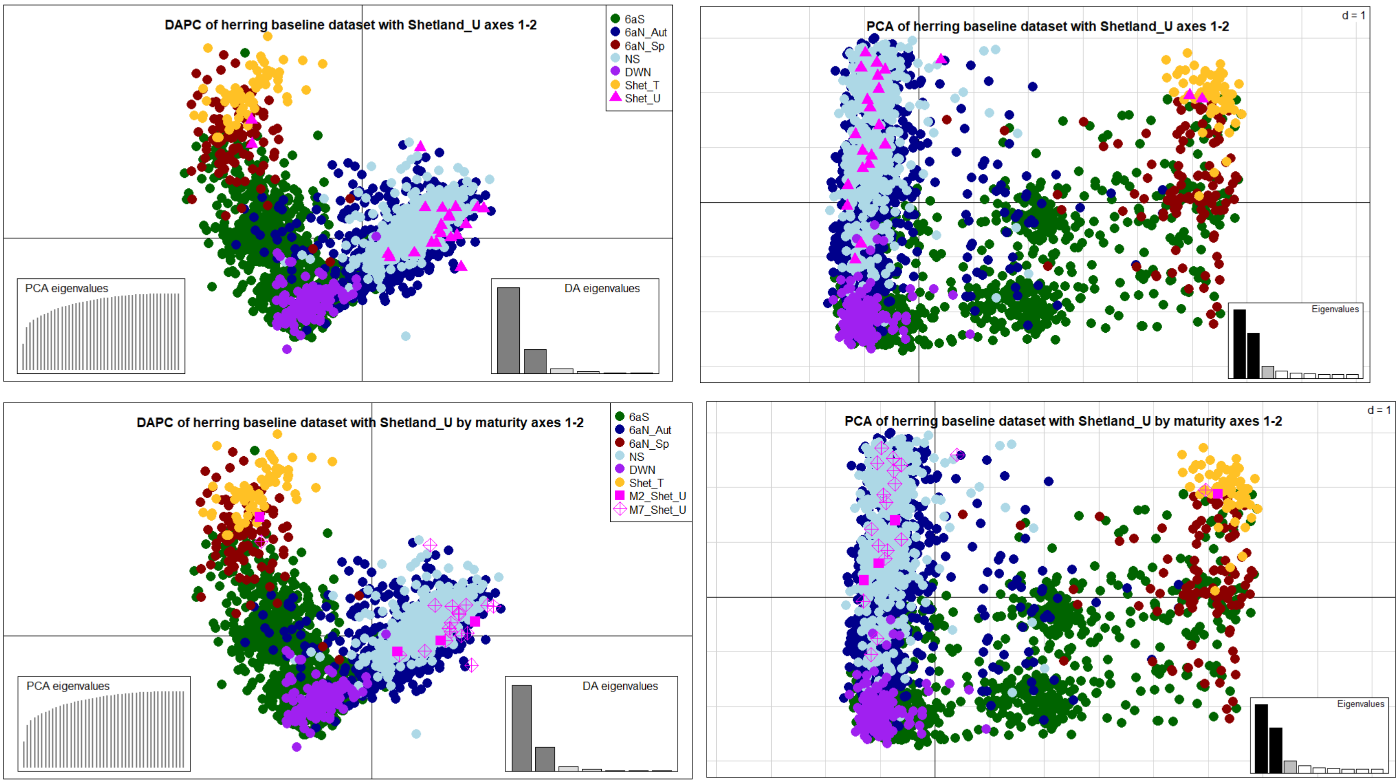
Appendix 6s: Sample S



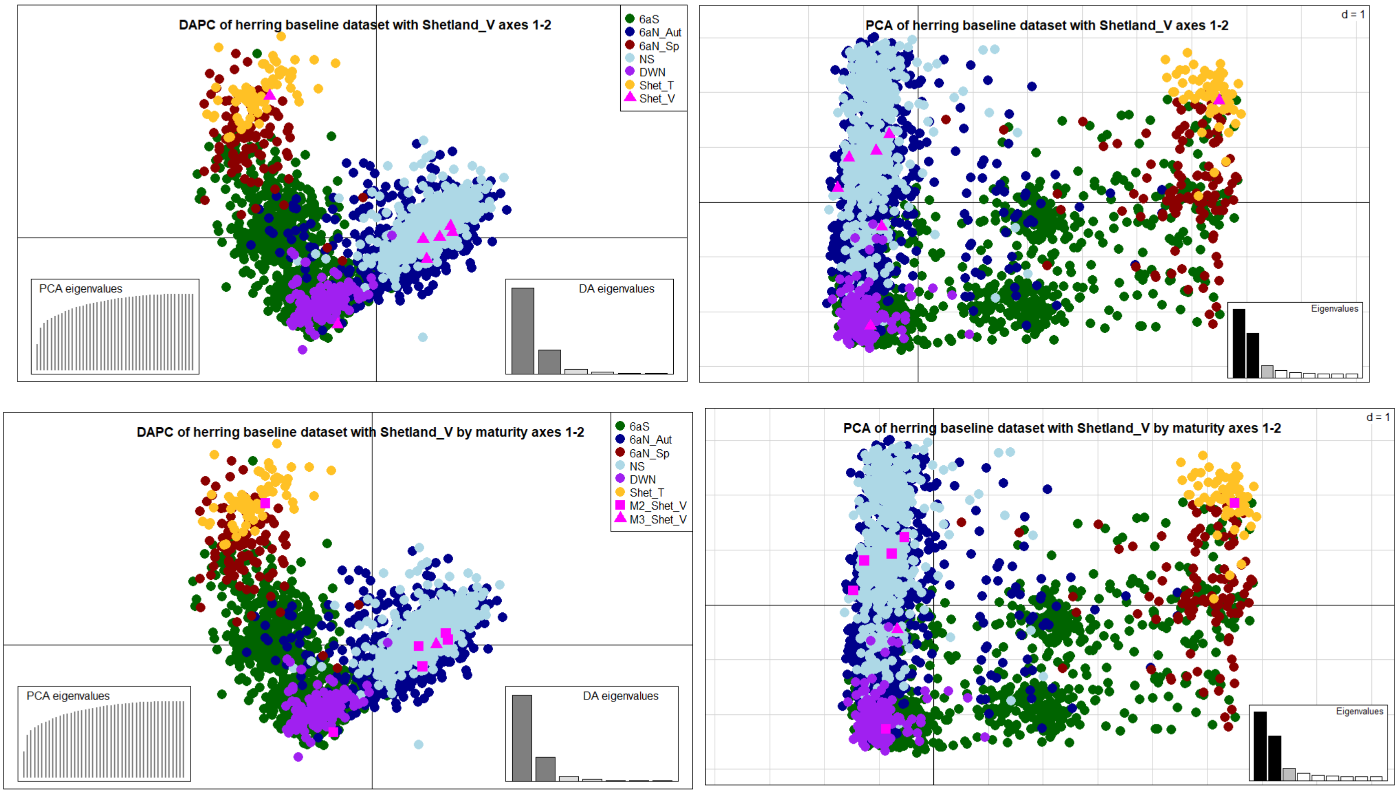
Appendix 6t: Sample T (non-spawners)



Appendix 6u: Sample U

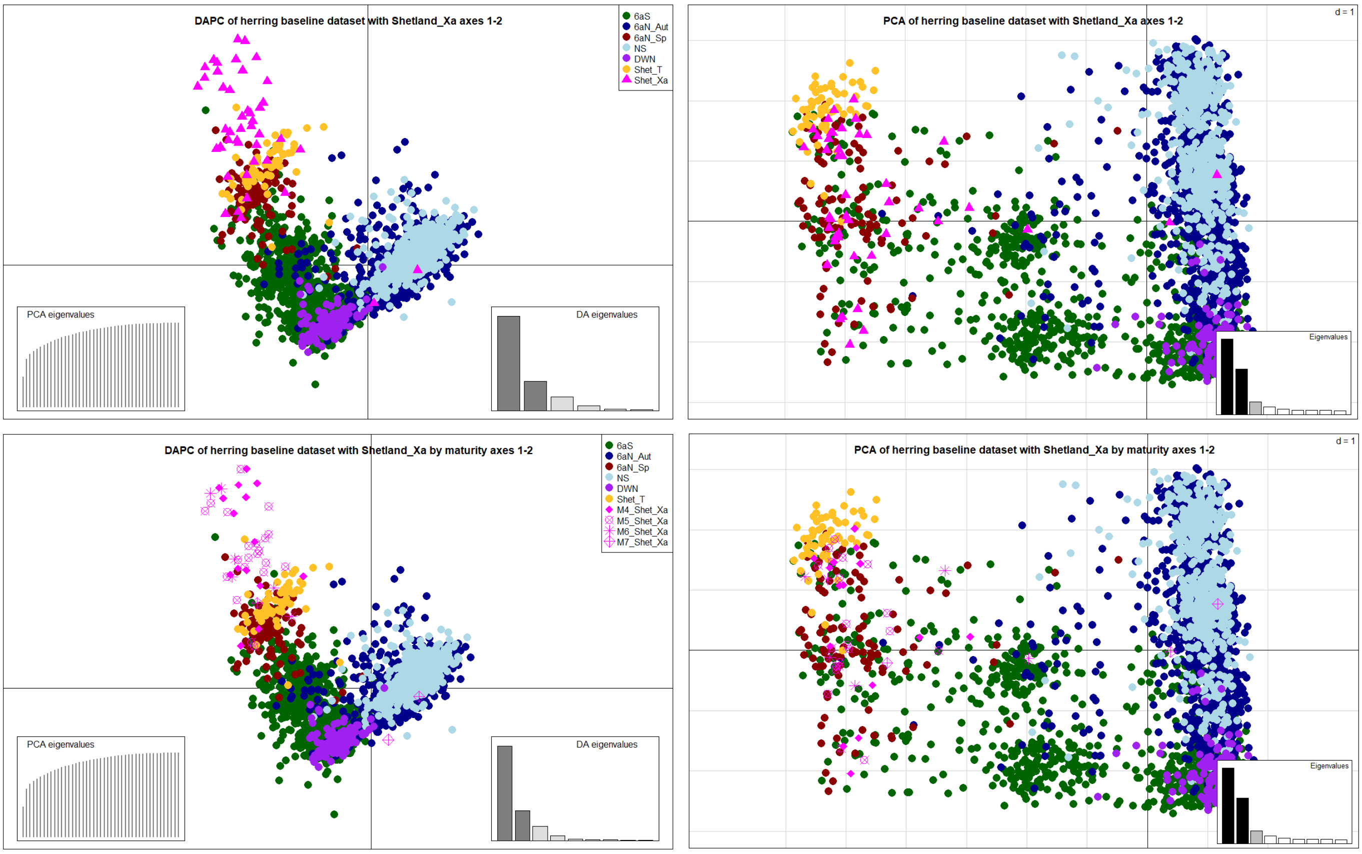


Appendix 6v: Sample V

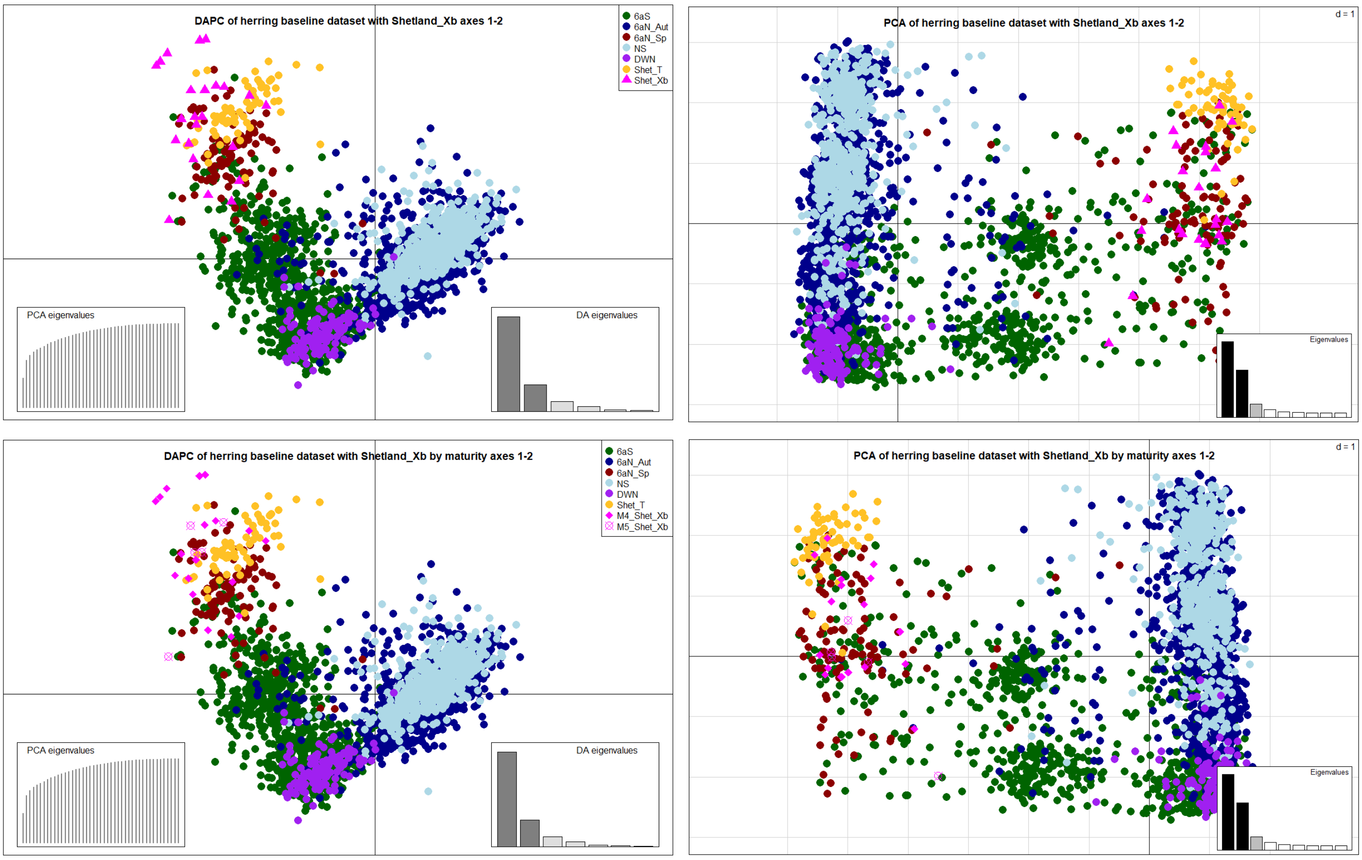


Appendix 6w: Sample W

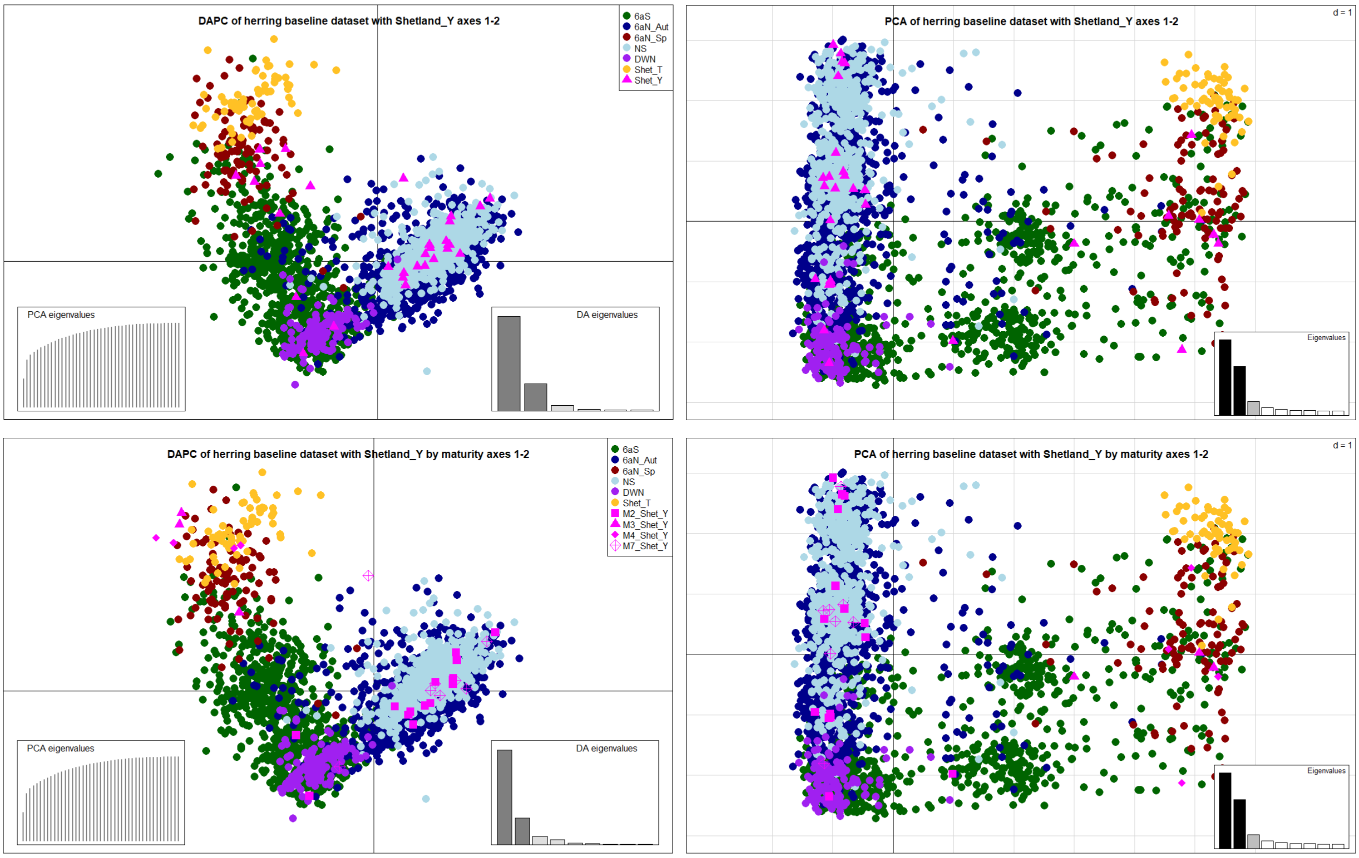
Appendix 6Xa: Sample Xa



Appendix 6Xb: Sample Xb



Appendix 6y: Sample Y



Appendix 6z: Sample Z