Genetic analysis of Zostera sp. samples from the East of England.

A regional case-study of the genetic diversity of Zostera sp. across the UK

April 2024

Natural England Commissioned Report NECR521

www.gov.uk/natural-england

NATURAL ENGLAND

About Natural England

Natural England is here to secure a healthy natural environment for people to enjoy, where wildlife is protected and England's traditional landscapes are safeguarded for future generations.

Further Information

This report can be downloaded from the Natural England Access to Evidence Catalogue. For information on Natural England publications or if you require an alternative format, please contact the Natural England Enquiry Service on 0300 060 3900 or email enquiries@naturalengland.org.uk.

Copyright

Catalogue code: NECR521

Report details

Author(s)

Aline Finger, Genetics and Conservation, Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh, EH3 5LR United Kingdom

Richard Lilley, Project Seagrass, Unit 1 Garth Drive, Brackla Industrial Estate, Bridgend, CF31 2AQ United Kingdom

Natural England Project Manager

Shannon McCloskey, Marine Lead Adviser, East Midlands Area Team, Ceres House, 2 Searby Road, Lincoln, LN2 4DT

Contractor

Project Seagrass, Project Seagrass, Unit 1 Garth Drive, Brackla Industrial Estate, Bridgend, CF31 2AQ United Kingdom

Keywords

Seagrass, Genetics, Biodiversity, Connectivity, Zostera marina, Zostera noltii

Acknowledgements

Tom Cameron and Alice Malcolm-McKay, University of Essex, Wivenhoe Park, Colchester CO4 3SQ

Anna Cucknell and Thea Cox, Zoological Society of London, Outer Circle Regents Park London, NW1 4RY United Kingdom

James Wood and Andy Jayes, Yorkshire Wildlife Trusts, 1 St. George's Place, York, YO24 1GN

William Manning, Environment Agency, Dragonfly House, 2 Gilders Way, Norwich NR3 1UB

Emma Fox, Project Seagrass, Unit 1 Garth Drive, Brackla Industrial Estate, Bridgend, CF31 2AQ United Kingdom

Citation

FINGER AND LILLEY. 2023. Genetic analysis of Zostera sp. samples from the East of England. NECR521. Natural England.

Foreword

Natural England commissioned this report to assist in the production of a genetic database for two seagrass species in the East of England: *Zostera marina and Zostera noltii*. The results of this work fill an important evidence gap by improving our understanding of seagrass connectivity and may be used to inform future restoration of seagrass habitats in the UK.

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Executive summary

There is increasing recognition and interest in seagrass restoration across the UK and globally. This is partly due to the historic losses identified, but also because the restoration of this habitat is known to be important for addressing the twin crises of climate change and loss of biodiversity, on account of the ecosystem services that this habitat provides (e.g., carbon sequestration, increased biodiversity, provision of fish nursery habitat, sediment stabilisation).

However, given the decimation of habitat extent in the UK, emerging restoration techniques are heavily reliant on wild seed harvesting and translocation of seed or seedling, sometimes over large distances. Genetic diversity is therefore an important consideration, and more information is required to inform and best manage seagrass restoration moving forward.

Initial work started in 2020 by Project Seagrass (in collaboration with the Royal Botanical Gardens Edinburgh and NatureScot) has used a geographically broad range of samples to identify genetic clusters of *Zostera marina* around the UK. However, for the East of England, only a single sample from Essex existed and this was identified as a genetic outlier, with the next nearest samples located in the Solent (to the south and west) and Lindisfarne (to the north).

In addition to this regional data paucity, no such genetics data currently exists for *Zostera noltii* anywhere in the UK.

An improved understanding of Zostera sp. genetic clusters around the UK will be fundamental in supporting appropriate seagrass restoration ambitions. Accordingly, although the English east coast holds high potential to support seagrass restoration, further sampling was required to add to the East of England *Z. marina* genetics to the database already started, and to develop a similar database for *Z. noltii.*

This project collected samples of both Zostera sp.. *Z. marina* samples were collected and analysed according to a pre-existing methodology to help identify potential geographic

limits for the apparently isolated genetic cluster identified in Essex. *Z. noltii* samples were collected and used to develop the methodology using different genetic markers, so that a similar UK wide database could be developed for this species.

Whilst sampling was focussed on the Norfolk and Suffolk area (and was dependent on tissue sample availability from existing seagrass beds), additional samples were collected from areas that are geographically adjacent to the target area, and of importance in the wider context of seagrass restoration across the UK.

This work has contributed to a spatially representative database of *Z. marina* genetic diversity across the UK, and to a methodology for testing of *Z. noltii* and the beginnings of a genetics database for this species. This has led to an improved understanding of genetic clusters and diversity of UK seagrasses, which will be fundamental in supporting informed seagrass restoration ambitions moving forward.

Contents

Introduction

Currently UK seagrass populations are considered degraded following significant declines (Green et al., 2021) and are an OSPAR threatened and / or declining habitat. Seagrass beds are a protected feature in a number of Marine Protected Areas (MPAs) in the northeast Atlantic.

Research has shown that restoring seagrass beds can also restore the associated ecosystem services that they provide (Orth et al. 2020), although protecting existing beds is a priority. An understanding of the genetic relatedness of Zostera sp. populations is required to inform restoration approaches, such as deciding on donor beds but also can inform spatial management such as which areas should be a priority for protection.

Seagrass (Zostera sp.) connectivity

Very little is known about seagrass (Zostera sp.) connectivity in the UK. Early genetic analysis of *Z. marina* has shown no significant differences between populations in Wales and Southern England (Nahaa and Bull, In Prep). Individual genetic assignment of floating, detached seed bearing reproductive shoots also indicate dispersal among populations ca 50 km apart (Reusch, 2002). However, no comprehensive assessment of seagrass genetic diversity and connectivity exists for seagrass beds in the north-east Atlantic.

Information on the genetic makeup of East of England seagrass will help inform site suitability assessments for potential seagrass restoration trials and the viability of seed translocation from other parts of the country. Furthermore, understanding how meadows are connected will help to inform MPA management (e.g., population declines, management unit and bottlenecks etc.).

Seagrass (Zostera sp.) distribution

Zostera sp. are widely, but patchily distributed throughout the British Isles. Major concentrations exist in south-west of England and along the west coast of Scotland, including the Hebridean islands. The species has been recorded in the majority of coastal vice-counties in Britain including the northern isles of Orkney and Shetland as well as the majority of coastal vice-counties in Ireland. Its distribution in Wales is relatively sparse in comparison to Ireland, Northern Ireland, Scotland and England (Kay, 1998). Zostera sp. have also been recorded in the Channel Islands, Isles of Scilly and the Isle of Man. Knowledge of the extent of Zostera sp. in the British Isles has always been and continues to be poor, where both a lack of in-depth studies and taxonomic confusion have contributed to this lack of understanding.

Zostera marina

Z. marina is a species of seagrass known by the common name ‘Eelgrass’ and it is the most dominant seagrass species in temperate northern hemisphere (den Hartog 1970). *Z.marina* is found throughout the north Atlantic, north Pacific and in the Mediterranean and Black Seas. *Z.marina* leaves can grow up to 12mm wide and may reach over 1.5m long. Morphological characteristics vary extensively across its range as a result of environmental conditions (Phillips and Menez, 1988), to the point at which it was previously thought there may be more than one species, giving rise to the name *Z. angustifolia* (Percival et al, 1996 ; Provan et al, 2008). However, it is currently thought that *Z.angustifolia* is merely a phenotypic variant of *Z. marina* (see Becheler et al, 2010) and thus an ‘ecotype’ (Den Hartog 1970 ; De Heij and Neinhuis, 1992 ; Becheler et al, 2010), rather than a distinct species.

Zostera noltii

Z.noltii is a species of seagrass known by the common name ‘Dwarf eelgrass’. It is found in shallow coastal waters and lagoons along the Atlantic Coast of Europe, in the Mediterranean Sea, Black Sea, Caspian Sea and Aral Sea and on islands in the Atlantic off the coast of northwest Africa. *Z.noltii* leaves grow up to 2mm wide and may reach 80cm in length when found subtidally in the lagoons of the French Mediterranean. However, in the UK they are found intertidally and more likely to be much shorter, often between 10-20cm in length.

Aim

The aim of this report is to describe the genetic diversity and differentiation of seagrass samples collected in the East of England, using both methods from Sweden (see Jahnke et al, 2020) and samples from the rest of the UK as reference material.

Method

Genetic sampling

All sites were accessed on foot and all seagrass tissue samples were collected between September 2022 and November 2022. A single seagrass leaf was taken from 40 plants at each site (32 were used in analysis but 40 provided 8 as a backup in case some DNA extractions failed). Leaves were only picked that were attached to the plant to allow certainty of origin. GPS coordinates were taken at the approximate centre of each site. Samples were collected evenly across the entire population (centre and extremities as far as possible on foot) to make sure that we capture a high proportion of the diversity while avoiding resampling the same individual. Where possible, and variable depending on population size, a distance of several meters between samples was allowed to avoid sampling clones.

After collection, leaves were rinsed in freshwater and raked with a sterilised razor blade to remove epiphytic algae. Each collected leaf was then placed into an empty teabag and the teabag marked with a unique number that linked to the sampled population (e.g., “Spurn Point, Leaf 23”). The teabag was then closed and the leaves dried. Drying was done either in a warm room or over a heater (warm but not hot, no direct sunlight). Samples were usually dried overnight. Alternatively, leaves could be dried using silica gel. Samples could also be stored without drying by freezing them immediately after collection. Once dried, the seagrass tissue samples were then sent to the Royal Botanic Garden Edinburgh for genetic analysis (RBGE).

Genetic analysis

DNA extraction, Polymerase Chain Reaction (PCR) amplifications, and genotyping
DNA was extracted from the leaves using the QIAGEN DNeasy 96 Plant Kit, following the manufacturer’s protocol. All samples were screened at a total of 40 nuclear microsatellite loci (described in Reusch 2002 and Jahnke et al. 2020) for *Z. marina* and 9 nuclear microsatellite loci for *Z. noltii* (described in Coyer et al. 2004). The forward primers of “Reusch 2002” and “Coyer et al. 2004” were labelled with fluorescent labels for later visualisation on a sequencer. The “Jahnke et al. 2020” markers were labelled using the M13-tag technique as described by Schuelke (2000).

PCR protocols initially followed the methodology described in Reusch 2002, Jahnke 2020, Coyer et al. 2004. To reduce the number of PCR we included several loci in the same PCR reaction and used the QIAGEN PCR Multiplexing Kit Qiagen multiplex kit, Qiagen, Hilden, Germany; (https://www.qiagen.com/us/products/discovery-and-translational-research/pcr-qpcr-dpcr/pcr-enzymes-and-kits/end-point-pcr/qiagen-multiplex-pcr-kit?catno=206145). We used the PCR cycling program recommended for the QIAGEN kit.

All PCR reactions were performed on a Biorad Tetrad 2 thermal cycler and then sent to Dundee Sequencing Services (https://dnaseq.co.uk/services/fragment-analysis) for

fragment analysis on an Applied Biosystems 3730 DNA analyser. LIZ-500 labelled internal size standard (Applied Biosystems, Foster City, California, USA) was added to each sample to size fragments. The data generated was analysed with the software GeneMarker (SoftGenetics, Pennsylvania, USA) scoring electropherograms manually.

Population genetic analysis

Of 40 tested nuclear microsatellite markers for *Z. marina*, 36 were polymorphic and were used for further analysis. All 9 markers were polymorphic and therefore used for *Z. noltii*.

Number of alleles (NA), number of effective alleles (NE), observed and expected heterozygosity (HO, HE), number of private alleles (PA) and inbreeding coefficients (FIS), pairwise comparisons between populations (Jost's D), and Principal Component analysis based on genetic distance were calculated using GenAlEx 6.5 (Peakall and Smouse 2006).

Clonal growth has not been analysed systematically, but when samples scored the same alleles at all 36 microsatellite markers for *Z. marina* and 9 for *Z. noltii*, it was assumed that these samples originated from the same genetic individual. For further analysis we discarded all clones from the dataset.

Results

A total of eight populations of Zostera sp. were sampled; two *Z.marina* populations and six *Z.noltii* populations.

Table 1. Sampling locations for *Z. marina and Z noltii*. For exact population locations see Fig. 2 and 3.

Location (North to South)	Date	Species
Spurn Point (East Riding of Yorkshire)	27th October 2022	*Z.noltii*
Wells-next-the-Sea (Norfolk)	24th September 2022	*Z.noltii*
Orwell (Suffolk)	1st September 2022	*Z.noltii*
Stour (Suffolk-Essex border)	2nd September 2022	*Z.noltii*
Foulness (Essex)	25th November 2022	*Z.noltii and Z.marina*
Seasalter (Kent)	18th October 2022	*Z.noltii and Z.marina*

Zostera noltii

A total of six populations and 192 leaves were analysed, with 32 leaves per population. Of these, 133 were identified as unique genotypes. Genetic analysis was therefore based on these 133 samples.

Table 2. Genetic variability of all *Z. noltii* populations using 9 microsatellite loci. Abbreviations: N_A, Mean number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P_A, total number of private alleles; F_{IS}, Inbreeding coefficient, * = p < 0.05.

Population	# Leaf samples	# Unique genotypes	N_A	H_O	H_E	P_A	F_{IS}
Seasalter	32	19	3.11 (±0.74)	0.28 (±0.10)	0.29 (±0.10)	2	0.02
Stour	32	19	4.11 (±0.70)	0.39 (±0.10)	0.37 (±0.08)	4	0.07
Wells-next-the-sea	32	32	4.11 (±0.51)	0.49 (±0.07)	0.50 (±0.07)	4	0.11
Spurn Point	32	22	1.89 (±0.31)	0.15 (±0.05)	0.19 (±0.07)	2	0.17*
Foulness	32	25	4.33 (±1.14)	0.33 (±0.10)	0.35 (±0.10)	2	0.04
Orwell	32	16	3.67 (±0.69)	0.49 (±0.12)	0.41 (±0.10)	2	-0.19*
All	**192**	**133**	**3.54 (±0.31)**	**0.35 (±0.04)**	**0.35 (±0.04)**	**NA**	**0.04**

Clonal growth is present in all populations, except Wells-next-the-sea, but this is not the dominant mode of reproduction. Highest clonal growth was found in Orwell, where only 16 of 32 analysed leaf samples were from unique genetic individuals. Using a larger number of microsatellite markers would allow for better data resolution and, particularly when genetic diversity is low, such an increased resolution might improve the ability to identify individuals and consequently decrease the number of (assumed) clones. The clonal data should therefore be treated carefully.

Genetic diversity (H_E and N_A) varies between sites and was lowest for population Spurn Point (lowest H_E). Highest diversity was found in populations Wells-next-the-Sea and Orwell (see Table 2). Significant inbreeding was only detected in population Spurn Point, while significant outbreeding was found for Orwell which could indicate substantial gene flow for that population. Private alleles (alleles that are only found in one population) were present in all populations, which demonstrates that populations keep unique genetic traits despite gene flow between populations.

Table 3. Pairwise Jost'd D values for 6 *Z. noltii* populations.

	Seasalter	Stour	Wells-next-the-sea	Spurn Point	Foulness	Orwell
Seasalter	0.00	0.08	0.18	0.39	0.03	0.10
Stour	0.08	0.00	0.16	0.32	0.04	0.06
Wells-next-the-sea	0.18	0.16	0.00	0.27	0.14	0.17
Spurn Point	0.39	0.32	0.27	0.00	0.28	0.28
Foulness	0.03	0.04	0.14	0.28	0.00	0.03
Orwell	0.10	0.06	0.17	0.28	0.03	0.00

Gene flow seems to be extensive across populations, particularly between the geographically close Seasalter, Stour, Foulness and Orwell populations as indicated by low pairwise Jost's D values (Table 3). Spurn point is the most differentiated population, showing the highest pairwise Jost's D values with all other population.

A principal component analysis groups the samples into three main genetic clusters, confirming the Jost's D pairwise values and the geographic location of populations (see Fig. 1). The three clusters represent 1: Seasalter, Stour, Foulness and Orwell, 2: Wells-next-the-sea and 3: Spurn Point.

While some genetic structure exists between populations roughly representing the geographic locations of populations, the data also indicates frequent gene flow between populations. Overall Jost's D values are low, and inbreeding is rare in populations which indicates that populations are not genetically isolated.

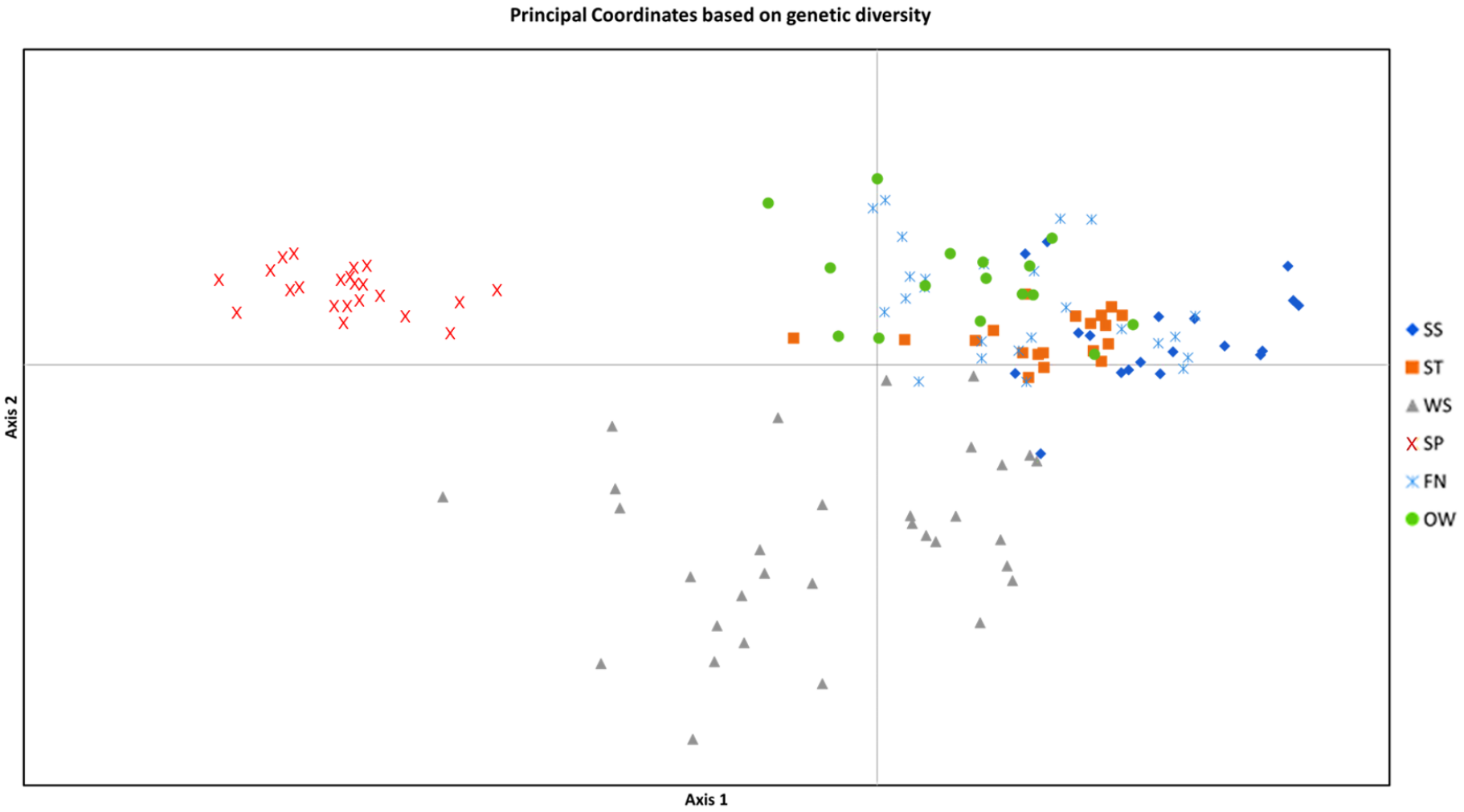

Figure 1. Principal component analysis of 6 *Z. noltii* populations. SS: Seasalter, ST: Stour, WS: Wells-next-the-sea, SP: Spurn Point, FN: Foulness, OW: Orwell

Figure 2. Map of sampled *Z. noltii* populations of and genetic clusters represented as different coloured circles. Geographically close populations Orwell, Stour, Foulness and Seasalter form one genetic cluster. Google Maps.

Zostera marina

Two populations from Seasalter (32 samples) and Foulness (11 samples) were analysed and included in the analysis of a wider dataset including populations from across the UK. Out of 32 collected leaf samples, 23 unique genotypes were identified for Seasalter, and three unique genotypes were identified out of 11 samples from Foulness. Both populations have a low genetic diversity compared to other UK population (H_E = 0.12 ± 0.03 SE and H_E = 0.22 ± 0.05 SE, respectively) and no inbreeding was detected in these populations.

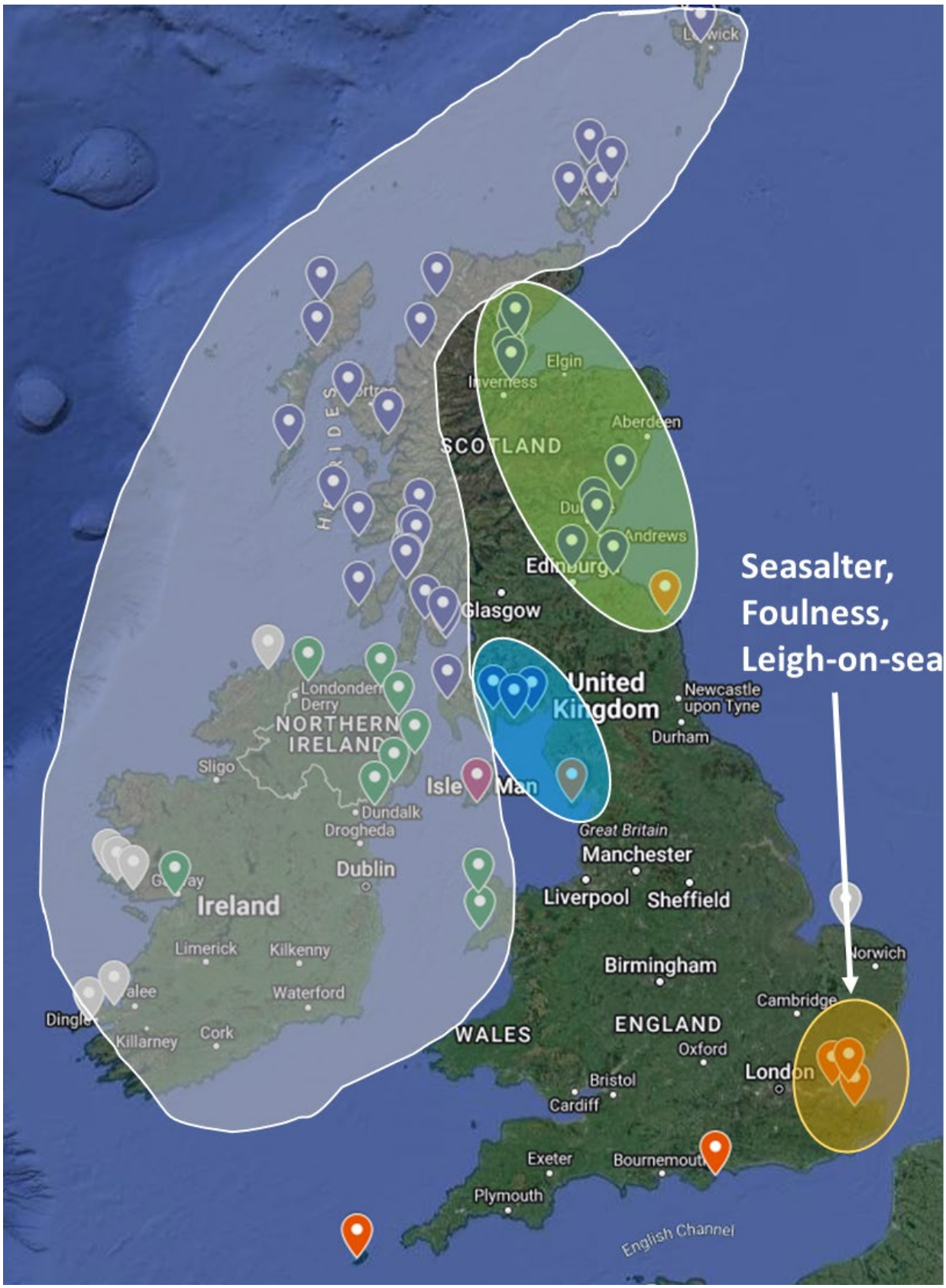

Figure 3. Map of sampled Z. marina populations (extended dataset from wider UK Z. marina study). Circled are the rough genetic clusters (preliminary analysis) across the UK including the southeast England populations, formed of Leigh-on-sea, and (for this study analysed) Seasalter and Foulness. Google Maps.

The full data cannot be presented as it's not yet available. However, a preliminary principal component analysis and pairwise Jost'd D values of the current UK dataset containing 54 populations groups the southeast England populations into one genetic cluster, representing the geographic location of Leigh-on-sea, Seasalter and Foulness (see Fig. 3).

More detailed results can be obtained from the authors once the final UK dataset has been analysed.

The addition of Seasalter and Foulness populations to the genetic database for *Z. marina* confirms that the south-east of England represents a unique genetic cluster compared to the rest of the UK.

Discussion

The results indicate that while there are smaller scale genetic structures for populations of both species representing their geographic location, gene flow is extensive between populations.

This is particularly true for *Z. marina*, for which the wider genetic dataset showed that all West Coast populations are genetically connected. Only the Solway Firth represents its own genetic cluster. East Coast populations can also be roughly grouped into one genetic cluster. It is important to highlight though that these groupings, and their representation on the presented maps, are for the purpose of visualising the genetic results and do not represent hard borders (e.g., there is gene flow between the "West" and the "East Coast" clusters). Then, likely due to the sparseness of *Z. marina* population along the Southeast coast of England, populations analysed there (Leigh-on-Sea, Seasalter and Foulness) form a fourth genetic cluster which might be genetically connected to populations along the continental European coast (e.g., Germany, Netherlands, or France - this has not been analysed yet). More detailed analysis of *Z. marina* genetics across the UK and beyond are currently underway and results will be published as soon as they are available.

The results for *Z. noltii* can't be compared to a UK wide dataset as this does not exist yet. However, this data shows that gene flow is frequent between geographically close populations e.g., Orwell, Stour, Foulness and Seasalter. Spurn Point was the most genetically distinct population, and also geographically furthest away. It is possible that gene flow rates are slightly lower for *Z. noltii* compared to *Z. marina* but a wider genetic study including more populations is required to draw a more robust conclusion. Genetic diversity varied between sites; it was very low for Spurn and relatively high for Orwell and Wells-on-the-Sea. Whilst these six populations, in isolation, provide the opportunity to explore local genetic connectivity and diversity, further work is required to create a similar UK wide data set like that which is being created for *Z.marina*. This project marks the beginnings of a genetics database for *Z.noltii*.

Populations of both species are not completely genetically isolated, but some populations have a very low genetic diversity. If restoration was to be planned the data from this study might be useful to determine source populations for restoration and whether to mix seeds from different geographic locations.

As populations are not isolated the disruption of potential local adaption by mixing seed sourced from different populations is unlikely. Nevertheless, seed and transplant collections for restoration should be prioritised within these groups to ensure that restoration sites will consist of plants best suited for their environment. If no collection can occur within the genetic cluster, seeds or transplants from the closest cluster should be prioritised.

Using either seeds or transplants for restoration should ideally focus on mixing different populations to maximise genetic diversity but also avoid sampling from populations with extensive clonal growth. The genetic data from this study can help to concentrate seeds collections on those populations that have the highest genetic diversity. Restoration sites using genetically diverse plants maximise the chances of creating new populations capable of adapting to environmental challenges such as climate change and disease.

We would like to emphasise that more data is required, particularly for *Z. noltii*, to allow interpretation of the data in a UK wide context. The *Z. marina* data presented in this report is preliminary data which will be reanalysed once the full dataset is complete. The authors are happy to share final datasets on both species once these are available.

References

Becheler, R., Diekmann, O., Hily, C., Moalic, Y. and Arnaud-Haond, S., 2010. The concept of population in clonal organisms: mosaics of temporally colonized patches are forming highly diverse meadows of Zostera marina in Brittany. Molecular ecology, 19(12), pp.2394-2407.

Coyer, J.A., Reusch, T. B .H., Stam, W.T., Serrao, E. A., Pearson, G., Procaccini, G., Olsen, J. L. 2004. Characterization of microsatellite loci in the dwarf eelgrass *Zostera noltii* (Zosteraceae) and cross-reactivity with *Z. japonica*. Molecular Ecology Notes 4, pp.497-499.

Den Hartog, C., 1970. The seagrasses of the world. North-Holland, Amsterdam.

Green, A.E., Unsworth, R.K., Chadwick, M.A. and Jones, P.J., 2021. Historical analysis exposes catastrophic seagrass loss for the United Kingdom. Frontiers in plant science, p.261

de Heij, H. and Nienhuis, P.H., 1992. Intraspecific variation in isoenzyme patterns of phenotypically separated populations of Zostera marina L. in the south-western Netherlands. Journal of Experimental Marine Biology and Ecology, 161(1), pp.1-14.

Jackson, E.L., Higgs, S., Allsop, T., Cawthray, A., Evans, J. and Langmead, O., (2011) Isles of Scilly Seagrass mapping. Natural England Commissioned Reports, Number 087

Jahnke, M., Moksnes, P.O., Olsen, J.L., Serra Serra, N., Nilsson Jacobi, M., Kuusemäe, K., Corell, H. and Jonsson, P.R., 2020. Integrating genetics, biophysical, and demographic insights identifies critical sites for seagrass conservation. Ecological Applications, 30(6), p.e02121.

Källström, B., Nyqvist, A., Åberg, P., Bodin, M. and André, C., 2008. Seed rafting as a dispersal strategy for eelgrass (Zostera marina). Aquatic Botany, 88(2), pp.148-153

Kay (1998) A review of the existing state of knowledge of the ecology and distribution of seagrass beds around the coast of Wales. Countryside Council for Wales

Orth, R.J., Lefcheck, J.S., McGlathery, K.S., Aoki, L., Luckenbach, M.W., Moore, K.A., Oreska, M.P., Snyder, R., Wilcox, D.J. and Lusk, B., 2020. Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. Science Advances, 6(41), p.eabc6434.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Phillips, R.C. and Menez, E.G., 1988. Seagrass. Smithsonian Contribution to The Marine Science No. 34. Smithsonian Institution Pres, Washington DC.

Provan, J., Wilson, S., Portig, A.A. and Maggs, C.A., 2008. The importance of reproductive strategies in population genetic approaches to conservation: an example from the marine angiosperm genus Zostera. Conservation Genetics, 9, pp.271-280.

Reusch, T.B., 2002. Microsatellites reveal high population connectivity in eelgrass (Zostera marina) in two contrasting coastal areas. Limnology and Oceanography, 47(1), pp.78-85

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233-234.

Glossary

Biodiversity - (also less frequent biological diversity) the existence of a large number of different kinds of animals and plants which make a balanced environment.

Genetic Diversity - Genetic Diversity refers to the range of different inherited traits within a species. In a species with high genetic diversity, there would be many individuals with a wide variety of different traits. Genetic diversity is critical for a population to adapt to changing environments.

Genome - The genome is the entire set of DNA instructions found in a cell. A genome contains all the information needed for an individual to develop and function.

Intron - An intron is any nucleotide sequence within a gene that is not expressed.

Intertidal - Intertidal relates to the part of the littoral zone above the low-tide mark

Microsatellite - Repetitive segments of DNA scattered throughout the genome in noncoding regions between genes or within genes (introns). They are often used as markers for linkage analysis because of their naturally occurring high variability in repeat number between individuals.

Neritic - The Neritic zone relates to the belt or region of shallow water found along the coast

Polymorphic - Polymorphism, as related to genomics, refers to the presence of two or more variant forms of a specific DNA sequence that can occur among different individuals or populations.

Subtidal – Subtidal relates to the part of the neritic zone lying below the low-tide mark but still shallow and close to shore

www.gov.uk/natural-england