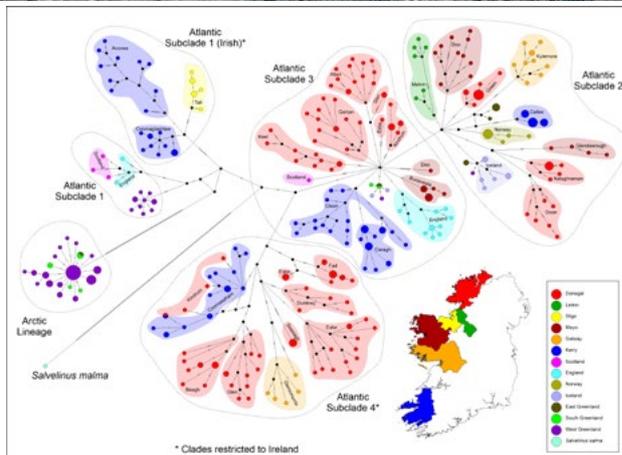


# Taxonomy and Phylogeography of the Irish Arctic Char (*Salvelinus alpinus*)

Authors: Noé Barthelemy, Rosaleen Hynes, George Hutchinson and Paulo A. Prodöhl



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2. Office of Environmental Enforcement
3. Office of Evidence and Assessment
4. Office of Radiation Protection and Environmental Monitoring
5. Office of Communications and Corporate Services

The EPA is assisted by advisory committees who meet regularly to discuss issues of concern and provide advice to the Board.

# Taxonomy and Phylogeography of the Irish Arctic Char (*Salvelinus alpinus*)

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## Identifying pressures

This research focuses on the conservation and management of the endemic salmonid Arctic char (*Salvelinus alpinus*) in Irish loughs, a species vulnerable to low oxygen levels, rising temperatures associated with global warming, threats from invasive species, and water abstraction activities. There is an urgent need for effective conservation and management of Irish Arctic char, as it is recognised as “vulnerable” in the Irish Red Data Book. The study aims to clarify their taxonomic status in Ireland, investigating whether there is scientific evidence supporting the existence of multiple species in different loughs, which is vital for protecting their unique biodiversity. It also explores the phylogeographical origins and population genetic structure of the species in Ireland, which is key to informing targeted conservation strategies to preserve their genetic diversity and unique populations. Finally, the study examines the role of scientific communication in influencing policymakers’ decisions on conservation, a crucial factor considering Arctic char’s sensitivity to environmental changes and their significance as indicators of climate change and ecosystem health.

## Informing policy

This research on Irish Arctic char provides a sound scientific foundation for their conservation. It challenges the species status of Arctic char populations based on Victorian-era taxonomy, thus enhancing public understanding and support for protecting unique genetic diversity and populations. Although the study found no genetic or morphometric evidence for the presence of multiple Arctic char species in Ireland, it highlighted the distinctiveness and richness of the Irish Arctic char’s genetic diversity, which has evolved in relative isolation since the Last Glacial Maximum. Remarkably, two of the four Arctic char genetic lineages identified are thought to be restricted to Ireland. The study also highlights how uncertainties in the conservation context can significantly affect policy decisions, emphasising the importance of well-informed conservation strategies. A key outcome is the development of a prioritisation list for Arctic char conservation that incorporates genetic and morphometric data along with other relevant information. This list serves as a practical guide for stakeholders, facilitating the implementation of effective conservation measures. In summary, the study’s insights contribute to more informed, effective conservation practices for Irish Arctic char, with broader implications for global biodiversity conservation.

## Developing solutions

This study’s distinctiveness lies in its comprehensive approach to understanding the genetic and morphometric diversity of Arctic char populations in Ireland. It analysed over 3200 specimens, including museum samples from the 1800s and 1900s, covering 82% of the loughs where the species has been recorded as naturally present, including those where it is now presumed extinct. Using modern genetic tools, including single nucleotide polymorphism (SNP) markers for both nuclear and mitochondrial DNA (mtDNA), along with complete sequencing of Arctic char mtDNA genomes, the research provided an in-depth examination of the species’ genetic composition across various loughs in Ireland. Challenging previous views, the study proposes that Ireland’s Arctic char populations do not represent multiple species. Instead, it reveals four distinct genetic lineages of a single species, with considerable mtDNA diversity, two of which might be exclusive to Ireland. This diversity, having evolved in isolation since the Last Glacial Maximum, emphasises the need to manage its preservation. The study recommends revising the species status of Arctic char, focusing on their unique genetic lineages rather than classifying them into different species. It also advocates for the further development and use of a prioritisation list for conservation needs in Ireland, based on genetic and morphometric data, alongside other lough information. This list is intended to guide effective conservation strategies. Overall, the study calls for tailored conservation efforts, underpinned by extensive genetic data, to safeguard the unique biodiversity of Ireland’s Arctic char.

**EPA RESEARCH PROGRAMME 2021–2030**

**Taxonomy and Phylogeography of the  
Irish Arctic Char (*Salvelinus alpinus*)**

**(2017-W-MS-29)**

**EPA Research Report**

Prepared for the Environmental Protection Agency

by

Queen's University Belfast

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## ACKNOWLEDGEMENTS

This report is published as part of the EPA Research Programme 2021–2030. The EPA Research Programme is a Government of Ireland initiative funded by the Department of the Environment, Climate and Communications. It is administered by the Environmental Protection Agency, which has the statutory function of co-ordinating and promoting environmental research. This project was also supported by COVID-19 pandemic relief funds awarded to Mr Noé Barthelemy (PhD candidate) by Queen’s University Belfast.

The authors would like to acknowledge the members of the project steering committee, namely Professor Colin Adams (University of Glasgow), Professor Colin Bean (University of Glasgow/NatureScot), Professor Mike Christie (Aberystwyth University), Dr Fran Igoe (Local Authority Waters Programme), Dr Fiona Kelly (Inland Fisheries Ireland) and Dr Neasa McDonnell (EPA), as well as Dr Cecilia Hegarty and Dr Karen Roche (Research Project Managers on behalf of the EPA).

The authors further acknowledge Inland Fisheries Ireland (IFI) and the members of the Irish Char Conservation Group (ICCG) for provision of all Arctic char samples, including digital photographic material used in this study, and both IFI and the EPA for provision of environmental metadata associated with several sampled loughs. We are also thankful to the research personnel of the Fish Genetics Laboratory at Queen’s who assisted with the laboratory training of Noé Barthelemy during this work, namely Dr Caroline Bradley, Dr Darren Conway and Dr Purnika Ranasinghe, in addition to the PhD candidates Ms Clíodhna Surgenor and Mr Ryan Magee. We are particularly thankful to Darren Conway for his assistance with the genomic libraries for the full mitochondrial DNA analyses and Dr John Taggart (University of Stirling) for his assistance with the preparation of the double-digest restriction-site-associated DNA sequencing (ddRADseq) libraries. The authors also acknowledge Professor Simone Cerroni, Rowan Callaghan-Creighton (MSc candidate), Dr Daniel Derbyshire, Dr Chloe McCallum, Dr Marios Zachariou, Joanna Mulholland and Dr Devon Pearse for their valuable assistance with the construction and dissemination of the online survey, and in the analyses of the resulting data. We are also thankful to Dr Peter Koene (University of Glasgow) and Dr Louise Chavarie (University of Glasgow) for assisting Noé Barthelemy with training in morphometric analyses. The authors extend their thanks to Dr Simon Loader, Dr James Maclaine and Dr Oliver Crimmen from the Natural History Museum in London, and to Dr Amy Geraghty from the National Museum of Ireland, for their enthusiastic support and the invaluable provision of tissue samples from unique specimens (holotypes/syntypes) of Arctic char from Ireland. Finally, the authors would like to thank Queen’s University Belfast, who provided financial support to Noé Barthelemy during the COVID-19 pandemic.

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This report is based on research carried out/data from August 2018 to February 2022. More recent data may have become available since the research was completed.

The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

**EPA RESEARCH PROGRAMME 2021–2030**  
Published by the Environmental Protection Agency, Ireland

ISBN: 978-1-80009-138-2

Novembre

Price: Free

2023 Online

version

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# Executive Summary

A comprehensive genetic analysis of Arctic char (*Salvelinus alpinus*) populations in Irish loughs was undertaken to address several aspects relevant to conservation and management. First, the study aimed to clarify the taxonomic status of this species, investigating whether there is scientific evidence supporting the existence of multiple char species in Ireland. Second, it sought to uncover the phylogeographical history and population structure of contemporary Arctic char populations in Ireland. Third, it assessed the impact of scientific communication on conservation decisions made by policymakers, using an environmental economics analytical framework. Finally, the study aimed to provide insights into the development of effective conservation measures for Arctic char in Ireland, using the genetic data generated in conjunction with other available information.

The study involved sampling Arctic char from 37 loughs in Ireland, representing 82% of the loughs where the species has been recorded as naturally present. Archived samples were also available from 18 additional loughs where Arctic char is now thought to be extinct. Limited samples from Scotland (UK), England (UK) and Sweden were included in the dataset for comparison. Historical specimens, collected during the late 1800s and early 1900s, were sourced from the Natural History Museum in London and the National Museum of Ireland. These included holotypes and syntypes associated with species previously described in Ireland by Günther (1862, 1863) and Regan (1908, 1911). In total, over 3200 Arctic char specimens were analysed during the study.

The analysis involved the examination of genetic data from both nuclear and mitochondrial DNA (mtDNA), in addition to morphometric data. Single nucleotide polymorphism (SNP) markers were developed for both nuclear DNA and mtDNA, based on a sample set of 288 individuals representing 29 Irish Arctic char loughs, along with specimens from England and Scotland for reference. Nuclear SNP markers were employed to screen the 3072 samples collected for the project. The results revealed that all Arctic char loughs hosted genetically distinct populations, with

varying degrees of divergence. There was no major correlation between geographical location and genetic similarity, indicating that geographical proximity is not an indicator of genetic similarity. Several loughs were found to host multiple genetically distinct populations, a phenomenon known as sympatric populations.

Comparative analysis of 342 Arctic char mitochondrial genomes unveiled 173 distinct mtDNA haplotypes. Results indicate that Arctic char populations in Ireland's loughs are represented by four genetically distinct, rich (i.e. represented by several genetic variants) evolutionary lineages. Genetic divergence among these lineages suggests separate evolution in distinct refugia prior to the Last Glacial Maximum. As the ice retreated between 20,000 and 13,000 years ago, these lineages independently colonised Irish freshwater habitats. Two of these lineages appeared to be unique to Ireland, possibly representing the first colonisers following the ice retreat. The remaining lineages were also found in other North Atlantic countries. Within-lough diversity explained most of the mitochondrial genetic diversity, further emphasising the lack of a geographical pattern. The disjunct contemporary geographical distribution of lineages indicates that the recolonisation of Ireland was complex, involving colonisation, extinction, recolonisation, and/or secondary contact and admixture among Arctic char populations representing different lineages.

The results of the study challenge the species status of Arctic char populations based on Victorian-era taxonomy. Neither genetic nor morphometric data support the existence of multiple char species in Ireland. While scientific evidence does not substantiate the presence of distinct species, the study highlights the uniqueness, richness and potentially endemic nature of mtDNA diversity among Irish Arctic char. Much of this diversity has evolved in isolation since the Last Glacial Maximum. This unique biodiversity warrants conservation attention and protection.

The results of the environmental economics analysis reveal that policymakers' conservation decisions are influenced by the inclusion or exclusion of

uncertainties in the conservation context. Building on these findings, to assist with the development of effective conservation measures, a prioritisation list ranking Arctic char conservation needs in Ireland was devised. This ranking system, based on a straightforward points-based approach, uses the novel

genetic and morphometric data generated by the study, combined with other available information on the loughs under investigation. This prioritisation list could be a valuable tool for relevant stakeholders, to assist with the development and implementation of sound conservation measures for Arctic char in Ireland.

# 1 Introduction: Rationale and Objectives

Arctic char (*Salvelinus alpinus*) was among the first fish species to colonise Irish freshwater habitats following the retreat of the ice cap that covered most of Ireland between 20,000 and 13,000 years ago (Callard *et al.*, 2020). In the northernmost part of their circumpolar range, Arctic char populations are anadromous, spawning in rivers and feeding as adults in the sea. Present-day Irish Arctic char populations, however, are considered glacial relicts of a more southerly distribution from the last Ice Age and, without exception, all reside in lakes (Ferguson *et al.*, 2019). Arctic char is one of the rarest fish species present in Ireland. Current information indicates that 34% of the 86 known populations are now extinct, and the status

of 15% is still unknown (Igoe *et al.*, 2001; Ferguson *et al.*, 2019). While once widespread, all extant Arctic char populations ( $N=45$ ) are primarily located in western Ireland (Figure 1.1). The species is listed as “vulnerable” (under threat of extinction) and as being of conservation concern in the Irish Red Data Book (King *et al.*, 2011).

Among salmonids, Arctic char is the most sensitive to decreased oxygen levels and increased temperature (Connor *et al.*, 2019; Ferguson *et al.*, 2019). Thus, both natural and human-mediated environmental pressures leading to eutrophication and climatic warming represent major threats to extant populations.

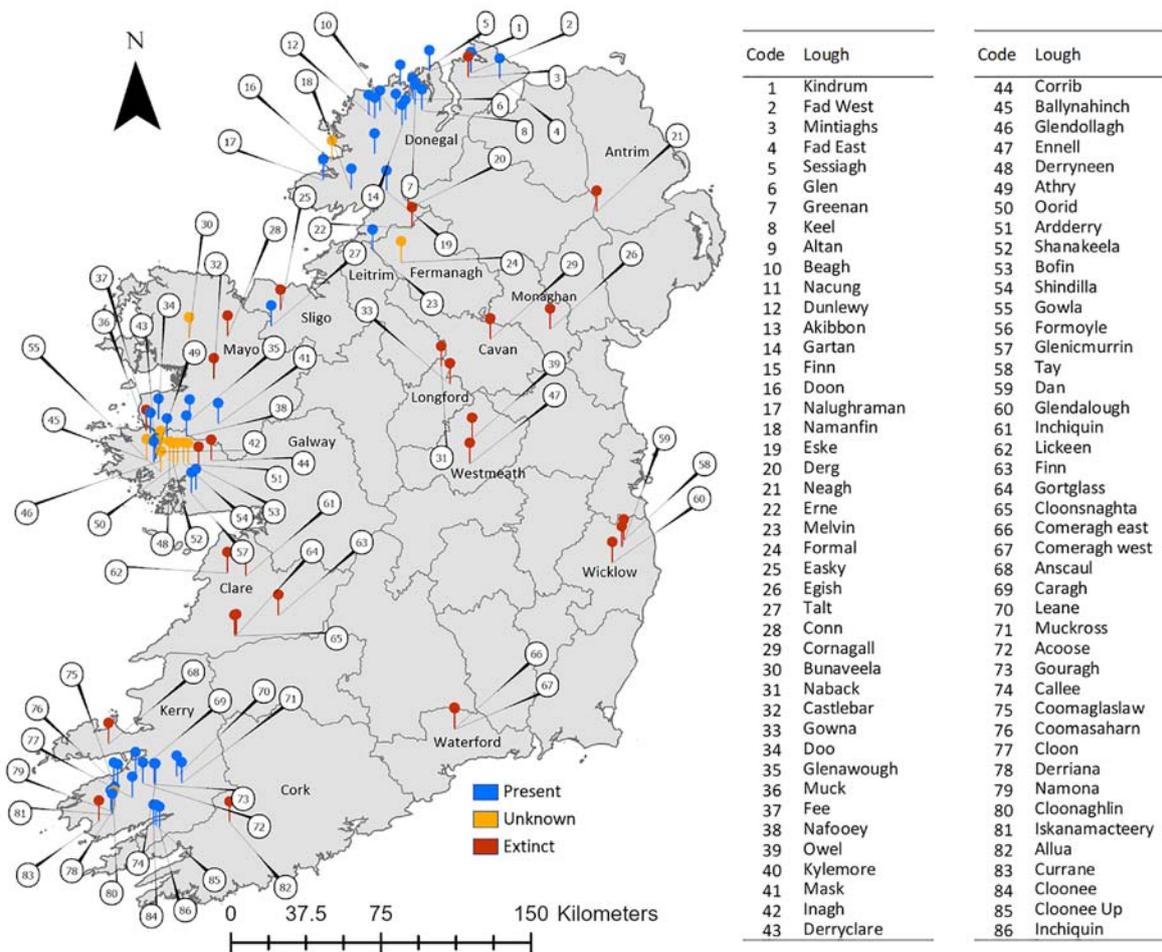


Figure 1.1. Distribution and status of all recorded Arctic char locations on the island of Ireland. ● Loughs with extant populations. ● Loughs where the status of Arctic char is unknown. ● Loughs where Arctic char is thought to be extinct. In all cases, the lough and associated county names are also displayed.

In Ireland, the effect of these threats on lake-resident populations can be exacerbated by the presence of non-native and/or introduced species (e.g. roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), pike (*Esox lucius*)), in addition to water abstraction activities that may negatively impact on breeding by exposing spawning grounds (Igoe and Hammar, 2004; Connor *et al.*, 2019). Despite their potential vulnerability, Arctic char populations are therefore ideal biomonitors of climate change, water quality and ecosystem services.

In common with other salmonids, the Irish Arctic char is renowned for its remarkable variation in morphology, coloration patterns, growth rate, age of maturation and migration, time and place of spawning, and feeding behaviour, in addition to other aspects of its life history. Thus, anadromous, semi-anadromous, fluvial-lacustrine or lacustrine Arctic char populations are common throughout the species range (Ferguson *et al.*, 2019). Similarly, lakes supporting non-interbreeding, genetically distinct sympatric populations, characterised by distinct morphology, feeding behaviour and other life history traits, are also common (Jonsson and Jonsson, 2001; Klemetsen *et al.*, 2003; Adams and Maitland, 2007).

This variation led to the description of several species of char/charr in European waters by 19th and early 20th century naturalists (reviewed in Behnke, 1972; Adams and Maitland, 2007; Kottelat and Freyhof, 2007). The recognition that Arctic char can change its morphology, ecology and behaviour under different environmental conditions (i.e. reflecting the phenotypic plasticity of a species gene pool) led 20th century fish taxonomists to lump these species into a single polytypic species, *S. alpinus*, which is widespread throughout the Palearctic and Nearctic regions (Ferguson *et al.*, 2019). This simplistic taxonomic approach could, however, potentially lead to the development and implementation of inadequate management and conservation strategies for otherwise unique genetic lineages. Indeed, there is increasing scientific evidence arguing for the existence of distinct Arctic char species and/or genetically unique lineages living even in the same lakes (Guðbrandsson *et al.*, 2019; Ferguson and Prodöhl, 2022).

In Ireland, six Arctic char species were previously described as what is now referred to as *S. alpinus*. Günther (1862, 1863) described two species: (1) *S. colii*, the most common species, which was

reported in Loughs Eske and Sessiagh (Donegal), Mask and Glenawough (Mayo), Ballynahinch, Corrib, Derrynreen, Glenicmurrin, Inagh and Kylemore (Galway), and Cloonee, Currane and Iskanamacteery (Kerry); and (2) *S. grayii*, which was thought to be restricted to Lough Melvin (Leitrim). Regan (1908, 1911) described a further four species: (1) *S. trevelyani*, restricted to Lough Finn (Donegal); (2) *S. fimbriatus*, restricted to Lough Coomasaharn (Kerry); (3) *S. scarfii*, restricted to Loughs Owel and Ennell (Westmeath); and (4) *S. obtusus*, restricted to Loughs Acoose (Kerry), Tay and Dan (Wicklow), and the Killarney lakes (Kerry). In a more recent review of the taxonomic status of Arctic char in Europe, Kottelat and Freyhof (2007) defended the contention that four species are present in Ireland: *S. grayii*, *S. obtusus*, *S. fimbriatus* and *S. colii*. Adams and Maitland (2007), however, argued that current evidence for full species status is poor and that, to ensure that relevant populations are adequately protected, a comprehensive review of the genetic and phenotypic populations in the island of Ireland and Great Britain (which includes the numerous smaller islands and island groups, such as the Hebrides, the Shetland Islands, the Orkney Islands, the Isles of Scilly and the Isle of Man) is required.

The cost-effective and sustainable management and conservation of freshwater fish biodiversity requires the correct identification of the unit to be conserved and/or managed. Thus, the common or rare status of one organism makes sense only if its species or population can be unambiguously defined and recognised. If a fish or population of fish is not recognised as distinct from other conspecifics in scientific terms, and subsequently becomes extinct, then the biological consequence of such loss could be deemed inconsequential. However, in terms of biodiversity, ecosystem resources and services, the loss is very real (Ferguson, 2004). These taxonomic uncertainties currently prevent the scientifically sound development and implementation of appropriate management/conservation planning, and seriously compromise the ability to assess the economic value of Arctic char as an important component of Irish biodiversity.

Using a comprehensive biological sample dataset representing over 82% of all extant Arctic char populations on the island of Ireland and a combination

of modern morphometrics and genetics-based methodologies, the study addresses this knowledge gap with the following five interlinked objectives:

1. investigate the taxonomic status of Arctic char in the island of Ireland;
2. investigate the phylogeographical history of the Arctic char populations in the island of Ireland and, in particular, test for pattern(s) of historical colonisation of putative distinct genetic lineages following the retreat of the ice at the end of the last Ice Age;
3. investigate patterns and levels of genetic diversity within extant populations;
4. use methods from environmental and behavioural economics to assess the importance of the conservation risk preferences of decision-makers and the effect of updated scientific communications on genetic diversity and unique evolutionary lineages on conservation decisions in the context of Arctic char in the island of Ireland;
5. discuss how information generated in this study can be used, in combination with other available data, to assist with identifying conservation priorities for Arctic char in the island of Ireland.

## 2 Methodological Approach and Results

### 2.1 Sampling for Geometric Morphometric and Genetic Analyses, and Lake Characteristics

The sampling of all contemporary biological material used in this study was independently undertaken by Inland Fisheries Ireland (IFI) and the Irish Char Conservation Group (ICCG) as part of routine lake surveys of Arctic char in Ireland carried out between 2004 and 2021. The sampling methodology for IFI-based fish surveys is described in Morrissey-McCaffrey *et al.* (2018), and involved the use of surface, pelagic and benthic multi-mesh survey gillnets. Biological samples used for DNA extraction consisted of dried scales stored in individual envelopes and/or biopsy tissue samples (e.g. fin clips, gill rakers, muscle tissue). Tissue samples were either frozen at  $-20^{\circ}\text{C}$  or stored in tubes (either 5 mL or 1.5 mL Eppendorf tubes) containing 99% molecular-grade ethanol. In total, material from 3019 Arctic char specimens was obtained from 55 loughs in Ireland, with 37 of these having extant populations and 18 being loughs where populations are potentially extinct (and therefore dried, archived scales were used).

Among the Arctic char loughs examined in this study (Figure 2.1), surface areas ranged from  $0.0006\text{ km}^2$  (0.06 ha) to  $176\text{ km}^2$  (17,600 ha), with a mean of  $10.4\text{ km}^2$  (s.d.  $46.2\text{ km}^2$ ). Average depths ranged from 1.3 to 29.9 m (mean of  $9.54 \pm 6.61$  m), and the loughs were located at elevations ranging from 4 to 328 m (mean of  $84.2 \pm 74.89$  m) above sea level. Surface area was not correlated with average lake depth (Pearson correlation test:  $r = -0.190$ ,  $p\text{-value} = 0.087$ ), but elevation was strongly and positively correlated with average lake depth (Pearson correlation test:  $r = 0.466$ ,  $p\text{-value} = 0.0002$ ). Additional sample details, including number of Arctic char samples obtained per lake, basic lake topographical and bathymetric features (i.e. elevation, area, depth), and information on whether the water body contained “a distinct species” (as described by Günther (1862, 1863) and Regan (1908, 1911)), are given in Table 2.1.

For several loughs, individual fish photographs, taken against an appropriate contrasting background

and including a measuring rule as a reference for length, were also available as part of both the IFI ( $N = 198$ ) and ICCG ( $N = 208$ ) Arctic char data (see Table 2.1 under “Digital photo”). These included two loughs (Lough Coomasaharn and Lough Melvin) in which *S. fimbriatus* and *S. grayii* had previously been reported, by Günther (1962, 1963) and Regan (1908, 1911), respectively. This opportunistic digital sample set was used to assess the validity of the old taxonomic system, which was entirely based on morphometric features.

In addition to the samples outlined above, an additional limited number of samples from England, UK (Lake Windermere,  $N = 84$ ), Scotland, UK (Loch Arklet,  $N = 27$ ), and Sweden (Lake Kilpisjärvi,  $N = 15$ ) were also available for analysis. These were used as outgroups for comparison with samples from Ireland. A very limited number of historical samples were also obtained, under licence, from the biological archive collections held by the Natural History Museum in London and from the National Museum of Ireland. These unique samples, collected in Victorian times and dating back to the late 1800s and early 1900s, included holotypes and syntypes for each of the species previously described in Ireland by both Günther (1862, 1863) and Regan (1908, 1911). These samples were used as baseline references for addressing the first objective of this study, namely to investigate the taxonomic status of Arctic char in Ireland.

### 2.2 Geometric Morphometric Analysis

#### 2.2.1 Rationale

As reviewed by Adams and Maitland (2007), much of the ongoing controversy associated with the taxonomic uncertainties linked to Arctic char in Britain and Ireland is linked to the original morphological criteria used by early Victorian taxonomists to describe what were presumed to be different species. While defending the need for, and the importance of, a full taxonomic review of Arctic char, Adams and Maitland (2007) argued that the assignment of full species status

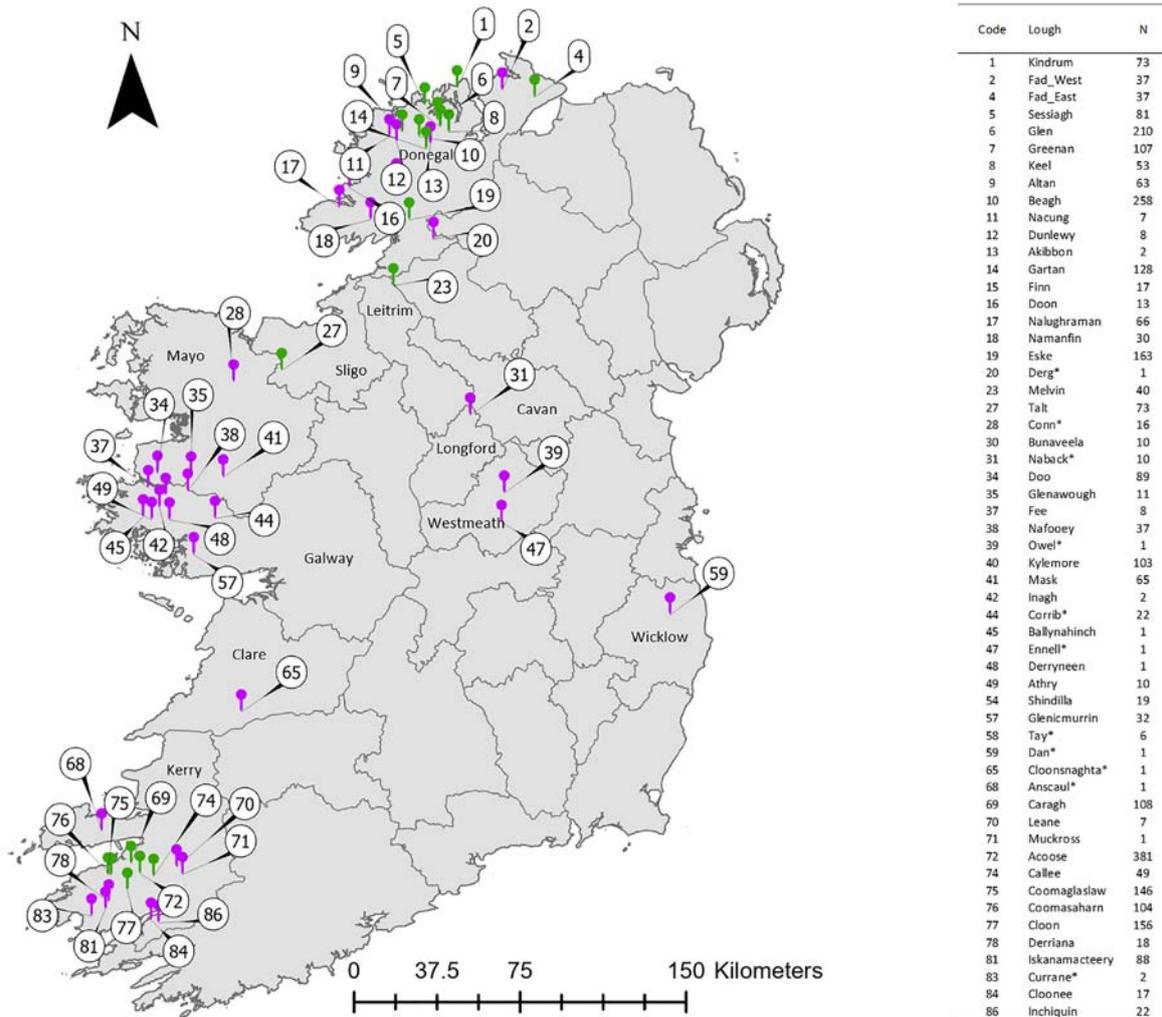


Figure 2.1. Location of the Arctic char loughs analysed in this study within the island of Ireland.

● Loughs initially selected for double-digest restriction-site-associated DNA sequencing (ddRADseq) and full mitochondrial DNA sequencing analysis. ● Loughs screened for both nuclear and mitochondrial DNA selected single nucleotide polymorphism (SNP) markers. \*Loughs where Arctic char is thought to be extinct. In all cases, the lough and associated county names are also displayed.

in many instances (e.g. by Wheeler and Fisheries Society of the British Isles (1992) and Kottelat and Freyhof (2007)) is not well supported by evidence. There are several areas where evidence is insufficient to support “full species” status, for example where only a few specimens were used by Victorian taxonomists in their original species description, despite the abundant evidence supporting the view that the considerable phenotypic variation observed in Arctic char populations can lead to incorrect classification. Another issue is the lack of consideration given to the fact that high levels of phenotypic variation within loughs is further exacerbated by the presence of sympatric populations in some water bodies – a common feature for Arctic char throughout its natural

distribution (Gordeeva *et al.*, 2014; Jacobs *et al.*, 2020; Brachmann, 2021). This has been shown to add further bias to the description of species based on a small number of specimens (Adams and Maitland, 2007). It is also worth noting that many of the characteristics commonly used to describe putative species were subjective and, hence, not easily quantified (e.g. shape of the snout, mouth and jaw, and size of scales).

In this component of the study, the opportune digital photo collection associated with several Arctic char loughs (Table 2.1) permitted a comparative assessment of the morphology of specimens belonging to different loughs using modern geometric morphometric analytical approaches.

**Table 2.1. Details of Arctic char samples**

Lough code	Lough name	EU lough code	Lat.	Lon.	County	N	Digital photo	Elevation (m)	Area (ha)	Lough depth (m) mean (max.)	Victorian species
1	Kindrum	IE_NW_38_670	55.24	-7.70	Donegal	73	27	7	61	2.5 (15)	-
2	Fad West	IE_NW_40_02	55.23	-7.38	Donegal	37	-	125	40	>4 (18)	-
4	Fad East	IE_NW_40_17	55.20	-7.15	Donegal	37	30	234	12	na	-
5	Sessiagh	IE_NW_38_61	55.17	-7.93	Donegal	81	4	26	24	3.5 (20.94)	<i>S. coli</i> (Günther)
6	Glen	IE_NW_38_22	55.11	-7.84	Donegal	210	47	23	168	4.8 (14.14)	-
7	Greenan	IE_NW_38_635	55.08	-7.82	Donegal	107	26	162	23	6.4 (25)	-
8	Keel	IE_NW_38_75	55.06	-7.76	Donegal	53	25	96	61	7.2 (25)	-
9	Altan	IE_NW_38_19	55.06	-8.09	Donegal	63	20	139	132	18.3 (51.7)	-
10	Beagh	IE_NW_38_80	55.04	-7.97	Donegal	258	37	39	260	9.2 (46.5)	-
11	Nacung	IE_NW_38_26	55.04	-8.18	Donegal	7	-	57	208	8.1 (52.1)	-
12	Dunlewy	IE_NW_38_683	55.02	-8.13	Donegal	8	-	59	110	7.1 (18.8)	-
13	Akibbon	IE_NW_39_11	55.01	-7.89	Donegal	2	-	66	45	1.3 (2.9)	-
14	Garfan	IE_NW_39_12	54.99	-7.92	Donegal	128	-	68	204	3.5 (24)	-
15	Finn	IE_SH_27_127	54.86	-8.13	Donegal	17	-	92	74	1.9 (10.6)	<i>S. trevelyani</i> (Regan)
16	Doon	n/a	54.83	-8.46	Donegal	13	-	61	0.1	n/a	-
17	Nalughraman	IE_NW_37_200	54.75	-8.53	Donegal	66	-	176	56	16.9 (45.5)	-
18	Namanfin	IE_NW_37_150	54.70	-8.31	Donegal	30	-	125	23	12	-
19	Eske	IE_NW_37_188	54.70	-8.04	Donegal	163	-	25	387	7.4 (30.4)	<i>S. coli</i> (Günther)
20	Derg	IE_NW_01_115	54.62	-7.87	Donegal	1	-	138	862	5.9 (26)	<i>S. coli</i> (Günther)
23	Melvin	IE_NW_35_160	54.43	-8.15	Leitrim	40	12	24	2197	7.8 (43)	<i>S. grayi</i> (Günther)
27	Talt	IE_WE_34_405	54.08	-8.92	Sligo	73	6	133	97	10.3 (40)	-
28	Conn	IE_WE_34_406b	54.03	-9.25	Mayo	16	-	6	4704	>4 (37.9)	-
30	Bunaveela	n/a	54.02	-9.55	Mayo	10	-	118	490	7 (18)	-
31	Naback	IE_NW_36_346	53.90	-7.62	Longford	10	-	125	12	4.4 (9.1)	-
34	Doo	IE_WE_32_490	53.65	-9.76	Mayo	89	25	30	155	>4 (46)	-
35	Glenawough	IE_WE_32_392	53.65	-9.53	Mayo	11	-	217	73	24.5 (61.1)	<i>S. coli</i> (Günther)
37	Fee	IE_WE_32_498	53.59	-9.82	Galway	8	-	44	174	11.2 (32)	-
38	Natfoeey	IE_WE_30_342	53.58	-9.55	Galway	37	-	26	248	15.6 (46.6)	-
39	Owel	IE_SH_26_703	53.58	-7.39	Westmeath	1	-	96	1031	7.2 (22.8)	<i>S. scharffii</i> (Regan)
40	Kylemore	WE_32_509b	53.56	-9.70	Galway	103	5	24	134	10.8 (30)	<i>S. coli</i> (Günther)
41	Mask	IE_WE_30_665	53.64	-9.31	Mayo	65	-	17	8218	5 (57)	<i>S. coli</i> (Günther)

**Table 2.1. Continued**

Lough code	Lough name	EU lough code	Lat.	Lon.	County	N	Digital photo	Elevation (m)	Area (ha)	Lough depth (m) mean (max.)	Victorian species
42	Inagh	IE_WE_31_223	53.51	-9.74	Galway	2	-	19	309	4.5 (22.2)	<i>S. coli</i> (Günther)
44	Corrib	IE_WE_30_666b	53.47	-9.36	Galway	28	-	5	16631	>4 (42)	-
45	Ballynahinch	IE_WE_31_228	53.47	-9.85	Galway	1	-	13	166	6.8 (37.2)	<i>S. coli</i> (Günther)
47	Ennell	IW_SH_25_188	53.46	-7.41	Westmeath	1	-	77	1156	6.4 (27.4)	<i>S. scharffii</i> (Regan)
48	Derryneen	IE_WE_31_1055	53.46	-9.67	Galway	1	-	36	5	n/a	<i>S. coli</i> (Günther)
49	Athry	IE_WE_31_126	53.46	-9.79	Galway	10	-	12	35	6 (24.3)	-
54	Shindilla	IE_WE_31_171	53.46	-9.57	Galway	19	3	37	66	6.3 (22)	-
57	Glenicmurrin	IE_WE_31_226	53.32	-9.50	Galway	32	6	23	163	2.9 (16)	<i>S. coli</i> (Günther)
58	Tay	IE_EA_10_25	53.11	-6.27	Wicklow	6	-	244	50	17.7 (35.1)	<i>S. obtusus</i> (Regan)
59	Dan	IE_EA_10_29	53.07	-6.28	Wicklow	1	-	201	106	17.1 (38)	<i>S. obtusus</i> (Regan)
65	Cloonsnaghta	IE_SH_27_54	52.68	-9.16	Clare	1	-	70	9	n/a	-
68	Anscaul	IE_SW_22_189	52.18	-10.07	Kerry	1	-	77	26	10.3 (27.3)	-
69	Caragh	IE_SW_22_207	52.05	-9.87	Kerry	108	9	17	491	10.9 (40.4)	-
70	Leane	IE_SW_22_185	52.04	-9.57	Kerry	7	-	17	1952	13 (60)	<i>S. obtusus</i> (Regan)
71	Muckross	IE_SW_22_184	52.01	-9.53	Kerry	1	-	17	267	29.9 (73.5)	<i>S. obtusus</i> (Regan)
72	Acoose	IE_SW_22_208	52.01	-9.81	Kerry	381	71	152	67	5.7 (19)	<i>S. obtusus</i> (Regan)
74	Callee	IE_SW_22_182	52.00	-9.72	Kerry	49	-	328	18	9.6 (26.3)	-
75	Coomaglaslaw	IE_SW_22_197	52.00	-10.02	Kerry	146	63	244	52	29.2 (77.4)	-
76	Coomasaham	IE_SW_22_205	52.00	-10.00	Kerry	104	19	164	80	24.4 (51.5)	<i>S. fimbriatus</i> (Regan)
77	Cloon	IE_SW_22_206	51.94	-9.89	Kerry	156	-	88	77	13.8 (31.4)	-
78	Derriana	IE_SW_21_449	51.89	-10.01	Kerry	18	-	116	239	13.4 (37)	-
81	Iskanamacteery	IE_SW_21_437	51.86	-10.03	Kerry	88	-	146	23	10.6 (48.6)	<i>S. coli</i> (Günther)
83	Currane	IE_WE_21_457	51.83	-10.12	Kerry	2	-	4	1032	7.9 (36)	<i>S. coli</i> (Günther)
84	Cloonee	IE_SW_21_446	51.82	-9.73	Kerry	17	-	28	94	2.6 (13.69)	<i>S. coli</i> (Günther)
86	Inchiquin	IE_SH_27_130	51.81	-9.68	Kerry	22	-	21	108	10.7 (29)	-

The geographical locations of loughs, by lough code, are shown in Figure 2.1. "Digital photo" refers to the number of samples in loughs with available digital photos of individual fish. The "Victorian species" column indicates whether or not species linked to the taxonomic work of Günther (1862, 1863) and Regan (1908, 1911) have been recorded in loughs (Went, 1945; Adams and Maitland, 2007).

Lat., latitude; Lon., longitude; N, number of samples; n/a, information not currently available.

The main objective here was to test, where possible (i.e. *S. fimbriatus* in Lough Coomasaham and *S. grayii* in Lough Melvin), whether the original, Victorian taxonomy of these Arctic char presumed to be different species is supported by modern morphometric analysis.

### 2.2.2 Methodological approach

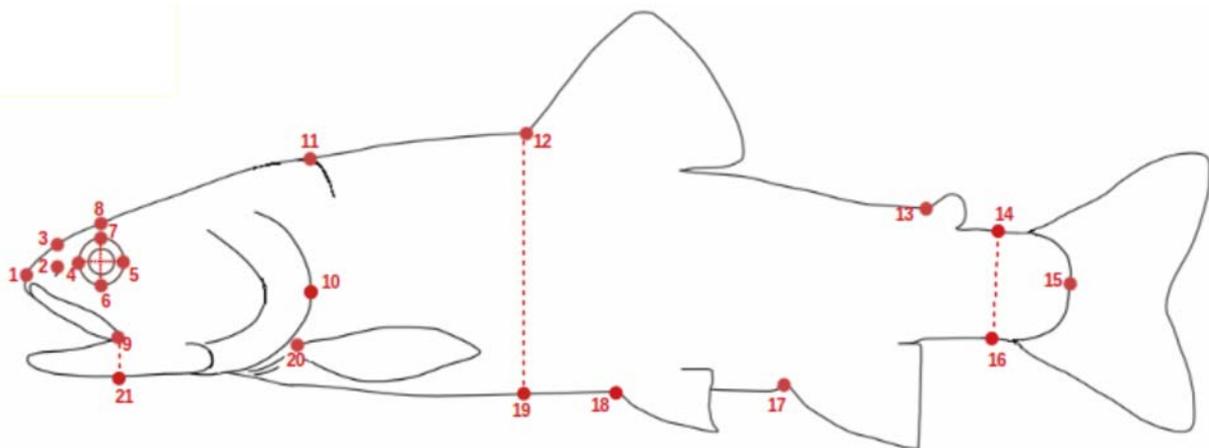
Following a preliminary assessment of the quality/resolution of the digitised images (based on light level, resolution and body condition), 435 were selected for further analysis. The following 18 Arctic char loughs are represented in this analysis: (1) Acoose, (2) Altan, (3) Beagh, (4) Caragh, (5) Coomaglaslaw, (6) Coomasaham, (7) Doo, (8) Fad East, (9) Glen, (10) Glenicmurrin, (11) Greenan, (12) Keel, (13) Kindrum, (14) Kylemore, (15) Melvin, (16) Sessiagh, (17) Shindilla and (18) Talt. See Table 2.1 and Figure 2.1 for further details. Biological metadata, including data on length, weight and sex, collected when the fish were photographed, were also available for analysis.

A set of 21 geometric morphometric landmarks were defined (Figure 2.2). Landmarks were digitised using the tpsUtil and tpsDig2 v2.16 software platforms (Rohlf, 2010, 2013). Geometric morphometric and

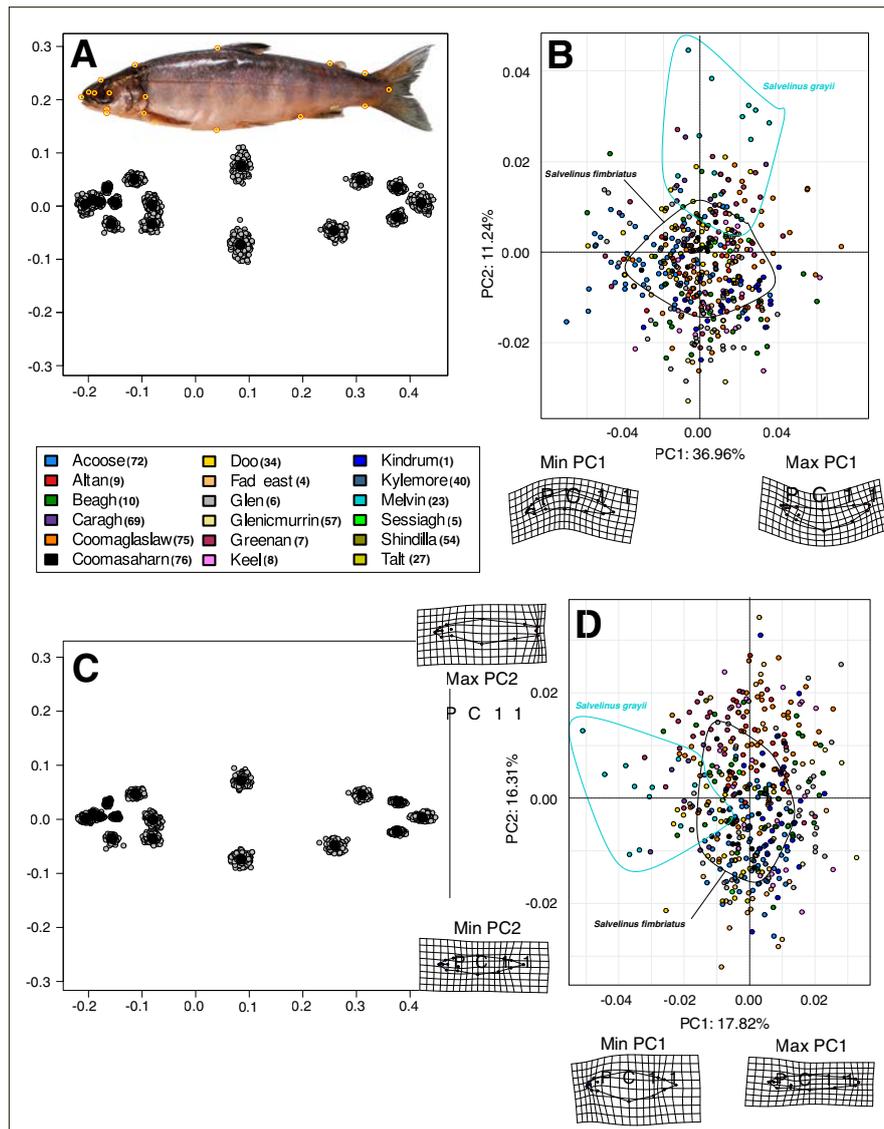
related statistical analyses were carried out with the “Geomorph” (Adams *et al.*, 2021), “RRPP” (Collyer and Adams, 2021) and “Morpho” (Schlager, 2017) packages on R version 4.2.0 (R Core Team, 2022). A generalised Procrustes superimposition analysis (Gower, 1975; Rohlf and Slice, 1990) was performed on the 21 landmarks, where all individuals were translated to the origin, scaled to centroid size and rotated. A preliminary analysis of the data indicated that four landmarks (landmarks 3, 6, 7 and 21) were redundant and were thus removed from further analysis.

Aligned Procrustes coordinates (PCs) were plotted using Kendall’s tangent space coordinates (Figure 2.3) (Dryden and Mardia, 1993; Rohlf, 1999). Variation in shape was investigated using principal component analysis (PCA), before and after removal of lunate distortion effect. To remove the effect of lunate distortion due to rigor mortis on the shape of individuals, PCs were regressed against the residuals of the affected PC, and the residuals of this regression used for further analysis (Hooker *et al.*, 2016; Koene *et al.*, 2019).

Within-population morphological variance was investigated by calculating variances in Procrustes scores of body shape per population, and between



**Figure 2.2.** Diagram illustrating landmarks collected for the geometric morphometric analysis. Landmarks were positioned as follows: (1) tip of the snout; (2) nostril; (3) 90° above landmark 2; (4–7) corners of the eyes (in a cross shape); (8) 90° above landmark 7; (9) posterior end of the maxilla; (10) “crack” in operculum; (11) posterior end of skull; (12) anterior base of dorsal fin; (13) anterior base of adipose fin; (14) dorsal point of shortest caudal peduncle distance; (15) posterior end of lateral line at base of caudal fin; (16) ventral point of shortest caudal peduncle distance; (17) anterior base of anal fin; (18) ventral anterior base of ventral fin; (19) 90° below landmark 12; (20) anterior base of pectoral fin; (21) 90° below landmark 9.



**Figure 2.3. Multiple plots illustrating residuals of Procrustes analysis and PCA of the two main axes before and after correction for lunate distortion (i.e. regression of Procrustes residuals on PC1). PCs of the 17 landmarks used for shape analysis before (A) and after (C) correction for lunate distortion, respectively. PCA plots describing the two main components of variance in the shapes of the 435 Arctic char specimens from 18 populations in Ireland used in this study, before (B) and after (D) correction for lunate distortion. Wireframe deformation grids represent extremes along each axis.**

each pair of populations. The power of the resulting dataset for the assignment of individuals to different predefined groups using shape information was assessed using canonical variate analysis (CVA) with 10,000 rounds, jackknife cross-validation and Benjamini–Hochberg correction of  $p$ -values (Darlington *et al.*, 1973). To explore the relationship between genetic and morphological distances, the pairwise Mahalanobis  $D_2$  statistic distance between populations was compared with the population pairwise  $F_{st}$  estimated for Arctic char from the target loughs, derived from the nuclear single nucleotide

polymorphism (SNP) data generated in section 2.3.2 (data not shown).

### 2.2.3 Geometric morphometric analysis results

#### Variation in morphology among and within populations

The post-mortem bending of individuals accounted for most of the variance in body shape (PC1: 36.96% proportion of variance explained (PVE); Figure 2.3B).

Following correction for post-mortem bending, the PVE values for PC1 and PC2 were similar (17.82% and 16.31%, respectively). Individuals with a high PC1 score tended to exhibit a longer and shallower body and head, much larger eyes and a mouth in terminal position. In sharp contrast, a low PC1 score indicated very small eyes, greater body depth, a longer and deeper caudal peduncle, and a mouth in inferior position. Differences between maximal and minimal PC2 values were concentrated in the length of the caudal peduncle and in the insertion of the caudal fin. However, high PC2 values also indicated an unusually deep belly. None of the 153 comparisons of least squares means distances between the 18 populations was significant (data not shown). However, 48 out of 153 comparisons were significant when examining variances in body shape among pairs of population (data not shown). Examination of within-population variances in body shape revealed that the smallest degree of variation was found in Lough Shindilla, and the highest was noted in Lough Fad East. Within-population variance was not correlated with any of the lake characteristics (Pearson correlation test:  $p$ -value > 0.05).

#### *Correlation between genetic and morphological distances*

Mahalanobis distances between pairs of populations ranged from 2.206 (Acoose–Beagh) to 7.203 (Melvin–Sessiagh). Pairwise  $F_{st}$  estimates from double-digest restriction-site-associated DNA sequencing (ddRADseq) data were available for 13 of the 18 populations investigated here (data not shown), and ranged from 0.0202 (Beagh–Glen) to 0.2262 (Caragh–Sessiagh). The Mahalanobis distance to  $F_{st}$  ratio averaged at 32.934, with a higher ratio indicating larger differences in morphology than in genetics. The minimum value of this ratio was found in the Keel–Kindrum population pair (16.756), while the maximum value was found in the Glen–Beagh pair, exceeding 5.7 times the average value (189.429; Figure 2.4). Finally, a medium positive correlation was found between the Mahalanobis distance and  $F_{st}$  estimates (Pearson correlation test:  $r=0.318$ ,  $p$ -value = 0.002245).

#### *Morphology, genetics and Victorian taxonomy*

As outlined earlier, two of the Arctic char populations examined in this study were described as full

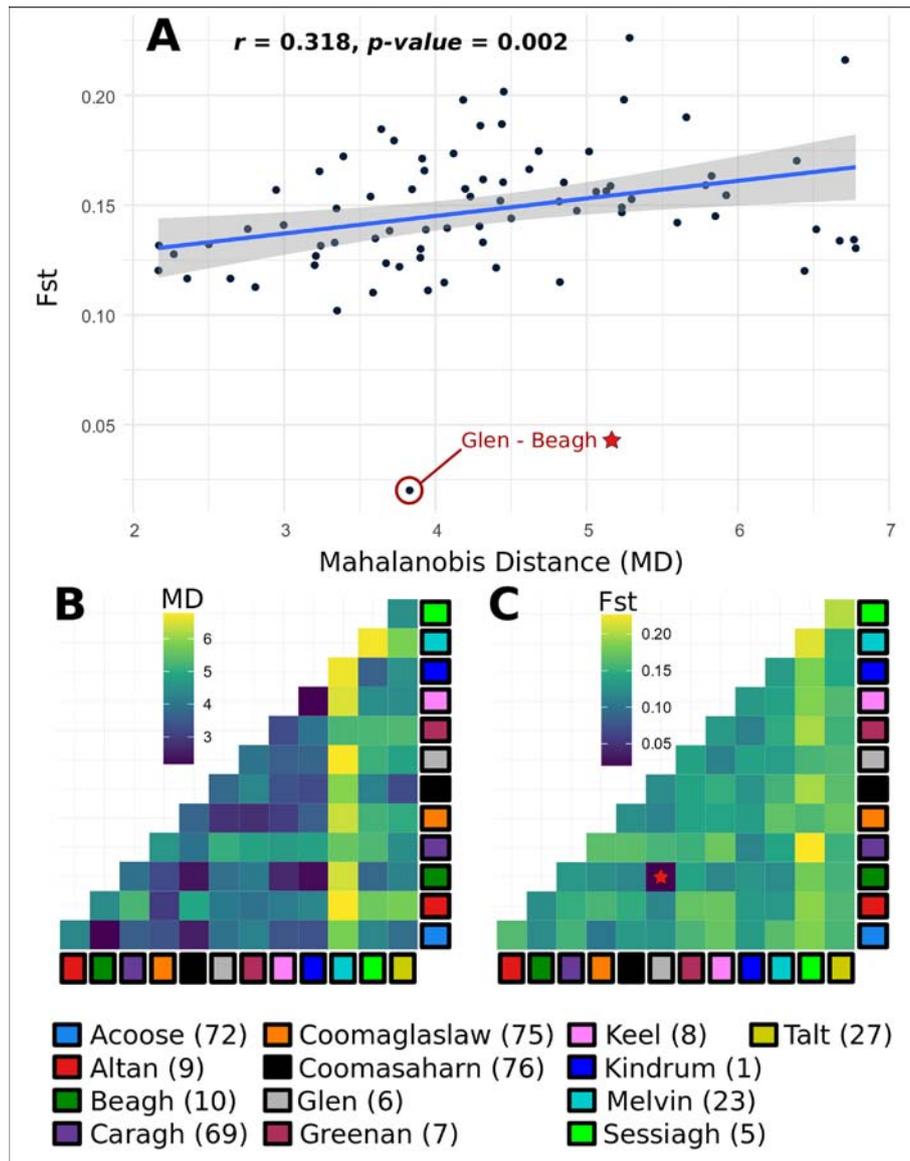
species by Günther (*S. grayii* in Lough Melvin, 1862) and Regan (*S. fimbriatus* in Lough Coomasaharn, 1908). The Arctic char population from Lough Melvin appeared as the most isolated cluster in the morphospace (Figure 2.3B). While not statistically significant, it is interesting to note that 14 of the 25 highest morphological distances observed between populations involved Arctic char from Lough Melvin (data not shown). Similarly, the Arctic char population from Lough Melvin was involved in 8 of the 10 population pairs with the highest Mahalanobis distance to  $F_{st}$  ratios, indicating disproportionate differences in morphology, despite relatively moderate levels of genetic divergence in comparison with other populations examined in this study. In contrast, the Arctic char population from Lough Coomasaharn did not show any extreme signs of morphological or genetic differentiation in comparison with other populations.

#### *Canonical variate analysis*

The CVA with the jackknife cross-validation procedure, based on body shape, was able to distinguish Arctic char individuals from several loughs with varying levels of successful assignment, ranging from 33% (Talt) to 100% (Caragh, Shindilla), with an average assignment success of 64.3% (Table 2.2). Jackknife cross-validation rates were not significantly correlated with sample size (Pearson correlation test:  $r=-0.323$ ,  $p$ -value = 0.0952).

#### **2.2.4 Main findings**

The results from the geometric morphometric analysis reported here confirm the remarkable diversity in the morphologies of Arctic char in Ireland, as previously reported by early fish naturalists (e.g. Regan and Günther). This high level of morphological variation, which is common in Arctic char throughout its distribution range, is thought to stem largely from a few key genes (Jacobs *et al.*, 2020; Salisbury and Ruzzante, 2022). Although some evidence correlating morphological and genetic distances is shown here, this is highly variable, as illustrated by the Glen–Beagh pair or in Lough Melvin, where high morphological differentiation was noted despite low genetic distance (Figure 2.4). If these significant morphological differences do not occur because of general genomic differentiation, they are likely to reflect the phenotypic plasticity of common genes



**Figure 2.4. (A)** Multiplot graph illustrating the relationship between morphological and genetic distances. Mahalanobis distances vs  $F_{st}$  (with regression line and confidence interval) for the 78 population pairs for which  $F_{st}$  estimates were available (data not shown). Note that the Glen–Beagh population pair is characterised by a very high Mahalanobis distance to  $F_{st}$  ratio. Heatmaps of Mahalanobis distances (B) and  $F_{st}$  (C).

triggered by different ecological conditions. Thus, despite the apparent connection between genomic divergence and morphology noted in some instances, the considerable variation around this relationship (brought about by environmental plasticity) significantly limits the use of geometric morphometric analysis as a stand-alone method for taxonomic assignment and/or the identification of conservation units of Arctic char in Ireland and, potentially, elsewhere.

Adams and Maitland (2007), in a review based on available information, questioned the validity of the Victorian taxonomy of Arctic char in the island of Ireland and Great Britain, particularly because it is based on the examination of a few specimens from a small number of loughs and also because it uses inconsistent and/or subjective characteristics. Based on the analyses of novel data presented here, there is no morphological evidence to support full species status for *S. fimbriatus* in Lough Coomasaham

**Table 2.2. Cross-validation assignment rates (%) of Arctic char individuals (classified by population in rows) to their population of origin (columns) based on a CVA on the residuals of PCs corrected for length of individuals and post-mortem bending**

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	None
Acoose (1)	<b>69</b>	0	6	0	7	4	1	0	1	0	0	1	1	0	0	0	0	3	6
Altan (2)	0	<b>95</b>	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
Beagh (3)	<b>19</b>	0	<b>38</b>	0	5	3	0	3	0	0	0	3	<b>16</b>	5	0	5	0	0	3
Caragh (4)	0	0	0	<b>100</b>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coomaglashlaw (5)	0	<b>13</b>	0	0	<b>59</b>	2	0	2	5	0	<b>10</b>	3	0	0	0	0	0	2	6
Coomasaham (6)	5	0	<b>11</b>	0	0	<b>68</b>	0	0	0	0	0	0	0	<b>16</b>	0	0	0	0	0
Doo (7)	0	0	0	0	0	4	<b>92</b>	0	0	0	0	0	4	0	0	0	0	0	0
Fad East (8)	0	7	3	0	<b>10</b>	0	0	<b>47</b>	7	7	3	0	3	0	0	0	0	0	<b>13</b>
Glen (9)	2	0	0	0	4	4	4	9	<b>57</b>	<b>11</b>	0	0	4	0	0	0	0	2	2
Glenicmurrin (10)	0	0	0	0	0	0	0	0	<b>33</b>	<b>67</b>	0	0	0	0	0	0	0	0	0
Greenan (11)	0	0	0	0	0	0	4	0	4	4	<b>81</b>	0	4	0	0	0	0	0	4
Keel (12)	0	0	4	0	0	0	0	0	4	0	<b>24</b>	<b>48</b>	<b>16</b>	0	0	0	0	0	4
Kindrum (13)	7	4	<b>11</b>	0	0	0	0	0	0	4	0	<b>11</b>	<b>59</b>	0	0	4	0	0	0
Kylemore (14)	0	0	0	0	0	0	0	0	<b>20</b>	0	0	0	0	<b>80</b>	0	0	0	0	0
Melvin (15)	8	0	0	8	0	0	0	0	0	0	0	0	0	0	<b>83</b>	0	0	0	0
Sessiagh (16)	0	0	0	0	0	0	0	0	0	0	0	0	<b>25</b>	0	0	<b>75</b>	0	0	0
Shindilla (17)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<b>100</b>	0	0
Talt (18)	0	0	0	0	0	<b>17</b>	0	0	0	0	0	0	0	0	0	0	0	<b>33</b>	<b>50</b>

The overall assignment rate was of 64.3%. Self-assignments have a grey background and misassignments above 10% are in bold.

and *S. grayii* in Lough Melvin. While no similarly quantifiable evidence was available for Arctic char in Ireland assigned full species status by Victorian taxonomists, this is also likely to be the case for these other species, based on the results presented here. In summary, the reinstatement of the Victorian-based taxonomy for Arctic char in Ireland as defended by Kottelat and Freyhof (2007) has no scientific support and, hence, this taxonomy should not be used. This finding is of particular relevance, as the Kottelat and Freyhof (2007) taxonomy is the current reference for Arctic char in the International Union for Conservation of Nature (IUCN) Red List (<https://www.iucnredlist.org/>), which, in turn, is used by governments to set conservation goals and assess losses related to extinction, among other biodiversity metrics. Thus, the results of the present study strongly argue for a review of the information related to Arctic char in the IUCN Red List of species and other species lists. As it stands, this information is incorrect and, hence, of no conservation value.

While of limited taxonomic value, results from the geometric morphometric analysis revealed important insights into the ecology of Arctic char in Ireland. Thus,

the significant differences in PCA extremes observed are in agreement with those displayed by benthic and pelagic morphs that are found in many Arctic char populations outside Ireland (Brachmann, 2021) and in other salmonids in general (Samways *et al.*, 2015; Piggott *et al.*, 2018; Koene *et al.*, 2020; Ferguson and Prodöhl, 2022). Benthic feeders typically have larger eyes, greater body depth, and a shorter and deeper caudal peduncle than pelagic feeders, and a mouth in inferior position. In contrast, pelagic feeders tend to display a longer and shallower body and head, smaller eyes, a longer and narrower caudal peduncle, and a mouth in terminal position.

It was not surprising to observe morphologies that are often related to resource usage among Arctic char populations from distinct loughs in Ireland. Arctic char is well known for its exceptionally generalist diet, often linked to individual specialisation. Thus, Arctic char can adapt so that it can feed in all available niches of lakes (Alekseyev *et al.*, 2002; Klemetsen, 2013), e.g. the littoral, limnetic and benthic (sometimes profundal) zones (Ferguson and Prodöhl, 2022). This adaptable life history trait is thought to have contributed to Arctic

char's success in being one of the first species to invade new freshwater habitats during deglaciation periods. This, in turn, has facilitated the occurrence of many instances of more or less isolated sympatric populations characterised by resource partitioning (Salisbury *et al.*, 2020). While the relatively small sample sizes used here do not allow for in-depth analyses (in particular within-lake comparisons), some interesting trends were noted. Thus, based on morphological features, Arctic char from Lough Coomasaharn appears to be predominantly planktivorous. In Lough Melvin, however, results, while not conclusive, seem to suggest the presence of both planktivorous and benthivorous Arctic char. Indeed, results from the genetic analyses of other loughs examined in this study seem to suggest the potential occurrence of trophic partition and, hence, that some of the Arctic char loughs in Ireland may harbour more than one population.

## 2.3 Genetic Analysis

DNA was extracted from Arctic char tissues using the Wizard SV Genomic DNA Purification System (Promega, UK), following the manufacturer's instructions. The resulting DNA quality was assessed through visual examination by gel electrophoresis. DNA concentration was determined using the AccuBlue Broad Range dsDNA Quantitation Kit (Biotium, USA) on a CLARIOstar plate reader.

Resulting genomic DNA quality and quantity were extremely variable among samples and different sources of tissue (e.g. scales, frozen tissue, ethanol preserved). Genomic DNA samples extracted from dried fish scales, with exceptions, were invariably of good quality (i.e. not degraded) but limited quantity (i.e. low yields). The exceptions (i.e. scales yielding DNA samples degraded to differing levels) were likely to be linked to suboptimal storage conditions for some of the scale samples (e.g. excess humidity). Substantial variation in degradation levels, however, was observed in DNA extracted from other tissues (either frozen or ethanol preserved). The most likely reason for this is related to the elapsed time between the death of the fish caught in the multi-mesh gillnets and the sampling of tissue for DNA work. This elapsed time could have reached several hours, thus potentially compromising the quality of the tissue for DNA extraction.

DNA extraction from museum-derived samples was particularly challenging. A special customised DNA extraction protocol based on the DNeasy Blood and Tissue Kit (Qiagen, Netherlands) was developed to ensure the recovery of DNA of optimal quantity and quality. While this protocol considerably improved both quality and yield, substantial levels of degradation were still evident in comparison with non-museum-derived samples. Resulting genomic DNA from all samples was grouped according to ad hoc quality criteria as follows: good, partially degraded and heavily degraded DNA. This facilitated the adjustment of subsequent genetic analysis protocols to improve results.

### 2.3.1 Nuclear DNA SNP marker development

In this first stage of the study, ddRADseq was used to analyse a limited number of samples ( $N=288$ ) from 18 Arctic char populations in Ireland and two outgroup populations – one from England (Windermere) and one from Sweden (Kilpisjärvi). The main objective of this work was to identify a set of informative SNP markers to be used in the genetic characterisation of Arctic char from loughs in Ireland.

To maximise the likelihood of identifying informative SNPs to differentiate potentially distinct types of Arctic char in Ireland, loughs were selected on the basis of prior knowledge (Went, 1945; Adams and Maitland, 2007; Kottelat and Freyhof, 2007) about the putative presence of the different species described by Victorian taxonomists and geographical coverage (see Table 2.1 and Figure 2.1). Genomic libraries were prepared using a modified version of the ddRADseq protocol of Peterson *et al.* (2012), as described in Maroso *et al.* (2018). Libraries were sequenced on the Illumina NextSeq 550 platform at the Genomics Core Technology Unit (Queen's University, Belfast) using paired-end 151 base pair (bp) sequencing.

Data processing, filtering and SNP identification followed an in-house customised bioinformatics workflow (further details are available from the authors; see Project Partners). To ensure the high quality of the final SNP dataset (minimising the occurrence of false SNPs), a stringent filtering multi-step process was carried out based on guidelines given in O'Leary *et al.* (2018). A graphical summary of the bioinformatic workflow, including multiple filtering steps, used to

identify nuclear SNPs from the ddRADseq raw data is provided in Figure 2.5.

A total of 315,060,260 sequence reads were obtained for the 288 individuals representing the 18 selected Arctic char loughs (average of 16 individuals per lake), with 84.69% exceeding a quality score of 30. During the demultiplexing step, 15.5% of these sequences were removed because the barcode could not be identified, with an additional 1.3% being removed because the RAD cutsite could not be identified. In total, 73.15% of the combined reads for all individuals processed successfully aligned to the Arctic char

reference genome. Variant calling using BCFtools resulted in the identification of 363,163 SNPs in the dataset. Following subsequent filtering steps (Figure 2.5) to remove false and/or non-informative SNPs, 1405 SNPs (referred to as the main dataset) were identified for further evaluation.

In the first stage of evaluation, the main ddRADseq dataset (1405 SNPs) was used to investigate the phylogenetic relationships among the Arctic char populations from the 18 selected loughs. A Bayesian-inferred phylogeny was produced using MrBayes v3.2.6 (Ronquist *et al.*, 2011), with the following

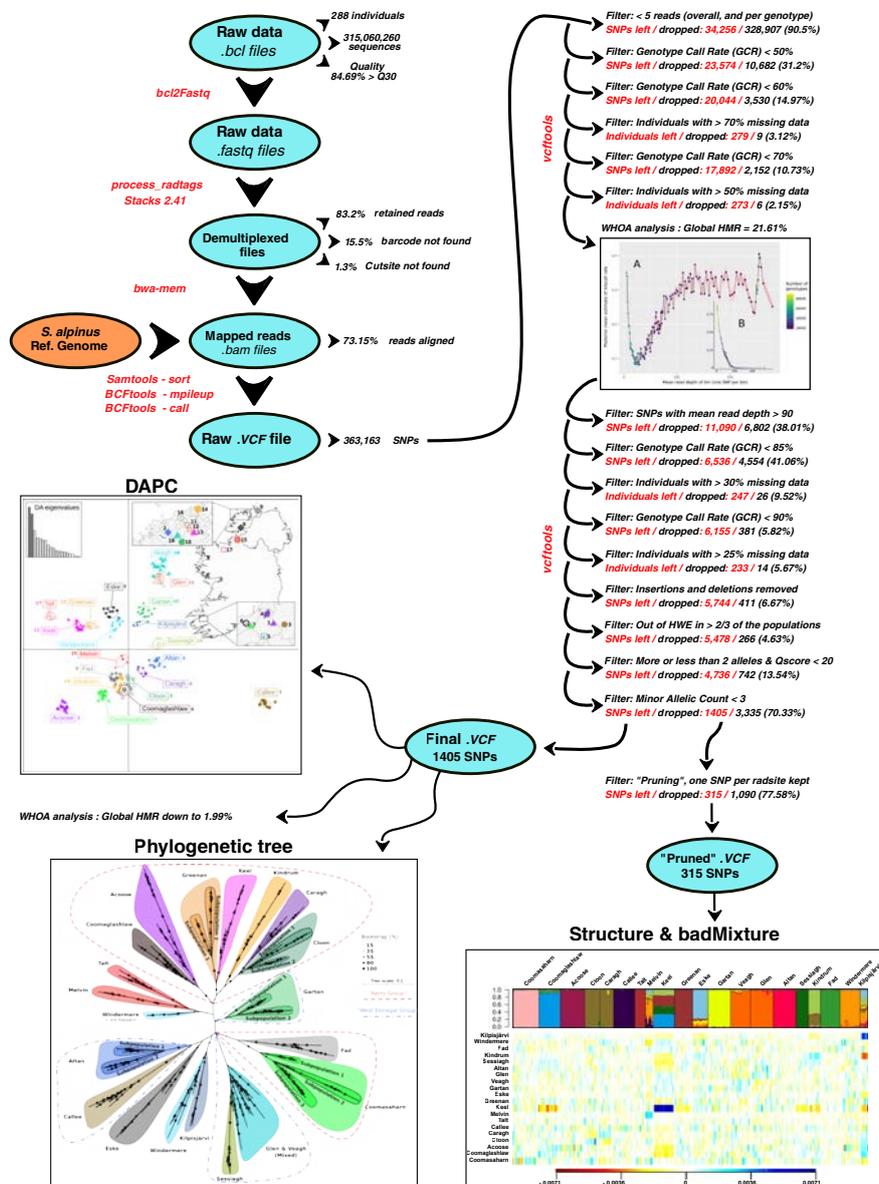


Figure 2.5. Graphical summary of the bioinformatics workflow used for the post-processing analysis of resulting ddRADseq raw genomic data from raw .bcl/ files to filtered SNPs, following guidelines given in O’Leary *et al.* (2018).

parameters: 15,000,000 generations; five runs with four chains each, allowing the Markov chain Monte Carlo (MCMC) procedure to sample across substitution models; and model of gamma-shaped rate variation across sites used. MrBayes runs were carried out using CIPRES Science Gateway (Miller *et al.*, 2010). Trees were imported, visualised and annotated using the Interactive Tree of Life online tool v5.5.1 (Letunic and Bork, 2007).

The resulting phylogeny is shown in Figure 2.6. At the highest level (i.e. internal tree nodes), the phylogeny is only weakly supported (i.e. low bootstrap values). At the terminal (lake) level, however, with the exception of Glen, Beagh and the two outgroups (Windermere

and Kilpisjärvi), most Arctic char loughs are relatively well defined. Glen and Beagh (lough codes 6 and 10; Figure 2.1) seemed to be admixed, suggesting a common genetic background and potentially some degree of gene flow. This finding was not surprising, as the loughs are geographically very close (~8 km apart) and directly connected by a river channel. Several broader Arctic char lake groupings were also evident from this analysis. Interestingly, in general, these broad groups were characterised by a geographically disjunct distribution.

Thus, the broader Kerry group and the West Donegal group comprise Arctic char loughs from both south-west (Kerry) and north-west (Donegal) areas of the

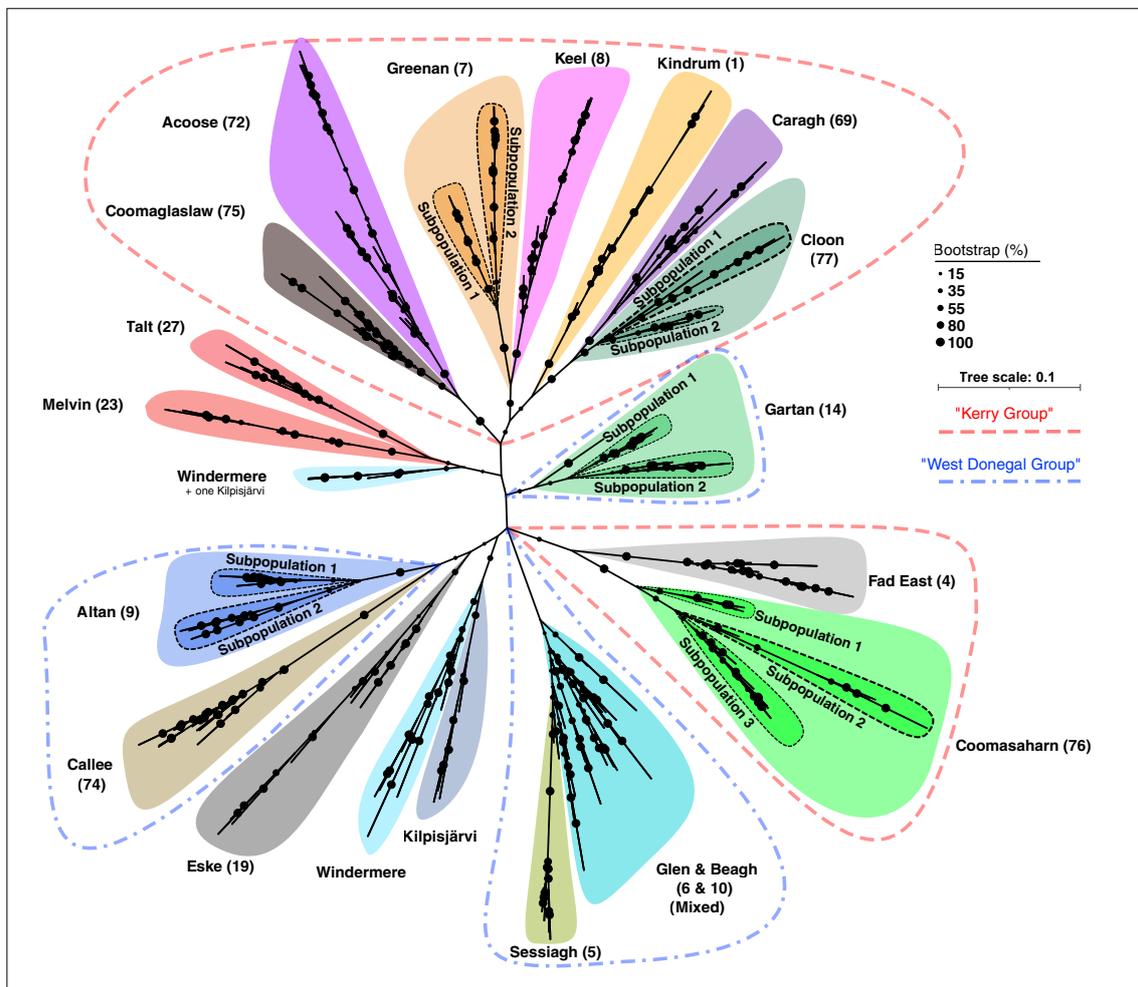


Figure 2.6. Bayesian phylogenetic tree representing Arctic char from 18 Arctic char loughs on the island of Ireland, and two outgroups (Kilpisjärvi, Sweden; and Windermere, England). The tree is based on the analysis of the 1405 SNPs identified in this study. Each branch represents an individual, and branch length reflects genetic distance. Bootstrap support values of more than 15% are represented by black dots of increasing size at the midpoint of each branch. Suspected population genetic sub-structuring within lake populations is highlighted.

island of Ireland. The Arctic char populations from Lough Melvin and Lough Talt appear to be more or less isolated. It is also interesting to note that the data seem to suggest further partitioning within some of the loughs (i.e. Gartan, Cloon, Altan, Greenan and Coomasaharn). This observation seems to support earlier results derived from the geometric morphometric analysis (section 2.2), indicating that more than one Arctic char population occurs in these loughs (sympatry). It is also worth noting that similar genetic partitioning was also observed among the outgroup samples (i.e. Windermere and Kilpisjärvi). In summary, the results of these preliminary analyses confirm the usefulness of these nuclear SNP markers for further investigations into the population genetics of Arctic char in Ireland.

Given the practical impossibility of obtaining data for all of the original 1405 SNPs for the > 3000 individuals in our sample, a subset of SNP markers was selected from the main 1405-SNP dataset for further genotyping. Selection criteria were primarily based on their informative value for ancestry inference (i.e. information content). This was estimated using the *Incalc* function of the *diveRsity* R package (Keenan *et al.*, 2013). A further criterion for selection was genome distribution. Thus, the most informative SNPs were selected on the basis of information content and genome representation. In the first selection round, 315 SNPs were identified, which were, on average, distributed throughout the Arctic char genome every 40,000 bp.

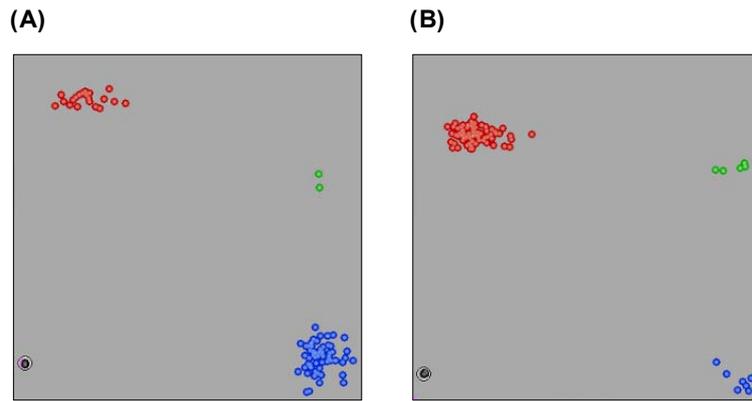
The filtered ddRADseq dataset comprising the 315-SNP subset for the 288 Arctic char individuals from the 18 selected loughs was used to test for the presence of potential outlier markers, that is, SNPs presumably under the influence of selection that should be removed from standard population genetic analysis (Luikart *et al.*, 2003). No outliers were identified using two distinct analytical approaches as implemented in *BayeScan* 2.1 (Foll and Gaggiotti, 2008) and *Outflank* (Whitlock and Lotterhos, 2015). Thus, all 315 SNPs behaved as neutral genetic markers and could be included in the subsequent step of evaluation.

Sequences containing the site variation for each of the 315 SNPs were used for the design of KASP assays (LGC Biosearch Technologies; [www.biosearchtech.com](http://www.biosearchtech.com)), which are based on competitive allele-specific polymerase chain reaction (PCR). Given the variable

quality of DNA available for analysis in this study (i.e. the different levels of degradation), assays were designed to minimise PCR product size. Thus, maximum effort was made to ensure that KASP assays resulted in PCR products of between 40 and 60 bp in length. In total, 110 assays met this criterion. Initial evaluation of the performance of these assays was carried out using a sample set of 92 Arctic char specimens of varying DNA quality representing different loughs in Ireland, and an in-house SNPLINE plate-based high-throughput genotyping platform ([www.biosearchtech.com](http://www.biosearchtech.com)). The aim here was to identify SNPs that would both be informative (polymorphic) and work consistently for all samples regardless of DNA quality. Examples of consistent SNP genotyping results generated during this evaluation step are illustrated in Figure 2.7. While 70% of the SNP markers tested ( $N=77$ ) resulted in a positive signal, considerable variation, linked to DNA quality, was noted for most. Thus, despite efforts to design assays compatible with poor-quality DNA, most of them failed to consistently generate genotypes for partially or heavily degraded DNA. Fifteen SNP markers, however, were found to consistently amplify DNA (i.e. produce a signal for genotyping) from all samples regardless of DNA quality. Close examination of the assay design for these SNP markers confirmed that they were linked to the smallest PCR products, within the very narrow window of 40–50 bp in length.

### ***2.3.2 Arctic char population genetic structuring based on nuclear SNP markers***

The 15 SNP markers identified were used to screen the 3072-sample collection obtained for this study, using the KASP assays and the LGC SNPLINE platform described above. The resulting raw nuclear SNP genotypes for all samples were assembled into an Excel database for quality control assessment. Individuals with more than 80% of data missing were removed. This resulted in a final dataset comprising 2606 specimens (i.e. 84% of the original samples), representing 42 Irish loughs and the outgroup samples (Scotland, England and Sweden), which was retained for further analyses. Given their limited sample size (e.g. one to seven specimens in each case) and/or excess missing data in the last filtering step, Arctic char individuals from the remaining 13 loughs (i.e. Akibbon, Anscaul, Ballynahinch, Cloonsnaghta,



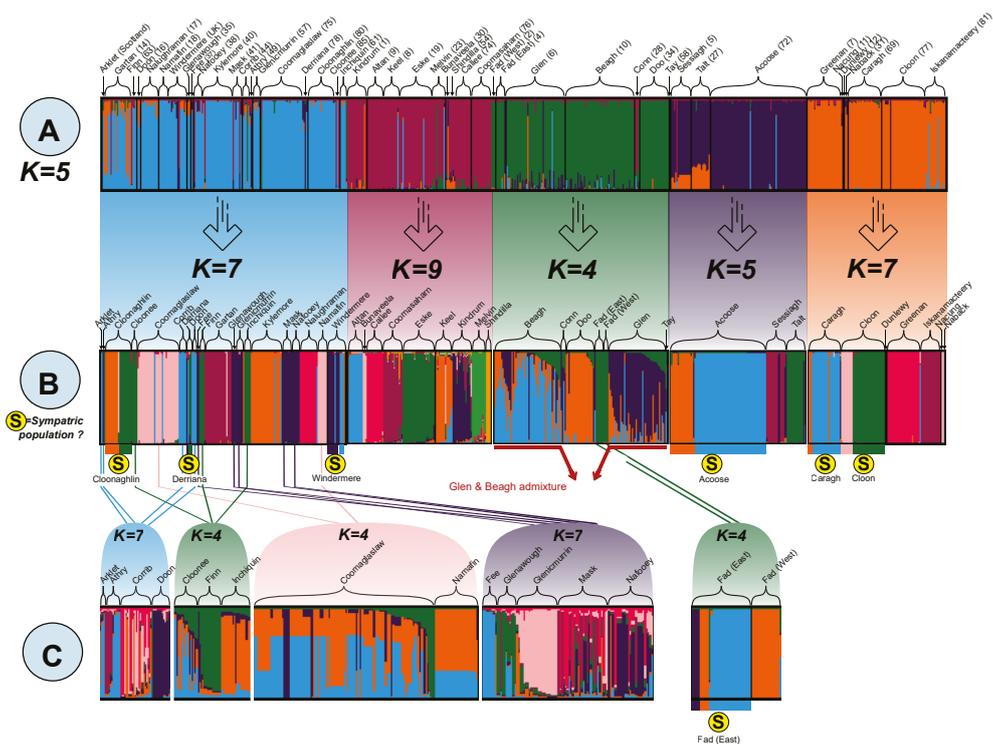
**Figure 2.7. Raw genotype plots for the (A) IAC\_01138 and (B) IAC\_1099 Arctic char nuclear SNP markers developed in this study, generated using an in-house LGC SNPline plate-based high-throughput genotyping platform. In each case, coloured circles represent individual genotypes. Homozygote individuals for alternative SNP variants are displayed as red and blue circles. Heterozygote individuals are displayed as green circles. Black circles represent non-template (i.e. no DNA) control samples.**

Currane, Dan, Derg, Derryneen, Ennell, Inagh, Leane, Muckcross and Owel) were removed from subsequent nuclear SNP analyses. While confirmation is still required, Arctic char is now thought to be extinct in five of the loughs in this group (i.e. Anscaul, Cloonsnaghta, Dan, Derryneen and Inagh) (Table 2.1).

The Bayesian individual-based clustering approach implemented in PopCluster (Wang, 2022) was used to investigate population structuring among Arctic char samples from the 51 loughs in Ireland and the outgroups (Scotland, England and Sweden). This software is particularly suited to exploring patterns of population genetic structure in datasets comprising samples of different sizes (i.e. unbalanced sampling), which is known to bias the outcome of analyses. PopCluster implements a “scaling” algorithm that balances the *a priori* assignment of an individual to a cluster based on the sample sizes among populations. In this study, PopCluster was run using the strong scaling parameter to minimise the effect of unbalanced sampling (i.e. varying sample sizes). Ten replicates were run to obtain each *K* value, which represents the number of genetic clusters that can best explain the data and was permitted to range from 2 to 50. The *FSTIS* method, which is based on Wright’s (1984) *F*-statistics, and is also implemented in PopCluster, was used in addition to visual inspection as a guide to identify the uppermost hierarchical level of population structuring within the dataset. To account for possible deep genetic divergence between clusters that could

hide subtle population structure patterns, PopCluster runs were carried out using a hierarchical approach aiming to identify major population groups within the data (i.e. potentially related by common ancestry), with the subsequent refinement of these (i.e. subsequent PopCluster runs involving samples from each previously identified group) down to single populations. Results of these analyses are shown in Figure 2.8.

The first hierarchical level of population structuring (Figure 2.8A) of the 42 Arctic char loughs (and the three outgroup samples) indicates the presence of five genetic clusters (*K*=5). Similarly to what is shown above (see Figure 2.6), results of this more comprehensive analysis confirmed the absence of geographical concordance between loughs and genetic cluster membership from nuclear data. Cluster 1 was predominantly represented by Arctic char loughs in Donegal, Galway, Kerry and Mayo, in addition to the outgroups in England and Scotland. The average elevation and area of the loughs within this cluster were 79.7 m and 159.5 ha, respectively. Cluster 2 (average lake elevation = 104.2 m; average area = 338.6 ha) comprised samples from Donegal, Kerry, Leitrim and Galway. Cluster 3 (average lake elevation = 90.2 m; average area = 55.09 ha) was predominantly composed of loughs in Donegal but included one lough in Mayo. Group 4 (average lake elevation = 138.7 m; average area = 42.24 ha) consisted of loughs in Donegal, Kerry, Sligo and Wicklow. Finally, Cluster 5 (average lake elevation = 91.8 m;



**Figure 2.8. PopCluster bar plots. (A) First hierarchical level of population structuring of the 43 Arctic char loughs (and outgroup samples), supporting the presence of five genetic clusters ( $K=5$ ). Individual specimens are represented as thin vertical coloured lines. In each case, different colours represent distinct genetic inferred lineages/clusters. Multicoloured individual vertical lines are indicative of introgression and/or genetic similarities between inferred lineages, clusters and/or populations. The lough sources for Arctic char individuals are named at the top of the bar plots. Second (B) and third (C) hierarchical levels of analyses resulting from independent PopCluster runs involving each of the five clusters identified in the first hierarchical level (A). While similar colours within each independent run represent similar genetic groups, similar colours between runs are unrelated. In total, 32 distinct genetic clusters were identified: seven, nine, four, five and seven from clusters 1, 2, 3, 4 and 5 in (A), respectively. “S” denotes loughs where more than one Arctic char population potentially occurs. Patterns of genetic introgression between Arctic char from Lough Glen and Lough Beagh are also highlighted.**

average area = 53.07 ha) was represented by loughs in Donegal, Mayo, Longford and Kerry.

The results of the second hierarchical level of PopCluster analyses (Figure 2.8B) indicate that, with exceptions, most Arctic char loughs contain genetically distinct populations. This was confirmed in the third and final hierarchical level of PopCluster (Figure 2.8C), where Arctic char from most loughs resolved into genetically distinct populations. The high level of genetic admixture (genetic introgression) observed between Arctic char populations from Lough Beagh and Lough Glen confirms previous results suggesting at least some level of contemporary and/or relatively recent gene flow between these two loughs. This finding was not entirely unexpected given that

both loughs are still connected by a natural river channel. Interestingly, no substantial level of genetic introgression was noted for the few other Arctic char loughs that are still connected by a river channel (e.g. Cloon and Caragh). For the remaining loughs, not surprisingly given their isolation history, only limited evidence of admixture was noted. Overall, Arctic char populations from the majority of the loughs are genetically distinct. It is important to note that these differences, as measured by the neutral SNP markers employed in this study, are likely to be the result of the effects of genetic drift on isolated populations of finite size.

One notable result from this stage of the analysis is the clear evidence for the potential presence of more

than one Arctic char population within some of the loughs. This is particularly the case for Arctic char from Loughs Cloonaghlin, Derriana, Acoose, Caragh and Cloon in Ireland, and Lake Windermere in England. It is interesting to note that previous studies (e.g. Child, 1984; Winfield *et al.*, 2008) have suggested the presence of at least two genetically distinct Arctic char populations in Lake Windermere, the largest natural lake in England. Results presented here seem to confirm this.

To further examine the genetic relationships among Arctic char populations from different loughs in Ireland, a neighbour-joining (NJ) phylogenetic tree, based on Nei's DA genetic distance (Nei *et al.*, 1983), was

constructed using POPTREE2 (Takezaki *et al.*, 2010). The results of this analysis are shown in Figure 2.9.

The low to moderate bootstrap support observed for most of the tree nodes was expected given the small number of SNP markers used in this analysis. Notwithstanding this, the overall pattern of the tree is similar to that observed for the partial sample dataset screened for the full complement of SNPs (1406). Thus, overall, there is little correlation between genetic and geographical groups (i.e. disjunct geographical distribution). The few exceptions involve loughs still sharing a connection through natural river channels (e.g. Dunlewey and Nacung, Caragh and Cloon). Interestingly, the Arctic char population from

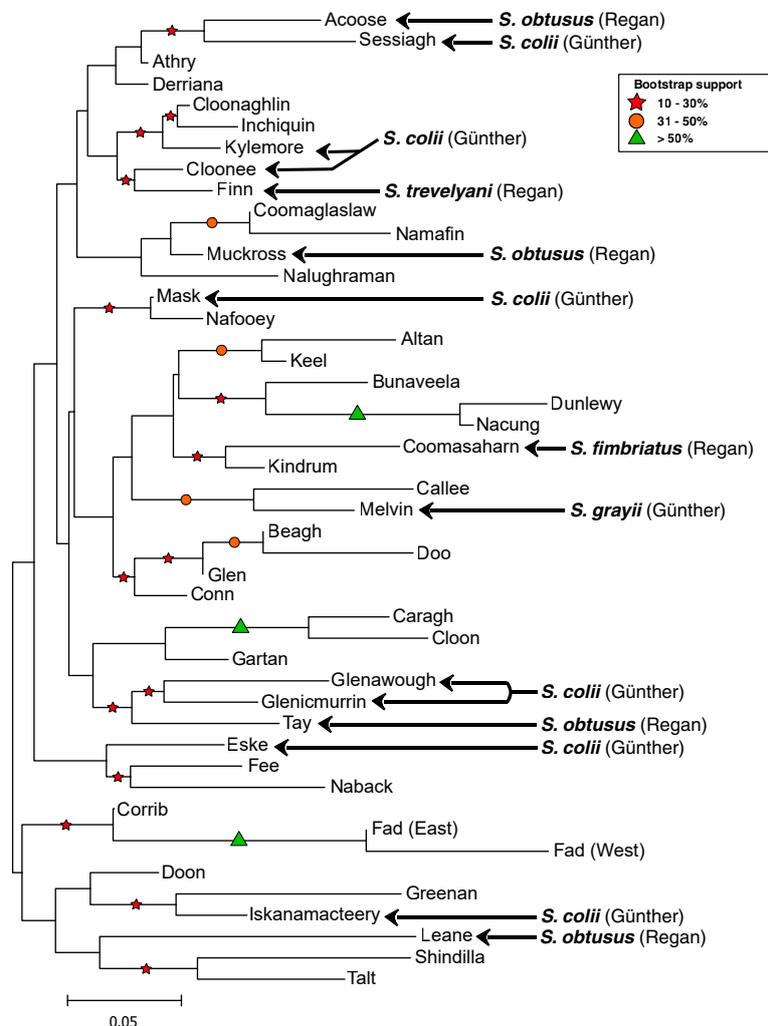


Figure 2.9. Unrooted NJ phylogenetic tree based on Nei's DA genetic distance (Nei *et al.*, 1983) illustrating the relationship among Arctic char populations from different loughs in Ireland. Bootstrap support for tree nodes is displayed using coloured symbols. Where species described by Victorian taxonomists have previously been reported for particular loughs (Adams and Maitland, 2007), this is also illustrated for information.

Tay (Wicklow), one of the few eastern Ireland loughs for which samples from a now extinct population were available, seems to group with Arctic char populations from loughs located in the mid-west area of Ireland (Glenawough in Mayo and Glenicmurrin in Galway). Similarly, the now extinct Arctic char population of Lough Naback (Longford) in central Ireland also appears to be related to Arctic char populations from the mid-west and northern loughs (Fee in Galway and Eske in Donegal). Again, in support of previous findings from this study, no evidence was found for the presence of species previously described by Victorian taxonomists in these loughs. Thus, no grouping of these species was observed in the phylogenetic tree.

### **2.3.3 *Insights into the phylogeographical history of Arctic char in Ireland from full mitochondrial genomes***

Rare and vulnerable genetic lineages represent distinct evolutionary units resulting from various environmentally driven processes. The identification and preservation of these evolutionary units is a key issue in biological conservation and, hence, of fundamental relevance for the development and implementation of sound biodiversity action plans. Phylogeographical inference has been successfully used as an analytical tool for identifying meaningful evolutionary units. While useful for answering population-based questions, the utility of nuclear DNA for phylogeographical inferences can be greatly compromised by recombination, which tends to reduce and/or confuse the evolutionary signal over time. Given that mitochondrial DNA (mtDNA) is inherited from mother to offspring without recombination, it retains its phylogeographical signal over time, even in the presence of population introgression (Avice, 2004). Hence, mtDNA has been used as a major tool for phylogeographical inference.

In this component of the study, analyses of the full mitochondrial genomes (mitogenomes) of 288 Arctic char individuals (4 to 10 individuals from 29 Irish loughs and outgroups from England and Scotland) were used to infer the phylogeographical history of Arctic char in Ireland. The selection criteria for individual samples were as follows: (1) the inclusion of samples representing the largest possible number of loughs in Ireland; (2) the selection of larger numbers of specimens for analyses from loughs suspected of

harbouring sympatric populations (see sections 2.3.1 and 2.3.2); and (3) the inclusion, where possible, of the sample specimens used in for ddRADseq (see section 2.3.2), for direct comparison.

#### *Generating Arctic char mitogenomes from Irish loughs and outgroups*

The complete protocol details used for sequencing the mitogenomes of the 288 selected Arctic char individuals are available on request from the authors. Briefly, nine PCR primer sets (pairs) were designed using Mitoprimer V1 (Yang *et al.*, 2011) to amplify the whole Arctic char mitogenome (~16.5 kilobases (kb)) from each sample. These PCR primer sets were designed based on conserved regions of the *S. alpinus* (AF154851.1) and *S. malma* (KJ746618.1) mitogenomes. For each individual, the resulting PCR products (ranging from 2770 to 3950 bp in length) were pooled in equal amounts (~450 ng of each PCR product), purified using Agencourt AMPure XP/SPRI select beads (following the manufacturer's instructions) and resuspended in 2 µL of double-distilled water. Genome library construction, based on a dual-index strategy (IDT8UDI), was carried out using the Kapa HyperPlus Kit (Kapa Biosystems), following the manufacturer's protocol. Following quantification, the pooled library was sequenced in-house on an Illumina MiSeq next-generation sequencing platform using paired-end chemistry with a read length of 150 bp.

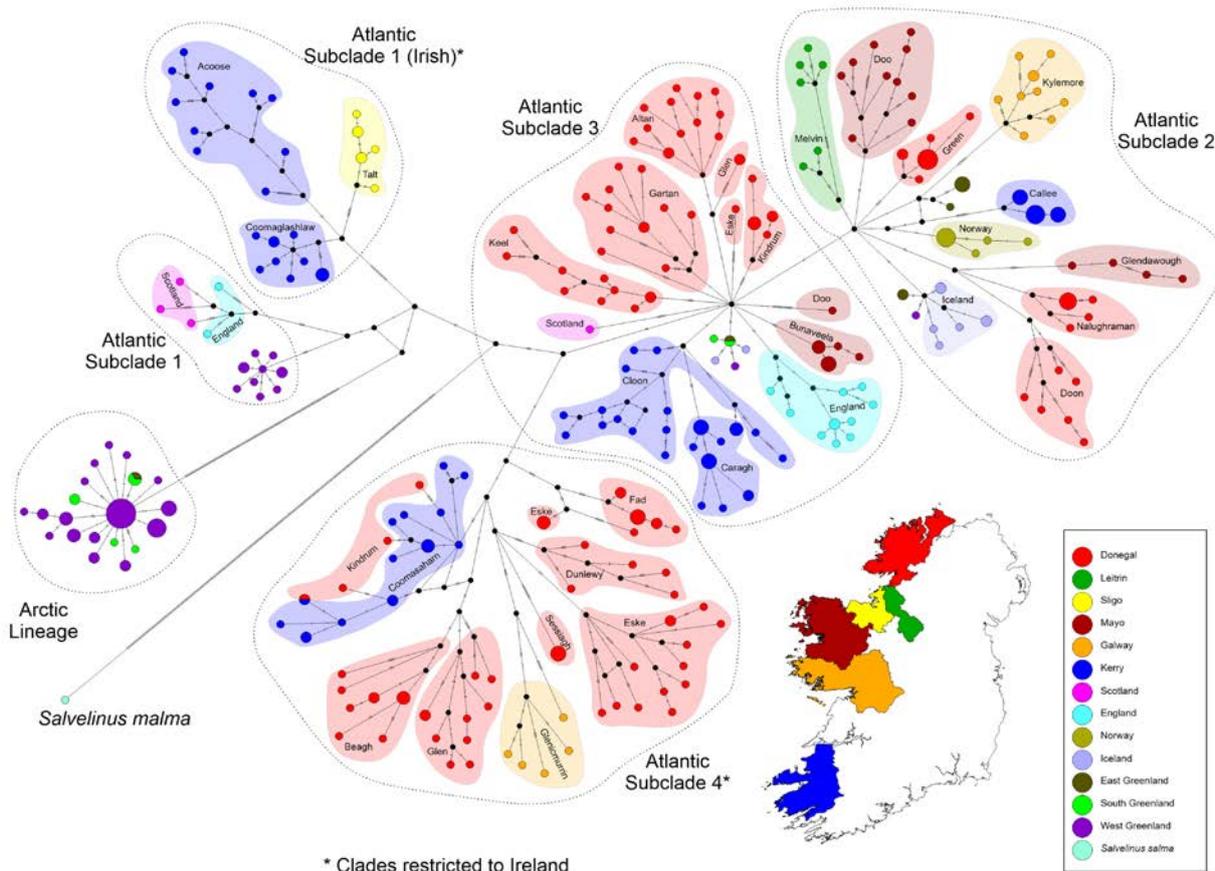
Raw sequence reads were demultiplexed and barcodes removed on the MiSeq reporter software (Illumina). Clean demultiplexed reads were then aligned using Bowtie2 with the "local" alignment option. Resulting SAM files were converted to BAM files and sorted using Samtools and one available reference mitogenome for *S. alpinus* (accession No.AF154851.1). Variant calling and consensus sequence construction was performed using Freebayes with a ploidy of 1 and a minimum alternate count of 2. Resulting files were converted to Fastq format using VCFutils and, subsequently, to FASTA format using seqtk (<https://github.com/lh3/seqtk.git>). All reconstructed mitogenomes were assessed for quality, and, subsequently, mitogenomes from 40 individuals were removed because of poor genome coverage and/or because more than 15% of data were missing. Sequencing alignment of resulting mitogenomes and

a reference genome was carried out in MEGA11 using the ClustalW algorithm with default parameters.

*Phylogenetic analysis of Arctic char mitogenomes*

For subsequent phylogeographical analysis, in addition to the novel 248 mitogenomes generated in this study, 94 Arctic char mitogenomes derived from a recent study by Jacobsen *et al.* (2021) were also included for comparison. These latter samples represent Arctic char originating from 10 sites across western and eastern Greenland, Iceland and Norway. The Jacobsen *et al.* study did not include Arctic char mitogenomes from Britain or Ireland, however. In different parts of the subsequent analyses, the mitogenomes *S. fontinalis*, *S. namaycush* and *S. malma* were also included for reference.

Several approaches were explored in an effort to investigate the phylogenetic relationships among the Arctic char mitogenomes from Ireland and elsewhere. First, a median-joining haplotypic network based on whole mitogenomes was constructed using PopArt version 1.7 (Leigh and Bryant, 2015). The resulting network is displayed in Figure 2.10. In total, 173 distinct mitogenomes (haplotypes) were identified among the 342 specimens included in the analyses. Not surprisingly, the haplotype (subsequently referred to as the mitogenome) of the outgroup *S. malma* (Dolly Varden trout) is highly divergent in comparison with that of Arctic char, with these species differing in over 360 fixed mutations. Within the Arctic char group, the more divergent lineage comprises samples from west and south Greenland (~162 unique mutations), referred to by Jacobsen *et al.* (2021) as the “Arctic lineage”. Several outgroup mitogenomes generated



**Figure 2.10. Median-joining network representing 378 complete Arctic char mitogenomes. Mitogenome sequence data (N=94) from Arctic char from Greenland, Iceland and Norway are derived from Jacobsen *et al.* (2021). The sources of all mitogenomes in Ireland and elsewhere are identified by colour codes. Black circles represent inferred haplotypes (not observed in datasets); small crosslines represent mutational steps.**

in this study, representing Arctic char from Scotland and England, can be grouped with equivalent mitogenomes from Jacobsen *et al.*'s (2021) Atlantic Subclade 1 lineage. Mitogenomes found only in Irish Arctic char from Loughs Acoose, Coomaglaslaw and Talt form a clearly distinct novel group, referred to here as Atlantic Subclade 1 (Irish) lineage. Jacobsen *et al.* (2021) grouped mitogenomes from Arctic char samples from east Greenland, south Greenland, Iceland and Norway into Atlantic Subclade 2. The additional phylogeographical resolution, provided by the inclusion of the novel mitogenome sequences generated in this study, suggests that this lineage can be further partitioned into Atlantic Subclade 2 and Atlantic Subclade 3. These two groups comprise Arctic char samples from Ireland, England, Scotland, south Greenland, Iceland and Norway. A final novel lineage, Atlantic Subclade 4, is similar to Atlantic Subclade 1 (Irish) lineage, comprising exclusively Arctic char samples from Ireland.

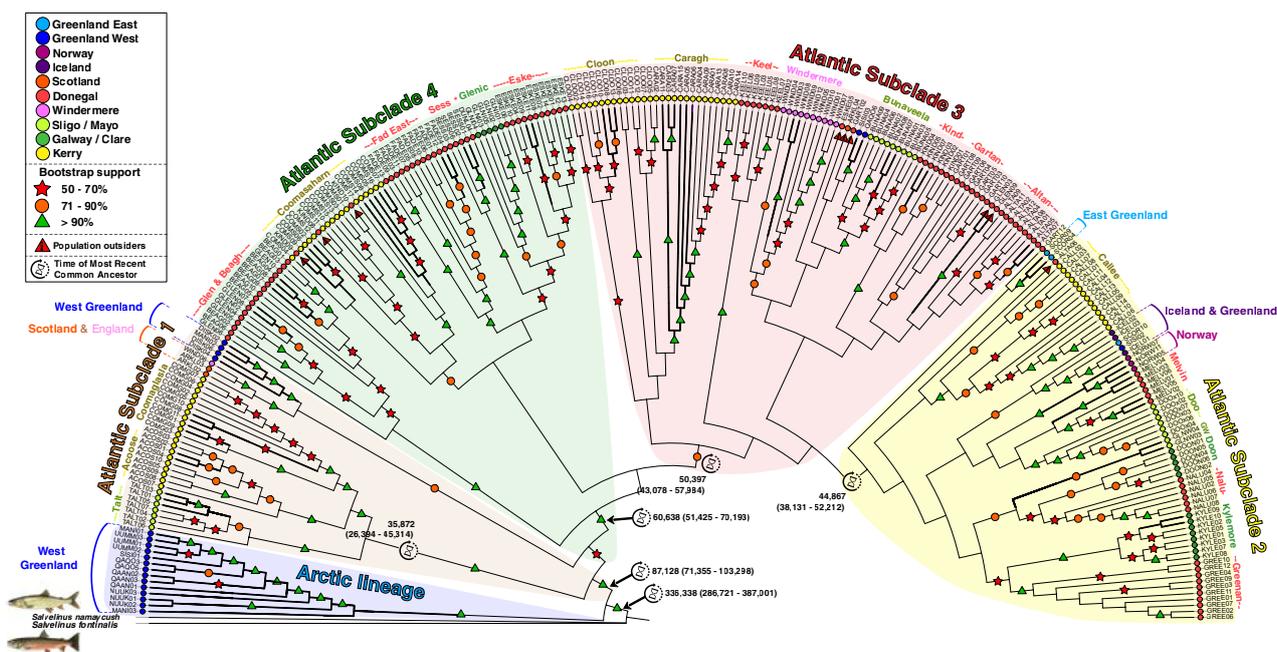
Two major features are clear from this mitogenome (haplotypic) network. The first is that, similarly to what has been noted from the analysis of nuclear DNA, there is no apparent correlation between geographical location and distribution of the several clearly distinct mtDNA lineages in Ireland and elsewhere. This holds true even for lineages that are restricted to Ireland. Thus, of the three Arctic char loughs representing Atlantic Subclade 1 (Irish), two are located in Kerry (Acoose and Coomaglaslaw) and one in Sligo (Talt).

Similarly, Atlantic Subclade 4 comprises Arctic char loughs in Donegal (Kindrum, Eske, Beagh, Glen, Fad, Sessiagh and Dunlewey), Kerry (Coomasaharn) and Galway (Glenicmurrin). A similar disjunct geographical distribution pattern is noted for the other lineages, with broader distribution in Ireland and elsewhere. The second major feature of the network is that, with exceptions, the mitogenomic diversity is explained by within-lough diversity. Thus, the majority of mitogenomes identified here are restricted to single loughs. Perhaps not surprisingly, given the genome coverage (~16.5 kb) per individual, some 45% of the mitogenomes represent unique haplotypes. Interestingly, within loughs most mitogenomes diverge by very few mutations. Indeed, in many cases, mitogenomes diverge by one to four mutations over the 16.5 kb genome coverage. This suggests that most diversification took place after Arctic char populations became isolated in these loughs following the retreat

of the ice 23,000 years ago. Some exceptions can be noted, however; mitogenomes belonging to both Atlantic Subclade 3 and Atlantic Subclade 4 are found within Loughs Kindrum, Eske and Glen. Similarly, mitogenomes belonging to both Atlantic Subclade 2 and Atlantic Subclade 3 co-occur within Lough Doo.

Following the network-based analysis, a maximum likelihood (ML) phylogenetic tree was constructed using a general time reversible model (GTR+G+I), with 100 bootstrap replicates. Branches with poor bootstrap support (i.e. less than 20%) were collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying the maximum parsimony method. A discrete gamma distribution was used to model evolutionary rate differences among sites (four categories + G, parameter = 1.0704). The rate variation model allowed for some sites to be evolutionarily invariable (+I, 63.26% of sites). Divergence times for main nodes were estimated using BEAST (Drummond and Heled, 2009). For this, a "birth-death" evolutionary model was used, with the MCMC run for 100,000,000 generations, and sampled every 10,000 generations. A strict molecular clock and a full mitogenome substitution rate of  $1.537 \times 10^{-8}$  were used, as estimated from whitefish (*Coregonus* spp.; Jacobsen *et al.*, 2012, 2021). Convergence was confirmed by the stationarity of the traces and the inspection of effective sample size values, as analysed in Tracer (Rambaut *et al.*, 2014). The time trees were summarised into a maximum-clade credibility tree using TreeAnnotator (Drummond and Rambaut, 2007), with mean heights for branches and with an initial "burnin" function of 10%. The final ML tree (Figure 2.11) was constructed and edited in FigTree v 1.3.1 (Andre Rambaut, University of Edinburgh, <http://tree.bio.ed.ac.uk/software/figtree/>).

With minor visual differences, the resulting ML phylogenetic tree confirms support for the findings of the results from the median-joining network analysis. Thus, all identified lineages (i.e. Arctic lineage, Atlantic Subclade 1, Atlantic Subclade 1 (Irish), Atlantic Subclade 2, Atlantic Subclade 3 and Atlantic Subclade 4) are well supported (bootstrap support for nodes > 90%). Furthermore, within each lineage there is strong evidence for further sub-lineages, often linked to particular loughs. Arctic char individuals from Loughs Acoose and Coomaglaslaw belonging to Atlantic Subclade 1 (Irish) provide a good example of this. Similarly, Arctic char individuals from Loughs



**Figure 2.11. ML phylogeny of 273 Arctic char mitogenomes. Node bootstrap support is shown by coloured symbols (nodes with less than 50% support are not shown). Coloured shaded areas in the tree represent the geographical regions indicated. Terminal branches were coloured according to the geographical region if neighbouring individuals came from the same broad area. The time since the Arctic char populations from the different loughs diverged from a common ancestral population is also noted in main tree nodes.**

Beagh and Glen appear to belong to the same well-supported sub-lineages within Atlantic Subclade 4. This observation is consistent with previous results linked to these loughs. A major informative feature of the ML phylogeny is linked to the estimated divergence times of the main tree nodes (i.e. estimated time of divergence of the Arctic char populations from the different loughs since they shared a common ancestor). The Arctic lineage, at ~336,300 years old, is the oldest group, followed by Atlantic Subclade 1 (~87,100 years), Atlantic Subclade 4 (~60,400 years), Atlantic Subclade 3 (~50,300 years) and Atlantic Subclade 2 (~44,800 years). Arctic char individuals from the loughs constituting Atlantic Subclade 1 (Irish) are estimated to have been diverging from the Jacobsen *et al.* (2021) Atlantic Subclade 1 lineage for some 35,000 years.

#### Mitochondrial DNA SNP marker development

Mitogenome sequencing alignments representing Arctic char populations from loughs in Ireland (see the section “Phylogenetic analysis of Arctic char mitogenomes”) were examined to identify

informative SNPs that could potentially be used for the development of KASP-based mtDNA SNP genotyping assays, using a similar rationale to the one employed for the development of the nuclear SNP KASP assays using ddRADseq (section 2.3.1). This selection process identified 914 SNPs throughout the complete Arctic char mitogenome (~16.5 kb) representing the Irish loughs examined. These SNPs were further categorised in terms of the frequency of the variant (SNP) in the alignment. In the first group, 443 SNPs met the 0.05% criterion (i.e. 5% of the individuals in the alignment displayed were polymorphic). The second, third and fourth groups consisted of 379 (0.1% criteria), 68 (0.15% criteria) and 24 (0.25% criteria) SNPs, respectively. SNPs from groups 3 and 4 were particularly useful for examining moderate to deep divergence, while those from groups 1 and 2 were useful for investigating shallow divergence.

SNPs belonging to groups 2, 3 and 4 were targeted for KASP assay design. All lineage-defining SNPs identified during median-joining network analysis were included in the selection. In total, 110 mtDNA sequences containing the site variation for these SNPs

were selected. The rationale for mtDNA KASP SNP assay design and empirical evaluation of resulting assays followed that provided in section 2.3.1 for the development of nuclear DNA SNP markers. Examples of consistent mtDNA SNP genotype plots generated during this evaluation step are shown in Figure 2.12. Forty-nine mtDNA SNP assays (44.5%) resulted in a positive and consistent signal regardless of sample DNA quality. The difference in success rate between nuclear and mtDNA SNP markers is likely to be associated with the larger molecule copy number of the latter. This result thus suggests that the mtDNA SNP markers may be particularly useful for studies where DNA quality may be an issue.

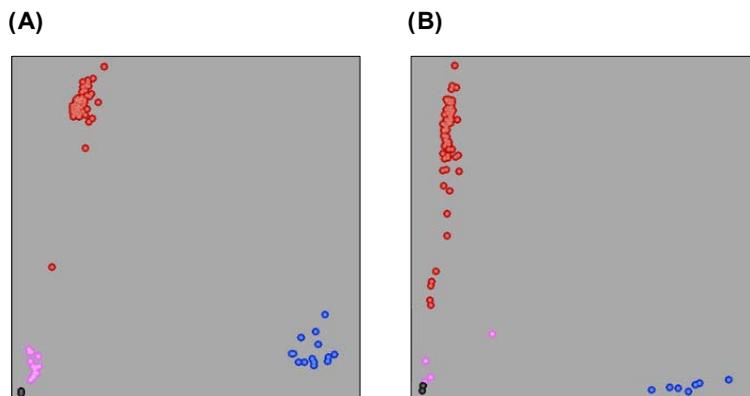
*Phylogenetic analysis of Arctic char in Ireland based on mitochondrial DNA SNP genotyping*

The 49 SNP markers identified above were used to screen the 3072-sample collection obtained for this study, and museum-derived samples, using the KASP assay and the LGC SNPLine SNP platform, as described in sections 2.3.1 and 2.3.2 for nuclear DNA SNP markers. Resulting raw mtDNA SNP haplotypes for all samples were assembled into an Excel database for quality control assessment. Data for three mtDNA SNPs were removed from further analysis owing to spurious haplotype calls and/or a high percentage of data missing. All individuals with more than 20% of data missing were also removed. The final mtDNA SNP dataset consisted of 1638 individuals (53% of original dataset) screened for 46 mtDNA

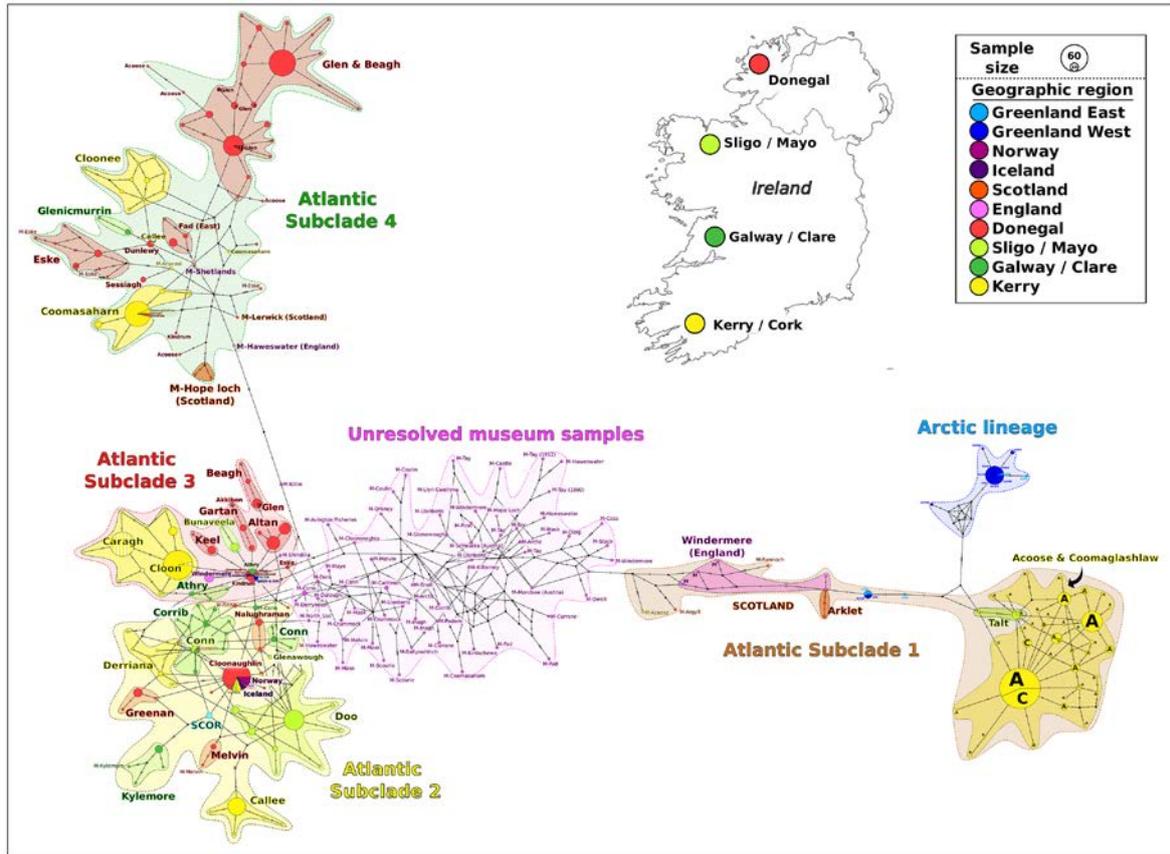
SNPs. These data were used to construct a median-joining mtDNA haplotype network using PopArt version 1.7 (Leigh and Bryant, 2015). For comparison, relevant SNP calls from selected samples representing the mitogenomes described by Jacobsen *et al.* (2021) were also included in the dataset.

The resulting network is displayed in Figure 2.13. In total, 283 mtDNA haplotypes were observed among Arctic char samples examined in this study. This represents a 50% increase in haplotype numbers in comparison with what was noted from the previous analyses of full mitogenomes based on a subset of the samples. This larger number of haplotypes was not unexpected given the much larger sample size involved in this analysis. The frequency distribution of the resulting mtDNA haplotypes was noticeably biased as follows: six mtDNA haplotypes were shared among 44% of all Arctic char samples examined, 16 were shared among 26% of the samples, 69 were shared among 17% of the samples and 192 were shared among 11% of the samples. The latter group (representing 67% of the observed mtDNA haplotypes) occurred in very low frequencies and were invariably restricted to single loughs.

Notwithstanding the additional number of mtDNA haplotypes, the selected mtDNA SNP assay panel successfully recovered all mtDNA lineages previously identified through the sequence analysis of complete mitogenomes. Thus, samples were readily resolved into Arctic lineage, Atlantic Subclade 1, Atlantic Subclade 1 (Irish), Atlantic Subclade 2, Atlantic



**Figure 2.12. Raw genotype plots for (A) mtDNA-00642 and (B) mtDNA-05944 Arctic char mtDNA SNP markers developed in this study and generated using an in-house LGC SNPLine plate-based high-throughput genotyping platform. In each case, coloured circles represent individual genotypes. The mtDNA haplotypes for alternative SNP variants are displayed as red and blue circles. Black circles represent non-template (i.e. no DNA) control samples. Pink circles represent failed samples.**



**Figure 2.13. Median-joining network derived from the screening of 1638 Arctic char samples for 46 mtDNA SNPs. Arctic char SNP data from Greenland, Iceland and Norway were derived from Jacobsen et al. (2021). The sources of all mitogenomes in Ireland and elsewhere are identified by colour codes.**

Subclade 3 and Atlantic Subclade 4. The positioning of many of the museum-derived samples was intriguing, forming an unresolved complex group between Atlantic Subclade 1 and Atlantic Subclades 2 and 3. It is important to note that many of these museum-derived Arctic char samples were not originally derived from the island of Ireland and were included as outgroups for this analysis. It is also worth noting that the museum-derived Arctic char samples that were originally collected from Irish loughs invariably linked to their correct lake. Thus, a Victorian-type *S. grayii* caught in Lough Melvin in 1895 was correctly linked to a contemporary Lough Melvin Arctic char individual. Other similar examples were noted, with museum specimens originally collected in Lough Eske also being linked to contemporary Lough Eske Arctic char samples. While these observations do not validate the Victorian species taxonomy, they do confirm the persistence of these lineages over time in these particular loughs.

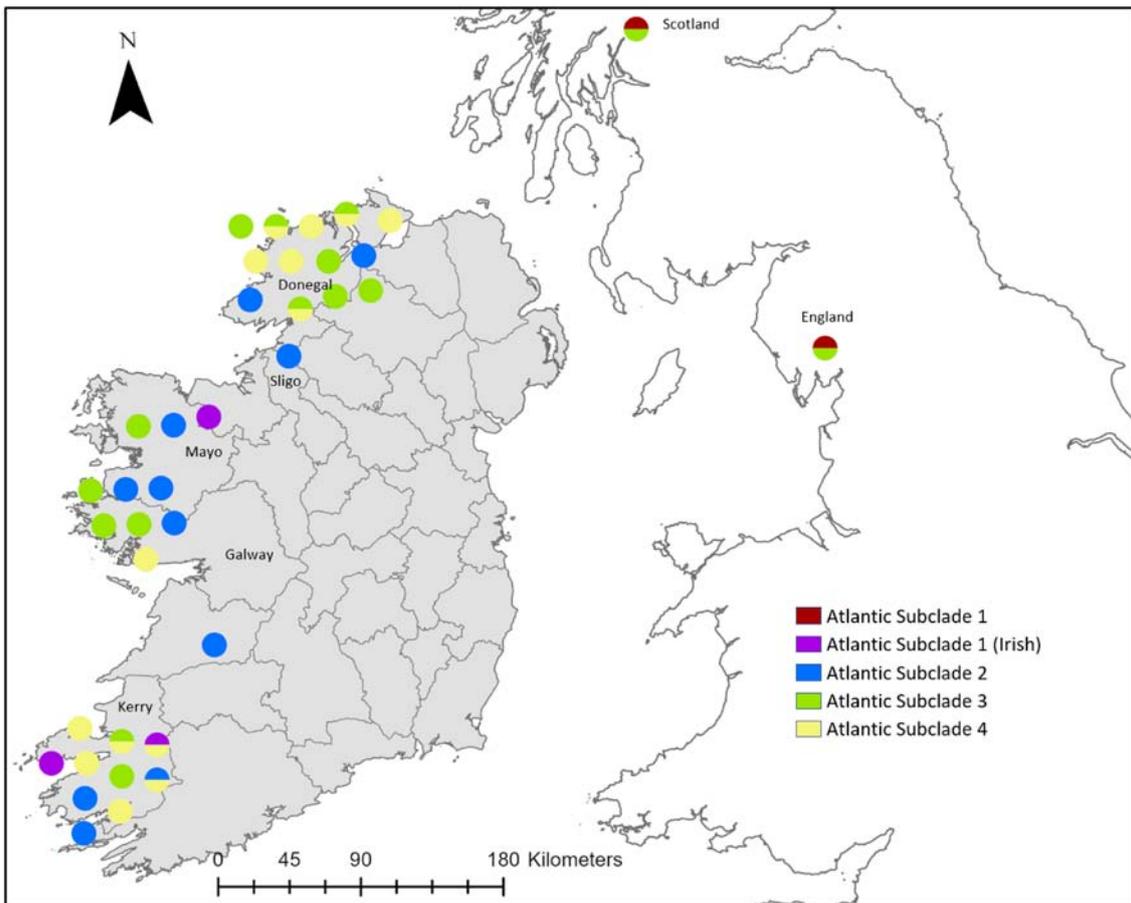
A relevant observation from the distribution of the resulting mtDNA haplotypes among Arctic char loughs in Ireland relates to the unusually large number of distinct haplotypes recorded in several loughs. Of particular relevance is the presence of 38 distinct mtDNA haplotypes in Lough Acoose. While this observation could be linked to the large number of samples examined for this particular lake ( $N=289$ ), this is unlikely to be the case.

Thus, 265 Lough Acoose Arctic char individuals shared 14 of these mtDNA haplotypes (i.e. haplotypes shared by two or more individuals). The remaining 24 haplotypes were singletons (i.e. occurred only once in the sample) and could be explained by sample size effect. It is interesting to note, however, that all singletons are related to one of the other 14, more common, haplotypes differing in one or two mutations. The presence of such a large number of mtDNA haplotypes is unusual. Assuming neutral evolution,

population diversity would be expected to reduce over time in populations of finite size. The presence of the large number of haplotypes observed is not consistent with this expectation. The most likely explanation for this is provided by the previous unusual results for Arctic char from Lough Acoose derived from the nuclear DNA SNP analysis (section 2.3.2). Thus, in agreement with what has been suggested by the results of the nuclear DNA SNP analysis, the independent evidence provided here further supports the existence of sympatric populations within Lough Acoose. This (i.e. the presence of sympatric populations) is also evident for several other Arctic char loughs. Therefore, while the Arctic char loughs investigated in this study are characterised by an average of 7.3 mtDNA haplotypes per lough, 13 loughs have from 7 to 38 haplotypes: Acoose (38), Beagh (27), Doo (15), Cloonee (13), Corrib (12), Derriana (11), Caragh (9), Cloonaghlin (8), Coomaglaslaw (8), Callee (7), Cloon (7), Coomasaharn (7) and Eske (7).

In summary, results from mtDNA analysis indicate that all major lineages evolved separately in several distinct refugia before the Last Glacial Maxima and independently colonised Ireland's freshwater habitats following the start of the retreat of the ice between 20,000 and 13,000 years before the present. The disjunct contemporary geographical distribution of lineages (Figure 2.14) suggests that the recolonisation of Ireland by Arctic char was complex and, in the case of several loughs, possibly involved secondary contact and admixture of Arctic char individuals representing different lineages.

Given the levels of genetic divergence and geographical distribution patterns, it is likely that contemporary Arctic char from loughs representing Atlantic Subclade 1 (Irish) (Acoose, Coomaglaslaw and Talt) are descendants from some of the first Arctic char to colonise the island of Ireland's freshwater habitats following the retreat of the ice. While



**Figure 2.14. Distribution of the main Arctic char genetic lineages identified in this study. Multicoloured pie charts indicate the presence of more than one lineage in a given lake (presence/absence only).**

characterised by a disjunct geographical distribution (Kerry and Sligo), these loughs are located at higher altitudes (an average of 174 m above sea level) than other loughs in Ireland. It is thus possible that the Arctic char populations in these loughs represent pioneer glacial relicts. That is, that they descended from the Arctic char that were first to arrive in Ireland. In many loughs, however, it is likely that these glacial relicts have been replaced by other, more widespread, lineages. Only the relict groups that experienced complete isolation, due to landmass uplift, were able to persist over time. Given that Arctic char individuals from Atlantic Subclade 4 also occur in Lough Acoose, and given that this particular lineage is restricted to Ireland, one hypothesis is that Atlantic Subclade 4 accounts for the second wave of colonisation. This is supported by the north–south (Donegal–Kerry) distribution bias of this lineage. Both Atlantic Subclade 2 and Atlantic Subclade 3 could possibly account for more recent colonisation events. This hypothesis is corroborated by the more even distribution of these subclades than of other subclades among Arctic char loughs in Ireland.

In summary, given the disjunct geographical distribution patterns observed for the other lineages, including the co-occurrence of haplotypes belonging to different lineages within several loughs, it is difficult to accurately reconstruct precise phylogeographical scenarios. Additional information on Arctic char from Britain and Scandinavia is required to address this. Notwithstanding this, results presented here do support the hypothesis that most of the contemporary, surprisingly rich mtDNA diversity (haplotypes) observed among Arctic char loughs in Ireland is endemic (potentially unique from Ireland), having arisen locally (in isolation) since the end of the Last Glacial Maxima. This potentially unique biodiversity merits proper protection and conservation.

## **2.4 Assessing the Impact of Decision-maker Risk Preference and Novel Scientific Knowledge on Conservation in a Context of Risk and Uncertainty: An Application of Environmental and Behavioural Economics Methods**

Biodiversity is currently declining at an alarming rate (Bongaarts, 2019), with most countries failing

to allocate sufficient resources to reverse this trend. Given strong budget constraints (Joseph *et al.*, 2009; Game *et al.*, 2013), in order to maximise biodiversity protection, stakeholders (e.g. conservation agencies and policymakers) must rely on available scientific evidence, produced by research scientists, for the development and implementation of effective environmental policies (Forest *et al.*, 2015; Tulloch *et al.*, 2015). While research scientists are usually not in charge of policy development, they often act as experts and may influence decisions made by policymakers (Game *et al.*, 2013). Thus, research scientists have a responsibility to effectively communicate scientific data to policymakers so that an optimal decision can be made that balances the risks and uncertainties of policy outcomes with the resources available from a constrained budget (Regan *et al.*, 2002).

Ineffective or inaccurate communication between research scientists and policymakers can lead to several negative outcomes, ranging from wasting precious resources to the loss of a conservation species population (Tulloch *et al.*, 2015). The transformation of pure scientific knowledge into conservation targets is often achieved through the prioritisation of conservation units (e.g. species, unique genetic lineages, unique populations). Many approaches are currently available to assist with the identification of meaningful conservation units (Game *et al.*, 2013). While some of these approaches include the use of phylogenetic information (Weitzman, 1998; Joseph *et al.*, 2009), they fail to incorporate individuals' preferences when it comes to making decisions based on risky or uncertain outcomes, a concept recognised in behavioural economics as risk preference (Van Harlow and Brown, 1990).

Given that, by default, our understanding of the variability of natural systems is always incomplete (i.e. based on currently available scientific evidence), no conservation decision can be assumed to have an absolutely certain outcome (Regan *et al.*, 2002). Thus, managing risk is an inherent part of the conservation decision-making process, and allocations of conservation funding will often be highly influenced by the risk preferences of decision-makers. Previously, the prevailing thought was that policymakers displayed risk-neutrality, meaning that they did not, on average, favour a certain outcome over an uncertain one if the results expected were the same. However, growing

evidence suggests that both the general public and decision-makers tend to lean towards risk aversion when making decisions about the environment and many other everyday decisions involving risk (Finnoff *et al.*, 2007; Tulloch *et al.*, 2015; Faccioli *et al.*, 2019; Canessa *et al.*, 2020).

While risk aversion intuitively leads to a more precautionary management approach, it has also been shown to reduce the expected benefits of conservation programmes (Stankey *et al.*, 2003; Borchers, 2005; Tulloch *et al.*, 2015). This is the case, for example, when policymakers are pushed away from *a priori* prevention methods in favour of more expensive *a posteriori* control methods when dealing with invasive species (Finnoff *et al.*, 2007). Another example is when decision-makers fail to recognise the relevance of highly endangered species because they are perceived as condemned (Tulloch *et al.*, 2015). To prevent this, and to ensure efficiency in conservation efforts, it may be best to focus on actions that offer the highest benefit-to-cost ratios, without being influenced by the risk preferences expressed by survey participants (i.e. respondents) or stakeholders (Tisdell and Wilson, 2006; Bottrill *et al.*, 2008). Thus, because risk aversion can make conservation decision-making less efficient, it is particularly important to elicit the risk preferences of decision-makers so that these can be taken into account.

An important aspect of measuring risk preferences is considering the potential differences in individuals' risk preferences across domains (e.g. financial versus health domains), and especially when comparing social and private risk scenarios (Weber *et al.*, 2002; Dohmen *et al.*, 2011; Bartczak *et al.*, 2013). If not assessed properly, risk preferences cannot be fully and precisely taken into account. In turn, this could lead to the misinterpretation of the risk behaviour of decision-makers and, ultimately, result in the development of flawed conservation plans. Thus, the elicitation of risk preferences in conservation should be anchored to a specific scenario that is relevant to the conservation issue that is being assessed, ensuring that the precise attributes of the scenario are included, to get accurate results (e.g. the species, genetic lineage or population, and its habitat and historical distribution, and regulations in place). Furthermore, domain specificity is known to influence even the best methods for eliciting risk preferences. Incentivised games can generally reward only positive risk outcomes, but

cannot easily impose negative risk outcomes if outright financial loss occurs. This is particularly true in the field of conservation, as incentivised games putting real populations at risk would be difficult to design, and, most importantly, implementing them would be completely unethical. For this reason, risk preference questions regarding the conservation of Arctic char populations were conducted by hypothetical surveys and not by fully incentivised games and experiments.

In Ireland, the problem of conservation risk is well illustrated by the case of Arctic char. The species is represented by at least 50,000 populations worldwide, most of which are located within the Arctic Circle. Several glacial relict populations, however, occur in cold post-glacial lakes in the Alps, and in Britain and Ireland. As previously summarised, these populations became landlocked in freshwater lakes following the last Ice Age. *S. alpinus* is the most sensitive of all salmonid species to decreased oxygen levels and increased temperatures. While the species is rare in Ireland and is of high scientific interest, it is relatively unknown to the general public. As summarised earlier, the taxonomy of the species remains unclear and, hence, making conservation decisions related to Arctic char is challenging. It is clear that the conservation requirements needed for one single common species inhabiting several lakes would be different if each lake (or groups of lakes) was inhabited by distinct evolutionary lineages, each an important and unique biodiversity component.

Policymakers are currently facing a difficult task, as, in many cases, decisions must be made with limited scientific evidence and are thus associated with a certain degree of uncertainty (Regan *et al.*, 2002). In this context, the aim of this component of the study was to explore how policymakers would behave if faced with realistic conservation decision-making situations relating to at-risk populations of Arctic char in the island of Ireland. To address this issue, a survey was designed to target a large sample of potential conservation stakeholders comprising scientific advisers and policymakers from the island of Ireland and other countries. Here non-incentivised multiple price list (MPL) tasks (Holt and Laury, 2002; Dohmen *et al.*, 2011) were used to explore respondents' risk preferences in two different domains: (1) personal finance and (2) conservation of Arctic char populations in Ireland. The aim was to try to identify potential domain specificities in the respondents' risk attitudes,

which was measured using respondents' constant relative risk aversion (CRRRA) coefficient values (Dave *et al.*, 2010).

The analytical design also included the use of hypothetical budget allocation games, to quantify the influence of updated, new scientific information about the genetic status (e.g. unique genetic lineages) of Arctic char in Ireland on the decision-making of such policymakers. The novelty of this experiment stems from the fact that the information about natural populations was provided in an asymmetric way, i.e. it was provided to only a fraction of the sample without revealing that no information was given to the other fraction of the sample. Only in a second round was all information transmitted to all respondents. This allowed us to study the change in respondents' behaviour resulting from updated scientific information. Given the sampling strategy employed, the expectation was that respondents would have a relatively strong background knowledge of Arctic char and, thus, would assign a high value to its presence in Ireland.

The following hypotheses were tested:

1. Given their interest in conservation, respondents would be more risk-averse when making decisions about natural Arctic char populations than when making decisions that would moderately impact on their own personal finances, because of their perception of the high value of natural Arctic char populations (it is well known that, as the financial stakes increase, subjects become more risk-averse in their decisions).
2. Respondents would allocate a higher proportion of a hypothetical budget to populations that are stated to be valuable in the scientific information given to them.
3. Respondents would allocate a lower proportion of that budget if told that the scientific information provided was uncertain or incomplete.

#### 2.4.1 Sampling

The data for this study were collected using an anonymous online survey between August 2021 and April 2022. The survey was designed and the results collected using Qualtrics software (Qualtrics, Provo, UT). The survey was approved by the Ethics Committee of the Faculty of Medicine and Life

Sciences at Queen's University Belfast and reviewed by a focus group comprising selected respondents with various levels of knowledge about Arctic char and conservation (a copy of the questionnaire is available from the authors on request). The survey was circulated to several groups of respondents, including postgraduate students in Biological Sciences at Queen's University Belfast; staff members of IFI, the Agri-Food and Bioscience Institute of Northern Ireland (AFBI) and the Environmental Protection Agency of Ireland (EPA); a group of experts in fish conservation in the UK; and various international researchers targeted for their particular knowledge on the subject (Table 2.3). As part of the survey, respondents were also encouraged to share the survey link with other potential respondents, resulting in untargeted respondents being included in the survey. The level of knowledge of each respondent about Arctic char and conservation in general was tested and quantified, as explained further in the next section.

#### 2.4.2 Survey design

The survey was composed of three parts, starting with a socio-demographic questionnaire. In the second part, three questions were asked that were designed to assess the level of respondents' prior knowledge about the conservation issues pertinent to this study, before any information about Arctic char was provided to them. Using a 1 to 10 scale, respondents were asked to rate how confident they were about the following statement: "This survey's results may be used by policymakers and could have real consequences for the conservation of Arctic char populations in Ireland". Using the same scale, respondents were asked to rate the significance of the conservation of Arctic char as a species in Ireland. The level of knowledge of respondents about Arctic char in Ireland was then quantified using a "true or false" exercise. Finally, respondents were asked about the number of publications related to Arctic char that they had read, as a measure of their academic knowledge on the subject. Before the third and main part of the questionnaire, respondents were provided with background information about Arctic char – namely about species biology, species distribution worldwide and in Ireland, and current threats to and legislation that protects the species. Respondents were informed that the survey would focus on the prevention of future

**Table 2.3. Survey respondent characteristics**

Variable	Total sample (N = 133)	Risk-averse (N = 101)	Risk-neutral (N = 14)	Risk-taking (N = 18)
<b>Gender (%)</b>				
Female	33	32	50	28
Male	66	68	50	67
Prefer not to say/other	1	0	0	5
<b>Age (years) (%)</b>				
18–30	23	22	43	17
31–60	67	67	57	72
61+	10	11	0	11
<b>Employment status (%)</b>				
Student	13	15	7	6
Employed	85	81	93	94
Other (retired, unemployed)	2	4	0	0
<b>Position (%)</b>				
Public research	45	49	50	28
EPA	13	10	14	28
Wildlife conservation agency	17	15	14	22
Other	25	26	22	22
<b>Highest level of education (%)</b>				
Undergraduate degree	18	73	64	67
Postgraduate degree	71	18	14	22
Other	11	9	22	11
<b>Experience in conservation (%)</b>				
Through work	73	74	64	72
Through studies	32	31	29	39
Through volunteering	39	29	36	39
None	9	9	14	6

The three simplified categories of risk attitudes (i.e. risk-neutral, risk-taking and risk-averse) depend on whether an individual demands an equal, a greater or a lesser expected value from a risky investment compared with an investment with a certain return when making conservation decisions about Arctic char populations in Ireland. Note that some percentages add up to more than 100%, as, with the conservation experience, some people cumulate different types of experience. Each variable is described as a percentage of the total sample.

illegal introductions of non-native species into Arctic char loughs, as this is currently thought to be the most important avoidable threat, in contrast to the impact of climate change, which cannot be easily mitigated at the national level (Morrissey-McCaffrey *et al.*, 2018). Respondents were also asked if they agreed or disagreed that Arctic char in Ireland was sufficiently protected by general regulations on water quality such that it was unnecessary to allocate a specific budget to its conservation. To measure the effect of providing information to survey respondents on this belief, the same question was asked at the very end of the survey.

In the third part of the survey, the following information was given to the respondents. A recent scientific study categorised the 45 remaining Arctic char populations in Ireland into two different risk categories, according to the likelihood that each population would become extinct because of the introduction of non-native fish species: (1) low immediate risk of extinction and (2) high immediate risk of extinction. Populations in the first category were characterised by the current absence of threat from the natural arrival of non-native fish in their respective loughs because physical barriers (e.g. dams or waterfalls) act as effective barriers (i.e. prevent non-native fish species from

entering the loughs). Populations in the second (high-risk) category were defined by the presence of mixed fish communities present in the same water body as the Arctic char population.

Respondents were provided with the following two additional scenarios: (1) there are 35 populations in the first category, and their risk of extinction related to non-native fish species is evaluated at X% within the next 10 years; and (2) there are 10 populations in the second category, and their risk of extinction – from the same cause – is Y% over the next 10 years. Risk estimates X and Y were chosen arbitrarily and for their statistical properties, as explained below. To test for the effect of these risk estimations on the budget allocation, respondents were split into two randomised groups. While respondents in the first group (Group A) were asked to consider 20% and 70% risk-of-extinction likelihoods triggered by non-native fish invasions (as above), respondents in the second group (Group B) were asked to consider risk-of-extinction likelihoods of 14% and 50% for the same scenarios.

Based on this context, respondents' risk attitudes regarding a conservation strategy for Arctic char populations were investigated using two questions, using a variant of the Holt and Laury (2002) MPL method. First, an MPL financial risk elicitation question was presented such that the respondent was faced hypothetically with a significant personal financial loss and had access to a risk-free or risky option to help mitigate that loss. Since conservation decisions typically focus on preventing biodiversity loss, the second MPL question presented offered the respondent a risk-free and a risky opportunity to reduce the loss of the Arctic char population. In the first MPL, respondents had to choose 10 times between two options: (1) risk-free option A, with a constant financial pay-off, and (2) option B, with higher risk levels and a higher potential financial pay-off. This exercise was performed twice. First, respondents were told that they had made a failing investment of £1000 and were asked to choose 10 times between a risk-free withdrawal option, where £500 is saved for certain (option A), and an option B with an X% chance of saving the entire £1000 and a 100–X% chance of losing everything, with X starting at 99%, then 90%, and then decreasing by increments of 10% (Table 2.4). Respondents were expected to choose the risky

option, option B, until their risk-tolerance threshold was met. The exercise was then repeated with a different context. Respondents were placed in the role of a policymaker and, instead of money, they were asked to make their choice based on the 10 “high and immediate risk” Arctic char populations being at stake. Option A was to save five populations for certain, and option B was to save X% of them, with the chances of losing every single population being 100–X%, with X starting at 99%, then 90%, and then decreasing by increments of 10% (Table 2.4).

The subsequent three scenarios were variations of a single budget allocation task. They differed in the amount of information provided and the associated varying risk of population extinction within the “low immediate risk” and “high immediate risk” categories. In the first stage, the respondents were provided with a tutorial in the form of explanatory slides, to maximise their engagement with different scenarios. Respondents were asked to act as policymakers whose role was to allocate a substantial conservation budget to Arctic char populations in Ireland. These exercises were carried out using the same context of populations split in two “risk of extinction” categories as previously outlined. The entire budget had to be spent, and respondents had to decide what proportion of the budget to allocate to each category using slider bars. Respondents were provided with the number of populations that were expected to become extinct as well as the number of populations that were expected to be safe from extinction as a result of their budget allocation. As respondents adjusted the slide bars to explore various allocation strategies, these numbers changed dynamically. This interactive approach enabled them to understand the task's mechanics and formulate a strategy aligned with their views on conserving specific Arctic char populations. In the first budget allocation task, the extinction risk estimations given to the two groups of respondents were designed in such a way that, given the numbers of populations in each category, the exact same numbers of populations were expected to become extinct regardless of the budget allocation, with up to seven Group A populations and up to seven Group B populations being lost. Thus, respondents were expected to base their first allocation on their conservation preferences (i.e. to protect a small number of highly endangered or a larger number of

**Table 2.4. Results of the MPL risk-attitude elicitation experiment undertaken in this study**

Round no.	Choice	Probability (%)	Pay-off (£)	Pay-off (populations)	CRRRA	Proportions of choices (money/populations)	Risk attitudes	Numerical coding of risk attitudes
1	A	100	500	5	0.98	4/6	Extremely risk-averse	5
	B	99	1000	10				
2	A	100	500	5	0.84	6/13	Highly risk-averse	4
	B	90	1000	10				
3	A	100	500	5	0.67	14/20	Very risk-averse	3
	B	80	1000	10				
4	A	100	500	5	0.48	21/26	Risk-averse	2
	B	70	1000	10				
5	A	100	500	5	0.26	24/11	Slightly risk-averse	1
	B	60	1000	10				
6	A	100	500	5	0	16/10	Risk-neutral	0
	B	50	1000	10				
7	A	100	500	5	-0.33	8/7	Risk-taking	-1
	B	40	1000	10				
8	A	100	500	5	-0.74	5/4	Very risk-taking	
	B	30	1000	10				
9	A	100	500	5	-1.33	2/1	Highly risk-taking	
	B	20	1000	10				
10	A	100	500	5	Infinite	1/2	Highly risk-taking	
	B	10	1000	10				

**This study used a variant of the Holt and Laury (2002) MPL method. Respondents were explicitly warned that the alternative outcome, for each round of choice, was the loss of all the money in the first option, and every population in the second. The 10 populations of the second experiment are the 10 Irish Arctic char populations of the high and immediate risk of extinction category presented earlier in the survey.**

less endangered populations), where, irrespective of their allocation, seven populations would be lost.

The second budget allocation task differed from the first in only two ways. First, the levels of risk of extinction were increased from 20% to 40% for the low immediate risk category and from 70% to 80% for the high immediate risk category. These increases were identical for the two respondent groups (Groups A and B), and therefore respondents were not divided into groups for the rest of the survey. Second, respondents were provided with updated scientific information (derived from this project and

from Connor *et al.* (2019)) reflecting recent real-life findings regarding Arctic char populations in Ireland, which can be summarised as follows. While the initial round of budget allocation implied that all Arctic char populations are of equal conservation status, the reality is more complex, as these different populations represent the descendants of multiple distinct genetic lineages that colonised Ireland at the end of the last Ice Age. Furthermore, some of these lineages are rarer than others, that is, they are represented by fewer extant populations. In a recent study involving 10 populations of the high immediate risk category

only, evidence was found for the presence of unique genetic lineages. The extinction of these populations, representing these unique lineages, would result in an irremediable loss of genetic diversity among populations of Arctic char, the oldest native fish species in Ireland.

Respondents were then warned that, unlike in the first allocation round, different allocations would result in a different number of populations being expected to become extinct. Indeed, 100% of the budget allocated to the high immediate risk or to the low immediate risk would result in 14 or 8 populations being lost, respectively. The aim here was to observe how respondents balanced the value that they attributed to these unique genetic lineages with the risk of losing a larger number of populations, mirroring the complexity of real-life conservation decisions. In particular, the objective was to investigate whether or not novel but also incomplete scientific information (i.e. only the high immediate risk populations were investigated in this scenario) could have a significant impact on decision-making.

During the third allocation round, no new information was provided; instead, the attention of the respondent was drawn to the incomplete nature of the information provided in the second budget allocation task. Thus, the respondents were made aware that, since the 35 populations of the low immediate risk category were not studied, it was likely that at least some of the populations in that category would also host unique genetic lineages in parallel to some of the high immediate risk populations. Furthermore, it was also possible that some of the populations in that category were genetically similar to the unique populations found in the high immediate risk category, thus potentially challenging their status of constituting unique lineages in Ireland. Thus, with this explanation, respondents were made aware that the information they had been provided with was incomplete. Of particular relevance is the fact that, at this stage, respondents realised that there was uncertainty around the conservation value of the low immediate risk populations.

It is often the case in the conservation of natural populations that information about the actual conservation targets is limited; hence, policymakers have to make decisions based on both certain and uncertain factors (Canessa *et al.*, 2020). This scenario

was reflected in the last allocation task, in which the objective was to assess respondents' attitudes towards the uncertainty surrounding new scientific information, particularly when this uncertainty could have dramatic consequences for the conservation of the populations at stake. For instance, in a situation where the low immediate risk populations contain few or no unique lineages, a policymaker's decision to allocate a large part of a budget to those populations would potentially compromise the protection of other, more deserving populations. Here, it was also important to ensure that any lack of change between the second and the third allocations was not simply because perceptive respondents recognised the incomplete nature of the information presented during the second allocation. To rule out this possible bias, respondents were asked simply if they had anticipated the fact that some of the low immediate risk populations could also be unique before they were informed of their status. Similar to any conservation strategy measure, attempts to protect Arctic char populations have a chance of failure. This risk of failure, however, is usually not accounted for in conservation plans, particularly when these plans are designed by stakeholders who have an interest in or a willingness to see them being implemented (Redford and Taber, 2000; Game *et al.*, 2013). Here, the risk of failure was conveyed by clearly informing respondents that the number of populations lost, as a result of their budget allocations, was only an estimate and, hence, subject to some variation.

#### 2.4.3 *Sample description and statistical analysis*

The resulting population sample is described in Table 2.3, in which sample statistics are given for each main risk attitude (risk-averse, risk-neutral, risk-taking). The sample was dominated by male respondents, with a vast majority of employed people, most of them being more than 30 years old and thus probably at an advanced stage of their career. The sample was dominated by academics, although a non-negligible proportion of respondents were from wildlife conservation and environmental protection agencies (Table 2.3). No patterns emerged when comparing the level of experience in conservation with the different risk attitudes. The largest category of respondents had read between 1 and 5 papers about Arctic char (34.6%), followed by 21 or more and 0 papers read (both 22.6%), between 6 and 10 papers read (14.2%),

and between 11 and 20 papers read (6%). The mean score in the true or false knowledge test exercise was significantly higher for experts (i.e. those who had read >21 publications) than in the rest of the sample.

The risk attitudes of respondents were elicited using the results of the two successive MPL tasks. As outlined earlier, respondents were categorised into groups based on their risk attitudes as inferred from their calculated CRRA (Table 2.4). The same risk attitude categories were used for the money CRRA and the population CRRA. Risk attitudes were integrated into the different econometric models of this study as continuous variables, as outlined in Table 2.5. Furthermore, risk attitudes were categorised with a numerical order. Respondents with any degree of risk-taking behaviour were merged into a single category (–1), and other respondents were put into the following categories: risk-neutral (0), slightly risk-averse (1), risk-averse (2), very risk-averse (3), highly risk-averse (4) and extremely risk-averse (5) (Tables 2.4 and 2.5). The aim of the econometric model used in this study was to examine which variables influenced the allocation that respondents made in the third allocation round. Thus, the data collected were fitted using a linear regression model with the percentage of the budget allocated to the high-risk populations for the third allocation round (i.e. HR3) as a dependent variable. All the variables

used in this study’s econometric model are described in Table 2.5. The model can be formulated as:

$$\begin{aligned} \text{AllocationHR3} = & \text{AllocationHR1} + \text{AllocationHR2} \\ & + \text{Group} + \text{Risk attitudes}_{\text{Populations}} \\ & + \text{CRRA shift} + \text{Char conservation} \\ & \text{interest} * \text{Age} + \text{Information} \\ & \text{anticipation} * \text{AllocationHR2} + E \end{aligned}$$

where E is the error term. All of the analyses were conducted using R v4.1.1 (R Core Team, 2022).

#### 2.4.4 Summary results and main findings

##### *Beliefs on the survey and on Arctic char conservation*

Respondents invariably agreed that the survey would have real consequences for the conservation of Arctic char in Ireland. They also had a high opinion of Arctic char’s scientific and conservation relevance, and 14% of them considered that Arctic char was the species of highest conservation interest in Ireland. Interestingly, this 14% mostly comprised respondents who were informed about Arctic char (i.e. had read a larger number of publications). Respondents disagreed that Arctic char populations in Ireland were sufficiently protected by general regulations on water quality

**Table 2.5. Variables used in the econometric model**

Variable	Description
<b>Socio-demographics and Arctic char-related beliefs</b>	
Age	Age simplified into three categories: (1) young, 18–30 years; (2) medium, 31–60 years; (3) old, 61+ years
Char conservation interest	Perceived interest in char populations in Ireland (1 to 10)
<b>Risk preferences</b>	
Risk attitudes (populations)	Individuals risk attitudes continuously coded from –1 to 5 (see Table 2.4), with all risk-taking attitudes merged into one category. The risk-neutral (0) attitude is taken as reference in the econometric models
CRRA shift (simplified)	Subtraction of the financial CRRA from the population CRRA, then simplified into three levels: (1) “more risk-averse” (i.e. in conservation than in finance); (2) “no change” (as reference level); (3) “more risk-taking”
<b>Allocation tasks</b>	
Allocation HR1	Percentage of budget allocated to populations of the “high and immediate risk” category during the first (HR1), second (HR2) and third (HR3) rounds
Allocation HR2	
Allocation HR3	
Group	One of the two groups in which respondents were randomly split for the first allocation round. The two groups had different estimates of risk of extinction (20–70% for the “low-risk”–“high-risk” categories in group A, and 14–50% in group B)
Information anticipation	Binary variable that indicates if the respondent anticipated the second information update or not

Further information is available from the authors on request.

and that no additional resources should be allocated to their conservation. When asked to state which attributes made Arctic char important for conservation or science, respondents mentioned those explicitly related to the following: (1) the conservation of Arctic char as a component of Irish ecosystems or as a climate change and ecosystem indicator, its natural history or simply its status as a living being (79% of the answers); (2) research interests, mainly as a unique subject for evolutionary studies, and for its high variability, ability to live in extremely cold environments and phylogeographical history (54.3% of the answers); (3) its general cultural value, its beauty and it being an important part of Irish natural history (41.3% of the answers); and (4) it being one of the few native fish species of Irish loughs that can be encountered by anglers and, to a lesser extent, it being an important fishery resource (16.7% of the answers).

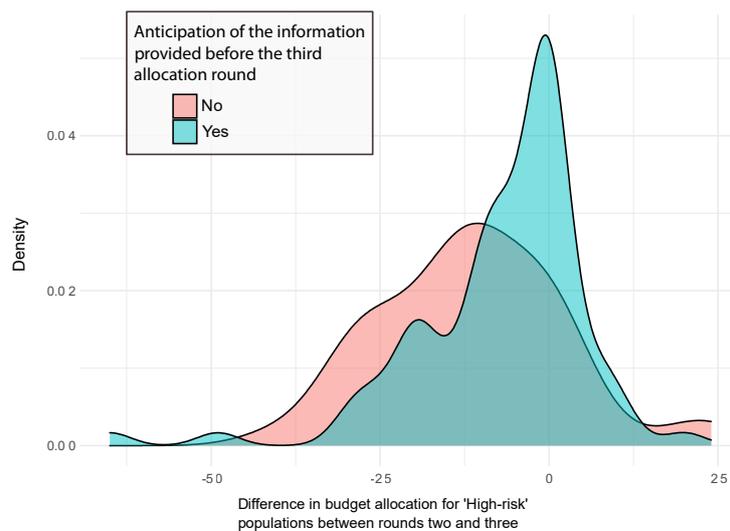
#### Risk preferences

Risk-averse attitudes were the norm regardless of the domain (personal finance or Arctic char conservation), with 69% and 79% of respondents with different degrees of risk aversion in terms of the financial and conservation questions, respectively (Table 2.4). Interestingly, respondents were notably more risk-averse with natural Arctic char populations than with their own money (although this was shown to change if

the financial stakes were greater and involved potential losses of more than the £1000 used in this study).

#### Budget allocations

During the first budget allocation exercise, where the allocation was considered to have no influence on the number of populations lost, respondents showed a significant preference for saving low-risk populations, with a mean allocation for high-risk populations of only 45.36% of the total budget. In contrast, respondents' preferences switched to high-risk populations in the second round, with a mean allocation of 54.39% of the budget. Finally, respondents switched back to favouring the low-risk populations in the third allocation round, with 45.90% of the budget allocated to high-risk populations. The majority of respondents (65%) stated that they had anticipated the information update provided after the second round. Thus, these respondents predicted that some of the populations in the low-risk category were potentially as unique and valuable as the high-risk populations. Notwithstanding this observation, however, 71.8% of respondents still changed their allocation between the second and third rounds, contradicting their declaration of anticipation (Figure 2.15). However, the change in allocation between the second and third rounds was still significantly lower among respondents who anticipated this information.



**Figure 2.15. Differences in budget allocations made by respondents between the second and the third allocation rounds. Different colours indicate whether the respondent anticipated (blue) or not (pink) the information provided before the third allocation round, that is, that populations from the low-risk category could be as valuable as high-risk populations.**

### **2.4.5 Main findings and concluding remarks**

Four main conclusions can be drawn from this study that are argued to be of value to relevant stakeholders and policymakers in developing and implementing conservation strategies in at-risk populations for many species.

1. This study has shown that, when provided with realistic conservation risk information, a large sample of biologically trained decision-makers demonstrate risk aversion in terms of conservation-related issues and systematically direct funding to low-risk strategies. This is likely to occur if decision-makers are presented with only extinction risk data for different populations, and where the uniqueness of different populations and lineages is not properly taken into account during the decision-making process. Background information is essential for the decision-making process and should cover all the aspects of an organism that is deemed worthy of conservation even if unknown or poorly understood by the general public.
2. Decision-makers with a strong aversion to risk may be unwilling to accept conservation strategies if the uncertainty of success is too great. There is good evidence (Tulloch *et al.*, 2015) to indicate that strong risk aversion in decision-makers can potentially lead to suboptimal conservation policies and an increased risk of species extinction. Thus, some level of risk acceptance may be required to make effective conservation decisions. Conservation plans should account for this and make explicit the degrees of risk of the different options.
3. Policymakers need to recognise the extent of the unknown in the conservation context, to quantify the uncertainty associated with different conservation plans. Failure to take this into consideration may lead to a biased weighting on the known part of the conservation context. From a conservation perspective, this could result in potentially disastrous consequences, in particular if that known part is significantly less important than the unknown part (e.g. by limiting protection to the single population, lineage or species that has been studied, at the expense of other, unstudied populations, lineages or species).

4. The unknown parts of the conservation context, if strongly suspected and highly relevant, should be mentioned explicitly in the decision-making context. For example, if species diversity is unknown in a given country but that species is exceptionally diverse elsewhere, the potential for high diversity in that given country should be accounted for. This study has shown that when a sample of decision-makers were updated with additional information on the diversity of lineages and populations, they were willing to consider reallocating conservation funding previously allocated without this information and on the basis of risk aversion.

## **2.5 Prioritisation of Arctic Char Populations for Conservation**

### **2.5.1 Rationale**

Genetic diversity is a basic requirement that allows species to respond (i.e. adapt) to changing environments (i.e. selective pressures brought about by, for example, a changing climate, shifting habitats or invasive species). Thus, a low level of genetic diversity increases the risk of extinction (Hellmair and Kinziger, 2014). Any sound conservation strategy should aim to maintain large gene pools to maximise the adaptive potential of species (Laikre *et al.*, 2016). A major challenge commonly encountered by policymakers is how to identify basic management units for actions to prevent biodiversity (genetic diversity) loss. It is often the case that prioritisation of effort is applied to species. Taxonomic uncertainties, however, often associated with species characterised by extensive phenotypic variation (in the broad sense), hinder the implementation of sound conservation strategies. Arctic char provides a good example of this type of problem, which, in Britain and Ireland, has been comprehensively reviewed by Adams and Maitland (2007).

While the study reported here found no evidence of the presence of different Arctic char species (i.e. as defined by Victorian taxonomists), it unambiguously demonstrated that Ireland harbours a surprisingly rich and endemic genetic diversity. Two of the four genetic lineages identified in Ireland (Atlantic Subclade 1 (Irish) and Atlantic Subclade 4) are potentially endemic. While the other two lineages also occur in

Britain and elsewhere in northern Europe, Ireland is represented by many genetic variants. Furthermore, results from independent analytical approaches (geometric morphometric and genetic analyses of both nuclear and mtDNA markers) clearly support the presence of sympatric Arctic char populations in several loughs (i.e. genetically distinct populations co-occurring in the same lake). Sympatric populations are often the outcome of allopatric and/or sympatric evolution. In the first instance, allopatric lineages (i.e. those that have diverged in separate refugia) can occur in sympatry as a result of independent colonisation. From this study, it appears that Loughs Acoose, Caragh, Glen and Kindrum fit into this category. Sympatric divergence is usually driven by feeding opportunities, with populations segregating as planktivorous, benthivorous and piscivorous ecotypes (“trophic polymorphism”), with further segregation occurring by feeding depth and body size. This mechanism also appears to be operating in at least some of the Arctic char loughs in Ireland (e.g. Lough Melvin). Regardless of the process, this unique biodiversity merits proper protection and conservation.

There is increasing science-driven awareness that, for species such as Arctic char, classical taxonomy based on morphology is not an appropriate basis for conservation efforts (Reist *et al.*, 2013). More recently, an integrative taxonomic approach for many organisms has been gradually gaining support (Dayrat, 2005; Ottenburghs, 2019; Hashemzadeh Segherloo *et al.*, 2021). Under this framework, different species concepts and information from multiple sources are integrated to reach a scientifically sound decision on the basic unit of management. Complementary sources of information include genomics, genetic markers and phylogeny, together with proven genetically based differences in morphology, behaviour, ecology and life history (Ferguson and Prodöhl, 2022).

Here, using the novel genetic and morphometric data generated in this study, in combination with other available information on the studied lakes, an attempt was made to assess multi-aspect diversity and pressures within Ireland’s Arctic char loughs, with the aim of producing a prioritisation list for conservation. This should provide a valuable tool to assist policymakers with the implementation of appropriate conservation measures for Arctic char in Ireland.

### 2.5.2 Design of the prioritisation procedure

The prioritisation criteria followed the approach proposed by Taylor *et al.* (2010) to rank natural populations of *Oncorhynchus mykiss* in British Columbia. The approach is based on the combined usage of information derived from morphological and genetic analyses (i.e. allelic richness (Ar), and within- and among-population divergence). The method also allows for different variables to be weighted differently. Thus, known and quantifiable threats for each population can also be included in the categorisation process in order to balance the risks and benefits in conservation decision-making.

Based on this rationale, a dual points-based scoring system was designed, where a multi-aspect diversity score and a pressure score were used in combination to rank Arctic char loughs in Ireland in order of conservation need. When available, genetic variation was accounted for by including nuclear Ar and mitochondrial lineage diversity. In addition, the diversity score included information on within-population morphological variance, indications of the presence of sympatric populations and historical interest.

The pressure score was developed to account for population viability (e.g. with effective populations sizes) and environmental pressures (e.g. presence of predators). While the exposure of Arctic char populations in Ireland to climate change is thought to be relatively uniform, their range shift potential and sensitivity to climate change-driven pressures are highly dependent on lake depth (Connor *et al.*, 2019). Thus, the specific vulnerability of populations to these threats was considered to account for risk of extinction. While somewhat arbitrary, the point system developed here has the significant advantage of being more transparent than sophisticated modelling methods, thus allowing policymakers to understand how the ranking was established and, hence, encouraging them to use it effectively (Coates *et al.*, 2018).

Here, however, an important novel parameter was included in the prioritisation criteria. The point system included an uncertainty score to account for unknown characteristics of each population in the conservation context under consideration. The inclusion of this score was thought to be of considerable importance given the results of the environmental economics analysis (section 2.4). Thus, policymakers are likely

to make significantly different conservation decisions when the unknowns of a conservation context are explicitly mentioned and when they are not mentioned.

### 2.5.3 Scoring system for the prioritisation procedure

The diversity score aimed to encapsulate the multifaceted nature of diversity and the significance of Arctic char across various loughs. For genetic diversity represented by  $A_r$ , the scoring was straightforward: low  $A_r$  received 0 points; mid- $A_r$  received 1 point; and high  $A_r$  was awarded 2 points. When considering mitochondrial lineage diversity, a population with a single Atlantic lineage earned 0 points. In contrast, the presence of two subclades earned 2 points. An additional point was added if both Atlantic Subclades 1 and 4 were present, highlighting their divergence and exclusive presence in Ireland. The score also included non-genetic features. Morphological variance within populations was divided into three categories: low variance (0 points), mid-variance (1 point) and high variance (2 points). Historical significance added a dimension; species documented by Victorian taxonomists in a lake contributed 1 point. Sympatric population evidence was integrated in various ways: 0.5 points from the full mitogenome-based phylogeny, acknowledging that lineage mixing can happen without nuclear genome changes; 1 point from ddRADseq-derived phylogeny; and 2 points from PopCluster analysis. An unusually high count of mtDNA haplotypes in a lough added 2 points, as did results from geometric morphometric analysis. Evidence from previous reports in the literature provided another point. Other information sources bringing unique conservation value were coded with an extra point. For instance, the documented local planktivory adaptation of char in Lough Coomasaham and the remarkable morphological diversity of char in Lough Melvin each contributed to the scoring system.

The pressure score summarised different factors that could potentially impact the mid- and long-term survival of Arctic char in loughs. In this score, higher values are indicative of lower degrees of threat. The following four factors were included in the pressure score:

#### 1. The effective population size ( $N_e$ ) derived from the ddRADseq data (data not shown).

This was included because populations with low

effective sizes have a higher risk of extinction. This was scored as follows:  $N_e < 50 = -1$  point;  $50 < N_e < 100 = 0$  points; and  $N_e > 100 = 3$  points.

2. **Maximum depth of a lake.** Deep parts offer refugia to Arctic char if the temperature increases, and in the presence of predators and competitors (Connor *et al.*, 2019). This was scored as follows: max. depth  $< 20$  m =  $-1$  point;  $20$  m  $<$  max. depth  $< 40$  m = 1 point;  $40$  m  $<$  max. depth  $< 60$  m = 2 points; and max. depth  $> 60$  m = 3 points).
3. **The current status of Arctic char in a given lake.** This was scored as follows: present = 1 point; unknown = 0 points; not recorded in recent surveys =  $-1$  point.
4. **The risk of extinction caused by the arrival of non-native fish species, as defined by Connor *et al.* (2019).** This was scored as follows: risk category 0 = 2 points (safe from risk without direct human influence); risk category 1 = 1 point (a mixed fish community species is present in a different sub-basin to the Arctic char lake, but with a physical barrier to their expansion); risk category 4 =  $-4$  points (high and imminent risk of extinction due to the presence of mixed fish communities in the lake). Other potential pressure factors (e.g. water quality, water abstraction, forestry, shoreline urbanisation) were counted as  $-1$  point.

It is important to note that, while Arctic char samples from some of the loughs were included in multiple analyses (i.e. ddRADseq, full mitogenome sequencing and morphometric analyses), this was not the case for Arctic char samples from other loughs. There was also variation with regard to the level of information related to pressures faced by Arctic char from different loughs (i.e. information was not consistently uniform).

As highlighted in the environmental economics section (section 2.4), the quantity of “unknown” in a conservation context should also be measured and reported to policymakers, in order to avoid bias in the allocation of conservation resources. In this context, any missing data for the score variables (namely, diversity and pressure) were coded as “uncertain”. These uncertain parameters were then grouped into two distinct uncertainty scores, corresponding to each of the original scores. The aim of this uncertainty indicator was to enable policymakers to fully consider how much information was or was not available

when making conservation decisions. The overall conservation score was calculated by subtracting the pressure score from the diversity score. The overall uncertainty score is the sum of the estimated uncertainties of both the diversity and pressure scores.

#### 2.5.4 Results

Summary results of the point-scoring system are shown in Table 2.6 (a detailed table with scoring values for the various parameters is available from the authors). While diversity scores ranged from 0 to 10 (average = 2.34), pressure scores ranged from -6 to 7 (average = 1.94).

The Arctic char loughs with the highest diversity scores (of potential conservation value) were Fad East, Glen, Beagh and Acoose. Arctic char populations in these loughs, however, are currently not under threat, as evidenced by their comparatively high pressure scores (which is indicative of low threat status). On the other hand, Arctic char populations from Loughs Ardderry, Inagh and Shindilla, while presumably of little conservation interest (very low diversity scores), are currently classified as potentially under high threat (very low pressure scores). In general, the diversity score was strongly and positively correlated with the pressure score (Pearson correlation test:  $r=0.304$ ,  $p<0.05$ ). This suggests that the most diverse Arctic char loughs identified in this study (i.e. potentially higher conservation value) are not currently under high threat (i.e. they have high pressure scores) as defined in this study. Interestingly, Arctic char loughs associated with the higher pressure scores (lower threat) tend to be located at significantly higher altitudes (Pearson correlation test:  $r=0.365$ ,  $p<0.01$ ). Thus, higher elevation Arctic char loughs are likely to be naturally more protected than Arctic char loughs located at lower altitudes. This is not particularly surprising given that those at lower altitudes are invariably more exposed to human-mediated factors (e.g. invasion of non-native species, water abstraction, agricultural waste).

Unsurprisingly, both the diversity and pressure scores were strongly correlated with sample size (Pearson correlation tests:  $r=0.731$  and  $r=0.308$ , respectively;  $p<0.01$ ). The overall uncertainty score was negatively correlated with both diversity and pressure scores. This was not unexpected and reflects the incomplete nature of the information available. As a balance

between these two scores, the overall conservation score suggests that the Arctic char loughs that should rank highest in the conservation prioritisation list were Acoose, Caragh, Fad East, Glen, Ardderry, Melvin and Inagh (Table 2.6). It is important to note that the overall conservation score was not geographically biased. Thus, the top 15 Arctic char loughs (out of 58) represented six of the eight counties where Arctic char is found (i.e. Kerry, Donegal, Galway, Leitrim, Sligo and Mayo).

#### 2.5.5 Summary of main findings

The purpose of the dual-scoring point system (diversity and pressure) used here aims to produce a clearly ranked list prioritising Arctic char loughs in Ireland. Lough Acoose, for instance, was characterised by a moderate pressure score and a very high diversity score. In contrast, Lough Ardderry was associated with a low (and uncertain) diversity score but an extremely negative pressure score. With these scores, both Arctic char loughs are at the top of the conservation prioritisation list. On the other hand, Arctic char from Lough Greenan, while displaying a moderate diversity score, was identified as not under threat, and, hence, this lough is currently placed lower in the ranking. The argument is that the ranking system used here provides a rational way of prioritising Arctic char loughs in Ireland for conservation. The system benefits from the use of information from multiple sources, allowing assessment of both the diversity merit (diversity score) and the potential threats (pressure score) currently facing a particular Arctic char lake. Thus, the use of morphometric data in the ranking is relevant because, even though the mechanisms leading to this diversity are unknown, morphologies reflect the interaction between the unique genomic background of the population and the environment it inhabits. This contributes to the species' diversity in the area, and partially reflects the adaptive potential of species (Taylor *et al.*, 2010). Similarly, the use of non-recombining mitochondrial lineages is important, as it may lead to more conservation resources being allocated to older, potentially unique lineages, or may be useful when multiple lineages are found in the same place.

The main benefit of the prioritisation process used here is that it considers diversity at different levels, hence highlighting the need to consider multiple

**Table 2.6. Scoring table for prioritising Arctic char loughs for conservation in Ireland**

Waterbody name	County	Diversity score	Uncertainty of diversity score	Pressure score	Uncertainty of pressure score	Overall uncertainty score	Overall conservation score	Prioritisation ranking
Lough Acoose	Kerry	8	0	1	0	0	7	1
Lough Caragh	Kerry	6	0	-1	1	1	7	1
Lough Fad (East)	Donegal	10	0	3	2	2	7	1
Glen Lough	Donegal	9	0	2	0	0	7	1
Ardderry Lough	Galway	0	3	-6	1	4	6	2
Lough Melvin	Leitrim	6	0	0	1	1	6	2
Lough Inagh	Galway	1	4	-4	1	5	5	3
Athry Lough	Galway	2	2	-2	1	3	4	4
Beagh (Veagh)	Donegal	9	0	5	0	0	4	4
Lough Shindilla	Galway	0	0	-4	1	1	4	4
Lough Mask	Mayo	3	3	0	1	4	3	5
Lough Nafoeoy	Galway	2	3	-1	1	4	3	5
Lough Talt	Sligo	2	0	-1	1	1	3	5
Bunaveela	Mayo	0.5	2	-2	2	4	2.5	6
Coomasaharn Lake	Kerry	6.5	0	4	0	0	2.5	6
Cloon Lough	Kerry	5	2	3	0	2	2	7
Derryclare Lough	Galway	0	4	-2	1	5	2	7
Derryneen Lough	Galway	2	3	0	3	6	2	7
Kindrum Lough	Donegal	7	0	5	0	0	2	7
Leane, Lough	Kerry	2	3	0	1	4	2	7
Oorid Lough	Galway	0	4	-2	2	6	2	7
Gartan Lough	Donegal	2.5	2	1	0	2	1.5	8
Cloonaghlin Lough	Kerry	2	2	1	2	4	1	9
Glenicmurrin Lough	Galway	3	1	2	1	2	1	9
Lough Keel	Donegal	5	0	4	0	0	1	9
Muckross Lake	Kerry	1	4	0	1	5	1	9
Lough Akibbon	Donegal	0	3	0	1	4	0	10
Ballynahinch Lake	Galway	1	4	1	2	6	0	10
Castle lake	Cavan	0	3	0	4	7	0	10
Derriana Lough	Kerry	4	2	4	1	3	0	10
Doon Lough	Donegal	0	2	0	4	6	0	10
Lough Formal	Fermana	0	4	0	1	5	0	10
Glendollagh Lough	Galway	0	4	0	3	7	0	10
Gowla, Lough	Galway	0	4	0	3	7	0	10
Shanakeela Lake	Galway	0	4	0	3	7	0	10
Lough Callee	Kerry	4	2	5	0	2	-1	11
Cloonee	Kerry	0	4	1	3	7	-1	11
Cloonee Lough (Upper)	Kerry	3	2	4	1	3	-1	11
Coomaglaslaw Lake	Kerry	5	0	6	0	0	-1	11
Lough Eske	Donegal	4	2	5	0	2	-1	11
Kylemore Lough	Galway	3	0	4	1	1	-1	11
Dunlewy Lough	Donegal	1	2	3	1	3	-2	12
Lough Formoyle	Galway	0	4	2	1	5	-2	12
Lough Namona	Kerry	0	4	2	1	5	-2	12
Sessiagh Lough	Donegal	4	0	6	1	1	-2	12
Lough Greenan	Donegal	4.5	0	7	0	0	-2.5	13

**Table 2.6. Continued**

Waterbody name	County	Diversity score	Uncertainty of diversity score	Pressure score	Uncertainty of pressure score	Overall uncertainty score	Overall conservation score	Prioritisation ranking
Lough Fad (West)	Donegal	0	3	3	1	4	-3	14
Lough Finn	Donegal	1	4	4	1	5	-3	14
Lough Namanfin	Donegal	0	3	3	2	5	-3	14
Doo Lough	Mayo	1	0	5	1	1	-4	15
Lough Fee	Galway	0	3	4	1	4	-4	15
Lough Glenawough	Mayo	2	2	6	1	3	-4	15
Lough Gouragh	Kerry	0	4	4	1	5	-4	15
Lough Inchiquin	Kerry	0	3	4	1	4	-4	15
Lough Nacung (Upper)	Donegal	1	3	5	1	4	-4	15
Lough Iskanamacteery	Kerry	1	3	6	1	4	-5	16
Lough Nalughraman	Donegal	1	2	6	1	3	-5	16
Lough Altan	Donegal	1	0	7	0	0	-6	17

**Loughs are ordered by overall conservation score. For each lough, the diversity score and the pressure score, in addition to the uncertainty points associated with these parameters, are provided.**

sources of information and ultimately a more holistic approach to conservation (Igoe and Hammar, 2004; Adams and Maitland, 2007; Taylor *et al.*, 2010). The scoring system is also flexible, allowing for a useful degree of subjectivity (uncertainties) where variables can be weighted differently (e.g. depending on the quality of the data). Among other advantages, the scoring system is extremely simple and thus transparent to policymakers. The scores can be easily integrated, along with other variables, by policymakers or by their scientific advisors, in order to get a more detailed assessment of conservation priorities (Hunter and Heywood, 2011). Policymakers, however, might have to weigh financial and political constraints and also take into account public opinion as a social factor influencing their decision (Hunter and Heywood, 2011).

Another strength of the proposed prioritisation system is that it gives policymakers the flexibility to adjust the weights of specific scores based on the best available information regarding the value and threats of Arctic char lochs. However, it is essential to note that these scores should not be ignored entirely. One approach could involve emphasising data on diverse populations, but not to the total exclusion of other populations. Another strategy might be to momentarily de-emphasise the uncertainty score and prioritise populations with clear data, with the understanding that, as further studies emerge, the conservation programme will undergo subsequent reassessments. Such a choice would be risky, as another research

project on Arctic char in Ireland, similar to the one conducted here, may not happen for another decade or more. In the meantime, unique populations may become extinct in the absence of minimal protection. Because the diversity and pressure scores were strongly correlated with sample sizes, it is clear that more investigations will lead to more unique diversity being discovered, thus reshuffling the ranking in the conservation prioritisation list. For these reasons, policymakers should be particularly aware of the uncertainty scores presented here and be cognisant of the elements at their disposal (or not) when making conservation decisions. While scientists are not responsible for deciding which populations should be prioritised in conservation programmes, they have an important role to play in providing scientific knowledge in a concise and transparent way to policymakers so that scientifically supported conservation decisions can be made. It is argued that the simple and readily applicable prioritisation system used here could be a valuable tool for use by relevant stakeholders, to assist with the development and implementation of sound conservation measures for Arctic char in Ireland.

The conservation prioritisation list presented for Irish Arctic char is an important first step towards ensuring science-informed conservation and management. It is essential to emphasise that this list requires additional examination. Through this initiative, the aim was to develop a science-based framework for prioritising populations by harnessing all available

data. The number and variety of these data directly influence the relevance and applicability of the rankings in conservation and management contexts. The prioritisation list provided is intended to be a

starting point for future reference. The continuous integration of additional relevant information about these char populations should ensure its effectiveness for policymakers and other key stakeholders.

### 3 Concluding Remarks and Recommendations

Based on the combined analyses of novel data presented here (morphometric and genetic analyses, and including holotype/syntypes), there is no evidence to support the full species status of *S. fimbriatus* in Lough Coomasaharn or *S. grayii* in Lough Melvin. While no similar quantifiable evidence was available for the other species previously described by Victorian taxonomists in Ireland, based on the results presented here, this is also likely to be the case for these species. In summary, the reinstatement of the Victorian-based taxonomy for Arctic char in Ireland as proposed by Wheeler *et al.* (2004) and Kottelat and Freyhoff (2007) has no scientific support and, hence, should not go ahead, as this taxonomy offers little and/or misleading value from a conservation perspective. It is important that the results of this research are used to inform changes to the IUCN Red List and other relevant global taxonomic master lists of species, including the Global Biodiversity Information Facility (GBFI) and FishBase, a global information system on fish species. Governments rely on these databases for assessing conservation goals, rate of biodiversity loss and/or other related metrics.

Results from extensive mtDNA-based analyses unambiguously show that Arctic char in Ireland's loughs is represented by four genetically distinct and diversity-rich evolutionary lineages. Two of these (Atlantic Subclade 1 (Ireland) and Atlantic Subclade 4) appear to be endemic in Ireland. The other two lineages (Atlantic Subclade 2 and Atlantic Subclade 3), while well represented in Ireland, are also found elsewhere, in Britain (England and Scotland) and in the North Atlantic (Iceland and Norway).

Results indicate that all major lineages evolved separately in several distinct refugia prior to the Last Glacial Maxima, and independently colonised Ireland's freshwater habitats following the start of the retreat of the ice between 20,000 and 13,000 years before the present. The disjunct contemporary geographical distribution of lineages suggests that the colonisation pattern was complex and, in several cases, possibly involved secondary contact and admixture of Arctic char of different lineages. This disjunct distribution should also be considered in any

re-introduction plans for Arctic char in Ireland. Thus, with very few exceptions involving still-connected loughs (e.g. Caragh and Cloon, and Glen and Beagh), geography is not a good indicator of genetic similarity.

Given the levels of genetic divergence and geographical distribution patterns, it is likely that Arctic char from loughs representing Atlantic Subclade 1 (Irish) (Acoose, Coomaglaslaw and Talt) were among the first Arctic char to colonise Ireland's freshwater habitats following the retreat of the ice. While characterised by a disjunct geographical distribution (Kerry and Sligo), these loughs are located at higher altitudes (average 174 m above sea level) than other loughs in Ireland. Arctic char populations in these loughs thus represent pioneering glacial relicts. That is, these populations are descended from the Arctic char that were the first to arrive in the island of Ireland after the ice retreated. In many loughs, however, these glacial relicts have been replaced by other, more widespread, lineages. Only those relict populations that became completely isolated, due to landmass uplift, have been able to persist over time.

Since Arctic char individuals from Atlantic Subclade 4 also occur in Lough Acoose, and since that particular lineage is also restricted to Ireland, one hypothesis is that Atlantic Subclade 4 would have been the second wave of colonisation. The interesting north–south (Donegal–Kerry) distribution for this lineage seems to support this hypothesis. Both Atlantic Subclade 2 and Atlantic Subclade 3 possibly account for more recent colonisation events. This hypothesis is corroborated by the more even distribution of these subclades among Arctic char loughs in Ireland. To fully reconstruct the phylogeographical history of Arctic char in Ireland, additional information on Arctic char from Britain and elsewhere in northern Europe will be required.

In Ireland, most of the mitogenomic diversity is explained by within-lake diversity. Given the observed levels of genetic divergence among genetic variants, most variants are likely to have evolved in isolation within those loughs since the end of the Last Glacial Maxima some 20,000 years before the present. It is clear that sympatric populations occur in several Arctic char loughs. Sympatric populations are often

the outcome of allopatric and/or sympatric evolution. In the first instance, allopatric lineages (i.e. those that have diverged in separate refugia) can occur in sympatry as a result of independent colonisation. Loughs Acoose, Caragh, Glen and Kindrum in Ireland, for instance, appear to fit into this category. Sympatric divergence is usually driven by feeding opportunities, with populations segregating as planktivorous, benthivorous and piscivorous ecotypes (“trophic polymorphism”), with further segregation occurring by feeding depth and body size. This mechanism also appears to be operating in at least some of the Arctic char loughs in Ireland (e.g. Lough Melvin).

Notwithstanding the generalisations made above about phylogeographical history and the presence of sympatric populations, the overall results of this study support the hypothesis that most of the contemporary, rich genetic diversity observed among Arctic char loughs in Ireland is endemic (potentially unique to Ireland), having arisen locally (in isolation) since the end of the Last Glacial Maxima. This potentially unique biodiversity merits proper protection and conservation.

Genetic diversity is a basic requirement that allows species to respond to changing environments. Thus, a low level of genetic diversity increases the risk of extinction. For Arctic char in Ireland and elsewhere, any sound conservation strategy should aim to maintain large gene pools, to maximise the adaptive potential of species. In this context, within this study we found that Arctic char populations with the highest genetic diversity were associated with loughs that had

superior water quality, such as Cloon, Coomasaharn, Coomaglaslaw, Caragh, Acoose and Eske. These loughs meet the “high status” water quality objective set by the EU Water Framework Directive. While water quality plays a primary role in the long-term sustainability of char, it is important to also take into account other influencing factors, such as water abstraction, the presence of invasive species and introduced predators.

It is clear that the species-based approach is unsuitable for the conservation of Arctic char. The simple, transparent and readily available conservation prioritisation system discussed in this study would provide a useful tool to assist with efforts to conserve Arctic char and, hence, should be further developed and assessed. While it is important to make efforts to conserve all char populations, prioritising the preservation of those with genetically distinct lineages unique to Ireland (and hence of international importance) would enhance conservation outcomes. Similarly, it is important to note that more detailed information is still lacking for many of the loughs surveyed in this study (because of the low sample size). Wherever possible, efforts should be made to fill this knowledge gap, to increase the value of the conservation prioritisation list. Likewise, it is important to assess the status of loughs where the presence of Arctic char still needs to be confirmed. This will help with the identification of any potential threats or challenges and allow for the timely development and implementation of interventions, to protect the species.

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# Abbreviations

<b>Ar</b>	Allelic richness
<b>bp</b>	Base pair
<b>CRRA</b>	Constant relative risk aversion
<b>CVA</b>	Canonical variate analysis
<b>ddRADseq</b>	Double-digest restriction-site-associated DNA sequencing
<b>EPA</b>	Environmental Protection Agency
<b>IFI</b>	Inland Fisheries Ireland
<b>IUCN</b>	International Union for Conservation of Nature
<b>ICCG</b>	Irish Char Conservation Group
<b>kb</b>	Kilobase
<b>MCMC</b>	Markov chain Monte Carlo
<b>Mitogenome</b>	Mitochondrial DNA genome
<b>ML</b>	Maximum likelihood
<b>MPL</b>	Multiple price list
<b>mtDNA</b>	Mitochondrial DNA
<b>Ne</b>	Effective population size
<b>NJ</b>	Neighbour-joining
<b>PC</b>	Procrustes coordinate
<b>PCA</b>	Principal component analysis
<b>PCR</b>	Polymerase chain reaction
<b>PVE</b>	Proportion of variance explained
<b>SNP</b>	Single nucleotide polymorphism

# An Gníomhaireacht Um Chaomhnú Comhshaoil

Tá an GCC freagrach as an gcomhshaoil a chosaint agus a fheabhsú, mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaoil a chosaint ar thionchar díobhálach na radaíochta agus an truaillithe.

## Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

**Rialáil:** Rialáil agus córais chomhlíonta comhshaoil éifeachtacha a chur i bhfeidhm, chun dea-thorthaí comhshaoil a bhaint amach agus díriú orthu siúd nach mbíonn ag cloí leo.

**Eolas:** Sonraí, eolas agus measúnú ardchaighdeán, spriocdhírthe agus tráthúil a chur ar fáil i leith an chomhshaoil chun bonn eolais a chur faoin gcinnteoireacht.

**Abhcóideacht:** Ag obair le daoine eile ar son timpeallachta glaine, táirgiúla agus dea-chosanta agus ar son cleachtas inbhuanaithe i dtaobh an chomhshaoil.

## I measc ár gcuid freagrachtaí tá:

### Ceadúnú

- > Gníomhaíochtaí tionscail, dramhaíola agus stórála peitрил ar scála mór;
- > Sceitheadh fuíolluisce uirbhig;
- > Úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe;
- > Foinsí radaíochta ianúcháin;
- > Astaíochtaí gás ceaptha teasa ó thionscal agus ón eitlíocht trí Scéim an AE um Thrádáil Astaíochtaí.

### Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- > Iniúchadh agus cigireacht ar shaoráidí a bhfuil ceadúnas acu ón GCC;
- > Cur i bhfeidhm an dea-chleachtais a stiúradh i ngníomhaíochtaí agus i saoráidí rialáilte;
- > Maoirseacht a dhéanamh ar fhreagrachtaí an údaráis áitiúil as cosaint an chomhshaoil;
- > Caighdeán an uisce óil phoiblí a rialáil agus údaruithe um sceitheadh fuíolluisce uirbhig a fhorfheidhmiú
- > Caighdeán an uisce óil phoiblí agus phríobháidigh a mheasúnú agus tuairisciú air;
- > Comhordú a dhéanamh ar líonra d'eagraíochtaí seirbhíse poiblí chun tacú le gníomhú i gcoinne coireachta comhshaoil;
- > An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaoil.

### Bainistíocht Dramhaíola agus Ceimiceáin sa Chomhshaoil

- > Rialacháin dramhaíola a chur i bhfeidhm agus a fhorfheidhmiú lena n-áirítear saincheisteanna forfheidhmithe náisiúnta;
- > Staitisticí dramhaíola náisiúnta a ullmhú agus a fhoilsiú chomh maith leis an bPlean Náisiúnta um Bainistíocht Dramhaíola Guaisí;
- > An Clár Náisiúnta um Chosc Dramhaíola a fhorbairt agus a chur i bhfeidhm;
- > Reachtaíocht ar rialú ceimiceáin sa timpeallacht a chur i bhfeidhm agus tuairisciú ar an reachtaíocht sin.

### Bainistíocht Uisce

- > Plé le struchtúir náisiúnta agus réigiúnacha rialachais agus oibriúcháin chun an Chreat-treoir Uisce a chur i bhfeidhm;
- > Monatóireacht, measúnú agus tuairisciú a dhéanamh ar chaighdeán aibhneacha, lochanna, uiscí idirchreasa agus cósta, uiscí snámha agus screamhuisce chomh maith le tomhas ar leibhéal uisce agus sreabhadh abhann.

### Eolaíocht Aeráide & Athrú Aeráide

- > Fardail agus réamh-mheastacháin a fhoilsiú um astaíochtaí gás ceaptha teasa na hÉireann;
- > Rúnaíocht a chur ar fáil don Chomhairle Chomhairleach ar Athrú Aeráide agus tacaíocht a thabhairt don Idirphlé Náisiúnta ar Gníomhú ar son na hAeráide;

- > Tacú le gníomhaíochtaí forbartha Náisiúnta, AE agus NA um Eolaíocht agus Beartas Aeráide.

### Monatóireacht & Measúnú ar an gComhshaoil

- > Córais náisiúnta um monatóireacht an chomhshaoil a cheapadh agus a chur i bhfeidhm: teicneolaíocht, bainistíocht sonraí, anailís agus réamhaisnéisiú;
- > Tuairiscí ar Staid Thimpeallacht na hÉireann agus ar Tháscairí a chur ar fáil;
- > Monatóireacht a dhéanamh ar chaighdeán an aeir agus Treoir an AE i leith Aeir Ghlain don Eoraip a chur i bhfeidhm chomh maith leis an gCoinbhinsiún ar Aerthruailliú Fadraoin Trasteorann, agus an Treoir i leith na Teorann Náisiúnta Astaíochtaí;
- > Maoirseacht a dhéanamh ar chur i bhfeidhm na Treorach i leith Torainn Timpeallachta;
- > Measúnú a dhéanamh ar thionchar pleananna agus clár beartaithe ar chomhshaoil na hÉireann.

### Taighde agus Forbairt Comhshaoil

- > Comhordú a dhéanamh ar ghníomhaíochtaí taighde comhshaoil agus iad a mhaoiniú chun brú a aithint, bonn eolais a chur faoin mbeartas agus réitigh a chur ar fáil;
- > Comhoibriú le gníomhaíocht náisiúnta agus AE um thaighde comhshaoil.

### Cosaint Raideolaíoch

- > Monatóireacht a dhéanamh ar leibhéal radaíochta agus nochtadh an phobail do radaíocht ianúcháin agus do réimsí leictreamaighnéadacha a mheas;
- > Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as tasmí núicléacha;
- > Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta;
- > Sainseirbhísí um chosaint ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

### Treoir, Ardú Feasachta agus Faisnéis Inrochtana

- > Tuairisciú, comhairle agus treoir neamhspleách, fianaise-bhunaithe a chur ar fáil don Rialtas, don tionscal agus don phobal ar ábhair maidir le cosaint comhshaoil agus raideolaíoch;
- > An nasc idir sláinte agus folláine, an geilleagar agus timpeallacht ghlan a chur chun cinn;
- > Feasacht comhshaoil a chur chun cinn lena n-áirítear tacú le hiompraíocht um éifeachtúlacht acmhainní agus aistriú aeráide;
- > Tástáil radóin a chur chun cinn i dtithe agus in ionaid oibre agus feabhsúchán a mholadh áit is gá.

### Comhpháirtíocht agus Líonrú

- > Oibriú le gníomhaireachtaí idirnáisiúnta agus náisiúnta, údaráis réigiúnacha agus áitiúla, eagraíochtaí neamhrialtais, comhlachtaí ionadaíochta agus ranna rialtais chun cosaint comhshaoil agus raideolaíoch a chur ar fáil, chomh maith le taighde, comhordú agus cinnteoireacht bunaithe ar an eolaíocht.

## Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an GCC á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóir. Déantar an obair ar fud cúig cinn d'Oifigí:

1. An Oifig um Inbhuanaitheacht i leith Cúrsaí Comhshaoil
2. An Oifig Forfheidhmithe i leith Cúrsaí Comhshaoil
3. An Oifig um Fhianaise agus Measúnú
4. An Oifig um Chosaint ar Radaíocht agus Monatóireacht Comhshaoil
5. An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tugann coistí comhairleacha cabhair don Gníomhaireacht agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

## EPA Research

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