Population genomics of a critically endangered data-deficient elasmobranch, the blue skate *Dipturus batis*

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Preface

This thesis was submitted in fulfilment of the requirements for the degree of Philosophiae Doctor (PhD) at the Faculty of Biosciences and Aquaculture (FBA), Nord University, Bodø, Norway. The original research presented in this thesis through three research papers, and an additional encyclopaedia chapter contribution, was carried out over a period of three years from August 1st, 2018 to August 31st, 2021. The candidate and the project were funded by Nord University. Partial funding for Paper III was provided by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft, UK, with whom the PhD project team collaborated closely.

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Paper II: Delaval A, Frost M, Bendall V, Hetherington SJ, Stirling D, Hoarau G, Jones CS, Noble LR. Population and seascape genomics of a critically endangered benthic elasmobranch, the blue skate Dipturus batis. Accepted in *Evolutionary Applications (Manuscript)*

Paper III: Delaval A, Bendall V, Hetherington SJ, Skaug HJ, Frost M, Jones CS, Noble LR. Can genomic data be used to estimate abundance and identify important habitats for a critically endangered benthic elasmobranch? Submitted to *Evolutionary Applications (Manuscript)*

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Delaval A, Wagner CI, Schwanck T, Wood FR, Jones CS, Hoarau G, Noble LR. Endangered coastal elasmobranchs of the North-East Atlantic. Accepted in: Goldstein MI, DellaSala DA. (Eds), Imperiled: The Encyclopedia of Conservation. Elsevier. (Manuscript)

Abstract

Many elasmobranchs have experienced pronounced population declines and local extinctions as a result of fishing pressure and habitat loss during the last century. Conservation efforts have been hindered by a lack of biological knowledge stemming from taxonomic confusion, misreporting of fisheries catches, and the impracticalities of studying rare, large, and mobile marine top-predators. Genomic approaches are among the novel research methodologies that can contribute to filling important knowledge gaps in elasmobranch biology.

This thesis aimed to utilize and test the applicability of novel genomic approaches to address fundamental knowledge gaps relevant to conservation efforts for a critically endangered and data-deficient elasmobranch, the blue skate *Dipturus batis*. With a patchy distribution largely restricted to the occidental seas around the British Isles, the viability of remaining *D. batis* populations remains questionable. In some areas, *D. batis* habitat coincides with socio-economically important fishing grounds, where it is frequently landed as accidental bycatch. Furthermore, *D. batis* populations inhabit a warming and increasingly acidic North-East Atlantic, which may have implications for their future sustainability.

Taxonomic confusion of large skates (family Rajidae) remains an important issue that complicates research and conservation efforts. *D. batis* was recognized as a cryptic species in 2010, but is still often confused with other large co-occurring rajids. Improved genomic resources will facilitate species identification and contribute to taxonomic revisions of the Rajidae in the coming years. As a contribution to this effort, the complete mitochondrial genome of *D. batis* was assembled using next-generation sequencing technology and uploaded to a growing database of elasmobranch mitogenomes. The mitogenomic resource would also facilitate genetic species identification in the event of taxonomic uncertainties in subsequent work during this thesis.

Conservation planning requires a fundamental understanding of a species' population structure, size, and future viability in the face of climate change. To address these questions, opportunistic samples were obtained from the widest available extent of *D. batis'* known distribution, and genotyped using a reduced-representation next-generation sequencing approach. The results suggested high levels of coastal connectivity along the British continental shelf from the Celtic Sea to Northern Scotland, which contrasted with the relative isolation of offshore populations at Rockall and the Faroe Islands. Effective population sizes were largest on the British continental shelf, dominated by a higher abundance in the Celtic Sea, but low enough to be of potential conservation concern in the northern and offshore parts of the range. One single nucleotide polymorphism (SNP) was significantly associated with environmental variables influenced by climate change (temperature, salinity, and pH). Although a paucity of well annotated elasmobranch genomes prevented assignment of a possible function to this SNP, these associations suggested climate change may be a selective agent on remnant *D. batis* populations, which could impact their ecological niche.

Robust demographic estimates, and identification and protection of biologically important sites such as nursery grounds, are imperative for elasmobranch conservation. Traditional fisheries-based approaches are often unsuitable for gathering samples of benthic elasmobranchs, as the data are subject to considerable sources of bias. This thesis aimed to test the suitability of genomic kin-identification to address these issues. The applicability of close-kin mark-recapture (CKMR), a novel demographic modelling approach, was tested on the Celtic Sea population of *D. batis*, making use of longitudinal survey data and reduced-representation genotyping technology. Despite limitations owing to the data-deficient status of *D. batis*, we produced the first estimates of adult breeding abundance, population growth rate, and survival rate for *D. batis* in the Celtic Sea using CKMR. Recommendations for the implementation of CKMR as a complementary population monitoring tool are discussed. In addition, the spatio-temporal position of sibling-pairs revealed strong site-attached behaviour, and a potential area of critical habitat west of the Isles of Scilly that could qualify for protection.

Overall, this thesis demonstrates the potential of using modern molecular approaches to access the genomic detail necessary to address fundamental knowledge gaps relevant to the conservation of a data-deficient and critically endangered elasmobranch. When incorporated into the conservation biology toolkit, genomic approaches have the ability to reveal complex patterns in elasmobranch biology that should be considered when developing conservation strategies.

1. Introduction

1.1 General introduction

Many elasmobranch species have undergone drastic population declines as a consequence of fishing pressure and habitat loss during the last century. As a result, almost one-third of elasmobranch species worldwide are threatened with extinction, yet nearly half remain too data-deficient to be assessed (Dulvy et al. 2017, 2014; IUCN 2021). In addition, climate change is expected to impact the niche of many elasmobranch species, and has already resulted in species distribution shifts and altered community assemblages. Elasmobranchs serve important ecological functions in marine food-webs, mostly as top-predators. They also have important roles in local fishery and recreational industries, and feature prominently in some traditional practices and in popular culture (Dulvy et al. 2017). Therefore, many elasmobranchs have become a management priority from both a conservation and socio-economic perspective.

The impact of anthropogenic activities on elasmobranch populations spans marine habitats, from coastal and continental (Dulvy et al. 2017) to deep offshore waters (Devine et al. 2006). Despite some regulatory measures such as marine protected areas (MPAs) and restrictions on fishing and international trade, a large number of elasmobranchs are still caught as by-catch. The issue is largely attributable to a lack of species-specific biological data and difficulties in discriminating among taxa (ICES 2020). Implementing appropriate conservation actions requires that better tools be developed to correctly identify species, and an improved understanding of contemporary elasmobranch population structure, demographics, and habitat requirements. The data-deficient nature of many elasmobranchs, combined with their increasing rarity and protected status, has meant that novel approaches are needed to address a range of pertinent questions in the least invasive manner possible. Achieving this requires a multi-disciplinary strategy within which molecular approaches are having an increasingly important role.

1.2 Elasmobranchs: general biology and population structure

Elasmobranchii is a subclass of the cartilaginous fishes (Class Chondrichthyes) that includes over 1,400 species of sharks, skates, rays, and sawfishes (Eschmeyer's Catalog of Fishes, www.calacademy.org). It is a tremendously diverse group, with species inhabiting a range of habitats and exhibiting a variety of life-history traits. What elasmobranchs generally have in common, however, is a K-selected life-history. This means that they tend to grow slowly, mature at an advanced age, and produce relatively few offspring per generation; these patterns are generally more pronounced for larger-bodied elasmobranchs. Such a slow population growth rate is not conducive to exploitation, and as such many depleted populations struggle to recover (Dulvy et al. 2017). The harvesting of elasmobranchs has led to a range of ecological impacts, such as changes in population sizes and age structures, shifts in relative species abundance, and indirect effects through trophic interactions, though these processes are complex and often lack well-documented evidence (reviewed by Stevens et al. 2000). A well-documented example is the decline in the number of larger-bodied elasmobranchs and relative increase in the number of smaller and biologically more productive elasmobranchs in the North Sea (Sguotti et al. 2016).

Studying elasmobranch population structure is not straightforward, and often requires the use of a combination of approaches. Generally speaking, as techniques such as population genetics and animal tagging have made significant advances in recent decades, they have uncovered more complex patterns than were previously known. Indeed, elasmobranchs exhibit a great diversity of life-history traits. Behaviours such as site-fidelity and natal philopatry (Corrigan et al. 2018; Feutry et al. 2017; Pardini et al. 2001; Thorburn et al. 2018), long-distance migration (Blower et al. 2012; Cameron et al. 2018; Corrigan et al. 2018), and aggregations among close relatives (Lieber et al. 2020; Thorburn et al. 2018) have been documented in an increasing number of species. Consequently, the patterns of population genetic connectivity also vary among species, from those that are considered panmictic across ocean basins (e.g. Lieber et al. 2020) to those composed of discrete sub-population units (e.g. Le Port and Lavery 2012). The diversity in elasmobranch life-history

traits and the resulting variation in habitat requirements highlight the importance of resolving conservation questions for individual taxa, and that a 'one-size-fits-all' approach may not be appropriate for elasmobranch conservation.

1.3 Elasmobranchs in the North-East Atlantic

The North-East Atlantic is home to a diverse range of elasmobranchs, several of which are endemic. A large proportion of these are threatened with extirpation, with the primary cause attributed to overfishing and habitat loss (for more details on the subject, see Annex 1). Although efforts have been made to assess the status of European elasmobranch populations and to protect them by enforcing fishing quotas, moratoriums, and marine protected areas (MPAs), these have been significantly hindered by inaccurate species identifications and insufficient knowledge of their biology (ICES 2020; IUCN 2015). The issue of taxonomic confusion has been especially problematic for large-bodied skates of the family Rajidae (Iglésias et al. 2010). For conservation actions to be effective, they need to adequately protect habitats that serve critical functions for important elasmobranch life stages, such as pupping, nursery and feeding grounds, and migration routes (IUCN 2015).

There is a growing body of research investigating the population structure, habitat use, and behaviour of a number of elasmobranchs of conservation priority in the North-East Atlantic, with the aim of informing conservation decisions. Such studies have benefited from long-term mark-recapture tagging programmes led by citizen science inputs (e.g. as used by Régnier et al. (2021)), and more recently from the development of sophisticated genetic and tagging methods. The combination of these approaches has enabled the identification of biological population units, aggregation sites, potential nursery grounds, and fine-scale movement patterns for priority species like spurdog *Squalus acanthias* (Thorburn et al. 2018, 2015), basking shark *Cetorhinus maximus* (Lieber et al. 2020), and tope *Galeorhinus galeus* (Thorburn et al. 2019). For one species in particular, the flapper skate *Dipturus intermedius*, a long-term tagging programme demonstrated that large

numbers of individuals were residents or frequent visitors to a seemingly important refuge habitat on the Scottish west coast (Benjamins et al. 2018a, 2018b; Little 1998; Neat et al. 2015; Wearmouth and Sims 2009). As a result, the Loch Sunart to the Sound of Jura MPA was designated for the protection of *D. intermedius* (Scottish Government 2016), and there is ongoing work assessing the efficacy of this MPA (Scottish Natural Heritage 2019). For other species like the blue skate *D. batis*, a relative of *D. intermedius* that has received considerably less research and media attention, systematic biological data collection has only recently begun (Bendall et al. 2018; Frost et al. 2020). The continued development and implementation of a variety of research methodologies is allowing us to gain a more thorough understanding of elasmobranch biology, which will aid in the development of effective conservation strategies.

1.4 Potential impacts of climate change

In addition to fishing pressure and habitat loss, climate change may represent a current and future threat to elasmobranch populations. With the exception of a few species that have endothermic capabilities (i.e. Lamnidae), most elasmobranchs are ectothermic, meaning their physiological functions largely depend on the temperature of their surroundings. Life in the marine environment also presents a particular set of challenges, namely in terms of osmoregulation and acid-base regulation, for which elasmobranchs have developed a range of adaptations (Claiborne et al. 2002; Hammerschlag 2006). Climate change is expected to affect ocean circulation patterns and local abiotic conditions. For the British continental shelf, most models predict that sea temperatures may rise by 1-4 $^{\circ}$C, salinity may decrease by up to ~0.5 PSU (Practical Salinity Units), and the increased influx of atmospheric CO_2 may lower pH by ~0.37 by the year 2100 (MCCIP 2020). Changes in species distributions in response to increased temperatures, dominated by poleward distribution shifts, have already been documented across a range of marine taxa (Barton et al. 2016; Brattegard 2011; Chaudhary et al. 2021; Perry et al. 2005).

The impact that these environmental changes will have on elasmobranchs remains relatively unknown. Early evidence suggests that some elasmobranchs may alter their depth distribution in search of thermal refugia (Perry et al. 2005). However, even related and functionally similar co-occurring species may have different thermal niche preferences (Frost et al. 2020), suggesting that responses to climate change may differ among species. The combined effects of warming and acidification have been demonstrated to affect elasmobranch hunting ability through impaired olfaction and higher energetic demands (Pistevos et al. 2015), affect skeletal development in juveniles (Di Santo 2019), lead to denticle corrosion (Dziergwa et al. 2019), and influence aerobic and anaerobic performance (Di Santo 2016). In contrast, some species living in areas that experience regular adverse conditions, such as hypoxia and elevated CO_2, have demonstrated physiological adaptations to cope with such conditions (Dziergwa et al. 2019; Heinrich et al. 2014). Therefore, climate change is likely to affect elasmobranch species differentially depending on their life-history traits and their ability to cope physiologically.

1.5 The era of population genomics

The rapid development of molecular methods in recent decades has resulted in an array of tools that are frequently used in marine population ecology. While most studies have focused on commercially important fishes, the smaller yet growing body of research on elasmobranchs has also made use of such methods, reviewed by Dudgeon et al. (2012) and Johri et al. (2019). To summarize, these studies have largely made use of Sanger (or chain-termination) sequencing techniques, in which genetic markers of interest are isolated and amplified using polymerase chain reactions (PCR), and subsequently sequenced via capillary gel electrophoresis. Sanger sequencing, which grew in popularity in the 1980s and 1990s, has been used to genotype samples of individuals across tens to hundreds of genetic markers like microsatellites, restriction-fragment length polymorphisms (RFLPs), mitochondrial DNA (mtDNA) markers, and single nucleotide polymorphisms (SNPs) to address biological questions across a range of evolutionary and spatial scales.

For some elasmobranchs, the use of a combination of marker types has revealed complex patterns that would have otherwise gone unnoticed. For example, several studies have documented high levels of nuclear (e.g. microsatellites and SNPs) gene flow contrasting with pronounced population structure across mtDNA markers. Because mtDNA is generally maternally inherited, this has demonstrated the occurrence of sex-differentiated dispersal and female natal philopatry in species like the white shark *Carcharodon carcharias* (Blower et al. 2012; Pardini et al. 2001). Although new technologies have emerged since, the sequencing of genetic markers by Sanger sequencing can still be very informative and has recently been used to explore population structure, patterns of relatedness, and aggregation behaviours in species like the short-tailed stingray *Dasyatis brevicaudata* (Le Port and Lavery 2012), spurdog *Squalus acanthias* (Thorburn et al. 2018) and basking shark *Cetorhinus maximus* (Lieber et al. 2020). However, these genetic approaches generally rely on large population sample sizes that may be unrealistic when studying rare species, leading to low resolution, and they can be technically challenging to implement on elasmobranchs (Dudgeon et al. 2012). Furthermore, some commonly used markers may simply lack sufficient levels of intraspecific variation to assess population structure, as demonstrated for the mtDNA control region in the flapper skate *Dipturus intermedius* (referred to as the northern *D. batis* clade in Griffiths et al. (2010)).

The past two decades have seen yet further advances in sequencing technologies, namely with the development of high throughput next-generation sequencing (NGS), which has grown in popularity and affordability since its introduction in 2005 (Dudgeon et al. 2012). In brief, NGS methods allow for the parallel sequencing of millions of DNA fragments which can then be assembled and analysed using bioinformatic tools. NGS technologies have led to the emergence of the '-omics' fields, where studies now explore patterns and processes across whole genomes rather than at a select few genetic markers. Genomic approaches represent a paradigm shift, opening doors to investigate a range of contemporary and historical evolutionary questions at a much higher resolution (Dudgeon et al. 2012; Johri et al. 2019).

Not only can genomic methods resolve fine-scale population structure, but they also allow for the identification of genes under selection, which can be used to interpret how and why a species might have adapted to its environment and to forecast its response to future external pressures. For example, full genome comparisons revealed adaptations involved in wound healing and genome stability in elasmobranchs (Marra et al. 2019), while transcriptomic analyses have identified positive selection in genes relating to brain and reproductive functions in tiger sharks (Swift et al. 2016). In addition, new fields have emerged in landscape (and seascape) genomics that can provide insights into population structuring and selection along environmental gradients (Balkenhol et al. 2017; Dudgeon et al. 2012; Riginos et al. 2016). These can be combined with other tools, such as tagging data, to provide high resolution information on individual migrations and habitat use (Michalsen et al. 2014). Furthermore, the increased resolution makes it possible to identify related individuals in large populations with higher precision, revealing patterns of reproductive behaviour (Feutry et al. 2017), and that can even be used to estimate population sizes (Bravington et al. 2016b).

A key objective of population genomic research lies in the identification of a multitude of informative genetic markers from across the genome. Ideally, the assembly of whole genomes would provide a corpus of information from which polymorphic markers within gene regions could be identified – ultimately, a better assessment of genomic architecture will permit a better understanding of the interplay between genomics and epigenomics, leading towards functional elucidation of gene-environment interactions. Current use of genomic data allows identification of useful genetic markers which can be assessed using large sample sizes at more reasonable costs; such a strategy has been used in wildlife monitoring programmes (e.g. Ekblom et al. 2018). However, whole-genome sequencing is costly and resource-heavy. Furthermore, elasmobranch genomes present some challenges that make genome assembly particularly challenging: they can be large (e.g. the whole genome of the cloudy catshark *Scyliorhinus torazame* is estimated at 6.7 Gbp, roughly twice that of *Homo sapiens*), complex, and consist of numerous repetitive units (Hara et al. 2018). This means that few full-genome resources exist for elasmobranchs, with only nine

species' genomes published to date (National Library of Medicine 2021). In contrast, mitochondrial genomes are far more readily sequenced, partly due to their abundance in eukaryotic cells and their small size (ca. 16,000 bp circular sequence). As a result, hundreds of elasmobranch mitogenomes have been sequenced (National Library of Medicine 2021), and this growing database now represents a tremendous resource in the fields of conservation genomics and phylogenomics.

In terms of nuclear genomic analyses, alternative genome-wide approaches may provide a better alternative to full-genome sequencing for non-model species facing urgent conservation questions. A group of approaches collectively referred to as reduced-representation sequencing (RRS) target single nucleotide polymorphisms (SNPs) spread out across the genome. While not as extensive as full-genome approaches, RRS methods are able to examine hundreds to hundreds of thousands of loci (Jones and Good 2016), generating resolutions high enough that sample sizes can reportedly be as low as 4-6 individuals in population genomic studies (Willing et al. 2012). This is a tremendous advantage when studying rare and protected species. Several RRS methods exist, and include among others those that target specific gene-rich regions with specially designed probes like target capture (although this requires some pre-existing knowledge of a species' genomic architecture; Jones and Good 2016; Li et al. 2013), and those using restriction enzymes such as restriction site associated DNA sequencing (RADseq, Andrews et al. 2016) and DArTseq™ (Kilian et al. 2012).

RRS methods have thus far seen success in several elasmobranch studies. Target capture has been applied in a phylogenetic examination of river sharks *Glyphis spp.* (Li et al. 2015) and to study the population history of blacktip reef sharks *Carcharhinus melanopterus* (Delser et al. 2016), while large SNP panels generated from RADseq methods have allowed for the inference of population structure and the identification of kin in school sharks *Galeorhinus galeus* (Devloo-Delva et al. 2019) and speartooth sharks *Glyphis glyphis* (Feutry et al. 2017), to resolve systematic uncertainty in the magpie fiddler ray *Trygonorrhina melaleuca* (Donnellan et al. 2015), and to estimate abundance in a

population of white shark *Carcharodon carcharias* (Hillary et al. 2018). The documented effectiveness of RRS methods in answering applied questions with high precision compared with earlier genetic tools, while bypassing the challenges of full-genome approaches, represents an opportunity to apply these methods to other elasmobranchs of conservation importance. Genomic approaches, when used in combination with other approaches, have the potential to significantly improve our understanding of elasmobranch biology.

1.6 The blue skate (*Dipturus batis*)

The 'common skate' or *Dipturus batis*-species complex was once widely distributed along the continental shelf (30-600 m deep) of the Eastern Atlantic from the Mediterranean to northern Norway and Iceland. Following local extinctions and population declines resulting from overexploitation, common skate are now reportedly range-restricted primarily around the British Isles and the North Sea (Griffiths et al. 2010; ICES 2020), and they qualify as critically endangered (Dulvy et al. 2006). It was only recently confirmed, through morphological and genetic investigations (Griffiths et al. 2010; Iglésias et al. 2010), that the common skate-complex comprises two species (Figure 1) with an overlapping range dictated by differences in their thermal niche: the larger flapper skate (*Dipturus intermedius*) predominantly occupies cooler and more thermally variable waters along the Scottish coast, whereas the smaller blue skate (*Dipturus batis*) predominantly occurs in the warmer waters of the Celtic Sea and Rockall (Frost et al. 2020; Annex 1). As a result of this taxonomic revision, the data-deficient nature of each species has worsened and their imperilment has likely been underestimated.

D. batis is thought to have a patchy distribution with areas of high local abundance in the Celtic Sea, Rockall, and along the Scottish west coast (Frost et al. 2020; Griffiths et al. 2010). A recent study has also confirmed the presence of *D. batis* in Iceland (Bache-Jeffreys et al. 2021). There are reports of common skate occurring in the Mediterranean Sea

(Benmeslem et al. 2019), however it remains unclear which of the two species occupied the historical range, or indeed if some records might involve other species. There has historically been a significant amount of taxonomic confusion among large hardnosed skates (family Rajidae) in northern Europe, namely with the other co-occurring species within the genus, the Norwegian skate *D. nidarosiensis* and the sharpnose skate *D. oxyrinchus*. This taxonomic confusion is still a problem in practice even among experts, and has thrown historical fisheries catch data into question (Iglésias et al. 2010; Lynghammar et al. 2013). Distinguishing among *Dipturus spp*. can be challenging, and sometimes requires genetic validation. More details about the general biology of the species complex, their distributions, and conservation status are presented in Annex 1.

Figure 1: External morphology of the flapper skate Dipturus intermedius *(left) and blue skate* D. batis *(right). Diagnostic features include dorsal colouration and patterning, disc (i.e. body) shape, and eye colour (iris olive green in* D. intermedius *vs. pale yellow in* D. batis*). Figure source: Francis Neat.*

Assessing the status of *D. batis* and developing effective conservation strategies requires that many knowledge gaps on its biology be filled. Specifically, there is an urgent need to assess the full extent of its current distribution by confirming species identifications, and to identify population units and their areas of preferred habitat. In addition, comprehensive monitoring of marine stocks (or population units) relies on an array of biological data for a species, namely of life-history traits such as reproductive biology, population growth and individual survival rates. These are severely lacking for *D. batis*, and indeed for European rajids as a whole, such that alternative approaches may be required.

Recent developments have meant that *D. batis* could potentially be considered as a model species for the conservation of endangered and data-deficient rajids. The species has recently received a lot of attention in the Celtic Sea, where reports from a local tangle-net fishery suggested the occurrence of a large number of common skate in the area. Following the 2009 common skate EU landing ban, concern was expressed regarding the conservation and socio-economic consequences of skate by-catch in the area. Initial surveys identified that common skate landings in the Celtic Sea were dominated by *D. batis*, and these could be taken in high numbers (Ellis et al. 2016; Figure 2). As a result, a longitudinal research survey was initiated in 2011 by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) to map the distribution of, and obtain abundance estimates for, *D. batis* in the Celtic Sea (Bendall et al. 2018). A parallel monitoring programme was also initiated by the Muséum national d'Histoire naturelle (MNHN) in collaboration with the French fishing fleet (Barreau et al. 2016). Tagging programmes conducted by both institutions have enabled the tracking of individual skate movements and ongoing reassessments of important species life-history traits.

Preliminary results from these surveys have suggested the occurrence of juveniles and adults in the area, and a high degree of local residency (Bendall et al. 2018). Furthermore, early genetic work has identified the occurrence of siblings within the Celtic Sea (Frost 2017), and of a genetic discontinuity between the British continental shelf and Rockall populations (Frost et al. 2017). This has led to further questions regarding the extent of

philopatric behaviour exhibited by *D. batis*, and whether the patterns of population structure extend across their wider (anecdotal) distribution range.

In this thesis, investigations into the biology of *D. batis* have been extended, making use of recent methodological developments in molecular biology, and of additional sample collections from across the species' contemporary distribution range. These developments have made it possible to address long-standing questions about *D. batis* that will help inform conservation measures for the species, and for endangered North-East Atlantic rajids as a whole.

Figure 2: Fishing crew disentangling a common skate from fishing gear in the Celtic Sea (Ellis et al. 2016).

2. Objectives and methods

The overall objective of this thesis was to apply novel genomic approaches to address questions of conservation importance for the blue skate *Dipturus batis*. More specifically, the thesis aimed to i) contribute to a growing mitochondrial genomic database for the Rajidae by sequencing the mitogenome of *D. batis*, ii) characterize the contemporary population structure and identify any patterns of local environmental adaptation for *D. batis* across its contemporary distribution range, and iii) estimate effective and total population sizes of *D. batis* in areas of conservation priority.

Taxonomic confusion remains problematic when it comes to large European rajids, and taxonomic re-assignments are expected to occur among the Rajidae with the increasing availability of genomic resources (Last et al. 2016). Mitochondrial DNA is often used to resolve phylogenetic relationships among taxa, identify species, and to study intra-specific genetic variation. Until now, only partial mtDNA sequences (e.g. barcoding sequences for the mitochondrial cytochrome oxidase subunit 1 (COI) and 16S ribosomal RNA genes) have been contributed for either *D. intermedius* or *D. batis* (Iglésias et al. 2010; Lynghammar et al. 2014), while only a handful of full rajid mitochondrial genomes have been sequenced (Vargas-Caro et al. 2016). The first aim of this thesis was to contribute to a growing database of rajid mitochondrial genomes by sequencing, validating, and describing the full mitogenome of *D. batis* (**Paper I**). This was achieved by shotgun sequencing genetic material from a *D. batis* tissue sample collected from Rockall on a next-generation sequencing (NGS) platform, assembling the mitogenome using bioinformatic resources, and uploading the sequence onto a publicly available genomic database (GenBank, NCBI). The mitogenomic resource would also serve as a reference for species identification in the event of taxonomic uncertainties during sampling for later chapters in this thesis.

An important consideration when implementing conservation management measures is to account for the population structure of a species. This translates to assessing levels of gene flow across a wide geographic range to identify population boundaries, characterizing the genetic diversity within and among populations, and estimating their effective

population sizes. In addition, identifying potential genes under selection and relating these to environmental variables may inform us about the adaptations of *D. batis* to local conditions, and indicate how they may respond to environmental change. The response to climate change has become of interest in light of recent findings suggesting that *D. batis* may tolerate a narrower thermal niche than its parapatric congener *D. intermedius* (Frost et al. 2020), and of oceanographic projections of a warming, freshening, and increasingly acidic North-East Atlantic by the end of the century (MCCIP 2020). This thesis has addressed these questions using a population and seascape genomics approach, whereby genome-wide SNPs obtained using DArTseq[TM] (Kilian et al. 2012) were used to assess population structure, and were related to environmental variables from across the species' contemporary geographic range (**Paper II**, Figure 3). DArTseq[TM] is a reduced-representation sequencing (RRS) approach similar to RADseq which combines complexity reduction methods, using a combination of restriction/ligation reactions that are optimized for each species, and NGS sequencing. The method was chosen because of its efficiency in genotyping hundreds of samples at genic regions spread across the genome, and its demonstrated success in an earlier study on *D. batis* (Frost 2017) with which the data could be combined to increase the sample size and geographic coverage. In addition, this study included new samples of opportunity from the Faroe Islands, allowing us to determine whether *D. batis* occurs in the area, and if so, how related they are to the neighbouring populations.

The Celtic Sea has become a priority area for the conservation of *D. batis*, as it reportedly hosts a relatively high abundance of skates as well as an important mixed-species fishery. As a result, the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) has undertaken regular surveys to map the distribution and relative abundance (based on catch per unit effort, CPUE) of *D. batis* in the area. Estimates of the absolute abundance, population growth rate, and survival rates of *D. batis* in the Celtic Sea are needed to evaluate current management regimes. Close-kin mark-recapture (CKMR) has emerged as a potential method of estimating these demographic parameters in data-deficient species. In CKMR, a one-time capture of an individual may 'recapture' its close

relatives as identified using NGS-derived genotypes (Bravington et al. 2016b), thereby bypassing the need to recapture marked individuals as is required with traditional mark-recapture approaches. So far, CKMR has seen successful applications to southern bluefin tuna *Thunnus maccoyii* (Bravington et al. 2016a), brook trout *Salvelinus fontinalis* (Ruzzante et al. 2019), Atlantic salmon *Salmo salar* (Wacker et al. 2021), white shark *Carcharodon carcharias* (Hillary et al. 2018), and grey nurse shark *Carcharias taurus* (Bradford et al. 2018) populations. Like other stock modelling approaches, CKMR requires a basic understanding of the life-history parameters of a population. Though still requiring validation, recent survey data has provided preliminary estimates of these parameters for *D. batis* (Barreau et al. 2016), to a level sufficient to implement a simple CKMR model. This thesis made use of a time series of genetic samples collected during CEFAS surveys from 2011 to 2017, DArTseq^{TM} genotyping, and the best available knowledge of *D. batis* life-history parameters to estimate the population size, growth rate, and survival rates of adult *D. batis* in the Celtic Sea using CKMR (**Paper III**). The suitability of the method as a monitoring tool for the stock was evaluated and compared to evolutionary analogous estimates of effective population size (N_e), genetic diversity, and catch per unit effort (CPUE), the latter of which is more familiar in fisheries science.
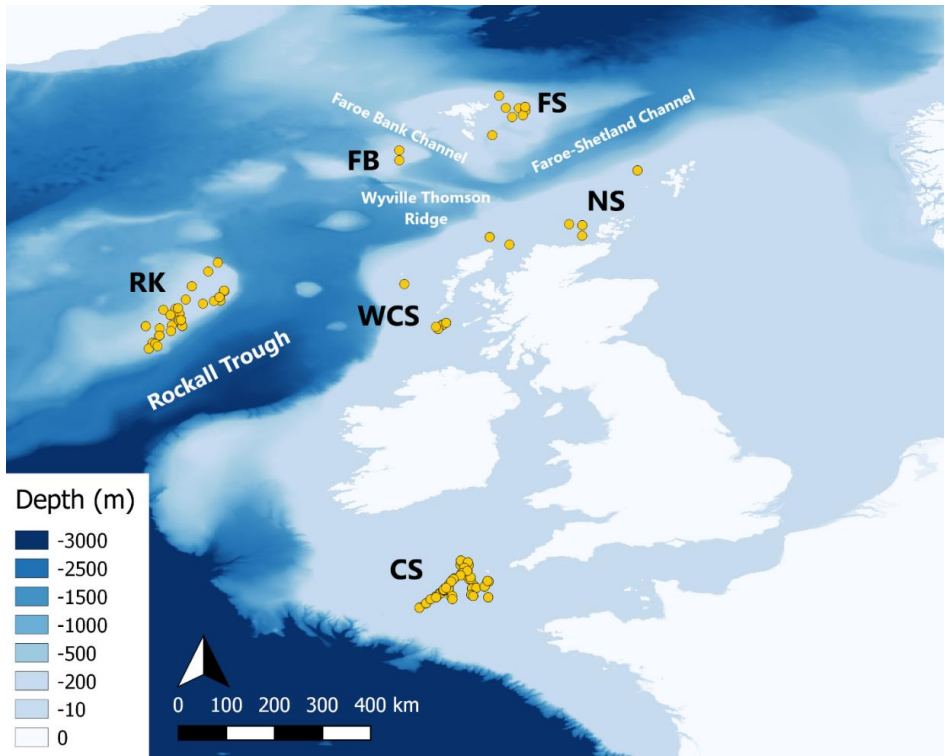
Figure 3: Sampling locations (yellow points) of Dipturus batis *across its confirmed geographic range. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), Faroe Bank (FB) and Faroe Shelf (FS). Prominent bathymetric features are shown. Map produced in QGIS (v 2.18.28) using bathymetry data from the Global Multi-Resolution Topography Data Synthesis (GMRT, www.gmrt.org).*

3. Main findings

Paper I: The complete mitochondrial genome of the blue skate *Dipturus batis*

As a contribution to a growing database of genomic resources for the Rajidae, which will facilitate species identification, phylogenetic and population genetic investigations, the complete mitochondrial genome of the blue skate *Dipturus batis* was sequenced, assembled and uploaded to GenBank (accession number MN820820), an open access database of DNA sequences. This was achieved by shotgun sequencing genetic material obtained from a *D. batis* tissue sample from Rockall (donated to the Natural History Museum in London, accession number NHMUK014391967). The mitogenomic resource was also intended to serve as a reference for species identification, in the event that genetic species confirmation would be required in subsequent work (**Papers II** and **III**).

The mitogenome of *D. batis* reported is similar in size (16,911 bp) and sequence order (Figure 4) to most vertebrates and to its *Dipturus* congeners. A phylogenetic analysis comprising the described mitogenome and the mitogenomes of 17 other Rajiformes (skates and rays) placed *D. batis* within the monophyletic rajid clade, thereby further validating the mitogenomic sequence.
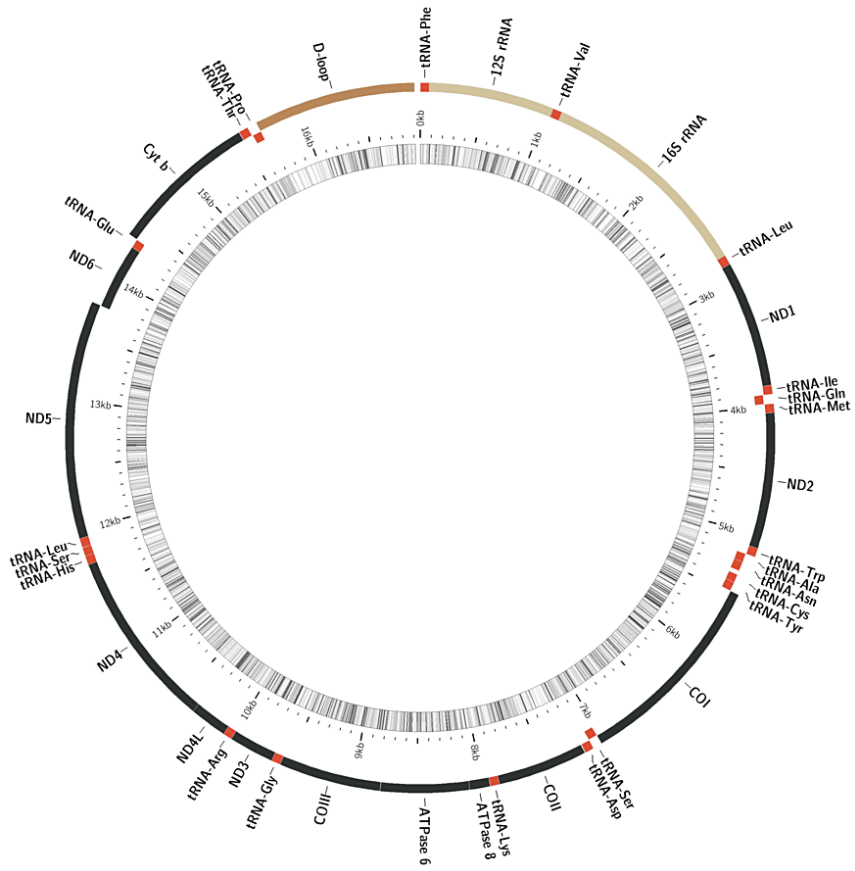
Figure 4: A representation of the mitochondrial genome of Dipturus batis*, generated in MitoAnnotator (Iwasaki et al. 2013). The 16,911 bp sequence follows the typical vertebrate mitogenomic structure.*

Paper II: Population and seascape genomics of a critically endangered benthic elasmobranch, the blue skate *Dipturus batis*

In this study, samples collected from the widest available extent of *D. batis*' known distribution were genotyped using DArTseq™ to assess the species' population genomic structure, estimate effective population sizes (N_e), and identify potential signals of selection along environmental gradients. The final dataset consisted of 503 individuals that were predominantly from the Celtic Sea (N=387) and Rockall (N=70), with additional opportunistic samples from along the Scottish coast and the Faroe Islands, collectively genotyped across 6,350 informative single nucleotide polymorphisms (SNPs).

The results revealed significant inshore vs. offshore genetic discontinuities, with high levels of gene flow along the British continental shelf from the Celtic Sea to Northern Scotland contrasting with the relative isolation of offshore populations at Rockall and the Faroe Islands. Geographic isolation was most pronounced across the >3,000 m deep Rockall Trough. Genetic diversity indices were comparable across sites, but were slightly lower at Rockall. In total we identified ten pairs of closely related individuals (full- or half-siblings) occurring in close proximity to one another, in the Celtic Sea, at Rockall, and on the Faroe Bank, a result indicative of site-attached behaviour.

The precision of N_e estimates varied among sample sites, and they had large confidence intervals for those sites with small sample sizes. After grouping individuals into three hypothetical populations inferred from the population genomic analyses, N_e estimates suggested that the largest population was from around the British Isles (~21,000 individuals across the aggregate of sites around the British Isles), followed by Rockall (~11,000 individuals) and the Faroe Islands (~2,300 individuals across the Faroe Shelf and Faroe Bank combined). Estimates for the British Isles population were dominated by a high abundance in the Celtic Sea.

Among the 6,350 SNPs were 21 under putative positive selection. One of these loci was significantly correlated with bottom current velocities, temperature, salinity and pH.

Latitude was also a significant predictor of the genotypes at this locus. On closer inspection, the Celtic Sea population, which experiences warmer, more saline, and more acidic conditions, was nearly fixed for the reference allele at this locus (96% of individuals). In contrast, the other sites had a mix of primarily reference homozygotes and heterozygotes. The results indicate that local environmental conditions may exert a strong selective force on *D. batis* populations in spite of gene flow, across a set of abiotic variables predicted to change increasingly under climate change scenarios. The paucity of well annotated elasmobranch genomes precluded us from identifying a putative function for this SNP.

This was the first time, to our knowledge, that common skate samples from Faroese waters were formally investigated following the taxonomic revision of the species complex in 2010. All Faroese samples genotyped in this study were genetically confirmed as *D. batis*.

Paper III: Can genomic data be used to estimate abundance and identify important habitats for a critically endangered benthic elasmobranch?

The aim of this study was to evaluate the potential of close-kin mark-recapture (CKMR), a novel demographic modelling approach based on the identification of close relatives (i.e. parent-offspring or half-sibling pairs) using high-throughput sequencing data, for estimating demographic parameters relevant to the management of a data-deficient skate population. Results from a pilot CKMR study performed using the genomic dataset from **Paper II**, and from early tagging experiments (Bendall et al. 2018), suggested that the Celtic Sea population would be a good candidate for CKMR without the need to sample thousands of individuals as is typical of CKMR projects. This was because the following criteria were met: the population could in theory be considered a 'closed population' of highly resident individuals, there was a relatively high probability of finding half-siblings, and rudimentary life-history trait data that are required for CKMR had recently become available for *D. batis*. Therefore, a half-sibling pair CKMR model could be implemented to produce the first demographic estimates for the *D. batis* population in the Celtic Sea. The final dataset included 662 individual skate samples collected during fishery-dependent trammel-net surveys performed by CEFAS in the Celtic Sea between 2011 and 2017, genotyped across 6,291 informative SNPs using DArTseq^{TM}. We identified two full-sibling and 17 half-sibling pairs among the samples, the latter of which were incorporated into the CKMR model.

The number of half-sibling pairs identified was relatively low compared to published CKMR studies, and generated large confidence intervals as a result. The breeding adult population size was estimated at ~19,000 individuals (95% CI: 3,000 – 115,000) for the initial model year (1996), however the population growth trajectory could not be determined with sufficient confidence. The abundance estimate was comparable to that of the evolutionary analogous estimate of N_e (14,832 individuals, 95% CI: 11,987 – 19,416). Annual adult survival rates were estimated at 0.78 (95% CI: 0.60 - 0.96).

The results of the CKMR study supported fisher's anecdotal information that *D. batis* is locally abundant in the Celtic Sea. The CPUE estimates and molecular estimates of N_e and genetic diversity, against which the CKMR results were compared, indicated a stable population across the years surveyed. The results were interpreted in light of the potential biases involved in each of the modelling approaches, and recommendations for the application of CKMR to a skate population were discussed.

In addition, the spatio-temporal position of sibling-pairs revealed strong site-attached behaviour, and a potentially significant reproductive or nursery site west of the Isles of Scilly. Of the 19 sibling pairs identified, four had been sampled in the same haul, and the remainder were sampled between two and 94 km apart; the majority of the sibling pairs included at least one individual sampled just west of the Isles of Scilly. This area corresponded to an area described as 'biologically important' by Bendall et al. (Bendall et al., 2018) where there were increased catches of immature and mature males and females, and the occurrence of sexually active males and egg-bearing females had been observed.

4. General discussion

4.1 Implications for rajid taxonomy and phylogenetics

Taxonomic confusion of large-bodied skates (Family Rajidae) has masked the true extent of their distributions and abundance in the North-East Atlantic, complicating biological studies and conservation efforts. The 'common skate' species-complex has only recently been resolved based on morphological and genetic analyses (Griffiths et al. 2010; Iglésias et al. 2010), and now consists of two species, the blue skate (*Dipturus batis*) and the flapper skate (*D. intermedius*). Despite a number of informative morphological characters (Iglésias et al. 2010), distinguishing among *Dipturus spp.* visually can be challenging even to the trained eye. Twelve individuals included in the present thesis (Paper II) were genetically identified as non-target species (data not presented) despite visual identification as *D. batis* during sampling by trained surveyors, which is a case in point. The DArTseq™ genotype data was powerful enough to identify and exclude these non-focal species prior to SNP calling for Paper II, and therefore subsequent identification using mitochondrial markers was deemed unnecessary. The risk of visual identification based on morphological characters in the field emphasizes the importance of complementary genetic species identification.

Early phylogenetic analyses based on partial mitochondrial DNA (mtDNA) sequences have indicated that *D. intermedius* and *D. batis* are not as closely related as one might expect; *D. intermedius* appears more closely related to the sharpnose skate (*D. oxyrinchus*) than to *D. batis* (Griffiths et al. 2010; Iglésias et al. 2010), while *D. batis* appears more closely related to the barndoor skate *D. laevis* (Bache-Jeffreys et al. 2021). Frequent changes have been made to the taxonomy and systematics of Rajidae in recent years, and further rearrangements are anticipated with the arrival of new genomic data, such as full-genome assemblies (Last et al. 2016). We contributed to a growing database of rajid genomic resources by publishing the first full mitogenome of *D. batis* (Paper I). It was not possible to evaluate the current phylogenetic relationship among *Dipturus spp.* using full

mitogenomes, since only a few mitogenomes were available at the time of writing. However, initial results suggest that all but one *Dipturus spp.* mitogenomes belong in one clade (Paper I). It should be noted that the genus *Zearaja*, of which four species lay within the clade, is now synonymized with *Dipturus* (Fricke et al. 2020). The findings highlight the value of molecular markers in resolving taxonomic and systematic questions. In future, as the mitogenomes of other skate species become available, the phylogenetic relationships among them can be reassessed in greater detail.

4.2 Blue skate distribution and population structure

The extent of *D. batis*' distribution has been called into question since the 'common skate' species-complex was revised. Doubt has also been cast over the species complex's historical distribution, as a result of taxonomic confusion among *Dipturus spp*. Until now, *D. batis* has been confirmed as occurring in the occidental seas around the British Isles, with an offshore population occurring at Rockall. A recent study has confirmed the presence of *D. batis* in Iceland (Bache-Jeffreys et al. 2021), while reports of their occurrence as far south as the Mediterranean Sea (Benmeslem et al. 2019) still require genetic validation. In the early stages of the project, we were made aware of occasional common skate landings around the Faroe Islands (H. Olsen, Faroe Marine Research Institute, Pers. Comm), though the exact species involved was uncertain. Photographs of 62 'common skate' landed at the Faroe Bank and the Faroe Shelf aboard fishing and research vessels in 2019 suggested that these were *D. batis*, based on the characters described in Iglésias et al. (2010), and this was confirmed by genomic analysis of 20 of these individuals (Paper II). Considering the limited and opportunistic sampling effort that was involved, the result does not rule out the occurrence of *D. intermedius* in Faroese waters.

The population structure of *D. batis* was previously investigated by Frost et al. (2017), who identified a population at Rockall that is genetically isolated from the British continental shelf based on DNA microsatellite markers. We expanded on this analysis

(Paper II) by incorporating new samples from the same geographic range and additional samples from the Faroe Islands, using a DArTseq^{TM} genotyping approach. The results corroborated the genetic isolation of Rockall blue skate, and further suggested significant, albeit less pronounced, isolation of *D. batis* on the Faroe Bank and the Faroe Shelf. These patterns contrasted with the high levels of gene flow that were apparent along the British continental shelf from the Celtic Sea to northern Scotland.

The patterns of population structure could largely be explained by the bathymetry of the area and by the behaviour of *D. batis*. The British continental shelf has a relatively homogeneous shallow bathymetry that would enable movement and gene flow on evolutionary time scales. In contrast, the Rockall Trough is a ~250 km wide channel reaching >3,000 m deep, well beyond the recorded depth range for the species (30-600 m for the common skate species-complex, Griffiths et al. (2010); 83-385 m for *D. batis*, Paper II). The Faroe Bank and Faroe Shelf are also separated from the British continental shelf by the ~1,200 m deep Faroe-Shetland channel. The proximity of the Faroe Shelf, Faroe Bank, and the Scottish shelf and the presence of shallow corridors connecting these sites, such as the Wyville Thomson Ridge (~400-1000 m deep, Figure 3), could facilitate some level of gene flow among these sites.

Being dorsoventrally flattened and adapted to a demersal lifestyle, skates are some of the least active swimmers among elasmobranchs. The inshore-offshore population genetic structure observed is also apparent in other coastal batoids (Le Port and Lavery 2012). Tagging studies have revealed that the majority of skates in the North-East Atlantic are highly resident, remaining within 50 km of their release locations, although some individuals have been observed to travel up to ~900 km from their release sites (Bird et al. 2020). High residency has also been observed in *D. intermedius* (Little 1998; Wearmouth and Sims 2009), a relative of *D. batis*, for which approximately 25% of individuals are considered 'transient' or migratory (Neat et al. 2015). The results of tagging experiments on *D. batis* suggest that their preferred habitat is more restricted than that of *D. intermedius*, with a maximum recorded recapture distance of 170 km (Bendall et al. 2018).

The proximity of close relatives (Papers II and III) supports this hypothesis. Therefore, *D. batis* appears to be highly site-attached and unlikely to undertake long-distance movements, though occasional migrants (perhaps as little as one migrant per generation (Mills and Allendorf 1996)) could facilitate gene flow along the British continental shelf, and potentially to offshore populations.

4.3 Patterns of relatedness

Advances in next-generation sequencing (NGS) technologies have enabled the identification of closely related individuals among samples from wild populations with higher precision. A few studies identifying relatives within elasmobranch populations have revealed that, for some species, family structuring and site-attached behaviour may play an important role in structuring populations and identifying which habitats serve important biological functions. For instance, genetic connectivity among sites where related individuals of basking shark *Cetorhinus maximus* aggregate has indicated a potentially important migration corridor for the species in the Irish Sea (Lieber et al. 2020). In contrast, the close proximity of full-siblings revealed strong juvenile site-fidelity and female reproductive philopatry in speartooth sharks *Glyphis glyphis*, influencing patterns of population subdivision among northern Australian rivers (Feutry et al. 2017).

Our results revealed that similar patterns may occur in *D. batis*. Not only did we fail to identify sibling pairs between sites, but all sibling pairs were sampled within proximity of one another within sites (<94 km), and in some cases within the same haul. One half-sibling pair was found at each of Rockall and the Faroe Bank (Paper II), while a total of two full-sibling and 17 half-sibling pairs were identified within the Celtic Sea (Papers II and III). A large proportion of individuals involved in these sibling pairs were collected in a relatively restricted area just west of the Isles of Scilly (Paper III), flagging the area as a potential reproductive or nursery ground, given that the individuals involved were mostly maturing juveniles or young adults. The results corroborate the observations of Bendall et

al. (2018), who reported ongoing reproductive activity in this area while collecting the samples used in this study (milting males, and sexually active and egg-bearing females).

The identification of a relatively high number of close-kin within the Celtic Sea population of *D. batis* (Paper II) led to the prospect of estimating abundance using close-kin mark-recapture (CKMR). To our knowledge, this was among the first applications of CKMR to a batoid population, and represents a valuable test of the method in the context of a monitoring programme for a data-deficient bycatch species.

4.4 Monitoring data-deficient skate populations

The estimation of demographic parameters such as abundance, population growth rates, and survival rates are a fundamental component of effective wildlife management and conservation programmes. In the marine environment, monitoring fish populations (or stocks) usually relies on catch data from commercial fisheries or from fisheries-independent surveys. These data can then be used to calculate catch per unit effort (CPUE), an often-used metric of relative abundance. However, CPUE can be prone to bias due to variations in catchability of animals and misreporting of catches (Maunder and Piner 2015). For a benthic elasmobranch like *D. batis*, CPUE-based estimates can be problematic. As dorsoventrally flattened animals that often rest on the sea floor, skates are able to evade capture by trawling methods unless more destructive methods such as beam trawling are used (S. Hetherington, Pers. comm.). In addition, various types of gear can be selective towards different age- and size-classes, as demonstrated by the low number of small juveniles sampled in trammel net surveys (Paper III). Consequently, CPUE trends can differ depending on the sampling gear used; whereas we noted stable CPUEs for *D. batis* in the Celtic Sea between 2014 and 2017 based on trammel net surveys, trawl-based CPUE data indicated a slight increase in abundance in between 2011 and 2014 (Barreau et al. 2016). Furthermore, taxonomic confusion remains an issue, in that *D. batis* is often confused with

other large rajids (Iglésias et al. 2010), thereby generating an additional confound in CPUE-based assessments.

An alternative approach to studying population dynamics is through mark-recapture based approaches (Cormack 1964; Jolly 1965; Seber 1965). Here, animals are captured, marked, and then released, representing a less destructive and consequently more favourable manner of studying critically endangered elasmobranchs. Then, the proportion of marked individuals in subsequent sampling events can be used to generate demographic estimates, such as absolute abundance. Mark-recapture approaches have generally suffered from low recapture rates for rajids, and have been particularly low (<2% of individuals are recaptured) for *D. batis* (Barreau et al. 2016; Bendall et al. 2018; Bird et al. 2020). Thus, any mark-recapture based approach may require considerable longitudinal sampling efforts for some skate species. This has been achieved for *D. intermedius* within the Loch Sunart to the Sound of Jura MPA following considerable long-term inputs from multiple stakeholders to monitor a local aggregation site, which have led to increasingly comprehensive biological knowledge of the species (Benjamins et al. 2018a, 2018b; Little 1998; Neat et al. 2015; Régnier et al. 2021; Wearmouth and Sims 2009).

The development of NGS technology has enabled the genotyping of hundreds of individuals at a relatively low cost, across a sufficient number of loci to estimate effective population size (N_e) and identify related individuals with relatively high precision. This development has enabled the application of CKMR, a demographic modelling approach based on the identification of close relatives (Bravington et al. 2016b), to wild populations. CKMR represented a promising approach to assess the status of the data-deficient *D. batis* population in the Celtic Sea for a number of reasons: i) a captured individual 'tags and recaptures' its close relatives, thereby avoiding the need for physical recaptures, ii) we can be certain of species identification based on their genetic profile, thereby avoiding the issue of taxonomic confusion, and iii) we could be confident in finding close relatives, having identified full- and half-sibling pairs within a modest sample size in Paper II.

The primary objective of paper III was to determine whether CKMR could be applied to the Celtic Sea population of *D. batis*, and to compare model outputs to CPUE and N_e estimates. The results demonstrated that CKMR can be successfully implemented for the population, provided that some sampling considerations are met. First of all, the reliance on trammel-net surveys meant that young juveniles were seldom sampled, thus reducing the chance of sampling parent-offspring pairs that could be used to estimate total population size. Second, although we were able to estimate adult demographic parameters based on the identification of half-siblings, we were unable to identify a sufficient number of pairs to generate precise estimates at this stage. Our estimates suggested that a model robust enough for the purposes of stock assessment (i.e. when ~45 half-sib pairs are found, Bravington et al. (2016b)) could be obtained following continued annual survey efforts until at least 1,100 individuals are genotyped (roughly double the sample size used in Paper III). Third, CKMR estimates rely on accurate life-history trait data (e.g. estimates of age-at-length), which are yet to be validated for *D. batis* and which could represent a significant confound in our CKMR estimates. Life-history trait data have only recently been reassessed for *D. intermedius*, following a considerable longitudinal mark-recapture sampling effort involving citizen scientists and anglers within an MPA (Régnier et al. 2021), paving the way for undertaking similar future reassessments for *D. batis*.

Nonetheless, the CKMR model was able to provide the first estimates of absolute adult abundance, population growth rate, and annual survival rate for *D. batis* in the Celtic Sea. Despite large confidence intervals, the CKMR-based adult abundance estimate was comparable to estimates of N_e, placing the abundance of breeding adult *D. batis* in the Celtic Sea in the order of ~20,000 individuals. Adult annual survival rates were estimated at ~0.78, which could be considered low for a large elasmobranch, but not unexpected considering the high rates of bycatch in the region (Barreau et al. 2016; Ellis et al. 2016; Enever et al. 2009).

Estimates of N_e suggested that *D. batis* occurs in substantially lower numbers outside of the Celtic Sea (approx. 2,000 – 11,000 individuals, or less; Paper II). These patterns are in line with anecdotal observations and the findings of Frost et al. (2020).

In summary, the genomic approaches explored in this thesis were able to address fundamental knowledge gaps and to estimate important demographic parameters for *D. batis*. Despite some limitations, CKMR and N_e represent a valid complementary approach to CPUE and mark-recapture approaches. Each method suffers from different sources of bias that are accentuated by the data-deficient nature of the species, stressing the importance of a combination of approaches to provide an overview of the population's status. The effective management and monitoring of data-deficient elasmobranchs may require a combination of these approaches, particularly in cases where management decisions could have significant conservation and socio-economic implications.

4.5 Implications of climate change

The potential impacts of climate change on North-East Atlantic skate populations has come into question following projections of a warming and acidifying region (MCCIP 2020) and growing evidence of the physiological costs of these changes for elasmobranchs (Pistevos et al. 2015; Di Santo 2016; Dziergwa et al. 2019). For *D. batis*, an inability to tolerate such conditions might necessitate a distribution shift, as has been observed in a range of taxa including elasmobranchs (Barton et al. 2016; Brattegard 2011; Chaudhary et al. 2021; Perry et al. 2005).

Our results (Papers II and III) and earlier observations indicate that *D. batis* is range restricted and may be highly site-attached to specific areas along the continental shelf. Analysis of population structure revealed an inshore *vs.* offshore pattern that was maintained when looking only at putatively adaptive loci, suggesting that selective processes may be occurring (Paper II). These patterns may be influenced by evolutionary processes other than selection, such as genetic drift, a genetic bottleneck following

fisheries induced mortality, or the observed lack of gene flow. However, our seascape genomics analysis (Paper II) revealed one locus that was likely under environmental selection irrespective of gene flow; the pattern was apparent along an environmental gradient despite high levels of gene flow from the Celtic Sea to Northern Scotland. The fact that 96% of Celtic Sea individuals were homozygous for the reference allele at this locus, and that this was linked to the warmer, more saline, and more acidic waters of the region, suggested that these environmental variables may exert a selective force on *D. batis* populations that is apparent at this locus.

The paucity of genomic resources for elasmobranchs prevented us from determining a putative function for this locus. Instead, it has led to a number of new questions and hypotheses. Does the locus in question convey a selective advantage to climate change conditions for *D. batis*? If so, would a northward distribution shift following warming waters imply a competitive edge over the more cold-water adapted *D. intermedius* that is currently more abundant in these regions (Frost et al. 2020)? Can the patterns in the Celtic Sea be used to predict the response of *D. batis* to climate change in the northern parts of the range? The increasing availability of annotated elasmobranch genomes and targeted genomic approaches may enable us to address these questions in the years to come.

5. Conclusions and future perspectives

Large hardnosed skates (Family Rajidae) in the North-East Atlantic have frequently been confused as a result of their morphological similarity and the occurrence of cryptic species, contributing to their data-deficient nature. The increasing availability of genomic resources in public databases has led, and will continue to lead, to phylogenetic re-assessments in the Family. The mitochondrial genome of the blue skate *Dipturus batis* published as part of this thesis (Paper I) represents a small, but valuable, contribution to a growing database that will enable the shift from phylogenetic to phylogenomic studies on elasmobranchs.

The data-deficient status of *D. batis* has meant that the full extent of its contemporary distribution, following fisheries-induced range restrictions and extirpations, remains unclear. This thesis has added Faroese waters to the *D. batis* distribution map (Paper II). However, samples from a wider distribution range, such as the recently confirmed *D. batis* from Iceland (Bache-Jeffreys et al. 2021) and the (anecdotally reported) *D. batis* from the Bay of Biscay and further south, should be included in future assessments of population structure.

Of particular relevance to current conservation strategies for *D. batis*, this thesis characterized the contemporary population structure of the species across most of its geographic range, and estimated population sizes in these areas. On an evolutionary time scale, patterns of gene flow revealed a clear inshore vs. offshore population structure, with populations at Rockall and the Faroe Islands each genetically isolated, albeit to varying degrees, from a larger population of *D. batis* on the British continental shelf (Paper II). In contrast, the identification of sibling pairs revealed more refined patterns of habitat preference and site-attached behaviour (Papers II and III) that may be more immediately relevant for conservation, particularly where these overlap with commercial fishing grounds. Careful consideration of the scale at which fisheries management and conservation strategies are designated is therefore recommended. The high susceptibility of *D. batis* to bycatch, combined with its highly resident nature and slow reproductive output, means that even the larger populations may be at considerable risk of extinction.

In the longer term, climate change may exert a selective pressure on *D. batis* populations (Paper II), although the exact mechanisms and their consequences remain hypothetical until functional genomic resources for elasmobranchs improve.

Finally, this thesis explored the use of a novel demographic modelling approach based on the identification of close-kin (CKMR, Paper III) for a data-deficient batoid. Using *D. batis* in the Celtic Sea as a case study, where the species has been the subject of an annual survey as part of local multi-stakeholder bycatch monitoring effort, the method generated the first estimates of absolute adult breeding abundance, growth rate, and survival rates for the population. Although significant data-gaps led to a considerable margin of error, the study demonstrated the potential of the approach, which provided useful comparisons with established stock monitoring approaches.

Overall, this thesis has demonstrated the ability of molecular approaches to address fundamental knowledge gaps relevant to the conservation of a data-deficient and critically endangered benthic elasmobranch. The results highlight the value of integrating a range of multidisciplinary and novel approaches to study and develop conservation strategies for elasmobranchs, within which population genomic methods play a crucial role.

6. References

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Paper I

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MITOGENOME ANNOUNCEMENT

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The complete mitochondrial genome of the blue skate *Dipturus batis*

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ABSTRACT

The complete mitochondrial genome of the blue skate *Dipturus batis* is described from shotgun sequencing on an Illumina next-generation sequencing platform. We report a 16,911 bp long sequence similar in size to other members of the genus, containing 13 protein-coding regions, 22 *tRNA* genes, 2 *rRNA* genes, and 2 non-coding areas. Phylogenetic analysis was performed using the complete mitochondrial genomes of 17 related species, placing *D. batis* within the Rajini tribe of the Rajidae family, consistent with current taxonomy. The new resource adds to a growing database of rajid mitogenomes which will help resolve phylogenetic relationships within the family.

The blue skate *Dipturus batis* (Family Rajidae, previously *Dipturus* cf. *flossada*) occurs on the continental shelf of the north-east Atlantic, primarily around the British Isles. *D. batis* has only recently received species status, after morphological and genetic investigations distinguished it from other large skates, particularly *D. intermedius* (Griffiths et al. 2010; Iglésias et al. 2010). Taxonomic confusion remains an issue among large skates, many of which are of conservation concern, and could benefit from additional tools.

We report the complete mitochondrial genome of *D. batis* from the fin clip (Natural History Museum London accession NHMUK014391967) of a male collected from the Rockall plateau (57°09′6′′N, 14°19′3W) on 10 September 2012 during a Marine Scotland Science survey. The individual was identified based on morphology including size of mature specimen, eye color, and dorsal patterning (Iglésias et al. 2010), and genetically (M. Frost pers comm). DNA was extracted using a DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany) and shotgun sequenced on an Illumina® NextSeq® 500 using paired-end sequencing after library preparation using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina (New England Biolabs®, Ipswich, MA, USA). The 32,180,856 raw reads generated were trimmed with BBDuk (30,920,799 reads remained) and mapped against the mitogenome of *D. oxyrinchus* (NC_037967) in Geneious version R9.1(Biomatters Ltd., Auckland, New Zealand). A total of 9077 reads mapped to the reference with 100% coverage (mean coverage depth 75.8), and produced a consensus sequence of 16,911 bp. To validate the consensus sequence we also performed a *de-novo* assembly using MINIA (kmer length 71), after trimming raw reads with TrimGalore and quality checking using

FastQC, and BLASTed this assembly against the reference-mapped consensus sequence from Geneious.

The 16,911 bp consensus mitogenome (GenBank accession number MN820820) we report is similar in size to other members of the genus (16,907–16,913). The consensus was annotated using MitoAnnotator (Iwasaki et al. 2013) and MITOS (Bernt et al. 2013). Gene order and structure was typical of vertebrate mitogenomes and to that of *Dipturus* relatives, containing 13 protein-coding regions, 22 *tRNA* genes, 2 *rRNA* genes, and two non-coding areas (control region and the origin of L-strand replication). Nucleotide frequencies were: 30.1% A, 26.8% C, 14.3% G, 28.8% T (A + T composition of 58.9%). This is comparable to that reported for other *Dipturus* species (Vargas-Caro et al. 2016).

We performed a phylogenetic analysis in Geneious using the full mitochondrial genome sequences of 17 related species. Sequences were aligned using MAFFT and the phylogenetic tree was constructed using Bayesian inference, as implemented in the MrBayes (Huelsenbeck and Ronquist 2001) plugin, using the GTR substitution model for 500,000 iterations, sampling every 500th iteration after a burn-in of 100,000. *Atlantoraja castelnaui* (NC_025942.1) was used as the out-group. The phylogenetic tree places *D. batis* within the monophyletic clade of hardnosed skates (Rajidae), and within the tribe Rajini, consistent with current taxonomy (Last et al. 2016) (Figure 1). Rajidae currently includes 159 validated species (Fricke et al. 2019) within which re-assignments of genera are likely to occur (Last et al. 2016). The mitogenome of *D. batis* adds to a growing database that can be used to resolve phylogenetic relationships in the future.
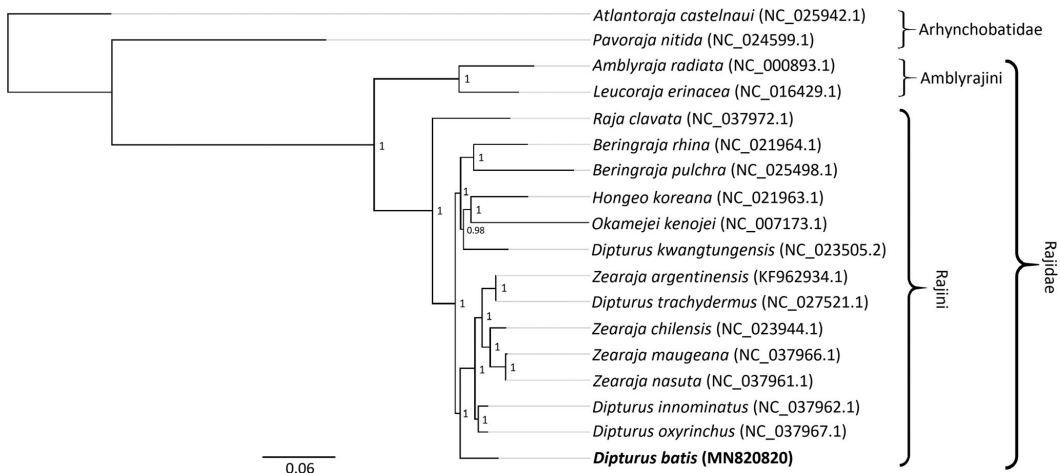
Figure 1. Phylogenetic tree of Dipturus batis (in bold) and 17 other species inferred from their complete mitogenomes. Scale bar shows the number of substitutions per site (thick black line), and posterior probabilities are shown for each node. Species names following Last et al. (2016) and GenBank accession numbers are given.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data that support the findings of this study are openly available in Genbank at https://www.ncbi.nlm.nih.gov/search/all/?term=MN820820, accession number MN820820.

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Paper II

Manuscript style adapted to the journal guidelines of

Evolutionary Applications

Population and seascape genomics of a critically endangered benthic elasmobranch, the blue skate *Dipturus batis*

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Abstract

The blue skate (*Dipturus batis*) has a patchy distribution across the North-East Atlantic, largely restricted to occidental seas around the British Isles following fisheries-induced population declines and extirpations. The viability of remnant populations remains uncertain, and could be impacted by continued fishing and bycatch pressure and the projected impacts of climate change. We genotyped 503 samples of *D. batis*, obtained opportunistically from the widest available geographic range, across 6,350 single nucleotide polymorphisms (SNPs) using a reduced-representation sequencing approach. These data were used to assess the species' contemporary population structure, estimate effective population sizes, and identify putative signals of selection in relation to environmental variables using a seascape genomics approach. We identified genetic discontinuities between inshore (British Isles) and offshore (Rockall and Faroe Island) populations, with differentiation most pronounced across the deep waters of the Rockall Trough. Effective population sizes were largest in the Celtic Sea and Rockall, but low enough to be of potential conservation concern among Scottish and Faroese sites. Among the 21 candidate SNPs under positive selection was one significantly correlated with environmental variables predicted to be affected by climate change, including bottom temperature, salinity, and pH. The paucity of well annotated elasmobranch genomes precluded us from identifying a putative function for this SNP. Nevertheless, our findings suggest that climate change could inflict a strong selective force upon remnant populations of *D. batis*, further constraining its already restricted habitat. Furthermore, the results provide fundamental insights on the distribution, behaviour, and evolutionary biology of *D. batis* that will be useful for the establishment of conservation actions for this critically endangered elasmobranch.

Introduction

Many elasmobranchs have experienced drastic population declines as a consequence of fishing pressure during the last century, representing a major conservation concern. Almost one-third of elasmobranch species globally are threatened with extinction, yet nearly half remain too data-deficient to be assessed (Dulvy et al., 2014, 2017; IUCN, 2021). Despite fishing restrictions, a large number are still caught as by-catch, particularly in unregulated coastal and continental waters (Dulvy et al., 2017) where they can be of significant socio-economic importance to local fisheries (Bendall et al., 2018; ICES, 2020). The K-selected life-history that most elasmobranchs exhibit exacerbates the impacts of exploitation; their characteristically slow growth, late onset maturity, and relatively low reproductive output limit population recovery potential (Dulvy et al., 2017). In addition, evidence is mounting on the consequences of climate change for elasmobranch fitness (Pistevos et al., 2015; Di Santo, 2016; Dziergwa et al., 2019). For many data-deficient elasmobranchs, instituting appropriate conservation actions requires a better understanding of their population structure and of their current and future realized niche in the face of environmental changes.

Elasmobranchs exhibit a range of life-history traits that translate to different degrees of population structuring. Some species demonstrate high levels of gene flow across ocean basins (Lieber et al., 2020), while others are divided into smaller sub-populations with limited gene flow (Le Port & Lavery, 2012; Thorburn et al., 2018). A wide range of behaviours such as site-fidelity and natal philopatry (Pardini et al., 2001; Feutry et al., 2017; Corrigan et al., 2018; Thorburn et al., 2018), long-distance migrations (Blower et al., 2012; Cameron et al., 2018; Corrigan et al., 2018), and aggregating behaviour among closely related individuals (Thorburn et al., 2018; Lieber et al., 2020) can shape patterns of elasmobranch population connectivity and genetic diversity. In addition, environmental discontinuities such as bathymetric barriers (Le Port & Lavery, 2012) and temperature gradients (Griffiths et al., 2010) can influence species distributions and population connectivity, especially for less vagile species. The diversity and complexity of elasmobranch life-histories are likely underappreciated due to issues such as taxonomic confusion (Iglésias et al., 2010) and mis-reporting of catches (ICES, 2020). Consequently, current conservation strategies that include marine protected areas (MPAs) have been suggested by some as over-simplified and ineffective (Dulvy et al., 2017; Dureuil et al., 2018), requiring more comprehensive species-specific assessments.

Climate change represents a major threat to global biodiversity. In particular, climatic extremes such as maximum temperatures may lead to higher probabilities of local extinctions for species that are unable to disperse or adapt to these conditions (Román-

Palacios & Wiens, 2020). In the North-East Atlantic, coastal waters are projected to experience temperature rises and acidification, and decreasing dissolved oxygen and salinity levels by the end of the century, while extreme oceanographic events are expected to increase in frequency and magnitude (MCCIP, 2020). Rising temperatures have already been associated with poleward distribution shifts for many species (Perry et al., 2005; Brattegard, 2011; Barton et al., 2016; Chaudhary et al., 2021), and have been linked to decreases in individual growth rates and fitness through the pejus effect (Morrongiello & Thresher, 2015). Behavioural changes have also been documented on a local scale, with some benthic elasmobranchs exploiting deeper thermal refugia (Perry et al., 2005). However, species that are more sedentary in nature may not be capable of undertaking spatial distribution shifts; in these cases, survival may depend upon physiological adaptation to a changing environment. For marine elasmobranchs, the projected environmental changes are likely to incur important physiological costs, particularly in relation to osmoregulation and acid-base regulation to maintain homeostasis. While some elasmobranchs have adapted strategies to cope with environmental extremes (Heinrich et al., 2014; Dziergwa et al., 2019), others are likely to suffer greater losses in individual fitness (Pistevos et al., 2015; Di Santo, 2016).

For non-model species that cannot be studied in-situ or experimentally, novel molecular approaches in the era of next-generation sequencing (NGS) can provide insights into the structure and local adaptation of wild populations. Ideally, the assembly and annotation of full genomes would provide a functional basis for genomic investigations of a species. However, genome assembly remains prohibitively costly and resource heavy to address urgent conservation questions at the scale of populations, especially given that elasmobranch genomes can be large and complex (Hara et al., 2018). Reduced-representation sequencing (RRS) methods provide an alternative approach, whereby thousands of genome-wide single nucleotide polymorphisms (SNPs) can be examined in the absence of a reference genome (Andrews et al., 2016). These generate high resolution data to estimate genetic differentiation even when sample sizes are small (Willing et al., 2012), which is an advantage for studies on rare species that rely on opportunistic sampling. Genome-wide SNPs can also be used to estimate effective population size (N_e), the evolutionary analogue of census population size (N_c) that is often used in conservation genetics (reviewed in Allendorf et al., 2013). Furthermore, genotype-environment studies have taken a leap forward with the arrival of NGS methods. Landscape (or seascape) genomics combines genomic and environmental data to investigate how genetic structuring may be driven by environmental variables, and can reveal candidate genes under selection in certain environmental conditions (Riginos et al., 2016; Roffler et al., 2016; Balkenhol et al., 2017).

In this study, we used a population and seascape genomics approach to investigate patterns of population structuring, abundance, and local adaptation in a critically endangered elasmobranch, the blue skate *Dipturus batis*. *D. batis* has only recently received species status after morphological and genetic investigations distinguished it from the parapatric flapper skate *D. intermedius* (Griffiths et al., 2010; Iglésias et al., 2010). Both species have become a conservation priority as a result of population declines and range restrictions, but are still caught as by-catch despite an EU landing ban (Ellis et al., 2016). In addition to the continual threat posed by fisheries-induced mortality, the ability of North-East Atlantic skates to adapt to environmental changes has become a pertinent question. For example, *D. batis* currently exploits a narrower thermal niche than *D. intermedius* (Frost et al., 2020), and consequently may respond differently to ocean warming. Using samples collected from the widest available extent of *D. batis'* range, obtained through a combination of research surveys and samples of opportunity, we applied a RRS approach (DArTseq^{TM}, Kilian et al., 2012) to i) assess the level of gene flow and levels of genetic diversity among extant *D. batis* populations, ii) estimate their effective population sizes, and iii) identify potential signals of selection in relation to environmental conditions. It is hoped that our findings will improve the current status of knowledge on *D. batis* and assist in conservation management, but also reveal patterns of structuring and local adaptation that may be relevant in other studies of data-deficient elasmobranchs facing similar threats.

Materials and Methods

i) Study species

The blue skate *Dipturus batis* is one of two rajids classified as Critically Endangered by the IUCN (Dulvy et al., 2006) formerly belonging to the cryptic common skate species complex (*D. batis*-complex). Presumed to have been widely distributed along the continental shelf (between 30-600m deep) of the eastern Atlantic from the Mediterranean to northern Norway and Iceland, common skate are now range-restricted primarily around the British Isles and the North Sea (Griffiths et al., 2010; ICES, 2020; Frost et al., 2020), although recent evidence suggests a wider contemporary distribution range (Bache-Jeffreys et al., 2021). Taxonomic confusion among large European rajids (Iglésias et al., 2010; Lynghammar et al., 2013) means the full extent of their distributions remains unclear without comprehensive species identification. At present, the confirmed geographic range of *D. batis* extends from the Celtic Sea to north of Orkney, with higher densities occurring in the Celtic Sea and Rockall (Griffiths et al., 2010; Frost et al., 2020), while their occurrence has recently been confirmed in Iceland (Bache-Jeffreys et al., 2021).

ii) Sample collection

Samples of *D. batis* were primarily obtained from fishery-dependent surveys conducted by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in the Celtic Sea during the autumn from 2011 to 2017 (Supplementary figure 1), and from fishery-independent surveys conducted by Marine Scotland Science along the Scottish coast and at Rockall. Faroese samples, obtained opportunistically from fisheries-independent surveys and the commercial fishing vessel 'Sandshavið', were donated by the Faroe Marine Institute. Gender and length (cm) data were collected from all samples except those from the Faroe Bank. Fin or muscle tissue samples were collected and stored in 96% ethanol or RNAlater®. A sample size of 4-6 individuals reportedly provides sufficient power for resolving population genomic structure when over 1,000 bi-allelic markers are used (Willing et al., 2012). To ensure sufficient power in our study, we selected at least ten individuals from different geographical areas where available, across the narrowest temporal range possible to minimise temporal structuring of our sample set. A total of 564 samples were selected for genomic analysis, from six locations: the Celtic Sea, the Scottish West Coast, Northern Scotland, Rockall, the Faroe Bank, and the Faroe Shelf (Figure 1, Table 1).

Table 1: Overview of sample sizes of *Dipturus batis* selected for genomic analysis from six geographical areas, and resulting sample sizes after SNP and sample filtering. The number of males and females after filtering (N=503) is shown together with their size range (length in cm).

Locality	Year	Sample size			Biological characteristics	
		Initial	Post-filtering	After removal of close relatives	No. males (size range)	No. females (size range)
Celtic Sea (CS)	2011-2017*	417	387	379	186 (66-138)	201 (69-148)
West Coast Scotland (WCS)	2012-2013	33	18	18	11 (28-108)	7 (21-106)
North Scotland (NS)	2013, 2019	14	9	9	2 (45-103)	7 (32-79)
Rockall (RK)	2012-2013	80	70	69	28 (33-123)	42 (30-127)
Faroe Shelf (FS)	2019	10	10	10	4 (132-152)	6 (46-152)
Faroe Bank (FB)	2019	10	9	8	NA (90-145)	
Total		564	503	493		
*Details of the Celtic Sea survey are shown in Supplementary figure 1.						
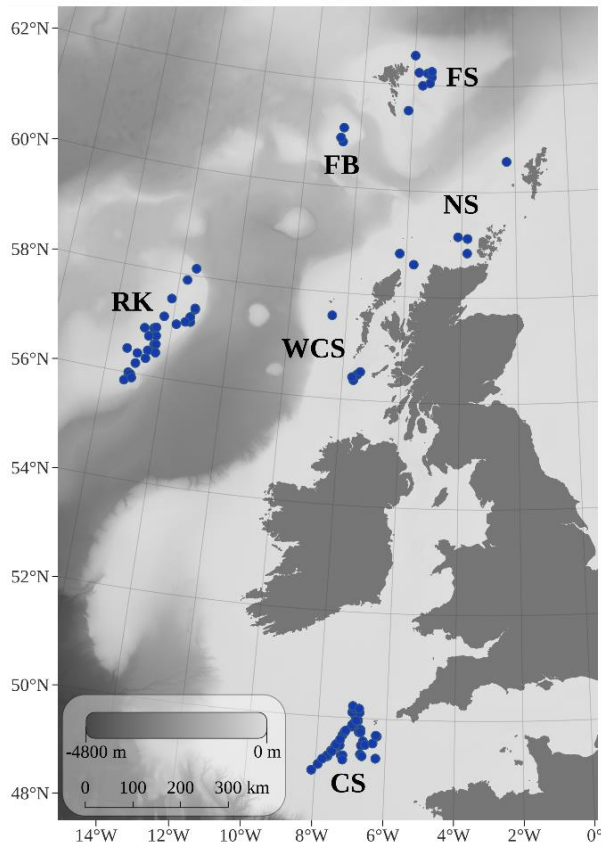
Figure 1: Sampling locations across the northeast Atlantic for 503 blue skate *Dipturus batis* that were used for population genomic analyses. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), the Faroe Bank (FB), and the Faroe Shelf (FS).

iii) DNA extraction and genotyping

  Genomic DNA was extracted using a DNeasy® Blood & Tissue kit (Qiagen). DNA concentrations were quantified on a Qubit fluorometer (ThermoFisher Scientific) and adjusted to 10-60ng/μL in preparation for sequencing. To assess the quality of DNA, we performed a mock digest of the samples in CutSmart® Buffer (New England Biolabs) for 2 hours at 37°C, and resolved all samples on a 0.8% TAE electrophoresis gel. In addition, two samples from each locality were visualised on a Genomic DNA ScreenTape® for a more detailed assessment of DNA quality. Genotyping was performed by Diversity Arrays Technology (DArT Pty. Ltd., Canberra, Australia) using DArTseq[TM] following standard protocols as described in Kilian et al. (2012). DArTseq[TM] combines complexity reduction

methods and next generation sequencing, and is optimised for each organism. Based on tests of several enzyme combinations for complexity reduction, DArT Pty. Ltd. applied the restriction enzyme combination PstI and SphI on the samples. Samples were sequenced (single read) on an Illumina® HiSeq® 2500, generating approximately 1.5 million sequences per sample. Sequences were processed using proprietary DArT Pty. Ltd. analytical pipelines. Twelve samples were identified as non-target species (data not shown) and were removed from the dataset before processing of raw sequences was repeated. This generated data for 17,620 sequences of 69 bp length, each containing a single nucleotide polymorphism (SNP).

iv) Data filtering

Ten samples were not reported by DArT Pty. Ltd. due to poor sample quality, and an additional sample was removed due to suspected genotyping error, as determined by visually scanning the raw data. SNPs were further filtered based on a call rate of 80%, and when duplicate loci were present, only the locus with the highest call rate was retained. After this step, the proportion of scored loci per sample was assessed, and all samples were considered to have a sufficiently high score rate to be retained (>88%). Monomorphic loci and loci with low minor allele frequencies (MAF < 0.05) were identified using *adegenet* (v 2.1.2, Jombart, 2008; Jombart & Ahmed, 2011), as implemented in R (v 3.6.2, R Core Team, 2019), and subsequently removed. Because human error could lead to sampling an individual multiple times or to contamination during molecular laboratory work, we looked for duplicate samples based on a threshold of 700 mismatching loci (roughly 10% of remaining loci) using the R package *CKMRsim* (Anderson, https://doi.org/10.5281/zenodo.820162). Where duplicates were found (i.e. >90 % genetically identical), the sample with the highest score rate was retained. Next, we tested for conformation of loci to Hardy-Weinberg proportions using the R package *pegas* (v 0.12, Paradis, 2010), performing an exact test based on Monte Carlo permutation of alleles (Guo & Thompson, 1992) with 1000 replicates for each of the six sampling locations and for the entire dataset (Supplementary table 2). After applying the false discovery rate (FDR) correction method of Benjamini and Hochberg (1995), loci were removed if they significantly deviated from Hardy-Weinberg proportions (at a significance threshold of α=0.05) in at least 2 sampling locations. We then tested for linkage disequilibrium among loci using the R package *snpStats* (v 1.36.0, Clayton, 2020) and removed one locus from each pair for which $R^2 > 0.80$. Following these filtering steps (summarised in Supplementary table 1), the resulting dataset contained 503 individuals genotyped at 6,350 loci (Table 1, Figure 1).

v) Finding related individuals

Because related pairs of individuals may introduce a bias in population genomic analyses, particularly when sample sizes are small, we looked for first-order (e.g. parent-offspring, full-sibling) and second-order (e.g. half-sibling) relatives in our dataset and removed one individual of each related pair for downstream analyses. Identifying related individuals also allowed us to observe any patterns of family structuring and habitat use. Related individuals were identified using *CKMRsim*, which simulates related pairs of individuals based on observed allele frequencies using a Monte Carlo approach. Using *CKMRsim*, we calculated the false positive and false negative rates at different log-likelihood thresholds for each pairwise hypothesis test involving parent-offspring (PO), full-sibling (FS), half-sibling (HS), and unrelated (U) relationship categories. Due to the large number of pairwise comparisons in relationship testing (503 samples imply 126,253 pairwise tests), this approach allowed us to identify appropriate log-likelihood thresholds when performing the relationship tests. Following *CKMRsim* recommendations, we aimed for a false-positive rate threshold of 100-times smaller than the reciprocal of the number of comparisons made (i.e. FPR $< 7.92 \times 10^{-8}$). We identified ten related pairs, and removed one individual from each pair such that population genomic analyses involved 493 individuals (Table 1).

vi) Population structure

Population- and locus-wide summary statistics were obtained using GenAlEx (v 6.5, Peakall & Smouse, 2006, 2012) with the exception of allelic richness, which was estimated using the R package *PopGenReport* (Adamack & Gruber, 2014). Spatial population structure was assessed using three approaches. First, we employed a Bayesian clustering algorithm using STRUCTURE (v 2.3.4, Pritchard et al., 2000). We used an admixture model with correlated allele frequencies, a burn-in length of 300,000 (more than enough to reach convergence) followed by 500,000 MCMC, and performed 5 iterations for each prior sub-population number K (ranging from K=1 to K=6). In order to avoid impractically long computation times and potential biases resulting from imbalanced sample sizes (Wang, 2017), we randomly sub-sampled 10 individuals from the Celtic Sea, West Coast Scotland, and Rockall, and included all samples from North Scotland (N=9), the Faroe Shelf (N=10) and the Faroe Bank (N=8), such that the total sample size for STRUCTURE analysis was 57. The most likely value of K was estimated using the delta-K method of Evanno et al. (2005) in STRUCTURE Harvester (Earl & VonHoldt, 2012), and summary plots for each K were produced using CLUMPAK (Kopelman et al., 2015). In order to justify the pooling of samples from different years in the Celtic Sea for the STRUCTURE analysis, we performed exploratory runs to test for genomic heterogeneity among all 379 Celtic Sea samples. We

found no evidence of genetic heterogeneity in the Celtic Sea between 2011 and 2017 (Supplementary figures 3 and 4).

Second, spatial genomic structure was assessed for all individuals (filtered dataset, N=493) using a discriminant analysis of principal components (DAPC) in *adegenet*. Missing data at any locus was replaced with the mean allele frequencies across all samples. Cluster identification was performed using the *find.clusters* function, with the optimal number of clusters evaluated using the Bayesian Information Criterion (BIC). Two DAPC plots were produced; one in which the prior grouping of individuals was based on the evaluated number of clusters, and one with prior groupings based on the six pre-defined geographic locations.

Third, we performed a principal component analysis (PCA) with the R function *prcomp*, using the pre-filtered dataset (N=503). The first two principal components from the PCA were used as co-variates for each individual in our seascape genomics analysis. Since the function does not allow for any missing data, we utilized 3,540 loci with a call rate of 100%.

Overall F-statistics (F_{IS}, F_{ST}) and pairwise F_{ST} (Weir & Cockerham, 1984) between sampling locations were estimated with the R implementation of *GenePop* (v 1.1.3, Rousset, 2008). *GenePop* was also used to perform overall and pairwise tests of genic differentiation, testing the null hypothesis that all alleles are drawn from the same distribution in all populations. Here, we applied an exact G-test (Fisher's method), using 1000 dememorizations, 100 batches, and 1000 iterations per batch.

vii) Effective population size

The effective population size (N_e) is a theoretical estimator of population size after accounting for genetic drift, that is useful in conservation genetics as it reflects the additive genetic variation, or evolutionary potential, of wild populations (reviewed in Allendorf et al. 2013). We estimated contemporary N_e for each sampling location and for each putative population (inferred from the preceding analyses) using the linkage-disequilibrium (LD) estimator (Hill, 1981; Waples, 2006; Waples & Do, 2010) in NeEstimator (v 2.1, Do et al., 2014). The estimate assumed random mating and was performed at critical values (i.e. MAF at which alleles should be excluded) of 0.05, 0.02, and 0.01. Confidence intervals were obtained using the Jackknife-over-individuals method.

viii) Seascape genomics and candidate loci under selection

To detect candidate loci under natural selection, we implemented a Bayesian outlier detection method using BayeScan (v 2.1, Foll & Gaggiotti, 2008), which identifies loci for which allele frequencies in the defined sub-populations deviate significantly from those of the total gene pool (i.e. all populations). We used BayeScan's default parameters (i.e. 20 pilot runs of length 5,000, and an additional burn-in length of 50,000), and applied a q-value threshold of 0.05.

We tested for associations between allele frequencies and environmental variables using multiple logistic regression, implemented in Samβada (v 0.8.1, Joost et al., 2007; Stucki et al., 2017). We obtained environmental variables that were representative of the skate's primarily epibenthic habitat. Monthly means for 4 physical (temperature, mixed layer depth, salinity, current velocity) and 7 biogeochemical (chlorophyll, dissolved oxygen, nitrate, phosphate, pH, primary production, light attenuation) variables were obtained from the Ocean Physical and Biogeochemical Reanalysis (NWSHELF_MULTIYEAR_PHY_004_009 and NWSHELF_MULTIYEAR_BGC_004_011) data products, available from the Copernicus Marine Service (https://marine.copernicus.eu/). Information was extracted for the near-bottom depth layer for each variable and for the 12 months preceding each individual's sampling date at a spatial resolution of ~7 km. Overall mean, maximum, and minimum values for each of these variables were retained for our seascape genomics analysis. In attempting to characterise the skates' year-round environment, such an approach assumed that the skates remained near their sampled locations in the 12 months prior to being sampled. Bottom depths for each sample were obtained from the EMODnet Bathymetry Consortium (2020). Five individuals from the Celtic Sea had erroneous or missing sample site information and were excluded from the analysis. Seven samples collected at the Faroe Bank by commercial fishermen also lacked precise sampling metadata, but are known to have been collected around late August-early September 2019 from the south-western part of the Faroe Bank shallower than 200 m (Faroe Marine Institute pers. comm.). To obtain environmental variables for these samples, the statistical rectangles from ICES subdivision 5.b.2 were used to subset the bathymetric layer. This subset was then binarized (> / < 200 m) and the centroid for the polygon above 200 m depth used as spatial coordinates for those records.

We performed a PCA to characterise the environmental variation among sites and to identify those variables responsible for this variation. We calculated Spearman's correlation coefficients among all variables, to allow for removal of those showing collinearity from the multiple logistic regression. In Samβada, the frequencies of alleles at each locus were tested for associations with latitude, longitude, depth, and the 11 physical

and biogeochemical variables (means, minima and maxima). To account for population structure, we employed a multivariate model, taking the first two principal components from a PCA performed on the genomic dataset as covariates for each individual. We computed P-values based on G and Wald scores for each test, and corrected for Type I error from multiple comparisons using Bonferroni correction at thresholds of 0.05 and 0.01.

The sequences containing the SNPs detected in BayeScan and in our seascape genomics analysis were BLASTed on NCBI (NCBI, 1988) to identify functional sequences. The blastn function was used to allow for comparisons across species, given the poorly annotated nature of elasmobranch genomes. We reported the top five BLAST hits with an E-value < 0.01.

Results

i) Sampled individuals

Sample sizes reflected sampling effort and corresponded to anecdotal reports of *D. batis* abundance. Most samples were obtained from the Celtic Sea and Rockall during scientific surveys (Table 1). Males and females were collected at all sites, and a higher number of females was collected overall. Gender data was not recorded for the Faroe Bank skates. A broad size range of individuals was collected for both sexes (ranging from 21 to 152 cm), representing a mix of juveniles and adults at most sites based on estimated ages at first maturity for *D. batis*-complex at 115 cm and 125 cm for males and females, respectively (McCully et al., 2012).

ii) Related individuals

The final panel of 6,350 SNPs was very informative for the detection of related individuals among 503 samples of *Dipturus batis*. False positive rates were considerably lower than our defined threshold for all six pairwise relationship tests (FPR ≤ 5.62×10^{-56} in each case, Supplementary table 3, Supplementary figure 2). We identified ten related pairs, including one full-sibling pair and seven half-sibling pairs from the Celtic Sea, one half-sibling pair from Rockall, and one half-sibling pair from the Faroe Bank (Table 2). All related pairs were therefore found at the same locality, and in four of these cases the pair was collected in the same haul (i.e. at the same time and place).

Table 2: Full-sibling (FS) and half-sibling (HS) pairs of blue skate *Dipturus batis* and their biological and sampling details. Note that Faroe Bank samples were obtained from a fishing vessel for which some sampling details are lacking.

Relationship	Locality	ID number	Sampled date	Length (cm)	Sex	Latitude (decimal)	Longitude (decimal)
FS	Celtic Sea	CEFAS2015-16-240	24.09.2015	121	M	49.317	-6.731
	Celtic Sea	CEFAS2017-C10-4-16	26.10.2017	122	F	49.197	-7.815
HS	Celtic Sea	CEFAS2017-C11-2-09	26.10.2017	94	F	49.092	-7.933
	Celtic Sea	CEFAS2017-C09-3-06	26.10.2017	84	F	49.270	-7.673
HS	Celtic Sea	CEFAS2017-C14-21-07	30.10.2017	97	M	50.225	-7.008
	Celtic Sea	CEFAS2017-C14-21-10	30.10.2017	115	M	50.225	-7.008
HS	Celtic Sea	CEFAS2017-C13-20-04	30.10.2017	113	M	50.178	-6.983
	Celtic Sea	CEFAS2017-C13-20-05	30.10.2017	129	M	50.178	-6.983
HS	Celtic Sea	CEFAS2017-C02-15-10	29.10.2017	120	M	50.105	-6.800
	Celtic Sea	CEFAS2014-80	17.09.2014	117	M	49.952	-6.835
HS	Celtic Sea	CEFAS2015-16-242	24.09.2015	74	M	49.317	-6.731
	Celtic Sea	CEFAS2017-C05-11-06	29.10.2017	78	F	49.725	-7.216
HS	Celtic Sea	CEFAS2015-18-253	24.09.2015	133	M	49.284	-6.682
	Celtic Sea	CEFAS2011-279	24.08.2011	123	F	49.967	-6.850
HS	Celtic Sea	CEFAS2011-271	24.08.2011	132	F	49.967	-6.850
	Celtic Sea	CEFAS2011-308	24.08.2011	116	M	49.967	-6.850
HS	Rockall	1413S-125	24.10.2013	74	M	56.610	-14.444
	Rockall	1413S-126	24.10.2013	112	M	56.610	-14.444
HS	Faroe Bank	Sandshavið-F1	2019	90	NA	NA	NA
	Faroe Bank	Sandshavið-F2	2019	102	NA	NA	NA

iii) Genetic diversity

Patterns of genetic diversity were generally comparable across sites, however, samples from Rockall exhibited lower genetic diversity (Table 3). Allelic richness, which unlike number of alleles corrects for differences in sample size, was lower at Rockall. Although higher at both Faroese sites, the Faroese samples had a slightly lower allelic richness when compared with the three British sites. The fixation index (F = 1-(H_o/H_e)), otherwise known as the inbreeding coefficient, was generally low across sites, but was higher at Rockall and West Coast Scotland. Negative values of F for individuals from North Scotland, Faroe Bank and Faroe Shelf corresponded with a slightly higher than expected level of heterozygosity at these sites, whereas the opposite was true of the Celtic Sea, Rockall, and West Coast Scotland. There was no evidence for the presence of unique loci at any of the sites, with no private alleles detected.

Table 3: Mean genomic summary statistics for *Dipturus batis* overall and across six sampling locations. Sample sizes (N), number of alleles (Na), observed (Ho) and expected (He) heterozygosity, fixation index (F), and number of private alleles (PA) are shown, as reported using GenAlEx (v 6.5, Peakall & Smouse, 2006, 2012). Mean allelic richness (Ar) is also shown, as reported by *PopGenReport* (Adamack & Gruber, 2014).

Locality		N	Na	Ho	He	F	PA	Ar
CS	Mean	375.107	2.000	0.284	0.296	0.045	0	1.662
	SE	0.137	0.000	0.002	0.002	0.002		
RK	Mean	67.747	1.974	0.251	0.264	0.062	0	1.590
	SE	0.040	0.002	0.002	0.002	0.002		
NS	Mean	8.925	1.906	0.292	0.283	-0.034	0	1.646
	SE	0.004	0.004	0.002	0.002	0.004		
WCS	Mean	17.503	1.968	0.269	0.287	0.061	0	1.648
	SE	0.015	0.002	0.002	0.002	0.004		
FB	Mean	7.928	1.861	0.288	0.274	-0.050	0	1.625
	SE	0.005	0.004	0.003	0.002	0.004		
FS	Mean	9.902	1.900	0.277	0.276	-0.010	0	1.629
	SE	0.006	0.004	0.002	0.002	0.004		
Overall	Mean	81.185	1.935	0.277	0.280	0.014	0	
	SE	0.682	0.001	0.001	0.001	0.001		

iv) Spatial population structure

Overall, the results suggest a clear barrier to gene flow between Rockall and all other sites, with Rockall demonstrating significant genomic differentiation across all analyses. There was high gene flow among British continental shelf sites (CS, WCS, and NS), whereas gene flow occurred to a more limited extent between the British shelf and the Faroese sites; results of the different analyses led to different conclusions regarding the degree of genomic differentiation among Faroese and British skates.

Results of the Bayesian clustering analysis implemented in STRUCTURE suggested that the most likely number of clusters was 2, as per the delta-K method of Evanno et al. (2005). The clustering clearly separated Rockall skates from the rest (Figure 2). When visualising the output of K=3, which had only a slightly lower mean log probability than K=2 (Supplementary figure 5), a third cluster consisting of Faroese skates was identified, with some proportion of assignment to the British cluster for a few samples from the Faroe Shelf (Figure 2).

The *find.clusters* function in the discriminant analysis of principal components (DAPC) suggested an optimal number of 2 clusters, based on the lowest BIC score after retaining all 493 principal components (Supplementary figure 7). Results were plotted using these inferred clusters as well as based on the six sampling locations using the first 350 principal

components (explaining 82% of the observed variance). For the two inferred clusters, all 69 individuals from Rockall formed one cluster while the remaining 424 samples were clearly differentiated into a second cluster, summarized by only one discriminant function (Figure 3).

When grouped by sampling location, the same distinction between Rockall and the rest of the samples could be seen, summarized most informatively by the first of five eigenvalues (Figure 3). However, in this case, samples from the Faroe Bank and the Faroe Shelf could be distinguished from all other sites, and from each other.

In order to assess the influence of loci under selection and the level of potentially adaptive population structure, DAPC was repeated using only the 21 outlier loci identified in Bayescan, and with a neutral dataset excluding these 21 loci. Despite weaker clustering that could be expected with the small number of loci, the DAPC still revealed genomic differentiation across the putatively adaptive loci (Supplementary figure 8), while the removal of these 21 loci from the total dataset did not affect the results (Supplementary figure 9). The principal component analysis (PCA) also showed a clear separation between samples from Rockall and the remaining sites, however the variation explained by each principal component was low ($\leq$ 1.2%, Supplementary figures 10 and 11).

Results of the G-test suggested significant genic differentiation across samples overall (P < 0.001), and between all pairwise site comparisons with Rockall (F_{ST}>0.038, P < 0.001, Table 4). Overall F_{ST} and F_{IS} were low (F_{ST}=0.026 and F_{IS}=0.043). The G-tests and F-statistics were repeated after grouping individuals into the three putative populations inferred from the previous results: British Shelf, Rockall, and Faroe Islands. After grouping, the overall G-test indicated significant genic differentiation overall and between all pairs of sites (P < 0.001), and there was a slight increase in overall F_{ST} and F_{IS} (F_{ST}=0.032 and F_{IS}=0.044).
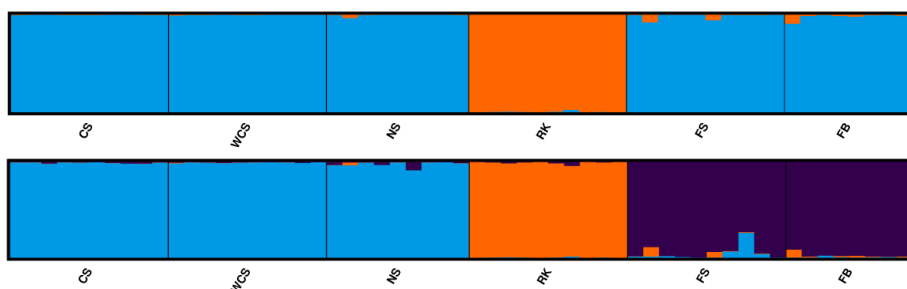
Figure 2: Results from the Bayesian clustering algorithm implemented in STRUCTURE, visualised using CLUMPAK for K=2 (top) and K=3 (bottom), for 57 *Dipturus batis* samples collected from the Celtic Sea (CS), West Coast Scotland (WCS), North Scotland (NS), Rockall (RK), the Faroe Shelf (FS), and the Faroe Bank (FB). Each individual is represented by a vertical line with the proportion of assignment to a cluster indicated by 2 or 3 colours.
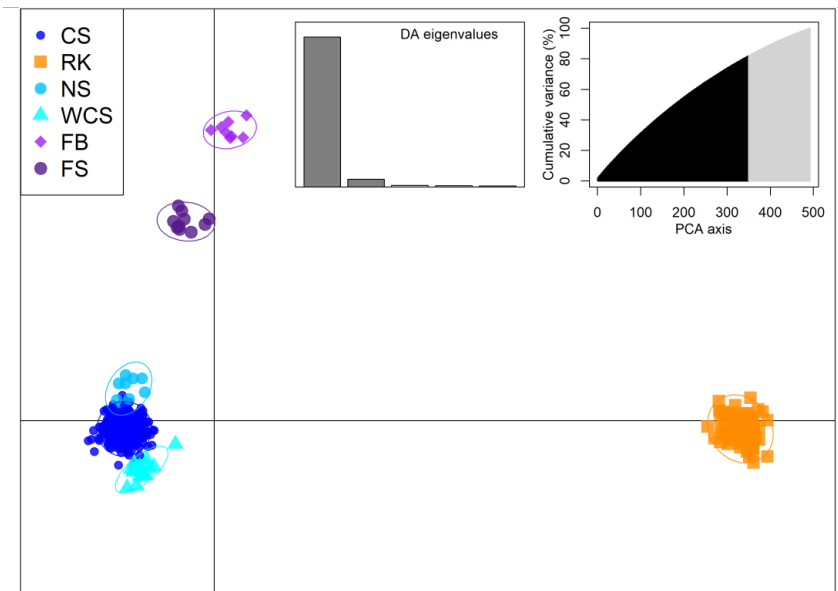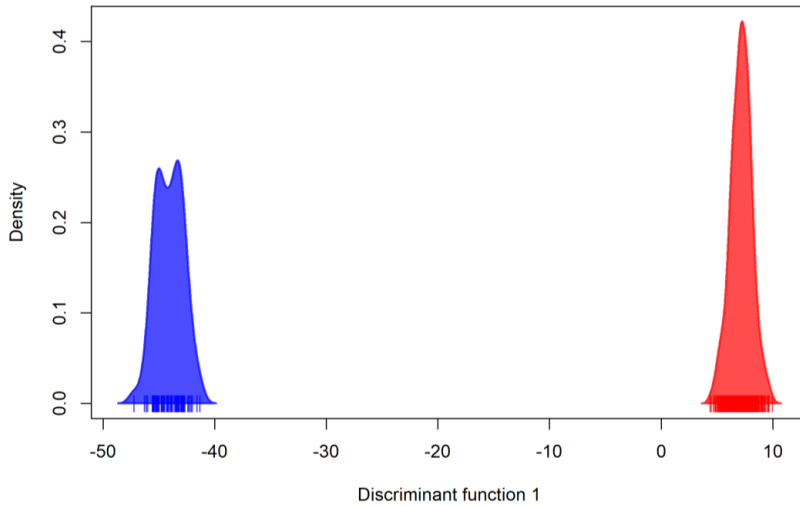
Figure 3: Discriminant analysis of principal component (DAPC, *adegenet*) plots depicting the variation among 493 *Dipturus batis* samples genotyped across 6,350 SNPs. Top: variation between two clusters inferred using *find.clusters*, where the blue (left) cluster contains all 69 samples from Rockall and the red (right) cluster contains the remaining 424 samples from the UK and Faroe Island sites. Bottom: variation among samples grouped by their sampling locations, with 95% inertia ellipses shown for each group. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), the Faroe Bank (FB), and the Faroe Shelf (FS).

Table 4: Pairwise F_{ST} for *Dipturus batis* among six sites. Values in bold indicate significant genomic differentiation (P<0.05) from pairwise G-tests.

	CS	RK	NS	WCS	FB	FS
CS	-					
RK	**0.0383**	-				
NS	0.0004	**0.0425**	-			
WCS	0.0013	**0.0420**	0.0003	-		
FB	0.0123	**0.0444**	0.0122	0.0133	-	
FS	0.0082	**0.0449**	0.0083	0.0089	0.0086	-

v) Effective population sizes

Effective population sizes (N_e) were estimated for each sample site and after grouping samples into the three putative populations. For the former, sample sizes were too small and N_e could not be estimated for some sites (Supplementary table 4). For the latter, the British Shelf population had the highest N_e (ca. 21,000, Table 5) whereas N_e for Rockall was as low as half of this (estimates ranging from ca. 11,300 to 19,000). The Faroese skates demonstrated the lowest levels of N_e (estimates ranging from ca. 2,300 to 3,500). In some cases estimates reached infinity, probably as a result of small sample sizes (Marandel et al., 2019) rather than very large population sizes.

Table 5: Estimates of effective population size (N_e) for *Dipturus batis* grouped into three putative population units, using the linkage-disequilibrium method in NeEstimator. Estimates are shown for three critical values (Crit = 0.05, 0.02, and 0.01), and 95% confidence intervals by Jackknifing over individuals are shown in parentheses. Sample sizes (N) are also shown.

Population	N	Crit=0.05	Crit=0.02	Crit=0.01
British Shelf	406	21,068 (17,141-27,313)	21,015 (17,110-27,213)	21,010 (17,128-27,150)
Rockall	69	11,299 (3,903-∞)	14,475 (3,810-∞)	18,983 (3,943-∞)
Faroe Islands	18	2,362 (1,362-8,824)	3,501 (1,798-63,597)	3,501 (1,798-63,597)

vi) Seascape genomics and candidate loci under selection

The PCA showed a clear differentiation among sites based on all 34 environmental variables. The first two principal components (PCs) explained 72.7% of the environmental variation among sites (Figure 4), with all variables contributing to at least one of these PCs (Supplementary figure 12). Overall, there was a clear difference between southern (Celtic Sea) and northern (all other sites) across PC1 and PC2, whereas a distinction could also be made between 'offshore' (Celtic Sea and Rockall) and 'inshore' (Scottish and Faroese, also including Faroe Bank) sites across PC2. The variation among sites was complex, but a

general pattern could be seen where the Celtic Sea was warmer, more saline, and more acidic, while Rockall was the deepest of the sites (up to 385m deep, Table 6). There was a strong correlation between primary productivity and chlorophyll concentration and between nitrate and phosphate concentrations (Spearman's correlation coefficients >0.9). Removing one variable from each collinear pair did not influence the results of our multiple logistic regressions, and actually reduced the environmental variation explained in the PCA. Therefore, we report results of logistic regression using all 34 environmental variables.

Testing for associations between allele frequencies (two alleles for each of 6,350 loci) and 36 environmental variables (including latitude and longitude as covariates) generated a total of 457,200 tests in Samβada. After Bonferroni correction, one allele (alternate allele at locus 100069553) was significantly associated with seven environmental variables (P<0.01 for both G-score and Wald-score). These were: latitude, mean current velocity, maximum pH, minimum bottom temperature, and mean, minimum and maximum salinity. This locus was also detected as one of 21 outliers under putative positive selection in Bayescan (Table 7, Supplementary figure 14). On closer inspection, we observed that the proportion of reference homozygotes at this locus was high in the Celtic Sea (96%) when compared with the other sites (33-80%). Only one homozygote for the alternate allele, a skate from West Coast Scotland, existed among all 503 genotyped skates.

The blastn search produced ambiguous hits for seven out of 21 outlier loci (Table 7). Percentage sequence identity ranged from 80-97%. The majority of the hits were against mRNA and ncRNA transcript variants for *Amblyraja radiata*, but there were also matches against the immunoglobin heavy chain gene region for *Raja eglantaria*.
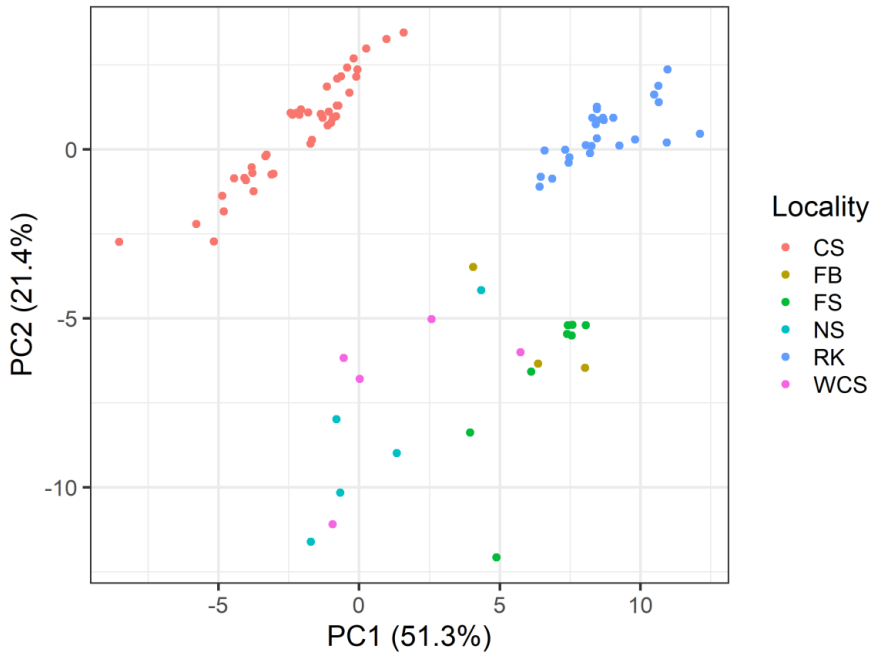
Figure 4: Principal component analysis depicting the variation among sites based on 34 environmental variables. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), the Faroe Bank (FB), and the Faroe Shelf (FS).

Table 6: Overview of environmental characteristics for each site, showing mean and range (in parentheses) across monthly means obtained for 12 months prior to sampling date. Data show bottom depth, bottom temperature (BT), mixed layer depth (MLD), salinity (Sal), current velocity (Cur), chlorophyll concentration (Chl), dissolved oxygen concentration (O_2), nitrate concentration (NO_3^-), phosphate concentration (PO_4^{3-}), pH, light attenuation (KD), and primary productivity (PP).

Locality	Year	Depth (m)	BT (°C)	MLD (m)	Sal (PSU)	Cur (ms^{-1})	Chl ($mg\ m^{-3}$)	O_2 ($mmol\ m^{-3}$)	NO_3^- ($mmol\ m^{-3}$)	PO_4^{3-} ($mmol\ m^{-3}$)	pH	KD (m)	PP ($mg\ C\ m^{-3}\ day^{-1}$)
Celtic Sea (CS)	2011-2017	115 (83-149)	10.9 (8.9-15.8)	51 (10-140)	35.5 (35.3-35.6)	0.01 (<0.01-0.03)	0.35 (<0.01-2.25)	249 (221-272)	6.3 (1.8-8.6)	0.4 (0.0-0.5)	8.06 (8.02-8.12)	0.11 (0.07-0.16)	0.60 (0.03-3.82)
West Coast Scotland (WCS)	2012-2013	142 (115-158)	9.6 (7.3-12.6)	46 (10-140)	35.1 (34.8-35.4)	0.04 (<0.01-0.11)	0.28 (<0.01-2.25)	254 (227-274)	7.8 (3.1-12.0)	0.5 (0.2-0.8)	8.09 (8.04-8.14)	0.1 (0.09-0.16)	0.36 (0-0.86)
North Scotland (NS)	2013, 2019	127 (93-149)	8.9 (6.3-9.4)	65 (11-128)	35.1 (34.7-35.4)	0.03 (<0.01-0.09)	0.14 (<0.01-0.55)	259 (238-279)	8.1 (2.2-11.6)	0.5 (0.1-0.7)	8.08 (8.04-8.16)	0.12 (0.09-0.17)	0.24 (<0.01-0.80)
Rockall (RK)	2012-2013	222 (168-385)	9.4 (8.5-10.1)	99 (13-284)	35.3 (35.3-35.4)	0.02 (<0.01-0.07)	0.02 (0-0.25)	235 (199-262)	12.3 (9.3-13.3)	0.8 (0.6-0.9)	8.05 (8.04-8.08)	0.09 (0.07-0.11)	0.05 (0-0.37)
Faroe Shelf (FS)	2019	178 (153-207)	7.5 (6.3-9.4)	95 (10-195)	35.1 (35.1-35.2)	0.03 (<0.01-0.10)	0.04 (<0.01-0.34)	258 (237-278)	10.5 (5.2-12.1)	0.7 (0.3-0.8)	8.08 (8.06-8.15)	0.09 (0.07-0.12)	0.07 (0-0.47)
Faroe Bank (FB)	2019	115 (115)	8.6 (7.3-9.8)	71 (11-127)	35.2 (35.1-35.2)	0.02 (<0.01-0.07)	0.26 (<0.01-0.84)	251 (234-258)	11.0 (8.5-12.9)	0.7 (0.5-0.9)	8.08 (8.06-8.11)	0.09 (0-0.11)	0.40 (0-1.23)
Overall	2011-2019	115 (83-385)	10.5 (6.2-15.8)	59 (10-283)	35.4 (34.7-35.6)	0.01 (<0.01-0.10)	0.3 (<0.01-2.30)	248 (199-279)	7.4 (1.8-13.3)	0.5 (0.0-0.9)	8.06 (8.02-8.16)	0.11 (0.07-0.17)	0.50 (0-3.82)

Table 7: 21 SNP loci identified as potentially under positive selection in *Dipturus batis*. Significance at P<0.05 (*) and P<0.01 (**) is shown for outlier detection in BayeScan. Loci associated with environmental variables in multiple logistic regression using Samβada following Bonferroni correction at P<0.05 (*) and P<0.01 (**) are also shown. Abbreviations refer to salinity (SAL) and bottom temperature (BT). The top BLASTn hits from NCBI with an E-value < 0.01 for each locus are reported.

Locus	Bayescan	Samβada	BLASTn search		
			Description	E-value	% identity
10967992	**				
10975590	*				
11001882	*				
11005420	**				
16778142	*				
16781304	**		Amblyraja radiata uncharacterized LOC116974003 (LOC116974003), ncRNA	1e-14	88.7
			Amblyraja radiata transmembrane protein 50A (tmem50a), transcript variant X2, mRNA	2e-13	87.3
			Amblyraja radiata beta-1,4-galactosyltransferase 3-like (LOC116974216), transcript variant X1, mRNA	2e-12	87.0
			Raja eglanteria clone 2113 Ig heavy chain (Vx, Dx1, Dx2, Jx, Cx1, and Cx2) gene region	2e-12	87.3
			PREDICTED: Amblyraja radiata SAM and SH3 domain containing 1 (sash1), transcript variant X8, mRNA	7e-12	85.9
16781498	**				
16782764	*		Amblyraja radiata solute carrier family 19 member 1 (slc19a1), mRNA	3e-05	97.1
16783348	**				
16783589	**				
16783836	**				
16785038	**				
16785054	*		Danio rerio genome assembly, chromosome: 25	2e-12	97.9
			Danio rerio strain Nadia (NA) genome assembly, chromosome: 3	2e-12	97.9
			Zebrafish DNA sequence from clone DKEY-106C17 in linkage group 3, complete sequence	2e-12	97.9
			Danio rerio strain Nadia (NA) genome assembly, chromosome: 7	5e-12	95.9
			Zebrafish DNA sequence from clone CH211-72D16 in linkage group 17, complete sequence	5e-12	95.9
16785163	**				
16785175	**		Amblyraja radiata uncharacterized LOC116974003 (LOC116974003), ncRNA	3e-16	89.0
			Amblyraja radiata transmembrane protein 50A (tmem50a), transcript variant X2, mRNA	4e-15	88.7
			Amblyraja radiata beta-1,4-galactosyltransferase 3-like (LOC116974216), transcript variant X1, mRNA	5e-14	88.4
			Raja eglanteria clone 2113 Ig heavy chain (Vx, Dx1,	5e-14	87.7

				E-value	Identity
			Dx2, Jx, Cx1, and Cx2) gene region		
			Amblyraja radiata SAM and SH3 domain containing 1 (sash1), transcript variant X8, mRNA	2e-13	86.3
16785304	**		Amblyraja radiata twinfilin actin binding protein 2 (twf2), transcript variant X4, mRNA	8e-18	92.9
			Amblyraja radiata RRN3 homolog, RNA polymerase I transcription factor (rrn3), transcript variant X1, mRNA	4e-15	90.0
			Amblyraja radiata transmembrane protein 50A (tmem50a), transcript variant X2, mRNA	5e-14	88.6
			Amblyraja radiata beta-1,4-galactosyltransferase 3-like (LOC116974216), transcript variant X1, mRNA	6e-13	88.2
			Amblyraja radiata uncharacterized LOC116976331 (LOC116976331), ncRNA	2e-12	87.1
100018343	*		Amblyraja radiata ATR interacting protein (atrip), transcript variant X4, mRNA	3e-11	91.1
			Amblyraja radiata ATR interacting protein (atrip), transcript variant X3, mRNA	3e-11	91.1
			Amblyraja radiata ATR interacting protein (atrip), transcript variant X2, mRNA	3e-11	91.1
			Amblyraja radiata ATR interacting protein (atrip), transcript variant X1, mRNA	3e-11	91.1
			Amblyraja radiata quinolinate phosphoribosyltransferase (qprt), transcript variant X1, mRNA	9e-11	90.9
100018549	*				
100024701	**		Amblyraja radiata gamma-glutamyl hydrolase-like (LOC116981285), transcript variant X3, mRNA	1e-08	84.1
			Amblyraja radiata gamma-glutamyl hydrolase-like (LOC116981285), transcript variant X2, mRNA	1e-08	84.1
			Amblyraja radiata gamma-glutamyl hydrolase-like (LOC116981285), transcript variant X1, mRNA	1e-08	84.1
			Amblyraja radiata uncharacterized LOC116978972 (LOC116978972), ncRNA	3e-04	80.7
			Amblyraja radiata zinc finger protein 516 (znf516), transcript variant X3, misc_RNA	3e-04	86.7
100034323	*				
100069553	**	CUR.mean** SAL.min** SAL.mean** SAL.max** Latitude** pH.max** BT.min**			

Discussion

The objectives of this study were to characterise the contemporary population structure, estimate effective population sizes, and investigate putative patterns of adaptation along environmental gradients in the critically endangered blue skate *Dipturus batis*. We identified a clear genetic discontinuity across the Rockall Trough contrasting high gene flow along the British continental shelf. The results corroborate the findings of Frost et al. (2017), who demonstrated this using microsatellite markers on a subset of the samples used in this study. With additional samples from these as well as Faroese sites, we identified another genetic discontinuity between the Faroe Islands and the British shelf. Effective population size estimates were relatively high in the Celtic Sea and Rockall, but sufficiently low in Scotland and the Faroe Islands to be considered a potential conservation concern. We also identified 21 candidate SNPs under selection, including one associated with environmental variables that are expected to shift in response to a changing climate, which may have implications for the future realized niche of *D. batis*.

The isolation of offshore populations of *D. batis*, which contrast the high level of coastal connectivity, is a recurring pattern for coastal elasmobranchs (Le Port & Lavery, 2012) that may be driven by the presence of bathymetric barriers. The Rockall Bank was the most genetically isolated population in *D. batis*, occurring on an offshore plateau ~250 km from the British continental shelf and surrounded by deep (>1,000 m) waters that exceed the species' reported depth range. Similarly, the Faroe Shelf and Bank are separated from the Scottish shelf by the Rockall Trough to the south and the ~1,200 m deep Faroe-Shetland Channel to the east. Several studies have attributed the genetic isolation of Rockall and Faroe Island populations of benthic and benthopelagic fishes (Mattiangeli et al., 2002; Gonzalez et al., 2014; Saha et al., 2015; Régnier et al., 2017; Johansen et al., 2020) and squid (Shaw et al., 1999) to these bathymetric barriers. Unlike these animals, skates are almost exclusively epibenthic throughout their life cycle, laying their egg cases (mermaid's purses) on the sea-bed (Last et al., 2016) and spending the majority of their life near the sea floor (Wearmouth & Sims, 2009). Therefore, the bathymetric barriers are unlikely to be readily overcome unless skates swim long distances high in the water column.

The patterns of population structure observed in *D. batis* are also likely influenced by the species' highly resident behaviour. The proximity of close relatives identified in this study is indicative of site-attached behaviour, and supports early tagging work in the Celtic Sea demonstrating that *D. batis* almost exclusively remain within relatively confined shallow areas (<200 m) of the continental shelf (Bendall et al., 2018). Nonetheless, the results indicate high levels of gene flow spanning over 1,000 km along the British continental shelf from the Celtic Sea to Northern Scotland. Tagging experiments investigating the related

and more extensively studied flapper skate *D. intermedius* along the Scottish coast may provide inference about movement patterns in *D. batis*. Despite high levels of residency (Little, 1998; Wearmouth & Sims, 2009), around 25% of *D. intermedius* are vagrant or 'transient' individuals (Neat et al., 2015) and have been recaptured up to 900 km from their release sites (Little, 1998). In contrast, the extent of *D. batis* movement appears to be more restricted (early results from Bendall et al. (2018) indicated a maximum recapture distance of 170 km), but the occurrence of transient individuals is conceivable. If the scarcity of samples from Scotland and their low effective population sizes are indicative of low abundances in this area, the occurrence of occasional breeding migrants from larger populations such as the Celtic Sea could explain the genomic homogeneity observed. It could be argued that the Scottish samples in our study were themselves transient individuals from the Celtic Sea, however these samples were predominantly juveniles (21-108 cm, Table 1) and are unlikely to have undertaken long-distance movements. In fact, individuals in the immature size range were collected at all sites (length at first maturity <115 cm for males, <125 cm for females, McCully et al. 2012), indicative of restricted resident populations utilizing nursery habitats at or near each of the sample sites.

Abundance estimates are vital to establish effective conservation strategies but are currently lacking for *D. batis*. Our pooled effective population size (N_e) estimates indicated that the British Shelf and Rockall had the highest N_e (~21,000 and ~11,000 individuals, respectively), though the former were dominated by high population densities in the Celtic Sea. The Faroe Islands had a much lower N_e (~2,300 individuals). The minimum N_e required to maintain the evolutionary potential of a population has been a subject of debate, with proposed conservation thresholds set between 500 and 5,000 individuals (reviewed in Allendorf et al., 2013), below which populations may be prone to the loss of genetic diversity, higher inbreeding, and the accumulation of deleterious mutations. Whereas the Celtic Sea had relatively high N_e, the estimates for the Scottish, Rockall, and Faroese populations could be construed as being of conservation concern. We note that N_e estimation from high-throughput sequencing data can be subject to bias due to violations to the assumptions of unlinked loci that are difficult to correct for without knowledge of the species' genomic architecture (Waples et al., 2016). Its interpretation also depends on the life history and demography of the species and should ideally be compared to census population size (N_c) (Waples et al., 2018; Lieber et al., 2020). Although these factors may affect the reliability of our N_e estimates, they provide an important first estimate of *D. batis* abundance across its distribution range and serve as initial indicators of their conservation status. The identification of close relatives among our samples suggests that N_c could be estimated using close-kin mark-recapture, provided an appropriate survey design (Bravington, Grewe, et al., 2016; Bravington, Skaug, et al., 2016).

The seascape genomics analysis revealed a significant association between one locus under putative positive selection and a number of abiotic variables expected to be influenced by climate change. Previous work has demonstrated that *D. batis* may be tolerant to a narrower thermal range than its parapatric congener, *D. intermedius*, where their ranges overlap (Frost et al., 2020); this may lead to differential shifts in fitness under projected climate change. Not only did our findings identify an association between a putatively adaptive locus and minimum bottom temperature, but also associated it with pH, salinity, and current velocity; a dominance of homozygotes for the reference allele was found in the Celtic Sea (96% of individuals, compared to 33-80% of individuals elsewhere), where bottom waters are warmer, more saline, more acidic, and have slower mean current velocities than the northern sites. The pronounced geographic pattern at this locus occurs despite apparently high levels gene flow between Scottish sites and the Celtic Sea, suggesting that a strong selective force may be acting on this locus or on a closely linked one.

Climate change is expected to lead to warmer, fresher, and more acidic waters around the British Isles by the end of the century, with an increasing frequency of extreme oceanographic events (MCCIP, 2020). These three abiotic variables are likely to have important biological consequences for a benthic elasmobranch. Osmoregulation and acid-base regulation are vital physiological functions for the maintenance of homeostasis in the marine environment. The implications of these changes on *D. batis* remain unclear. The closest comparison can be drawn from experimental work on little skate *Leucoraja erinacea* from the western North Atlantic, in which individuals from northern parts of the range (colder and less thermally variable) were more sensitive to acidification and warming, as indicated by a lower aerobic capacity and slower recovery following anaerobic activity (Di Santo, 2016). Other studies have demonstrated that warming and acidification could impact elasmobranch hunting ability through impaired olfaction and increased energetic demands (Pistevos et al., 2015), influence juvenile skeletal development (Di Santo, 2019), and lead to denticle corrosion (Dziergwa et al., 2019). The association suggested with mean current velocity could relate to gas exchange rates during respiration when resting on the sea-floor, or to the energetic costs of swimming. Due to a lack of reference genomes for *D. batis* and its relatives, BLAST searches for this and the 20 other putatively adaptive loci did not produce any unambiguous hits. Whereas this locus could be part of a functional sequence, it could also be physically linked to a gene under selection. Future investigations will benefit from more targeted approaches as genomic resources for elasmobranchs improve.

Our results provide an updated assessment of *D. batis*' population structure, regional abundance, and movement patterns that will have immediate relevance for the

development of conservation actions for this critically endangered species. What's more, it may be necessary to consider the long-term efficacy of such conservation strategies, with the results suggesting that climate change may affect populations of *D. batis* differentially across their distributional range based on their adaptive genomic variation. Should environmental changes incur a physiological cost to which they cannot adapt, a move to greater depths as observed in other species could constrain *D. batis* habitat to the fringes of the continental shelf, whereas a northward distribution shift may be confined by the northern extent of the continental margin and by direct competition with other large rajids inhabiting this range. Given the highly residential nature of *D. batis*, their slow population turnover rates, and their high susceptibility to accidental bycatch, the results support the urgent need to implement comprehensive management measures in order to conserve the adaptive variation and long-term viability of the species across the few remnant populations. Our findings may have similar implications for other threatened and data-deficient coastal elasmobranchs in the North-East Atlantic.

Acknowledgements

Author contributions

LRN and CSJ coordinated the study, and designed the study together with AD. VB and SJH coordinated sampling efforts in the Celtic Sea and contributed biological knowledge on *D. batis*. AD and MF prepared the DNA samples for sequencing. DS gathered the environmental data. AD and MF performed the data analysis under the supervision of LRN, CSJ, and GH. AD wrote the first version of the manuscript. All authors contributed to the final version of the manuscript.

Data Accessibility

Sample information and genotype data are available at: *to be completed after manuscript is accepted for publication*.

Conflict of interest

The authors declare no conflicts of interest.

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Supplementary Material

i) Further details of sample provenance from the Celtic Sea

Samples of blue skate from the Celtic Sea were obtained during a fishery-dependent common skate survey performed by CEFAS in collaboration with the fishing industry in 2011, and from 2014-2017. Surveys were run during the late summer to autumn (August-October). Fixed trammel nets were deployed along a transect of stations running 12-80 nm to the south and west of Newlyn, Cornwall, UK, with additional exploratory stations outside the main transect area also surveyed (Supplementary figure 1). Further details of the sampling protocol are described in Bendall et al. (2018).

Supplementary figure 1: Trammel net haul locations where blue skate (Dipturus batis) samples were collected in 2011, 2014, 2015, and 2017 in the Celtic Sea, as part of a CEFAS monitoring programme.
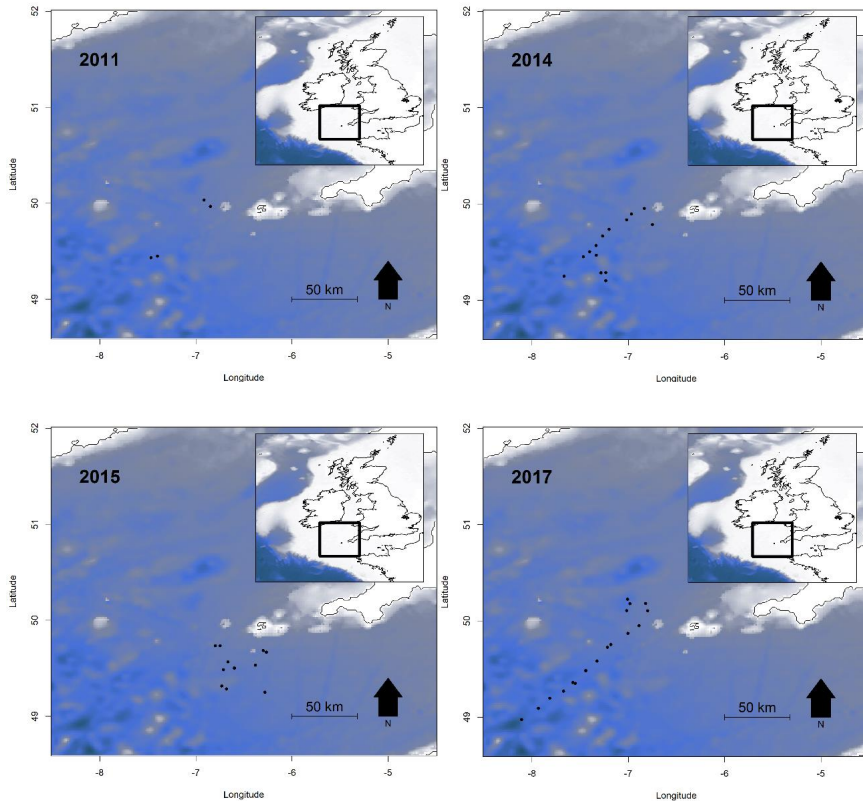
ii) Filtering of the SNP dataset.

Supplementary table 1: Summary of filtering steps for single nucleotide polymorphism (SNP) data obtained using DArTseq on blue skate *Dipturus batis*

Filtering step	No. loci removed	No. loci remaining
Raw data		17,621
Call rate <80%	3,329	14,292
Duplicate loci	1,352	12,940
Monomorphic loci	77	12,863
Minor Allele Frequency (MAF) < 0.05	5,800	7,063
Loci out of Hardy-Weinberg proportions in at least 2 populations	521	6,542
Loci in linkage disequilibrium	192	6,350

Supplementary table 2: Results of exact test for conformation of loci to Hardy-Weinberg proportions, based on Monte Carlo permutation of alleles (Guo and Thompson 1992) with 1000 replicates, implemented using the R package *pegas*. Number of loci out of Hardy-Weinberg are shown before and after Benjamini and Hochberg (1995) false-discovery rate (FDR) correction. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), the Faroe Bank (FB), and the Faroe Shelf (FS).

Sampling location	No. of tests involved	No. of loci out of HW	No. loci out of HW after FDR correction
CS	42,378	2170	1509
WCS	42,378	516	63
NS	42,378	128	3
RK	42,378	1323	610
FB	42,378	99	3
FS	42,378	197	6
All samples	7,063	2528	2092

No. of loci out of HWE in at least 1 sampling location: 1640
No. of loci out of HWE in at least 2 sampling locations: 521
No. of loci out of HWE in at least 3 sampling locations: 33
No. loci out of HWE in at least 4 sampling locations: 0

iii) The power of relationship inference based on 6,350 SNP loci, and decision making for log-likelihood thresholds in relationship tests. A Monte Carlo simulation approach was implemented using *CKMRsim* (Anderson, https://doi.org/10.5281/zenodo.820162), simulating 10,000 relationship pairs (parent-offspring PO, full-sibling FS, half-sibling HS, and unrelated U) based on observed allele frequencies in our genotype data from 503 *Dipturus batis* individuals.

Supplementary table 3: False positive rates (FPR) and associated log-likelihood (logl) thresholds in each of six pairwise relationship tests (involving parent-offspring PO, full-sibling FS, half-sibling HS, and unrelated U pairs) based on simulated data, at false-negative rates of 0.001.

| Test	FPR	logl threshold
PO/U	2.23×10^{-294}	667
FS/U	7.02×10^{-268}	606
HS/U	5.62×10^{-56}	118
PO/FS	3.94×10^{-286}	95
PO/HS	2.87×10^{-289}	192
FS/HS	4.53×10^{-255}	164
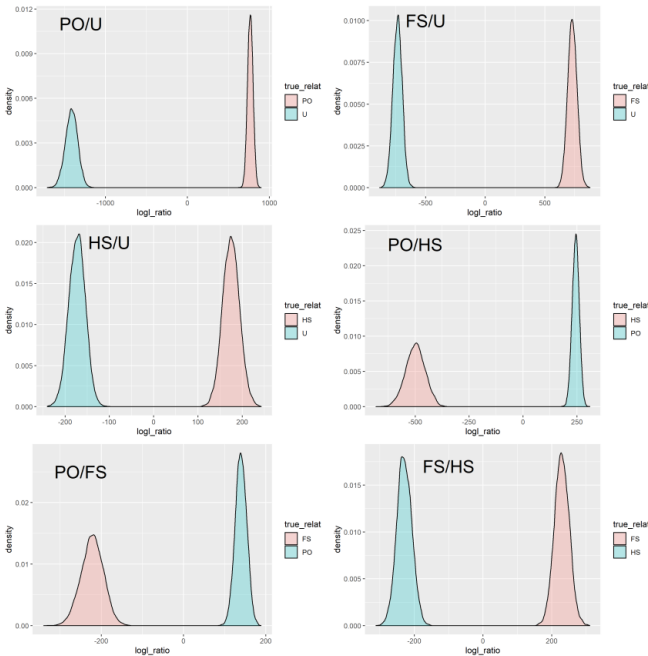
Supplementary figure 2: Density plots depicting the log-likelihood ratios of simulated relationship pairs in each pairwise test involving parent-offspring (PO), full-sibling (FS), half-sibling (HS), and unrelated (U) pairs. The distributions were used by CKMRsim to calculate false-positive and false-negative rates associated with each log-likelihood ratio.

iv) Exploratory analysis of population structure for 379 *D. batis* sampled in the Celtic Sea between 2011 and 2017. Analysis was performed using the Bayesian clustering algorithm implemented in STRUCTURE (Pritchard et al., 2000), and results interpretation using STRUCTURE Harvester (Earl & VonHoldt, 2002) and CLUMPAK (Kopelman et al., 2015). We used an admixture model with correlated allele frequencies, a burn-in length of 100,000 followed by 50,000 MCMC runs, and performed 3 iterations for each prior sub-population number K (ranging from K=1 to K=5).
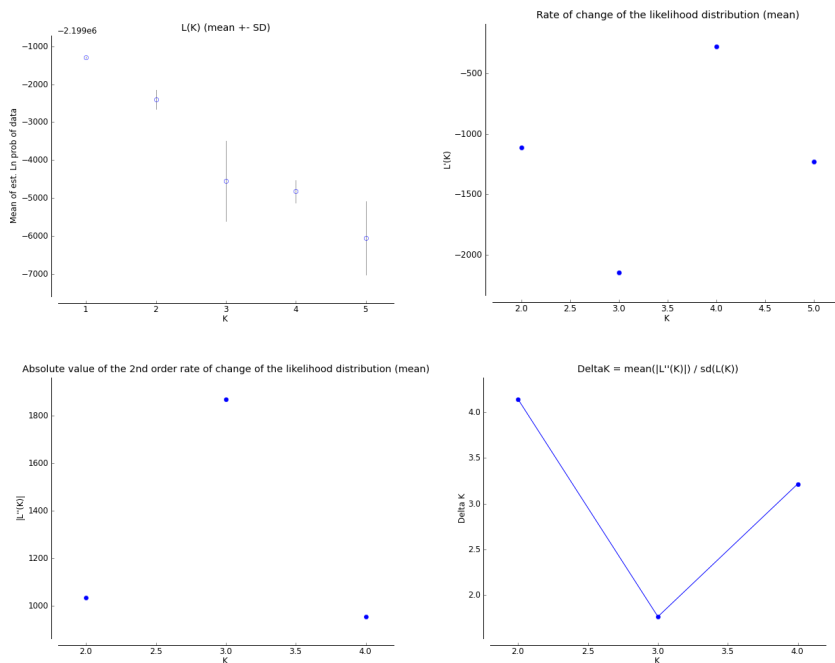
Supplementary figure 3: Interpreting the results of STRUCTURE runs on 379 Celtic Sea D. batis. The likelihood of the data and various transformations to calculate delta-K using the Evanno et al. (2005) method implemented in Structure Harvester (Earl and vonHoldt, 2012) are shown.

K=2
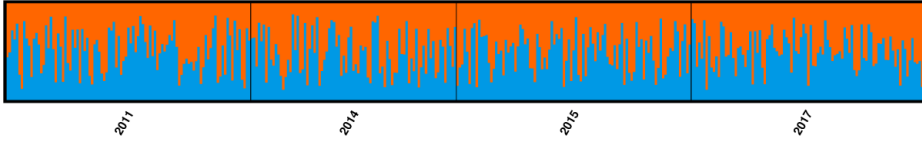
K=3
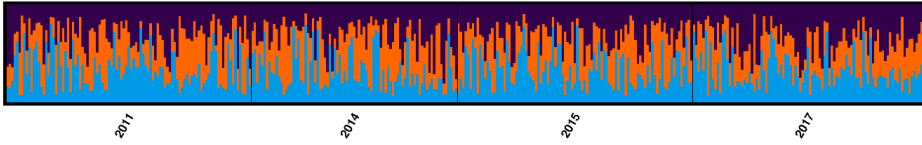
K=4

K=5

Supplementary figure 4: Summary of clustering for K=2 through K=5 generated in CLUMPAK (Kopelman et al. 2015), for 379 D. batis samples collected from the Celtic Sea in 2011, 2014, 2015 and 2017.

v) Final analysis of population structure for 57 *D. batis* from the study's entire geographic range. Analysis was performed using the Bayesian clustering algorithm implemented in STRUCTURE (Pritchard et al., 2000), and results interpretation using STRUCTURE Harvester (Earl & VonHoldt, 2002) and CLUMPAK (Kopelman et al., 2015). We used an admixture model with correlated allele frequencies, a burn-in length of 300,000 followed by 500,000 MCMC runs, and performed 5 iterations for each prior sub-population number K (ranging from K=1 to K=6).


Supplementary figure 5: The likelihood of the data and various transformations to calculate delta-K using the Evanno et al. (2005) method implemented in Structure Harvester (Earl and vonHoldt, 2012).
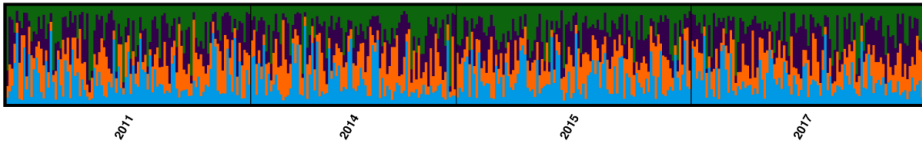
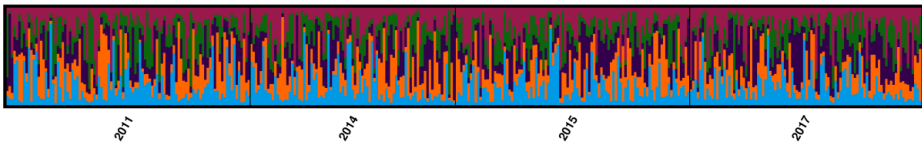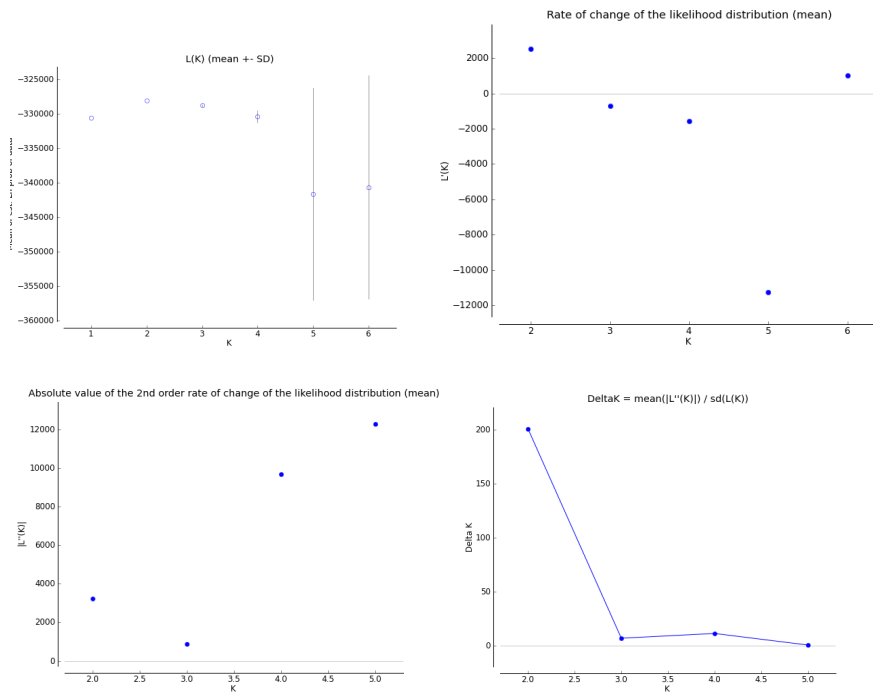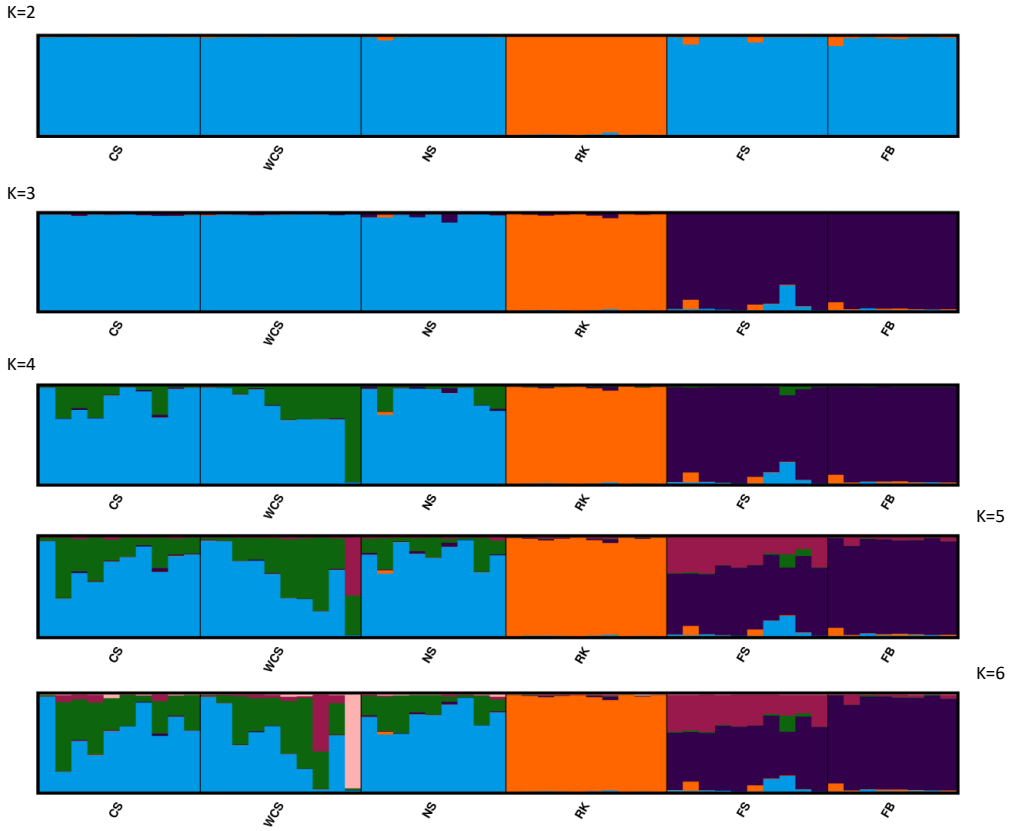Supplementary figure 6: Summary of clustering for K=2 through K=6 generated in CLUMPAK (Kopelman et al. 2015) for 57 *Dipturus batis* samples collected from the Celtic Sea (CS), West Coast Scotland (WCS), North Scotland (NS), Rockall (RK), the Faroe Shelf (FS), and the Faroe Bank (FB). Each individual is represented by a vertical line with the proportion of assignment to a cluster indicated by 2 to 6 colours.

vi) Analysis details, and further analyses, using discriminant analysis of principal components (DAPC).
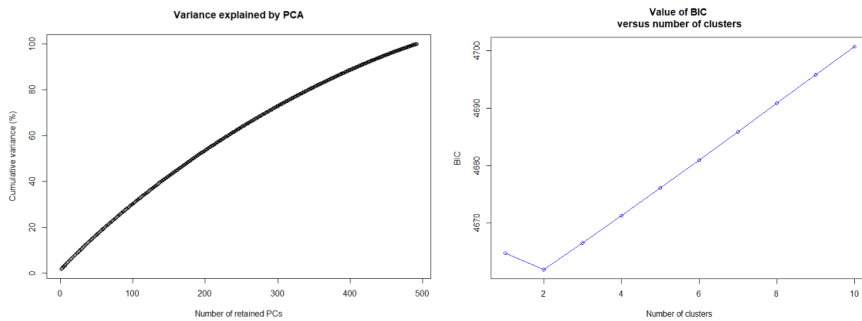
Supplementary figure 7: Analysis plots for a discriminant analysis of principal components (DAPC) on 6,350 SNPs from 493 individuals of Dipturus batis, using the find.clusters function implemented in the R package adegenet. The figures depict the cumulative variance (%) explained by 493 principal components, and the Bayesian Information Criterion (BIC) for each inferred number of clusters ranging from 1 to 10.
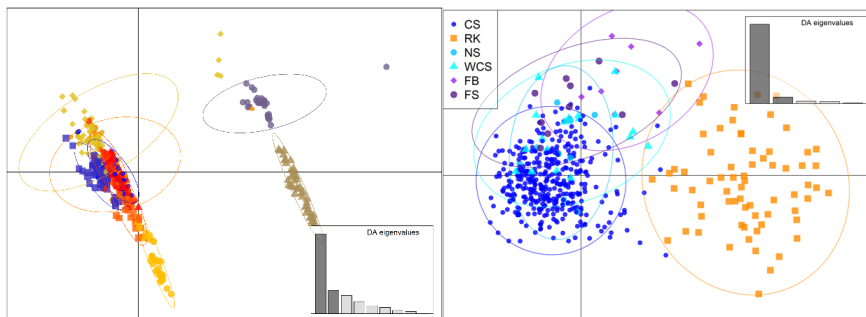
Supplementary figure 8: Discriminant analysis of principal components (DAPC) depicting the diversity between 493 blue skates in 10 inferred groups (left) and 6 groups defined by geographical location (right), using only 21 outlier loci. DAPC results were plotted using all 21 PCs (explaining 100% of variance), after retaining 9 and 5 discriminant functions of inferred and pre-defined groups respectively. 95% inertia ellipses are shown. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), the Faroe Bank (FB), and the Faroe Shelf (FS).
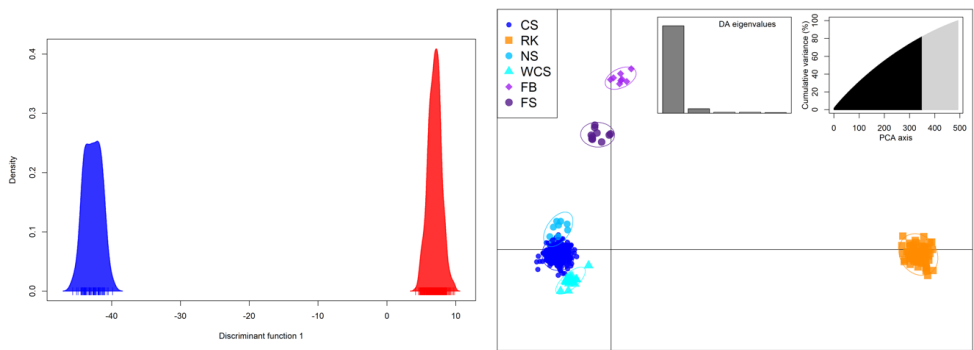
Supplementary figure 9: Discriminant analysis of principal components (DAPC) depicting the diversity between 493 blue skates in 2 inferred groups (left) and 6 groups defined by geographical location (right), using only 6,329 neutral loci, after removal of 21 putative outlier loci. The plots were produced after retaining 350 PCs (82% of variance) and 1 and 5 discriminant functions for inferred and pre-defined groupings respectively. 95% inertia ellipses are shown. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), the Faroe Bank (FB), and the Faroe Shelf (FS).

vii) Results of principal component analysis (PCA) of 503 individuals of *Dipturus batis* genotyped across a subset of 3,540 SNPs, using the R function *prcomp*.
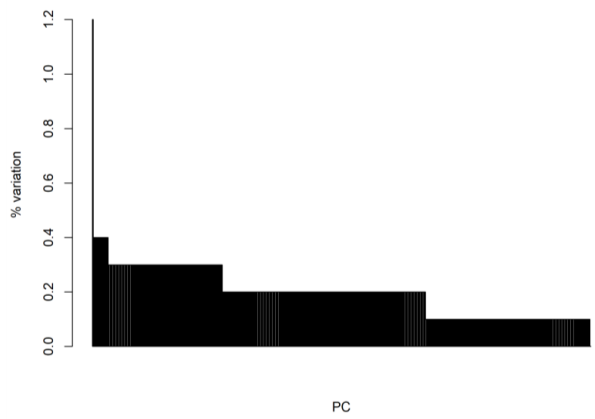
Supplementary figure 10: Percentage of variation explained by each of 503 principal components
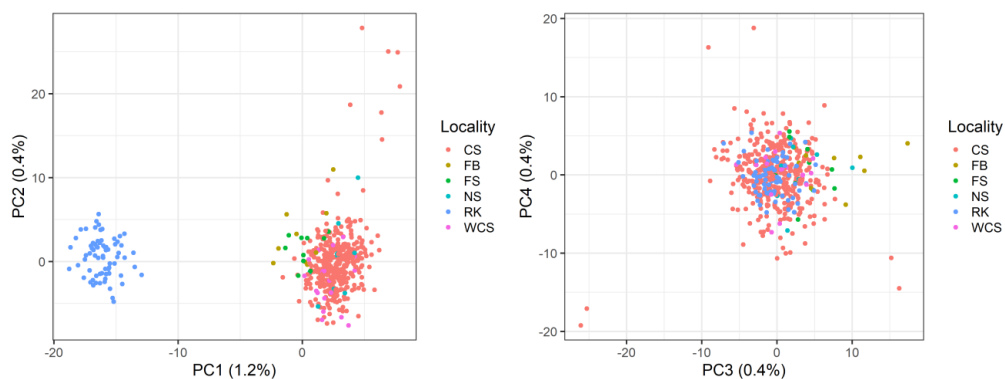
Supplementary figure 11: Principal component analysis of 503 D. batis from 6 sampling locations across 3,540 SNP loci, showing variation across the first 4 principal components. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), the Faroe Bank (FB), and the Faroe Shelf (FS).

viii) Estimates of effective population size (N_e) for *Dipturus batis* at individual sampling sites

Supplementary table 4: N_e for *D. batis* at 6 sampling locations, estimated using the linkage-disequilibrium method in NeEstimator. Estimates are shown for three critical values (Crit = 0.05, 0.02, and 0.01), and 95% confidence intervals by Jackknifing over individuals are shown in parentheses. Sample sizes (N) are also shown. Site names are abbreviated for the Celtic Sea (CS), West Coast Scotland (WCS), Northern Scotland (NS), Rockall (RK), the Faroe Bank (FB), and the Faroe Shelf (FS).

Sample site	N	Crit=0.05	Crit=0.02	Crit=0.01
CS	379	20,887 (17,312-26,307)	21,011 (17,550-26,161)	21,001 (17,551-26,127)
RK	69	11,299 (3,903-∞)	14,475 (3,810-∞)	18,983 (3,943-∞)
NS	9	∞	∞	∞
WCS	18	5,526 (328-∞)	∞ (330-∞)	∞ (330-∞)
FB	8	∞	∞	∞
FS	10	∞	∞	∞
All	493	1,137 (880-1,572)	1,146 (894-1,566)	1,146 (894-1,566)

ix) Environmental characterisation and seascape genomics analysis
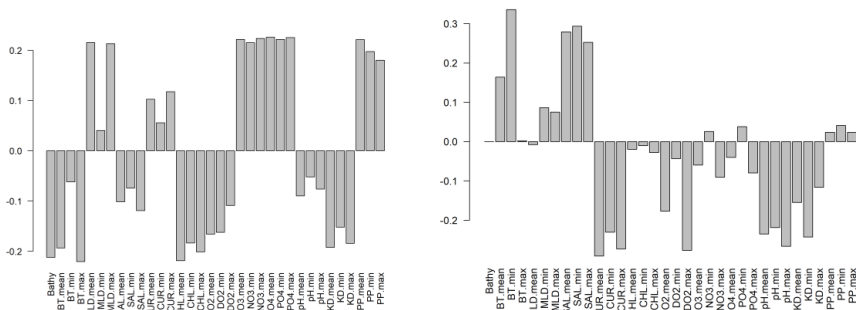
Supplementary figure 12: PCA rotation values, depicting the relative contribution of each environmental variable to PC1 (left) and PC2 (right). Variable names are abbreviated for bottom depth (bathy), bottom temperature (BT), mixed layer depth (MLD), salinity (SAL), current velocity (CUR), chlorophyll concentration (CHL), dissolved oxygen concentration (DO2), nitrate concentration (NO3), phosphate concentration (PO4), pH, light attenuation (KD), and primary productivity (PP).
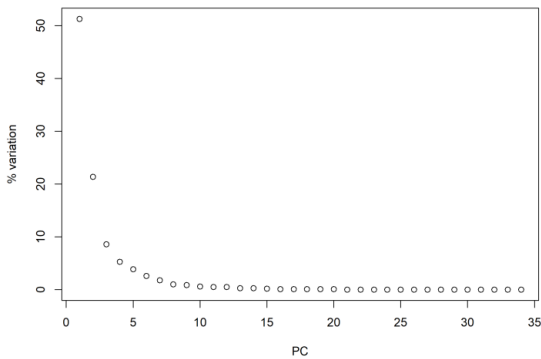
Supplementary figure 13: Percentage variation among sites per principal component in a PCA with 34 environmental variables.

Supplementary table 5: The number of significant allele-environment tests before and after Bonferroni correction for P-values derived from G and Wald scores.

Criteria	Gscore	Waldscore
All tests	457,200	457,200
P<0.05	28,143	25,820
P<0.01	6,683	5,757
Bonferroni P<0.05	8	7
Bonferroni P<0.01	8	7

x) Outlier loci
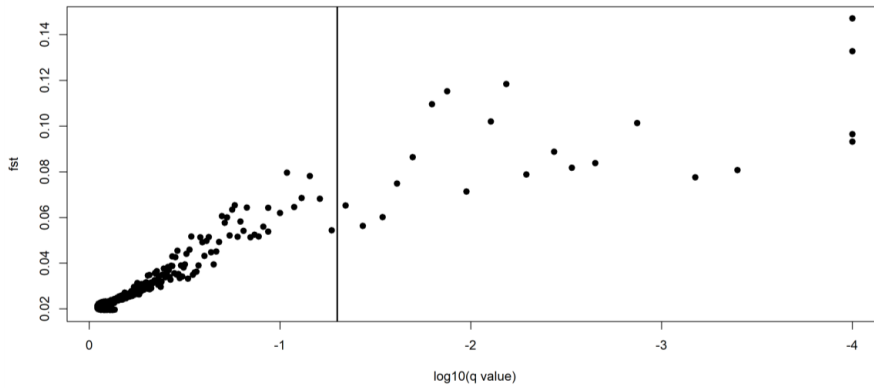
Supplementary figure 14: Detection of outlier loci in Bayescan, using a dataset of 493 Dipturus batis from 6 sampling locations, genotyped at 6,350 loci. Twenty-one loci under putative positive selection are shown to the right of the FDR threshold of 0.05 (vertical line).

Paper III

Manuscript style adapted to the journal guidelines of

Evolutionary Applications

Can genomic data be used to estimate abundance and identify important habitats for a critically endangered benthic elasmobranch?

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Abstract

Estimating and monitoring population demographic parameters is a crucial component of elasmobranch conservation programmes. For benthic elasmobranchs such as skates, traditional fisheries-independent approaches are often unsuitable as the data may be subject to various sources of bias, while mark-recapture programmes can suffer from low recapture rates. Close-kin mark-recapture (CKMR), a novel demographic modelling approach based on the identification of close relatives within a sample that does not require physical recaptures, represents a promising alternative approach to monitor a critically endangered data-deficient elasmobranch population. We evaluated the suitability of CKMR as a monitoring tool for the Celtic Sea population of blue skate (*Dipturus batis*) using samples collected during fisheries-dependent trammel-net surveys that ran from 2011 to 2017. We identified 19 sibling pairs among 683 skates, which were genotyped across 6,291 genome-wide single nucleotide polymorphisms (SNPs), 16 of which were cross-cohort half sibling pairs that could be implemented in a CKMR model. Despite limitations owing to a lack of validated life-history trait parameters for the species, we produced the first estimates of adult breeding abundance ($N^A_{t=1996} \approx 19{,}000$; 95% CI ≈ 3,000 to 115,000), population growth rate (λ = 0.01; 95% CI ≈ -0.15 to 0.17), and annual adult survival rates (φ^A = 0.78; 95% CI ≈ 0.60 to 0.96) for *D. batis* in the Celtic Sea. The results were compared to estimates of genetic diversity, effective population size (N_e), and to catch per unit effort (CPUE) estimates from the trammel-net survey. Recommendations for the implementation of CKMR as a conservation tool for benthic elasmobranchs are discussed. In addition, the spatio-temporal distribution of the 19 sibling pairs revealed strong site-attached behaviour in *D. batis*, and supported field observations suggesting that an area of critical habitat that could qualify for protection might occur near the Isles of Scilly.

Introduction

Estimating population census size (N_c) and other demographic parameters such as population growth and mortality rates is a fundamental problem in conservation biology and fisheries management. The status of marine fish populations is often assessed by estimating their relative abundance based on catch per unit effort (CPUE), obtained from commercial landings data and/or fisheries-independent surveys. However, CPUE estimates can be prone to bias due to misreporting of catches and variations in catchability of the animals with different sampling gears (Maunder & Piner, 2015). Data limitations are particularly pertinent to elasmobranchs, for which assessments are often hindered by taxonomic confusion (Iglésias et al., 2010) and insufficient knowledge of their biology (IUCN 2015; ICES 2020). As a result, many elasmobranchs have suffered population declines and local extinctions, largely driven by fishing pressure, which has been exacerbated by their typically K-selected life-history traits (Dulvy et al., 2017) such as slow growth and late-onset maturity.

Alternatively, abundance can be estimated using mark-recapture approaches (Cormack, 1964; Jolly, 1965; Seber, 1965), with the added benefit that they can reveal patterns of animal movement and habitat use. Although mark-recapture studies have been a popular approach to study several elasmobranch species (Neat et al., 2015; Biais et al., 2017; Corrigan et al., 2018), applications to benthic elasmobranchs such as batoids have generally suffered from low animal recapture and tag recovery rates (Bendall et al., 2018; Bird et al., 2020). For elasmobranch populations facing methodological challenges such as these, novel approaches are urgently needed to assess population status and inform marine conservation decisions.

The rapid advances in molecular approaches in the last few decades have provided novel means of assessing population trends. Close-kin mark-recapture (CKMR) has recently emerged as a method of estimating demographic parameters such as absolute abundance, population growth rates, and survival rates (Bravington et al., 2016b). CKMR builds on mark-recapture approaches by making use of genetic data obtained from small biopsies (e.g. muscle or fin-clips) to identify closely related individuals within a population; here, genotypes can be considered as 'tags' and individuals' relatives in a sample can be considered as 'recaptures' based on the principles of Mendelian inheritance. The principles of mark-recapture are conserved in CKMR, in that a higher proportion of recaptures reflects a smaller population size. Since the method requires only a single capture of individuals, circumventing the need for recaptures as well as the additional stress this would inflict on the animals, it represents a promising approach to monitor a critically endangered elasmobranch population.

The idea behind CKMR is by no means new. Skaug (2001) and Nielsen et al. (2001) initially proposed that individual genotypes can function as genetic tags from which abundance can be estimated. However, genotyping and identifying close-kin among large populations has only become feasible following recent advances in sequencing technologies, such as next generation sequencing (NGS), which have enabled the genotyping of large numbers of samples across the large number of loci required to accurately identify close-kin. Thus far, CKMR has been successfully applied to populations of a handful of species, including southern bluefin tuna *Thunnus maccoyii* (Bravington et al., 2016a), brook trout *Salvelinus fontinalis* (Ruzzante et al., 2019), Atlantic salmon *Salmo salar* (Wacker et al., 2021), white shark *Carcharodon carcharias* (Hillary et al., 2018), and grey nurse shark *Carcharias taurus* (Bradford et al., 2018).

In addition to facilitating a novel demographic modelling approach, the identification of close-kin can also provide insights on elasmobranch behaviour and habitat use, which can reveal areas that may qualify for additional protective measures. For example, the identification of cross-cohort siblings of speartooth sharks *Glyphis glyphis* across three Australian river systems revealed patterns of adult movement and breeding behaviour (Feutry et al., 2017), while the high degree of genetic relatedness within aggregations of basking sharks *Cetorhinus maximus* in the North-East Atlantic revealed kin-associated behaviour and identified potentially important migratory corridors for this species (Lieber et al., 2020).

The blue skate (*Dipturus batis*) is a large-bodied rajid with a patchy distribution across the North-East Atlantic (Frost et al., 2020). *D. batis* was only recently differentiated from its larger congener, the flapper skate (*D. intermedius*), following recent morphological and genetic investigations (Griffiths et al., 2010; Iglésias et al., 2010). Much of the presumed knowledge on both species is being re-assessed and still lacks detail, and as a result management will continue on the basis of a single common skate-complex (*D. batis*-complex) until species-specific assessments improve (ICES, 2020). At present, both species are classed as Critically Endangered by the IUCN (Dulvy et al., 2006). Current management implies a total landing ban on common skate in EU waters since 2009, though post-ban landings and bycatch are still reported (Simpson & Sims, 2016; ICES, 2020).

Anecdotal reports have indicated high rates of common skate bycatch in the Celtic Sea, representing an important conservation and socio-economic concern in the context of a multi-species fishery. Initial surveys identified that common skate bycatch in the Celtic Sea is dominated by *D. batis* (Bendall et al., 2018). In an effort to evaluate current management regimes, longitudinal fisheries-dependent surveys have been initiated in collaboration with British (Bendall et al., 2018) and French (Barreau et al., 2016) fishing fleets in order to

develop demographic models and identify biologically important sites for the species. Initial results from these surveys suggest the occurrence of juveniles and adults in the area, displaying a high degree of local residency, while a population genomic study has identified the occurrence of siblings in the area (Delaval et al. *in review*). Altogether, these findings suggest that the Celtic Sea may host important reproductive and/or nursery sites for *D. batis*.

The Celtic Sea *D. batis* population represents a unique opportunity to test the suitability of CKMR as a monitoring tool for a data-deficient benthic elasmobranch; the apparently resident nature of *D. batis* in the Celtic Sea, the occurrence of close-kin, and the longitudinal samples available from standardized surveys make it an ideal candidate for CKMR. In this study, we applied a genome-wide genotyping approach (DArTseq^{TM}, Kilian et al., 2012) on samples collected during Centre for Environment, Fisheries and Aquaculture Science (CEFAS) fishery-dependent surveys in the Celtic Sea between 2011 and 2017 (see Bendall et al. (2018)) to identify half-siblings and generate the first absolute estimates of the number of breeding adults (N_A), adult population growth rates, and adult survival rates for the population using CKMR. To evaluate the suitability of CKMR as applied to this population, these estimates were compared to molecular estimates of N_e (the evolutionary analogue of N_A, see Waples et al. (2018)) and genetic diversity, and to relative abundance estimates (CPUE) obtained from the survey. In addition, the spatio-temporal distribution of close-kin would allow us to evaluate skate movements and identify potential areas of biological interest that may qualify for additional protection.

Methods

a) Sample collection and genotyping

Fishery-dependent common skate surveys were performed in the Celtic Sea by CEFAS in collaboration with the fishing industry in 2011, and from 2014-2017. The surveys, which ran in early autumn (August-October), sampled using fixed trammel nets along a transect of stations running 12-80 NM to the south and west of Newlyn, Cornwall, UK (Figure 1). Additional exploratory stations outside the transect area were also surveyed to assess the extent of *D. batis'* distribution. Further details on the sampling protocol are described in Bendall et al. (2018). Across survey years, biopsies (fin or muscle clips) were taken from 1,140 individuals and stored in 96% ethanol or RNAlater®. Skates were also sexed and measured (total length, cm). A pilot study was performed to determine the power of relationship inference using DArTseq^{TM} genotyping, and the probability of obtaining parent-offspring or half-sibling pairs among the samples (Appendix 1). The results revealed that parent-offspring pairs were unlikely to be found, as samples were mostly comprised of

young adults, whereas half-sibling pairs could be identified with high levels of precision. Based on these results, we opted for a half-sibling pair (HSP) CKMR approach. Samples selected for genotyping were primarily juveniles and young adults, but spanned as wide a size range as possible to maximise the number of cohorts included in the model.
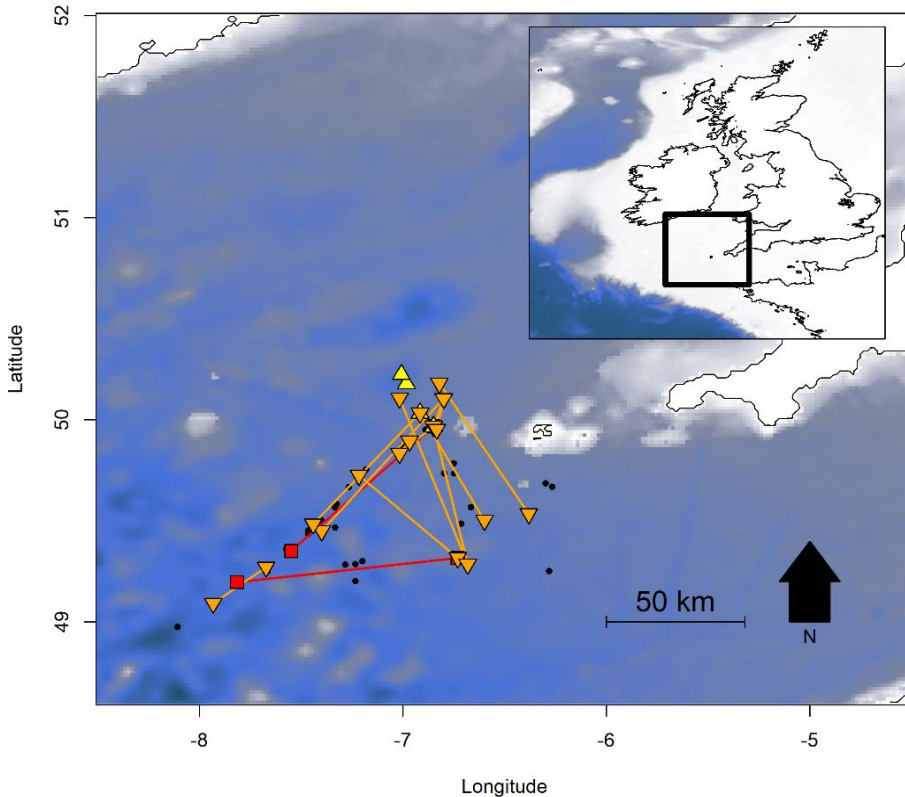
Figure 1: Sampling locations of all *Dipturus batis* in this study (black points), full siblings (red squares), and half-sibling pairs (orange and yellow triangles). Straight lines are drawn between full sibling (red) and half-sibling (orange) pair capture locations. Half-siblings captured in the same haul are indicated by yellow triangles. Latitude and longitude are in decimal degrees.

Of the available samples, 683 were selected for genotyping. Genomic DNA was extracted using a DNeasy® Blood & Tissue kit (Qiagen), quantified on a Qubit fluorometer (ThermoFisher Scientific) and adjusted to 10-60 ng/µL prior to sequencing. To assess the suitability of the DNA for restriction enzyme digestion, we performed a mock sample digest in CutSmart® Buffer (New England Biolabs) for 2 hours at 37°C, and resolved all samples on 0.8% TAE electrophoresis gels. In addition, 33 samples were visualised on a Genomic DNA

ScreenTape® for more detailed visualization of DNA quality. DNA was then sent to Diversity Arrays Technology (DArT) Pty. Ltd. for genotyping using DArTseq[TM] technology.

Genotyping was performed following standard protocols as described in Kilian et al. (2012). DArTseq[TM] combines complexity reduction methods and next generation sequencing platforms, and is optimised for each organism. Based on tests of several enzyme combinations for complexity reduction, DArT Pty. Ltd. applied the restriction enzyme combination PstI and SphI on the samples, which were sequenced (single read) on an Illumina® HiSeq® 2500, generating approximately 1.5 million sequences per individual. Sequences were processed using proprietary DArT Pty. Ltd. analytical pipelines, generating data for 25,131 sequences of 69 bp length, each containing a single nucleotide polymorphism (SNP).

b) SNP filtering

SNPs were filtered based on a call rate of 95%, and when duplicate loci were present, only the locus with the highest call rate was retained. After this step, the proportion of scored loci per sample was assessed, and all samples were deemed to have a sufficiently high score rate to be retained (≥90 %). Monomorphic loci and those with low minor allele frequencies (MAF < 0.05) were identified using *adegenet* (v 2.1.2, Jombart, 2008; Jombart & Ahmed, 2011), as implemented in R (v 3.6.2, R Core Team, 2019), and subsequently removed. Next, we tested for conformation of loci to Hardy-Weinberg proportions using the R package *pegas* (v 0.12, Paradis, 2010), performing an exact test based on Monte Carlo permutation of alleles (Guo & Thompson, 1992) with 1000 replicates. After applying the false discovery rate (FDR) correction method of Benjamini and Hochberg (1995), loci were removed if they deviated significantly from Hardy-Weinberg equilibrium (at a significance threshold of α=0.05). We then tested for linkage disequilibrium among loci using the R package *snpStats* (v 1.36.0, Clayton, 2020) and removed one locus from each pair of loci for which $R^2 > 0.80$. Because human error could lead to sampling an individual multiple times or to contamination during molecular laboratory work, we looked for duplicate samples based on a threshold of 629 mismatching loci (roughly 10% of remaining loci) using the R package *CKMRsim* (Anderson, https://doi.org/10.5281/zenodo.820162). Where duplicates were found (i.e. >90% genetically identical), only the sample with the highest score rate was retained. Following these filtering steps, summarised in Table 2, the resulting dataset contained 662 individuals genotyped at 6,291 loci (Table 1, Figure 1).

Table 1: Final panel of 662 *Dipturus batis* from the Celtic Sea incorporated into a close-kin mark-recapture model

Year sampled	Sample size (male, female)	Mean length in cm (range)	Mean estimated age (range)
2011	158 (69, 89)	122 (75, 148)	9 (3, 27)
2014	155 (84, 71)	121 (75, 147)	9 (3, 23)
2015	204 (109, 95)	115 (66, 146)	8 (2, 21)
2017	145 (66, 79)	113 (69, 142)	8 (2, 16)
Total	662 (328, 334)	118 (66, 148)	8 (2, 27)

Table 2: Summary of SNP filtering steps

Filtering step	# Loci removed	# Loci remaining
Raw data		25,131
Call rate < 95%	7,857	17,274
Duplicate loci	1,081	16,193
Monomorphic loci	3,346	12,847
Minor allele frequency (MAF) < 0.05	2,744	10,103
Loci out of Hardy-Weinberg proportions.	3,674	6,429
Loci in linkage disequilibrium	138	6,291

c) Identification of kin-pairs

Related individuals were identified using *CKMRsim*, which simulates related pairs of individuals based on observed allele frequencies using a Monte Carlo approach. *CKMRsim* calculates the false positive and false negative rates at different log-likelihood thresholds for pairwise hypothesis tests involving different relationship categories (e.g. parent-offspring PO, full-sibling FS, half-sibling HS, first-cousin FC, and unrelated U). Due to the large number of pairwise comparisons in relationship testing (662 samples imply 218,791 pairwise tests), this approach enables the user to identify an appropriate log-likelihood threshold when performing the relationship tests. Following *CKMRsim* recommendations, we aimed for a false-positive rate threshold of 100-times smaller than the reciprocal of the number of comparisons made (i.e. FPR < 4.57×10^{-8}). For the sake of comparison with other relatedness-finding methods, we also searched for related pairs using *ML-relate* (Kalinowski et al., 2006) and calculated pairwise relatedness (r) using the Wang estimator in the R package *related* (v. 1, Pew et al., 2015).

d) Close-kin mark-recapture

Close-kin mark-recapture (CKMR) builds upon traditional mark-recapture (MR) abundance estimation by integrating information on the relatedness of individuals. In short, CKMR consists of building a population dynamics model by comparing all pairs of individuals in a sample and determining the prior probability that they are related (in this case as half-siblings) given their life-history parameters. These parameters, if known for the species, can include sex-specific growth rates, age at maturity, and fecundity-at-age. This component of the model is flexible and can be adapted to the species in question by the addition or removal of life-history parameters (Bravington et al., 2016b). Models for data-deficient species are by necessity simplified, requiring a number of assumptions to be made. After accounting for the observed related pairs inferred from the genetic data, the population dynamics model then feeds into a demographic model from which initial population size and population growth rate can be estimated (e.g. by maximum likelihood or Bayesian modelling). In a half-sibling pair (HSP) model, adult survival rates can also be estimated using information on time interval between siblings' birth years.

For the HSP approach, the population dynamics model requires at least knowledge of the age of the individuals at the time of sampling. When sampling non-lethally in the field, this implies estimating an individual's age based on its size. The data-deficient nature of *D. batis* has resulted in a lack of understanding of its life-history traits. Through a mark-recapture experiment, Barreau et al. (2016) were able to use a sclerochronological approach using vertebral growth readings to propose an age-at-length model for *D. batis*, using the Von Bertalanffy (1938) growth equation (Equation 1) which is commonly used to model fish size at age. We estimated the age (in years t) of each individual based on their length (in cm L) using this equation, rounding down to the year.

$$Equation\ 1\ (\text{Barreau et al. 2016}): L_t = 149\left[1 - e^{-0.18(t+0.49)}\right]$$

We recognize that the parameters of this equation still require validation from further recaptures (Barreau et al., 2016), however they offer the best method currently available to estimate age-at-length for *D. batis*. Owing to a lack of further available life-history data, we opted for a sex- and age- aggregated population dynamics model (Equation 2). That is, we assumed equal growth rates for both males and females, and constant adult survival rates over time. This approach is similar to that applied to other elasmobranchs to date (Bradford et al., 2018; Hillary et al., 2018), which also lacked precise life-history parameters. Furthermore, for simplicity in this first iteration of CKMR for *D. batis*, we assumed a closed population in the Celtic Sea with equal probability of capture across space and time. Indeed, the closed population assumption may be valid, given that migration in and out of the Celtic Sea is apparently limited based on genetic (Delaval et al. *in review*) and tagging (Bendall et

9

al., 2018) data. Regarding probability of capture, the distribution of *D. batis* in the Celtic Sea is characterized by biological hotspots and variable CPUEs across space (Bendall et al., 2018), however we selected samples randomly across survey stations and years to minimize any potential bias.

In our half-sibling population dynamics model (Equation 2), the probability that the relationship *K* between any two individuals *i* and *j* is half-sibship (HSP), given their respective birth years (*b*) and that *j* was born after *i*, is dependent on the number of potential parents (i.e. number of breeding adults N^A) in the year of *j*'s birth and on the adult survival rate (φ) between *i* and *j*'s years of birth.

$$\textit{Equation 2 (adapted from Hillary et al. 2018)}: P\left[K_{ij} = HSP | b_i, b_j; b_j > b_i\right] = \frac{4}{N^A_{b_j}} \times (\varphi^A)^{(b_j - b_i)}$$

In the demographic model (Equation 3), the number of adults in any given year *t* (N^A_t) is dependent on the number of adults in the initial model year (*t=0*, i.e. the birth year of the first cohort in our sample set) and the population rate of growth (λ).

$$\textit{Equation 3}: N^A_t = N^A_{t=0} e^{\lambda t}$$

Our aim was to estimate initial adult breeding abundance ($N^A_{t=0}$), population growth rate (λ), and adult survival rates (φ^A), and to model adult population sizes (N^A_t) over time. We used two approaches to estimate these three parameters. First, we used a maximum-likelihood approach, implementing the *optim* function in R to maximise the log-likelihood of the model across the parameter space of the three variables and estimate 95% confidence intervals. We also used a Bayesian approach, allowing us to set biologically meaningful prior probability distributions for each parameter and to visualize the credible intervals *a-posteriori*. For the Bayesian approach, we implemented a Metropolis-Hastings Markov-Chain Monte Carlo (MCMC) model in R, whereby the log-posterior probability was calculated as follows:

$$\textit{Equation 4}: lnP\left(N^A_{y=0}, \lambda, \varphi | K_{ij} = HSP, Binom\right)$$
$$= lnP\left(K_{ij} = HSP | N^A_{y=0}, \lambda, \varphi, Binom\right) + lnP\left(N^A_{y=0} | \mu, \sigma, N\right) + lnP(\lambda | -1, 1, U)$$
$$+ lnP(\varphi | 0, 1, U)$$

i.e. the log-posterior joint probability of the three estimates equal the sum of the log-likelihoods that each pair of individuals is a HSP under a binomial distribution (true or false for sibship), and the log-prior probabilities of population size (assuming a normal probability density function PDF), population growth rate (assuming a uniform PDF between -1 and 1), and adult survival rate (assuming a uniform PDF between 0 and 1). We experimented with modelling parameters using short runs of 100,000 iterations, evaluating the performance of

the models in Tracer (v 1.7.1, Rambaut et al., 2018) by assessing the distribution of parameter estimates 'sampled' by the Markov chain, and assessing the effective sample size (ESS) for each parameter. The final MCMC was run for 1 million iterations, sampling every 100th iteration, and discarding the first 100,000 iterations as burn-in. New variables for $N_{y=0}^A$, λ, and φ were proposed using a sliding-window approach at each iteration, and accepted based on the posterior acceptance ratio approach. Details of the final MCMC parameters are provided in Appendix 3. We plotted 100 iterations at random for visualization purposes.

e) Genetic diversity and effective population size

Population- and locus-wide summary statistics were obtained using GenAlEx (v 6.5, Peakall & Smouse, 2006, 2012). We estimated effective population sizes (N_e) using the linkage-disequilibrium (LD) estimator (Hill, 1981; Waples, 2006; Waples & Do, 2010) in NeEstimator (v 2.1, Do et al., 2014). N_e is the evolutionary analog to census size (N_c) that reflects the degree of genetic drift and, thereby, the evolutionary potential of wild populations; conservation thresholds of N_e have generally been set to 500 or 5,000 individuals to mitigate the loss of genetic diversity and inbreeding depression, though these thresholds are debated (reviewed in Allendorf et al., 2013). The LD estimate assumed random mating and was performed at critical values (i.e. MAF at which alleles should be excluded) of 0.05, 0.02, and 0.01. Confidence intervals were obtained using the Jackknife-over-individuals method. N_e was calculated for the samples overall, and for each sampling year.

f) Catch per unit effort (CPUE)

In order to contextualize our estimates of population size and growth trends obtained from CKMR, we estimated relative abundance by calculating catch per unit effort (CPUE), a metric that is more familiar to fisheries management.

We calculated annual CPUE rates from *D. batis* captured during fishery-dependent common skate surveys performed in the Celtic Sea by CEFAS in collaboration with the fishing industry from 2014-2017, as in Bendall et al. (2018). All *D. batis* caught during the survey were counted and measured. Individual weights W_T (g) were estimated from the parameters given by Silva et al. (2013):

$$W_T = 0.0038 \times L_T{}^{3.1201}$$

Fishing effort was defined as kilometre-hours (km.h) of net soaked:

$$Unit\ Effort = Length\ of\ net\ (km) \times Soak\ time\ (hours)$$

CPUE was calculated for each station, as abundance (individuals.km^{-1}.h^{-1}) and biomass (kg.km^{-1}.h^{-1}), based on the number of individuals N_T and summed weights converted to kg respectively:

$$Abundance = \frac{N_T}{Length\ of\ net\ (km) \times Soak\ time\ (hours)}$$

$$Biomass = \frac{\Sigma W_T(kg)}{Length\ of\ net\ (km) \times Soak\ time\ (hours)}$$

For each year surveyed, mean abundance and biomass were calculated for (a) all stations fished, and (b) for prime stations that were fished each year (2014–2017). Where four stations were fished multiple times, the average was calculated for the four stations prior to averaging across the four stations.

As female immature and mature stages could not be recorded without internal examination of reproductive organs (which was not feasible during surveys), *D. batis* catch data by maturity stage were calculated using published estimates of the length at 50% maturity (L$_{50}$) (Iglésias et al., 2010).

Results

a) Identification of kin-pairs

A power analysis with *CKMRsim* suggested that the panel of 6,291 SNPs offered high precision in pairwise relationship testing, with false-positive rates (FPRs) ≤ 1.89x10^{-9}, well below our target (FPR < 4.57x10^{-8}) at corresponding false-negative rates (FNR) of 0.0001 (Appendix 2). Using *CKMRsim*, we identified two full-sibling pairs and 17 half-sibling pairs among the 662 genotyped individuals. When comparing the three kin-finding approaches, *CKMRsim, ML-relate,* and *related*, the results were largely consistent. However, *ML-relate* identified eight additional half-sibling pairs and categorized one of the existing half-sibling

pairs as full-siblings, and so was less conservative. On assessing the relatedness values (*r*) among these additional pairs of samples, we noticed the additional related individuals from *ML-relate* generally had lower *r* values than the rest (Appendix 2, Supplementary table 2). By including tests for first-cousins in *CKMRsim*, we identified 51 pairs of individuals that were likely to be distant relatives (e.g. third-order relatives such as cousins). As *ML-relate* does not test for third-order relatives, it may have falsely identified a number of third-order relatives as half-siblings. A conservative approach was adopted, whereby the two full-sibling and 17 half-sibling pairs identified using *CKMRsim* were retained for downstream analysis.

Of the 19 sibling-pairs identified, four had been captured together in the same haul. The other sibling pairs had been captured between two and 94 km apart (Figure 1, Table 3). Two individuals were each involved in multiple sibling-pairs. The majority of sibling-pairs involved at least one individual captured just west of the Isles of Scilly (Figure 1).

Table 3: Sampling and biological details of two full-sibling (FS) and 17 half-sibling (HS) pairs of *Dipturus batis* sampled in the Celtic Sea. Samples involved in multiple sibling pairs are in bold.

ID1	Year	Lat, Long (decimal)	Sex	Length (cm)	ID2	Year	Lat, Long (decimal)	Sex	Length (cm)	Kinship	Distance apart (km)
2015-16-240	2015	49.32 N 6.73 W	M	121	2017-C10-4-16	2017	49.20 N 7.82 W	F	122	FS	80
2017-C08-6-11	2017	49.35 N 7.55 W	F	142	2011-264	2011	49.97 N 6.85 W	M	132	FS	85
2017-C11-2-9	2017	49.09 N 7.93 W	F	94	2017-C09-3-6	2017	49.27 N 7.67 W	F	84	HS	27
2017-C14-21-7	2017	50.23 N 7.01 W	M	97	2017-C14-21-10	2017	50.23 N 7.01 W	M	115	HS	0
2015-15-144	2015	49.50 N 6.60 W	F	136	2011-44	2011	50.03 N 6.92 W	F	141	HS	63
2017-C02-15-10	2017	50.11 N 6.80 W	M	120	**2014-80**	2014	49.95 N 6.83 W	M	117	HS	17
2017-C13-20-4	2017	50.18 N 6.98 W	M	113	2017-C13-20-5	2017	50.18 N 6.98 W	M	129	HS	0
2015-16-242	2015	49.32 N 6.73 W	M	74	2017-C05-11-6	2017	49.73 N 7.22 W	F	78	HS	57
2011-268	2011	49.97 N 6.85 W	F	127	**2014-80**	2014	49.95 N 6.83 W	M	117	HS	2
2011-39	2011	50.03 N 6.92 W	M	107	2011-42	2011	50.03 N 6.92 W	M	123	HS	0
2011-302	2011	49.97 N 6.85 W	F	122	2014-20	2014	49.83 N 7.02 W	M	127	HS	19
2014-45	2014	49.89 N 6.97 W	M	125	2014-14	2014	49.83 N 7.02 W	F	123	HS	8
2017-C02-15-4	2017	50.11 N 6.80 W	F	134	2011-214	2011	49.45 N 7.40 W	M	121	HS	85
2015-18-253	2015	49.28 N 6.68 W	M	133	2011-279	2011	49.97 N 6.85 W	F	123	HS	77
2017-C02-15-6	2017	50.11 N 6.80 W	F	136	2011-274	2011	49.97 N 6.85 W	F	139	HS	16
2015-20-321	2015	49.53 N 6.38 W	M	119	2017-C01-22-3	2017	50.18 N 6.82 W	F	125	HS	78
2017-C07-7-6	2017	49.48 N 7.44 W	M	125	**2011-39**	2011	50.03 N 6.92 W	M	107	HS	72
2015-18-250	2015	49.28 N 6.68 W	F	105	2017-C13-19-2	2017	50.11 N 7.02 W	F	127	HS	94
2011-271	2011	49.97 N 6.85 W	F	132	2011-308	2011	49.97 N 6.85 W	M	116	HS	0

b) Close-kin mark-recapture

We identified 17 half-sibling pairs that could be used for the CKMR model, which was fewer than the 21 we expected based on a pilot study (Appendix 1). The age of the samples, estimated using the von Bertalanffy growth parameters proposed by Barreau et al. (2016), ranged from two to 27 years of age. The equation generated significant uncertainty when estimating the age of larger individuals. Whereas the majority of samples (N=656) were estimated at between two and 15 years of age, the six largest individuals (length 142-148 cm) were estimated at between 16 and 27 years of age. The oldest skate involved in a half-sibling pair was estimated at 15 years of age. Therefore, in order to minimize bias and imprecision in our CKMR model while retaining as much pairwise relatedness information as possible, we excluded the six individuals more than 15 years old from our analyses. One half-sibling pair involved two individuals from the same cohort (i.e. same estimated birth year). Because CKMR only makes use of cross-cohort comparisons, this half-sibling pair and other unrelated same-cohort pairs were excluded from the analysis. The final analysis involved 197,466 pairwise comparisons, down from the original 218,791, of which 16 were half-sibling pairs. The cohorts (and hence the modelled years) spanned 1996 to 2015.

The maximum-likelihood and Bayesian MCMC models both generated similar parameter estimates. As a result of finding relatively few half-sibling pairs (16 HSPs such that coefficient of variation CV=0.25), the estimates involved fairly large confidence intervals (in the case of maximum-likelihood) and credible intervals (in the case of MCMC, Table 4, Figure 2). Initial adult breeding abundance ($N_{t=1996}^A$) was estimated at approximately 19,000 individuals in the Celtic Sea (95% CI: 3000 to 115000). The high uncertainty in growth rate estimate precluded us from drawing conclusions, though the mean estimate suggested a slightly increasing population size at a rate of approximately 0.01 (95% CI: -0.15 to 0.17). Annual adult survival rate was estimated at approximately 0.78 (95% CI: 0.60 to 0.96).

Table 4: CKMR estimates of initial adult breeding abundance, growth rate, and survival rate, using Maximum-likelihood and Bayesian MCMC. Point estimates are shown, with 95% confidence intervals (for ML) or credible intervals (for MCMC) shown in parentheses.

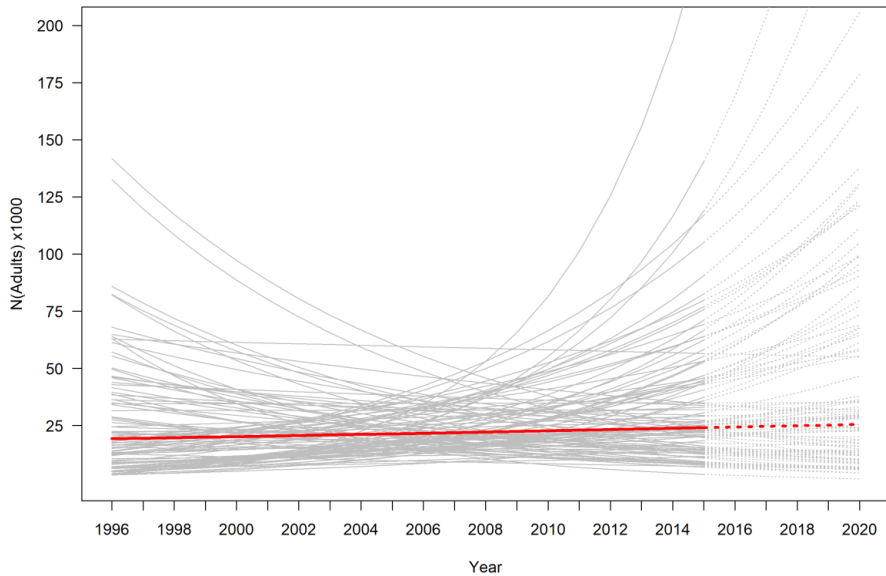| Parameter	Max. Likelihood	Bayesian MCMC
Initial adult abundance, $N_{y=0}^A$	18,613	19,220
	(2,978 − 116,316)	(3,115 − 114,909)
Growth rate, λ	0.009	0.012
	(-0.152 − 0.170)	(-0.145 − 0.175)
Adult survival rate, φ^A	0.782	0.778
	(0.596 − 0.968)	(0.604 − 0.959)

Figure 2: Mean (red line) modelled adult breeding abundance of *D. batis* in the Celtic Sea, and trend from 1996 to 2015, estimated using CKMR in a Bayesian MCMC framework. 100 random iterations from the model are plotted (grey lines), and the models are projected forward to 2020 (dotted lines).

Table 5: Mean genomic summary statistics for *Dipturus batis* across sampling years, and overall across years. Sample sizes (N), number of alleles (Na), observed (Ho) and expected (He) heterozygosity, fixation index (F) are shown, calculated in GenAlEx (v 6.5, Peakall & Smouse, 2006, 2012).

Sampling year		N	Na	Ho	He	F
2011	**Mean**	157.729	2.000	0.311	0.312	0.004
	SE	0.010	0.000	0.002	0.002	0.001
2014	**Mean**	154.016	2.000	0.301	0.311	0.031
	SE	0.031	0.000	0.002	0.002	0.001
2015	**Mean**	203.825	2.000	0.309	0.311	0.007
	SE	0.009	0.000	0.002	0.002	0.001
2017	**Mean**	144.852	2.000	0.315	0.313	-0.005
	SE	0.007	0.000	0.002	0.002	0.001
Overall	**Mean**	165.105	2.000	0.309	0.312	0.009
	SE	0.144	0.000	0.001	0.001	0.001

Table 6: Estimates of effective population size (N_e) of *Dipturus batis* in the Celtic Sea, for each sampling year and overall, using the linkage-disequilibrium method in NeEstimator (v 2.1, Do et al., 2014). Estimates are shown for three critical values (Crit = 0.05, 0.02, and 0.01), and 95% confidence intervals by Jackknifing over individuals are shown in parentheses. Sample sizes (N) are also shown. 'Inf' denotes infinity.

Sample year	N	Crit=0.05	Crit=0.02	Crit=0.01
2011	158	13,752 (7,930-51,118)	13,987 (8,057-52,351)	14,037 (8,074-53,073)
2014	155	19,663 (8,642-Inf)	19,604 (8,631-Inf)	19,650 (8,642-Inf)
2015	204	28,191 (19,981-47,793)	29,563 (21,442-47,545)	29,563 (21,442-47,545)
2017	145	8,489 (4,467-79,490)	8,811 (4,565-114,074)	8,811 (4,565-114,074)
All	662	14,832 (11,987-19,416)	14,823 (11,990-19,380)	14,823 (11,990-19,379)

c) Genetic diversity and effective population size

Genetic diversity was stable across all sampling years (2011-2017), with the population of *D. batis* maintaining relatively constant levels of observed heterozygosity of 0.31 across years (Table 5). Overall effective population size (N_e) was estimated at 14,832 individuals (95% CI: 11,987 – 19,416, Table 6). N_e estimates varied across sampling years, with an increase from approximately 14,000 individuals in 2011 to 28,000 individuals in 2015, before a drop to 8,500 in 2017. However, confidence intervals were much larger for yearly N_e estimates (Table 6).

d) CPUE

Across the 2014–2017 survey period, the mean CPUE by abundance (individuals.km^{-1} h^{-1}) and biomass (kg.km^{-1}.h^{-1}) for *D. batis* remained relatively stable. Mean CPUE ranged from 0.44–0.49 individuals.km^{-1}.h^{-1} and, in terms of biomass, from 3.96–5.66 kg.km^{-1}.h^{-1}, with notable standard deviations of the mean (Table 7; Figure 3).

Only four stations were fished in all four survey years (C03, C04, C07 and C09, see Bendall et al. 2018), and the mean CPUE at these sites ranged from 0.68–0.77 individuals.km^{-1}.h^{-1} and 6.09–9.27 kg.km^{-1}.h^{-1} (Table 8; Figure 3). The lowest recorded mean CPUE (biomass) was recorded in 2016, at 3.96 kg.km^{-1}.h^{-1} for all stations fished, and 6.09 for kg.km^{-1}.h^{-1} for the four stations that were fished each year.

Table 7: Annual catch rates of *D. batis* for all stations fished (see Bendall et al., 2018 for detailed survey & CPUE information)

Year	Number of stations fished with trammel nets	Mean (± SD) CPUE (abundance)	Mean (± SD) CPUE (biomass)
2014	19	0.49 (± 0.40)	5.66 (± 4.71)
2015	16	0.44 (± 0.31)	4.93 (± 3.43)
2016	15	0.47 (± 0.40)	3.96 ± (3.63)
2017	23	0.46 (± 0.34)	4.81 (± 4.30)

Table 8: Annual catch rates of *D. batis* at four survey stations sampled in all years (see Bendall et al., 2018 for detailed survey information)

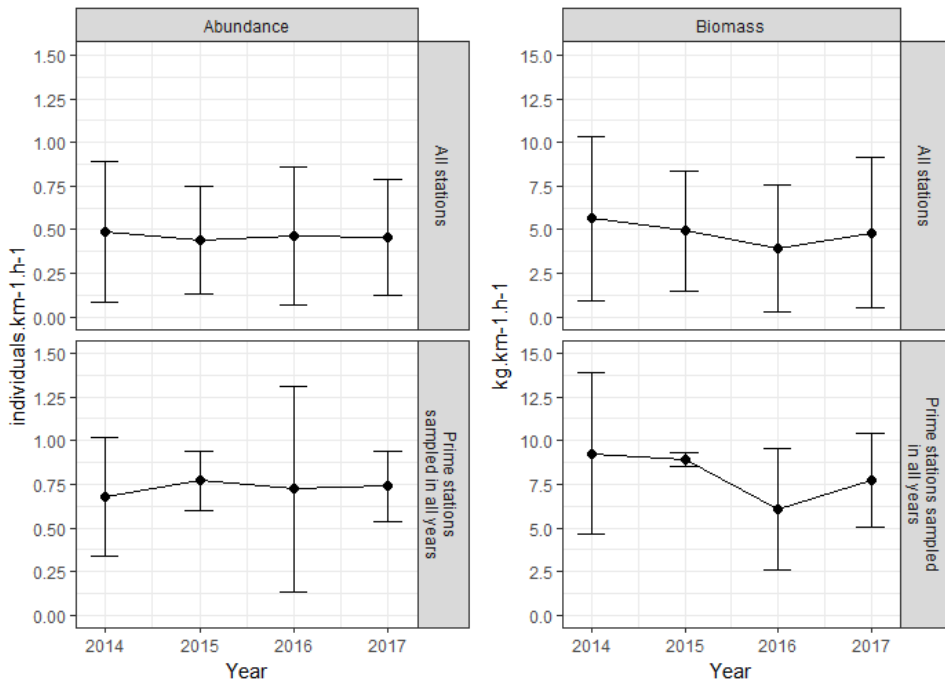| Year	Total number of trammel net deployments	Mean (± SD) CPUE (abundance)	Mean (± SD) CPUE (biomass)
2014	7	0.68 (± 0.34)	9.27 (± 4.62)
2015	4	0.77 (± 0.17)	8.90 (± 0.41)
2016	5	0.72 (± 0.59)	6.09 (± 3.48)
2017	7	0.74 ± (0.20)	7.72 (± 2.70)

Figure 3. Temporal changes in CPUE of *D. batis* (left panel: abundance; right panel: biomass) for all stations fished (top) and for four stations sampled each year (bottom) during fishery-dependent common skate surveys in the Celtic Sea by CEFAS in collaboration with fishing industry from 2014-2017.

Discussion

In this study, we evaluated the suitability of close-kin mark-recapture (CKMR) as a demographic modelling tool for a data-deficient and critically endangered benthic elasmobranch population, the Celtic Sea population of blue skate *Dipturus batis*. Using samples collected during fishery-dependent common skate surveys from 2011 to 2017, we implemented a half-sibling pair (HSP) CKMR model to generate the first estimates of adult breeding abundance (N_A), population growth rate, and annual survival rates for the population. In addition, the spatio-temporal distribution of sibling pairs supported evidence that *D. batis* are highly site-attached, and revealed a potential area of critical habitat near the Isles of Scilly. Despite limitations owing to the limited number of kin-pairs identified and the data-deficient nature of *D. batis*, CKMR represents a promising demographic modelling tool for the species, as demonstrated by comparing results from it with molecular estimates of effective population size (N_e), genetic diversity, and catch per unit effort (CPUE), the last a more familiar estimate in fisheries science. We discuss the limitations of these estimates,

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and evaluate the potential of CKMR as a conservation tool for this and other data-deficient elasmobranch populations.

The results support anecdotal information that *D. batis* is locally abundant in the Celtic Sea. CPUE estimates remained consistent both in terms of biomass and abundance, indicating a stable population in the short term from 2014 to 2017; estimates of genetic diversity also remained stable across sampling years. The CKMR results suggested that this level of relative abundance corresponds to an adult breeding population in the order of ~19,000 individuals. However, the CKMR estimates should be interpreted with caution since they were based on the identification of only 16 HSPs, which is less than the ~45 recommended by Bravington et al. (2016b) for fisheries stock assessment purposes; this resulted in large confidence intervals around our estimates. The only other known estimates of abundance for the population come from Barreau et al. (2016)'s estimates of CPUE, which were based on fishery-independent bottom trawl data. The authors reported a moderate increase in CPUE from 2009 to 2014. Altogether, the results from both molecular and CPUE approaches seem to suggest a stable, and possibly increasing, population of *D. batis* in the Celtic Sea following the 2009 landing ban, at least until 2017.

Estimates of N_e were in the order of ~15,000 individuals, which is above accepted conservation thresholds (e.g. of 500 or 5,000, see Allendorf et al. 2013) and suggests that the Celtic Sea population of *D. batis* is at a relatively low risk of inbreeding depression. We noted a decline in N_e in 2017, however our N_e estimates should be interpreted with caution: estimates were derived from a mixed-age group of individuals and therefore do not reflect N_e per generation, which could have been assessed by estimating N_e for each cohort should sample sizes have allowed it (e.g. as in Waples et al. 2018), and the high-throughput sequencing data from which they were derived may be subject to bias due to violations to the assumptions of unlinked loci (Waples et al., 2016).

The CKMR estimates suggested an annual survival rate of 78% (95% CI: 60-96%) for adult *D. batis* in the Celtic Sea. This is higher than the 64% estimated for the larger flapper skate (*D. intermedius*) based on the results of a mark-recapture programme on the west coast of Scotland (Neat et al., 2015), although this estimate included juveniles which likely experience higher natural mortality rates than adults on account of their smaller size. The higher mortality of *D. intermedius* might also reflect a greater susceptibility to fishing mortality for this species (Brander, 1981). In comparison with existing elasmobranch CKMR studies, the adult survival rate estimates for *D. batis* were lower than for adult white shark *Carcharodon carcharias* (Hillary et al., 2018) and grey nurse shark *Carcharias taurus* (Bradford et al., 2018), both estimated at over 90%. Such a high survival rate might be expected for large oceanic predators given their higher trophic position. It is worth noting

that mortality rates for *D. batis* discards (accidental catches) in commercial tangle-net and trawl fisheries have been estimated at 38.5% (Ellis et al., 2016) and 33.5% (Barreau et al., 2016), respectively, while skates in general experience mortality rates as high as 45% following capture in UK trawl fisheries (Enever et al., 2009). The potential rates of mortality that can be inflicted on skate populations by commercial fisheries may therefore be consequential, supporting the need for rigorous monitoring and implementation of targeted conservation actions.

There is growing evidence of philopatric, site-attached (e.g. Neat et al., 2015; Feutry et al., 2017), and aggregating behaviour (Thorburn et al., 2018; Lieber et al., 2020) exhibited by elasmobranchs, which may relate to preferential feeding, and reproductive or nursery grounds that could qualify as conservation areas. The full- and half-sibling pairs identified in this study were sampled in relatively close proximity to one another (<100 km apart), even when sampled several years apart, indicative of highly site-attached behaviour. Of particular interest was the high density of sibling pairs that occurred in the waters west of the Isles of Scilly. This area corresponded to an area thought to be 'biologically important' by Bendall et al. (2018) where there were increased catches of immature and mature males and females, and the occurrence of sexually active males and egg-bearing females had been observed. Our results therefore support earlier observations, and identify a site warranting further investigation, one that could perhaps benefit from targeted conservation actions.

This study allows us to evaluate sampling-design considerations for improved implementations of CKMR on *D. batis* and other elasmobranchs sharing similar life-history characteristics. We could establish that the sampling effort required for a large-bodied skate might conceptually lie between that of a 'teleost fish' (e.g. Bravington et al. (2016a) identified 45 parent-offspring pairs among 14,000 genotyped southern bluefin tuna, estimating an adult abundance of ~2 million) and a 'shark' (e.g. Hillary et al. (2018) identified 21 HSPs among 100 genotyped white sharks, estimating an adult abundance of 280-650). Though we effectively maximised the genotyped sample size in our study based on DNA quality and population dynamic considerations, we estimate that in order to obtain CKMR estimates at the precision required of fisheries stock assessments (i.e. with a CV of 0.15, corresponding to ~45 kin-pairs, according to Bravington et al. (2016b)), sampling should continue for the Celtic Sea *D. batis* population until ~1,100 individuals have been genotyped, based on the assumption that the number of kin-pairs identified increases exponentially with sample size (Bravington 2016a; Appendix 1). Furthermore, the site-attached behaviour of *D. batis* suggests that sampling effort should be increased longitudinally and across several stations in order to avoid sampling litter mates (i.e. siblings born in the same year), which complicate CKMR as they violate the assumption of independent samples (Bravington et al., 2016b).

The variable catchability of elasmobranchs by different sampling gears is also an issue to consider. The trammel-net surveys captured mostly sub-adults and young adults (L_T = 66-148 cm; Table 1). The relative lack of small juveniles may have limited the number of cohorts sampled and might explain the absence of parent-offspring pairs in our samples, which would have enabled total adult abundance estimation using a parent-offspring pair CKMR approach (Bravington 2016a, b). In contrast, the bottom trawl data used in the CPUE estimates of Barreau et al. (2016) contained a wide range of size classes (L_T = 20-140 cm), including an abundance of juveniles. These patterns suggest that alternative sampling methods such as trawling may yield a wider size (and age) range of skates that could be advantageous for CKMR abundance estimation, although they may be more destructive.

We further highlight the preliminary age-at-length key used in this study (Barreau et al. 2016), which has yet to be validated but provides the best available age estimator for *D. batis* at the time of writing. Accurate age estimation remains a challenge in elasmobranch population assessment, often requiring lethal sampling to obtain vertebral growth readings. However, novel statistical approaches provide an opportunity to model age based on individual size, though these require considerable sampling effort (Régnier et al., 2021).

In summary, our CKMR and N_e estimates provide a first approximation of the number of breeding adult *D. batis* in the Celtic Sea, but they would benefit from further validation by other methods. At present, experimental validation of CKMR has been limited to parent-offspring pair models in riverine brook trout (*Salvelinus fontinalis*) populations using mark-recapture methods (Ruzzante et al., 2019; Marcy-Quay et al., 2020). The long-term tagging of *D. batis* in the Celtic Sea, initiated by Bendall et al. (2018) and Barreau et al. (2016), may provide a useful dataset to validate our CKMR estimates once a sufficient number of individuals are recaptured.

The results improve the status of knowledge for what is likely one of the few remaining populations of *D. batis*. Despite its apparent local abundance in the Celtic Sea, *D. batis* is relatively scarce across the rest of its distributional range (Frost et al. 2020; Delaval et al. *in review*), which has implications for the management of a local by-catch fishery. Despite the limitations discussed, CKMR has proven to be a relatively cost-effective method of estimating abundance, complementing other monitoring approaches. The results demonstrate the additive value of SNP-based genomic approaches to elasmobranch conservation research, which, with continued longitudinal sampling efforts involving a combination of analytical approaches, will lead to continuous improvements in our understanding of these endangered data-deficient species and lead to better conservation outcomes.

Acknowledgements

Author contributions

LRN and CSJ led and coordinated the project. AD, LRN, VB, SJH, and HJS designed the study. VB and SJH coordinated sampling efforts and contributed biological knowledge on *D. batis*. AD and MF prepared the DNA samples for sequencing. AD performed the data analysis with inputs from all authors. AD wrote the first version of the manuscript. All authors contributed to the final version.

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Appendices

In order to investigate the feasibility of CKMR in our study system and design an optimal sampling protocol given the samples available, we performed a preliminary analysis on 387 blue skates from the Celtic Sea collected during CEFAS surveys between 2011 and 2017, which were DArTseq™-genotyped in a recent study of population structure (Delaval et al. *in review*). This allowed us to i) establish the level of precision with which the genotyping panel can identify related individuals (i.e. calculate false-positive and false-negative rates), ii) tailor our research questions, and iii) design an optimal sampling strategy to address those questions.

Among the 387 skates we identified one full-sibling pair and seven half-sibling pairs, and false-positive rates in relationship tests were very low (FPR ≤ 5.62×10^{-56} using the R package CKMRsim, Delaval et al. unpublished data), suggesting the genotyping panel identifies kin-pairs with high confidence. Kin-pairs were found in close proximity to one another, and there was limited gene flow with neighbouring populations (Delaval et al. *in review*). Therefore, for the purposes of the CKMR model, we assumed the Celtic Sea population to be a closed population.

CKMR generally consists of two approaches. The first identifies parent-offspring pairs to estimate total adult abundance, while the second identifies half-sibling pairs to estimate the number of breeding adults (Bravington et al., 2016b). The more related pairs are identified, the higher the precision of the model, and an ideal CKMR study is reportedly one in which the coefficient of variation (CV) is 0.15, which is achieved when a dataset includes at least 45 kin-pairs (Bravington et al., 2016b). Having primarily identified half-sibling pairs in our dataset, we opted for a half-sibling pair (HSP) CKMR model. Seven HSPs implies a CV of 0.38, so sampling effort would need to be increased to obtain precise estimates of population size. To achieve a CV of 0.15 would in theory require genotyping 1,034 individuals (equations shown below).

The maximum number of individuals we could genotype at this stage, due to budgetary constraints and DNA quality of the available material, was 683 individuals. With this sampling effort we might expect to find 21 HSPs (CV = 0.22). We focused our efforts on the HSP approach by primarily genotyping juveniles and young adults, while including a wide size range of individuals to minimize sampling bias and maximise the number of cohorts covered in our model.

Useful equations:

Coefficient of variation (CV):

$$CV = \frac{1}{\sqrt{H}}$$

where H is the number of related pairs found in the data.

Number of related pairs expected for a given sample size:

$$H_2 = \frac{H_1}{N_1 * (N_1 - 1)/2} * \frac{N_2 * (N_2 - 1)}{2}$$
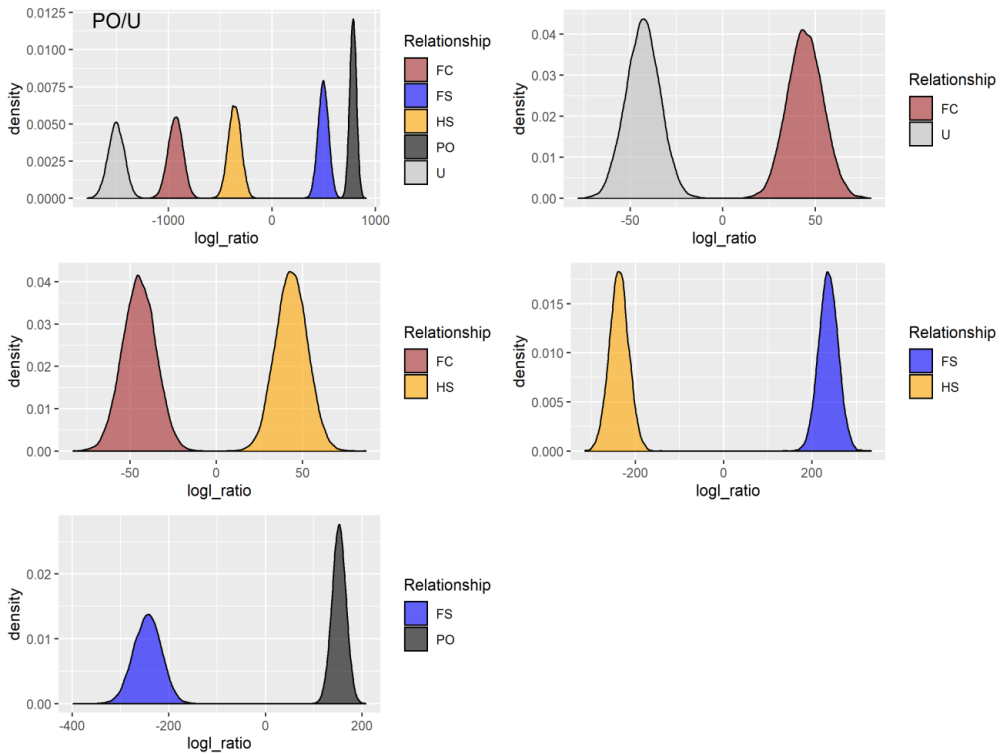
Where H_1 is the number of pairs found initially, H_2 is the number of pairs expected with change of sample size, N_1 is the initial sample size, and N_2 is the new sample size.

Appendix 2: Finding related individuals

Using the R package *CKMRsim* (Anderson, https://doi.org/10.5281/zenodo.820162), we simulated 20,000 related pairs of each of the following relationship categories using observed allele frequencies: unrelated (U), first-cousin (FC), half-sibling (HS), full-sibling (FS), and parent-offspring (PO). The simulations were used to define log-likelihood thresholds in relationship tests at desirable false-positive (FPR) and false-negative (FNR) rates. With 662 samples implying 218,791 pairwise comparisons of individuals for each test, we aimed for FPRs less than 4.57×10^{-8}. We performed tests comparing all possible relationship comparisons, but ultimately made our decisions based on the four following tests: FC vs. U, HS vs. FC, FS vs. HS, and PO vs. FS. In this order, we could a) identify which individuals were related versus unrelated, and b) progressively filter pairs of individuals categorically from less related (FC) to most related (PO), based on the defined log-likelihood thresholds for each test.

All tests produced desirably low FPRs associated with FNR thresholds as low as 0.0001. FNRs could not be reduced further as these represented computational challenges for the package.

Supplementary table 1: Decision making thresholds in four relationship tests involving unrelated (U), first-cousin (FC), half-sibling (HS), full-sibling (FS) and parent-offspring (PO) pairs, showing false-positive rates (FPR) associated with false-negative rates (FNR) of 0.0001, log-likelihood ratio (logl) cut-offs, and the number of positive hits for each test.				
Test	FNR	FPR	logl cut-off	No. pairs found
FC/U	0.0001	1.89×10^{-9}	11.9	51
HS/FC	0.0001	2.18×10^{-49}	9.29	19
FS/HS	0.0001	5.01×10^{-265}	155	2
PO/FS	0.0001	5.85×10^{-279}	98.4	0

Supplementary figure 1: Density distributions in log-likelihood (logl) space of 5 simulated relationships categories (20,000 simulated pairs for each category: unrelated U, first-cousin FC, half-sibling HS, full-sibling FS, and parent-offspring PO). The top-left panel depicts the relative positions of all simulations in a test of PO vs. U, for illustrative purposes. The remaining panels depict the density distributions used for each of four relationship tests, from which logl thresholds were determined (Supplementary table 1).

Supplementary table 2: Summary of relationship pairs among genotyped *Dipturus batis* from the Celtic Sea, identified using *CKMRsim* and *ML-relate*, and the Wang relatedness score (r) for those pairs calculated using *related*.				
ID1	ID2	*CKMRsim*	*ML-Relate*	r (*related*)
2015-16-240	2017-C10-4-16	FS	FS	0.5687
2017-C08-6-11	2011-264	FS	FS	0.4596
2017-C11-2-9	2017-C09-3-6	HS	FS	0.4351
2017-C14-21-7	2017-C14-21-10	HS	HS	0.3716
2015-15-144	2011-44	HS	HS	0.2533
2017-C02-15-10	**2014-80**	HS	HS	0.2625
2017-C13-20-4	2017-C13-20-5	HS	HS	0.3407
2015-16-242	2017-C05-11-6	HS	HS	0.2495
2011-268	**2014-80**	HS	HS	0.2454
2011-39	2011-42	HS	HS	0.2359
2011-302	2014-20	HS	HS	0.2586
2014-45	2014-14	HS	HS	0.2413
2017-C02-15-4	2011-214	HS	HS	0.2102
2015-18-253	2011-279	HS	HS	0.2169
2017-C02-15-6	2011-274	HS	HS	0.2159
2015-20-321	2017-C01-22-3	HS	HS	0.2219
2017-C07-7-6	**2011-39**	HS	HS	0.2104
2015-18-250	2017-C13-19-2	HS	HS	0.2342
2011-271	2011-308	HS	HS	0.2025
2015-15-231	2017-C02-15-6	U	HS	0.1553
2015-18-253	2011-41	U	HS	0.149
2011-53	2017-C12-1-4	U	HS	0.1519
2017-C09-3-8	2015-18-253	U	HS	0.1553
2011-310	2011-309	U	HS	0.1634
2015-20-412	2011-277	U	HS	0.1907
2017-C13-20-5	2017-C03-17-7	U	HS	0.1907
2014-50	2011-13	U	HS	0.1441

Appendix 3: Markov-Chain Monte-Carlo (MCMC) model parameters and outputs

Starting parameters:

$N^A_{y=0}$ = ln(10000)

λ = 0.01

φ^A = 0.90

Probability density functions (PDFs) used for each parameter:

$N^A_{y=0}$: Normal PDF with μ = ln(20000) and σ = ln(10000)

λ: Uniform PDF with lower bound of -1 and upper bound of 1.

φ^A: Uniform PDF with lower bound of 0 and upper bound of 1.

Method of updating priors (starting parameters):

$N^A_{y=0}$: Sliding window with window size of ln(2000)

λ: Sliding window with window size of 0.4

φ^A: Sliding window with window size of 0.4

MCMC parameters:

Number of iterations = 1 million

Sampling frequency = every 100 runs

Burn-in = 100,000

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Many elasmobranch species have experienced pronounced population declines during the last century, representing an important conservation and socioeconomic issue. Instituting appropriate conservation measures for elasmobranchs relies on a thorough understanding of their contemporary population structure, the identification of important habitat areas, and estimates of demographic parameters such as population size. Unfortunately, conservation efforts have been hindered by a lack of biological knowledge stemming from issues such as taxonomic confusion, misreporting of catches, and methodological constraints. Population genomic approaches are among the novel research methodologies that can help fill important knowledge gaps for data-deficient and critically endangered elasmobranchs.

In this thesis, we used population genomic approaches to address knowledge gaps relevant to the conservation of the Critically Endangered blue skate (*Dipturus batis*), one of Europe's largest benthic elasmobranchs. First, we sequenced the full mitochondrial genome of *D. batis*, a resource that will facilitate species identification and help resolve taxonomic uncertainties among hardnosed skates (Family Rajidae). Second, we used a genome-wide genotyping approach to assess the population structure of *D. batis*, estimate effective population sizes, and investigate patterns of selection along environmental gradients. Lastly, we evaluated the suitability of a novel demographic modelling approach (close-kin mark-recapture) as a monitoring tool for the Celtic Sea population of *D. batis*, and produced the first absolute abundance estimate for the species. In doing so, we also identified a potential area of critical habitat based on the spatio-temporal distribution of close relatives. Overall, this thesis revealed important biological patterns for *D. batis* that will have direct implications for its conservation, and demonstrates the value of genomic approaches in elasmobranch conservation research.